BIOMASS PRODUCTION OF ENERGY WILLOW UNDER UNFAVOURABLE FIELD CONDITIONS

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Abstract. The production of woody energy crops may offer a real alternative for the utilisation of unfavourable cropping sites during the coming years. An experiment with woody energy crops on rust-brown forest soil was set up in 2007 at the Crop Production and Biomass Utilisation Demonstration Centre of Szent István University in the town of Gödöllő. The experiment was aimed at studying five willow varieties (Tora, Tordis, Inger, Sven, Csala) at three different nutrient levels (control, fertilisers, compost). We were seeking for the nutrient treatment and the variety that would produce the best results in a two-year harvesting schedule.In 2009 the average (50.8 t/ha) of the plots where fertilisers were applied exceeded the control yield (38.6 t/ha) by 31.6% which was even exceeded by the plots where compost was applied (40.6 t/ha) by 5.2%. In 2011 the yields after the application of fertilisers (51.0 t/ha) and compost (49.2 t/ha) exceeded the control yield (37.5 t/ha) by 36.0% and 31.2%, respectively. In 2009 a 22.9% while in 2011 a 49.7% difference was found between the average yields of the two different groups of varieties, respectively. The Tordis and Sven varieties fell short of, while Csala, Inger and Tora exceeded the 40 t/ha two-year biomass yield in both 2009 and 2010. In view of the impacts of the different growing seasons further studies will need to be carried out in order to be able to choose the variety that is best suited to the given site.

Key words: *short rotation coppice, biomass, willow, fertilizer, compost*

Introduction

Owing to the scarcity of primary energy sources some 70% of Hungary's energy consumption is supplied from imports.

Renewable energy sources covered a mere 8.1% of Hungary's total energy consumption in 2009 according to Eurostat data. The European Union has set itself a target of covering an average of 20% of the energy sources of the 27 member states from renewable energy sources by year 2020 (Eurostat, 2012). The European Environmental Agency estimated that by 2030 some 20-30% of the total agricultural area of the European Union may be used for the production of energy crops (EEA, 2006). Hungary has also set itself a similar target (National Action Plan, 2010) but according to Eurostat's projection not more than about 13 percent should be expected to be achieved by 2020 (Eurostat, 2012).

Hungary has great potentials in terms of renewable energy sources. Particularly, the use of biomass and the utilisation of geothermal energy may play an important role in a longer term. Biomass production is currently the dominant form of Hungary's utilisation of renewable energy sources, as over 90% of the country's energy production from renewable sources originates from some form of biomass source (Szajkó, 2009; National Action Plan, 2010). During the forthcoming years this ratio will decrease but biomass will continue to be the most important renewable energy source in Hungary. The share of hydro and wind power in Hungary's energy economy is not likely to materially increase in the near future (Varga and Homonnai, 2009).

Three categories of biomass harvested from arable land for energy generation are distinguished: by-products of cropping, herbaceous and woody energy plants.

The utilisation of by-products of cropping for energy generation is limited by the growing shortage of organic matter in arable fields, as it is far more important to incorporate such materials in the soil either directly, or indirectly after utilisation as litter in livestock production in the form of farmyard manure, to maintain the quality and state of arable soils (Birkás et al., 2009).

Sites not suitable for economically efficient production of feeds or crops for human consumption are the areas that can be primary used for the production of herbaceous and woody energy crops. Decentralised power plants, relying on input materials that can be economically produced locally, may offer the best potentials for Hungary (Gyuricza, 2008). Such facilities may also have an important effect in the way of rural development, contributing to the restructuring of cropping in Hungary and to the creation of new jobs (Jolánkai, 2009).

The energy that can be produced on a unit area by growing energy crops is many times over the energy produced by growing cereals (Aylott et al., 2008).

Thanks to its continental climate Hungary has favourable conditions for the production of woody plant species such as for example acacia (*Robinia sp.*), willow (*Salix sp.*) and poplar (*Populus sp.*) (Tobisch et al., 2003; Ivelics, 2006; Barkóczy et al., 2007). These wood species are a source of a considerable amount of energy, e.g. about 19-20 MJ/kg energy can be generated from the soft-wood willow species after the wood has dried out (Demo et al., 2011; Mcelroy and Dawson, 1986; Mészáros et al., 2007).

Another argument in favour of the establishment of plantations is that apart from an occasional willow rust infection (Dawson and McCracken, 1995; McCracken and Dawson, 1998; PEI et al., 1993) and damage caused by willow leaf beetles (Ahman, and Lövgren, 1995; Larsson, 1998; Peacock et al., 1999; Sylvén and Lövgren, 1995) they do not tend to be attached by pathogens or pests, and in the majority of cases no crop protection activities are required in such plantations. Moreover, these plantations absorb considerable amounts of carbon-dioxide (Lemus and Lal, 2005; Galbraith et al., 2006; Sims et al., 2006).

The sizes and the total output of such of energy wood plantations in Hungary are way below the desirable: according to records kept by the National Food Chain Safety Office (NÉBIH) as many as 430 plantations had been established by end-2012 on a total area of 2169 hectares, with a 5.0 hectare available plantation size (NÉBIH, oral information).

Research on energy wood plantations has been varied in Hungary during the recent decades. Methods and technological variants that could be most securely and reliably applied in sites of different ecological conditions were worked out (Bai et al., 2008; Liebhard, 2009). Research has been focused on the choice of species and variety, the establishment of the optimum plant density, continued development of various vegetative propagation techniques, improvement of the planting technologies crop treatment and protection, methods and effects of crop nutrition and the storage, drying and other forms of utilisation of the harvested wood material (Ivelics, 2006; Barkóczy et al., 2007; Gyuricza et al., 2011).

The increasing frequency of weather extremes however, necessitates further studies to ensure optimum nutrient supplies for energy wood plantations established in unfavourable growing sites. Our study was aimed at showing the biomass outputs of an energy willow experiment on brown forest soil near the town of Gödöllő, in the case of different nutrient treatments.

Material and methods

Our woody energy plantation experiment was set up in 2007 at the Crop Production and Biomass Utilisation Demonstration Centre of Szent István University in the town of Gödöllő. According to the genetic soil classification system applied in Hungary the soil of the site of the experiment is a rust-brown forest soil on a sandy basis for the most part (Luvic Calcic Phaeozem). The rust-brown forest soil sub-type developed on tertiary sand and marl belongs to the 'Rannan' brown forest soil type. The processes of soil degradation have created a variety of a medium-depth fertile layer of low humus content.

The area is exposed to erosion, the soil's physical type is sand-loam, which is prone to settle. The top 20 cm layer is comprised of 53% sand, 26% loam and 20% clay. The 35 cm topsoil layer contains 26% clay, it has favourable water conductivity, in contrast to the poor water conductivity of the subsoil layer. The topsoil has a low humus and nitrogen content, but it is adequately supplied with potassium and phosphorous. Some of the key data of the soil of the experimental plot are summarised in *Table 1.*

***Table 1**. Major pedological data*

Genetic soil horizon	pH (H_2O)	K_A	humus	$CaCO_3$	Total salt	Total nitrogen	AL-P_2O_5	AL-K_2O
			%			mg·kg^{-1}		
A (0–40 cm)	6.76	30	1.32	0.00	0.044	16.8	371.1	184.0
B (40–60 cm)	7.08	40	1.04	0.00	0.052	11.9	33.0	112.0
BC (60–70 cm)	7.66	61	0.88	0.00	0.060	2.0	123.0	127.1
C (70–100 cm)	8.10	60	0.54	5.57	0.075	16.8	107.5	110.8

The climate in the area of the experiment is continental, with frequent weather extremes. The multi-year average temperature is 9.7 ºC. The average annual precipitation is 550 mm, two thirds of which falls in the form of rain during the growing season. Data of the weather during the years of our experiment (2007-2011) are presented in *Table 2*.

The experiment was of a two-factor type, in random block arrangement, in three iterations. The following five different willow variants and clones:

1. Csala (*Salix triandra* x *Salix viminalis 'Csala'*)
2. Tora (*Salix* schwerinii x *Salix viminalis 'Tora'*)
3. Tordis (*Salix* schwerinii x *Salix viminalis 'Tordis'*)
4. Inger (*Salix triandra* x *Salix viminalis 'Inger'*)
5. Sven (*Salix schwerinii* x *Salix viminalis 'Sven'*)

were used in the experiment, with three different nutrient supply levels in each case: 1 - surface cover with compost (50 t/ha); 2 - nitrogen fertiliser in the spring (50 kg/ha); and 3 - control without added nutrients. The compost and the fertiliser were both applied in early May, in the pairs of rows. The applied technology was one of a twin-row type, with 70 cm distances between the rows and 2.5 metres between the twin-rows. The space between the plants was 40 cm in the case of four variants (Tora, Inger, Sven and Tordis), while in the case of Csala, a variant of less vigorous growth, it was 30 cm. 25 cm cuts without roots were used for planting the trees, manually, in mid-April. Chemical weed control - with pendimethaline as active agent - took place in the year of planting in the twin-rows. This was supplemented by mechanical weed control using a rotary cultivator twice between the twin-rows. From year 2008 on, the spaces between the twin-rows were tilled with the rotary cultivator twice a year. There was no need for chemical control of pests and pathogens.

***Table 2**. Meteorological data of years of experiment (Gödöllő, 2007-2011)*

(Rainfall (mm)

Years	**April**	**May**	**June**	**July**	**August**	**September**	**Total (April - September)**	**Annual rainfall**
2007	5.8	44.0	63.2	21.8	69.0	46.0	249.8	518.2
2008	34.4	59.6	66.8	200.8	28.6	82.0	472.2	688.2
2009	2.0	28.0	54.0	18.0	27.0	4.0	133.0	392.2
2010	40.4	161.4	172.0	43.0	38.0	106.6	561.4	757.4
2011	4.6	25.2	45.8	59.0	4.6	1.0	140.2	272.8

Temperature (°C)

Years	**April**	**May**	**June**	**July**	**August**	**September**	**Average (April – September)**	**Annual average**
2007	13.7	18.6	22.6	24.1	22.9	14.1	19.3	12.1
2008	11.9	17.5	21.6	21.6	21.9	15.5	18.3	11.7
2009	15.4	17.6	18.2	22.6	21.8	18.3	19.0	11.2
2010	11.1	15.2	20.2	22.3	20.3	13.4	17.1	9.7
2011	11.6	16.4	19.9	19.9	21.2	19.0	18.0	10.8

The plantation was cut on 26 February 2008, after the year of planting, to encourage bud production. The complete two-year's growth was then harvested on 18 February 2010 and on 12 January 2012, when we also measured the quantity of the biomass. The dry mass and the moisture content was established after desiccating until reaching a constant mass at 105 ºC.

Statistical evaluation was carried out with the help of the SPSS.

Results and discussion

In the case of the two-year growth in 2009 the largest amount of biomass was harvested from the plot where fertiliser had been applied (*Table 3*). The two-year growth produced a 50.8 t/ha wet mass. The yield on the plot where fertiliser had been applied differed significantly ($SD_{5\%}$=4.4) from the biomass produced on the plot with compost and the control plot. There was no difference at a 5% significance level between the compost-covered plot and the control area: the former produced a yield 5.1% more than the latter. The modest yield-increasing effect of the 50 t/ha compost was explained by the fact that as little as 392.2 mm precipitation was recorded in the experimental area in 2009 - 133.0 mm of which fell in the form of rain during the period of April to September, the most important period for the growth of willow - so the nutrients contained in the compost could not decompose and pass down to the root zone. In a rainy year the retarded nutrient supply from the decomposing of compost may even be an advantage, since in this case hardly any of the nitrogen is bleached out of the soil (Adegbidi and Briggs, 2003).

The average biomass yield of the plots treated with fertiliser exceeded the control and the compost-covered plots by 31.6% and 25.1%, respectively. The easily dissolved fertiliser reached the root zone despite the shortage of soil moisture, where it could be taken up by the plants, increasing their growth. Moreover, by improving the water uptake of the plants the nutrient so utilised alleviated the symptoms caused by the drought. This is all the more important because the most intensive period of growth and water uptake often coincides in the case of willow species with the driest periods of the year (Hall and Allen, 1997; Lindroth and Bath, 1999).

Under unfavourable site conditions the yield of the energy wood plantation harvested every other year reached in 2009, even in the control plots, the levels measured in some international experiments (Cannel et al., 1987; Mcelroy and Dawson, 1993; Kowalik and Randerson, 1994; Labercque et al., 1997; Aylott et al., 2008,) and exceeded those measured in other studies (Bullard et al., 2002a; Bullard et al., 2002b).

***Table 3**. Analysis of variance of biomass according to nutrient level in 2009*

	Sum of Squares	Degree of freedom	Mean Square	F value	Calculated significance
Between groups	1166.760	2	583.380	14.033	0.000
Within groups	1746.041	42	41.572		
Total	2912.801	44			

Nutrient level		**Mean difference**	**Standard error**	**Calculated significance**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Control	**Fertilizer**	-12.22933*	2.35435	0.000	-16.9806	-7.4781
	Compost	-3.99133	2.35435	0.097	-8.7426	0.7599
Fertilizer	**Control**	12.22933*	2.35435	0.000	7.4781	16.9806
	Compost	8.23800*	2.35435	0.001	3.4867	12.9893
Compost	**Control**	3.99133	2.35435	0.097	-0.7599	8.7426
	Fertilizer	-8.23800*	2.35435	0.001	-12.9893	-3.4867

* significance level 0.05

In the case of the two-year growth harvested in 2009 the largest biomass was produced by the Inger variety (48.2 t/ha), at a 5 percent confidence level its biomass yield significantly ($SD_{5\%}$=3.9) exceeded the yield of the Tordis (39.6 t/ha) and the Sven (39.2 t/ha) varieties (by 21.7% and 22.9%, respectively) (*Table 4*). Its biomass yield did not, however, statistically exceed that of the Csala (43.2 t/ha) and the Tora (46.4 t/ha) varieties at a 5% confidence level. No significant differences were found in the biomass yields of the other varieties. One reason for this is that the Tora, the Tordis and the Sven varieties have a largely common genetic background (Stackeviciene et al., 2010; Jereková et al., 2011).

Though Labecque and Teodorescu (2005) harvested a larger amount of growth in Canada a one-year harvest cycle calculated for a two-year period, however, the average annual precipitation there is 952 mm, i.e. they had more rain during the growing season only, than in Hungary during the whole of year 2009.

Table 4. *Analysis of variance of biomass according to varieties in 2009*

	Sum of Squares	Degree of freedom	Mean Square	F value	Calculated significance
Between groups	573.422	4	143.356	2.136	0.094
Within groups	2684.672	40	67.117		
Total	3258.094	44			

Varieties		Mean difference	Standard error	Calculated significance	95% Confidence Interval	
					Lower Bound	Lower Bound
Csala	Tora	-3.13556	3.86197	0.422	-10.9409	4.6698
	Tordis	3.68556	3.86197	0.346	-4.1198	11.4909
	Inger	-4.87778	3.86197	0.214	-12.6831	2.9276
	Sven	4.06444	3.86197	0.299	-3.7409	11.8698
Tora	Csala	3.13556	3.86197	0.422	-4.6698	10.9409
	Tordis	6.82111	3.86197	0.085	-0.9842	14.6265
	Inger	-1.74222	3.86197	0.654	-9.5476	6.0631
	Sven	7.20000	3.86197	0.070	-0.6053	15.0053
Tordis	Csala	-3.68556	3.86197	0.346	-11.4909	4.1198
	Tora	-6.82111	3.86197	0.085	-14.6265	0.9842
	Inger	-8.56333*	3.86197	0.032	-16.3687	-0.7580
	Sven	0.37889	3.86197	0.922	-7.4265	8.1842
Inger	Csala	4.87778	3.86197	0.214	-2.9276	12.6831
	Tora	1.74222	3.86197	0.654	-6.0631	9.5476
	Tordis	8.56333*	3.86197	0.032	0.7580	16.3687
	Sven	8.94222*	3.86197	0.026	1.1369	16.7476
Sven	Csala	-4.06444	3.86197	0.299	-11.8698	3.7409
	Tora	-7.20000	3.86197	0.070	-15.0053	0.6053
	Tordis	-0.37889	3.86197	0.922	-8.1842	7.4265
	Inger	-8.94222*	3.86197	0.026	-16.7476	-1.1369

* significance level 0.05

Similarly to the preceding harvest cycle in the case of the two-year growth cut in 2011 the largest biomass was produced in the fertilised plot (51.0 t/ha), 36.0% more than in the control plot (37.5 t/ha). There was no statistically confirmed difference ($SD_{5\%}$=3,5) between the average of the fertilised and that of the compost-covered plot (49.2 t/ha), in that two-year period the plot with the fertiliser applied produced only 3.6% more biomass that did the control. Compost produced 31.2% more biomass than the control plot (*Table 5*).

The 21.2% yield increase in 2011 in the plot with the compost in comparison to 2009 must have been enabled by the fact that the nutrients contained in the compost layer had decomposed and found their way to the root zone by that time. Under the unfavourable site conditions and in the soil that tends to settle the process took somewhat more time

(Epsein et al., 1976; Aggelides and Londra, 2000), though it was accelerated by the higher than average precipitation (757.4 mm) in 2010. Although year 2011 was a very dry year (272.8 mm precipitation), with only 140.2 mm rainfall during the growing season, the required moisture content was available from the preceding year's surplus.

A 0.4% yield increase was recorded in the fertilised plot in comparison to 2009. Since no nutrient had been applied in the control plot during the whole of the experiment, the biomass harvested in 2011 was 2.8 percent below the yield in 2009.

Table 5. *Analysis of variance of biomass according to nutrient level in 2011*

	Sum of Squares	Degree of freedom	Mean Square	F value	Calculated significance
Between groups	2505.439	2	1252.720	23.642	0.000
Within groups	2225.409	42	52.986		
Total	4730.848	44			

Nutrient level		Mean difference	Standard error	Calculated significance	95% Confidence Interval	
					Lower Bound	Lower Bound
Control	**Fertilizer**	-15.78547*	2.65797	0.000	-21.1495	-10.4215
	Compost	-15.87134*	2.65797	0.000	-21.2353	-10.5073
Fertilizer	**Control**	15.78547*	2.65797	0.000	10.4215	21.1495
	Compost	-0.08587	2.65797	0.974	-5.4499	5.2781
Compost	**Control**	15.87134*	2.65797	0.000	10.5073	21.2353
	Fertilizer	0.08587	2.65797	0.974	-5.2781	5.4499

* significance level 0.05

The year 2011 yield was highest among the varieties in the case of the one called Csala (53.6 t/ha), significantly higher than those of Tordis (39.6 t/ha) and Sven (39.2 t/ha) ($SD_{5\%}$=4.3) (*Table 6*). Tora's yield was 46.4 t/ha. The best variety (Csala) yielded 49.7% more than the least well performing variety (Tordis). The substantial difference was caused by the fact that the high performing Swedish varieties are characterised by poor drought tolerance (Lindroth and Bath, 1999; Wikberg and Ogren, 2004; Cochard et al., 2007).

Inger, which produced the largest biomass in 2009 yielded a 51.5 t/ha two-year growth in 2011. Though its yield was 4.0% below that of Csala, the difference between Inger and the other varieties could not be statistically proven at a 5% confidence level. Both varieties comprise the *Salix triandra* parental line (Stackeviciene et al., 2010; Jereková et al., 2011) which must have provided the higher resistance and biomass, but owing to Csala's slower initial growth could not appear in 2009 but was already manifested in 2011.

Csala's yield increased by 204.0% by 2011 in comparison to 2009. The yield increase was 6.8% in the case of Inger and 6.0% in the case of Tora. The Sven variety's biomass output increased by only 0.3%, while that of Tordis dropped by 9.5%. The reason for this may lie in the fact that for the clones produced in Sweden on the basis of the *Salix schwerinii* parental line it takes a shorter period of time to deliver their maximum biomass yield. As a consequence of the loss of foliage caused by unfavourable environmental conditions even the self-shading of the vigorously growing stand may result in a loss of yield (Bullard et al., 2002b).

Table 6*. Analysis of variance of biomass according to varieties in 2011*

	Sum of Squares	Degree of freedom	Mean Square	F value	Calculated significance
Between groups	2242.830	4	560.707	6.930	0.000
Within groups	3236.381	40	80.910		
Total	5479.211	44			

Varieties		Mean difference	Standard error	Calculated significance	95% Confidence Interval	
					Lower Bound	Lower Bound
Csala	Tora	4.39470	4.62909	0.348	-4.9610	13.7504
	Tordis	10.37654*	4.62909	0.031	1.0208	19.7323
	Inger	2.09553	4.62909	0.653	-7.2602	11.4513
	Sven	11.00125*	4.62909	0.022	1.6455	20.3570
Tora	Csala	-4.39470	4.62909	0.348	-13.7504	4.9610
	Tordis	5.98184	4.62909	0.204	-3.3739	15.3376
	Inger	-2.29917	4.62909	0.622	-11.6549	7.0566
	Sven	6.60655	4.62909	0.161	-2.7492	15.9623
Tordis	Csala	-10.37654*	4.62909	0.031	-19.7323	-1.0208
	Tora	-5.98184	4.62909	0.204	-15.3376	3.3739
	Inger	-8.28102	4.62909	0.081	-17.6368	1.0747
	Sven	0.62471	4.62909	0.893	-8.7310	9.9805
Inger	Csala	-2.09553	4.62909	0.653	-11.4513	7.2602
	Tora	2.29917	4.62909	0.622	-7.0566	11.6549
	Tordis	8.28102	4.62909	0.081	-1.0747	17.6368
	Sven	8.90572	4.62909	0.062	-0.4500	18.2615
Sven	Csala	-11.00125*	4.62909	0.022	-20.3570	-1.6455
	Tora	-6.60655	4.62909	0.161	-15.9623	2.7492
	Tordis	-0.62471	4.62909	0.893	-9.9805	8.7310
	Inger	-8.90572	4.62909	0.062	-18.2615	0.4500

* significance level 0.005

Conclusions

The majority of unfavourable sites that cannot be economically used for the production of other crops are suitable for energy wood plantations in Hungary. This is a costly investment therefore it is key that the species and variety that is the most productive under the given conditions should be planted at the different sites. Though there is ample and detailed literature on the theme, there is a relative shortage of experimental results produced specifically in circumstances prevailing in Hungary.

Our experiments show that willow plantations under the unfavourable site conditions of the Gödöllő region can produce the amounts of biomass described in reports on foreign experiments even in extremely dry years. The Swedish varieties used in our experiment (Tora, Tordis, Inger and Sven) produced the yields observed in their own genetic centres even in the Carpathian basin.

An amount of 50 kg/ha nitrogen fertiliser was enough in both years to significantly increase the biomass growth. It is recommended to be delivered to the fields concerned in the spring after harvest.

The yield increasing effect of compost was not observed to a statistically confirmed degree before 2011. The reason for this was that its nutrient content took longer to reach the root zone but in view of its soil protecting effects its use is always recommended right from the time of plantation.

In both of the two-year growing cycles (2008-2009 and 2010-2011) there was one dry and one rainy year, therefore in view of the year effects there is a need for further studies to make it possible to choose the willow variety best suited to a given site.

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CLIMATE CHALLENGES AND SOLUTIONS IN SOIL TILLAGE

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Abstract. The aim of this paper was to study the effects of climate induced phenomena occurred in arable soils in the first half of year, 2013. The problem was investigated in a long-term trial on a preserved Chernozem soil and further assessments were performed on Luvisols and a Gleysol which have seriously suffered from climate extremes. Eight types of the soil deterioration were studied from the formation to the state that has changed for the worse. These phenomena were as follows (1) dust formation in the soil surface as the effect of the periodic frost, (2) silting of the dust, due to the periodic and heavy rains, (3) dust leaching into the soil and extending the former compacted layer, (4) soil settling due to the repeated rainfall, (5) strong crust formation in the silted surface both in soils to be unsown and in the rows of the crops. (6) remaining the crusty structure in long-term, (7) over compacting of the seedbed-base, (8) limited crop rooting in the over settled soils. Soil condition improvement may be expected to take place after the passage of 2-3 years, in the wake of soil conservation tillage practices.
Keywords: *Dust formation, surface silting, crusting, dust leaching, soil settling*

Absztrakt. Klímakihívások és talajművelési megoldások. A tanulmány a 2013. év első félévében csernozjom, erdő és glejes talajokon bekövetkezett klímakárok értékelésére vállalkozik. Nyolc káros klíma jelenség kerül bemutatásra a kialakulástól a súlyosbodásig, amelyek (1) felszínporosodás a fagyok hatására, (2) felszín eliszapolódás az ismétlődő esők nyomán, (3) por lemosódás a legközelebbi tömör rétegig, (4) erős ülepedés, (5) káros felszín kérgesedés, (6) a kérgesedés tartós fennmaradása, (7) a magágy-alap túltömörödése, (8) gyökér deformálódás. A cikk a kár megelőzés és gyógyítás módszereit is bemutatja.

Introduction

Extreme climate phenomena, for instance drought stress, water deficit or water-logging, hail storms etc. have been afflicted the soils of the Carpathian basin in the last decade. The fundamental appearance of the climate damage is extreme shortage or abundance of precipitation, causing over drying and/or over wetting of the soils even within a single growing season (Garamvölgyi and Hufnágel, 2013; Ladányi et al., 2003). As Szalai and Lakatos (2013) noted, the tendencies in the precipitation sums, the number of precipitation events with threshold values, especially the more intense rain events shows tendencies having serious effects on the available water amount and the surface water balance. Classic authors have long been preoccupied with climate extremes having adverse impacts on agricultural production (e.g. Milhoffer, 1897). From the aspect of cropping the damage caused by the underlying factor is also affected by the site parameters, the actual soil fertility, nutrient supply and water regime (Várallyay, 2013). Research findings show that agricultural activities have contributed to climate change and that at the same time agriculture is one of the sectors adversely affected by climate damage (Jolánkai et al., 2013). Soil is an environmental element labelled by a variable state and quality, renewed or degraded (Várallyay, 2011). This renewal capability of the soil can be maintained by continuous treatment carefully aligned to the prevailing circumstances. Therefore the primary task of tillage is to maintain favourable soil quality and fertility and to prevent climate sensitiveness.

Birkás (2011) found, that the state of soils is another modifying factor in the degree of the climate stress. Some of the soil condition defects are visible to the naked eye, e.g. the dust formation, surface silting, surface crusting, clod and dust formation, however it does not help prevention since consequences are not being considered to be as serious as they actually are (Kalmár et al., 2013). Defects occurred in the soil, e.g. dust being leached to the compacted layer close to the surface, growing thickness of the compacted layer, extending of the crust on the top of the soil, increased settling of the loosened soil, are assumed to have been caused by lack of knowledge of their existence or their consequences (Kalmár et al., 2011; Birkás et al., 2012).

As authors (Baumhardt et al., 2004; Birkás, 2012; Kalmár et al., 2013; Várallyay, 2013) have already stressed, maintaining good soil quality and soil condition may reduce the effects of climatic extremes and degree of the damage.

Materials and methods

The problem referred to in this paper was studied in a long-term trial that has been underway since 2002 in a field of the Experimental and Training Farm of the Szent István University, located in Heves county, near to the town Hatvan (47°41’N, 19°36’E, 136 m a.s.l.). The research site is flat and the soil – Chernic Chernozem soil by WRB (2006) with a clay loam texture – is moderately sensitive to compaction (Csorba et al., 2011, 2012). Soil assessment was extended to the surrounding area of an approx. 10 km radius with similar type of soil. In this site the long-term annual precipitation is 580 mm. The last five years’ precipitation figures are as follows: dry (2011: -283 mm, 2012: -286 mm) and rainy (2010 +371 mm, 2013 in the first half of year +100 mm). Year 2009 was dry in the growing season. The experiment was of the single-factor type, in random stripe arrangement in four replications (Sváb, 1981), in which the following six treatments were applied: direct drilling (DD), disking (15 cm, D), shallow and medium deep cultivator (15 cm SC, 22 cm C) ploughing (32-33 cm, P) followed by surface consolidation and loosening (40 cm, L). *Table 1* presents types of the soil phenomenon and the time of the measurements.

Table 1. *Assessed soil state phenomena and place and time of measurements*

Phenomenon	**Soil**	**County**	**Time**
Dust formation by frost	Chernozem	Heves	March 2010, 2013
Surface silting	Chernozem	Heves	April 2013
Dust leaching and extending former compacted layer	Chernozem	Heves	Growing season 2010, 2013
Soil settling	Chernozem, forest, gley	Borsod-Abaúj-Zemplén; Baranya, Virovitica-Podravina	November 2012-May 2013
Water-logging			
Extending of seedbed base			
Crust formation, Crust thickness	Chernozem	Heves	September 2012-July 2013

The measurements were completed and evaluated in accordance with the applicable standards (Csorba et al., 2011); Soil Sampling Protocol, JRC, 2010). Classic soil state measurement methods (Dvoracsek et al., 1957) were also taken into account. Soil surface silting and crusting occurred by rains and drying was recorded by visual assessment on a notation grid. The areas observed were 50 cm x 50 cm per variants and by using a quadrate device in six replications per plot. The thickness of the crusts and extension of seedbed base were measured on a soil cube (edge of 30 cm) extracted from the concerned areas. Dust leaching and soil settling were also measured on soil cubes and by using a handheld Szarvas-type penetrometer having a 1.0 cm^2 cone and a 60^o apex, at soil depths of 55 cm at each 5 cm increment, in at least six repetitions.

Further assessments were performed in other counties on forests (Luvisols) and gley (Gleysol) soils which have seriously suffered from extreme climate in the first half of year 2013 (*Table 1*).

The data were evaluated with the method of variance analysis, by Sváb (1981) and by using the Microsoft Excel 2010 program.

Results and discussion

Climate induced phenomena occurred in arable soils in the first half of year, 2013

Soil deterioration has strongly been occurred mainly in three periods, that is at the end of the winter, in the beginning of the spring (thereafter rainy period) and in the beginning of the summer (following May rains).

Dust formation in the soil surface as the effect of the periodic frost

The strong dust formation by the frost effect in the cold period is considered to the climate induced damage. The frost effect is regrettably misunderstood in the practice, because the “frost-crumbs” seemed to crumbs, however catching some frost-crumbs they turn to dusts. The dusting effect of the frost depends on the length of the cold period, the repeat of the frost, and largeness of the soil surface exposed to the frost. A large, cloddy soil surface is highly suffered from the frost effect than a smaller, levelled soil surface. For this reason, the ratio of the dust that can be found in the surface layer at the very beginning of the spring is also referred to the autumnal soil tillage quality.

The ratio of the dust formation can be ranked by the degree of the damage, that is:

<10 %: slight (negligible)
11-30%: conspicuous
31-50 %: risky
51-70 %: serious
71-100 %: very serious

Fig. 1 presents data of the dust ratio measured in soil surface in case of different tillage methods and after a cold and a mild winter.

There were significant differences between two types of winter’ effect and between tillage variants. The dust formation was found higher after a cold winter included frost phenomenon in number and mainly in ploughed soil left it in cloddy state in autumn. Moreover, the degraded soil (Pdegr) seems in danger to a high degree than soil that has been preserved in long term. In the trial the P variant is also ploughing, but the surface is usually decreased by packer just when ploughed, and for this reason the frost effect has been succeeded to a lesser extent. References were no found in concerning literature about pulverisation effect of the frost. However, Dagesse (2013) proved a lower aggregate stability when freeze and thaw changed in cycles. Others, e.g. Sinha and Cherkauer (2010) threw on further threat, that is increasing the frequency of freeze-thaw cycles may increase the risk of soil erosion in the arable sites.

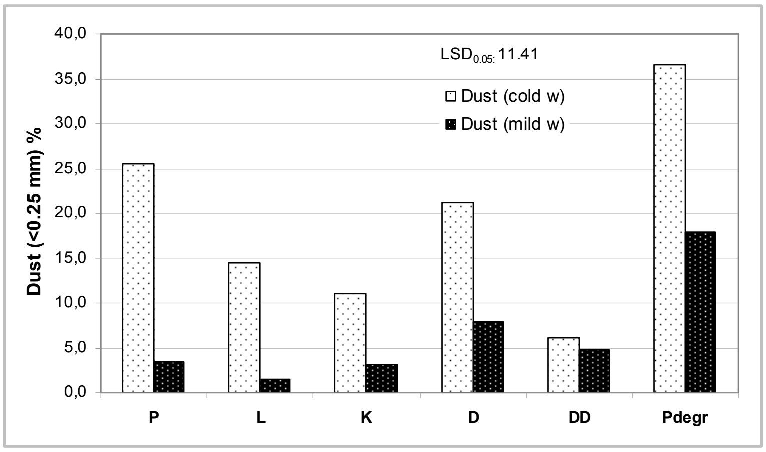

Figure 1. *Dust ratio in soils following a cold (2013) and a mild (2010) winter (Hatvan) Legend: P: ploughing and levelling, L: loosening. K: cultivator use, D: disking, DD: direct drilling, Pdegr: ploughing, no levelling, in degraded soil*

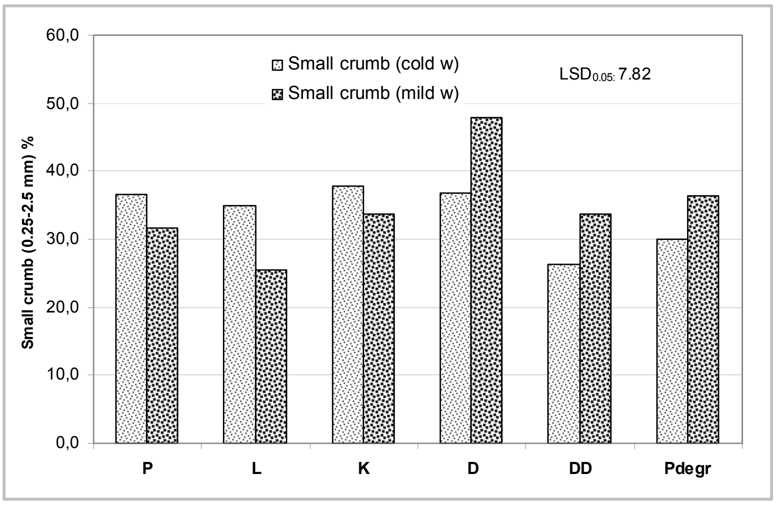

Figure 2*. Small crumb ratio in soils following a cold (2013) and a mild (2010) winter (Hatvan) Legend: see Figure 1.*

Fig. 2 shows ratio of the small crumbs assessed in soil surface at different tillage methods and after two types of winter. The small crumb formation, on account of pulverisation requires higher attention following critical periods (e.g. winter, rainy spring or summer). According to the climate and farming circumstances the small crumbs may transform to crumb or, in case of the unfavourable influences they pulverise by progressive stages. The frost effect was higher at the D variant that is in disked soil, and after a mild winter, and lowest in the loosened (L) soil following a cold winter. The small crumb ratio was showed a normal level (while greater part of the fractions transformed to dust) in the degraded soil (Pdegr) in both period.

Silting of the dusty soil

The silting is the outcome of the dust formation in recurrent rainy periods and after-effect of this phenomenon is the crusting in warm and dry days. The degree of the silting, out of the precipitation depends on the soil quality (that is degraded or preserved), and on the covering state of the surface. As Morris et al. (2010) outlined that soil surface exposed to rainfall often leads to the breakdown of aggregates. This process leads to the displacement of small soil particles forming a more continuous structure (mud film) that creates a surface seal and later on a surface crust.

The ratio of the surface silting can be ranked by the degree of the damage, similarly to the dust formation (e.g. <10 % is slight, and 31-50 % is risky, and 51-70 % is serious etc.). *Fig. 3* represents data measured in silted soil surface in the end of the rainy periods. On the basis of the ranking the degree of the damage at the variant bare surface and degraded soil is considered to be serious, at the variant of bare surface and preserved soil and the covered and degraded soil to be risky, however, at the covered surface and the preserved soil is seemed negligible. Surface silting was significantly decreased by the surface cover and in the same way a significantly lower ratio of silting occurred in the preserved soil (*P > 0.01*).

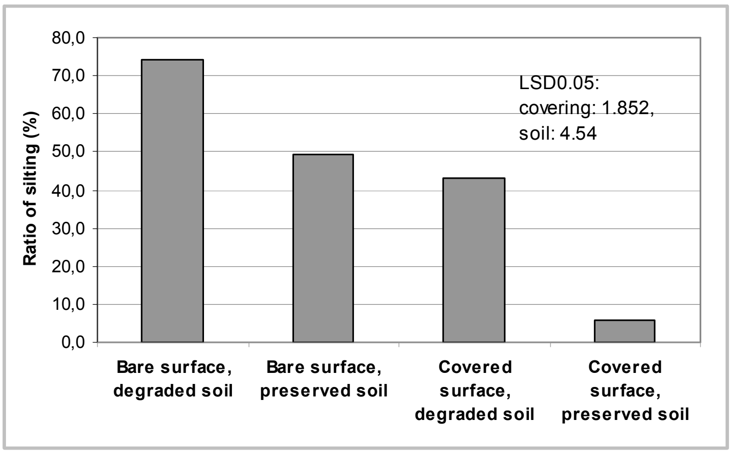

Figure 3. *Impacts of surface cover and soil quality on soil surface silting in a rainy period (Hatvan, April, 2013)*

Dust leaching

Dust formation in the soil surface has become a noteworthy phenomenon considering the combined effect of the multi-traffic tillage and the extreme climate. The dust in soil surface is usually labile matter that may remove by wind or rainwater (Baumhardt et al., 2007). Some parts of the dust remain in the surface as a silt film and other parts leach into the soil and agglomerate to the nearest compact layer and increase thickness of this layer (*Fig. 4*).

The leached dust mixed with soil mineral particles constitutes a most firm condition (Dexter, 1988; Kalmár et al., 2011). This phenomenon explains the formation of large clods at any loosening tillage and higher resistance of soil. Close coherence was found between dust ratio of the surface soil layer and the extension of the soil compaction (*Fig.4*). After all the increase of the dust ratio in the surface layer and increase of the leached ratio that aggravate the thickness of the former compacted layer. Birkás (2012) and Gao et al. (2012) proved that compacted layer is considered to be serious when that increases soil penetration resistance and causes water stagnation above the consoled layer and it extends of 20-25 mm. It was found that soil cover is assumed to have played an important role in retaining the moisture moving up from the deeper layers of soil – without any compact layers that would have impeded water transport – towards the surface.

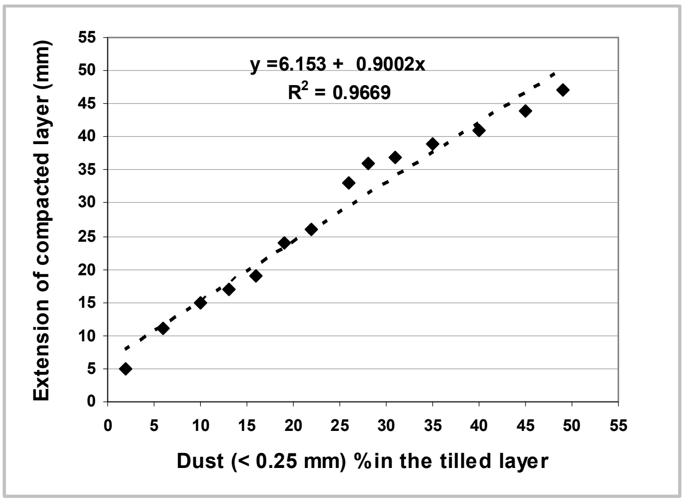

Figure 4. *Relations between surface dust ratio and the extension of the compacted layer*

Soil settling

Soil settling has become a typical phenomenon in the regional soils in the first half of year, 2013 but it strongly depended on the soil quality. A quite intensive settling effect was found on degraded soils and on soils having low organic matter content (*Table 2*). According to Lamandé and Schjønning (2011) the transmission of stresses in the soil profile is leading to either persistent deformation or elastic deformation.

Table 2*. Degree of the soil settling (mm) in different sites (November, 2012 – April, 2013)*

Chernozem soil (Hatvan)		**Forest soil** (Northern Hungary)		**Forest soil** (South-Hungary)		**Gley** (Slavonia)	
Prevented	Degraded	Prevented	Degraded	Prevented	Degraded	Prevented	Degraded
11	37	19	43	21	50	19	36

The extension of the soil settling, as compared to the soil state at sowing or after surface levelling, can be ranked by the degree of the damage, that is:

<15 mm: slight (normal)
16-25 mm: conspicuous
26-45 mm: risky
46-75 mm: serious
75-100 mm: very serious

On the basis of the ranking the degree of the settling was slight (11 mm) in the prevented soil (in field trial), and showed a remarkable stage (19-21 mm) in other prevented soils. However the degree of the settling was found to be risky and serious at the degraded soil variants.

Over-consolidation of the seedbed-base

In April, 2013 the seedbed preparation was completed in soils that were dry and crusty in the surface and wet below 5-6 cm and for this reason seedbed-base turned to over-compacted. The natural drying of the soils was lasted considering the hard and thick crust in the surface and by this means seedbed preparation tools have inevitably kneaded and thickened the seedbed base. Similar findings were cited by Fang et al. (2007), Guo and Wang (2013), and by Mueller et al. (2013).

***Table 3**. Depth of crops rooting at different soil condition (July, 2013)*

Crop	Roots	Extension of seed-bad base (cm)				
		≤ 5.0	5.1 – 10.0	10.1 – 15.0	15.1-20.0	≥25 (extreme)
W. oilseed rape	main	48.2	45.2	41.1	32.8	26.7
	lateral	25.8	22.0	20.1	16.3	12.8
Maize	main	32.1	29.4	27.4	25.1	24.5
	lateral	22.7	20.3	17.3	15.5	12.2
Sunflower	main	40.6	38.6	36.3	32.8	30.5
	lateral	24.1	22.5	20.5	18.1	15.2

n=70/crop

Due to the rainy period after crops sprouting, the main roots have penetrated the compacted layer (*Table 3*). However the lateral roots has grown horizontally (mostly above the seedbed base). This phenomenon is free from risk in a normal season when precipitation soaks the soil every 7-10 days. This is highly risky in a long-term dry period while main root is capable only to water intake from the deeper soil layers. For this reason, crops are more sensitive to the drought than in case of the normal rooting state.

Crust formation

Crust formation in the silted surface considering the frequency pays more attention because no similar serious damages occurred in the last decades. In spring 2013 crusts have been formed both in soils to be unsown and in the row spacing of the winter and spring crops. Crust development follows several stages under the effects of cumulated rainfall and this phenomenon has followed with appropriate attention in the international research (e.g. Fang et al., 2007; Gallardo-Carrera et al., 2007; Mueller et al., 2013).

The degree of the crusting can be ranked by the ratio of the damaged surface in a unit area, that is:

<10 %: slight (negligible)
11-30%: conspicuous
31-50 %: risky
51-70 %: serious
71-100 %: very serious

Table 4 shows the changing of the crusted are in a long term preserved soil (in the experimental site) and in a soil to be in degraded state. The crust formation remained under risky degree over periods due to long-term soil conservation and carbon preserving tillage. However, a large crusted area was occurred at disking (D) variant after rainy period in the beginning of the growing season. This damage has gradually decreased parallel with the crop growth.

More serious crusting occurred at the Pdegr variant not only in stubble phase but during growing season as well. This finding may suppose that the cause of the crusting is the combined effect of the soil deterioration, and the soil silting in rainy periods (Birkás et al., 2012; Kalmár et al., 2013).

Table 4. *Ratio of the crusted area at different soil tillage variants (Hatvan, Sept, 2012 – July, 2013)*

Time	**P**	**L**	**K**	**D**	**DD**	**Pdegr**
24 09 2012, covered stubble	1	1	0.5	1	0	36.2**
22 10 2012, after primary tillage	0	0	0	0	0	0
08 03 2013, before sowing	5.10	3.45	1.80	15.18	1.06	11.05
08 03 2013, after sowing	0	0	0	0	0	0
16 04 2013*, after rainy period	0.65	0.49	1.43	84.05	0.03	86.25
10 05 2013, sunny and windy weather	4.08	2.95	1.25	22.65	0.11	74.15
13 06 2013, dry weather	3.72	2.57	1.65	11.32	1.17	72.35
05 07 2013, dry and windy weather	3.13	2.38	1.20	9.85	0.88	65.05
13 07 2013, very dry and windy weather	10.52	7.10	3.13	13.41	2.29	50.75

Legend: P: ploughing and levelling, L: loosening. K: cultivator use, D: disking, DD: direct drilling, Pdegr: ploughing, no levelling, in degraded soil,
**: between spring barley rows; **: bare surface*

Crust thickness

Both ratio of the crusting and crust thickness is a suitable soil quality indicator and they may give information about progress of the degradation (Dvoracsek et al., 1957). The crust is typically appeared in acidic, alkaline, and clay soils, however it is endangered other soil types (chernozem, medium heavy forest, ameliorated meadow soils) if the degradation process has begun started (Gallardo-Carrera et al., 2007). Serious crust has occurred in soils desiccated rapidly in April 2013 after a long-term rainy period when soil silting was unavoidable.

The crust thickness can also be ranked by the extension of the crust, that is:

<5 mm: slight
6-15 mm: conspicuous
16-25 mm: risky
26-35 mm: serious
>35 mm: very serious

Assessing the crust thickness at different soil quality and surface state (*Fig. 5*) a low degree of the damage can be stated at preserved soil variant (DD, K, L), mainly in stubble phase when surface was fully covered by stubble residues. The impact of disking (D) variant on crust formation shows the real risk of the applying disk tillage in long-term. Most serious crust formation was found at the Pdegr variant which calls attention again to the soil conservation requirements.

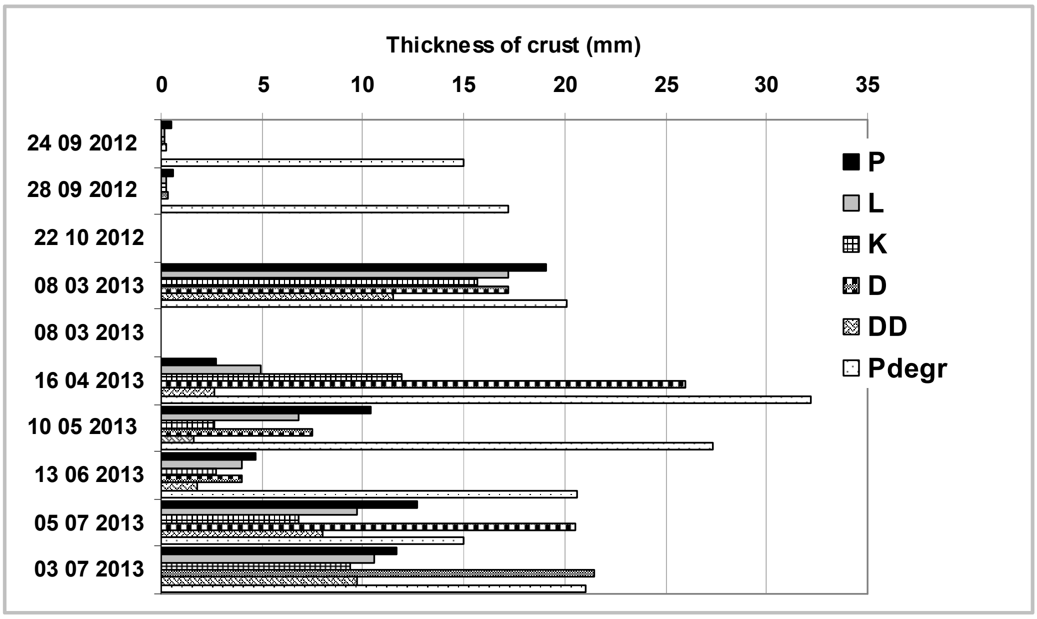

Figure 5. *Extension of the surface crust in preserved and in degraded soils (Hatvan, 2013)*

Water-logging

In the first half of year, 2013 a natural induced water logging has been occurred in lowlands and along the underground water veins. It can be stressed that this phenomenon is out of the farming negligence. However, the tillage induced water-logging has also been occurred both in the surface of the over settled soils and above the compacted pan layers. Trautner and Arvidsson (2003) and Gao et al (2012) described that this phenomenon is typical after-effect of the defective intervention in the soil condition and of tillage performing under constraint.

Conclusions

Soil structure is an important factor of agricultural soil quality, and its preservation and improvement are keys to preserving soil functions. As Dexter (1988) stressed, tillage alone cannot remedy a soil which has suffered severe structural degradation.

Tillage can be the first step in soil quality improvement. Next are based on findings derived from long term trials and from monitoring on soils that are suffered from climate induced damages in the first half of year, 2013.

The most important task to avoid frost-dust formation is the reduction of the tilled surface before wintering. The long-term crumb conservation combined with the soil organic material preservation and applying the carbon conserving tillage may prevent the dust formation that prior stage to the siltation in critical periods. Covering the surface in the required rate – min. 45 % but it may vary between 45-65 % – helps to maintain crumb formation and to prevent surface runoff. In a soil containing low ratio of dust, silting process may also lesser and crust formation appears at most sporadically.

Preventing the dust formation and the dust leaching which extends the former compaction layer are the most important steps in the alleviation steps. The depth of the inter-row cultivation is quite shallow and by this means it disturbs slightly the compacted seedbed base. That's all the more reason for the next sowing periods avoiding the over-consolidation of the seedbed-base in wet soil.

The natural induced water-logging is to be managed by regional water regulation. The solution of the farming induced water stagnation requires local and field level management.

The further tasks are to estimate the degree of the probable damage and then taking the initial steps in prevention and later in soil remediation.

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PHYTOSTABILIZATION OF MOSABONI COPPER MINE TAILINGS: A GREEN STEP TOWARDS WASTE MANAGEMENT

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Abstract. Bare metal mine tailings are prone to erosion as the very young top soils develop on unstable materials with low cohesion and also cause environmental pollution through leaching of toxic metals to surroundings. An appropriate vegetation cover may reduce the erosion and immobilize toxic metals through phytostabilization. The abandoned copper mines in Mosaboni (Jharkhand, India) left huge amount of untreated tailings containing high concentration toxic metals and became a source of metal pollutants. So, through a pot scale study an attempt was made to determine the potential of Cymbopogon citratus in phytostabilization of copper tailings with and without amendment. Although, limited plant growth was observed on pure tailings (T); addition of chicken manure (M) or soil-manure mixture (MS) caused manifold increase in plant growth. Application of M caused more accumulation of toxic metals in aerial parts indicating possible threat of entry of toxic metal into the food chain. However, use of MS reduced metal accumulation in plant tissue. So, in the present case, where there is no option for continuous harvesting and disposal of plant biomass in the secured landfill, phytostabilization is more appropriate than phytoextraction, and lemon grass could be used in combination with MS to stabilize bare tailings.

Keywords : Copper tailings, phytostabilization, pot experiment, heavy metal

Introduction

Mining activities such as crushing grinding, washing, smelting and all the other processes used to extract and concentrate metals generate a large amount of waste rocks and tailings. Bare tailings are very prone to erosion and often cause leaching of toxic metals from tailings to soil and water [1, 2]. Metal contaminated soil and water pose serious environmental and health problems worldwide because of bio-magnifications of toxic heavy metal in food chain [3]. The problem list also includes the loss of cultivated land, forest and the overall loss of production. [4]. These will eventually lead to loss of biodiversity, amenity & economic wealth [5].

Excavation of contaminated soil and storing the same in engineered landfill is a common practice to manage such metal rich soil or substrate. Such technology in one hand is very costly and at the same time cause dramatical disturbence to the existing landscape [3]. These problems are may be partially solved by an emerging new technology ‘Phytoremediation’. It is also receiving attention lately as the results from field trials indicate a cost savings compared to conventional treatments. The term

'Phyto-remediation' (Phyto=plants; remediation= correct evil) is relatively new, coined in 1991. Phytoremediation is the name given to a set of technologies that use different plants for containment, destruction, or an extraction technique.

Mine spoil or tailing dumps usually have barren surfaces, with rare plants that show signs of suffering such as stunted growth, chlorosis, nacrosis and anomalous development of roots with respect to shoots [6]. But also a number of plant species endemic to metalliferous soils have been found to accumulate metals at extraordinarily high levels (> 1% and up to 10%) in contrast to normal concentrations in plants called hyperaccumulators. So, far approximately 400 metal hyper accumulators have been identified [7]. The term hyper accumulator was first used by Brooks *et al.*, [8] in relation to plants containing more than 1000-10000 mg kg^{-1} of Ni in dry tissue. The idea of using plant which hyperaccumulate heavy metals for remediation of metal contaminated soil was first introduced in the 1980s [9] and in recent years this has been developed as an effective technique [10,11]. Hyper accumulator plants possess genes that regulate the amount of metals taken up from the soil by roots and deposited at other locations within the plant. The depositions vary between hyperaccumultor species. There are a number of genes contributing to the hyper accumulation trait. These genes govern processes that can increase the solubility of metals in the soil surrounding the roots [2] either by acidification of the rhizosphere through the action of plasma membrane proton pumps or by secreting ligand capable of chelating metal. As well as there are other gene codes for proteins that moves metals into root xylem. Actually plat have evolve this process to liberate essential metals from the soil but in case of soil with high concentration of toxic metals will release both essential and toxic metals. Basically the metals are introduced into xylem after passing the plasma membrane. The toxic metal when present in high concentration cross the membrane through pump and channels intended to transport essential elements.

The present study was carried out to determine the physico-chemical properties of copper tailing of Mosaboni copper-mines, comparison of growth, metal accumulation and distribution in *Cymbopogon citratus* grown on pure and amended tailings. Lemon grass was chosen for this experiment as it has ability to draw large quantities of water, rapid growth over the entire season and persistence for several seasons, easy establishment and also to withstand dry and wet conditions.

Study area

The Mosaboni underground Cu-mine is located at the Singhbhum shear zone in the Eastern India, Jharkhand (*Fig. 1*) and has produced enormous amounts of copper along with of Mo, Ni, Au, Ag, Pt, Co etc as byproducts. Copper ores occur as stringers and disseminations associated with host rocks quartz-chlorite-biotite schistose variants of Proteozoic age. The Singhbhum copper mines were in operation from 1850's to 1995 where complete copper refinement from mining of raw ore to smelting of refined ingot of copper along with considerable amounts of byproducts recovery was integrated on a very large scale. The deposit was exploited by underground mining formed by a series of quartz veins. Mineralization is mainly in the form of sulphides, Chalcopyrite ($CuFeS_2$) and pyrites are two major sulphides associated with smaller quantities of sulphides of Ni, Co and Mo etc. Magnatite with minor amount of Ilmenite and rutile constitute the main oxide minerals. The veins are located between schist, migmatitites and granites. The tailing was collected and brought from Mosaboni during December 2004 and the same was used for pot experiment.

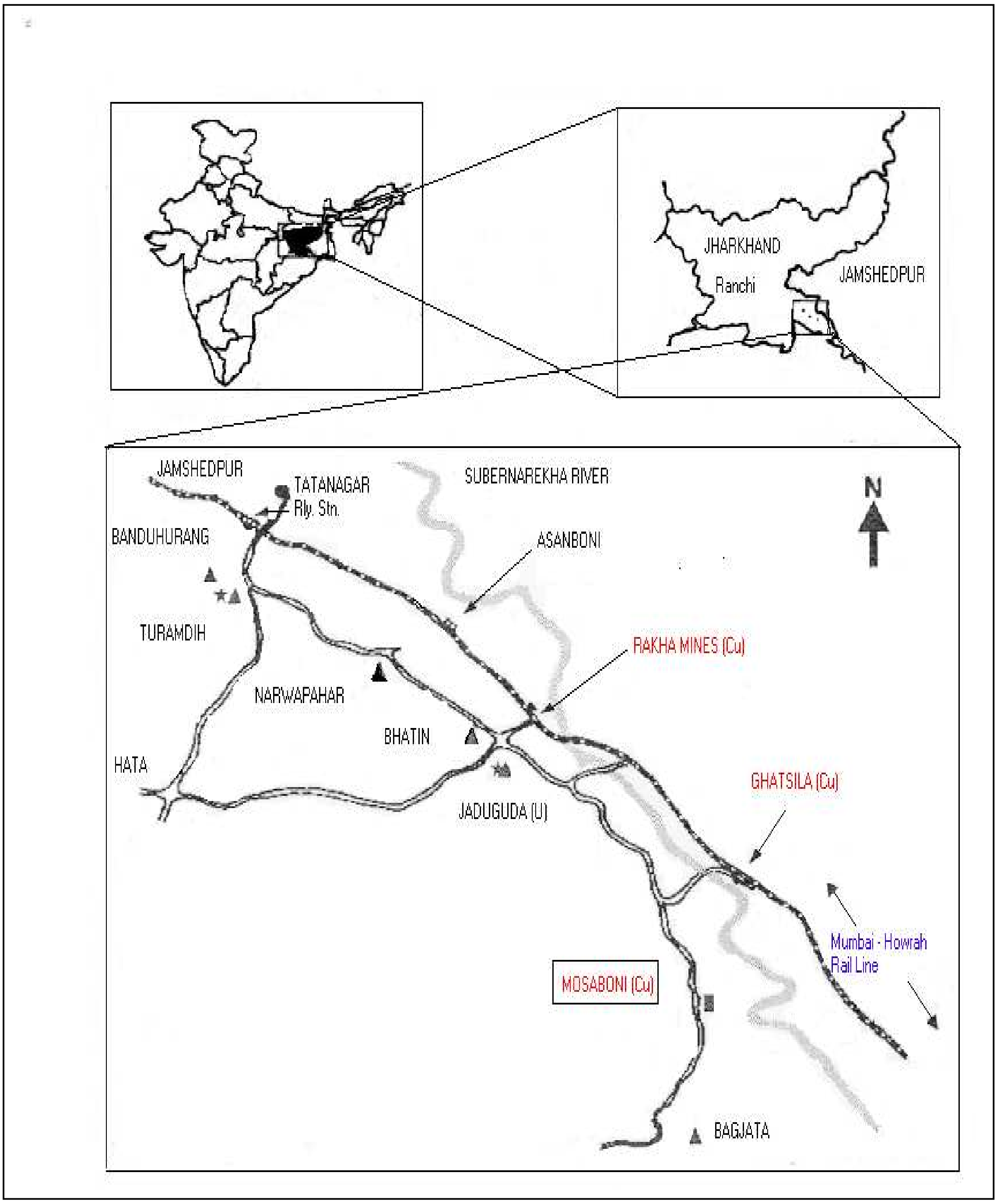

Figure 1: *Location map of Mosaboni Cu-mine area*

Materials and methods

Pot experiment

The pot experiment was carried out at Centre of Mining Environment, Indian School of Mines University, Dhanbad. Two different additives to copper tailings were used in the study: chicken manure and manure-soil mixtures. Soil was collected from the

Mosaboni area (crop field soil) whereas chicken manure was bought from local farmers of Dhanbad.

Three combinations of tailing and amendments (*Table 1*) and the grass species *Cymbopogon citratus* were used for the pot experiment.

***Table 1**. Composition of growth medium used for pot experiment*

Treatment	Composition (%)		
	Tailing (T)	Chicken manure (M)	Soil (S)
T	100	-	-
TM	97.5	2.5	-
TMS	97.5	1.25	1.25

Old clumps of Lemon grass growing on natural soil were collected and after proper washing and removal of the dried part, single clump was planted in each of the pot (3 replicates for each treatment). Experimental pots were then placed in the ISM garden under the nurture of nature.

Tailings, soil and chicken manure analysis

Tailings, soil, chicken manure and mixtures (used in pot experiment) were air-dried separately and then oven dried at 80^oC for 2 hours and sieved through 2mm mesh. A fraction of sieved samples was used for determination of pH, electrical conductivity, organic carbon, available nitrogen, cation exchange capacity [12]. Another fraction of dried samples was processed for environmentally available [13] and bioavailable Cu, Ni and Pb [14] content in samples. Metal contents in processed samples were determined by using Flame Atomic Absorption Spectrophotometer (FAAS), Avanta, GBC, Australia.

Plant sample analysis

After 6 months of growth experiment, plants were harvested carefully from each pot. The soil (tailings/sediments) loosely adhered to roots were gently shaken off and the rhizosphere soil adhering to roots were separated by hands. Individual plants were divided into two components: root and shoot, carefully washed in deionized water until visual inspection revealed that no solid particles remained adhering to the roots or shoots. Cleaned plant samplers were then oven dried at 80^0C overnight. Dried plant samples (shoots and roots) were ground to powder form in a mixer grinder. The powdered samples were digested with concentrated 10 ml conc. HNO_3 and conc. $HClO_4$ (5:1, v/v) as suggested by Yang *et al.* [15]. Metal concentrations in digests were determined by FAAS.

Result and discussion

General characteristics of tailing and additives used for pot experiment

The general properties of the tailings material are shown in *Table 2*. The tailing was slightly acidic in nature (pH 5.03) having low organic carbon (0.27%) and low available nitrogen (0.007%) content. The acidic nature of the tailing may be due to the release of protons produced by oxidation of sulphide minerals present in the Cu tailings. Low available nitrogen can be explained by the small contribution made by the organic matter (low content and low negative charge due to the acidity of the samples), as well as lack of microorganism, which make it bioavailable. The low cation exchange capacity [2.63 c mol (+) Kg^{-1}] may be due to the lack of inorganic colloids (sandy and sandy loam textures) and also of low organic carbon.

When comparing environmentally available metal (same as total metal excluding metal that bound in silicate matrix, which is not usually mobile in the environment), Cu (154 mg kg^{-1}) appeared as the most abundant heavy metal, followed by Ni (136 mg kg^{-1}) and Pb (9.9 mg kg^{-1}).

The analysis of the potentially bioavailable metal fraction is probably more significant than the analysis of total or environmentally available metal, because the former allows prediction of the risk of metal uptake by plants and its mobility in the system [6, 16]. In the present study, Cu was found most abundant metal in the bioavailable form followed by Ni and Pb, a sequence that is similar to that of environmentally available metal content in tailings. The bioavailable forms of Cu, Ni and Pb in tailings contributed about 12.5%, 0.8% and 8% to the environmentally available fraction of these metals, respectively.

The physico-chemical properties of different additives, used for pot experiment are also summarized in the *Table 2*. Chicken manure was slightly alkaline in nature and having elevated level of electrolytes, high nutritional status and low heavy metal contents. Soil material had intermediate nutritional status and almost neutral pH.

***Table 2**. General characteristics of Mosaboni Cu-tailings; Soil and Chicken manure*

Properties	**Mosaboni Tailing**	**Natural Soil**	**Chicken manure**
pH	5.03 ± 0.04	7.19 ± 0.07	7.45 ± 0.1
EC [dS/m]	0.15 ± 0.0	0.25 ± 0.02	7.69 ± 0.05
OC (%)	0.27 ± 0.02	1.43 ± 0.08	17.1 ± 0.007
Available Nitrogen (%)	0.007 ± 0.003	0.02 ± 0.004	0.379 ± 0.002
CEC(cmol (+) kg^{-1})	2.63 ± 0.03	11.9 ± 0.06	60.1 ± 0.040
Env available metal (mg kg^{-1})			
Cu	154.3 ± 1.9	25.9 ± 0.62	69.8 ± 0.31
Ni	136 ± 1.8	19.7 ± 0.23	17.4 ± 0.27
Pb	9.9 ± 0.9	46.3 ± 0.63	18.8 ± 0.16
DTPA available metal (mg kg^{-1})			
Cu	19.3 ± 0.78	0.5 ± 0.03	16.9 ± 0.37
Ni	1.1 ± 0.56	1.8 ± 0.01	1.6 ± 0.02
Pb	0.79 ± 0.01	1.19 ± 0.04	1.0 ± 0.02

Comparision of plant biomass

The biomass productions of lemon grass in tailing and different amended tailing are summarized in the *Table 3*. Use of additives like chicken manure or a mixture of soil and manure caused more biomass production compare to untreated tailings. Addition of chicken manure accounted 10 times increase in biomass production whereas tailings treated with soil-manure mixture produced about 8 times more biomass than pure tailings. In terms of number of tillers, treated tailings shown much better response than pure tailings.

***Table 3**. Comparison of plant growth in different amended tailing*

Treatment	Biomass, DW (g/m2)	No. of tillers/plant
T	27 ± 2	2.3 ± 0.6
TM	289 ± 42	7.3 ± 0.6
TMS	215 ± 26	6.3 ± 1.5

Comparison of metal accumulation

Regarding metal accumulation in root, lemon grass grown on pure tailing accumulated more Cu and Ni than the same species grown on the amended tailing. Whereas, shoot accumulation of Cu, Ni and Pb in the lemon grass, grown on amended tailing found higher than pure tailings (*Fig. 2*).

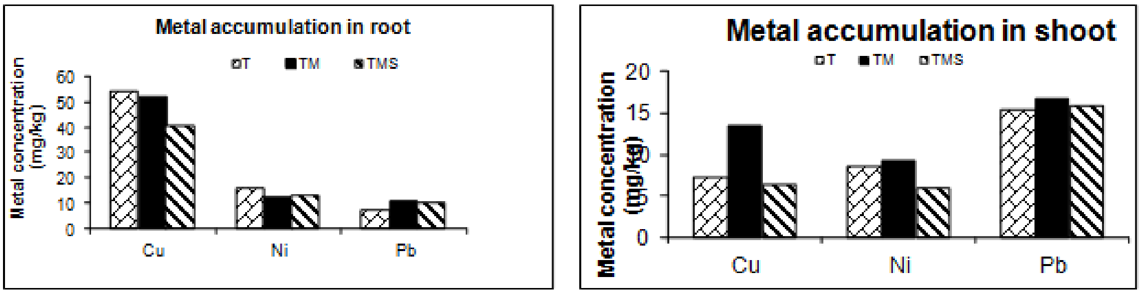

***Figure 2.** Metal accumulation shoot and root of lemon grass grown on pure and amended tailings.*

Conclusion

Copper tailing of Mosaboni mine was found acidic in nature and had low cation exchange capacity, elevated concentration of toxic metals and low nutrient contents. The most abundant heavy metal in both environmentally available and bio-available fraction was Cu followed by Ni and Pb. Concentration of Pb in both environmentally-available and bio-available fraction of normal soil was found higher than Cu-tailings. In all the samples concentration of total Cu and Ni were found exceeding the toxicity threshold limit [17]. Pot experiment revealed that despite of chemical limitations; lemon grass could grow on copper tailing but with limited growth. On the other hand, use of additives like chicken manure and soil could increase the growth (in terms of biomass and no. of tillers) in many folds. Addition of only chicken manure caused increase in metal accumulation by plant shoot but the opposite trend was found in metal accumulation by plant root. While, use of a mixture of chicken manure and soil decreased metal (except Pb) accumulation in both root and shoot of the lemon grass. Higher biomass production means, it would give rise to more addition of organic matter in tailing, and hence increase the moisture content which ultimately facilitate the growth of other native plants and stabilize (phytostabilization) the unstable tailings. High metal accumulation (phytoextraction) leads to removal of metals from tailing but it may results entrance of toxic metal into the food chain. So, in the present case where there is no option for continuous harvesting of plant biomass and disposal of the same in the secured dumping site, phytostabilization is more appropriate than phytoextraction, and lemon grass can be used for this purpose with addition of some additives. Use of a mixture of soil and chicken manure is more effective rather than only chicken manure because the former mixture results in high biomass production with lower accumulation of metals in plant's aerial parts.

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ANTHROPOGENIC ACTIVITIES AS A SOURCE OF HIGH PREVALENCE OF ANTIBIOTIC RESISTANT *STAPHYLOCOCCUS AUREUS* IN THE RIVER GANGA.

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Abstract.The presence, distribution and antibiotic profile of staphylococci was determined in River Ganga in Uttarakhand region (India). A total of 128 staphylococcal strains were obtained from 32 sites which corresponded to three species namely S. aureus, S. hominis and S. aegilis. S. aureus dominated the staphylococcal population comprising of 68.75% of the total staphylococci. The sensitivity of staphylococcal isolates against twelve antibiotics was determined. Majority of the isolates were resistant for erythromycin, while 17.6 % of S. aureus were resistant to methicillin. Interestingly, 93% of methicillin resistant Staphylococcus aureus (MRSA) was observed to be in the lower regions of river Ganga of Uttarakhand, facing severe anthropological activities. Water of the River Ganga is extensively used for drinking, religious bathing and cleaning purposes. However, the presence of pathogenic multiple drug resistant staphylococci indicate that direct consumption of untreated Ganga water and bathing in this stretch poses a great health risk.

Keywords: *S. aureus, Ganga, antimicrobial resistance, methicillin resistant Staphylococcus aureus (MRSA)*

Introduction

The surface waters in populous countries have become reservoirs of antimicrobial-resistant pathogenic microbes due to indiscriminate use of antimicrobials in human and veterinary medicine and addition of faecal contamination through point as well as non-point sources, storm drain infrastructure and malfunctioning septic trenches [1]. The number of antimicrobial-resistant (AMR) bacteria in the environment increases exponentially with the misuse of antimicrobials, as a result of increasing selective pressure on bacterial populations [25, 39]. Furthermore, reports of AMR are increasing continuously, and its spread between various distantly related bacterial strains in different habitats is well established [17, 40, 41]. The spread of AMR into environments where antibiotics are not used is a possibility that has not yet been well researched, although it has been postulated that water can be a potential source for dissemination of AMR [42]. The presence of AMR in aquatic sources is now considered an area of serious concern for human public health [20]. Recently, a dramatic increase in the resistance against antibiotics routinely used in human as well as in veterinary medicine has been recorded for the members of the genus *Staphylococcus*. Development of resistant or multiresistant staphylococci strains causes considerable therapeutic problems [29]. In fact, staphylococci are predominant pathogens in hospital-acquired infections, as hospital outbreaks due to methicillin-resistant *Staphylococcus aureus* (MRSA) have become a major problem in nososcomial infections [9, 32]. More recently, community-acquired MRSA has been documented among healthy individuals without any predisposing risk factors [16]. The recent increase in the resistance of staphylococcal isolates against antibiotics is alarming. Studies have established that contaminated sea and river water may serve as potential reservoirs of healthcare and community-associated MRSA, if such water sources become contaminated with these organisms [38].

The river Ganga is a major river of Indian subcontinent traversing 2510 km across the country. The river and its tributaries provide 40% of water requirement of the country for various purposes including irrigation, daily use and drinking [18]. In addition mass bathing in sacred water of Ganges is an age-old ritual in India. Organized outdoor bathing is an important *in situ* utilization of water bodies, which demands water quality requirements for drinking as well as bathing purposes [31]. There are several reports concentrated on the detection and survival of coliforms in the water of river Ganga [22]. Also, presence of multiple antimicrobial resistant *E. coli* has been reported from selected locations of river Ganga [28]. Several other members of *Enterobactericeae* and potential pathogenic genera in river Ganges of Uttarakhand (India) was reported [35] However; there is paucity of information on the presence and concentration of staphylococci and associated antimicrobial resistance in river Ganga. Therefore, this study aimed to isolate and characterize staphylococci, contextualize the dissemination of species diversity, along with determination of their antimicrobial-resistance profile along river Ganga in northern India (Uttarakhand).

Materials and methods

Study area and Sample Collection

The Gangetic River System of Uttarakhand was intensively surveyed to select different sites for sample collection. The study area was divided into three different stretches i.e. upper, middle and lower stretch. While Bhagirathi and Alaknanda both comprised upper and middle stretches, one in each tributary, the lower stretch was predominantly of Ganga, i.e. downstream to Devprayag (Sangam) till Haridwar (*Figure 1*). The total stretch covered in this study was 440 Km, out of which Alaknanda comprised a stretch of 200 Km, Bhagirathi comprised a stretch of 170 Km and Lower Ganga comprised a stretch of 70 Km.

The study was performed along 32 towns (*Figure 1*) situated on the banks of river Ganga in the state of Uttarakhand having the geographical coordinates of latitude in between 29°58′–31°03′N and longitude 78°13′–79°57′E (*Table 1*). The water samples were collected from selected 32 sites, in triplicate from margins and in the middle of the river; about 30 cm depth in 1000 mL sterilized bottles and were transported on ice to the laboratory. Physicochemical parameters such as pH and water temperature were determined using standard methods (4).

Isolation, enumeration and characterization of staphylococci

The strains were isolated by the direct plating (DP). Samples were serially diluted in sterile 0.85% saline and aliquots of 0.1ml were surface plated on mannitol salt (MS) agar [36] and Baird Parker (BP) agar medium [5], incubated at 37ºC for 24 h. After 24 h presumptive colonies were counted. Grey to black colonies, with or without halos, from each BP plate, and off white cream to yellow coloured colonies from MS agar were selected and transferred to slants of Tryptic Soy Agar, incubated overnight at 37ºC.

Table 1. *Different sites of sample collection and physical characteristics of water at respective sites*

Site Number	Name	Geographic location	pH	Temperature (°C)
1.	Badrinath	79º32' E; 30º44' N	7.2	1.2
2	Hanuman Chatti	79º31' E; 30º42' N	6.5	1.4
3	Pandukeshwar	79°34' E; 30°39' N	6.8	1.6
4	Vishnu Prayag	79°57' E; 30°57' N	7.4	5.6
5	Chamoli	79°21' E; 30°24' N	7.2	8.7
6	Nand Prayag-Nandakini	79°19' E; 30°19' N	7.8	20.7
7	Nand Prayag-Sangam	79°19' E; 30°19' N	7.8	17.6
8	Karan Prayag-Alaknanda	79°15' E; 30°16' N	7.6	17.6
9	Karan Prayag-Pinder	79°15' E; 30°16' N	7.5	22.8
10	Karan Prayag-Sangam	79°15' E; 30°16' N	7.6	20.1
11	Rudra Prayag-Alaknanda	78°58' E; 30°16' N	7.6	19.1
12	Rudra Prayag-Mandakini	78°58' E; 30°16' N	6.4	20.8
13	Rudra Prayag-Sangam	78°58' E; 30°16' N	7.4	19.2
14	Srinagar	78°46' E; 30°13' N	7.4	21.6
15	Kirtinagar	ND	7.4	19.2
16	Dev Prayag-Alaknanda	79°49' E; 30°74' N	7.6	19.8
17	Gangotri	78°55' E; 30°58' N	7.0	-1.6
18	Harsil	78°73' E; 31°03' N	7.4	1.1
19	Gangnani	78°67' E; 30°91' N	7.2	6.7
20	Maneri	78°53' E; 30°75' N	6.9	8.2
21	Maneri Dam	78°53' E; 30°75' N	7.2	8.1
22	Uttarkashi	78°45' E; 30°73' N	6.9	10.1
23	Chinyali Saur	77°49' E; 30°20' N	6.9	12.4
24	Tehri Dam	78°29' E; 30°23' N	7.1	12.1
25	Dev Prayag-Bhagirathi	79°49' E; 30°74' N	7.4	20.5
26	Dev Prayag – Sangam	79°49' E; 30°74' N	7.4	20.3
27	Kodiyala	79°04' E; 30°53' N	8.0	22.8
28	Shivpuri	78°38' E; 30°15' N	7.7	18.1
29	Rishikesh-Ramjhula	78°31' E; 30°11' N	7.8	19.2
30	Rishikesh-Lakshman Jhula	78°31' E; 30°11' N	7.8	19.0
31	Haridwar	78º13' E; 29°58' N	7.6	21.0
32	Haridwar-Har Ki Pauri	78°13' E; 29°58' N	7.6	20.1

ND: not defined

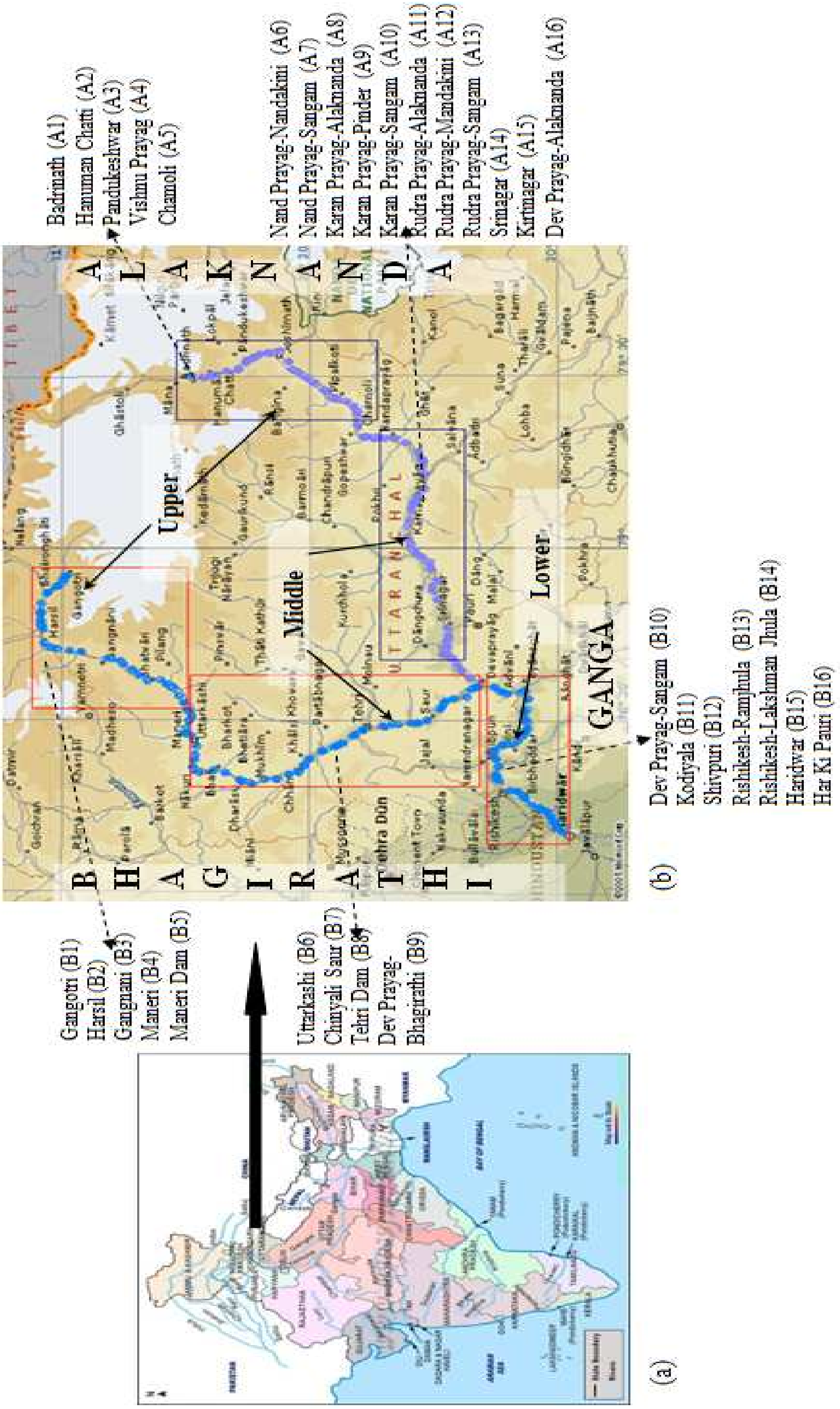

Figure 1. Location map of the study area

Each culture was submitted to Gram stain and tested for production of catalase and coagulase. All catalase positive Gram positive cocci were subjected to complete biochemical identification as described for the genus *Staphylococcus* in *Bergey's Manual of Systematic Bacteriology* [14].

Antimicrobial susceptibility testing

Antibiotic susceptibility was determined by disc diffusion assay on Mueller Hinton agar using Kirby Bauer method [7]. The strains were separately inoculated in nutrient broth and incubated at 37°C. Log phase cultures ($\approx 10^8$ cells ml^{-1}) were used for inoculation. Himedia octodiscs were used (Himedia, G-XIV and G-X-plus plus OD050) as per standard procedures. The following antibiotics were used erythromycin (10mcg), fusidic acid (10mcg), chloramphenicol (30mcg), vancomycin (30mcg), methicillin (10mcg), novobiocin (5mcg), streptomycin (25mcg). After incubation (37°C for 48 h), the plates were observed for the zone of inhibition surrounding the discs. The diameter of zone of inhibition was measured using a ruler. The zones of inhibition of test strains to various antibiotics were compared to those obtained for reference strain (procured from MTCC, IMTECH). *Staphylococcus aureus* MTCC 9542 was used as positive control in the study.

Amplification of ribosomal DNA

Strains were subjected to 16 S rDNA sequence analysis for establishment of their genotypic position. Genomic DNA was isolated using standard procedures [30]. Universal eubacterial primers 8F (5'-agagtttgatcctggctcag-3') and 492R (5'-gggttaccttgttacgactt-3') were used for amplification of 16S rRNA gene. The thermal cycling conditions were: initial denaturation of 3 min at 95°C followed by 35 cycles of denaturation of 1 min at 95°C, annealing of 56°C for 1 min, extension of 1 min at 72°C and a final extension of 10 min at 72°C. A 50 ml reaction mixture included 100 ng of bacterial DNA as template, 1 mM of each primer, 1.5 U of Taq DNA polymerase and 200 mM dNTPs. Amplified DNA was visualized in 1% agarose.

DNA sequencing and phylogenetic analysis

The 16S rDNA sequence was determined using ABI PRISM 377 DNA sequencer. The 16S rDNA nucleotide sequences obtained in this study was submitted in GenBank database. Searches in the EMBL/Gen bank/DDBJ/PDB data libraries were performed using BLAST (blastin) [2] search algorithm in order to identity of the isolate. Sequences of the close relatives were retrieved and aligned with the newly determined sequences. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 software [37] and phylogenetic tree was constructed by the maximum parsimony method.

Results and discussion

S. aureus has been well described as one of the most widely spread human pathogens. This could be as a result of its minimal growth requirements, ability to survive long in most unfavorable environments and to find a susceptible host. In present study, several staphylococci were isolated from river Ganga, that transverse through difficult terrain on mountains and faces mass bathing as an age-old ritual in Uttarakhand, India.

Prevalence and distribution of staphylococci in study area

As a result of rigorous sampling and careful culturing strategies, *Staphylococcus* species were isolated from 28 (87%) out of total 32 sites covering study area. In general, *S. aureus* is known to grow between 7 and 47°C, with an optimum of 30-37°C (24). However, in present study, coagulase positive-staphylococci were isolated from sites like Hanuman Chatti, Pandukeshwar, Vishnu Prayag, and Gangnani where water temperature was in range of 1.4-5.6°C. It is relevant to mention that human population is very low in these regions, and human interference is negligible because of heavy flow of water. Further, there were other regions completely devoid of staphylococci, like Gangotri and Harsil where water temperature was very low (i.e. -1.6°C and 1.1°C respectively). All the three sites at Karanprayag (Karanprayag-Alaknanda, Karanprayag-Pinder, Karanprayag-Sangam), were found free from staphylococci, in spite of the fact that the temperature at Karanprayag was suitable for its growth and survival (17.6°C, 20.1°C and 22.8°C).

Earlier, Bilgrami and Kumar [8] studied the bacterial contamination in water of the River Ganga at other regions like Bhagalpur, and found a marked correlation between bacterial density and physico-chemical quality of water including temperature and pH. In this study, the pH of water at the time of sampling (April, 2008) ranged from 6.4 to 8.0, while the temperature ranged from -1.6°C to 22.8°C for different sites. Further, Bilgrami and Kumar [8] also isolated several *Staphylococcus aureus* along with several other pathogenic bacterial strains from Ganga. Similarly, in present work, staphylococci were isolated from 87% of the sampling sites, while 62.5% of the samples had *S. aureus*. Therefore it may be concluded that direct consumption of untreated Ganga water and bathing, in stretch of flow may possess serious health risk.

Isolation, enumeration and characterization of staphylococci

The viable counts for *Staphylococcus* spp. ranged from 100 cfu/ml to 1900 cfu/ml (*Table 2*). The highest value was recorded from Haridwar-Har ki Pauri (1900 cfu/ml) on BP agar. These sites witness holy dip and mass bathing by a large number of pilgrims as an old age ritual in India, which is a constant source of contamination of water bodies [31]. Hence, the higher counts at lower stretch of the study area might be accounted to anthropogenic activities due to which high numbers of pathogens are introduced in the river water. Other ten less populated sites had viable counts as low as 100 cfu/ml, which support the hypothesis.

Baird Parker agar medium was observed to be a better medium for isolation of staphylococci from river water as compared to mannitol salt agar as evidenced by low cfu counts obtained on MS agar. Further, BP agar medium gave excellent recovery of *Staphylococcus* spp. and was inhibitory to other organisms. Baird-Parker agar has been recommended for the isolation of staphylococci from recreational water resources for its greater diagnostic qualities [33]. Also its value in ecological studies on the occurrence and development of *S. aureus* has been confirmed by other workers [10]. Interestingly, BP and MS, both media were found to be biased for the isolation of staphylococci at several sites. As example, no colonies were obtained on BP medium, but appeared on MS agar medium at sites like Hanuman Chatti, Tehri Dam and DevPrayag-Sangam.

Conversely, at sites like Rudra Prayag-Alaknanda, Kirtinagar, Dev Prayag-Alaknanda and Dev Prayag-Bhagirathi, no colony appeared on MS medium, while staphylocooci were isolated on BP agar. This may possibly be because of the fact that species of *Staphylococcus* are known to exhibit different growth pattern on BP and MS agar respectively. In this study, species other than *S. aureus* were isolated from different sites of the study (*Table 2*). Earlier, Bannerman [6] also observed that staphylococci other than *S. aureus* grow well on the BP agar medium.

A total of 128 staphylococcal strains were isolated from River Ganges. Majority of them belonged to three species of staphylococci, the most common being *S. aureus* (88, 68.75%) detected from 20 sites, followed by *S. hominis* (21, 16.4%) from 14 sites and *S. aegilis* (8, 6.25%) from 8 sites. Few strains could not be assigned to species level, therefore grouped as ‘other staphylococci’. Earlier also, *S. aureus* have been isolated from rivers and other aquatic habitats [19] even so that it was found in correlation to total coliforms for estimation of sewage pollution in northern coast of the Saronicos Gulf in Greece [11]. Also, *S. aureus* have been reported from other regions of river Ganga, along with other bacterial pathogens [8], though antibiotic sensitivity profile received little attention.

In addition, there are many reasons for potential concern over the presence of *S. aureus* in recreational and drinking water resources as *S. aureus* has ability to survive for longer period in water [3]. Although *S. aureus* is an important pathogen, many healthy people carry it as part of the normal population of micro-organisms associated with the nose, throat, perineum or skin and the nasal passages are also reported to harbour *S. aureus* in 10-50% of the healthy population [15]. Semwal and Akolkar [31] proposed that a considerable percentage of pilgrims visiting holy places and sacred Himalayan Rivers for bathing, carry skin and other communicable diseases. Additionally, some of these pilgrims settle nearby river banks and their daily routine near the water course becomes a constant source of contamination of the water bodies. The high population of *S. aureus* in River Ganga can be accounted to these anthropogenic activities. *S. aureus* in drinking water may also serve as a source for colonizing residents exposed to contaminated water [19].

***Table 2**. Quantification of Staphylococci species from the different sites of River Ganga by direct plating (cfu/ml) method*

Site Number	Viable count (cfu/ml)		*Species
	Baird Parker agar medium	Mannitol salt agar medium	
1	200	100	Sae
2	-	100	Sau
3	800	300	Sau; Sho; Ssp
4	300	200	Sau
5	600	700	Sau; Ssp
6	100	100	Sho
7	1200	300	Sae; Sho
8	-	-	-
9	-	-	-
10	-	-	-
11	100	-	Sau

12	1300	500	Sau; Sho
13	900	300	Sae; Sau; Sho; Ssp
14	1000	700	Sae; Sau; Sho
15	100	-	Sho
16	700	-	Sae; Sau
17	-	-	-
18	-	-	-
19	600	400	Sau; Sho; Ssp
20	1200	100	Sae; Sau; Sho
21	900	100	Sau
22	500	200	Sho; Ssp
23	100	200	Sau; Sho
24	-	100	Ssp
25	300	-	Sau; Ssp
26	-	200	Sae
27	800	700	Sau
28	1100	1000	Sae; Sau; Sho; Ssp
29	1200	100	Sau; Sho
30	1400	1100	Sau; Ssp
31	700	600	Sau; Sho; Ssp
32	1900	1400	Sau; Ssp

- Absent
*Sae: *Staphylococcus aegilis*; Sau: *S. aureus*; Sho: *S. hominis*; Ssp: other staphylococci

Incidence and distribution of MRSA

Results of antibiotic susceptibility testing showed multidrug resistance and variability in sensitivity and resistance patterns. Earlier also other workers have observed similar results for susceptibility patterns for staphylococci [13, 21, 26]. Prevalence of multidrug resistant staphylococci was recorded in river Ganga in present study. All the *S. aureus* isolates were resistant to either one or more of the antibiotics used in this study and complete sensitivity was not observed for any of the antimicrobial agent tested (*Figure 2a*). Majority of the isolates were resistant for erythromycin, while 17.6 % of *S. aureus* were resistant to methicillin. The number of isolates resistant to each antimicrobial agent were erythromycin (n = 69), augmentin (n = 49), streptomycin (n = 43), tetracycline (n = 41), fusidic acid (n = 39), novobiocin (n = 38), gentamicin (n = 28), chloramphenicol (n = 27), penicillin-G (n = 24), ciprofloxacin (n = 22), methicillin (n = 20) and vancomycin (n = 5). Thus the *S. aureus* from River Ganga in Uttarakhand, India showed high resistance to multiple drugs. Earlier, Tolba et al. [38] compared the survival ability of MRSA between saline and river waters, and concluded that MRSA may not survive well in river water as compared to sea water. Therefore, presence of 17.6% MRSA should be supposed as underestimation of contamination status due to its poor viability in river water, as actual count may be higher than obtained. Although the number of methicillin resistant *S. aureus* was low, the detection of such strains from river Ganga is alarming. Pertaining to the fact that prevalence of MRSA in hospitals has been increasing in India [27], its presence in river Ganga presents the possibility of dissemination in large community. Smith et al. [34] also emphasized that MRSA, besides having established itself as a major hospital pathogen, is now beginning to prevail in a wider community. Despite the development and introduction of several antimicrobial agents, MRSA infections remain an important cause of concern for the general public and physicians alike [23].

Further the isolation of MRSA from lower stretch of the study area was quite high as compared to those from higher stretch of the study area. Infact 93% of MRSA were isolated from the lower stretch of the study area. The prevalence of MRSA in the lower stretch of the study area can be also attributed to the fact that the lower sites experience higher anthropogenic activities in the form holy baths. So, these resistant strains might be introduced in the river water due to such activities. Asymptomatic carriage of MRSA in individuals using such waters allows the contamination of such waters with MRSA organisms. Such contaminated water may subsequently act as a transient environmental reservoir for MRSA, thus allowing the spread of MRSA among the population who use such sources for recreational and/or religious purposes [38]. Further the staphylococci isolated from the upper regions showed 90-95% sensitivity to most of the antimicrobials used in this study. This indicates that the strains indigenous to the Ganges have not acquired the resistance genes. Also, the low temperature conditions in the upper regions do not support the growth and survival of MRSA. However, if the contamination of river remains unchecked, soon the number of MRSA might increase the number of sensitive *S. aureus*. Regecová et al. [29] suggested that a rational use of antibiotics, complying with preventive measures in environmental hygiene and monitoring of existing resistance to antibiotics are very important weapons against spreading of antibiotic resistance.

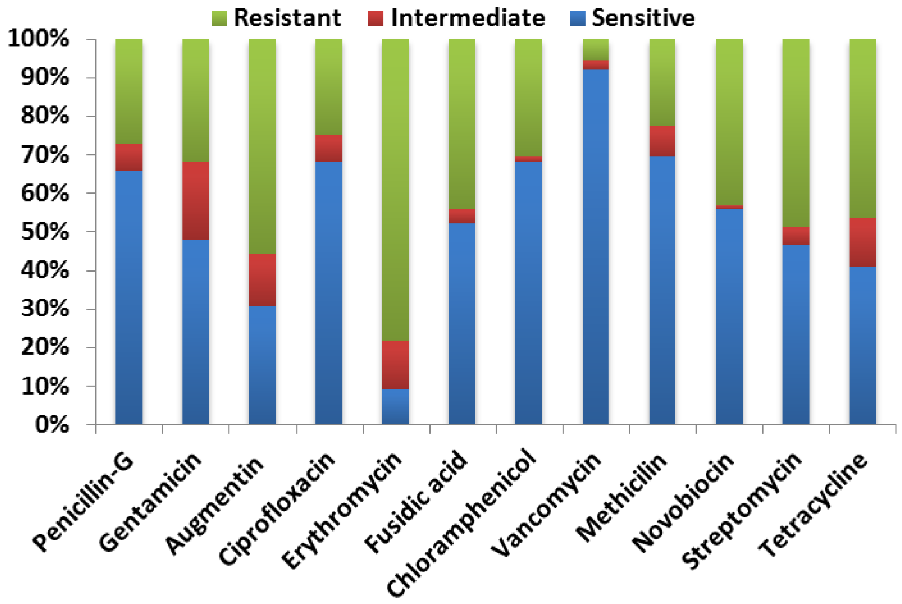

***Figure 2a**. Antibiotic susceptibility pattern of S. aureus isolates from River Ganga*

As a matter of fact, most of the *S. aureus* strains are known to be resistant to Penicillin-G [15], still very low resistance to Penicillin-G was observed in our study. The high sensitivity of *S. aureus* from river Ganga to this antibiotic might be attributed to non-pathogenicity and/or natural site of isolation of these strains. Although the vast preponderance of interest has been centred about the change from drug-sensitive to drug-resistant forms, certain epidemiological experiences suggest that drug-resistant bacteria sometimes revert to sensitive or are later crowded out by originally sensitive forms [12].

Antimicrobial susceptibility of S. hominis, S. aegilis and other staphylococci

Approximately 10% of the *S. hominis* strains were resistant to methicillin (*Figure 2b*). All the strains were sensitive to vancomycin and ciprofloxacin. The number of strains resistant to each antimicrobial agent were penicillin-G (n = 4), gentamicin (n = 2), augmentin (n = 11), erythromycin (n = 12), fusidic acid (n = 9), chloramphenicol (n = 11), methicillin (n = 3), novobiocin (n = 7), streptomycin (n = 7) and tetracycline (n = 8). On the other hand all the 8 strains of *S. aegilis* were sensitive to methicillin, vancomycin, augmentin and penicillin-G (*Figure 2c*). Further lesser strains exhibited resistance for rest of the antibiotics. The number of strains resistant to each antimicrobial agent were gentamicin (n = 2), erythromycin (n = 3), ciprofloxacin (n = 2), novobiocin (n = 3), streptomycin (n = 2) and tetracycline (n = 1). The other staphylococci showed complete sensitivity to gentamicin, erythromycin, vancomycin and methicillin (*Figure 2d*). 55% and 35% resistance was observed for tetracycline and novobiocin respectively.

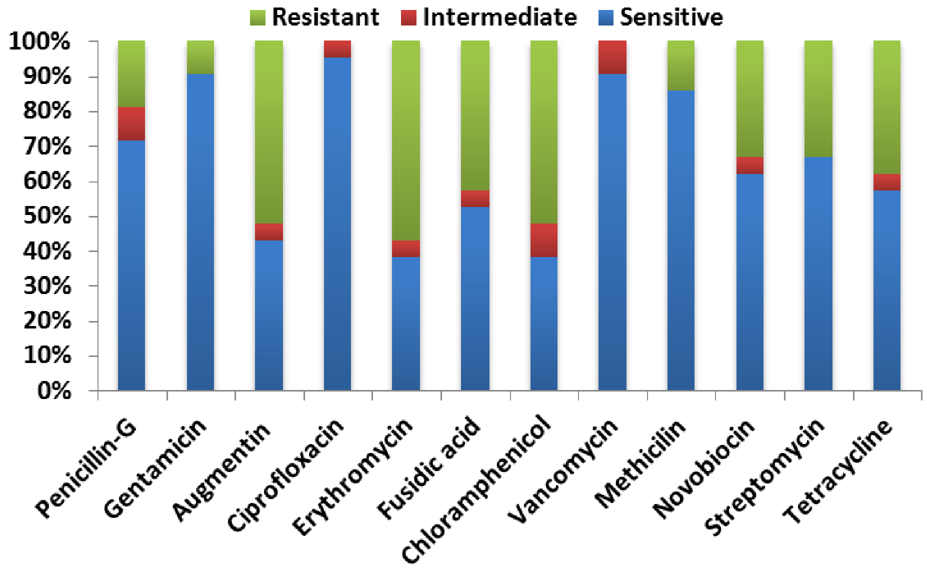

Figure 2b. *Antibiotic susceptibility pattern of* S. hominis *isolates from River Ganga*

Phylogenetic characterization of selected multiple drug resistant staphylococci

Selected multidrug resistant staphylococci isolated in this study (WB1P, RA3U1 and WB3T3) were subjected to 16S rDNA based molecular analysis to establish their phylogenetic position. These strains were isolated from Gangotri, Pandukeshwar and Gangnani respectively, all sites from upper region of the Ganges. A phylogenetic tree (*Figure 3*) was constructed based on evolutionary distances that were calculated with the Kimura two-parameter model. Alignment positions with insertions or deletions were excluded from the calculations. 16S rRNA gene sequence (NCBI GenBank accession numbers - EU882824, EU882823, EU882825 respectively) analysis indicated that the strains WB1P, RA3U1 and WB3T3 were phylogenetically related to members of the genus *Staphylococcus*; however the three strains clustered together in a separate minor clade. Other genera of family *Staphylococcaceae* were also taken into account in phylogenetic analysis, which formed a separate mega cluster. The biochemical properties of the strains are presented in *table 3*.

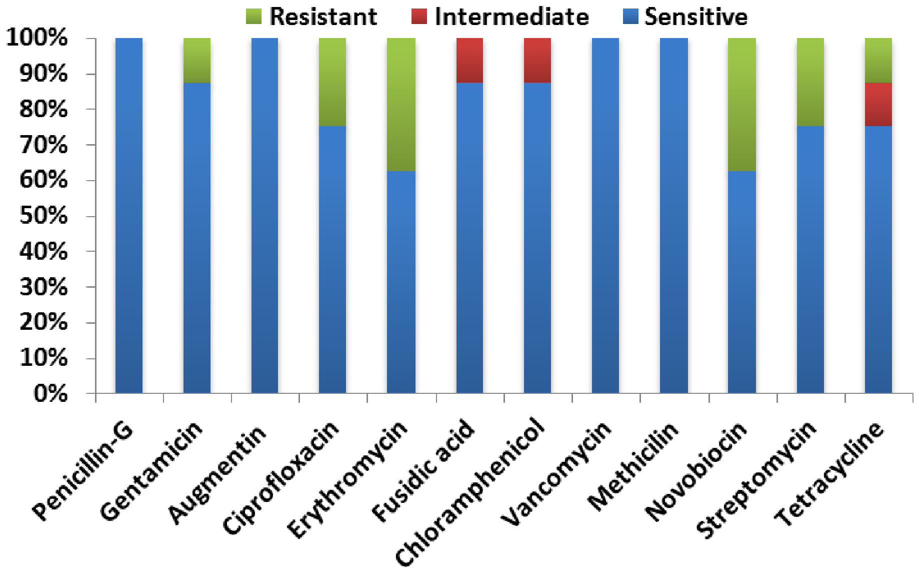

Figure 2c. *Antibiotic susceptibility pattern of* S. aegilis *isolates from River Ganga*

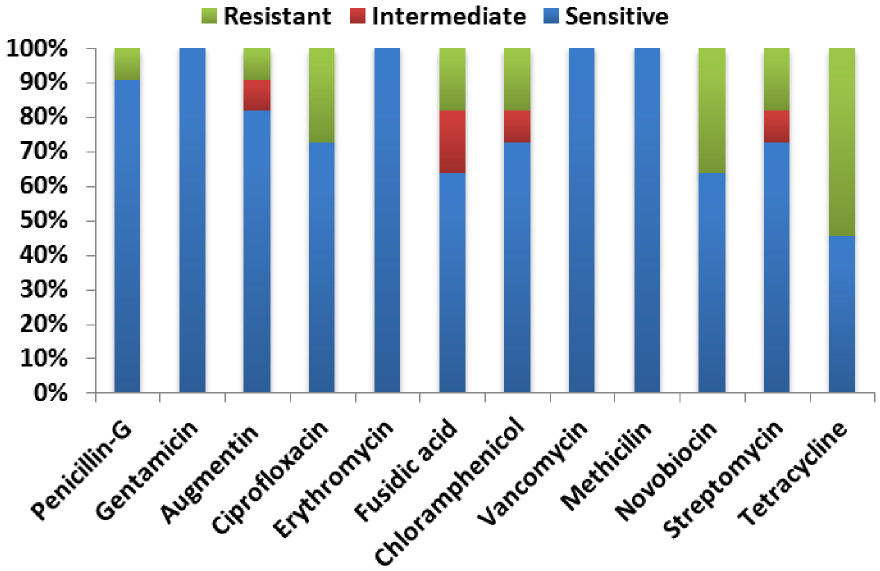

Figure 2d. *Antibiotic susceptibility pattern of other staphylococci isolated from River Ganga*

These strains were resistant to methicillin, penicillin-G, tetracycline, streptomycin and genatmicin. The high resistance of these staphylococci for most of the antimicrobial agents should be a matter of concern as these were isolated from less-populated upper region of River Ganges. Hence it is difficult to predict whether these strains were indigenous to this river system or introduced through human activities.

Table 3. *Morphological and physiological characteristics of selected multiple drug resistant staphylococci isolated from River Ganga*

Characteristic	***Staphylococcus* sp. RA3U1**	***Staphylococcus* sp. WB1P**	***Staphylococcus* sp. WB3T3**	***S. aureus* (MTCC 9542)**
Gram reaction	+	+	+	+
Shape	cocci	cocci	cocci	cocci
Indole Test	+	–	–	+
Methyl Red Test	–	+	+	+
Voges Proskauer Tests	–	+	+	–
Citrate Utilization	+	–	–	–
Gas Production from Glucose	+	–	+	+
Casein Hydrolysis	–	–	–	–
Urea Hydrolysis	+	–	+	–
Nitrate Reduction	+	+	+	+
H_2S Production	–	–	–	–
Coagulase	+	–	–	+
Cytochrome Oxidase	–	–	–	–
Catalase Test	+	+	+	–
Oxidation/Fermentation (O/F)	Fermentative	Fermentative	Fermentative	Fermentative
Gelatin Hydrolysis	+	+	–	+
Arginine dihydrolase	–	–	–	–
Lysine decarboxylase	–	–	–	–
Ornithine decarboxylase	–	–	–	–
Carbon source utilization				
Adonitol	+	–	–	–
Arabinose	+	+	+	+
Cellobiose	–	–	–	–
Dextrose	+	+	+	+
Dulcitol	–	+	–	+
Fructose	+	–	–	+
Galactose	–	–	+	+
Inositol	–	–	–	–
Lactose	–	+	+	+
Maltose	+	+	–	+
Mannitol	+	–	+	+
Melibiose	–	–	+	+
Raffinose	+	–	–	+
Rhamnose	+	+	+	+
Salicin	–	+	–	+
Sorbitol	–	–	–	+
Sucrose	+	+	+	+
Trehalose	+	+	–	+
Xylose	+	–	–	+
Temperature tolerance	15°C – 45°C	10°C – 37°C	15°C – 45°C	25°C – 37°C
pH range	5.0 – 8.0	5.7 – 8.5	5.0 – 9.0	5.7 – 8.0
NaCl tolerance upto	8.0%	9.0%	11%	10.0%
Growth under anaerobic condition	–	–	–	–

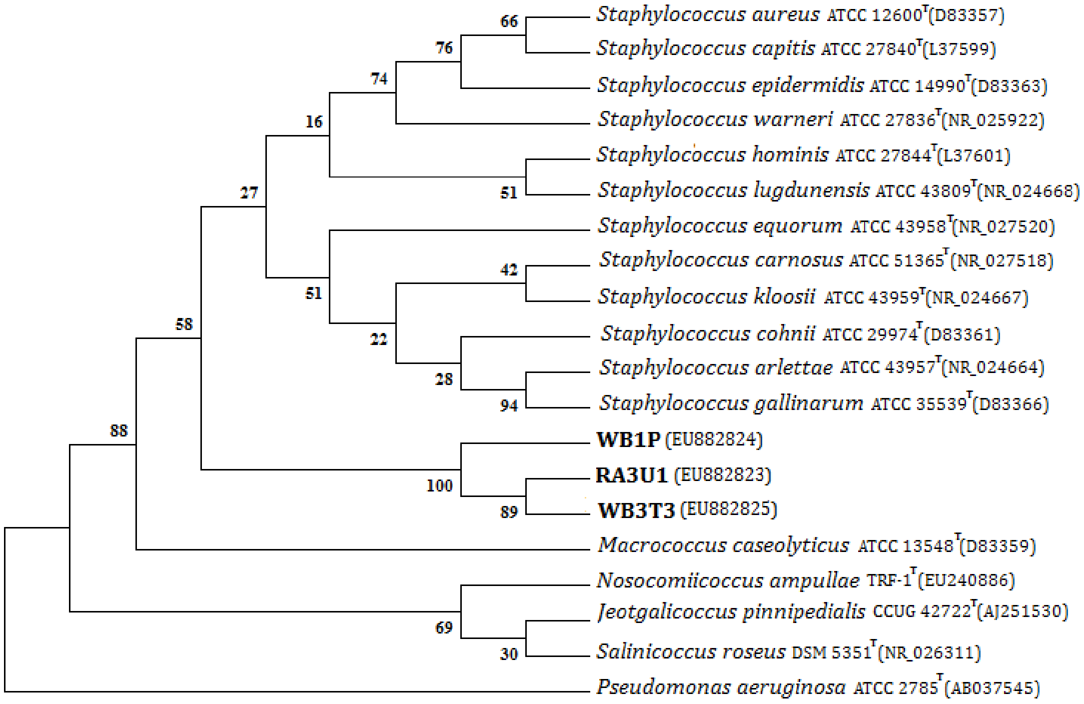

***Figure 3**. Phylogenetic relationship between RA3U1; WB1P; WB3T3 and representative Staphylococcus species based on partial 16S rDNA sequences constructed by using the maximum parsimony method. Sequence accession numbers are given in parentheses. Numbers at branching points refer to bootstrap values (1000 resamplings). Pseudomonas aeruginosa was used as outgroup*

Although there are some reports on the detection of *S. aureus* from river Ganga [8, 35], to the best of our knowledge this is the first report on the isolation, prevalence and antimicrobial susceptibility pattern of staphylococci from the Ganga river system of Uttarakhand comprising of 32 sampling sites. Considering the facts that multiple antibiotic sensitive *S. aureus* is one of the most widely spread human pathogen, the havoc it causes on life and subsequently on the economy; it is imperative to determine its incidence and antibiogram in our environment for adequate control and treatment.

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A FOURTEEN-YEAR MONITORING IN A PHENOLOGICAL GARDEN, STUDY OF PLANT SPECIES, CLIMATE TRENDS AND THEIR RELATIONSHIPS IN CENTRAL ITALY

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Abstract. The plant adaptation to climate trends appears as a main research field in the recent past. In the Mediterranean environment a 14-year (1997-2010) phenological study was realized inside a phenological garden to investigate the climate/ plant relationships. The average phenological data thus obtained provide a mean model of development for the different species in relation to the 14-year period of observation (1997-2010). Meteorological recordings showed June and July as the months with the highest temperature increases during the central period of the study period. The phenological data showed a double-trend behaviour during the historical series considering the first two growth phases (V3, V5) that was not seen for the later phases (V7, V8). Moreover, different leaf presence periods on the tree were calculated for some of the plant species, and commonly the clearest trends were seen for V3 to V7 with a decreasing period length from 1997 to 2002-2003 and a successive quite constant behaviour. The lowest correlations between annual vegetative phases and temperature variations were manifested above all by two species (*Sambucus nigra* L. and *Robinia pseudoacacia* L.) for which the first leaf development phases appeared probably influenced by photoperiod.

Keywords: *central Italy, climatic trend, phenological garden, phenology*

Introduction

Phenological events of the vegetation, such as bud breaking, flowering and leaf colouring, are closely associated with lower atmospheric conditions as the seasons change (Zhao and Schwartz, 2003). Phenology in its present meaning is the study of events that lead to the manifestation of phenomena that are associated with the functioning of plant organs or of the plant as a whole. Detecting the growing season variability of terrestrial vegetation is crucial for the identification of responses of ecosystems to recent climate change over seasonal and inter-annual time scales (Chen et al., 2005). The great advantage of phenological observations is that they are particularly suitable to illustrate and communicate the ongoing impact of climate change.

In temperate zones, the reproductive cycle of plants is largely controlled by temperature and day length, while at lower latitudes, rainfall and evapo-transpiration also need to be taken into account (Menzel et al., 2001; Estrella et al., 2007; Orlandi et al., 2009, 2013; Bonofiglio et al., 2009). Given these relationships, a significant increase in global temperature should also be visible along series of long-term phenological observations. Indeed, changes in plant phenology are considered to be the most sensitive and observable indicators of plant responses to climate change, as has been demonstrated by a large number of studies (Fornaciari et al., 1998, 2000; Chmielewski and Rötzer, 2001; Chmielewski et al., 2004; Estrella et al., 2006). Further studies have linked inter-annual variability of plant phenology to large-scale weather features, such as the North Atlantic Oscillation and the El Nino Southern Oscillation (Beaubien and Freeland, 2000; Menzel et al., 2005; Avolio et al., 2008).

In climatology and ecology, phenology and syn-phenology approaches are used to evaluate the degree of climate change that has occurred, and also to consider the potential consequences on living organisms in general and in particular on the vegetable kingdom with phyto-phenology (Kramer et al., 2000; Mutke et al., 2003; Orlandi et al., 2005a, b). Numerous studies using phenological records have documented phenological changes throughout the northern hemisphere (including Europe), and they have supported the same general conclusion: the growing season is beginning earlier (Cook et al., 2005). Such an altered pattern of seasonal progress might also influence, for example, the food chain, animal migration, and cross-breeding between populations (Walther et al., 2002).

Another important application of the phenological models is the evaluation of the spatial-distribution-pattern of a species under the hypothesis of future climate change. Phenological studies can interpret the reproductive success of a plant population each year, and the growth and survival probability of individual plants, along with their fitness under particular climate conditions (Cleland et al., 2007).

The present study was carried out in a phenological garden that is located near Perugia, in central Italy, and which contains indicator species that are common to all international phenological gardens (IPGs) (Orlandi et al., 2007). This phenological garden also contains indicator species that are common to the Italian phenological gardens and that are representative of this geographical area. The aims of the present study were therefore to determine and analyse the mean development trends of the plant species considered, and to show the plant adaptability to the Mediterranean environment, over a 14-year period (1997-2010). In addition, plant phenology was used as a tool to investigate the climate/ plant relationships.

Materials and methods

The phenological garden is located near to the city of Perugia, in central Italy (coordinates, 42°60' N, 12°18' E; altitude, 265 m a.s.l), and it is one of the few Italian sites that is a member of the European network founded in 1957 by F. Schnelle and E. Volkert, within the Phenology Study Group of the International Society of Biometeorology. The plant species in the phenological garden were obtained from mother plants that were received from the German Weather Service, the European coordinator for the distribution of IPG clones. The National Working Group for Phenological Gardens selected the species that were to be adopted as indicator species from those proposed by the IPG. As all of these species are typically from northern European climates, which are characterised by cold winters, mild summers and abundant rainfall, the group selected species that would adapt easily to the Mediterranean climate.

The tree indicator species that were examined were those suggested by the IPG network:

1) *Cornus sanguinea* L. Common name: dogberry, dogwood;
2) *Corylus avellana* L. Common name: hazel;
3) *Ligustrum vulgare* L. Common name: privet;

4) *Robinia pseudoacacia* L. Common names: robinia, acacia;
5) Common IPG species, such as *Salix acutifolia* Willd. Common name: willow;
6) *Sambucus nigra* L. Common name: elder.

The phenological sampling was carried out according to the basic phenological criteria (e.g. every phenological stage should interpret a distinct biological event, and the data must be objective so that they can be compared with those of other studies), using phenological keys that have been described in various previous studies (e.g. Chmielewski and Rötzer, 2001). In particular, for the vegetative cycle, the following phenological phases were considered: V3, bud break and leaf unfolding; V5, young unfolded leaf; V7, adult leaves; and V8, beginning of leaf colouring.

The observations were conducted on three individuals for each plant species, to limit the random variability that can be seen even in genetically similar plants. The mean date for the onset of each phenophase was mathematically calculated considering together the three plants (phenoids) of the same species. The average dates thus obtained provided a mean model of the plant development in relation to the species and the year of observation. The mean values of the phenological data were computed for the different species over the 14-year period of observation (1997-2010), to obtain the mean development in the study area.

For each plant species, the differences between the phenological data of year x+1 and year x were calculated to determine any delay or advancement of the phases over the study years. Moreover, these differences were parameterised considering the positive values always as 1, and the negative values always as -1 (not considering the entity of variation), for an unbiased comparison of the behaviours of the different species across the different phenological periods.

Afterwards, the yearly variations (-1, +1, or 0 if the dates were the same) were summarised, to calculate the overall trend line for the identification of the phenological behaviour throughout the investigation period. Linear trend lines were constructed on the basis of the yearly variations, to interpret the 14-year tendency of each species for each vegetative phase. This methodology was utilized in place of a 'canonical' one, to reveal any consecutive variations year on year.

Moreover, to determine the relationships between spring and summer temperatures and the vegetative plants development, the daily temperature values were elaborated calculating growing degree days (GDDs). For the calculation of the GDDs, a single sine method was used. This method evaluates the hourly temperature trends according to the means of the single sine function (Zalom et al., 1983). Moreover, the GDD formulae were calculated using 7 °C as the temperature threshold for vegetative development of all of the plant species considered.

To interpret the climate variations over the 14-year study period, the yearly totals of the daily GDDs were evaluated and also compared year by year every 4 weeks from 1 January to the end of August, with the construction of polynomial trend lines.

The same parameterisation for the phenological data was carried out from a climate point of view. The different temperatures were interpreted in terms of annual increases (GDD total in the year x+1 higher that in the year x) and decreases. These differences were parameterised, and the parameters obtained were summarised, to show the potential increasing or decreasing trends over the study period.

Moreover, the yearly whole periods of leaf presence on the tree were considered for the evaluation of any potential variations that were mainly caused by the erratic appearance dates from the first leaves with photosynthesis activity (V3) to the leaf withering and loss of assimilation (V8): the leaf assimilation period. These periods were evaluated on the basis of their annual duration over the study period, with the calculation of the weeks between the different vegetative development phases: i.e. weeks from V3 to V8, from V5 to V8, from V3 to V7, and from V5 to V7.

Results and Discussion

The temperature trends of the study area were analysed through the GDD calculations, and these trends over the 14-year period were interpreted through non-linear regression analysis. *Table 1* shows the coefficients of the second degree polynomial trend lines, to reveal the main trends in the GDDs over the first 8 months of each of the study years. The main characteristics of these curves are shown by the evaluation of the sign of the ‘a’ coefficient (parabolic convexity) and by its value. All of the curves corresponding to the moving GDDs had negative ‘a’ coefficients, with the maximum in the central years of the study, and an increasing parabolic curvature, as testified by the Δa parameter. The months that showed the highest temperature increases during the central period of the study years were those of June and July. In particular, the highest GDDs were those recorded during 2003.

Table 1. *Coefficients of the second degree polynomial trend lines (ax2 + bx + c = 0) of average GDD amounts calculated every 5 weeks from January to July.*

Week	Coefficient a	Δa	Coefficient b	Coefficient c	R^2
5	-0.38		6.6	48.5	0.23
10	-0.66	-0.28	11.0	95.5	0.25
15	-1.43	-0.77	24.2	164.3	0.39
20	-2.38	-0.95	38.3	413.6	0.41
25	-3.72	-1.34	58.6	860.0	0.42
30	-4.72	-1.00	77.6	1369.5	0.37

The annual parameterised phenological delays or advancements are shown for all the plant species (*Fig. 1*). The annual variations related to the first two phenological phases (V3, V5) for all these overall totals, showed a delay tendency (highest values) from the first study years (2000) to the mean period (2003-2005), and then an advance tendency (lowest values) during the following 5-year period (2006-2010). On the other hand, the V7 and V8 phases appeared to follow yearly advancements to 2004-2005, and then year by year these phases showed progressive delay.

Figures 2 to *5* show the plant species that underwent significant delay or advancement trends over the study period according to the polynomial trend lines (i.e. showed R2 values ≥0.4). Only three plant species had significant trends for their phase V3 variations over the 14-year period (*Fig. 2*). In particular, the parameterised annual variations for Corylus, Robinia and Ligustrum showed a tendency towards delay (although in the last few years, Robinia showed a tendency to phenophase advancement).

In the following two Figures, again, four plant species (Cornus, Corylus, Robinia and Sambucus) showed significant phenological trends for their vegetative V5 and V7 phases (*Figs. 3, 4*). These phases showed the clearest general trends, with a tendency to advancement considering the ‘young unfolded leaf’ phase (V5), and on the other hand, a tendency to delay for the ‘adult leaves’ phase (V7). In particular, the analysis of phase V5 in Figure 3 shows that the clearest advancements were recorded from 2005-2006, with the phase V7 delays generally apparent from about the same period (*Fig. 4*).

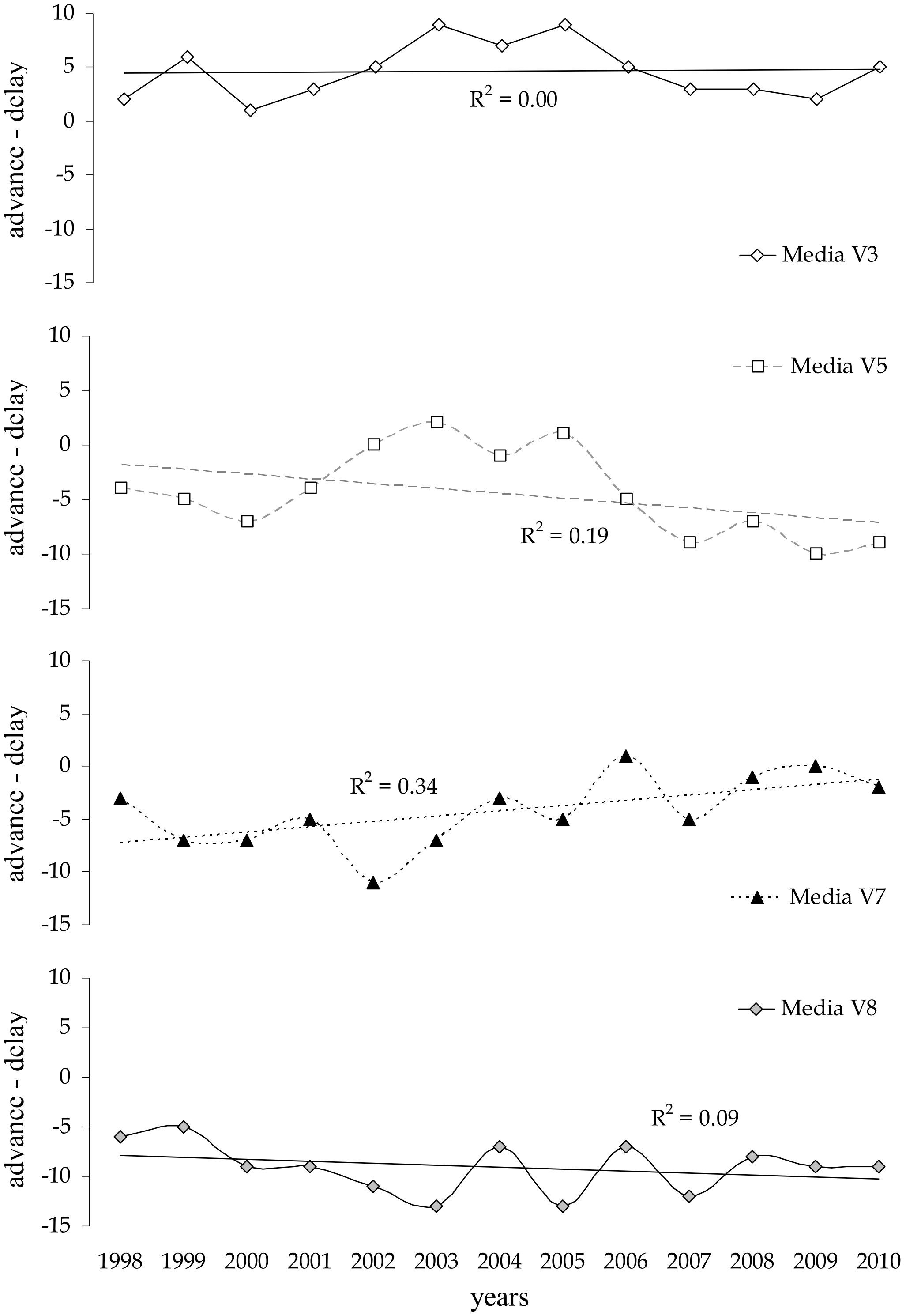

Figure 1. *Trends of normalised date delay or advancement for each of the four phenological phases, considering all of the plant species together.*

There were only two plant species that showed particular trends for the ‘beginning of leaf colouring’ of phase V8 (*Fig. 5*; Corylus and Cornus) and their behaviours were different: Corylus showed clear advancement of this final vegetative phase over the study years, while Cornus showed a delay (*Fig. 5*).

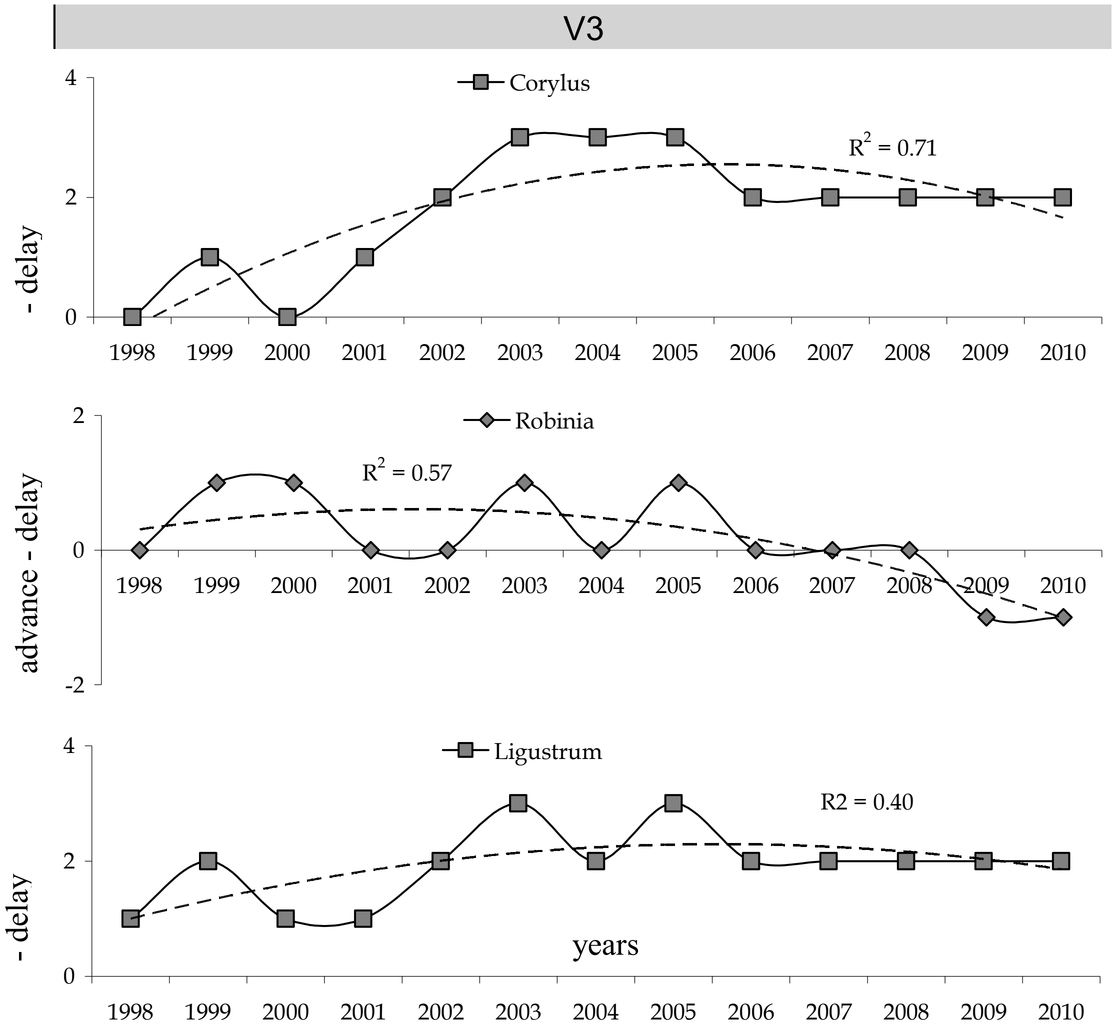

Figure 2. *Species showing significant trends of normalised date delay or advancement, considering the V3, bud break and leaf unfolding, phase.*

Following the calculation of the parameterised annual variations of these phenological phases, the annual phenological data for the different plant species were analysed for statistical correlations between these biological trends and the temperatures (GDDs). *Table 2* gives the correlation data for each of the four vegetative phenological phases for the plant species that showed significant phenological trends. The highest correspondence was characterised by positive signs that showed particular relationships between phenological delay–GDD increase, and phenological advancement–GDD decrease, above all for the V7 (mean R^2, 0.86) and V8 (mean R^2, 0.75) phases, with the lowest correlations seen for V5 (mean R^2, 0.43). In this sense, high correlation values can be interpreted as a strong relationship between vegetative development and temperature. These show a strong dependence on the temperature variations of all the vegetative phases, but particularly of adult leaves and the start of leaf colouring. The lower relationship of the initial vegetative phases would probably be related to other variables, such as the photoperiod (V3) or the water availability (V5) (Olmsted, 1951; Kramer, 1994, 2000).

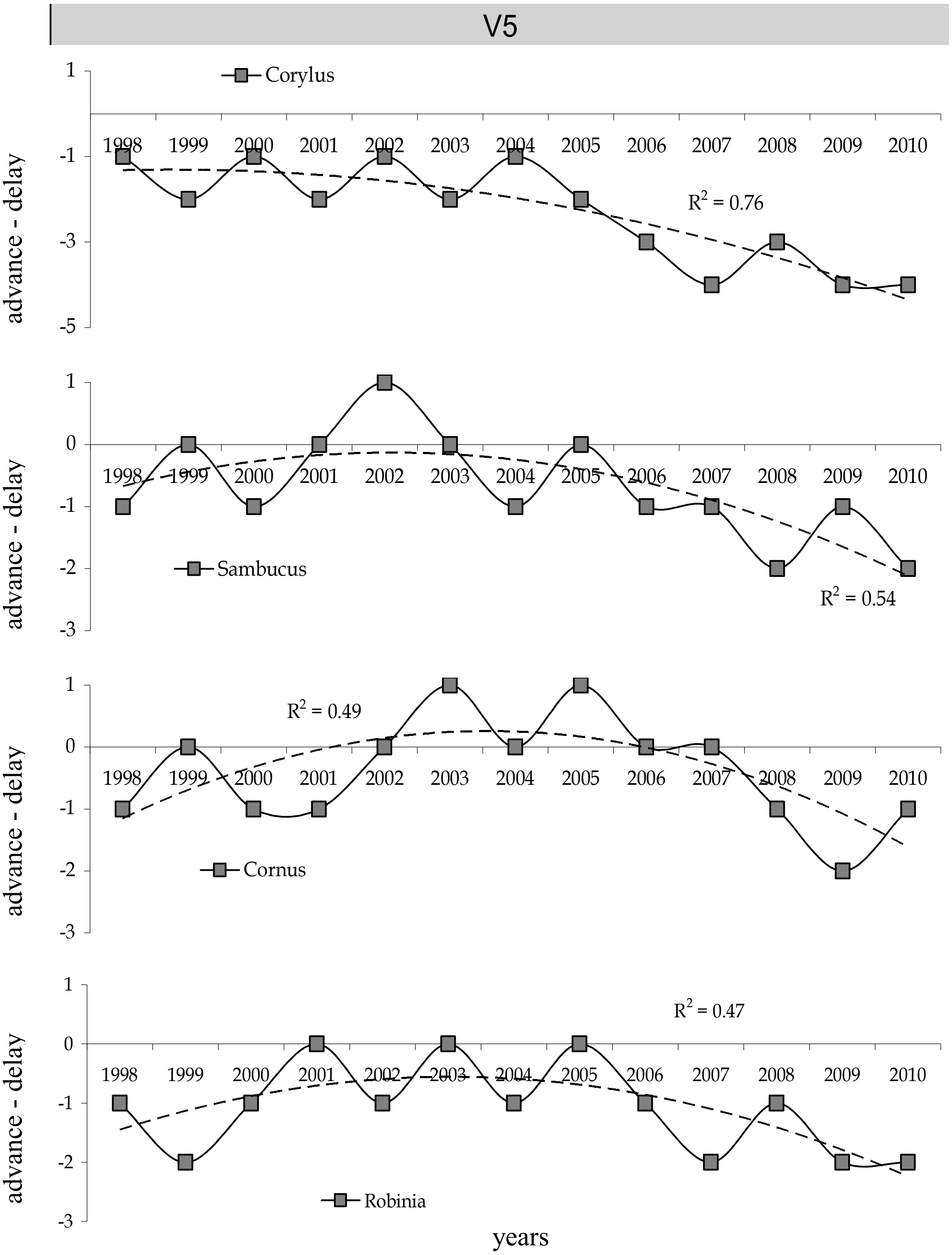

Figure 3. *Species showing significant trends of normalised date delay or advancement, considering the V5, young unfolded leaf, phase.*

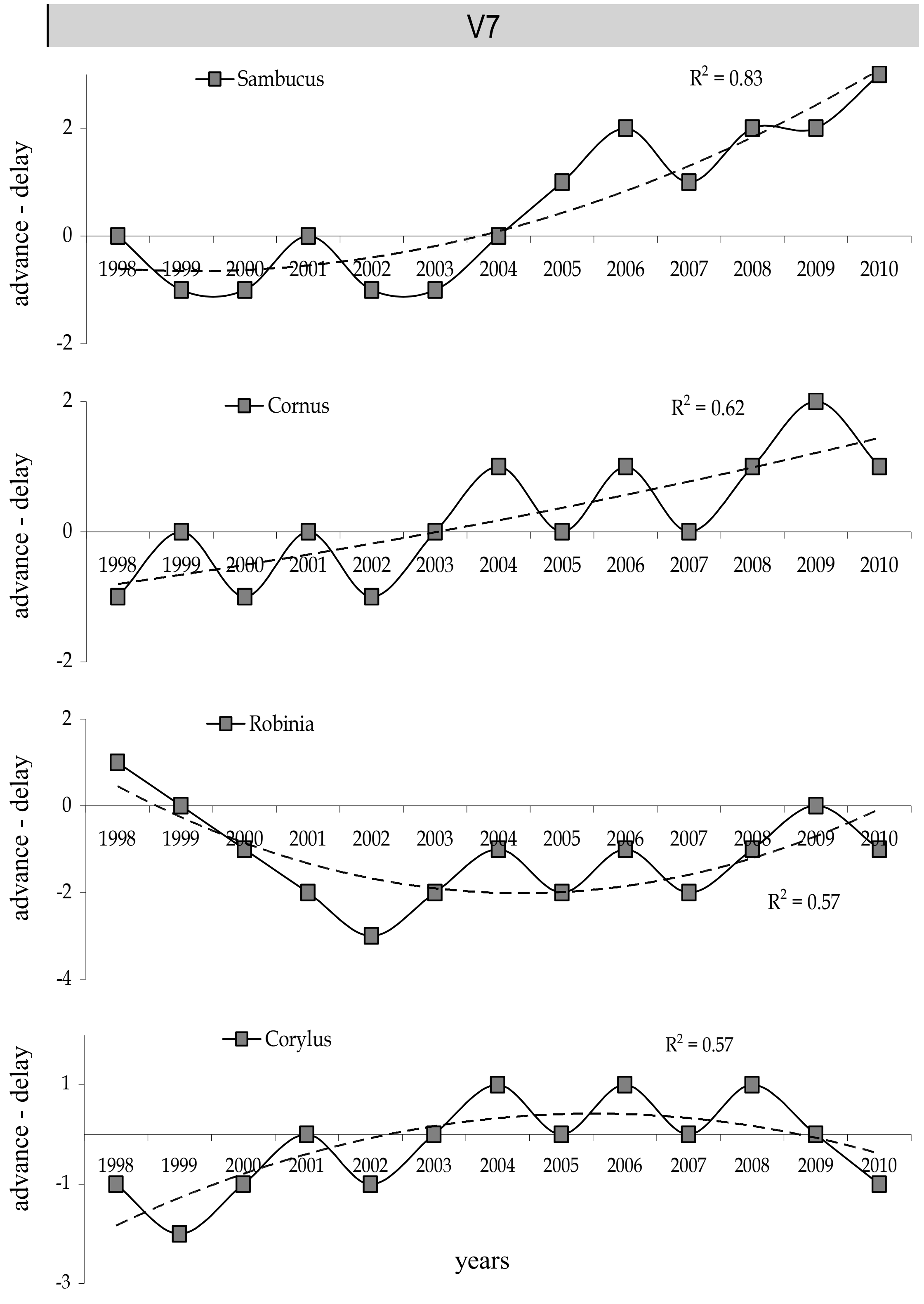

__Figure 4.__ Species showing significant trends of normalised date delay or advancement, considering the V7, adult leaves, phase.

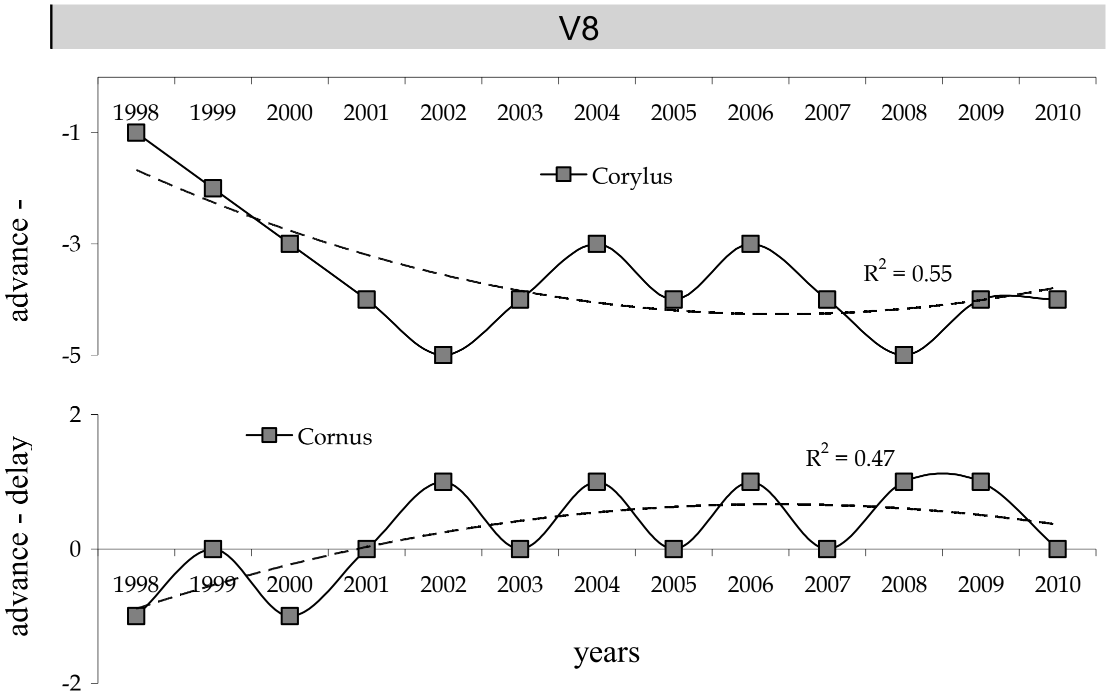

Figure 5. *Species showing significant trends of normalised date delay or advancement, considering the V8, beginning of leaf colouring, phase.*

Table 2. *Correlation data between the phenological dates and the GDD variations for each phenological phases for the plant species that showed significant phenological trends.*

Species	Correlation[a]	Mean correlation[a]
	V3	
Ligustrum	0.50	0.58
Corylus	0.58	
Robinia	0.65	
	V5	
Corylus	0.46	0.43
Sambucus	0.31	
Robinia	0.30	
Cornus	0.66	
	V7	
Corylus	0.82	0.86
Sambucus	0.82	
Robinia	0.87	
Cornus	0.91	
	V8	
Corylus	0.72	0.75
Cornus	0.78	

[a] *Correlation between phenological delay or advancement versus GDD increase or decrease.*

Moreover, the different periods of leaf presence on the tree were calculated for those plant species that showed significant trends across all of these four vegetative phenological phases, and thus for *Corylus*, *Robinia* and *Cornus*. The “leaf assimilation” period that manifested the clearest trend was that calculated from V3 to V7, as reported in *Figure 6*.

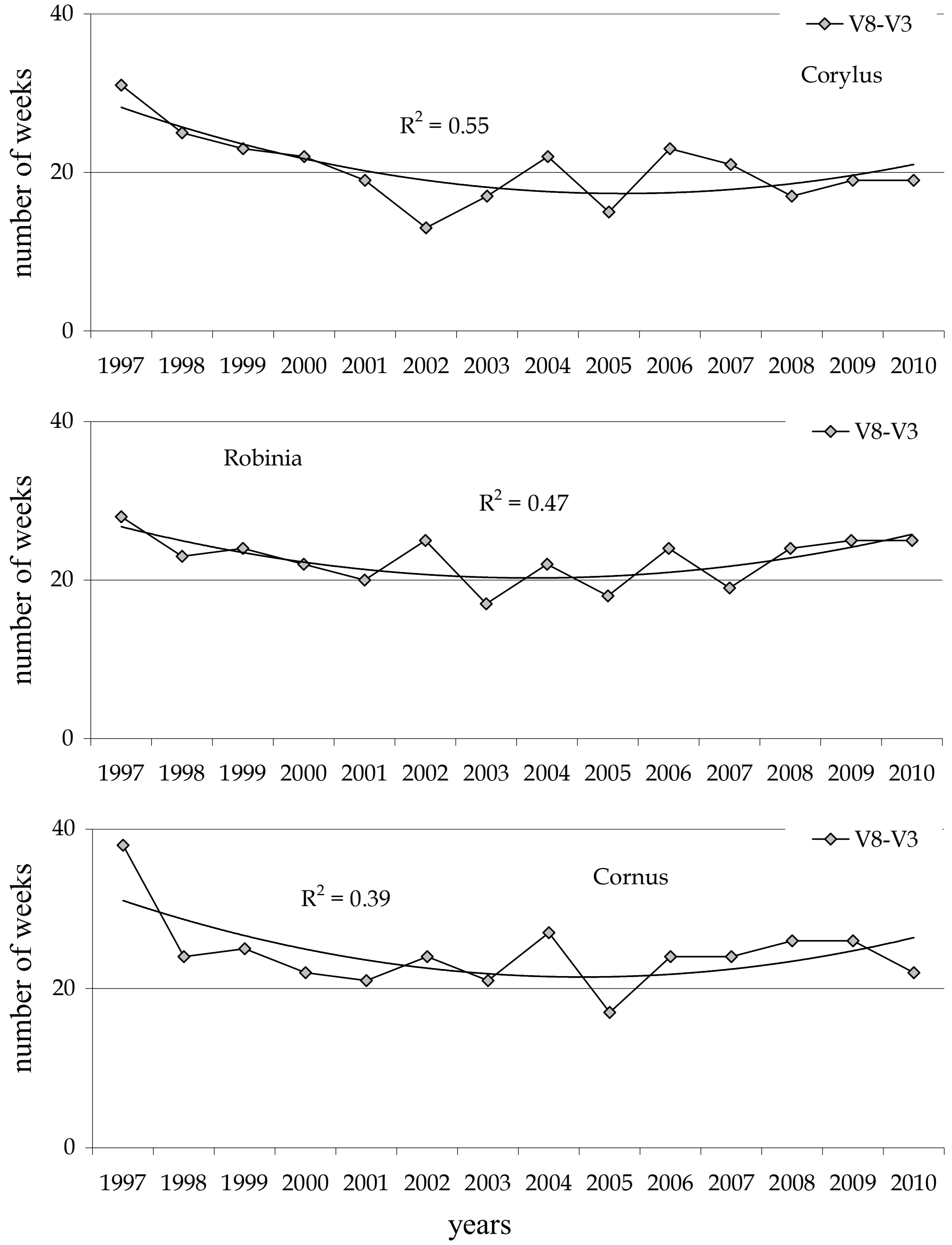

Figure 6. *Species showing significant trends of annual leaf assimilation periods (weeks from V3 to V7) over the 14-year study period.*

In physiological terms, this V3-to-V7 period represents the more important photosynthetic assimilation period for the different species, considering that the molecular chlorophyll degradation processes start from the central summer period (Farooq et al., 2009, Jaleel et al., 2009) with the leaf senescence that precedes the leaf-colouring phase (V8). Here, the V3-to-V7 leaf-assimilation periods in these three plant species considered started from about mid-March and finished at about the end of July, and overall these showed decreasing trends from 1997 to 2002-2003 for both *Corylus* and *Robinia*, and to 2000-2001 for *Cornus*. Following the decreases, these V3-to-V7 leaf-assimilation periods then showed a relatively constant phase, in which at least for *Cornus*, the last study year (2010) marked one of the lowest values, due particularly to the advancement of the V7 phase.

Conclusions

In general, the relative invariance in the start of the open-bud phase (V3) that was accompanied by the advancement of the young unfolded leaves phase (V5), which occurred particularly from 2006, led to a shortening of the leaf-opening period. In contrast, the beginning of the autumn leaf-colouring phase (V8) tended to remain constant, with the exception of the opposite trends of *Corylus* and *Cornus*, confirming that in contrast to spring events, the signal for leaf colouring in autumn is relatively ambiguous and less evident (Menzel et al., 2006). With their dependence on the meteorological features, the adult leaves show a tendency to remain on the trees longer. This will be as a consequence of the warmer season and milder temperatures favouring a delay in the successive vegetative phases, thus postponing the seasonal rest period of the plant.

At the level of the single species, it can be seen that the plants that suffered fewer phenological date variations during the study period were *Ligustrum* and *Salix*. *Corylus*, *Robinia*, *Sambucus* and *Cornus* showed the greatest variations, which are interpretable according to the phenological tendencies that depend on the variations in temperature.

These four phenological phases (V3, V5, V7, V8) showed different trends in terms of their annual delay or advancement over these 14 years of the study, with the clearest results shown in particular for the V5-to-V7 phases, while the V3 and V8 phases did not show particular trends over the study period, when all of the plant species were considered.

The lowest correlations between the annual vegetative phenological phases and the temperature variations (as GDD) were shown by *Sambucus* and *Robinia* in particular, for which the first leaf development phases appeared probably influenced by the photoperiod. Other studies have reported that the sensitivity of leaves to the photoperiod increases with aging of the expanding leaves, reaching a maximum when they are half expanded (which here corresponds to about V5, the young unfolded leaf stage) and decreasing in the later leaf development (Khudairi and Hamner, 1954; Withrow, 1959).

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SEASONAL DYNAMICS IN CARBON DIOXIDE FLUXES OF THE HERBACEOUS LAYER OF A MOIST KENYAN SAVANNAH

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Abstract. Strong seasonal variability in African carbon source/sink relationship is considered the continent's most significant contribution to the global carbon cycle. Alternating dry and wet periods dictate ecosystem carbon exchange and productivity of tropical African savannah. We examined the seasonal and daily trends in ecosystem CO_2 exchange in the herbaceous layer of a humid Kenyan savannah devoid of grazing. Microclimate, soil moisture, soil and tissue nitrogen, aboveground biomass and carbon dioxide fluxes were measured. The ecosystem carbon dioxide fluxes followed the seasonal rainfall pattern and were strongly correlated to soil water content. Peak mean carbon dioxide fluxes were 3.21±0.99, 8.21±1.02 and 4.67±1.06 $\mu molm^{-2}s^{-1}$ during drought and -13.86±1.48, 15.03±0.98, and 27.73±1.47 $\mu molm^{-2}s^{-1}$ during wet periods for net ecosystem carbon dioxide exchange, ecosystem respiration and gross primary production respectively. At daily scale, net ecosystem carbon dioxide exchange increased with increasing photosynthetic photon flux density under wet but decreased with increasing vapour pressure deficit under dry conditions. Ecosystem respiration increased with increasing soil temperature during wet but decreased with increasing soil temperature during drought. Our results point to the overriding role of soil moisture in the ecosystem carbon dioxide exchange processes of this savannah.

Keywords: *African savannah, CO2 chambers, ecosystem respiration, net ecosystem exchange, soil moisture*

Introduction

Increasing atmospheric concentration of CO_2 is the largest contributor to global climate warming and associated extreme weather events (IPCC, 2007). This makes the global carbon cycle highly important to the "stabilization of greenhouse gas concentrations in the atmosphere at a level that would prevent dangerous anthropogenic interference with the climate system" the sole objective of the United Nations Framework on Climate Change (UNFCC). In the global carbon (C) cycle, atmospheric CO_2 is mainly taken up by the terrestrial biosphere via plant photosynthesis or gross primary production (GPP). This photosynthetic phenomenon underscores the important role played by plants in regulating atmospheric C concentrations. In Africa, the savannah ecosystem that covers about two-thirds of the continent's land surface (Adams, 1996) must be a major player in the regional and global C balances (Grace *et al.,* 2006).

Ecosystem carbon exchange in African savannah though previously poorly studied is currently receiving substantial attention (Williams *et al*., 2007; Ardö *et al.,* 2008; Bombelli *et al.,* 2009; Ciais *et al*., 2009; Otieno *et al*., 2010; 2011). However, despite the renewed interest in the carbon budget of African savannah in the second half of the last decade, the continent's savannah is extensive and both spatially and temporally variable (Ciais *et al*., 2009; Williams *et al*., 2007). Such great variability in the continent's largest ecosystem is greatly impacting its carbon balance since exchanges of CO_2 between land surface and atmosphere in savannah are highly modulated by ecosystem characteristics such as structure, species and physiological functions (Beringer *et al*., 2011). The variability of the African carbon balance has been noted and is estimated to account for about 50% of inter-annual variability in global atmospheric CO_2 (Williams *et al*., 2007). The high variability coupled to complex interactions between biotic and abiotic factors that control plant productivity may be responsible for the continent's large contribution to the uncertainty in its C budget and needs to be understood. Clear understanding of the temporal variability in African savannah is needed for better parameterization of the models estimating the continent's carbon exchanges and consequently unraveling the uncertainties in its C balance.

Savannahs are characterised by two different plant functional types, namely the grass herbaceous layer and the tree/shrub over-storey in regions with alternating wet and dry seasons (Grace *et al*., 2006; Scholes & Archer, 1997). The dry and wet seasons are associated with strong seasonal changes in soil moisture and temperature regimes (Kanniah *et al.,* 2010; Scholes & Archer, 1997) that influence nutrient availability, vegetation growth, fire occurrence and decomposition (Scholes, 1990; Scholes & Walker 1993; Rodriguez-Iturbe *et al*. 1999; Ciais *et al*., 2011). The different plant functional forms in savannah may however, not respond in a similar way to this climatic seasonality further creating a source of ecosystem variability. In particular the shallow rooted herbaceous vegetation has their productivity synchronised to the wet season when soil water content is high but senesce during drought (Scholes & Walker, 1993; Xu & Baldocchi, 2004). This makes the herbaceous layer a more variable component of the savannah ecosystem and thus a better candidate for variability study.

Closely tied and driving these seasonal climatic variations in the African savannah, are daily variations in weather patterns. African savannah experiences daily variations in solar radiation and water vapour pressure leading to variations in temperature, photosynthetic photon flux density (PPFD), and vapour pressure deficit (VPD). Such daily changes in weather patterns result in daily variation in ecosystem CO_2 fluxes (Maherali *et al.,* 2003; Flanagan & Johnson 2005; Hastings *et al.,* 2005; Risch *&* Frank, 2010). In particular, PPFD (Dai *et al.,* 2004; Hastings *et al.,* 2005) and VPD (Maherali *et al.,* 2003) determine ecosystem photosynthesis whereas environmental temperature (Joffre *et al.,* 2003; Flanagan & Johnson, 2005; Li *et al.,* 2008) largely influences ecosystem respiration.

Despite the strong link between daily weather and seasonal variations in microclimatic factors and their great influence on ecosystem CO_2 fluxes in African savannah, the seasonality of CO_2 fluxes in the continent's savannah is poorly studied (Ardö *et al.,* 2008). Concurrent analysis of daily, intra annual and inter-annual carbon exchange trends enhances the understanding of the physical and physiological controls of carbon fluxes and functioning of ecosystems. In-depth understanding of the factors regulating ecosystem C dynamics of African savannah will further help refine and validate the models currently used to estimate the continent's C balance and to inform policies related to the role of Africa in regional and global carbon cycle.

The two known methods for direct measurements of ecosystem CO_2 exchange are the eddy covariance (EC) and the chamber techniques (Hall *et al.* 1992; Wohlfahrt *et al.* 2005) both of which have been compared in detail by Wohlfahrt *et al.* (2005). The greatest effort to assess the African carbon budget has been that of the CarboAfrica project (http://www.carboafrica.net), using the EC method (Ardö *et al.* 2008; Merbold *et al.* 2008). The chamber method whose results compare favourably with eddy data, when both are used in parallel (Li *et al.* 2008) is useful in heterogeneous landscapes and when the focus is to separate fluxes from the different ecosystem patches. The chamber method has widely been used for CO_2 measurements in the temperate (Li *et al.* 2008), Peatlands (Otieno *et al.* 2009) and in the African savannah (Otieno *et al.* 2010; 2011) ecosystems. We used the chamber method to measure the ecosystem CO_2 exchanges in the herbaceous layer of a moist African savannah.

In this study, we examined how climatic seasonality affects biomass development and ecosystem CO_2 exchange of a moist savannah. How do the ecosystem CO_2 fluxes change with season at both daily and seasonal scale? What drives the daily and seasonal CO_2 fluxes in a moist savannah? The study singles out the herbaceous layer (the most seasonal component) of the moist savannah. Given the distinct droughts and wet periods of African savannahs (Otieno *et al.*, 2010; 2011; Scholes & Archer, 1997; Kutsch *et al.*, 2008), we divided the year into three periods of different phases of soil moisture (wet, intermediate and dry) as dictated by rainfall distribution.

Materials and Methods

Study Site

The study was conducted at the Kenya National Youth Services (NYS) station (00^0 36' 28" S & 34^0 15' 24" E) in Lambwe valley, Nyanza province of Kenya during the year 2009. The NYS station borders Ruma National Park. The altitude of the valley ranges between 1200 and 1400 m above sea level. The terrain is mainly rolling grassland with tracts of open woodland and thickets dominated by *Acacia ancistroclada, A. gerarrdii, Rhus natalensis*, *R. vulgaris*, *Pilliostigma thonningii* and *Belanites aegyptica* trees. The dominant grass species was *Themeda triandra* while the soils are vertisols according World Reclamation Bureau (WRB). The climate is hot and humid with a mean air temperature of 22.5°C. Mean annual rainfall for the last 15 years is 1346 mm with a bimodal distribution pattern between April-June and September-November. The months of January and February are the driest and hottest.

The NYS station has about 40 ha land in which they rear livestock and grow crops. The studies were conducted in a 70 m x 100 m area that was previously grazed, but had a 2 m high fence erected to exclude the grazing livestock for three years prior to the commencement of the study.

Experimental Design

The study aimed at determining the seasonal variations in ecosystem CO_2 exchange and productivity of the herbaceous layer of a moist savannah. The year was divided into three periods namely wet (April-June and September-November), intermediate (July-August) and dry (January-March) with different phases of soil moisture. The transitions from short rains (September-November) to the dry period at the study site are rather abrupt making December to either be a wet or dry month depending on when the rains cease. The periods/months were the treatments. The 70 m x 100 m fenced block was divided into approximately three equal parts from east to west resulting into strips (30 m x 70 m) running north-south down-slope. Each of the three strips represented the replicates and was randomly sampled for measurements taking care to avoid areas under the tree canopies and close to termitaria which had been found to influence CO_2 exchange (Otieno et al., 2011). The collars/frames measuring 39.5 cm by 39.5 cm used in carbon flux measurements were used as quadrats for biomass harvesting. Measurements were conducted every month.

Measurements

Micrometeorology

A microclimate station was set up in an open area of the study site at a height of 2 m. Rainfall (RG3 HOBO pedant rain gauge, HOBOware, Eichstetten, Germany), air temperature and humidity (FUNKY-Clima, ESYS, Berlin, Germany), and global radiation (HOBO pedant, HOBOware, Eichstetten, Germany) were the weather parameters measured. Measurements were taken every 30 seconds and averaged and stored every 30 minutes into in-built data loggers. In addition soil temperature at 10 cm depth was measured within the chambers (see net ecosystem CO_2 exchange below) using digital thermometers (Einstichthermometer, Conrad, Hirschau, Germany) during carbon dioxide flux measurements. Vapour pressure deficit (VPD) was computed from ambient air temperature and relative humidity. Saturation vapour pressure (VP_{sat}) was first computed from the Arrhenius equation. Actual partial pressure of water vapour (VP_{air}) was then computed from saturation vapour pressure and relative humidity:

$$VPair = {VPsat * RH}/{100} \qquad \text{(Eq. 1)}$$

Vapour pressure deficit (VPD) was then computed as the difference between VP_{sat} and VP_{air}.

Soil water content

Soil samples for gravimetric soil water content (SWC) were obtained every month from 0-10 cm, 10-20 cm and 20-30 cm depths using a soil augur. The soils were weighed immediately after sampling, oven dried at 105^0C to constant weight and then re-weighed to determine its water content. Gravimetric soil water content was determined as the relative change in weight between fresh and dry soil samples.

Soil and plant carbon and nitrogen determination

Carbon (C) and nitrogen (N) concentration in the soil, root and shoot were determined. Soil and shoot samples were obtained from part of soil moisture and shoot biomass samples respectively. Root samples were obtained monthly using a root augur.

Samples (shoots, roots, and soil) were then dried and homogenised in a ball mill. The homogenised samples were re-dried in a desiccator to eliminate all water. Approximately 5 g of the dried soil and 1 g of plant samples were then analysed to determine their C and N concentrations (%) using elemental analysis according to Markert (1996).

Net ecosystem CO2 exchange (NEE)

Net ecosystem CO_2 exchange (NEE) was measured using a portable, temperature-controlled 40 cm x 40 cm x 54 cm transparent (≡light) closed chamber system (Droesler, 2005; Otieno et al. 2010). The light chamber was constructed from a 3 mm thick Plexiglas XT type 20070, with >95% light transmittance. To ensure closed-air circulation, plastic collars with base area of 39.5 cm x 39.5 cm and 10 cm height, externally fitted with a 3 cm wide platform (3 cm from the top) were inserted approximately 5 cm into the soil at the beginning of the season. Chambers were sealed to the plastic frames/collars with a flexible rubber gasket and the chamber firmly secured using elastic straps fastened onto the ground from two sides. Extension bases were used to adjust chamber height to the canopy height. Tests were done to check that leakage did not occur, however, this could not be examined regularly in the case of systematic field measurements and each set of data was scrutinised for abnormalities.

The chamber temperature was maintained within 2^0C of the ambient using frozen cool packs and air inside the chamber was mixed using three fans. Air temperature within and outside the chamber was continuously monitored and recorded during the flux measurements to check against wide variations. Sudden rise in pressure within the chamber was avoided by having a 12 mm diameter vent at the top of the chambers. This vent was opened during the placement of the chamber on the collars and replaced soon after the chamber was secured on the collars but before the onset of CO_2 flux measurement.

Carbon dioxide concentration inside the chamber was measured using a portable infrared gas analyser (IRGA, LI-820, LI-COR, USA) connected to the chamber via flexible 0.32cm diameter inflow and outflow tubes (Droesler, 2005). A battery-driven pump maintained a constant airflow rate through the IRGA-chamber system. Once a steady state was attained in the chamber system, CO_2 concentration (ppm) was recorded every 15 s for a period of 2½ minutes before shifting to the next collar. Photosynthetic photon flux density (PPFD) above the grass canopy inside the chamber was measured using a PAR sensor (LI-190, LI-COR, USA) and logged alongside the CO_2 concentrations. Soil temperature within the chambers at 10 cm depth was recorded, at the start and end of the CO_2 concentration measurements, from digital thermometers (Einstichthermometer, Conrad, Hirschau, Germany).

Changes in CO_2 concentration within the chamber headspace was calculated by linear regression from a linear portion of the plot of CO_2 against time for the duration of the measurement. CO_2 fluxes were calculated according to Risch & Frank (2010).

$$CO_2 flux = \frac{\partial CO_2}{\partial t} \times \frac{PV}{ART} \qquad \text{(Eq. 2)}$$

where $\frac{\partial CO_2}{\partial t}$ = rate of change in CO_2 concentration with time; V = volume of headspace within the chamber; P = atmospheric pressure; A= ground area enclosed by chamber; R= gas constant; T= air temperature (K).

A functional relationship between PPFD and NEE, also known as the 'light response curve' described by a rectangular hyperbola (Gilmanov et al., 2007) (Eq. 3) was employed to parameterize NEE response to light, using Sigma Plot 8.0.

$$NEE = -\frac{\alpha\beta Q}{\alpha Q+\beta}+\gamma 0 \tag{Eq. 3}$$

where Q is PPFD (µmol m^{-2} s^{-1}), NEE (µmol CO_2 m^{-2} s^{-1}), α is an approximation of the canopy light utilization efficiency (µmol CO_2/µmol photon), β is the maximum CO2 uptake rate of the canopy (µmol CO_2 m^{-2} s^{-1}) and $\gamma 0$ is an estimate of the average ecosystem respiration (Reco, µmol CO_2 m^{-2} s^{-1}) occurring during the observation period.

Ecosystem respiration (R_{eco})

A similar chamber system to that used for NEE measurement, but translucent (dark) was used to measure ecosystem respiration (R_{eco}). The dark chamber was made of opaque PVC and further covered with an opaque insulation and reflective layer of aluminium. The light and dark chamber measurements were conducted cyclically on hourly intervals from early in the morning (08:00 hr) to evening (18:00 hr), resulting in 8-10 measurement cycles per day.

Gross primary production

Gross Primary Production (GPP) was estimated from the general equation (Li et al. 2008):

$$GPP = R_{eco} - NEE \tag{Eq. 4}$$

where R_{eco} = ecosystem respiration (µmol CO_2 m^{-2} s^{-1}) and NEE = net ecosystem CO_2 exchange (µmol CO_2 m^{-2} s^{-1}).

Aboveground Biomass determination

During each CO_2 fluxes measurements, the collars (39.5 cm x 39.5 cm) were used as quadrants in sampling grass biomass that was then harvested. The harvested biomass was separated into standing/live biomass and dead/litter biomass. All aboveground shoots made up the live, while all shed-off litter made up the dead biomass. The separated live and dead biomasses were oven dried at 75^0C for 48 hr to determine their dry weights.

Statistical analysis

Statistical analysis was performed using GenStat Discovery Edition 3 (VSN International Ltd: http://www.vsi.co.uk). Monthly and periodic means were compared using ANOVA, with month/period as the fixed effects at significance level of $P \leq 0.05$. We applied multivariate linear mixed-effects model in order to correct for the repeated measures in the different plots while interaction between factors were obtained from Generalized Liner Model (GLM). We checked the normality of the model residuals visually by normal probability plots, and we assured the homogeneity of variances and goodness of fit of the models by plotting residuals versus fitted values. Post hoc test for pair-wise comparison of means was conducted with Tukey-LSD test ($P<0.05$) while correlation was tested using Pearson correlation test.

Results

Daily weather patterns

Both vapour pressure deficit (VPD) and photosynthetic photon flux density (PPFD) increased from early morning to daily maximum values before declining (*Figs. 1a and b*) irrespective of the soil moisture phase. However, while PPFD peaked at midday, VPD peaked later between 13:00 and 15:00 hrs. Soil temperature also increased from early morning (*Fig. 1c*) but did not peak unlike VPD and PPFD.

As expected vapour pressure deficit in the dry period was significantly higher than that of the wet and intermediate periods. Vapour pressure deficit of the wet and intermediate periods only differed significantly after midday. There was no definite seasonal trend on daily PPFD. The dry periods had significantly higher soil temperature than the wet which in turn was higher than that of the intermediate periods. Largest fluctuations in soil temperature (6 ± 1^0C) during the day occurred during the dry periods.

Seasonal microclimate and soil conditions

The rainfall pattern was bimodal with two peaks March to July and August to December (*Fig. 2a*). The total annual rainfall for 2009 was 1000 mm which was lower than the 15 year average of 1346 ± 54 mm. Mean monthly air and soil temperatures were highest during the dry period (January-March) and lowest during the wet months (April-May) and intermediate (July) (*Fig. 2b*). The mean monthly air and soil temperatures were 24.5^0C and 25.7^0C respectively. Annual variations in temperature were small, while daily fluctuations were large.

Gravimetric soil water content (SWC) within the 0-30 cm soil profile followed the rainfall pattern, increasing during the wet and declining during the dry months (*Fig. 2c*). SWC was lowest in January-February averaging ≈ 20% and highest in April-May at ≈ 40%.

Both total soil nitrogen (N) and carbon (C) were lowest (0.226±0.01% and 2.77±0.19% respectively) during the dry period (February-March) and increased with the rains to peak at the end of the rains (0.235±0.01% and 3.39±0.19% respectively) in June and (0.283±0.01% and 3.96±0.19% respectively) in November (*Fig. 3a*). Shoot N was lowest (0.342±0.16%) during the dry period, peaked (1.10±0.15%) at the onset of the rains in April and then declined with declining SWC to 0.55±0.15% in August before increasing again to another peak (1.01±0.15%) in November during the short rains (*Fig. 3b*). Root N increased slightly with the onset of rains from 0.573±0.09% in

March to 0.696±0.07% in April but thereafter declined to 0.445±0.07% in May while the shoot N remained high at 1.05±0.15%.

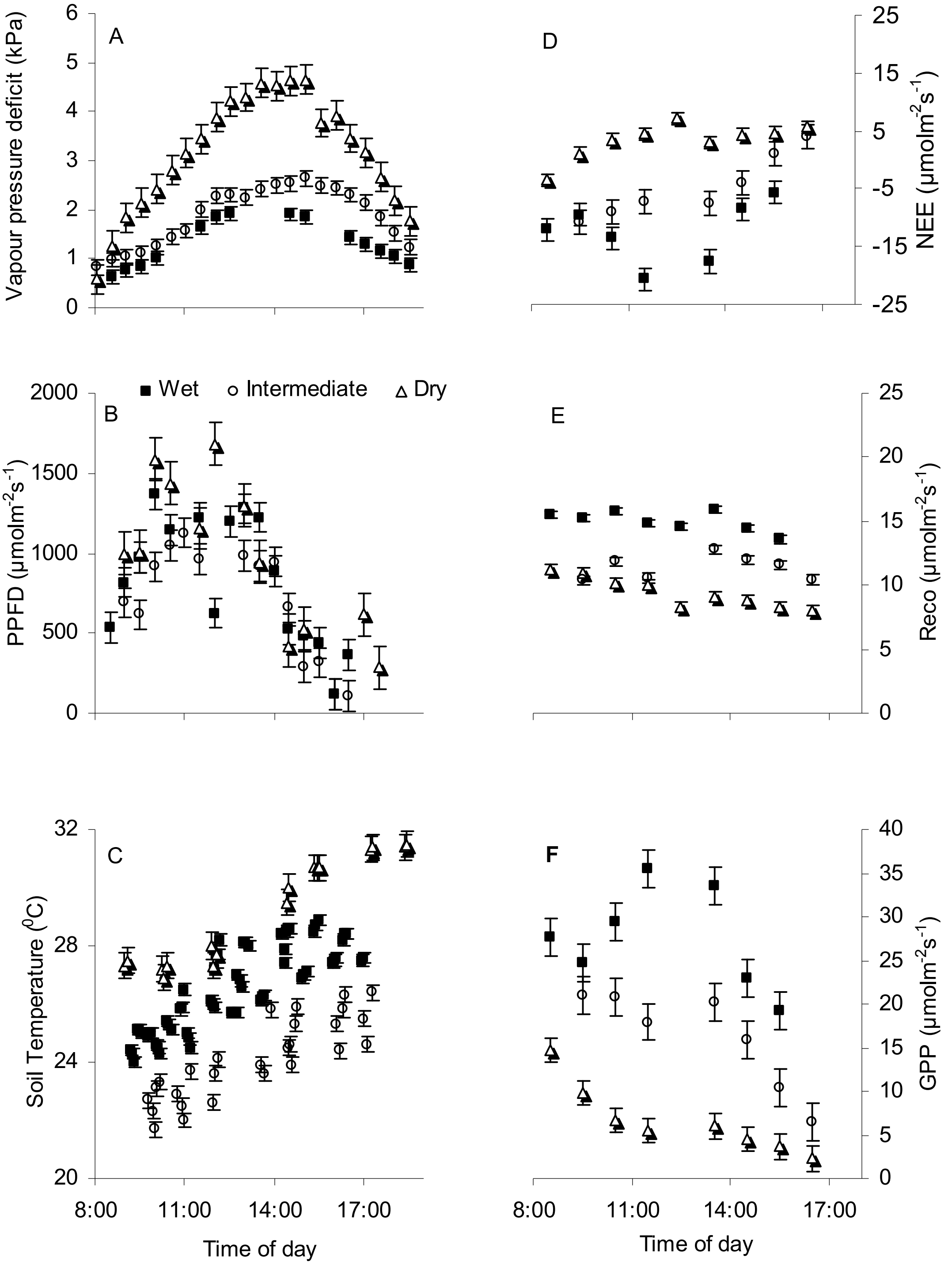

***Figure 1**: Daily trends of vapour pressure deficit (VPD) (a), photosynthetic photon flux density (PPFD) (b); soil temperature at 10 cm depth (c) net ecosystem CO2 exchange (NEE) (d); ecosystem respiration (e) and gross primary production (f) for dry, intermediate and wet, soil conditions. Values are means (± SE)*

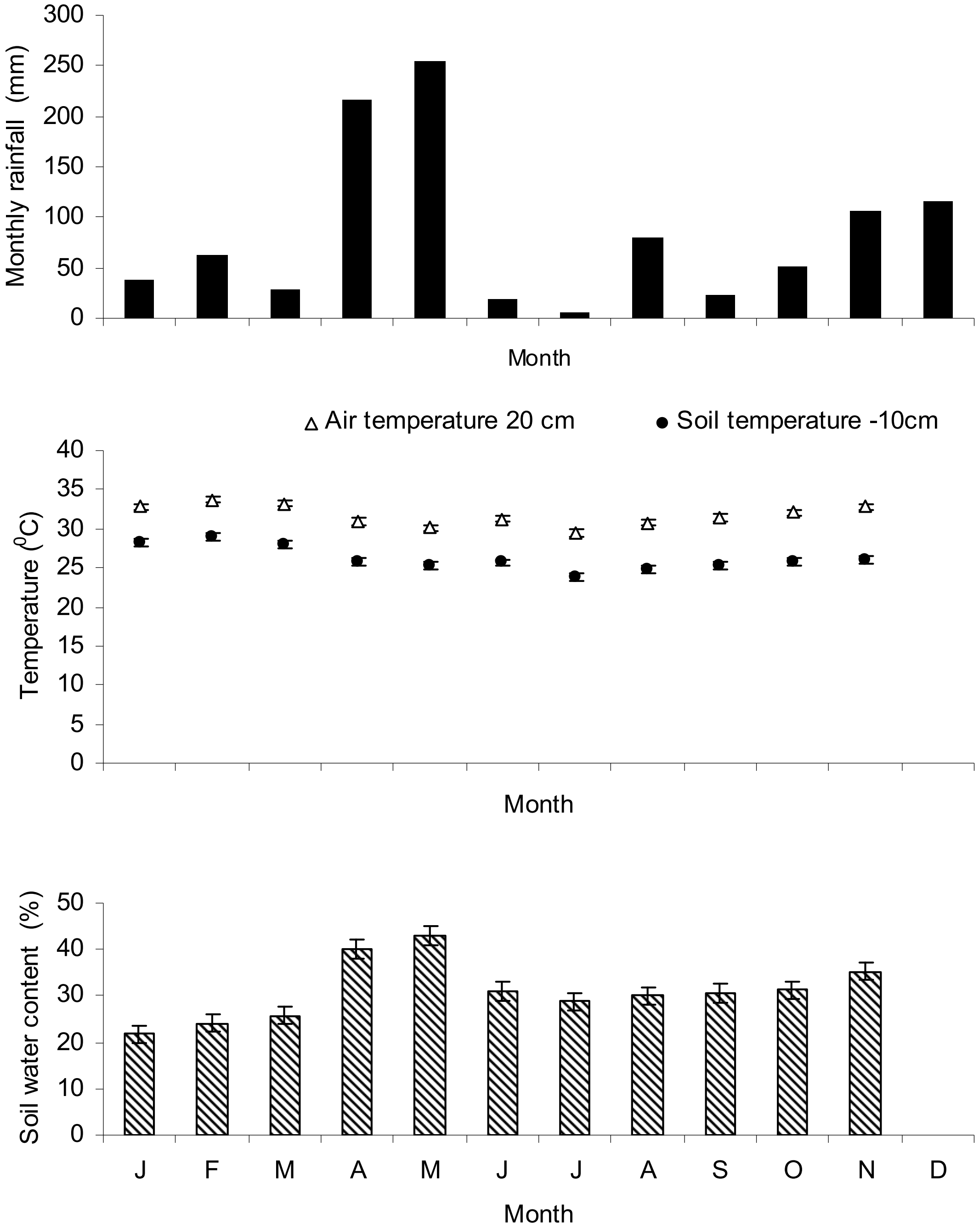

Figure 2. *Monthly rainfall (a), mean soil and air temperature (b) and mean soil water content (SWC) during 2009.*

As shoot N declined between May and August the root N levels increased by 50% from 0.445±0.07% to 0.880±0.07%. The roots had higher N levels (0.573±0.09% and 0.880±0.07%) compared to the shoots (0.342±0.16% and 0.55±0.15%) during February – March and August respectively. Shoot carbon to nitrogen (C:N) ratio was low during wet months when conditions are conducive for growth and high during drought (*Fig. 3c*).

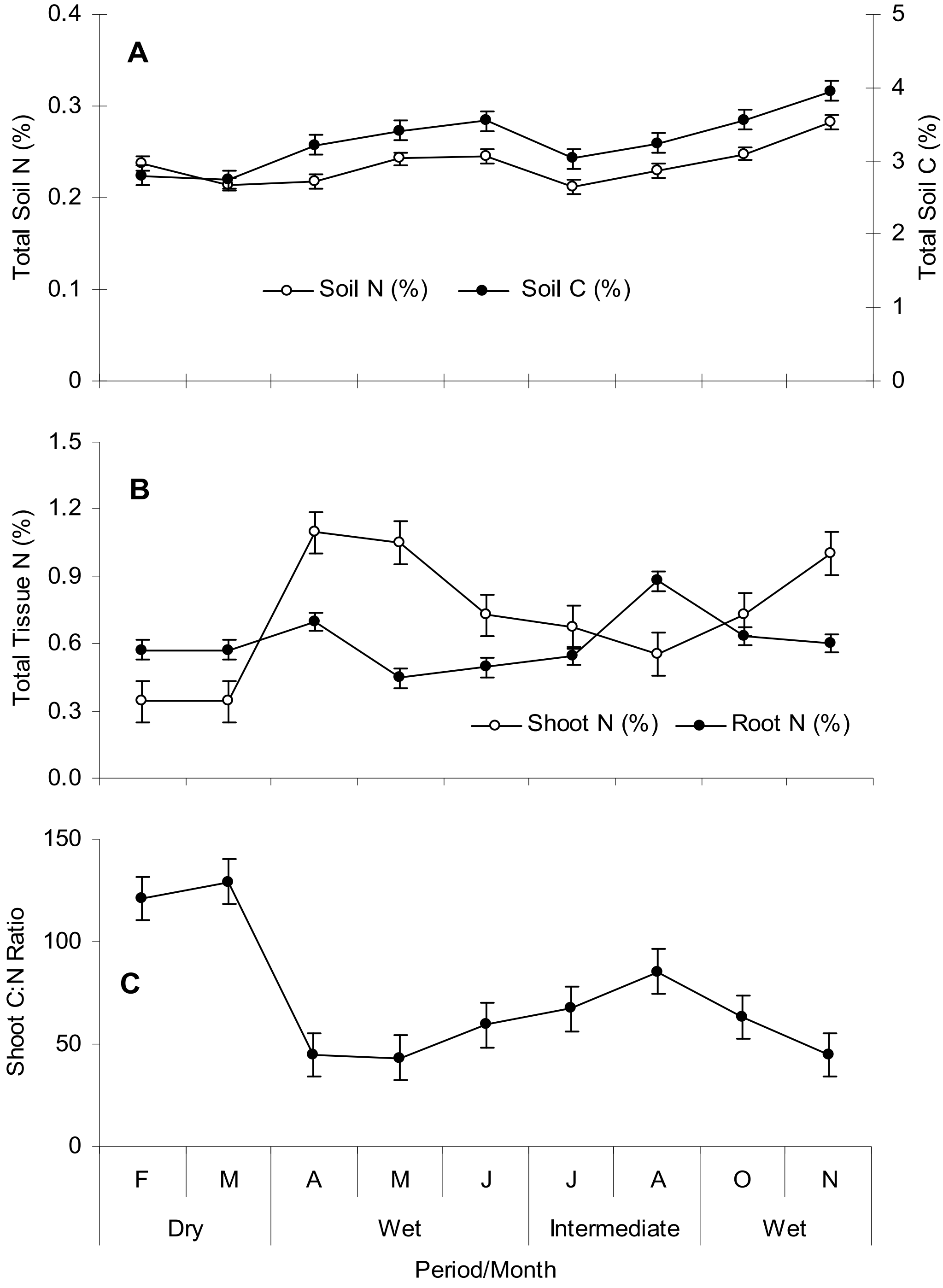

***Figure 3**. Total soil nitrogen (N) and carbon (C) (a), shoot and root N (b) and shoot C to N ratio (c). Values are means (± SE)*

Aboveground biomass

Both the total aboveground and dead/litter biomasses were highest (1426.1±71.3 and 553.4±31.1 gm^{-2} respectively) during drought at the beginning of the year (February – March) (*Fig. 4a*). Total aboveground and dead biomasses dropped to 529.5±64.3 and

154.0±28.5 gm^{-2} respectively at the onset of the rains in April and then gradually increased to peak again at the end of the growing season in July-August (887.2±71.3 and 290.6±31.1 gm^{-2}).

Carbon dioxide fluxes

Daily carbon fluxes

Daily trends of GPP, NEE and R_{eco} differed with the soil water status (*Figs. 1d, e, f*). When water was readily available (wet), GPP and NEE increased from 27.65 and -12.10 $\mu molm^{-2}s^{-1}$ respectively in the morning to daily maximum values of 35.49 and -20.65 $\mu molm^{-2}s^{-1}$ respectively around midday before declining gradually to 19.24 and -5.66 $\mu molm^{-2}s^{-1}$ respectively by around 17:00 hrs (*Figs. 1d, f*). This trend was similar to that of PPFD but GPP and NEE peaked earlier than VPD by about 2 hrs. During the periods of dry and intermediate soil water condition, GPP was highest (>10.00 and >20.00 $\mu molm^{-2}s^{-1}$ respectively) in the morning (08:00 hr) and decreased as the day progressed to 2.28 and 6.46 $\mu molm^{-2}s^{-1}$ respectively after 16:00hrs. Net ecosystem CO_2 exchange of the dry and intermediate periods followed a similar trend decreasing from <-3.00 and < -10.00 $\mu molm^{-2}s^{-1}$ respectively in the morning to >5.00 and > 3.00 $\mu molm^{-2}s^{-1}$ respectively after 16:00 hrs. The dry period NEE values were always positive implying the ecosystem was a net CO_2 source, while those of the wet period were always negative indicating a net CO_2 sink, during these periods. The daily trends of NEE and GPP for the intermediate soil conditions were in between those of the dry and wet conditions.

Ecosystem respiration (R_{eco}) varied little in the course of the day compared to GPP and NEE (*Fig. 1e*). It was almost unchanged in the morning (15.56 $\mu molm^{-2}s^{-1}$ at 08:00 and 15.86 $\mu molm^{-2}s^{-1}$ at 13:30 hrs) before decreasing to 13.58 $\mu molm^{-2}s^{-1}$ after 16:00 during wet period. It increased from 10.37 $\mu molm^{-2}s^{-1}$ at 9:00hrs to 12.78 $\mu molm^{-2}s^{-1}$ at 13:30 hrs before decreasing to 10.44 $\mu molm^{-2}s^{-1}$ after 16:00 hrs in the intermediate period. During the dry period, R_{eco} decreased from 11.19 $\mu molm^{-2}s^{-1}$ at 08:00hrs to 8.02 $\mu molm^{-2}s^{-1}$ by after 16:00 hrs.

Seasonal carbon fluxes

Net ecosystem CO_2 exchange (NEE), ecosystem respiration (R_{eco}), and gross primary production (GPP) followed the seasonal pattern of rainfall and SWC (*Fig. 4b*). Maximum net ecosystem CO_2 exchange, $R_{eco\ max}$ and GPP_{max} were lowest at 3.21±0.99, 8.21±1.02 and 4.67±1.06 $\mu molm^{-2}s^{-1}$ respectively during drought (February-March) at the beginning of the year, increased (became more negative for NEE_{max}) with the onset of the rains to -13.86±1.48, 15.03±0.98, and 27.73±1.47 $\mu molm^{-2}s^{-1}$ respectively in April, and gradually declined as the SWC decreased to reach the second low levels of -4.72±1.05, 6.59±1.02 and 9.16±1.62 $\mu molm^{-2}s^{-1}$ respectively in July. Maximum net ecosystem CO_2 exchange, $R_{eco\ max}$ and GPP_{max} again increased thereafter from August as the rains resumed.

The seasonal fluctuations in $R_{eco\ max}$ were relatively limited compared to those of the NEE_{max} and GPP_{max}. Maximum net ecosystem CO_2 exchange (NEE_{max}), $R_{eco\ max}$ and GPP_{max} were positively correlated with SWC with $R^2 = 0.79$, 0.63 and 0.89 respectively (*Fig. 4c*). Maximum R_{eco} ($R_{eco\ max}$) was strongly correlated ($R^2 = 0.68$; $P< 0.001$) to, and a linear function of GPP_{max} (*Fig. 4d*). The three periods of different phases of soil moisture distinctly stood out in the GPP_{max} vs. $R_{eco\ max}$ correlation.

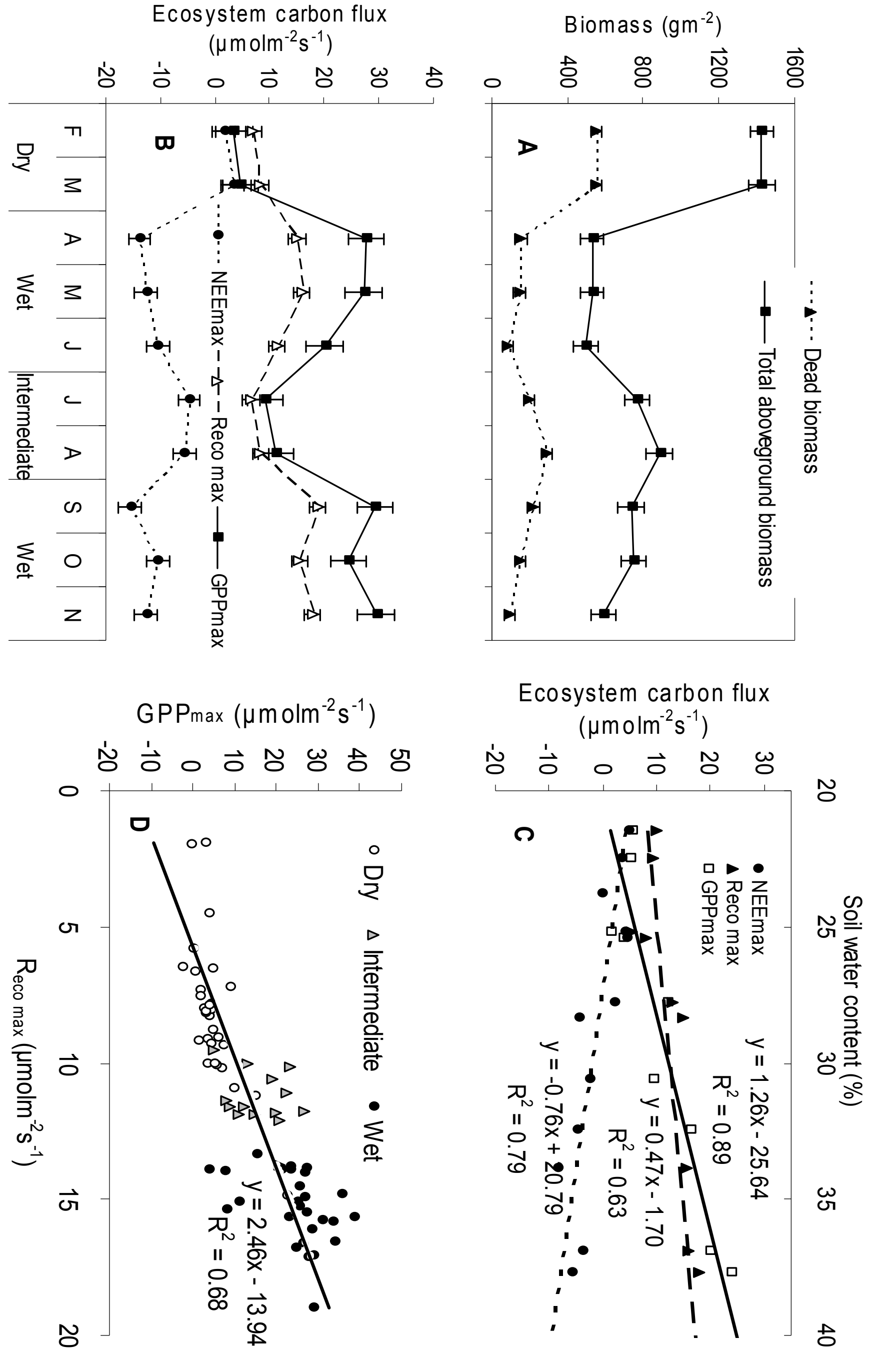

***Figure 4**. Total aboveground and dead biomass (a); Maximum net ecosystem CO2 exchange (NEEmax), ecosystem respiration (Reco max) and gross primary production (GPPmax) (b); and linear regressions, of SWC and NEEmax, Reco max, GPPmax (c), and of GPPmax and Reco max (d). Values are means (± SE)*

Net ecosystem CO_2 exchange was second-order polynomial non-linear function of VPD. It increased with increasing VPD when water was not limiting but decreased with increasing VPD under intermediate and dry soil conditions (*Fig. 5a*). The NEE Vs VPD correlations were moderate (R^2 = 0.50) under readily available soil water but strong under intermediate and dry conditions (R^2 = 0.84, 0.92 respectively).

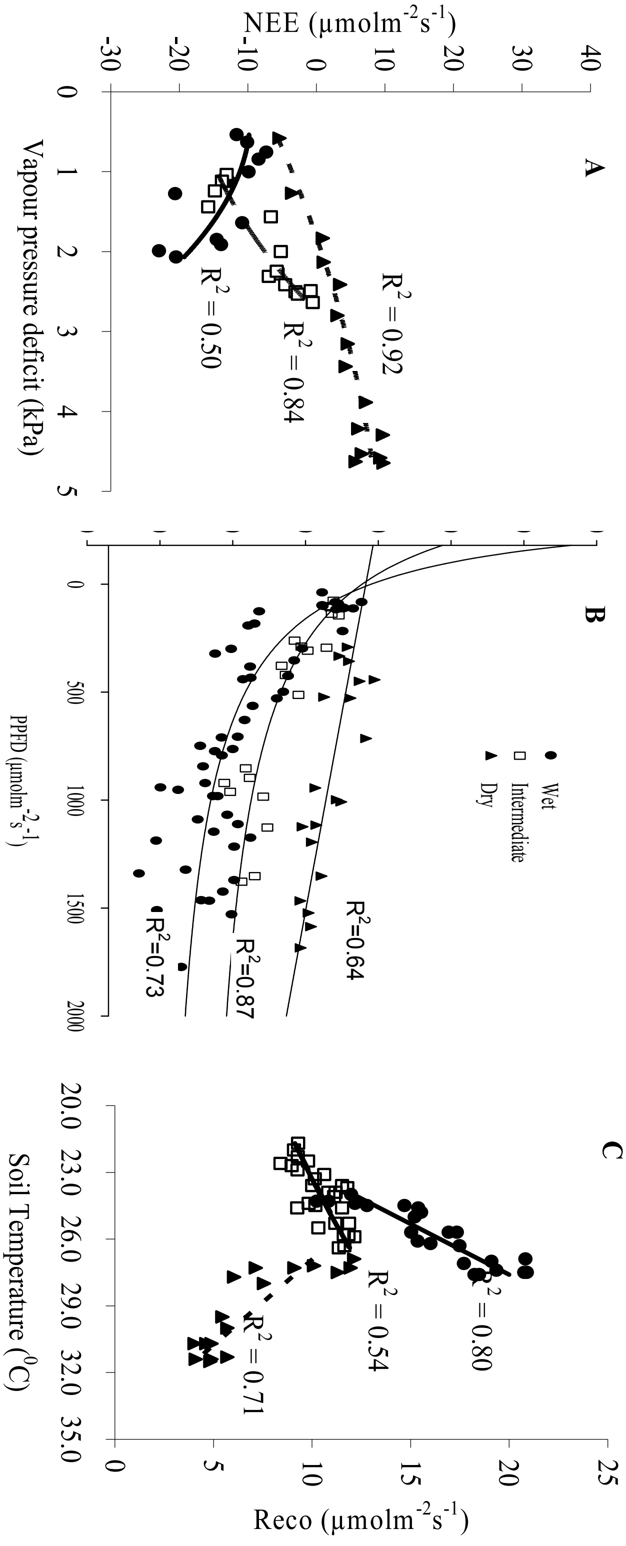

Figure 5: *Correlation between net ecosystem CO2 exchange (NEE), and vapour pressure deficit (a), and photosynthetic photon flux density (PPFD) (b): and between ecosystem respiration (Reco) and soil temperature (c) for dry, intermediate and wet, soil water status. Values are means (± SE)*

The rectangular hyperbolic light response curve (Eqn.3, Gilmanov *et al.*, 2007) best described the correlation between PPFD and NEE under all the three SWC conditions, with R^2 = 0.64, 0.87, and 0.73 for dry, intermediate and wet conditions respectively (*Fig. 5b*). Fitted parameters from the regression between NEE and PFD showed that light utilization efficiency (α) and maximum CO_2 assimilation rate of the canopy (β) decreased with decreasing soil moisture (*Table 1*). Light utilization efficiency (α) was -0.08 ± 0.04, -0.04 ± 0.02 and -0.01 ± 0.01 while maximum CO_2 assimilation rate of the canopy (β) was -30.39, 23.93 and 18.88 for the wet, intermediate and dry periods respectively.

Ecosystem respiration was linearly correlated to soil temperature with R^2 = 0.71, 0.54 & 0.80 for dry, intermediate and wet soil conditions respectively (*Fig. 5c*). It increased with increasing soil temperature under wet and intermediate soil water conditions but decreased with increasing soil temperature under dry soil conditions.

Table 1. Best-fit parameters of the empirical hyperbolic light-response model (Eqn 3) derived from NEE data grouped according to the different phases of soil moisture and their statistics.

Soil moisture condition	α	β	$\gamma 0$	SE α	SE β	SE $\gamma 0$	R^2	P
wet	**-0.08**	**-30.39**	**9.02**	**0.04**	**2.60**	**3.37**	**0.73**	**<0.0001**
Intermediate	**-0.04**	**-23.93**	**7.78**	**0.02**	**3.39**	**2.25**	**0.87**	**<0.0001**
Dry	**-0.01**	**-18.87**	**8.28**	**0.01**	**3.14**	**2.27**	**0.64**	**0.0003**

Discussion

Soil and plant nitrogen

Soil moisture levels as measured by SWC followed the rainfall pattern and were highest in the wet and lowest in the dry periods. The seasonal variation of rainfall and hence SWC determined soil C and N levels. The increments in total soil N at the onset of the rains most likely resulted from enhanced mineralization of easily decomposable organic matter that had been accumulated during the dry season (Wedin, 1996; Kutsch *et al.*, 2008). This is consistent with the rapid decline in the dead biomass at beginning of the rains in April. In addition, there was a strong positive correlation (R^2 = 0.80; $P < 0.001$) between soil C and N in this ecosystem.

The positive correlation of soil C and N is typical of the biological mineralization pattern of ecosystems in which soil nutrient levels are largely dependent on the soil litter content (Wedin, 1996; Ries & Shugart, 2008). The synchrony in soil C and N levels to soil moisture regimes underscores the significant role that soil moisture plays in nutrient cycling in this savannah. Our observations are in agreement with the conclusion by Wang *et al.* (2009) that soil water availability largely determines the patterns and rates of nutrient cycling in African savannah.

Shoot and root N levels increased from the onset of rains implying a strong interrelation between nutrient release from soil and uptake by grass plants in this ecosystem. Similarly enhanced uptake of N following onset of rains had been noted among perennial grasses (Joffre, 1990; Wedin, 1996). The shoot and root N levels were tightly inter-linked in this ecosystem. Shoot N was high and root N low during the periods of peak active growth and biomass accumulation (May) but shoot N declined as root N increased by 50% at the end of the growing season pointing to possible relocation and accumulation of N in the roots (Baruch & Gómez, 1996; Wedin, 1996). Furthermore the plant roots had higher N levels than the shoots during drought and shoot C:N ratio was high during drought but low during wet periods with active growth implying transportation of N out of the shoots possibly to the roots. Enhanced root accumulated N would enable rapid regrowth of the grasses the next time environmental conditions become favourable. The synchronised N release and uptake and its probable relocation from the shoot and accumulation in the roots are strategies that restrict N losses from the ecosystem (Wedin, 1996). Such strategies are highly beneficial in N-limited ecosystems like savannah.

Ecosystem productivity and carbon fluxes

Aboveground biomass development pattern followed the seasonal changes in soil moisture availability, with peak biomass coinciding with the end of the growing season. Similar biomass development trends had previously been noted in tropical savannah (Bourliére & Hadley, 1970; Scholes & Walker, 1993).

Net ecosystem CO_2 exchange, GPP, light use efficiency (α) and maximum canopy CO_2 assimilation rate (β) increased simultaneously with soil moisture peaking in the wet months. This is typical of most grassland, where gross and net photosynthesis are strongly correlated to green biomass (leaf area index) (Xu & Baldocchi, 2004; Li *et al.*, 2005; Otieno *et al.*, 2009; Beringer *et al.*, 2011). Given that shoot N also peaked in the wet season, the combined increase in green biomass (photosynthetic surface area), shoot N and light utilization efficiency facilitated rapid CO_2 uptake resulting into the high GPP and NEE.

At a daily scale, GPP, NEE and PPFD increased in tandem, their maximum values coincided at midday, and NEE was strongly correlated to PPFD ($R^2 = 0.73$, $P < 0.001$) during wet periods. Gross primary production and NEE were most likely being driven by photosynthetic photon flux density when water was not limiting (Dai *et al.*, 2004; Hastings *et al.*, 2005) as has previously been reported in savannah (Ardő *et al.*, 2008; Kutsch *et al.*, 2008; Risch & Frank 2010). During drought, the daily trends of GPP and NEE were reversed and they both decreased as VPD and PPFD increased. The decline in GPP and NEE during drought is attributed to either stomatal regulation by VPD (Eamus *et al.*, 2001; Maherali *et al.*, 2003; Li *et al.*, 2005) or their direct limitation by soil moisture (Kanniah *et al.*, 2010). Under drought with relatively high VPD, increasing VPD decreases stomatal conductance and hence GPP and NEE (Maherali *et al.*, 2003; Li *et al.*, 2005) in order to restrict plant water loss. Stomatal sensitivity to VPD is also known to increase with dropping leaf water potential in response to low soil moisture (Jones, 1998; Li *et al.*, 2005). Kanniah *et al.* (2010) however, reported canopy light use efficiency to be more strongly correlated to SWC at 10 cm depth than the atmospheric VPD. It therefore implies that both low SWC and high VPD may work in synergy to lower the gross and net photosynthesis under dry soil conditions.

There was no evidence of direct GPP and NEE responses to ambient temperature. Instead, mean daily temperatures were lowest during the rainy seasons, when GPP and

NEE were highest, and highest during drought when GPP and NEE were lowest. It is however, noteworthy that air temperature did not vary much over the year at our site.

Since soil N concentration did not differ over the year, we conclude that the seasonal differences in NEE and GPP must have resulted from the seasonality of soil moisture that caused the differences in green biomass (photosynthetic surface area), shoot N, light utilization efficiency and maximum canopy CO_2 assimilation rate. Similar driving role of soil moisture on gross and net photosynthesis of savannah had previously been reported (Xu & Baldocchi, 2004; Merbold *et al.*, 2008; Otieno *et al.*, 2010).

Seasonally we observed no influence of soil temperature on ecosystem respiration instead, increasing temperatures were associated with drying soils and reduced R_{eco} over the year. This contradicted other studies (Lloyd & Taylor 1994; Xu *et al.*, 2005; Flanagan & Johnson, 2005; Li *et al.*, 2008) but was consistent to previous work of Otieno *et al.* (2010) that reported minimal soil temperature control over R_{eco} at the same study site. It is attributed to soil moisture controlling R_{eco} directly through its effects on soil microbial activity (Bowden *et al.*, 1993, Hanson *et al.,* 2000) and indirectly through assimilation and transport of assimilates to the respiring surfaces and into the soil (Kuzyakov & Cheng, 2004). The indirect influence of soil moisture on R_{eco} via C assimilation is supported by our strong positive correlation between R_{eco} and GPP (R^2 = 0.68, P ≤0.001).

Contrary to our seasonal data and work of Otieno *et al.* (2010) at the same site, the daily trends revealed controlling effects of soil temperature on R_{eco}. Ecosystem respiration increased with increasing soil temperature under wet and intermediate soil water conditions but decreased with increasing soil temperature during drought and was strongly correlated to soil temperature (R^2 = 0.71 and R^2= 0.80 for dry and wet periods respectively). The trend under wet soil conditions is in agreement to the observation that when water is not limiting, R_{eco} is largely governed by the soil temperature (Joffre *et al.,* 2003; Flanagan & Johnson, 2005; Li *et al.,* 2008).

The decoupling of R_{eco} to soil temperature as noted during drought is not new having previously been reported (Flanagan & Johnson, 2005; Tang & Baldocchi, 2005) and is attributed to decline in the sensitivity of respiration to temperature with increasing soil temperature and decreasing soil moisture (Xu & Baldocchi, 2004; Flanagan & Johnson, 2005). We speculate that the mismatch between R_{eco} and soil temperature during drought may have resulted from, (i) restrained autotrophic respiration due to low carbon source for respiration as photosynthetic rates declined in response to high temperature, extremely high VPD, and low soil moisture; and (ii) decreased sensitivity of water stressed microbial decomposers (a major contributor to soil respiration) to high temperatures (Lloyd & Taylor, 1994; Tang & Baldocchi, (2005).

We attribute the anomaly in the seasonal and daily R_{eco} correlations with soil temperature to the greater daily (5 to7^{0}C) compared to annual temperature fluctuations (2 to3^{0}C) at our study site. The daily fluctuations in temperature were evened out over time (seasonally) leaving the seasonally significantly different soil moisture to determine the ecosystem respiration. Such overriding role of soil moisture on ecosystem respiration relative to temperature is not new having been reported by others (Xu & Baldocchi, 2004; Tang & Baldocchi, 2005; Otieno *et al.*, 2010).

Highest mean carbon fluxes were 3.21±0.99, 8.21±1.02 and 4.67±1.06 μmolm^{-2}s^{-1} during drought and -13.86±1.48, 15.03±0.98, and 27.73±1.47 μmolm^{-2}s^{-1} during wet periods for NEE, R_{eco} and GPP respectively. The ecosystem was a net C sink over most of the year except at the beginning when SWC fell below 25% and NEE was positive.

This is contrary to the reports from semi arid savannah of Sudan (Ardő *et al.*, 2008) which indicated a sink albeit a weak one even during the dry spell. The difference could stem from the fact that unlike Ardő *et al.* (2008) who integrated NEE from both the trees and herbaceous layer, we only measured the latterr. The herbaceous layer ceases growth and senesces (Eamus *et al.,* 2001; Baldocchi *et al.,* 2004) during drought, while the deep rooted trees would still continue accessing soil moisture and hence photosynthesising.

Conclusion

Seasonally, the ecosystem C fluxes and biomass production of this moist savannah were largely determined by rainfall and hence available soil moisture. Soil N, and shoot N levels, NEE, R_{eco}, and biomass development followed a seasonal pattern governed by the seasonality of rainfall. N mobility among its pools of soil, plant roots and shoots were tightly coupled in a manner that served to restrict its loss from the ecosystem and to enhance its availability and utilization by plants.

The daily ecosystem C fluxes and their relationship to the daily weather parameters were also determined by soil moisture levels as dictated by rainfall. However, on a daily scale, Gross primary production and net ecosystem CO_2 exchange were regulated by photosynthetic photon flux density when water was readily available and vapour pressure deficit during drought. Ecosystem respiration was controlled by soil temperature. We recommend intense measurements during periods of greatest temperature variations at the site (July-January) to rule out seasonal influence of temperature on ecosystem respiration.

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PHYTOPLANKTON BASED ASSESSMENT OF ECOLOGICAL STATUS OF BULGARIAN LAKES AND COMPARISON OF METRICS WITHIN THE WATER FRAMEWORK DIRECTIVE

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Abstract. Taxonomic composition and abundance of summer phytoplankton were studied in 78 lakes in Bulgaria. The Hungarian Assemblage index was applied for the first time on various Bulgarian lake types and 27 phytoplankton functional groups were identified. The dominant functional groups differed based on lake type and trophic status. Functional groups' frequency distribution showed that high relative abundance of assemblages L_0, Y, MP, N, E, X3, X_{ph} indicated high or good status, regardless of lake type. Simultaneously undesirable assemblages, linked to the declined water quality, were L_M, J, M, S1 and H1. We also examined the relationship between the Assemblage index and a number of phytoplankton metrics: Total biomass, % Cyanobacteria, Transparensy, Chlorophyll *a*, Algal bloom and Algae Group Index. The two applied indices (Hungarian Assemblage index and Algae Group index) were highly correlated ($p<0.01$) . The Assemblage index had also strong relationship ($p<0.001$) with Total biomass, % Cyanobacteria, Chlorophyll *a*, as well as with the assessed overall ecological status. Assemblage index and Algae Group Index resulted in similar assessment and were applicable to all three Alpine and mountain, Deep semimountain and Small and middle sized lowland lake groups. Assemblage index is recommended especially for lentic ecosystems in extreme cases, e. g. hyperhaline lakes, for its flexibility and more adequate assessment.

Keywords: *Assemblage index, Q index, Algae Group Index, Functional groups, Biomass*

Introduction

Studies on phytoplankton communities and their relationship with water quality status have a long history (Padisák et al., 2006; Cheshmedjiev et al., 2010a). A number of phytoplankton based indices have been developed for the purpose of Water Framework Directive (WFD). Among them six were applied in South Europe: Algae Group Index (AGI, Catálan Index) in Spain and Italy (Agència Catalana de l'Aigua, 2003; Marchetto et al., 2009); ITP or Barbe Index in France (Philippe et al., 2003); Brettum index in Austria (Dokulil and Teubner, 2006); Phytoplankton Trophic Index (PTI) in Italy (Salmaso et al., 2006); Mediterranean Phytoplankton Trophic Index (MedPTI) in Italy (Marchetto et al., 2009), and Assemblage index (Q index) in Spain (Becker et al., 2010). According to Marchetto et al. (2009) indices can be differentiated into two groups. The first one (Brettum and MedPTI), based on species trophic preference, evaluates abundance of each taxa and adjudges trophic points/values. The second group (AGI and Q) applies percent biomass share of particular algae.

Assemblage index was developed for the assessment of lakes in Hungary within the WFD (Padisák et al., 2006). It requires species level determination and combines relative share of species assemblages with factor numbers for particular lake types (Becker et al., 2010). Phytoplankton functional group (FG) concept outlined in Reynolds et al. (2002) was followed for ‘‘functional groups’’ establishment, as well as physiological, morphological and ecological attributes of the species that may potentially dominate or co-dominate in a particular type water body. Subsequently, the approach was further developed (Padisák et al., 2003; 2009). At present, 38 FGs are described, identified by numeric character codes (codons). The Q index is a reliable instrument to assess ecological status and trends (Padisák et al., 2006; Becker et al., 2010). Besides Hungarian lakes, Q index was also successfully tested at shallow tropical, deep subtropical and deep Mediterranean reservoirs (Crossetti and Bicudo, 2008; Becker et al., 2009; 2010). A major advantage of Q index is that unlike other indices, it reflects general anthropogenic pressure.

Algae Group Index (AGI) is the first index developed particularly for Mediterranean sites (Agència Catalana de l'Aigua, 2003) and was calibrated on the basis of Spanish reservoirs data. Algae Group Index was founded on proportion between biomass of two algae units. In view of high levels of taxa determination, AGI should be applied carefully, because its algal groups include species with different ecological preferences (Marchetto et al., 2009).

New phytoplankton functional classifications, based on a morphological approach, were reported recently (Salmaso and Padisák, 2007; Kruk et al., 2010). Classification of Salmaso and Padisák (2007) is based on morphological and functional criteria (size and shape, mobility, potential mixotrophy, buoyancy) and divides phytoplankton into 31 morpho-functional groups (MFGs) with different adaptive strategies. Classification of Kruk et al. (2010) is clearly only morphological and seven morphologically based functional groups (MBFGs) are differentiated. Selected morphological characteristics correlated well with functional properties, such as growth and sinking rate, vulnerability to consumption and also with the population size and biomass attained in the field. Among the above concepts, the FG concept (within Q index) and MFG concept within Functional Traits Index (FTI) (Phillips et al., 2011) are elaborated in the context of WFD.

Our study had three main aims. Firstly, we attempted to apply the Q index for assessment of the water bodies from existed lake types in Bulgaria according to the accepted typology in 2009. Moreover, the Q index was tested on water bodies significantly differentiated by origin, altitude, salinity and mixing and stratification. Secondly, we examined the relationships between several existing phytoplankton metrics to explore their potential for assessment of water bodies from existed lake types in Bulgaria, among them specific ecosystems. Thirdly, we evaluated the overall ecological status of selected lakes.

The research was a logical continuation of previously published results on revision of typology and ecological status/potential of the lakes in Bulgaria (Cheshmedjiev et al., 2010a; 2010b).

Materials and methods

Study area and sites description

Seventy-eight lakes were studied during 2009 in Bulgaria (*Fig. 1*). The water bodies differed in altitude characteristics (from 0 to 2375 m a.s.l.), origin (natural, artificial, glacial, tectonic, karst, liman, lagoon), morphometry (all classes), mixing and stratification (glacial monomictic, shallow polymictic and deep dimictic) and trophic state (from ultra oligotrophic to hypertrophic).

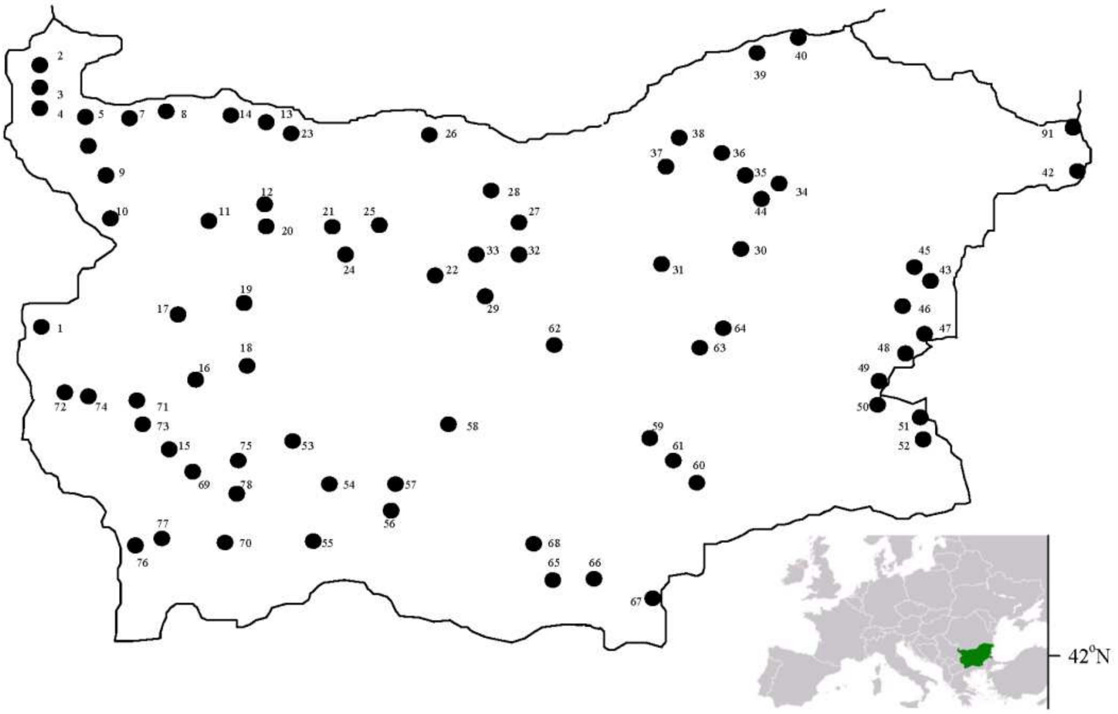

Figure 1. *Location of the studied lakes. Legend (national lake type written in parenthesis): 1 - Yarlovtsi Reservoir (L2); 2 - Kula Reservoir (L12); 3 - Poletkovtsi Reservoir (L12); 4 - Rabisha Reservoir (L4); 5 - Drenovets Reservoir (L16); 6 - Hr. Smirnenski Reservoir (Lomtsi) (L16); 7 - Rasovo Reservoir (L16); 8 - Kovachitsa Reservoir (L16); 9 - Ogosta Reservoir (L14); 10 - Srechenska bara Reservoir (L2); 11 - Dabnika Reservoir (L16); 12 - Tri kladentsi Reservoir (L16); 13 - Barsina Reservoir (L16); 14 - Asparuchov val Reservoir (L16); 15 - Beli Iskar Reservoir (L1); 16 - Iskar Reservoir (L11); 17 - Pancharevo Reservoir (L12); 18 - Ognyanovo Reservoir (L2); 19 - Bebresh Reservoir (L2); 20 - Devets Reservoir (L16); 21 - Enitsa Reservoir (L16); 22 - Sopot Reservoir (L12); 23 - Kruchovitsa Reservoir (L16); 24 - Telish Reservoir L16); 25 - Gorni Dabnik Reservoir (L14); 26 - Valchovets Reservoir (L16); 27 - Alexandrovo Reservoir (L16); 28 - Kamenets Reservoir (L16); 29 - Hr. Smirnenski Reservoir (Gabrovo) (L2); 30 - Yastrebino Reservoir (L12); 31 - Yovkovtsi Reservoir (L2); 32 - Al. Stambolijski Reservoir (L11); 33 - Krapets Reservoir (L12); 34 - Beli Lom Reservoir (L12); 35 - Lomtsi Reservoir (L12); 36 - Kavatsite Reservoir (L12); 37 - Boika Reservoir (L12); 38 - Baniska Reservoir (L12); 39 - Antimovo Reservoir (L16); 40 - Srebarna Lake (L5); 41 - Durankulak swamp (L7); 42 - Shabla lake (L7); 43 - Eleshnitsa Reservoir (L12); 44 - Saedinenie Reservoir (L12); 45 - Tsonevo Reservoir (L14); 46 - Acheloy Reservoir (L16); 47 - Poroy Reservoir (L16); 48 - Pomorijsko Lake (L10); 49 - Atanasovsko Lake (L10); 50 - Mandra-east Reservoir (L7); 51 - Alepu Lake (L8); 52 - Yasna polyana Reservoir (L12); 53 - Belmeken Reservoir (L13); 54 - Batak Reservoir (L3); 55 - Toshkov chark Reservoir (L3); 56 - Vacha Reservoir (L11); 57 - Krichim Reservoir (L11); 58 - Pyasachnik Reservoir (L15); 59 - Daskal Atanasovo Reservoir*

(L12); 60 - Ovchi kladenets Reservoir (L12); 61 - Ovcharitsa Reservoir (L12); 62 - Koprinka Reservoir (L11); 63 - Zhrebchevo Reservoir (L11); 64 - Asenovets Reservoir (L13); 65 - Kardzhali Reservoir (L11); 66 - Studen kladenets Reservoir (L11); 67 - Ivaylovgrad Reservoir (L11); 68 - Borovitsa Reservoir (L13); 69 - Redzhepsko Lake (L1); 70 - Bezbog Lake (L1); 71 - Studena Reservoir (L3); 72 - Pchelina Reservoir (L4); 73 - Dyakovo Reservoir (L13); 74 - Choklyovo marshland (L4); 75 - Chernoto Lake (L1); 76 - Stoykovtsi Reservoir (L13); 77 - Bistraka Lake (L6); 78 - Gyorgiysko Lake (L1)

National typology

Bulgaria belongs to ecoregions № 12 Pontic province and № 7 Eastern Balkans according Appendix № XI, map A of Water Framework Directive 2000/60/EC (EC Parliament and Coincil, 2000). Lake typology in Bulgaria was based on the obligatory factors (4 altitude zones, size typology based on surface area, depth and geology) and optional factors (mixing characteristics, salinity, residence time, presence of profundal zone). Seventeen lake types were identified, among them seven reservoir types, represented by heavily modified and artificial water bodies without any natural lake template within the country or region (Cheshmedjiev et al., 2010b). There are a small number of natural lakes in the country, most of them under srong anthropogenic impact. Four coastal lake types with various salinity (from freshwater <0.5‰ to hyperhaline >40‰) have been reviewed as transitional waters.

Sample collection

Integrated sampling from the deepest lake zone was carried out by Ruttner batometer during July-September 2009. The depth of the euphotic zone, was defined as 2.7 times the Secchi depth (Cole, 1994). Fixed median horisonts were sampled in alpine lakes due to their high transparency (0 m, 1 m, 2.5 m, 5 m, 7.5 m, 10 m and 15 m), whereas the whole water column was collected in shallow polymictic lakes (<3.0 m). Physico-chemical parameters of lake water: temperature, transparency according to Secchi, electrical conductivity and pH were measured *in situ*. Additionally NH_4-N, NO_2-N, NO_3-N, soluble reactive phosphorus (SRP: P-PO_4), total nitrogen and phosphorus were analyzed just after sampling according to Strickland and Parsons (1972). Chlorophyll *a* concentration was determined spectrophotometrically, after filtration through GF/B Whatman glass fiber filter and extraction in 90% acetone, following Jeffrey and Humphrey (1975).

Phytoplankton analysis

Phytoplankton taxonomic composition was determined by a light microscope Amplival (1000 x) on live and formalin-fixed samples. Phytoplankton was counted at 400x using an inverted microscope following the method of Utermöhl (1958). For numerous species, at least 100 specimens were counted (Lund et al., 1958). The units (cells, colonies or filaments) were counted in random plots. Biovolume was determined following the geometric forms formulas (Hillebrand et al., 1999). Transfomation of biovolume into biomass was done by 1 mm^3 l^{-1} = 1 mg l^{-1} (Wetzel and Likens, 2000). Total biovolume (biomass) at each sample was presented as a sum of biovolume of all taxa. The ecological status assessment was based on 5 main (total phytoplankton biomass (mg l^{-1}); Assemblage index; Algae Group Index, transparency according to Secchi (m), chlorophyll *a* (µg l^{-1})) and 3 additional metrics (% Cyanobacteria (in biomass); “bloom” (intensity) and presence of “bloom” toxic species (*Microcystis,* *Aphanizomenon, Cylindrospermopsis* and others). The intensity of the phytoplankton „bloom” was assessed on the basis of total biomass on a 5-degree scale, modified after Sirenko and Gavrilenko (1978): I degree ≤ 2.5 mg l^{-1}; II degree: 2.5 ÷ 10 mg l^{-1}; III degree: 10 ÷ 500 mg l^{-1}; IV degree: 500 ÷ 5000 mg l^{-1}; V degree („hyperbloom”) > 5000 mg l^{-1}. In calculating % Cyanobacteria, some species/genus for oligotrophic waters have been excluded, focusing on toxic species and eutrophic indicators (according WFD Intercalibration technical report, Part 2 – Lakes, Section 3 – Phytoplankton biomass metrics Annexes).

Phytoplankton-based Indices

Assemblage index was calculated after Padisák et al. (2006) for a selection of 19 lakes, representing 25% of all studied lakes and allocated to 4 representative groups. Water bodies from dominant type within the groups formed of lakes in identical ecological status were selected, whereas representative water bodies into different classes of ecological quality were chosen in the rest of the groups. Q index accounted relative share of FGs into the total biomass and factor number (F) determined for each FG in each lake group.

The Algae Group Index (Catálan Index) based on the percentage of biovolume of the algae groups was calculated following the formula (Agència Catalana de l'Aigua, 2003).

Classification system for ecological status according to phytoplankton

Two different scales for the basic metrics (*Table 1* and *Table 2*) have been used in the ecological assessment and interpretation of phytoplankton data, modified for the relevant lake types in Bulgaria. The scale for AGI was modified after WFD Intercalibration technical report, Part 2 – Lakes, Section 3 – Phytoplankton composition metrics (2008); those for Chlorophyll *a* and transparency were after Cardoso (2001). Q index scale followed Padisáк et al. (2006).

Table 1. *Oligotrophic type of „lakes” (L1, L2, L3, L11, L12, L13) – classification system.*

EQR (AGI)	Total biomass (mg l^{-1})	Q	AGI	Transparency (m)	Chl *a* (µg l^{-1})	% Cyanobacteria	“Bloom” (intensity)	“Bloom” toxic species
<0.998	**<1**	**4÷5**	**<0.9**	**>4**	**<4**	**<4**	**÷**	**no**
0.995÷0.998	**1÷5**	**3÷4**	**0.9÷2**	**2÷4**	**4÷10**	**4÷15**	**÷**	**no**
0.975÷0.995	**5÷8**	**2÷3**	**2÷10**	**1.5÷2**	**10÷15**	**15÷20**	**I**	**no/yes**
0.95÷0.975	**8÷10**	**1÷2**	**10÷20**	**1÷1.5**	**15÷50**	**20÷50**	**II÷III**	**yes**
<0.95	**>10**	**0÷1**	**>20**	**<1**	**>50**	**>50**	**III÷V**	**yes**

Statistical analysis

Data analyses using package Canoco ver. 4.5 were conducted (Ter Braak and Šmilauer, 2002). Canonical ordination technique (CCA) was carried out on data for 5 environmental variables (altitude, depth, transparency acc. to Secchi – SD, phytoplankton biomass and chlorophyll *a*) measured at 78 lakes, total number of occurrences 375, to study the role of variables related to lake's type. The data were transformed (x' = log (x + 1)), automatically centered and standardized by the Canoco analysis program. Monte Carlo permutation tests (number of permutations 499) and forward selection were used within CCA to detect significant ($p = 0.05$ probability threshold level) and independent environmental variables. Correlation coefficients (r) among the various phytoplankton metrics at studied lakes were calculated (correlation matrix).

Table 2. *Mesotrophic type „lakes" (L4, L5, L6, L7, L8, L9, L10, L14, L15, L16, L17) – classification system.*

EQR (AGI)	Total biomass (mg l^{-1})	Q	AGI	Transparency (m)	Chl *a* (µg l^{-1})	% Cyanobacteria	"Bloom" (intensity)	"Bloom" toxic species
<0.998	<1.5	4÷5	<1	>4	<4	<4	÷	no
0.994÷0.998	1.5÷7	3÷4	1÷2.5	2÷4	4÷10	4÷15	I	no/yes
0.975÷0.994	7÷15	2÷3	2.5÷10	0.5÷2	10÷20	15÷20	II	yes
0.95÷0.975	15÷25	1÷2	10÷20	0.6÷1	20÷50	20÷50	III	yes
<0.95	>25	0÷1	>20	<0.6	>50	>50	IV÷V	yes

Results

Lake groups division

The relationships between environmental characteristics of the studied 78 lakes were identified using CCA (*Fig. 2*). The sum of all canonical eigenvalues was 0.187. The first CCA axis explained 52% of the variance of the data. All five environmental parameters tested were significantly correlated with the lake type ($p<0.01$). Chlorophyll *a* and biovolume, significantly correlated with the negative part of the first axis, were shown to be the parameters exerting greatest influence, explaining together 64% of the variance. Second axis explaining together with first axis 59.6% of the variance of lake types, was most strongly correlated with altitude. Studied lakes were grouped as follows. Group I at the upper right side of the CCA ordination diagram incorporated 11 alpine and mountain lakes at an altitude > 800 m above sea level. Alpine lakes over 2240 m (I.1, i.e. No 69, 70, 75 and 78) formed a distinguished subgroup among them (I.1). The second group (II) included 27 deep semi mountain and lowland lakes, at less than 800 m a.s.l. and over 8.5 m maximal depth. Axis 2 separated the above two groups oligotrophic lakes at the right side of the plot from Group III, combining 38 mesotrophic small and middle sized lowland lakes at an altitude < 200 m a.s.l. and size up to 10 km^2. Two lakes from national type L10 Black Sea hyperhaline coastal lakes, included at Group III: Pomorijsko (No 48) and Atanasovsko lakes (No 49), differ strongly in salinity (> 42‰). Thus they were discussed as separate Group IV.

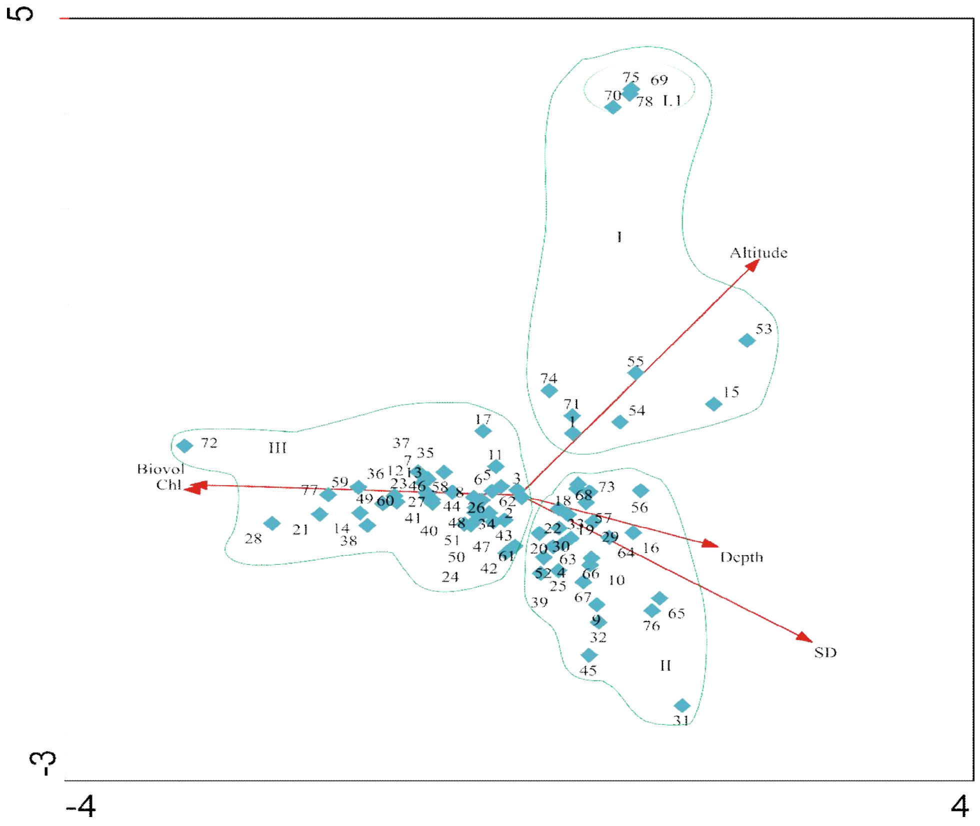

Figure 2. CCA ordination diagram of 5 environmental variables measured at 78 studied lakes from all lake types. Explanations: Arrows - relevant variable value, starts from the origin with average values and extends toward higher values (Chl = Chlorophyll a; Biovol = Biovolume, SD = Transparency according to Secchi); diamonds - relevant sampling site according Fig. 1; lake groups: I.1 – Alpine lakes; I – Mountain lakes; II – Deep semi mountain and lowland lakes; III Small and middle sized lowland lakes

Physico-chemical water quality analysis

Data for physico-chemical parameters of lake water were presented at *Table 3*. Average temperature and pH increased in comformity from Group I (Alpine and Mountain lakes) to Group III and IV (lowlands lakes), while transparency decreased. Electrical conductivity and salinity were highest at lakes from Group IV due to their transitional character.

Table 3. *Range of environmental variables for the different "lake" groups in 2009. Legend: Groups – according Fig. 2; T, Temperature; DO, Dissolved oxygen; OS, Oxygen saturation; SD, Secchi depth; Cond, Conductivity; N-NH4+, Ammonium nitrogen; N-NO2-, Nitrite nitrogen; N-NO3-, Nitrate nitrogen; SRP, Soluble reactive phosphorus; TP, Total phosphorus; TN, Total nitrogen; Chl a, Chlorophyll a; S, Salinity; Ls, Lake size.*

	Group I n=11		**Group II** n=27		**Group III** n=38		**Group IV** n=2	
	Interval	*Mean*	Interval	*Mean*	Interval	*Mean*	Interval	*Mean*
T °C	3.0-20.9	***13.5***	10.8-28	***19.0***	9.2-29.2	***19.6***	16.9-18.4	***17.7***
DO (mg l^{-1})	5.8-8.0	***7.4***	6.4-9.8	***7.9***	1.1-18.4	***7.9***	4.9-5.8	***5.4***

OS (%)	65-94	***82.5***	66-109	***85.4***	28.9-214	***85.6***	52.3-58.6	***55.5***
SD (m)	1.6-16.0	***7.10***	1.1-7.5	***3.3***	0.3-2.3	***1.0***	0.25-0.7	***0.5***
pH	7.2-8.4	***7.8***	6.7-9.0	***8.2***	7.7-9.6	***8.6***	8.4-8.9	***8.7***
Cond (µS cm^{-1})	9.7-308	***86.2***	22-536	***244***	176-1864	***591***	64000-105003	***84502***
N-NH$_4^+$ (mg l^{-1})	0.01-0.22	***0.039***	0.006-0.09	***0.026***	0.008-1.55	***0.15***	0.17-0.065	***0.118***
N-NO$_2^-$ (mg l^{-1})	0.002-0.005	***0.003***	0.002-0.04	***0.006***	0.002-0.067	***0.02***	0.007-0.025	***0.016***
N-NO$_3^-$ (mg l^{-1})	0.035-0.07	***0.157***	0.08-0.45	***0.212***	0.066-0.80	***0.27***	0.2-0.6	***0.4***
SRP (mg l^{-1})	0.01-0.083	***0.027***	0.005-0.034	***0.012***	0.001-0.317	***0.04***	0.023-0.059	***0.041***
TP (mg l^{-1})	0.002-0.263	***0.075***	0.008-0.091	***0.024***	0.010-0.364	***0.107***	0.07-0.096	***0.083***
TN (mg l^{-1})	0.50-0.50	***0.50***	0.4-1.9	***0.762***	0.378-4.10	***1.128***	1.9-3	***2.5***
Chl *a* (µg l^{-1})	<0.2-6.5	***2.65***	<0.2-14.5	***2.98***	0.2-119.3	***24.68***	2.61-31.2	***16.91***
S (‰)	< 0.5		< 0.5		< 0.5		42.7-63.4	***53.1***
Ls (km^2)	0.02-22.08	***3.11***	0.03-30.0	***7.68***	0.04-13.0	***2.42***	9.5-16.9	***13.2***

Assemblage index (Q)

Functional groups

A total of 27 phytoplankton FGs were identified on the basis of selected 19 lakes from the dataset (*Table 4* and *Table 5*).

Two lakes were included in the Lake Subgroup I.1 – Alpine lakes (*Table 4*). Chernoto and Bezbog Lake are small sized (<0.15 km^2) glacial lakes located at altitude above 2240 m. They were characterized by high transparency and ultra oligotrophic conditions. Six FGs were recoreded: **L$_0$, Y, F, MP, E** and **X3** (*Tables 4, 5*). FG **L$_0$** dominated as 2/3 from biomass was represented by unicellular dinoflagellates with large size: *Peridinium cinctum* – 77.2% (Chernoto Lake) and *Gymnodinium palustre* – 73.7% (Bezbog Lake). Colonial mucilaginous chlorococcocales from FG **F**: *Oocystis lacustris* – 5.3% (Chernoto Lake) and *Radiococcus nimbatus* – 5.2% (Bezbog Lake) were also dominant species with biomass > 5%.

Three reservoirs were selected as representative for the Lake Group I – Mountain lakes: Belmeken, Beli Iskar and Toshkov chark reservoirs. They are small to middle sized deep reservoirs, situated at above 1400 m a.s.l. Transparency over 3.5 m and oligotrophic conditions were assessed. Besides the above six FGs at alpine lakes, another five groups were determined: **B**, **C**, **G**, **N** and **W2** (*Table 4* and *Table 5*). The following groups were dominant in biomass: **L$_0$**, **E**, **N** and **C** (*Table 4*). Dinoflagellates from **L$_0$**: *Peridinium cinctum* (53.1%), *Gymnodinium* sp. (31.6%) and *Gymnodinium* sp. (59.2%) were first dominant taxa at Belmeken and Beli Iskar. Second dominants were large filamentous desmid *Spondylosium planum* (8.9%) from **N** at Belmeken Reservoir, and colonial chrysophycean *Stichogloea doederleinii* (22.4%) from **X2** at Beli Iskar Reservoir. Flagellate chrysophyceans from FG **E** *Dinobryon divergens* (27.8%), *Mallomonas* sp. (22.2%), *Mallomonas caudata* (11.1%) and colonial diatom from **C** - *Asterionella formosa* (11.1%) were dominant taxa at Toshkov chark Reservoir.

Lake Group II – Deep semi mountain and lowland lakes included 8 reservoires, located between 118 (Ivaylovgrad Reservoir) and 529 m a.s.l. (Vacha Reservoir) and characterized by presence of thermocline in summer and profundal zone. A total of 21 FGs were designated – in addition to 11 groups at Mountain lakes, 10 new groups were recorded: **J**, **X1**, **P**, **W1**, **D**, **H1**, **X2**, **Z**, **U**, **S1** (*Tables 4, 5*). Dominant position of **L_0**, **E**, **N** and **C** at Mountain lakes was replaced by **F**, **P**, **X3**, **B**, **H1** and **J**. Taxa from group **F** were first dominant at some of reservoirs (Studen kladenets, Kardzhali and Ivaylovgrad) (*Table 4*). Group **F** was represented from colonial mucilaginous chlorococcocales *Oocystis lacustris* (9.5%) and *Sphaerocystis planctonica* (9.5%) at Kardzhali Reservoir, from *Oocystis ecballocystiformis* (26.7%) at Studen kladenets Reservoir and *Oocystis solitaria* (67.8%) and *Planctococcus sphaerocystiformis* (7.9%) at Ivaylovgrad Reservoir. At other water bodies, first dominant were species from group **P**, e.g. at Krichim Reservoir colonial diatom *Fragilaria crotonensis*, monodominant (85.5%), colonial diatom *Aulacoseira granulata* (67.8%) at Borovista Reservoir, unicells desmids *Staurastrum teliferum* (23.7%) and *Staurastrum gracile* (20.5%) at Devets Reservoir. Only at Srechenska bara Reservoir species from FG **L_0** was first dominant - unicellular dinoflagellate with large size *Gymnodinium* sp. (61.4%). Vacha Reservoir was an isolated case in the group Deep semi mountain and lowland lakes. Regardless of its highest altitude, first and second dominant in the total biomass were species from FGs **J** – *Crucigeniella rectangularis* (36.7%) and **H1** - *Aphanizomenon flos-aquae* (33.6%). Overall assessment revealed worst ecological status among other water bodies in the group.

Third lake group Small and middle sized lowland lakes covered one swamp and 4 reservoirs. Durankulak swamp belongs to national lake type L7 (Black Sea freshwater coastal lakes, salinity < 0.5 ‰), reservoirs to L12 (Eleshniza and Ovcharitsa) and L16 (Poroj and Acheloy). Their altitude ranged between 0 m (Durankulak swamp) and 142 m (Acheloy Reservoir). Except for Durankulak swamp (Depth $_{max}$ - 1.70 m), which is shallow polymictic, the rest water bodies have average depth (Depth $_{max}$: 11.3 ÷ 22.3 m), and thermocline in the summer season. All lakes were in mesotrophic to eutrophic conditions. Twenty-five FGs were registered (*Tables 4, 5*). Additionally to the 21 FGs for Lake Group II, **L_M**, **X_{Ph}**, **K**, **T**, **M**, **S2** were recoreded at Lake Group III. At lowland lakes no particular dominants were established, possibly due to the particular extent of athropogenic pressure. Almost half of the biomass was formed by group **J** (44.2%), represented from coenobial chlorococcocales *Crucigeniella crucifera*, *Crucigenia quadrata*, *Pediastrum boryanum*, *Scenedesmus disciformis* and *S. opoliensis* at Durankulak swamp. Second dominant was colonial cyanobacteria *Microcystis aeruginosa* (9.0%) - group **M**. At Eleshniza Reservoir with equal share were picoplanctonic green algae *Stichococcus minutissimum* (12.5%) from **X3,** colonial chlorococcocales *Coelastrum polychordum* (12.5%) from **J**, and *Phacotus coccifer* (12.5%) from **X_{ph}**. First dominant at Poroj Reservoir was from **X2** (*Chlamydomonas* sp. - 33.3%), second from **B** (*Cyclotella* sp. -13.7%). *Ceratium furcoides* from **L_M** was first dominant (38.4%) at Ovcharitsa Reservoir together with *Microcystis aeruginosa*, second from **P** (*Aulacoseira granulata* - 37.5%). Biomass at Acheloy Reservoir was formed almost only from eutrophic species cyanobacteria, belonging to **S1** (*Limnothrix* *redekei* - 64.3%, *Pseudanabaena limnetica* - 19.7%) and **H1** (*Aphanizomenon flos-aquae* - 5.7%).

From Lake Group IV (Black Sea hyperhaline coastal lakes) was studied Atanasovsko Lake (national type L10 Black Sea hyperhaline coastal lakes, Transitional Waters), with salinity > 40 ‰. Only 4 FGs were registered: **X2, L_0**, **D** and **MP** (*Tables 4, 5*). About 90% of biomass was formed by group **X2,** to which belonged flagellates green algae *Provasoliella ovata* (52.4%) and *Pyramimonas tetrarhynchus* (35.5%).

Table 4. *Main phytoplankton species (>5% of the total biomass) with their taxonomic and functional groups. In parenthesis is given the total number of the studied lakes.*

Name of the Locality	Lake Type	Phytoplankton Species	Relative biomass (%)	Taxonomic Group	Functional Group
Lake Subgroup I.1– Alpine lakes (n=4)					
Chernoto Lake	L1	*Peridinium cinctum* (O.F.M.) Ehr.	77.2	Dinophyceae	L_0
		Cryptomonas sp.	7.0	Cryptophyceae	Y
		Oocystis lacustris Chod.	5.3	Chlorophyceae	F
Bezbog Lake	L1	*Gymnodinium palustre* Schilling	73.7	Dinophyceae	L_0
		Diatoma mesodon (Ehr.) Kütz.	7.4	Bacillariophyceae	MP
		Radiococcus nimbatus (De-Wild.) Schmidle	5.2	Chlorophyceae	F
Lake Group I – Mountain „lakes" (n=7)					
Belmeken Res.	L13	*Peridinium cinctum* (O.F.M.) Ehr.	53.1	Dinophyceae	L_0
		Gymnodinium sp.	31.6	Dinophyceae	L_0
		Aphanothece clathrata W. et G.S. West	5.1	Cyanoprokaryota	L_0
		Spondylosium planum (Wolle) W. et G.S. West	8.9	Zygnemaphyceae	N
Beli Iskar Res.	L1	*Gymnodinium* sp.	59.2	Dinophyceae	L_0
		Stichogloea doederleinii (Schmidle) Wille	22.4	Chrysophyceae	X2
Toshkov chark Res.	L3	*Dinobryon divergens* Imh.	27.8	Chrysophyceae	E
		Mallomonas sp.	22.2	Chrysophyceae	E
		Mallomonas caudata Iwanoff	11.1	Chrysophyceae	E
		Asterionella formosa var. *gracillima* (Hanztsch in Rabenh.) Grun.	11.1	Bacillariophyceae	C
		Aphanocapsa delicatissima W. et G.S. West	5.6	Cyanoprokaryota	L_0
		Sphaerocystis planctonica (Korshikov) Bourrelly	5.6	Chlorophyceae	F
		Cyclotella sp.	5.6	Bacillariophyceae	B
		Pandorina morum (O.F. Müller) Bory	5.6	Chlorophyceae	G
		Trachelomonas volvocina Ehr.	5.6	Euglenophyceae	W2
Lake Group II – Deep semi mountain and lowland "lakes" (n=27)					
Srechenska bara Res.	L2	*Gymnodinium* sp.	61.4	Dinophyceae	L_0
		Chrysococcus punctiformis Pasch.	22.8	Chrysophyceae	X3
		Cyclotella sp.	7.5	Bacillariophyceae	B
		Aulacoseira sp.	5.0	Bacillariophyceae	P
Kardzhali Res.	L11	*Cyclotella* sp.	16.7	Bacillariophyceae	B
		Oocystis lacustris Chod.	9.5	Chlorophyceae	F
		Sphaerocystis planctonica (Korshikov) Bourrelly	9.5	Chlorophyceae	F
		Aphanothece clathrata W. et G.S. West	9.5	Cyanoprokaryota	L_0
		Uroglena articulata Korshikov	9.5	Chrysophyceae	U
		Schroederia spiralis (Printz) Korš.	7.1	Chlorophyceae	X3
		Chroomonas acuta Uterm.	7.1	Cryptophyceae	X2
		Euglena sp.	9.5	Euglenophyceae	W1
Studen kladenets Res.	L11	*Oocystis ecballocystiformis* Iyengar	26.7	Chlorophyceae	F
		Tetrachlorella alternans (G.M. Smith) Korš.	25.0	Chlorophyceae	X3
		Rhopalodia gibba (Ehr.) O. Müll.	17.2	Bacillariophyceae	MP
		Cymatopleura solea (Brébisson) W.Smith	14.6	Bacillariophyceae	MP
Ivaylovgrad Res.	L11	*Oocystis solitaria* Wittr. in Wittr. & Nordst.	67.8	Chlorophyceae	F
		Coelastrum polychordum (Korš.) Hind.	10.9	Chlorophyceae	J
		Planctococcus sphaerocystiformis Korš.	7.9	Chlorophyceae	F
Devets Res.	L16	*Staurastrum teliferum* Ralfs	23.7	Zygnemaphyceae	P
		Staurastrum gracile Ralfs	20.5	Zygnemaphyceae	P
		Euastrum sp.	20.5	Zygnemaphyceae	N
		Chroococcus sp.	12.7	Cyanoprokaryota	L_0
		Amphora sp.	7.0	Bacillariophyceae	MP
		Chrysococcus rufescens Klebs	5.0	Chrysophyceae	X3
Krichim Res.	L11	*Fragilaria crotonensis* Kitt.	85.5	Bacillariophyceae	P
Borovista Res.	L11	*Aulacoseira granulata* (Ehrenb.) Sim.	67.8	Bacillariophyceae	P
		Radiococcus planctonicus Lund	7.3	Chlorophyceae	F
		Volvox aureus Ehrenberg	5.5	Chlorophyceae	G
Vacha Res.	L11	*Crucigeniella rectangularis* (Näg.) Kom.	36.7	Chlorophyceae	J
		Aphanizomenon flos-aquae (L.) Ralfs	33.6	Cyanoprokaryota	H1
		Sphaerocystis planctonica (Korshikov) Bourrelly	11.4	Chlorophyceae	F
		Radiococcus planctonicus Lund	9.1	Chlorophyceae	F

Lake Group III – Small and middle sized lowland “lakes” (n=38)					
Eleshnitsa Res.	L12	*Coelastrum polychordum* (Korš.) Hind.	12.5	Chlorophyceae	J
		Stichococcus minutissimus Skuja	12.5	Chlorophyceae	X3
		Phacotus coccifer Korschikoff	12.5	Chlorophyceae	X_{Ph}
		Staurastrum sp.	10.4	Zygnemaphyceae	P
		Ceratium hirundinella (O.F.Müller) Dujardin	6.3	Dinophyceae	L_0
		Cosmarium sp.	6.3	Zygnemaphyceae	N
Poroj Res.	L16	*Chlamydomonas* sp.	33.3	Chlorophyceae	X2
		Cyclotella sp.	13.7	Chlorophyceae	B
		Gymnodinium sp.	10.2	Dinophyceae	L_0
		Golenkinia radiata Chod.	6.8	Chlorophyceae	J
		Euglena limnophila Lemm.	6.8	Euglenophyceae	W1
		Aulacoseira granulata (Ehrenb.) Sim.	5.0	Bacillariophyceae	P
Ovcharitsa Res.	L12	*Ceratium furcoides* (Levander) Langhans	38.4	Dinophyceae	L_M
		Aulacoseira granulata (Ehrenb.) Sim.	37.5	Bacillariophyceae	P
		Carteria multifilis (Fresenius) Dill	15.1	Chlorophyceae	G
Durankulak swamp	L7	*Crucigeniella crucifera* (Wolle) Kom.	13.4	Chlorophyceae	J
		Crucigenia quadrata Morr.	11.7	Chlorophyceae	J
		Scenedesmus opoliensis P.G. Richt.	7.9	Chlorophyceae	J
		Pediastrum boryanum (Turpin) Meneghini	6.2	Chlorophyceae	J
		Scenedesmus disciformis (Chod.) Fott & Kom.	5.0	Chlorophyceae	J
		Microcystis aeruginosa (Kütz.) Kütz.	8.9	Cyanoprokaryota	M
		Navicula sp.			MP
		*Anabaena spiro*ides Kleb.	5.0	Cyanoprokaryota	H1
Acheloy Res.	L16	*Limnothrix redekei* (Van Goor) Meffert van Goor	64.3	Cyanoprokaryota	S1
		Pseudanabaena limnetica (Lemmermann) Komárek	19.7	Cyanoprokaryota	S1
		*Aphanizomenon flos-aq*uae (L.) Ralfs	5.7	Cyanoprokaryota	H1
Lake Group IV – Black Sea hyperhaline coastal lakes (n=2)					
Atanasovsko Lake	L10	*Provasoliella ovata* (Jac.) A.R. Loebl.	52.4	Chlamydophyceae (Chlorophyta)	X2
		Pyramimonas tetrarhynchus Schmarda	35.5	Prasinophyceae (Chlorophyta)	X2
		Gymnodinium splendens Lebour	5.4	Dinophyceae	L_0

Factor numbers (F)

Determination of F was based on expert knowledge, existing literature data and our background with F calibration at lakes under reference conditions, as well as at lakes with assessed indisputable overall status. The F values per lake groups and types were presented at *Table 5*, as well as literature data. It has to be noticed that Q was calculated on a set of 19 lakes from the total database of 78 water bodies, thus F was valid only for national types L1, L2, L3, L7, L10, L11, L12, L13, L16 (*Table 5*).

Table 5. *Factor number (F) to the phytoplankton functional groups per lake groups and types. *Functional groups covering dominant species (biomass >5% from the total biomass). L: Lake type. References: A - Padisák et al. (2006); B - Crossetti and Bicudo (2008); C - Becker et al. (2009); D - Becker et al. (2010).*

	Lake Groups				
Functional groups	**I.1 Alpine lakes L1**	**I Mountain lakes L3, L13**	**II Deep semi mountain and lowland lakes L2, L11**	**III Small and middle sized lowland lakes L7, L12, L16**	**IV Black Sea hyperhaline coastal lakes L10**
B		*3	*5	*5 A 5	
C		*2	3 C 2 D 3	5 A 5	
D			2 C 2 D 2	2,5 A 3 B 2	2,5

N		*5	*5 C 5 D 5	*5 A 5 B 5	
P			*3 C 2 D 0	*5 A 5 B 2	
MP	*5	5	*3 D 1	*3 A 3	3
T				5 A 5 B 5	
S1			0 C 0 D 0	*0 A 0 B 0	
S2				0 A 2	
Z			5		
X3	5	5	*5 D 5	*5 A 4 B 4	
X2			*5 C 5	*3 A 3,5 B 5	*3
X1			2,5 C 2 D 3,5	2,5 A 3 B 5	
X_{Ph}				*3,5 A 3,5	
E	5	*5	5 C 5 D 5	5 A 5 B 5	
Y	*3	3	3 C 3 D 3	3,5 A 3.5 B 3	
F	*2	*2	*3 C 2 D 2	*5 A 3 B 5	
G		*2	*3	*3 A 4	
J			*2 C 2 D 2	*3 A 3 B 5	
K				3 A 2 B 3	
H1			*0 C 0 D 0	*1 A 1 B 1	
U			*4		
L_0	*5	*5	*5 C 5 D 4	*5 A 5 B 5	*5

L_M				*0	
				A 0	
M				*0	
				A 0	
				B 0	
W1			*0	*1,5	
			C 0	A 2	
			D 0	B 0	
W2		*1	1	3	
			C 1	A 3	
			D 1	B 1	
Total	*4/6	*8/11	*13/21	*16/25	*2/4

Ecological status assessment

The assessement of ecological status was based on main and additional phytoplankton metrics (*Table 6*). Individual class boundaries were in concordance with scales for oligo and mesotrophic lake types (*Table 1* and *Table 2*). Both water bodies in Lake Subgroup I.1 (Alpine lakes) were in reference conditions. Three oligotrophic mountain reservoirs (Lake Group I) were also in high status, except for AGI and transparency values at Toshkov chark Reservoir.

High to moderate status dominated within Lake Group II. Srechenska bara drinking water reservoir was evaluated in high status based on all metrics. Q index, AGI and transparency resulted in one class lower status in comparison with biomass at Kardzhali Reservoir. Reservoirs Studen kladenets, Ivaylovgrad and Devets, under the urban impact were in good status after main metrics (*Table 6*), except for Q index at Ivaylovgrad Reservoir coincided with first degree bloom of *Oocystis solitaria* (1.4 mg l^{-1}). At Krichim Reservoir separate metrics differed, but biomass and Q index revealed good status. AGI, transparensy and chlorophyll *a* were in lower ranges due to bloom of colonial diatom *Fragilaria crotonensis* (4.1 mg l^{-1} – second degree). Reservoirs Borovista and Vacha, under eutrophication, had moderate status, since metrics reflecting species composition (Q, AGI and % Cyanobacteria) are more sensitive than biomass. Diatom *Aulacoseira granulata* (2.96 mg l^{-1} - II degree) bloomed at Borovista Reservoir. Metric % Cyanobacteria (33.6) was increased due to eutrophic *Aphanizomenon flos-aquae* at Vacha Reservoir. Both indices resulted in lower status then biomass in half of the water bodies within the group.

Lake Group III included Eleshnitsa Reservoir in reference conditions and Poroj Reservoir in good status. Ovcharitsa Reservoir was assessed in moderate status based on bloom of colonial diatom *Aulacoseira granulata* (2.39 mg l^{-1} – II degree) and green flagellat *Carteria multifilis* (0.96 mg l^{-1} – I degree). Durankulak swamp was evaluated in poor status, regardless most main metrics average levels. High % Cyanobacteria (25.56 %) and second degree bloom of *Microcystis aeruginosa* (2.65 mg l^{-1}) were registered. Acheloy Reservoir was in bad status in the view of Q index, AGI and exclusively high percent eutrophic Cyanobacteria (92%) from FGs **S1** (*Limnothrix redekei*, *Pseudanabaena limnetica*) and **H1** (*Aphanizomenon flos-aquae*) – *Table 4*.

Hyperhaline Atanasovsko Lake (Lake Group IV) had contradictory evaluation after applied metrics. Biomass, Q index and second degree bloom of green flagellats *Pyramimonas tetrarhynchus* (4.43 mg l^{-1}) and *Provasoliella ovata* (6.53 mg l^{-1}) resulted in moderate status. AGI was neglected as unrepresentative metric since does not enlisted green flagellats, as well as metric % Cyanobacteria indicative of freshwater ecosystems.

Table 6. *Ecological status of the lakes according to phytoplankton. Legend: Ltc – Lake Type Code; Bm – Biomass; Q – Assemblage Index; AGI – Algae Group Index; EQR – ecological quality ratio; SD – Transparensy; Chl a – Chlorophyll a; % Cyano - % Cyanobacteria; Bi – "Bloom" (intensity); Chl – Chlorophyceae; Ba – Bacillariophyceae; Cy – Cyanoprokaryota; Bts – "Bloom" (toxic species); ES – Ecological Status; H – high; G – good; M – moderate; P – poor; B – bad.*

Name	**Ltc**	**Main metrics**						**Additional metrics**			**ES**
		Bm (mg l^{-1})	**Q**	**AGI**	**EQR (AGI)**	**SD (m)**	**Chl *a*, (µg l^{-1})**	**% Cy**	**Bi**	**Bts**	
Lake Subgroup I.1 – Alpine lakes											
Chernoto	L1	0.57	4.9	0.55	0.999	> 15.5	< 0.2	0.00	no	no	H
Bezbog	L1	0.95	4.5	0.48	0.998	> 7.0	< 0.2	0.74	no	no	H
Lake Group I – Mountain „lakes"											
Belmeken	L13	0.79	4.9	0.51	0.999	4.0	2.60	0.00	no	no	H
Beli Iskar	L1	0.76	4.7	0.57	0.999	6.5	1.15	0.00	no	no	H
Toshkov chark	L3	0.18	4.1	1.04	0.998	3.5	2.20	0.00	no	no	H
Lake Group II – Deep semi mountain and lowland "lakes"											
Srechenska bara	L2	1.01	5.0	0.40	0.999	4.5	1.34	0.00	no	no	H
Kardzhali	L11	0.42	3.9	1.27	0.997	3.6	1.82	0.00	no	no	H
Studen kladenets	L11	1.16	3.6	1.04	0.998	2.4	2.44	0.00	no	no	G
Ivaylovgrad	L11	2.02	2.8	0.92	0.998	2.6	4.02	2.18	I Chl	no	G
Devets	L16	2.44	3.6	1.73	0.996	2.0	4.07	0.00	no	no	G
Krichim	L11	4.77	3.2	8.37	0.979	1.1	14.50	0.00	II Ba	no	G
Borovitsa	L13	4.37	2.9	6.04	0.985	1.5	6.93	0.14	II Ba	no	M
Vacha	L11	2.29	1.6	6.24	0.985	1.3	3.50	33.6	no	yes	M
Lake Group III – Small and middle sized lowland "lakes"											
Eleshnitsa	L12	0.48	4.1	1.17	0.997	1.2	0.98	0.00	no	no	H

Poroj	L16	1.77	3.6	1.03	0.998	1.10	3.56	5.08	no	no	G
Ovcharitsa	L12	6.37	2.6	1.2	0.997	2.3	7.32	2.97	II Ba	yes	M
Durankulak	L7	7.63	2.3	6.72	0.983	0.43	15.63	25.56	II Cy	yes	P
Acheloy	L16	14.28	0.4	44.1	0.890	0.65	27.5	92.1	III Cy	yes	B
Lake Group IV – Black Sea hyperhaline coastal lakes											
Atanasovsko	L10	12.47	2.9	0.4	0.999	0.25	31.2	0.00	II Chl	no	?M

Both main and additional phytoplankton metrics showed correlation, as Q index and AGI were highly correlated (*Table 7*). Assemblage index had also strong relationship ($p<0.001$) with Total biomass, % Cyanobacteria, Chlorophyll *a*, as well as with the assessed overall ecological status (ES).

Table 7. *Correlation matrix for all 8 metrics at four groups lakes. Level of significance: *** - $p < 0.001$, ** - $p < 0.01$, * - $p < 0.05$.*

	AGI	**EQR (AGI)**	**Q**	**Q Status**	**Biomass**	**Chl *a***	**% Cy**	**ES**
AGI		**-1.00*****	-0.73**	0.71**	0.87***	0.87***	0.94***	0.76***
EQR (AGI)			0.73**	-0.71**	-0.88***	-0.88***	-0.94***	-0.77***
Q				**-0.97*****	-0.82***	-0.75***	-0.79***	-0.88***
Q Status					0.80***	0.70**	0.78***	0.85***
Bm						**0.96*****	0.83***	0.92***
Chl *a*							0.80***	0.84***
% Cy								0.83***
ES								

Discussion

Assemblage index (Q index)

Functional groups (codons)

Species from functional assemblages $\mathbf{L_0}$, **Y** and **MP** were first and second biomass dominants at ultra oligotrophic alpine lakes (*Table 4*). Codons $\mathbf{L_0}$ (mostly dinoflagellates) and **Y** (large size cryptomonads) occur in broad variety of habitats and are able to survive in all lentic ecosystems conditions (Padisák et al. 2009, Kruk et al. 2010). Our study confirmed these groups were specific not only for alpine lakes. As dominant species from Codon $\mathbf{L_0}$ were registered also at mountain Belmeken and Beli Iskar Reservoirs, deep semi mountain and lowland Srechenska bara, Kardzhali and Devets Reservoirs and lowland Eleshnitsa Reservoir (*Table 4*). Since all the above water bodies were in high or good status, we assumed that Codon $\mathbf{L_0}$ included reference species for national types L1, L2, L11, L12, L13 and L16. Species from Codon **Y** were also registered at all lake groups but in biomass under 5% (*Table 4* and *Table 5*). Codon

MP was suggested to unify all the meroplanktonic autotrophic organisms that can be accidentally found in phytoplankton samples, independently of lake type (Padisák et al. 2009). During our study we found that **MP** species were dominant also in deep oligotrophic semi mountain Studen kladenets and Devets Reservoirs (*Table 4*). Assessed high and good status demonstrated that codon **MP** can also be applied as indicative for undisturbed conditions.

At three oligotrophic mountain lakes in reference conditions, dominant species were from assemblage **L_0**, **N**, **E** and **C** (*Table 4* and *Table 6*). Codon **N** (planctonic desmids and diatom *Tabellaria*) is related to summer season in unimpacted lakes in continental zone (Reynolds et al. 2002). Our results showed codon **N** was dominant in epilimnion of deep mountain Belmeken Reservoir. Moreover **N** was established at other lake types such as deep semi mountain Devets and lowland Eleshnitsa Reservoir, all reservoirs in high or good status (*Table 4* and *Table 6*). Codon **E** (Chrysophyceans - silica scaled flagellates) developes usually in small, shallow, base poor lakes or heterotrophic ponds (Reynolds et al. 2002). Species are tolerant to low nutrients and sensitive to CO_2 deficiency. Codon **E** was registered in high relative abundance at mountain Toshkov chark Reservoir (*Table 4*), which is a natural dystrophic, peaty lake.

Eight water bodies were selected among deep semi mountain and lowland lakes. Their ecological status varied from high to moderate, and first and second dominants were taxa from codons **L_0**, **X3**, **B**, **F**, **J**, **P**, and **H1** (*Table 4*). Based on frequency distribution of the FG, two reservoirs groups were outlined. First group included Studen kladenets, Kardzhali and Ivaylovgrad Reservoirs with first dominant species from codon **F** (mucilaginous colonial green algae). The assemblage is character for oligotrophic epilimnion, tolerant to low nutrients, but susceptible to shaded habitats (Reynolds et al. 2002). Our study confirmed codon **F** is characteristic to wide range of unimpacted lakes, but is with high relative abundance mainly in deep oligotrophic lake indicating good status (*Table 4* and *Table 6*). Second reservoir group (Borovitsa, Krichim and Devets) was situated at higher altitude (419-529 m), and had lower transparency (SD: 1.1-1.5 m). Obviously they were under the impact of stronger eutrophication and dominant was codon **P** (colonial diatoms and desmids), typical to eutrophic epilimnnia (Reynolds et al., 2002). Colonial diatom *Fragilaria crotonensis* was monodominant with 85.5% from the total biomass at Krichim Reservoir. In Borovitsa Reservoir *Aulacoseira granulata* accounted for 67.8% of the biomass, while unicells desmids *Staurastrum teliferum* and *Staurastrum gracile* for half of the biomass in Devets Reservoir. Assemblage **P** high relative abundance is indicative for ecological status alteration towards moderate conditions at deep semi mountain reservoirs.

Small and middle sized lowland lakes covered a shallow, polimictic swamp and 4 middle deep, stratified in summer reservoirs. Amnog twenty-five FG presented, specific for the particular lake group were codons **L_M**, **X_{Ph}**, **K**, **T**, **M**, **S2**. Various athropogenic pressures influenced dominant species and assemblages (*Table 4* and *Table 6*). Reference Eleshniza Reservoir had as dominant codon **X3**, which grows in oligotrophic conditions, presented by *Stichococcus minutissimum*. *Chlamydomonas sp.* was first dominant at Poroj Reservoir, from mesotrophic assemblage **X2,** typical to shallow, clear mixed layers in meso-eutrophic lakes (Reynolds et al., 2002). *Ceratium furcoides* from codon **L_M**, together with *Microcystis aeruginosa* dominated at Ovcharitsa Reservoir. At the assemblage *Ceratium* was associated with *Microcystis*, and reflected higher trophic status, based on eutrophic to hypertrophic, small to middle sized habitat pattern of **L_M**. Thus three codons, single or together with *Ceratium* and *Microcystis* development, connection with eutrophication trend $\mathbf{L_0} \rightarrow \mathbf{L_M} \rightarrow \mathbf{M}$ reported previously by Padisák et al. (2009) was confirmed. At Durankulak swamp codon **J** was established and accounted for a half of the biomass (*Table 4*). In conformity with exsiting habitat description from Reynolds et al. (2002), the group inhabited shallow, mixed, with high nutrient content waters. Group **M** (*Microcystis aeruginosa*) was registered at these eutrophic conditions. At Acheloy Reservoir in eutrophic conditions and low transparency (0.6 m), dominants were shade-adapted cyanoprokaryotes from codon **S1** (*Limnothrix redekei* and *Pseudanabaena limnetica*), which formed more than 80% of the biomass (*Table 4*). High S/V proportion determined their tolerance to limited light availability (Naselli-Flores and Barone, 2007). Eutrophic assemblage **H1** (dinitrogen-fixing Nostocaleans) was subdominant and incorporated *Aphanizomenon flos-aquae*.

Studied modified section of Atanasovsko Lake was shallow, polimictic, with high salinity (63.4‰) and conductivity (105,003 μS cm^{-1}). Almost 90% of biomass was formed by **X2** (*Table 4*). Both *Provasoliella ovata* and *Pyramimonas tetrarhynchus* bloomed intensively during the study (Table 6). *Provasoliella ovata* refers to bird-manured pools and other organically enriched waters (John et al., 2003). The registered boom probably can be related to natural organic enrichment from birds and lack of specialized zooplankton. Padisák et al. (2003) reported dominance of **X2** (as well as Y) in zooplankton lacing habitats.

The analysis of FG frequency distribution showed high relative abundance of assemblages $\mathbf{L_0}$, **Y**, **MP**, **N**, **E**, **X3**, $\mathbf{X_{Ph}}$ (depedant on lake type) indicated high or good status. At oligotrophic stratified reservoirs and naturally mezoeutrophic lowland lakes undesirable assemblages were $\mathbf{L_M}$, **J**, **M**, **S1** and **H1**.

Factor number (F)

Factor number (F) pre-determination according to the existing typology is the most crucial step, which defines the index (Q) impartial assessment (Padisák et al., 2006; Crossetti and Bicudo, 2008; Becker et al., 2009; 2010). Following the existing knowledge of Q implementation, we defined F for each FG. Thus higher F values were allocated to FGs typical of pristine conditions, whereas lower values were set for undesirable ones.

Since F has to be specified for each lake type, the lack of paleolimnological data for most of the national types caused perplexity. Major emphasis was given on to floristic and taxonomic surveys in Bulgaria (Vodenicharov and Vodenicharov, 2000).

Determination of F per FGs in Alpine lakes followed background for seven glacial lakes (Beshkova, 2000). Dominant species reported during 1995 – 1996, belonged to the same groups established in the current study: $\mathbf{L_0}$**, E, Y** and additionaly **N**. This finding verified reference character of the above groups for summer period in Bulgarian glacial lakes. Vodenicharov and Vodenicharov (2000) reported dominance of Bacillariophyceae and Desmidiales in such lentic ecosystems. Species from Chlorococcales appeared also, indicating eutrophication mainly due to touristic activities. Based on that FG **F** received the lowest value 2 of Factor number (*Table 5*).

Factor number at Lake Group II (Deep semi mountain and lowland lakes) was based on Becker et al. (2009; 2010) researched Faxinal Reservoir and Sau Reservoir. Similar to them is national lake type L11 (Large deep reservoirs), included in Lake Group II. Regardless of similarities we took into account that F values have to be set according to the typology (Padisák et al., 2006) and adapted F as followed: **P** - 3; **B** - 5; **F** – 3; $\mathbf{L_0}$ – 5 and **X1** – 2,5 (*Table 5*). Adaptation was based on Q calculation for reservoirs at which all phytoplankton metrics resulted in equal ecological status assessment. For example at Devets Resererervoir all basic metrics determined good ecological status (*Table 6*). Simulatneously if F values cited by Becker et al. (2009; 2010) were applied, Q index assessed unadequate lower status.

Lake Group III (Small and middle sized lowland lakes) was compared to F rates for Hungarian type 7 after Padisák et al. (2006) and shallow Garças Reservoir after Crossetti and Bicudo (2008), which appeared most similar to our national types. Calibration of F was based on Poroj Reservoir, which was in good ecological status according Biomass, AGI and % Cyanobacteria (*Table 6*).

Factor number calibration was complicated at Lake Group IV (Black Sea hyperhaline coastal lakes). Atanasovsko Lake has been artificialy modified and there are no paleolimnological records before modifications. Thus F values were based totally on species autecology, i.e. flagellates green algae *Provasoliella ovata* distribution in small nutrient rich lakes (John et al., 2003). Therefore F = 3 was determined for group **X2** which was formed up to 90%. Since no autecological data were available for the rest of the species, the particular groups received the same F values as in Lake Group III, but these rates need further verification.

Algae Group Index (AGI, Catálan Index)

Several groups such as Zygnemaphyceae, Euglenophyceae and single-celled green flagellates (Order Polyblepharidales, Tetraselmidales, Chlamydomonadales) are not included in AGI calculation. High coefficient of Cyanobacteria illustrates that AGI assess mainly eutrophication.

Comparative assessment of ecological status based on two indices

Both indices gave equal assessmet in four lakes within the first Lake Group - Alpine and Mountain lakes (*Table 6*). All metrics illustrated ultraoligo- and oligotrophic regerence conditions except for Toshkov chark Reservoir evaluated in good status according to AGI. Q index and AGI evaluated 5 lakes in identical status within Lake Group II – Deep semi mountain and lowland lakes, similar to common European L-M7 type. Both indices assessed lower status in four cases in contrast to Total biomass, which confirmed that Q index evaluates an ecological status one category lower than the classical biomass-based qualification (Padisák et al., 2006). Three cases of equal status assessed by two indices were found at third Lake Group – Small and middle sized lowland lakes.

In general, within Lake Groups I, II and III, Q index and AGI had good correlation in assessment. In non-conformity with biomass, two indices qualified lower category lake status, since they are based not only on biomass, but on phytoplankton taxa, thus they are more sensitive to changes in species composition.

AGI cannot be applied properly in Lake Group IV – Black Sea hyperhaline coastal lakes, where single-celled green flagellates consisted up to 90% of the total biomass and are not included in the AGI calculation. According Becker et al. (2009), because of high sensitivity of Q to species appearance, it can give realistic evaluation of ecological status, especially in ecosystems with specific conditions, such as haline lakes or naturaly eutrophic lakes with increased phosphorus levels. Moreover, AGI is not recommended in lentic ecosystems with excessed Euglenophyceae growth, e.g. riverine marshes (Bulgarian L5 type; unpublished data).

Conclusion

Assemblage index and Algae Group Index resulted in similar assessment and were applicable to all three Alpine and mountain, Deep semi-mountain and Small and middle sized lowland lake groups. Assemblage index is recommended especially for lentic ecosystems in extreme cases, e. g. hyperhaline lakes, for its flexibility and more adequate assessment. Important taxonomic groups, which can be dominant to biomass in particular lake types and which are not included in AGI, determined index restricted applicability. There is no constraint on the Q index based assessment except that the F number has to be correctly determined for each lake type. When paleolimnological data are missing, empirical approach to calibrate F based on lakes under reference conditions can be recommended.

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METALS IN SEDIMENT AND *PHRAGMITES AUSTRALIS* (COMMON REED) FROM TISZA RIVER, SERBIA

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Abstract. In this study the concentrations of metals were investigated in Phragmites australis (Cav.) Trin. eh Steud. 1841 and sediment samples from the bed of the River Tisza in Serbia. Al, As, B, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sr and Zn levels were analyzed using inductively coupled plasma – optical emission spectrometry. The sediment samples contained elevated concentrations of cadmium, chromium, copper, nickel and zinc. The increased concentration of these metals had no significant effect on wildlife of the river Tisza as the determined concentrations of the metals tested in the comon reed do not exceed the threshold of phytotoxicity. The research proved a strong positive correlation between the concentrations of metals in the sediment and all common reed organs (rhizome, stem and leave). Accumulated metals in the common reed are not distributed evenly, but there are target organs for bioaccumulation. Concentrations in belowground organs were usually higher than aboveground organs, and the general decreasing trend of element content was rhizome>leaves>stems.

Keywords: *metals, sediments, plant organs, River Tisza.*

Introduction

Knowing the mechanism of accumulation, distribution and metabolism of metals in aquatic macrophytes is of great ecological, scientific and practical importance (Kastori *et al.*, 1997). From the environmental point of view, the importance is reflected in the fact that aquatic macrophytes could be used as biological indicators of pollution (Pall *et al.*, 1996; Ravera, 2001; Balanson and Mal, 2005; Borišev and Stanković, 2006; Bonanno, 2011). Increased concentration of an element accumulated in the organs of a plant indicates an increasing load caused by this element in the nearby habitat, so the data on the concentration of certain chemical elements in different parts of the plant tissue can be used as indicators of chemical influences on natural resources (Gerloff and Krombholz, 1966; Pajević *et al.*, 2002). Significant bioconcentration of certain elements in plant tissue occurs as a result of the lack of regulatory mechanisms concerning the uptake of metals, particularly when the concentration of an element in the environment is markedly increased (Stanković *et al.*, 2000; Pajević *et al.*, 2002). In order to assess the effect of excessive metal concentration in natural resources, it is necessary to assess the correct amount of nutrients needed for the metabolism of plants, so that the increased accumulation of certain elements in the organs could be attributed to their increased concentration in the surrounding environment (Gerloff and Krombholz, 1966; Pajević *et al.*, 2002). The value of macrophytes as bioindicators in river systems is reflected in the fact that they always stay in one place, they are usually abundant, their sampling is easy, and taxonomic affiliation can be easily determined (Olivares-Rieunont *et al.*, 2007). Due to the fixed positions of the rooted submerged and floating hydrophytes, as well as emersal species, they have a role as bioaccumulators and biofilters, which is manifested mainly in shallow waters, in coastal areas of rivers, canals and lakes, as well as in parts of the river where the water flows more slowly (Westlake, 1975). Different types of aquatic macrophytes adopt and accumulate metals with different intensity. The differences are especially noticeable in species that grow in habitats that are anthropogenic or naturally rich in polluted metals (Pall *et al.*, 1996; Ravera, 2001; Balanson and Mal, 2005; Borišev and Stanković, 2006).

The accumulation of metals in tissues and cell compartments of the same species also differs, because the plants can attain metals through different organs (root, rhizome, leaf) (Welsh and Denny, 1980; Ward, 1987; Bishop and DeWaters, 1988; Levine *et al.*, 1990). Metals, which are particularly non-essential in the roots of aquatic macrophytes, accumulate more intensively than in the aboveground plant parts. Rooted aquatic plants have fibrinous root system with large contact area (Wang *et al.*, 1997). When they enter the root system, nutrients are transported through the plant and placed into cells. Transport within the body of a plant is done by the water flow through xylem or cytoplasmic fibers, and is strictly conditioned by environmental factors, such as temperature, humidity, light intensity and light quality. Toxic components are then deposited in the vacuoles, leaves, etc. (Agbaba *et al.*, 2008). The intensity of adoption depends not only on the type of metal, but also of their involvement in biochemical reactions, while mobility, in connection with the distribution of metals in plants, often depends on their ability to form chelate complexes together with the components of the xylem sap (Kastori *et al.*, 1997).

In aquatic ecosystems, rooted macrophytes such as reeds *P. australis* (Cav.) Trin. et Steud. 1841 are under greater influence from metals coming from sediments than those coming from the water, and therefore bioaccumulation is greater if the sediment contains higher concentration of metals (Zwolsman *et al.*, 1993). Numerous studies have pointed to a positive correlation between the metals in the sediment and various reed organs (Peverly *et al.*, 1995; Wang *et al.*, 1997; Bonano and Lo Giudice, 2010; Bonano, 2011). By studying the concentration of microelements in the rhizome, root, stem and leaf of *P. australis* in Italy, Bonanno (2011) found a strong positive correlation between the elements identified in plants and sediments, and he also found out that the metals remain mostly in reed rhizome. Peverly *et al.* (1995) point out that the reed rhizome plays the role of the filter, thus protecting the above ground parts from increased metal concentrations (Peverly *et al.*, 1995). Wang *et al.* (1997) also found that heavy metals are accumulated mainly in the roots with little translocation to the aboveground organs (Wang *et al.*, 1997). As a good bioacumulator of chemical elements, (Duman *et al.*, 2007; Bragato *et al.*, 2009), the common reed has found a wide application in the treatment of municipal and industrial wastewater loaded with metals (Bragato *et al.*, 2009; Lesage *et al.*, 2007; Vymazal *et al.*, 2007).

In this paper Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sr and Zn levels are determined in the rhizome, stem and leaves of the common reed and sediment samples from the bed of the Tisza River. Therefore, the objectives of this paper are: i) to give a pilot screening of metals content in aquatic plants from various sites on the Tisza River, ii) to provide a preliminary insight into the bioaccumulation patterns, and iii) to determine the degree of contamination of the Tisza River.

Materials and Methods

Study area

This study analyzed metals in *P. australis* (Cav.) Trin. et Steud. 1841 and the sediment from the Tisza River in Serbia (45°08'04.45"- 46°04'06.31"N, 20°16'54.09"- 20°04'07.52"E). The basin of the River Tisza is one of the largest natural river systems in southeastern Europe, and it is located almost exactly in the geographical centre of Europe and crosses the new boundaries of the European Union. The River Tisza originates in the Zakarpatian Mountains in western Ukraine and flows into the Danube by Slankamen (Serbia). With respect to its length of 966 km, the River Tisza forms the largest tributary of the Danube River. Anthropogenic influences cause permanent pollution from communal, industrial or agricultural activities. In Serbia the waste water from factories and municipal sewage discharges represent sources of inorganic pollution. In the past, the Tisza had suffered a large number of polluting accidents. The Tisza is an international river and, from its confluence with the Danube, the pollution is transmitted towards the Black Sea.

Sample collection

All plant material was sampled at four sites on the Tisza River in Serbia. The first sampling point (*Fig. 1*) was on the 3rd km of the river flow near the village Titel (45°8'20.44"N – 20°16'8.68"E). The second and the third sampling points were on the 58th km and 72th km of the river flow near the city of Novi Bečej (45°35'17.44"N – 20°7'45.34"E). The fourth sampling point was on the 153rd km of the river flow near the city of Kanjiža (46°4'15.66"N – 20° 3'59.79"E). All marks 3rd km, 58th km, 72th km and 153rd km of the river flow refer to the upstream distance from the confluence with the Danube River.

In each sampling point, 30 samples of *P. australis* were collected within a 5 x 2 m^2 plot. All the plant species had the same habitat with an average height of 2 m. Sampling of the plant material was conducted in areas bordering the river bank, and subject to periodic flooding. After collection, the plant samples were put in sealed plastic bags to avoid extraneous contamination. Climatically identical days were chosen in order to exclude the influence of weather conditions. The month of September was chosen because it coincides with the peak of the vegetative period of the common reed during which trace element concentrations generally show the highest values in the plant organs (Bonano, 2011).

Sediment samples were collected from 25 locations along the Tisza River on the territory of the Republic of Serbia (*Figure 1*) using a plastic corer. After sampling sediments were packed in polyethylene bags and transported to the laboratory.

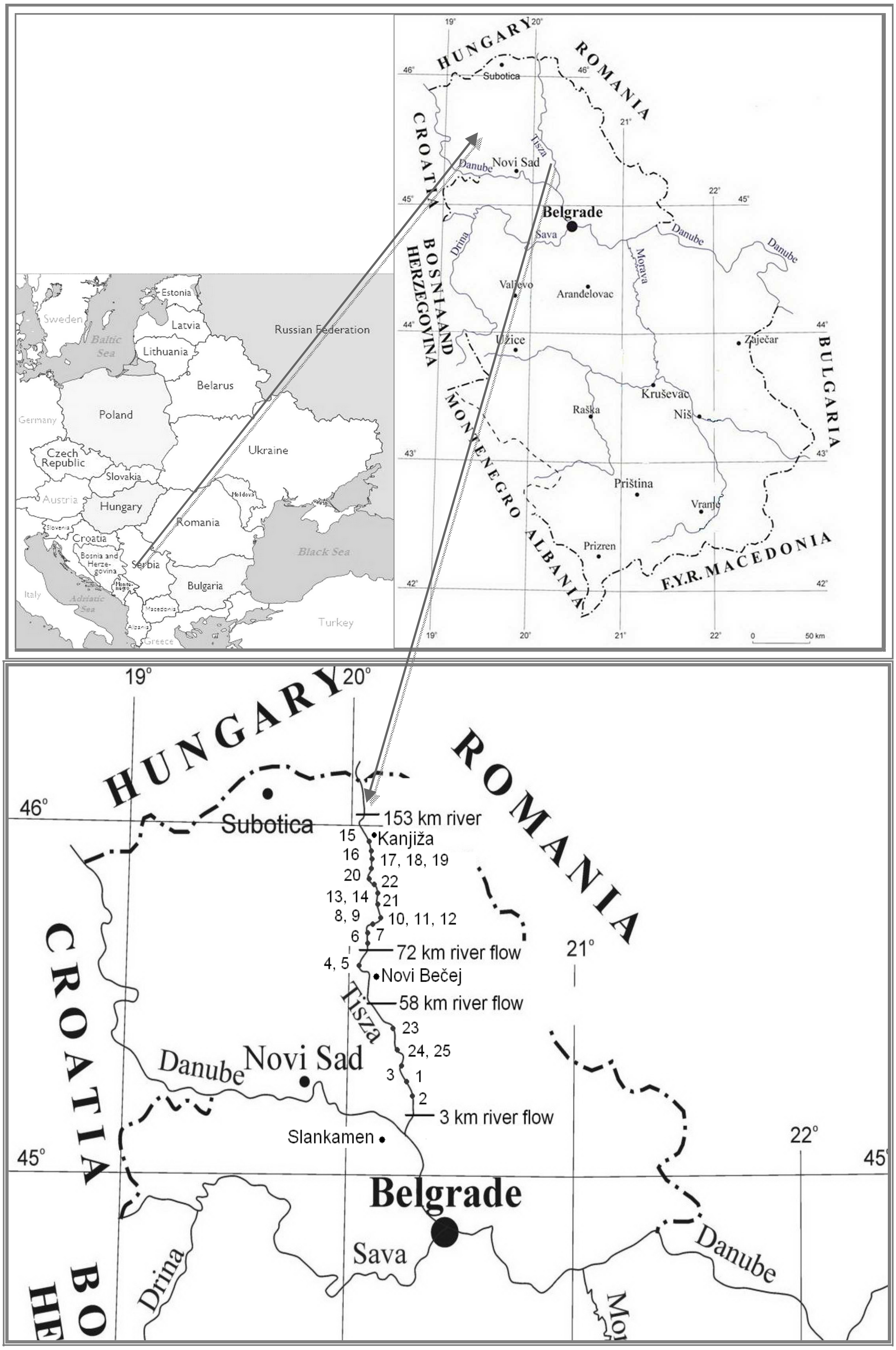

***Figure 1.** Study area. Four sites of plant samples marked with: 3rd km, 58th km, 72th km and 153rd km river flow. Twenty-five sites of sediment samples marked with numbers: 1-25.*

Sample analysis

Plant samples were preliminarily dissected into rhizomes, stems and leaves. In particular, stem samples were prepared by considering the whole stem. Rhizomes, stems and leaves from the same localities were homogenized in an electric blender and stored in plastic bags until analysis. To prepare analytical samples 0.5 g of samples were digested in a microwave digester (ETHOS 1, Advanced Microwave Digestion System, MILESTONE, Italy) using 7 ml of 65% HNO_3 with 1 ml of 30% H_2O_2 and 1 ml H_3PO_4 and 0.5 ml HF of analytical reagent grade (Carlo Erba, Italy). After cooling to room temperature, digested samples were diluted with distilled water to a total volume of 25 ml. All the plastics and glassware were washed in nitric acid for 15 min and rinsed with distilled water before use. High purity argon was used as inert gas. Analysis was performed by inductively–coupled plasma optical spectrometry (ICP/OES, Thermo Scientific iCAP 6500 Duo Instrument, Thermo Fisher Scientific, Cambridge, UK), and comprised assessment of the concentrations of 14 metals (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sr, and Zn). The following wavelength lines of the ICP-OES analysis were used: Al 167.079 nm, As 189.042 nm, Cd 228.802 nm, Co 237.862 nm, Cr 267.716 nm, Cu 224.700 nm, Fe 240.488 nm, Hg 184.930 nm, Mn 257.610 nm, Ni 231.604 nm, Pb 220.353 nm, Se 206.279 nm, Sr 421.552 nm, Zn 202.548 nm. All metals concentrations were expressed in μg/g.

In the laboratory the sediment samples were wet–sieved through a 63 μm sieve. Drying was conducted at room temperature. The dried samples were pounded gently in a porcelain mortar and pestle to break up to the aggregates. The samples were then split into smaller aliquots by coning and quartering. Sediments underwent two stages of preparation consisting of drying and screening. Sediments were dried at 60°C to minimize loss of volatile elements (eg. Hg). Samples were then handled, dried and screened in an area dedicated for these media to avoid contamination from more mineralized rock and core samples. Screening consists typically of two stages comprising extraction of the desired elements into a solution and element determination by instrumental analysis of the solution. Extraction was total to measure the total abundance of the elements from all minerals in the sample. Analysis was performed by ICP-MS.

Data analysis

Bioconcentration Factor (BCF)

The *BCF* provides an index of the ability of the plant to accumulate the metal with respect to the metal concentration in the substrate. The *BCF* was calculated using Equation 1. A larger ratio implies better phytoaccumulation capability.

$$BCF = (\mathrm{P} / \mathrm{E})_{\mathrm{I}} \tag{Eq.1}$$

Where $_i$ denotes the metal concerned, *BCF* is the dimensionless bioconcentration factor, P represents the element concentration in plant organs (μg/g), E represents the initial concentration in the sediment (μg/g dry mass) (Gakwavu *et al.*, 2012).

Translocation ability (TA)

The translocation ability was calculated by dividing the concentration of an element accumulated in the root by that accumulated in the shoots. *TA* was calculated using Equation 2.

$$TA = (Ar/As)I \qquad \text{(Eq.2)}$$

Where $_i$ denotes the metal concerned, *TA* is the translocation ability and is dimensionless, A_r represents the amount of element accumulated in the roots (μg/g), and A_S represents the amount of element accumulated in the shoots (μg/g) (Gakwavu *et al.*, 2012). *TA* > 1 indicates that plants efficiently translocated metals between different organs (Baker *et al.*, 1994).

Statistical analysis

The correlation of metal content between sediment and plant organs was tested by Pearson r coefficient. A one-way ANOVA, followed by the Tukey post-hoc test analysis, used to ascertain whether metal concentrations were significantly different among various organs and various localities. Statistical confidence was set at α=0.05. A statistical analysis of data was carried out using the software SPSS version 15.0 of Statistical Software Package (SPSS Inc. Chicago, USA).

Results

Contents of metals in sediment

Contents of metals (in μg/g of dry mass) in the sediment of the Tisza River bed are given in *Table 1*. The greatest contents were found for Al, Fe and Mn. The decline trend in the sediment is following: Al > Fe > Mn > Zn > Sr > Cr > Cu > Ni > Pb > Co > As > Cd > Se > Hg.

The obtained concentrations of metals in the sediment of the Tizsa River were compared with Serbian Regulation of limit values pollutant substances into surface water, groundwater and sediments and the deadlines for their attaining (SRLVsS). Despite its source metal concentration varies in sediment samples with the % of organic matter (OM) and clay. Therefore, limit values should not be presented as a single value but should be calculated for each sample. This is also a part of the Serbian regulation. In assessing sediment quality limit values for the sediment were corrected according to the measured % of OM and clay. Corrected thresholds were compared with the measured concentrations of metals in the investigated sediment. To correct limits of metal concentration depending on the % of clay and OM in the investigated sediment following correction formula was used (SRLVsS):

$$GVK = GVST * (A + B * \%\ clay + V * \%\ OM / A + B * 25 + V * 10) \qquad \text{(Eq. 3)}$$

where: GVK – corrected threshold for a sediment when the % of clay and OM are considered; GVST – limit values for standard sediment with 25% clay and 10% OM

(*Table 2*); % clay –mineral fractions < 2 µm in the examined sediment expressed in percentage of the dry weight; % OM – measured OM content in the investigated sediment expressed in percentage of the dry weight and A, B i V – constants depending on the type of metal (*Table 3*) (SRLVsS).

Table 1. *Metal concentrations (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sr, Zn) in sediments (in µg/g of dry mass).*

Sampling sites	Al	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Sr	Zn
1	80947.00	15.10	2.00	18.20	123.20	77.60	44832.70	0.26	851.90	50.00	44.60	0.50	140.70	299.00
2	80418.00	17.60	2.60	20.20	130.00	77.00	46441.30	0.36	619.60	45.80	48.90	0.50	144.10	307.00
3	85765.00	16.40	2.40	20.80	130.00	86.10	48469.60	0.25	464.70	51.30	51.80	0.50	143.10	315.00
4	79994.00	17.40	2.40	18.30	130.00	81.30	44832.70	0.23	1394.00	47.00	50.00	0.50	131.60	364.00
5	80947.00	17.30	2.80	18.20	130.00	80.20	45042.50	0.27	1548.90	46.40	51.90	0.50	145.40	335.00
6	76976.00	14.80	1.60	16.50	130.00	67.80	42244.80	0.17	1471.50	44.10	43.60	1.00	138.30	287.00
7	80629.00	15.00	1.60	18.70	123.20	67.70	44692.80	0.18	1626.30	47.20	42.00	0.50	150.00	253.00
8	77188.00	14.10	2.00	18.00	123.20	67.90	41965.10	0.30	1394.00	45.50	42.70	0.50	136.10	301.00
9	79200.00	17.40	2.60	17.20	136.80	84.00	43923.40	0.20	1161.70	45.90	56.00	0.50	137.40	349.00
10	81318.00	15.30	1.90	17.30	116.30	74.30	44762.70	0.22	1394.00	49.30	44.40	0.50	148.80	282.00
11	79571.00	18.50	2.50	18.70	136.80	88.90	44692.80	0.26	1316.60	49.60	50.40	0.50	137.80	346.00
12	80629.00	14.30	2.50	17.50	130.00	76.50	44413.00	0.31	1239.10	46.00	46.80	0.50	138.70	303.00
13	81741.00	19.00	2.10	18.00	130.00	82.00	46721.10	0.28	1471.50	48.10	48.50	0.80	131.20	336.00
14	86824.00	18.70	1.40	18.40	123.20	82.90	48119.90	0.21	1471.50	51.70	45.80	0.50	143.90	287.00
15	75600.00	17.10	2.10	17.20	123.20	75.30	40496.30	0.30	1394.00	50.50	50.90	0.50	132.40	337.00
16	79200.00	17.00	1.90	16.60	130.00	61.60	43433.80	0.18	1548.90	47.50	43.90	0.50	135.90	281.00
17	80629.00	14.30	2.50	17.50	130.00	76.50	44413.00	0.31	1239.10	46.00	46.80	0.50	138.70	303.00
18	81635.00	17.40	1.50	17.00	123.20	68.00	46371.40	0.18	1394.00	47.40	44.50	0.50	134.40	284.00
19	78459.00	17.80	2.20	17.10	123.20	76.00	41895.10	0.25	1626.30	47.10	49.70	0.50	131.90	349.00
20	83382.00	16.90	1.80	17.20	130.00	72.90	45881.80	0.14	464.70	50.20	47.30	0.50	144.90	279.00
21	83806.00	17.40	1.90	17.90	130.00	73.70	45392.20	0.26	1394.00	53.70	51.20	0.50	117.20	354.00
22	84441.00	16.80	1.70	18.10	123.20	78.10	47420.50	0.20	1394.00	50.40	44.60	0.50	145.60	273.00
23	82535.00	16.30	20	18.50	116.30	78.00	45392.20	0.19	1548.90	51.60	47.40	0.50	131.00	311.00
24	85235.00	16.30	1.50	19.90	123.20	78.20	46930.90	0.20	1239.10	51.80	45.50	0.50	141.80	287.00
25	78935.00	14.10	2.60	15.80	130.00	67.30	43224.00	0.22	1548.90	43.00	47.40	0.50	127.50	284.00

Table 2. *Limit values for standard sediment with 25% clay and 10% OM (SRLVsS).*

Substance	LV – limit values	MLC – maximum legal concentration	RV – remediation values
Metals (in µg/g DW)			
Arsenic	29.00	42.00	55.00
Cadmium	0.80	6.40	12.00
Chromium	100.00	240.00	380.00
Copper	36.00	110.00	190.00
Mercury	0.30	1.60	10.00
Lead	85.00	310.00	530.00
Nickel	35.00	44.00	210.00
Zinc	140.00	430.00	72.00

Table 3. *Constants depending on the type of metal (SRLVsS).*

Substance	Constants		
	A	B	V
Metals (in µg/g DW)			
Arsenic	15.00	0.40	0.40
Cadmium	0.40	0.007	0.21
Chromium	50.00	2.00	0
Copper	15.00	0.60	0.60
Mercury	0.20	0.0034	0.0017
Lead	50.00	1.00	1
Nickel	10.00	1.00	0
Zinc	50.00	3.00	1.50

In *Table 4* are given Serbian limit values and maximum legal concentrations for As, Cd, Cr, Cu, Hg, Pb, Ni and Zn.

Contents of metals in common reed

The contents of metals (in µg/g of dry mass) in rhizome, stem and leaf of the common reed (*Table 5*) at different localities revealed a trend of decline as follows:

- In rhizome: Fe > Al > Mn > Zn > Cr > B > Pb > Ni > Cu > Sr > As > Se > Cd > Co > Hg;
- In stem: Fe: > Al > Mn > Zn > Sr > Pb > Cr > Cu > Ni > Se > Co > As > Cd > B > Hg;
- In leaf: Fe > Al > Mn > Sr > Zn > Pb > Cu > Cr > Ni > Se > Co > B > As > Cd > Hg.

Declining trend Fe > Al > Mn > Zn was recorded in rhizome and stem, whereas in the leaf it is: Fe > Al > Mn > Sr > Zn.

Considering the average values for the contents of metals, bioaccumulation was the greatest in the rhizome of the common reed, and least in the stem. ANOVA revealed difference between various organs of the common reed for the bioaccumulation of metals, using the average contents in them, in total $F_{(2,9)} = 12{,}34$, P = 0,003.

Table 4. *Serbian limit values and maximum legal concentrations for metals (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn) (in µg/g of dry mass) in sediment for each sediment sample, depending on the % of OM and clay which are defined in the Serbian Regulation of limit values pollutant substances into surface water, groundwater and sediments, and the deadlines for their attaining. LV – limit values. MLC – maximum legal concentration.*

Sampling sites	As		Cd		Cr		Cu		Hg		Ni		Pb		Zn	
	LV	MLC	LV	MLC	LV	MLC	LV	MLC	LV	MLC	LV	MLC	LV	MLC	LV	MLC
1	26.58	38.49	0.88	7.02	103.54	248.50	33.23	101.54	0.29	1.57	36.77	46.23	78.94	287.89	133.56	410.23
2	25.11	36.36	0.84	6.70	96.60	231.84	30.94	94.55	0.28	1.50	33.30	41.86	75.26	274.49	122.85	377.31
3	28.39	41.12	0.92	7.35	112.90	270.96	35.90	109.70	0.31	1.65	41.45	52.11	83.48	304.46	147.40	452.72
4	23.43	33.94	0.80	6.38	88.20	211.68	28.44	86.90	0.27	1.43	29.10	36.58	71.08	259.23	110.27	338.69
5	23.80	34.46	0.81	6.46	89.88	215.71	29.02	88.66	0.27	1.44	29.94	37.64	71.99	262.56	112.90	346.76
6	20.96	30.35	0.73	5.83	76.70	184.08	24.56	75.03	0.25	1.32	23.35	29.35	64.90	236.68	92.37	283.71
7	22.40	32.44	0.77	6.17	83.18	199.63	26.86	82.08	0.26	1.38	26.59	33.43	68.50	249.82	102.63	315.23
8	22.15	32.08	0.76	6.11	82.04	196.90	26.47	80.88	0.26	1.37	26.02	32.71	67.88	247.55	100.85	309.74
9	22.23	32.19	0.77	6.13	82.42	197.81	26.58	81.23	0.26	1.37	26.21	32.95	68.07	248.24	101.42	311.49
10	25.23	36.54	0.84	6.73	97.14	233.14	31.15	95.17	0.28	1.51	33.57	42.20	75.58	275.63	123.72	379.99
11	24.00	34.76	0.81	6.48	91.16	218.78	29.26	89.42	0.27	1.45	30.58	38.44	72.50	264.40	114.61	352.03
12	22.67	32.83	0.78	6.24	84.26	202.22	27.32	83.47	0.26	1.39	27.13	34.11	69.17	252.26	104.45	320.80
13	24.18	35.02	0.81	6.51	92.18	221.23	29.52	90.19	0.27	1.46	31.09	39.08	72.95	266.07	116.07	356.49
14	22.97	33.26	0.79	6.30	85.68	205.63	27.78	84.89	0.26	1.40	27.84	35.00	69.92	254.99	106.64	327.52
15	24.78	35.89	0.83	6.63	95.10	228.24	30.43	92.97	0.28	1.49	32.55	40.92	74.45	271.51	120.49	370.09
16	24.83	35.96	0.83	6.64	95.32	228.77	30.52	93.25	0.28	1.49	32.66	41.06	74.58	272.00	120.86	371.22
17	20.00	28.96	0.69	5.51	73.58	176.59	22.77	69.58	0.24	1.28	21.79	27.39	62.49	227.90	86.42	265.43
18	24.01	34.77	0.81	6.48	91.28	219.07	29.27	89.43	0.27	1.45	30.64	38.52	72.52	264.50	114.74	352.43
19	20.43	29.59	0.72	5.78	73.38	176.11	23.90	73.04	0.24	1.29	21.69	27.27	63.58	231.88	87.90	269.99
20	27.17	39.35	0.89	7.09	107.06	256.94	34.02	103.95	0.30	1.60	38.53	48.44	80.43	293.34	138.44	425.22
21	29.05	42.08	0.93	7.44	116.74	280.18	36.79	112.41	0.32	1.68	43.37	54.52	85.14	310.50	152.76	469.19
22	25.71	37.24	0.85	6.79	99.94	239.86	31.79	97.13	0.29	1.53	34.97	43.96	76.78	280.00	127.62	391.97
23	27.64	40.02	0.90	7.21	109.06	261.74	34.78	106.26	0.30	1.62	39.53	49.69	81.59	297.56	141.68	435.15
24	28.85	41.78	0.93	7.45	115.00	276.00	36.62	111.90	0.31	1.67	42.50	53.43	84.62	308.62	150.68	462.81
25	25.31	36.66	0.84	6.74	97.64	234.34	31.26	95.51	0.28	1.51	33.82	42.52	75.79	276.40	124.41	382.12

Table 5. *Metal concentrations in rhizome, stem and leaf of common reed at different localities in µg/g of dry mass.*

Localities	**Al**	**As**	**B**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**Sr**	**Zn**
3 river km–rhizome	688,68	3,27	0,00	0,54	0,00	8,68	4,74	519,17	0,11	69,75	3,42	6,34	1,44	2,86	22,05
3 river km–stem	32,32	1,78	0,00	0,31	0,97	2,15	1,93	32,38	0,09	10,12	1,06	3,03	1,31	2,72	9,10
3 river km–leaf	115,22	0,98	2,10	0,35	0,89	2,81	4,34	99,21	0,09	60,31	1,17	15,69	1,61	28,12	14,38
58 river km- rhizome	427,66	1,52	0,00	0,59	0,49	11,94	3,65	380,02	0,09	35,68	4,59	8,27	1,20	2,66	20,28
58 river km – stem	37,79	1,37	0,00	0,51	1,66	2,77	1,71	34,67	0,06	5,65	1,42	2,56	1,91	2,79	13,77
58 river km –leaf	69,60	0,61	0,17	0,34	1,09	3,29	4,42	90,12	0,10	71,63	1,43	10,89	1,32	42,61	16,66
72 river km-rhizome	593,55	0,53	0,00	0,57	0,30	18,27	5,26	522,50	0,08	26,01	7,57	3,56	0,98	2,91	27,93
72 river km–stem	60,56	0,23	0,00	0,31	0,86	3,08	2,54	53,64	0,08	6,49	1,47	3,58	0,86	5,36	21,02
72 river km–leaf	107,33	0,00	0,39	0,38	1,09	6,49	4,83	123,09	0,11	19,52	2,02	12,33	1,09	31,94	20,08
153 river km-rhizome	833,99	6,56	0,13	0,86	1,42	10,66	4,18	1480,62	0,12	167,69	4,31	5,28	0,89	6,54	17,28
153 river km–stem	38,03	0,53	0,00	0,34	1,16	2,48	1,70	57,56	0,10	37,88	1,42	2,80	0,92	8,01	17,98
153 river km–leaf	83,70	0,38	0,16	0,36	1,10	3,73	3,66	134,54	0,09	109,02	1,50	6,18	1,04	26,29	20,45

The greatest average content of metals in the common reed was recorded at the 153rd river km, and the least at the 58th river km. The greatest accumulation in common reeds at all localities were those of Al, Fe, Mn, Zn, Sr i Pb, but certain metals accumulated the most at particular localities, e.g., Pb at the 3rd river km locality, Al, Fe i Mn at the one at 153rd river km, and Sr at the 58th river km locality. ANOVA revealed no difference between localities in the average contents of metals in common reeds either in total $F_{(3, 8)} = 0,31$, $P = 0,82$, or for any of the metals.

Bioconcentration Factor (BCF)

The greatest *BCF* values were recorded for Se and the lowest for Al and Fe (*Table 6*).

Table 6. *Biological Concentration Factor.*

Metals	**3rd km river flow**			**58th km river flow**			**72th km river flow**			**153rd km river flow**		
	Rhizome	**Stem**	**Leaf**	**Rhizome**	**Stem**	**Leaf**	**Rhizome**	**Stem**	**Leaf**	**Rhizome**	**Stem**	**Leaf**
Al	0,01	0,00	0,00	0,01	0,00	0,00	0,01	0,00	0,00	0,01	0,00	0,00
As	0,19	0,10	0,06	0,09	0,08	0,04	0,04	0,02	0,00	0,38	0,03	0,02
Cd	0,21	0,12	0,13	0,21	0,18	0,12	0,36	0,19	0,24	0,41	0,16	0,17
Co	0,00	0,05	0,04	0,03	0,09	0,06	0,02	0,05	0,07	0,08	0,07	0,06
Cr	0,07	0,02	0,02	0,09	0,02	0,03	0,14	0,02	0,05	0,09	0,02	0,03
Cu	0,06	0,03	0,06	0,05	0,02	0,06	0,08	0,04	0,07	0,06	0,02	0,05
Fe	0,01	0,00	0,00	0,01	0,00	0,00	0,01	0,00	0,00	0,04	0,00	0,00
Hg	0,31	0,25	0,25	0,33	0,22	0,37	0,47	0,47	0,65	0,40	0,33	0,30
Mn	0,11	0,02	0,10	0,02	0,00	0,05	0,02	0,00	0,01	0,12	0,03	0,08
Ni	0,07	0,02	0,03	0,10	0,03	0,03	0,17	0,03	0,05	0,09	0,03	0,03
Pb	0,13	0,06	0,32	0,16	0,05	0,21	0,08	0,08	0,28	0,10	0,06	0,12
Se	2,88	2,62	3,22	2,40	3,82	2,64	0,98	0,86	1,09	1,78	1,84	2,08
Sr	0,02	0,02	0,20	0,02	0,02	0,29	0,02	0,04	0,23	0,05	0,06	0,20
Zn	0,07	0,03	0,05	0,06	0,04	0,05	0,10	0,07	0,07	0,05	0,05	0,06

Pearson's Correlation Coefficient

All metals we investigated revealed a strong positive correlation between their levels in various organs of common reeds and in the sediment at all localities, except Se, which revealed a negative correlation.

Translocation ability (TA)

Translocation ability (*TA*) forthe majority of metals is of a value mainly less than 1. It is greater between the rhizome and leaf, than between the rhizome and stem. Co, Sr, Se, Hg, Zn, Cd and Pb revealed the greatest *TA* value between the rhizome and stem, whereas Sr, Pb, Co, Se, Hg i Cu revealed the greatest *TA* value between the rhizome and leaf (*Table 7*). Generally, Co, Sr, Se, Pb i Hg were the most mobile metals between the rhizome and both other emerged organs.

Table 7. *Translocation ability (TA) between the rhizome and emerged organs of reeds (stem and leaf) for particular metals at all four localities.*

Metals	**Rhizome/sediment**	**Rhizome/stem**	**Rhizome/leaf**
Al	0.01	0.07	0.15
As	0.17	0.33	0.17
Cd	0.32	0.53	0.56
Co	0.03	2.1	1.89
Cr	0.10	0.21	0.33
Cu	0.06	0.44	0.97
Fe	0.02	0.06	0.15
Hg	0	0.80	1
Mn	0.06	0.20	0.87
Ni	0.10	0.27	0.31
Pb	0.12	0.51	1.92
Se	1.13	1.11	1.12
Sr	0.03	1.26	8.62
Zn	0.06	0.71	0.82
Average values	0.16	0.58	1.26

Discussion

Based on the results obtained, conclusions can be made about the concentrations of the tested metals in the ecosystem of the river Tisza. Contents of metals (in μg/g of dry mass) in the sediment of the Tisza River bed are given in *Table 1*. The declining trend of metal concentrations in the sediment is the following: Al > Fe > Mn > Zn > Sr > Cr > Cu > Ni > Pb > Co > As > Cd > Se > Hg. Al followed by Fe had the highest accumulation values for all sites. This is expected since Al and Fe are the most common weathering products. Which correspond to a classical weathering product in research areas where the sediments are mainly composed of Al and Fe.

In *Table 4* are presented Serbian limit values and maximum legal concentrations for metals (As, Cd, Cr, Cu, Hg, Pb, Ni and Zn) in sediment for each sediment sample, depending on the % of OM and clay as discussed above. The sediment samples for all sites contained elevated concentrations of Cd, Cr, Cu, Ni and Zn with regard to SRLVsS. Concentrations of Cd, Cr, Cu and Zn are below maximum allowed legal concentration, except Ni which concentrations are above maximum legal concentration. Research area has a strong agriculture activity and the main metal sources are impurities from fertilizers, pesticides and sewage sludge.

However, the increased concentration of these metals had no significant effect on wildlife of the river Tisza as the determined concentrations of the metals tested in the reed do not exceed the threshold of phytotoxicity (*Table 5*).

The research proved a strong positive correlation between the concentrations of metals in the sediment and all reed organs (rhizomes, stems and leaves), except Se, which revealed a negative correlation. A positive correlation indicates that the value of one variable can be determined when we know the exact value of the other.

Accumulated metals in the common reed are not distributed evenly, but there are target organs for bioaccumulation. The underground organ (rhizome) shows a higher storage capacity than the above ground parts (stems and leaves). Based on many years of research, the tendency of bioaccumulation in plant organs has been established, by which metal concentrations decrease according to the following order: rhizome > leaf > stem (Vymazal *et al*., 2007; Bonanno and Lo Giudice, 2010), which has been confirmed by the research conducted (*Table 5*). The concentrations of metals accumulated in the organs of the common reed may vary during the growing season, as a result of a change in the levels of metal concentration in the surrounding medium, and, on the other hand, the changes in their bioavailability (Schierup and Larsen, 1981; Hardej and Ozimek, 2002). All common reed organs show a little *BCF* for the tested metals. The largest biological accumulation factor was found for selenium, and the lowest for aluminum, mercury, iron and manganese (*Table 6*). Although aluminum, iron and manganese are present in the highest concentrations in the sediment of the bed of Tisza River and in common reed (*Tables 1 and 4*), these metals show the smallest BCF (*Table 6*). The results obtained confirm the earlier assumption that the metals in the sediment originating from the decomposition of rocks are usually connected in the form of chemical compounds that are not easily bioavailable to wildlife (Schropp *et al*., 1990).

Examining the effect of the site on the bioaccumulation of metals in the common reed, it was found that most metals accumulate on the 153rd km of the river flow, but the effect of the site on the bioaccumulation of metals in the investigated area has no statistical significance. The results can be explained by the fact that the concentrations of metals in sediments along the entire flow of the river Tisza are generally consistent.

The concentration of aluminum in the common reed (*Table 5*) is below the threshold of phytotoxicity, which according to Kabat-Pendias (2001) is 1000–3000 μg/g. The *BCF* of aluminum in the common reed was the lowest (*Table 6*). Metals in sediments originating from the decomposition of rocks, such as aluminum and iron are usually connected in the form of chemical compounds that are not easily bioavailable to wildlife. Aluminium is mainly accumulated in the rhizome of the common reed and possesses low mobility to aboveground organs ($TA < 0.2$), which is consistent with the previous studies showing that aluminum and iron mainly accumulate in the root of metallophyte (Baker *et al*., 1994) (*Table 7*).

The values obtained for arsenic in the sediment of the bed of the river Tisza (3.8–19 μg/g) (*Table 1*) are below the values wich prescribed SRLVsS (*Table 4*). Data relating to the bioaccumulation of arsenic in the river is little known. Aquatic organisms adopt arsenic directly from the water or through the food chain. It accumulates in primary producers (plants and algae), but its concentration does not increase when invertebrates and fish (consumers) are concerned (Štajn *et al*., 2007).

The content of cadmium in the sediment of the river Tisza (0.2 - 2.8 μg/g) (*Table 1*) increased compared to values wich prescribed SRLVsS (*Table 4*), while the concentration of cadmium in rhizome, leaves and stems of the reed are significantly lower than the phytotoxic range (5–100 μg/g) which is suggested by Chaney (1989). According to Allen (1989), the concentrations of cadmium in the plant in an unpolluted environment ranges from 0.01–0.3 μg/g, which approximately corresponds to the values obtained in this study (*Table 5*). The *BCF* of cadmium in rhizome, stem and leaves is < 1. Cadmium is a highly toxic, non-essential element that affects the growth, metabolism and regime of water in a plant (Divan *et al.*, 2009). One reason is the high toxicity of its interaction with other elements. In addition to toxicity, oxidative stress is caused by releasing free radicals which causes the damage to the membrane lipids, proteins, pigments and nucleic acids (Hernandez *et al.*, 1998). *P. Australis* is a cadmium tolerant species, whose defense strategy is based on the increased activity of antioxidant enzymes (Ederli *et al.*, 2004).

Cobalt concentrations in the rhizomes, stems and leaves of the reed are far lower than the phytotoxic range (15–50 µg/g) which is suggested by Kabat-Pendian (2001). Plants on average acquire small amounts of cobalt, *BCF* is low (*Table 6*). Acquired over the roots by a transpiration stream, it is transported to the aboveground organs where it is accumulated in the peripheral part and the top of the leaf, and it is accumulated in a greater amount in generative than in vegetative organs (Kastori, 1983). The transfer factor for cobalt between the rhizomes and stems and leaves of the common reed is > 1 (*Table 7*). Plants can acquire cobalt through leaves. It is a regular ingredient in plant cells. Its role in life processes is not well known, but it is emphasized in the symbiotic fixation of atmospheric nitrogen and the oxidation processes.

Chromium content in the sediment of the river Tisza (116.3 - 136.8 µg/g) (*Table 1*) was higher than the values wich prescribed SRLVsS (*Table 4*), and the concentration of chromium in the common reed rhizome (*Table 5*) is higher than the threshold of phytotoxicity (0.5 µg/g), which is given by Allen (1989). For plants, chromium is not an essential element, it belongs to the group of elements that are harmful, and plants do not accumulate it in great amounts. It is believed that those plants that have the ability to accumulate iron, accumulate chromium as well (Kastori, 1995).

Copper concentration in the sediment of the river Tisza (25.6 - 88.9 µg/g) (*Table 1*) is higher than the values wich prescribed SRLVsS (*Table 4*), while the value of the copper in the common reed is below the threshold of phytotoxicity (25–40 µg/g), according to Chaney (1989). Siedlecka *et al.* (2001) found a tendency to accumulate copper in the roots with very little mobility to the aboveground organs. Thus, it was pointed out that the roots behaved as filters, since the concentrations of copper found in the plant were 70% less than the concentrations determined in the root (Siedlecka *et al.*, 2001). However, the concentrations of copper in this study show the same values in the rhizome and the leaf (*Table 5*). Kastori (1995) explains this phenomenon saying that plants can acquire copper ions over the leaf surface. Otherwise, copper shows a low potential to bioaccumulate itself in plants and there is no evident biomagnification. Ascending copper transport as well as its redistribution is highly dependent on the level of the plant's copper supply. It is one of the essential elements needed for the various enzymatic activities of plants.

Concentrations of iron found in the common reed (*Table 5*) were below the threshold of toxicity, ranging from 1000 to 3000 µg/g (Kabata-Pendian, 2001). Similar to aluminum, iron has a low *BCF* because in the sediment it originates from the decomposition of rocks, and it is not easly bioavailable to the wildlife of aquatic ecosystems (*Table 6*). According to the research of Szymanowska *et al.* (1999) the iron content in the sediment is linearly correlated to its presence in the plant organs (Szymanowska *et al.*, 1999), and roots show very high values, while mobility through the tissues of *P. australis* is low (Bonanno, 2011), which was confirmed in this study (*Table 7*).

Values for mercury in the sediment of the bed of the river Tisza (0.11 to 0.36 µg/g) (*Table 1*) are below the values wich prescribed SRLVsS (*Table 4*). Data relating to the bioaccumulation of mercury in the river is little known. Different studies have shown that the roots of various aquatic macrophytes accumulate higher concentrations of mercury than other plant parts, e.g. the amount of mercury accumulated in the roots of *Eichhornia crassipes* is 2–3 times higher than in shoots (Jana, 1988), which is not in accordance with the results obtained. Mercury concentrations in rhyzome, stem and a leaf of the common reed share approximately equal values (*Table 5*).

The content of manganese in the common reed (*Table 5*) was below the threshold of toxicity for plants which is 50–500 µg/g according to Allen (1989). The mechanism of uptake of manganese is specific. During the first phase, it is absorbed into the root surface, actually the apparent free space of the root, and then, depending on the metabolic processes inside the cell, it is activelly transferred. In this regard the dynamics of accumulation of manganese in the plants during the growing season has its own distinctive flow and depends on a number of internal and external factors. The movement of manganese is especially fast towards the meristem tissue and reproductive organs, thus younger plant organs, whoseh metabolic processes are more intensive, are usually richer in manganese than older plants (*TA* between the stem and leaves is $\approx$ 1) (*Table 7*).

The obtained values for nickel in the sediment of the river Tisza (38.4 - 53.7 µg/g) (*Table 1*) were higher than the values wich prescribed SRLVsS (*Table 4*), and only the concentration determined in the reed rhizome can be considered dangerous for the plant (*Table 5*). According to Allen (1989), nickel concentrations above 5 µg/g are considered phytotoxic. Recently, nickel was recognised as one of the necessary elements for higher plants, because it was determined that it represents a component of enzymes important for normal life processes. Low concentrations of nickel are essential for plants, but at higher concentrations its toxic effects have been proved (Parida *et al.*, 2003; Demirezen *et al.*, 2007). The intensity of uptake of nickel, and its accumulation in plants under natural conditions depends on its concentration in the environment as well as on the pH value of the environment.

The lead content in the sediment of the river Tisza (13.6 – 56 µg/g) (*Table 1*) was lower than the values wich prescribed SRLVsS (*Table 4*), while the concentration of lead in the common reed (*Table 5*) was far smaller than the phytotoxic range which is 30–300 µg/g (Roos, 1994). The concentrations found in the leaves are, on average, greater than the value determined in the rhizomes and stems (*Table 5*), which can be explained by the fact that the leaves accumulate lead by deposition from the air, which directly affects aboveground organs, as well as the values of *TA* (*Table 7*). *TA* between the stem and leaves is > 1. Once it gets into the leaves, lead can be returned to the environment either directly, through secretion from the leaves (Burke *et al.*, 2000; Batty and Younger, 2004, Weis *et al.*, 2004) or indirectly by decay which thereby increases its bioavailability.

The values obtained for selenium in the common reed are higher than those in the sediment (*Table 1 and 5*). Selenium is the only metal that shows a negative linear correlation between the concentrations in the common reed and the sediment, as well as the largest *BCF* (*Table 6*). *TA* for selenium between all common reed organs is higher than 1, which confirms the equal representation of this metal in the common reed (*Table 7*). Selenium is a very important micronutrient, necessary for the proper functioning of the enzyme systems, and therefore it is expected to be adopted in a larger amount.

The values obtained for strontium (*Table 5*) in the common reed are below the threshold of phytotoxicity which is 1000 µg/g according to Kabat-Pendias (2001). The high ratio of rhizome / leaf *TA* shows the interesting mobility of this element (*Table 7*). Thus, by doing this research, we have found out that the largest concentration of strontium is determined exactly in the highest parts of the plant, which is in accordance with Kabat-Pendias (2001).

The concentrations of zinc in the sediment of the river Tisza's bed (76 - 364 µg/g) (*Table 1*) were higher than the values wich prescribed SRLVsS (*Table 4*), while the concentration of zinc in the common reed (*Table 5*) is below the threshold of phytotoxicity which is 500–1500 µg/g as proposed by Chaney (1989). So far, works published concerning the distribution of zinc in the plant's tissue are generally not the same, sometimes they are even contradictory. Since zinc is an essential element it can be found in all organs and tissues of plants. On the basis of the published results regarding the distribution of zinc in plants, it can be concluded that it is accumulated to a greater extent in the roots and leaves (Kastori, 1990), which was confirmed by the survey (*Table 5*). As for translocation, zinc belongs to the group of elements whose mobility in plants is not great (*Table 7*).

Conclusions

On the basis of the research the following conclusions can be drawn:

a) The sediment samples for all sites contained elevated concentrations of Cd, Cr, Cu, Ni and Zn. Concentrations of Cd, Cr, Cu and Zn are below maximum legal concentration, except Ni which values are above maximum legal concentration based on the Serbian Regulation of limit values pollutant substances into surface water, groundwater and sediments, and the deadlines for their attaining, but without impact on wildlife because the established concentrations of metals in the common reed do not cross the threshold of phytotoxicity;

b) The common reed is a good indicator of the condition of the environment because a stron positive correlation was found between the concentrations of metals in the sediment and all reed organs (rhizomes, stems and leaves);

c) Accumulated metals in the common reed are not distributed evenly, the underground organ (rhizome) shows a higher storage capacity than the above ground parts (stems and leaves);

d) Due to the uniform concentration of metals in the ecosystem (biotope and biocenosis) of the Tisza, there are no differences in bioaccumulation in different localities.

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SPECIES LEVEL PHOSPHORUS ACQUISITION AND INTERNAL UTILIZATION EFFICIENCY AND THEIR RELATION WITH BOTTOM-UP AND TOP-DOWN FACTORS

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Abstract. Ecosystem level nutrient use efficiency (NUE) generally increases with the richness of species or life forms in a community, though, discrepancies may exist at species level. Existence of such discrepancies, phosphorus acquisition efficiency (PAE), phosphorus internal utilization efficiency (PUTIL), phosphorus use efficiency (PUE), quotient of phosphorus utilization (QUTIL) and phosphorus harvest index (PHI) were assessed at species (Corchorus depressus) level with various spatial and temporal factors within the arid area of the Thar Desert, India. These P variables were ordinate and regressed with various bottom up (species richness, relative importance of C. depressus, Shannon and Wiener index, Simpson index, evenness, seed output, percent cover of C. depressus, root length and root collar diameter) and with top down factors (soil organic carbon, nitrogen, moisture, pH and electric conductivity). The relative importance of PAE and PUTIL in the PUE as well as the QUTIL and PHI in the PUTIL were also assessed. ANOVA analysis revealed that all the factors undertaken in the present study (i.e. spatial and temporal events and their interaction) brought significant variation ($P<0.001$) in PAE, PUTIL, PUE and QUTIL. However, for PHI spatial and temporal events individually brought significant variations, their interaction was non-significant. It emerged that for PUE at low P environmental conditions, PAE was 10-37 times more important than PUTIL. Similarly for PUTIL, QUTIL was 17-56 times more important than PHI. Kaiser-Meyer-Olkin test (0.597) and Bartlett's test of sphericity indicated appropriate use of factor analysis (PCA) and significant correlation between variables in the present study, respectively. Ordination analysis showed lack of correlation between PAE and PUTIL, indicating that selection of one of these should not affect the other. Percent cover of plant, seed output, root length and root collar diameter exhibit various relationships with different P variables. Regression analysis between P variables and community factors revealed that at species level dominance of C. depressus reflects its higher P acquisition efficiency; however as the community diversity (richness, Shannon and Wiener index and evenness) increases the P internal utilization and PUE were inhibited. It can be concluded that P internal utilization and PUE of C. depressus are largely influenced by temporal factors (increase and decrease of community diversity during pulse and non-pulse events, respectively). As a result this species achieved effective nutrient use through temporal partitioning, through which it fulfilled P requirements during low resource availability. Among the soil variables soil nitrogen supported PUTIL, PUE and QUTIL, while soil moisture and soil pH favoured PHI and soil N and PHI showed negative relationship.

Keywords: *Nutrient Use Efficiency, Ecosystem level, Species Level, Ordination, Species Diversity, Plant and Soil Factors*

Introduction

Efficiency concepts in plant mineral nutrition have been defined based on the process by which plants acquire, transport, store and use the nutrient in order to produce dry matter or grain, at low or high nutrient supply (Ciarelli *et al.,* 1998). Nutrient acquisition efficiency and nutrient internal utilization efficiency are the two major components of plant nutrient use efficiency. These two components are related to the ability of the plant to acquire nutrient from the soil and to plants internal ability to produce yield units per unit nutrient in plant (Good *et al.,* 2004). Bridgham *et al.,* (1995) defined nutrient uptake efficiency as the proportion of available soil nutrients acquired by plants and the nutrient use efficiency, as total net primary productivity (PR) per quantity of nutrients acquired during the same periods. Hiremath and Ewel (2001) considered ecosystem nutrient use efficiency as the ratio of net primary productivity to soil nutrient supply. They further summarized how the ecosystem level nutrient use efficiency increases with the richness of species or life forms in a community and what the relative roles of species traits and nutrient supplying capacity of the soil in determining nutrient use efficiency are.

Nutrient use efficiency is studied by ecologists at different scales (Mathur, 2013). At the leaf level, nutrient use efficiency is the ratio of photosynthetic rate to concentration of nutrient in the leaf lamina (Field & Mooney 1986); at the plant level, it is the ratio of growth to nutrient uptake (Hirose, 1975). Plant level nutrient use efficiency depends on productivity per unit of nutrient in the plant and mean residence time of nutrient in the plant (Berendse & Aerts, 1987). There are two ways through which nutrient use efficiency of an individual can influence nutrient use efficiency of the whole system. The first is through its influence on competitive interaction among species. Plant with high nutrient use efficiency should be able to tolerate lower nutrient availabilities: thus, they should be effective competitors in diverse communities where nutrients are in short supply (Tilman *et al.,* 1997). A system made up of such individuals should therefore have a higher productivity per unit of nutrient supplied by the soil than one made up of individuals with low nutrient use efficiencies. The second way that plant nutrient use efficiency can influence the ecosystem nutrient use efficiency is through its influence on litter nutrient return (Hobbie, 1992).

Effective uptake can be achieved through four different mechanisms (a) temporal partitioning such that one species takes up nutrients at a time when others do not (b) spatial partitioning such that one species takes up nutrients from portions of the habitat that are inaccessible to other species (c) uptake of nutrients in different proportions, or (d) uptake of different forms of the same nutrient (for example plant root absorb P as either $H_2PO_4^-$ or HPO_4^{2-}).

It follows, therefore, as suggested by Tilman *et al.,* (1997) in the context of diversity and ecosystem productivity and by Hooper (1998) in the context of diversity and nutrient retention, that ecosystem nutrient use efficiency depends upon the identification of the species making up the system, and not on a greater diversity of species per se. A complete assessment of ecosystem productivity and nutrient dynamics requires measuring the above and below ground patterns of biomass increment, nutrient content and turnover. In fact, a proper evaluation of nutrient use efficiency requires data at the whole-plant level, because patterns of aboveground utilization efficiency are not necessarily similar to whole-plant utilization efficiency (Aerts & Chapin, 2000). However most of the previous studies have focused exclusively on the nutrient utilization efficiency (NUE) of fine-litter production (Vitousek, 1984; Silver, 1994; Yasumura *et al.,* 2002; Paoli *et al.,* 2005). One whole plant NUE study from semiarid grassland in northern China was conducted by Yuan *et al.,* (2006). Destructive methods of NUE evaluation are generally criticized, therefore nutrient solution techniques have been used as important tools in short-term experiments to select and identify nutrient efficient plants (Furlani and Furlani, 1988; Ciarelli *et al.,* 1998; Spehar & Souza, 1999). In addition, these non destructive techniques might be useful for those species which have low germplasm availability in nature (Furlani *et al.,* 2002).

Woody perennials exhibit a characteristic time course of phosphorus acquisition and internal P redistribution during their life cycle (Fageria *et al.,* 2011 and White and Veneklaas, 2012). Phosphorus efficiency can be divided into P acquisition efficiency (PAE) and P utilization efficiency (PUE). PAE refers to the ability of plants to take up P from soils, whereas PUE is the ability to produce biomass or yield using the acquired P (Wang *et al.,* 2010). Enhancement of P efficiency in plants can be achieved through improving P acquisition and/or utilization. However, the contribution of PAE or PUE to plant P efficiency varies with species and environmental conditions (Wang *et al.,* 2010). A higher P internal utilization efficiency has attributed to a higher grain yield per unit of P in the grain (quotient of utilization) and to higher ability to transfer nutrient from shoot to grain, called P harvest index (Baligar & Fageria, 1997).

To check the variability in species level nutrient use efficiency and its associated variables, present study was conducted with two objectives. (1) to determine the relative importance of P acquisitions and internal utilization in the P use efficiency and relative importance of P quotient of utilization and the P harvest index, in the P internal utilization efficiency at different spatial and pulse events and (2) to find out the relationship of different efficiency variables (P acquisition efficiency, P internal utilization efficiency, P use efficiency, quotient of P utilization and P harvest index) with bottom-up (species richness, Relative Importance value of *Corchours depressus*, diversity parameters, percent cover of plant, seed output, root length and root collar diameter and top-down factors (Soil organic carbon, soil nitrogen, moisture, soil pH and electric conductivity).

Corchorus depressus (Linn.) belongs to family Tiliaceae, distributed in almost all parts of the world. It is a prostrate, woody perennial with branches radiating from a woody crown, closely addressed to the ground, and the plant is regarded as a good sand binder in the desert (Mathur, 2005). Fruit is a capsule, 8 to 15-mm long, often curved upward from the underside of the branches, cylindrical, beaked, four-valve, and septet between the seeds. Seeds are minute and brown colored. Various medicinal uses of this plant for general weakness, gonorrhea, diabetes, treachery troubles, and improve sexual vigour have been reported (Chopra and Nayar, 1956; Shekhawat, 1986; Kumar *et al.,* 2003). The clinical validation, phytodiversity, and phytochemistry studies have been carried out by Mathur (2005).

Material and Methods

Site selection and their status

Five different sites were selected with-in in the 16 km radius of the Jodhpur city of the Rajasthan state, Indian (*Table 1*). Each site differing in respect of soil composition, land uses and vegetation status. During the study period mean annual precipitation ranged from 0.004 to 260 mm, winter (December) temperature varied from 10.7 to 23^0C, summer (May) temperature varied from 28.7 to 42.2^oC and relative humidity ranges from 31 to 91% (Morning) and 08 to 68% (Evening).

Biomass estimation

The above and below-ground biomass of the *C. depressus* was estimated by collecting random samples from different sites. Fifteen plants were uprooted and gently washed under tap water. The plants were air-dried and weighed using an electronic balance (accuracy 0.001 g.). As per the resources hypothesis (Goldberg & Novoplansky, 1997) the sampling were carried out during rainy (July), winter (December) and summer (May) periods for evaluation of the impacts of pulse, inter-pulse and non-pulse events.

Nutrient Quantification

Phosphorus was estimated by spectroscopic method (Allen *et al.,* 1976) based on the development of molybdenum blue color. The standard was prepared with KH_2PO_4.

Nutrient Efficiency Indices

Different nutrient efficiency indices were calculated following Parentoni and Junior-Souza, (2008). Two groups of efficiency variables were obtained for each site in each pulse event. The first group comprised of the variables P acquisition efficiency (PAE) and P internal utilization efficiency (PUTIL), which were used to obtain the P use efficiency (PUE). While, the second comprised of two variables, quotient of utilization and P harvest index

(1) Phosphrus Acquisition Efficiency (PAE)

$$\text{PAE} = \frac{\textit{Phosphorus in Plant}}{\text{Phosphorus in soil}}$$

(2) Phosphorus Internal Utilization Efficiency (PUTIL)

$$\text{PUTIL} = \frac{\textit{Seed dry matter produced (Seed Biomass)}}{\text{Phosphorus in Plant}}$$

(3) Phosphorus Use Efficiency (PUE)

$$\text{PAE X PUTIL}$$

(4) Quotient of Phosphorus Utilization (QUTIL)

$$\text{QUTIL} = \frac{\text{Seed Biomass}}{\textit{Phosphorus in Seed}}$$

(5) Phosphorus Harvest Index

$$\text{PHI} = \frac{\text{Phosphorus in Seed}}{\textit{Phosphorus in Plant}}$$

The relative importance of PAE and PUTIL over PUE was investigated according to Moll *et al.,* (1982). This methodology was developed to investigate the relative importance of two variables (PAE and PUTIL), obtained experimentally, over a third variable (PUE) which is obtained by the multiplication of PAE and PUTIL. This information could be valuable for comparative evaluation of each of two variables (PAE and PUTIL) that to be used in selection program related with P use efficiency. The same also exercised for two variables related with P internal utilization efficiency and PHI

The analysis of variance (ANOVA) was carried out in a two way strip – plot design, which sacrifices precision on the main effects of both factors. The interaction is measured more accurately by this method compared to randomized complete block or split-plot design (Gomez & Gomez, 1984).

Multivariate Analysis

Bartlett's test of sphericity and Kaiser-Meyer-Olkin (KMO) were carried out to assess the suitability of factor analysis. Principal Component Analysis (PCA) was carried out as a data reduction technique. PCA was performed with Pearson correlation coefficient. The main objective of PCA was to find out relationship of different efficiency variables (PAE, PUTIL PUE, QUTIL of P utilization and P harvest index) with bottom-up (species richness, Relative Importance value of *C. depressus*, diversity parameters, percent cover of plant, seed output, root length and root collar diameter) and top-down factors (Soil organic carbon, soil nitrogen, moisture, soil pH and electric conductivity).

Appropriate regression equations were selected on the basis of probability level significance and high R^2 value. Path analysis was carried out with Curve Expert software, 2001.

Results and Discussion

Most of the sites were located on the older alluvial plains (with higher proportion of sand), followed by younger alluvial plain, and piedemonts (*Table 1*). Herbaceous covers were dominated by *Dactyloctenium aegyptium, Eragrostis ciliaris, Aristida funiculata,* grasses that represent the sub-climax stage of habitats (Saxena & Aggarwal, 1983). Different plant, soil and community parameters are presented in *Table 2*. Coefficient of variance of biomass data at five sites and during different seasonal events indicates that at most of the sites comparatively higher biomass was recorded during pulse event except at site 2 where it was recorded maximum during non-pulse event (*Figure 1*). The greatest P concentration was recorded in seed collected from site 1 during the pulse event. The analysis of variance revealed that the factors studied in the present investigation caused P to vary at the 99% probability level (*Table 3*).

A significant proportion of P released in the present study is probably due to effects of drying and rewetting on the microbial biomass as reported by the Sparling *et al.,* (1985) and Qiu & Mccomb (1995). Qiu & Mccomb (1995) attributed the entire increase in soil P on air-drying to killed microbial cells. He & Zhu (1998) reported that of the adsorbed P transformed by microbes from soil, 17-34% was water soluble and available P. This clearly indicates microbial turnover to be the cause of increase in available P during wet season. The results are in agreement with those reported by Rao & Tarafdar (1992) where maximum available P was recorded during pulse event in soils.

PUTIL, which exhibit the relationships between seed dry matter productions with relation to P in plant, exhibits higher during inter-pulse events followed by non-pulse event. Thus, during medium and low resources conditions, compared to other modules, plant invests their resources more in their reproductive part.

The result revealed that PUE is largely affected with spatial factor. At site 1, 2 and 3 higher PUE was recorded during non-pulse event, while at remaining sites it was recorded maximum during inter-pulse event (*Table 4*). Thus these results revealed that at moderate to low resource condition nutrient uptake of this species seems better.

***Table 1**. GPS locations, habitat types and other attributes of sampling sites*

Site No.	Coordinates		Habitat types	Soil Textures				Sub-Climax species (arrange in descending order of RIV)
	N	E		Clay	Silt	Sand	Gravel	
1	$26^0\ 12'\ 48.4''$	$73^0\ 4'\ 7.8''$	Old alluvium plains	26.39	17.89	35.58	20.13	*Dactyloctenium aegyptium* *Eragrostis ciliaris*
2	$26^0\ 11'\ 33.4''$	$73^0\ 3'\ 6.1''$	Younger alluvium and river bed terrain	17.09	25.43	23.53	33.54	*E. cilliaris, Lepidagathis cristata*
3	$26^0\ 14'\ 47.01''$	$73^0\ 0.0'\ 58.9''$	Old alluvium plains	28.72	21.31	31.29	18.80	*D. sindicum ,I. cordifolia*
4	$26^0\ 14'\ 12.4''$	$73^0\ 01'\ 24.2''$	Old alluvium plains protected	29.18	18.56	43.3	10.26	*E. cilliaris*
5	$26^0\ 21'\ 54.5''$	$73^0\ 03'\ 48.9''$	Younger alluvium and river bed terrace	25.35	15.48	37.46	21.47	*E. cilliaris, A.funiculata*

***Table 2.** Various parameters at C. depressus locations during study period*

Parameters		Range
Plant Variable	Percent Cover of *C. depressus* (Sq. M)	0.061-0.645
	Seed Output	10.98-28.34
	Root Length (Cm)	7.5-31.18
	Root Collar Diameter (Cm)	0.5-2.07
Soil Compositions	Organic Carbon (mg $100g^1$)	61.4-432.5
	Total Nitrogen (mg $100g^1$)	18.95-112.65
	Moisture	0.59-12
	pH	7.32-9.08
	Electric Conductivity	0.11-1.202
Community Composition (1X1 m) quadrate	Richness	4-12
	Shannon Weiner Index (H')	1.19-2.38
	Relative Importance Value of *C. depressus*	6.25-49.17
	Evenness	0.77-1.202
	Simpson Index	0.008-0.33

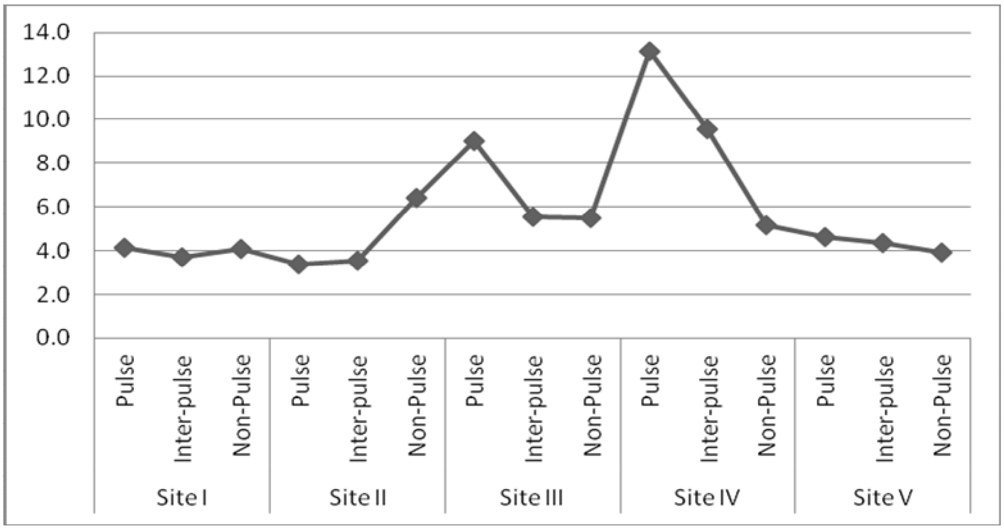


***Figure 1**. Coefficient of variance of biomass during various spatial and temporal events*

Table 3*. Range of Phosphorus (mg g-1) in various modules of C. depressus*

Modules	Range	Modules with higher
Root	1.12-9.86	Seed/I/Pulse Event
Stem	4.72-15.04	
Leaves	6.53-19.41	
Seed	8.09-38.37	
ANOVA results/Source of	Mean squares	Computed F Value
Sites	3789	40.19**
Season	139E+07	2017.89**
Sites x Season	960	164.52**
Plant Part	131E+07	225**
Sites x Plant Part	3300	56.59**
Season x Plant Part	216.11	370.60**
Sites x Season x Plant Part	2180	37.39**

**P < 0.01

QUTIL, ratio of seed biomass and the P content in seed, exhibits higher spatial and temporal variability's. At site 1, 3 and 5 it was recorded higher during non-pulse events while at other sites it was recorded maximum during inter-pulse event (*Table 4*). Thus moderate to low resources supports the seed biomass. Phosphorus Harvest Index (PHI), ratio of seed P to plant P recorded maximum during pulse events at most of the sites.

Analysis of variance revealed that all the factors taken in the present study (i.e., sites, events, and the interactions between them) caused PAE, PUTIL, PUE and QUTIL to vary at the 99% probability level (*Table 5*). However, for PHI sites and events individually brought significant variation, but their interaction was non- significant.

Results indicate that with the set of different temporal and spatial impacts, for phosphorus use efficiency, PAE was 10-37 fold more important than PUTIL, 3-9 time fold at low P environment (non-pulse events) and 2-10 fold more during moderate (inter-pulse) and high P environment (pulse event), respectively. Similarly, the related importance of QUTIL and PHI in PUTIL indicates that QUTIL was 17-56 folds more important than PHI at low P environment and 1-6 fold higher during high P condition. Greater importance of PAE to the tune of 94-100 % in P use efficiency at low or high P level in soil has also been reported in wheat (Manske *et al.,* 2001).

Multivariate Analysis

Result of Kaiser-Meyer-Olkin (KMO) and Bartlett's test of sphericity are presented in *table 6.* The Kaiser-Meyer-Olkin (KMO) is an index used to examine the appropriateness of factor analysis. A high value (between 0.5 and 1.0) indicates that the factor analysis is appropriate, value below 0.5 imply that factor analysis may not be appropriate. In this study KMO was 0.597, which indicates appropriate use of factor analysis. For Bartlett's test of sphericity there are two levels to interpret this test (a) H0: There is no correlation significantly different from 0 between the variables and Ha: at least one of the correlations between the variables is significantly different from 0. As the computed p-value is lower than the significance level = 0.05, one should reject the null hypothesis H0 and accept the alternate. In other words it can be conclude that there are significant relationships between studied variables (*Table 6*).

The PCA analysis was performed with the use of Pearson correlation coefficient, and the results are presented in *Figure 2*. The interpretation of the correlation circle was carried out using the following criteria, when two variables are far from the centre.

Table 4. *Different Phosphorus variables at various pulse events and spatial levels.*

Variables	Site 1			Site 2			Site 3			Site 4			Site 5		
	Pulse Event	Inter-pulse Event	Non-pulse Event	Pulse Event	Inter-pulse Event	Non-pulse Event	Pulse Event	Inter-pulse Event	Non-pulse Event	Pulse Event	Inter-pulse Event	Non-pulse Event	Pulse Event	Inter-pulse Event	Non-pulse Event
PAE	51.28	39.16	109	26.75	50.32	117.22	68.97	17.132	51.65	37.4	23.92	279.6	37.5	29.993	136.82
PUTIL	7.406	12.01	10.01	10.68	9.272	4.8793	3.324	18.946	16.603	2.23	34.7	11.88	2.15	17.951	17.163
PUE	228.2	157.2	491.9	598.9	294	649.1	288.3	616.6	872.9	332.6	893.2	709.2	304.95	864.65	816.7
QUTIL	6.74	12.65	17.8	14.2	16.26	10.823	5.851	22.165	53.646	2.09	61	25.32	2.44	28.903	31.047
PHI	1.099	0.95	0.57	0.752	0.57	0.4508	0.568	0.8548	0.3095	1.07	0.569	0.469	0.88	0.6211	0.5528

Table 5.*Analysis of Variance of Phosphorus Variables*

Variables	Sites			Events			Sites x Events		
	Sum of Square	Mean Square	Computed F Value	Sum of Square	Mean Square	Computed F Value	Sum of Square	Mean Square	Computed F Value
PHI	21998.7	5499.69	8854.84**	102159.8	51079.9	189513.9**	69032.96	8629.11	26455.74**
PUTIL	309.99	77.49	569.38**	1349.19	674.59	1183.59**	1309.43	163.67	2700**
PUE	815488	203861	81544**	969761	48488.5	19395**	10193	12741	33978.6**
QUTIL	221.50	552.87	3924.74**	4618.86	2309.43	7166.2**	6003.3	750.42	4813.99**
PHI	0.702	0.1754	9.3**	1.44	0.72	43.89**	0.7235	0.009	5.15^{NS}

Table 6. *Bartlett's sphericity and Kaiser-Meyer-Olkin sampling adequacy tests*

Chi-square (Critical value)	202.51
DF	171
p-value	0.09
Alpha	0.05
KMO	0.597

If they are close to each other, they are significantly positively correlated (r close to 1); if they are orthogonal, they are not correlated (r close to 0); if they are on opposite sides of the centre, then they are significantly negatively correlated (r close to 2). Squared cosines were used to link the variable with the corresponding axis; the greater the squared cosine, the greater the link with the corresponding axis. PCA were considered (*Table 7* and *Fig. 2*) useful if their cumulative percentage of variance approached 80% (Wei-Giang *et al.,* 2008).

In the present investigation cumulative percentage indicates that the first four axes together accounted for 80.98% variability in the data set (*Table 7*) with their individual contribution being 49.82%, 15.53%, 8.94%, and 6.68%, respectively. From present study correlation circle (*Fig. 2*) as well as *Table 8*, revealed that PUTIL related with

PUE (r = 0.69**) and with QUTIL (r = 0.88**). Similarly PUE related with QUTIL (r = 0.80**) and with PHI (-0.65**).

Table 7. *Eigen value analysis and other attributes obtained from Principal Component Analysis*

	F1	F2	F3	F4
Eigenvalue	9.467	2.951	1.699	1.271
Variability (%)	49.828	15.530	8.943	6.687
Cumulative %	49.828	65.358	74.301	80.988

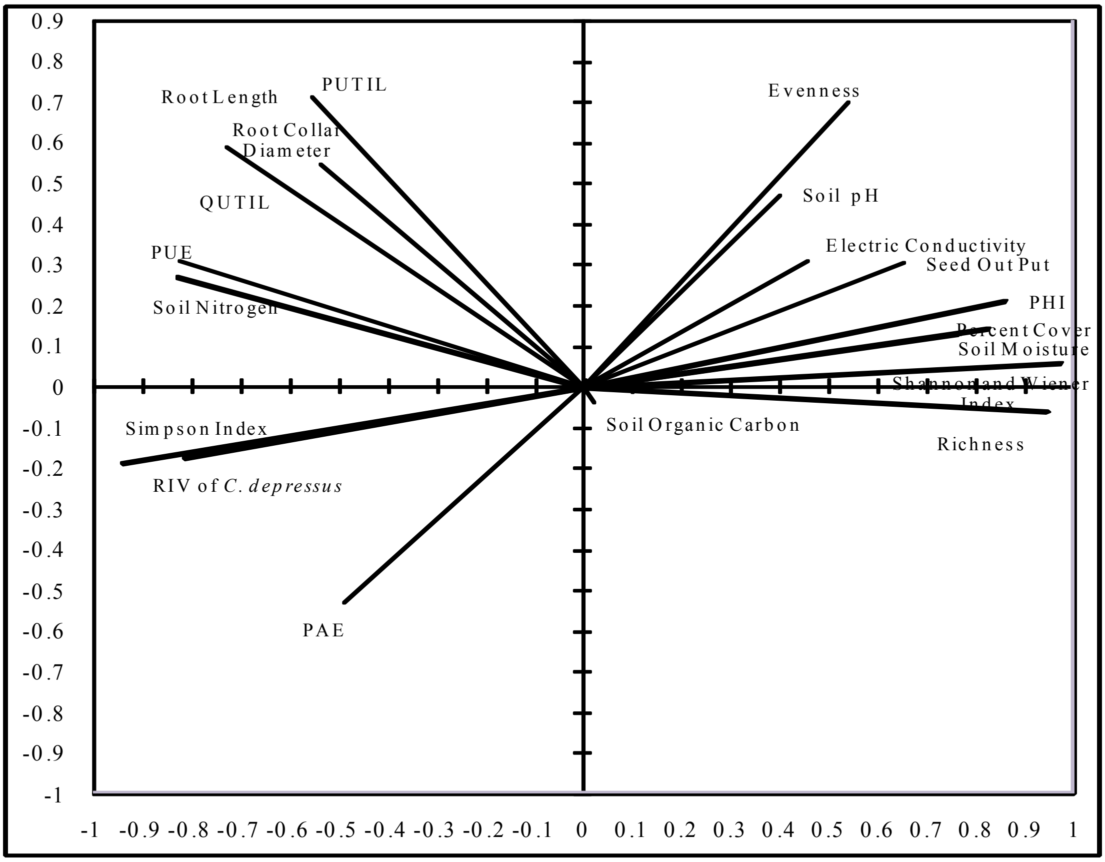


Figure 2. *Bi-Plot Analysis of Principal Component Analysis*

QUTIL was also negatively related with PHI (r = -0.58**). These significant correlations were observed at 0.01% (**) and 0.05% (*) levels. Correlation was not found between PAE and PUTIL, indicating that these variables are independent. Correlation between nitrogen acquisition and nitrogen internal utilization efficiency has also been not found in wheat, triticale and maize (Anderson, 1985 and Parentoni & Junior-Souza, 2008).

Table8. *Correlation Matrix Emerged from Principal Component Analysis*

	Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	PAE																		
2	PUTIL	-0.122																	
3	PUE	0.212	**0.696**																
4	QUTIL	0.019	**0.889**	**0.805**															
5	PHI	-0.442	-0.311	**-0.656**	**-0.589**														
6	Percent Cover	-0.404	-0.418	**-0.677**	**-0.532**	**0.787**													
7	Seed out put	**-0.643**	-0.032	-0.283	-0.213	**0.566**	0.497												
8	Root length	0.098	**0.517**	0.419	**0.569**	-0.273	-0.229	-0.428											
9	Root Collar Diameter	0.101	**0.521**	0.421	**0.572**	-0.275	-0.234	-0.430	**1.000**										
10	RIV of *C. depressus*	**0.667**	0.271	0.515	0.434	**-0.667**	**-0.544**	**-0.739**	0.419	0.424									
11	Richness	-0.327	**-0.601**	**-0.767**	**-0.715**	**0.792**	**0.833**	**0.539**	-0.445	-0.449	**-0.771**								
12	Simpson Index	**0.539**	0.337	**0.703**	**0.565**	**-0.824**	**-0.827**	**-0.618**	0.418	0.421	**0.786**	**-0.895**							
13	Shannon and Wiener Index	-0.467	-0.462	**-0.775**	**-0.666**	**0.853**	**0.826**	**0.586**	-0.471	-0.474	**-0.801**	**0.951**	**-0.980**						
14	Evenness	**-0.529**	0.199	-0.253	0.013	**0.583**	**0.660**	0.500	0.085	0.085	-0.445	0.447	**-0.696**	**0.571**					
15	Electric conductivity	-0.431	-0.142	-0.243	-0.127	0.476	0.246	0.212	-0.181	-0.176	-0.430	0.363	-0.405	0.432	0.316				
16	Soil Organic Carbon	-0.065	-0.110	-0.104	-0.194	-0.070	-0.223	0.014	0.036	0.040	-0.034	-0.115	0.044	-0.090	0.091	-0.075			
17	Soil Moisture	-0.300	-0.389	-0.507	-0.450	**0.537**	**0.555**	**0.647**	-0.294	-0.294	**-0.723**	**0.806**	**-0.709**	**0.726**	0.481	0.264	0.220		
18	Soil Nitrogen	0.222	**0.674**	**0.996**	**0.786**	**-0.674**	**-0.672**	-0.294	0.408	0.408	0.506	**-0.765**	**0.706**	**-0.776**	-0.275	-0.288	-0.116	**-0.520**	
19	Soil pH	-0.171	0.047	-0.257	-0.068	**0.515**	0.288	0.223	0.068	0.077	-0.125	0.319	-0.406	0.388	**0.531**	**0.606**	0.172	0.436	-0.330

Values in bold are different from 0 with a significance level alpha=0.05

In the present study, the lack of correlation between P acquisition and P utilization efficiency indicates that the selection of one of these should not affect the other, which would facilitate simultaneous selection of these traits, in the set of environmental studies. The main selection criteria for P internal utilization efficiency should be toward reducing the seed P concentration (inverse of the quotient of utilization) and in this case a negative weight should be used in the selection of species for ecosystem stability. Since this species is largely consumed along-with seed for its aphrodisiac properties, hence reduction in seed P concentration would have a positive impact on nutrition. Since seed P is stored as the anti-nutritional factor phytate; and it would also reduce environmental pollution from higher P manures produced by large animal feeding lots. However the strategy of reducing seed P concentration should have a limit, since seed P is needed for its germination and initial establishment (Philip & Veneklaas, (2012).

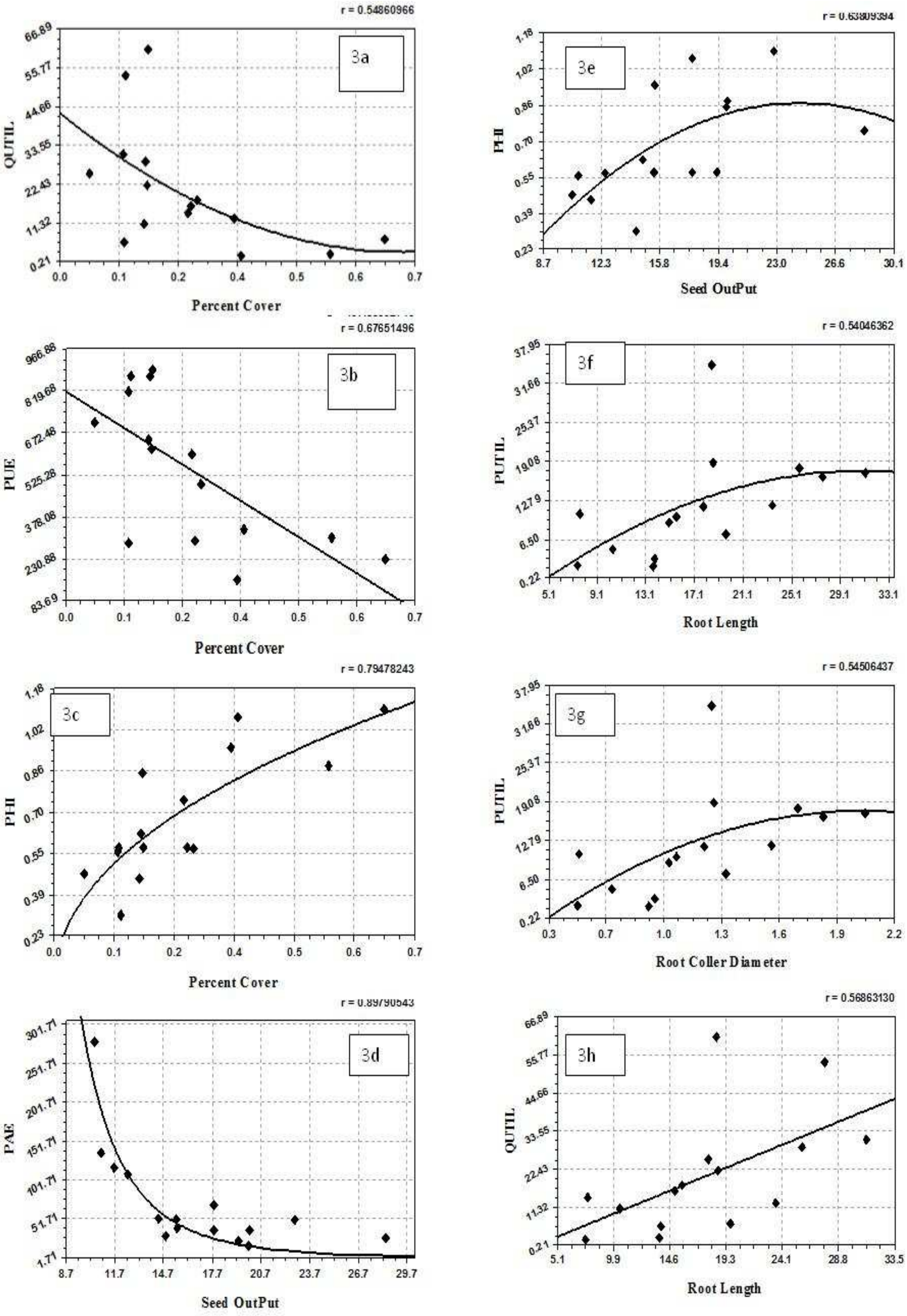
Path Analysis

P Variables and Plant Parameters

Polynomial negative relation was observed between percent cover and QUTIL (QUTIL = 43.05+-117.74 Percent Cover +86.38 Percent Cover$^{\wedge 2}$, R^2 = 0.548*, *Fig. 3a*). Percent cover also shows negative linear relation with PUE (PUE = 819.08+-1087.95Percent Cover, R^2 = 0.67**, *Fig.3b*). On the other hand, percent cover support Phosphorus Harvest Index in power fashion (PHI = 1.319Percent Cover ^0.448, R^2 = 0.79**, *Fig.3c*). Percent cover calculated through the two dimension of the plant that do not positively reflect in seed biomass, but this parameter certainly favour the P content in seed and this ultimately reflects with its initial establishment and subsequent growth (Nadeem *et al.,* 2011a).

Seed output depletes the PAE in power fashion (PAE = 2646787 Seed Out Put^-4.0, R^2 0.89**, *Fig.3d*). Seed output exhibits polynomial relation with PHI (PHI = -0.528+0.115 Seed Out Put+-0.002 Seed out Put$^{\wedge 2}$, R^2 0.63*, *Fig.3e*). This result revealed that the highest level of PHI was recorded at intermediated seed output level. Since PAE is the ration of P in plant to P in soil, and on the other hand this plant is under the category of R selection species (produce high number of seed) that ultimately pumps the more P in seed compare to other plant part. Thus this parameter negatively related with PAE but positive for PHI.

PUTIL exhibits positive polynomial relation with both root length (PUTIL = -7.007+1.586 Root Length +-0.0255 Root Length$^{\wedge 2}$, R^2 = 0.54*, *Fig. 3f*) and with root collar diameter (PUTIL = -7.725+24.328Root Collar Diameter+-5.956 Root Collar Diameter$^{\wedge 2}$, R^2 = 0.54*, *Fig. 3g*). Similarly QUTIL shows linear positive relation with root length (QUTIL = -4.424+o1.414 Root Length, R^2 = 0.56*, *Fig. 3h*) and with root collar diameter in polynomial positive fashion (QUTIL = -8.21+28.228 Root Collar Diameter+-2.729 Root Collar Diameter$^{\wedge 2}$, R^2 = 0.57*, *Fig. 3i*). PUTIL and QUTIL are the ratio of seed biomass to P in plant and seed biomass to P in seed, respectively, root morphological parameters like root length and root collar diameter supports seed biomass.



***Figures 3a to 3h**. Relationships between various P variables and plant parameters*

It is often observed that phosphate uptake by roots is regulated systemically by plant P status (White, 2012). It has been suggested that sucrose transported in the phloem from the shoot to the root acts as a systemic signal to regulate phosphate uptake by roots (Hammond & White, 2008).

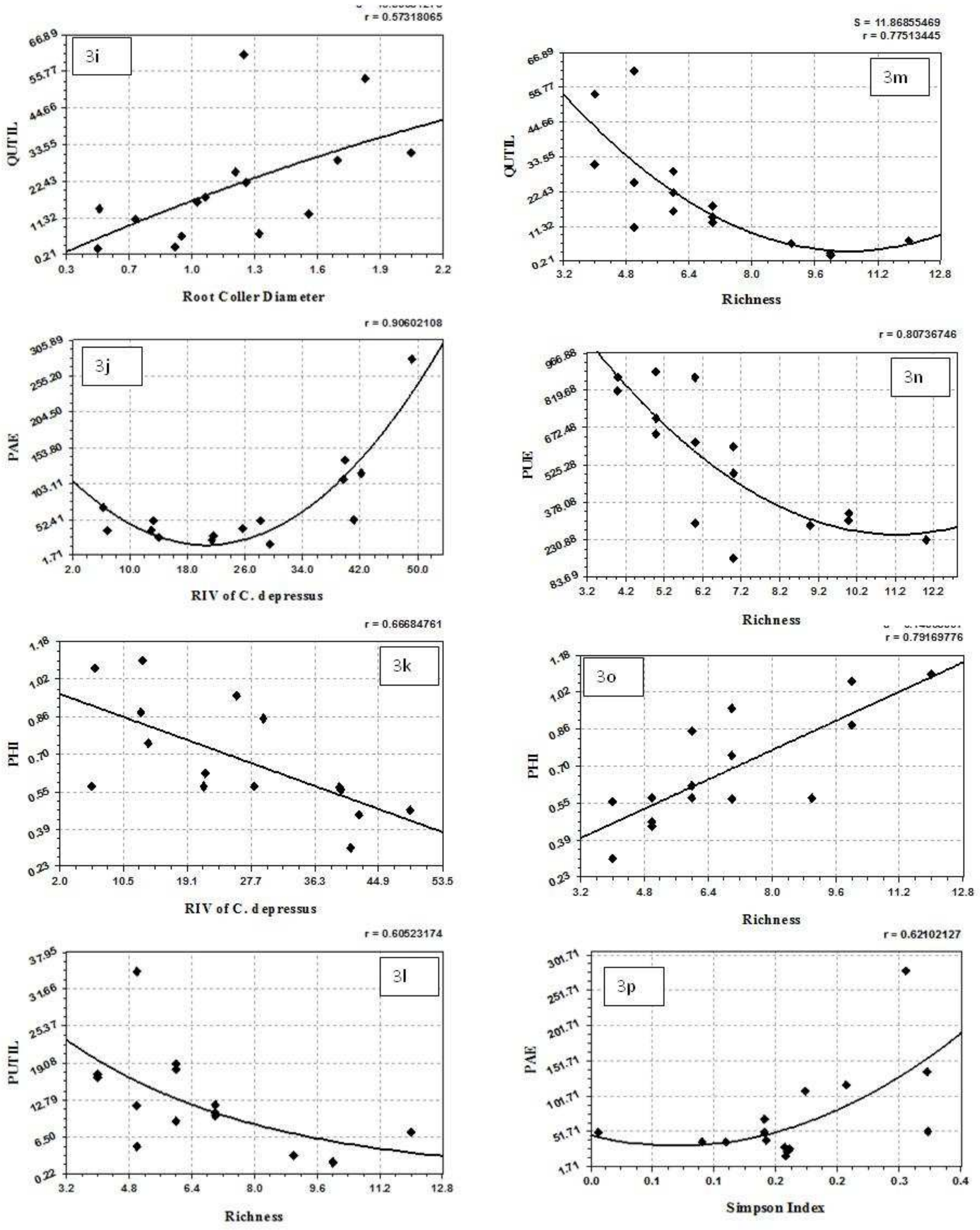
P Variables and Community Factors

Relative Importance Value (RIV) of *C. depressus* shows a positive polynomial relation with its phosphorus acquisition efficiency (PAE = 126.76+-10.749 RIV of *C. depressus*+0.262 RIV of *C. depressus*^^{2}, R^2 = 0.90**, *Fig.3j*) while on the other hand this ecological parameters held back the PHI of this plant in a linear fashion (PHI = 0.980+-0.011 RIV of *C. depressus*, R^2 = 0.66**, *Fig.3k*). Community richness inhibits PUTIL exponentially (PUTIL = 44.15e^ $^{(-0.203\ \text{Richness})}$, R^2 = 0.60*, *Fig.3 l*) and polynomial to QUTIL (QUTIL = 107.796+-19.977 Richness + 0.955 Richness^^{2}, R^2 = 0.77**, *Fig.3m*) and to PUE (PUE = 1752.03+-267.130+11.890Richnes^2, R^2 = 0.80**, *Fig.3n*). But it supports seed production (PHI) in linear fashion (PHI = 0.148+0.0782 Richness, R^2 = 0.79**, *Fig.3o*).

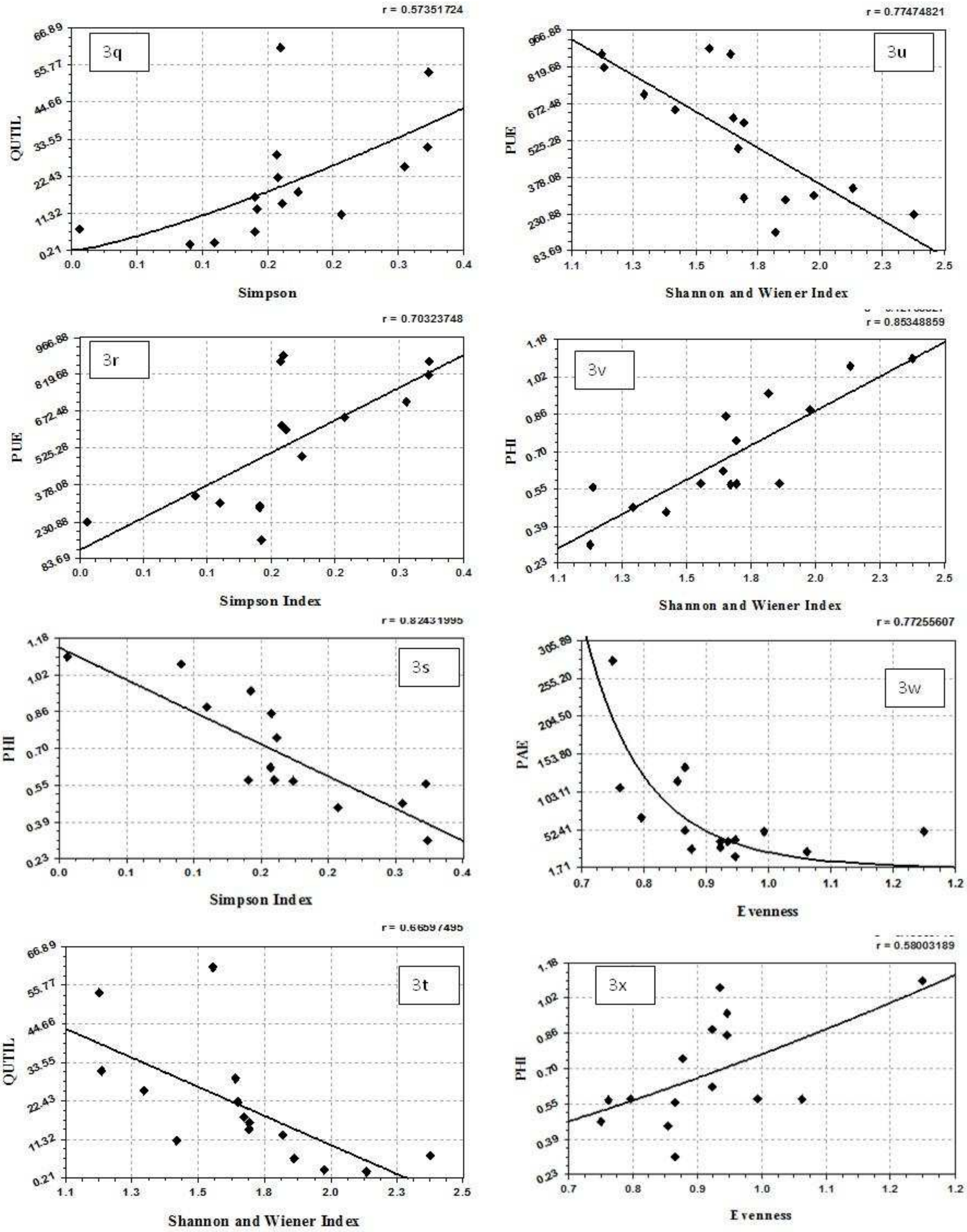
Different diversity indices gave the valuable information about community status. Simpson index measures the dominance of species and it ranges from 0 to 1. It measures the probability that two individuals selected at random from a sample will belong to the same species. Because Simpson index is actually an index of dominance, and then tend to be inversely to evenness and richness, but in some cases these two indices are independent from each other (Hill, 1973). Shannon-Weiner index (1949) is based on information theory.

The information content is a measure of the amount of uncertainty. It generally falls between 1.5 and 3.5, and rarely exceeds 4.5 (Margalef, 1972). Higher Shannon Weaver index values indicated that many species are represented by the same number and low value showed complete dominance of one species. In other words high value indicates high phytodiversity at particular stand. Evenness or equitability represents the distribution of individuals among the species. It is sometimes defined as the ratio of observed diversity to maximum diversity (Margalef, 1958). When all species are equally abundant, an evenness index would be at a maximum and decrease toward zero as the relative abundance of the species diverges away from evenness (Ludwig & Reynolds, 1999).

In the present investigation Simpson index supports PAE (PAE = 46.559+-351.419 Simpson Index+2074.70 Simpson Index^^{2}, R^2 = 0.62*, *Fig.3p*), QUTIL (QUTIL = 156.24 Simpson ^ 1.270, R^2 = 0.57*, *Fig.3q*), and PUE (PUE = 116.51+2154.85 Simpson Index, R^2 = 0.70**, *Fig.3r*) in polynomial, power and linear fashion, respectively. By contrast, this diversity parameters showed negative linear relation with PHI (PHI = 1.139+-2.300 Simpson Index, R^2 = 0.82**, *Fig.3s*). Shannon and Weiner Index (H') inhibits QUTIL (QUTIL = 80.97+-35.27 Shannon and Weiner Index, R^2 = 0.66**, *Fig.3t*) and PUE (PUE = 1585.09+-610.97 Shannon and Weiner Index, R^2 = 0.77**, *Fig.3u*) linearly but supports its PHI in a linear fashion (PHI = -0.361+0.613 Shannon and Weiner Index, R^2 = 0.85**, *Fig.3v*). Evenness shows two contrasting results with PAE and PHI of this species. It inhibits PAE (PAE = 20.100 Evenness^-8.802, R^2 = 0.77**, *Fig.3w*) but support PHI (PHI = 0.788Evenness^1.619, R^2 = 0.580*, *Fig.3x*), both in power fashion. Thus, from this study it can be concluded that at species level dominance (RIV of *C. depressus* and Simpson index) of this plant reflects its greater phosphorus acquisition efficiency, however, as the community diversity (richness, Shannon and Weiner index and evenness) increases the phosphorus internal utilization and phosphorus use efficiency inhibited.



***Figures 3i to 3p**. Relationships between various P variables and community parameters*



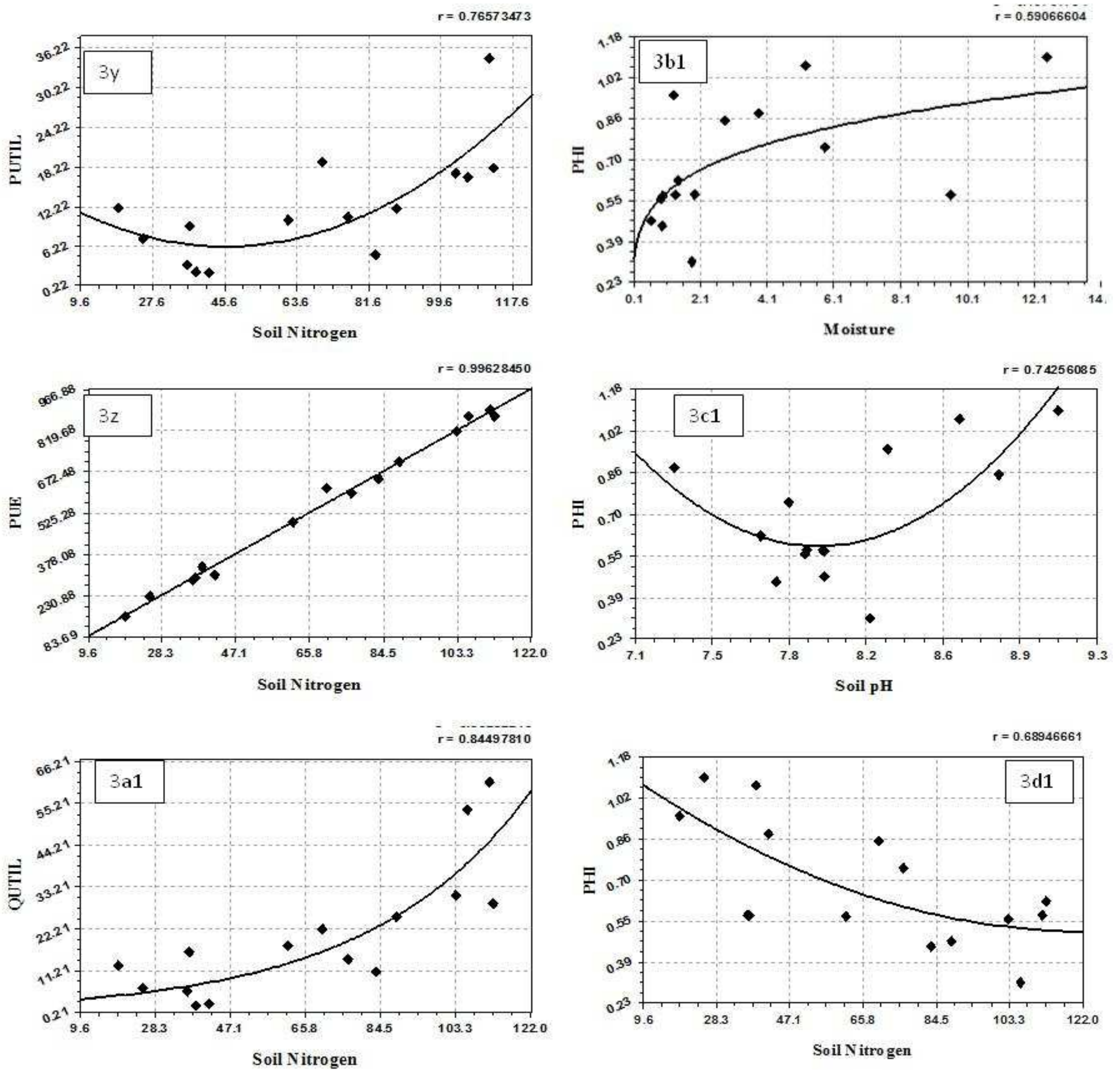
***Figures 3q to 3x**. Relationships between various P variables and community parameters*

Both P internal utilization and PUE of *C. depressus* are largely influenced by temporal factors (increase and decrease of community diversity during pulse and non-pulse events, respectively) and it also indicates the adjustments made by the species for their PUE and PUTIL according to their resources availability. Thus this species achieved its effective nutrient use through temporal partitioning, through which it full-fill its P requirements at low resource availability and that is ultimately reflected through its dominance (RIV) in community. Identification of such species are crucial for habitat stability, especially in rehabilitation programmes as supported by the findings of Aronson *et al.,* (1993) and Wortman *et al.,* (2012).

Regarding PHI, dominance of this species in community do not allow to allocate more P in seed, while as the community diversity increases plant switched on their P allocation in seed. This strategy could be the main approach to compete with their associates and this statement can be support by the findings of Nadeem *et al.,* (2011a, b) and White and Veneklaas, (2012) who reported that seed P is the only P source available to sustain the initial establishment and subsequent growth of seedling.

P Variables and Soil Factors

Soil nitrogen supports PUTIL (PUTIL = 14.341+-0.359 Soil Nitrogen+0.0039 Soil Nitrogen^^{2}, R^2 = 0.76**, *Fig.3y*), PUE (PUE = 13.05+7.844 Soil Nitrogen, R^2 = 0.996**, *Fig.3z*) and QUTIL (QUTIL = 2.897e^ (0.024 Soil Nitrogen), R^2 = 0.84**, *Fig.3a1*) in polynomial, linear and exponential fashion, respectively.



Figures 3y to 3d1. *Relationships between various P variables and soil parameters*

On the other hand both soil moisture and soil pH support PHI in power (PHI = 0.5729 Moisture^0.2062, R^2 = 0.59*, *Fig.3b1*) and polynomial (PHI = 32.656+-8.036 Soil pH+0.503 Soil pH$^{\wedge 2}$, R^2 = 0.74**, *Fig.3c1*) fashion, respectively. However, soil nitrogen inhibits PHI in polynomial pathway (PHI = 1.169+-0.0108+Soil Nitrogen+4.440 Soil Nitrogen$^{\wedge 2}$, R^2 = 0.68**, *Fig.3d1*).

Soil moisture and soil pH were recorded higher during the pulse event and that also related with bio-availability with soil P. During the high resource availability this species invests more P in seed compared to other plant modules. These results are in close agreement with those reported by George *et al.,* (2005). Due to increment in of plant growth, the P requirement is expected to increase. It may be a reason to get more PUTIL and PUE to meet the plant requirement when N availability and uptake enhances. Sanginga *et al.,* (2000) and Gao (2009) also reported higher relation of PUTIL and PUE with soil N in two different lines of cowpea.

Conclusion

Nutrient use efficiency at ecosystem level revealed that NUE increases with richness of species or life forms in a community. However this relation at species level has not been worked out, particularly in arid areas where resources are released in pulses. Present study shows that at species level dominance of *C. depressus* reflects with its higher P acquisition efficiency; however as the community diversity increases the P internal utilization and PUE are inhibited. Conclusively, dominance of this species inhibits the allocation of P in seed, in contrast as the community diversity increases this species switched on their P allocation in seed. This mechanism could be associated with its initial establishment and subsequent growth.

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THE RESPONSE OF AQUATIC PLANTS TO CATCHMENT LAND USE FOR DIFFERENT TYPES OF LOWLAND RIVERS

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Abstract . The article deals with the problem of ecological quality of rivers in relation to catchment-scale threats. We aimed to detect the reaction of the river plants to the land use in a catchment in different spatial scales. The response of different ecological groups was considered as well as specific reactions of individual species. The study was based on surveys of 116 river sites in Poland representing three major European lowland river types: small sandy siliceous, stony siliceous and large siliceous. Field surveys were undertaken according to the standard monitoring approach including assessment of macrophyte abundance within 100 m river stretch. Land use was estimated for each survey site in five different spatial scales. The results showed that macrophytes strongly react to land use. The observations revealed that the width of a river corridor that is most strongly connected to macrophytes composition is related to an analysed river type. In small sandy and large siliceous this was a 500 m and 1000 m corridor, in stony – a 100 m and 500 m corridor. Several river type-specific reactions of macrophyte functional groups' development, as the reaction to catchment deterioration, were also revealed. Moreover, a relation between development of individual species and land use was found based on canonical correspondence analysis.
Keywords: *macrophytes, monitoring, Water Framework Directive, ecology, aquatic plants ecology, river types, River Habitat Survey*

Introduction

The development of macrophytes is highly influenced by abiotic and biotic parameters. Among different parameters which influence growth of plants in a channel, the most important ones are biogenic concentration (Westlake 1975, Robach et al. 1996, Szoszkiewicz et al. 2006a), flow (Westlake 1975, Dawson 1988), hydrological regime (Westlake 1975, Haslam 1987), alkalinity and hardness (Tremp & Kohler 1995), shading (Westlake 1975, Dawson & Kern-Hansen 1979, Remy 1993) and hydromorphological modifications (O'Hare et al. 2006, Szoszkiewicz et al. 2006, Careya et al. 2011). All these parameters are strongly related to catchment land use structure (Hynes 1975, Worral & Burt 1999, Wilcock et al. 2004).

Understanding the relationships between catchment and the ecological status of rivers is very important for managing river quality and undertaking efficient protective actions (Allan & Flecker 1993, Bastian & Bernhardt 1993, Johnson & Gage 1997 Benini et al. 2010). Analyses of the relations between land use and ecological status can be efficiently supported by GIS systems such as CORINE Land Cover 2000 (European Environmental Agency 2000) which provide an opportunity to conduct extensive analyses of the land use structure. Based on such tools, it is possible to conduct spatial analyses and to find interactions between different environmental factors and biological elements (Richards & Host 1994, Xiang 1995, Allan & Johnson 1997, Johnson & Gage 1997, Cao et al. 1999). An extensive analysis of the relations between catchment land use and ecological status of rivers was conducted by Streyer (2003). His results showed a strong relation between these two factors.

Rivers are strongly influenced by their surroundings, perhaps most strongly by conditions at the land-water interface (Naiman & Decamps 1990) but also by the entire catchment (Naiman et al. 1995). As Hynes (1975) so effectively argued in an early synthesis of landscape-stream interactions, “In every respect, the valley rules the stream.” Increasingly today, we are experiencing the negative consequences of this relationship, as human degradation of landscapes is reflected in the deterioration of streams conditions (Naiman et al. 1995).

This article deals with the problem of ecological quality of rivers in relation to catchment-scale threats. On the basis of the macrophyte survey and GIS databases, we aimed to detect the reaction of river plants to the land use in a catchment in different spatial scales. The response of different ecological groups was considered as well as specific reactions of individual species.

The aim of the current water protection policy in the European Union is to reach a good ecological status in every catchment. To achieve this ambitious and difficult task, several problems associated with spatially related issues must be solved, first of which is the reduction of diffuse pollution (Karr & Schlosser 1987). Effective management of the entire catchments must be undertaken as well (Osborne & Wiley 1988, Delong & Brusven 1991, Johnson et al. 1997).

Material and methods

Site selection and river typology

The presented macrophyte records have been collected as a result of a 4-year survey campaign (2005-2008). Each survey was supplemented by hydromorphological, hydrochemical and land use data. It resulted in gathering a homogeneous database consisting of over 600 sites representing all river types observed in Poland. For the purpose of this paper, 116 survey sites were chosen (Table 1), representing a wide range of geographical and environmental gradients (Fig. 1). The chosen survey sites represented only three river types: small sandy, stony and large siliceous ones. They were also diverse in the terms of land use structure of the catchment.

All of the analysed rivers are of lowland type (location of the survey site is lower than 200 m above sea level). Surveyed sites represent small (catchment area 10-100 km^2) and large rivers (catchment area larger than 1000 km^2). The geology of all analysed rivers was siliceous. The substrate of the channel bed was divided into two types: sandy and stony. The number of river sites in each type is presented in Table 1.

Field surveys

Macrophyte surveys have been undertaken according to the Polish monitoring macrophyte method (Szoszkiewicz et al. 2010). The survey is conducted along a 100 m river stretch. All aquatic species are recorded according to a 9-point scale.

Hydromorphological assessment of every analysed river site was conducted using the British River Habitat Survey (RHS) method (Environment Agency 2003). The RHS records contain land use information related to the land use structure in a 50 m distance from a river bank and along a 500 m river stretch. The data about the land use structure along RHS survey site was included in further analyses.

Table 1. *Number of survey sites in analysed macrophyte types.*

Macrophyte type	Number of sites	Catchment size	Channel substrate
Small sandy rivers	49	10-100 km^2	Sand, silt
Stony rivers	41	10-100 km^2	Gravel, pebble
Large siliceous rivers	26	>1000 km^2	Sand, silt, gravel, pebble

Figure 1. *Location of survey sites*

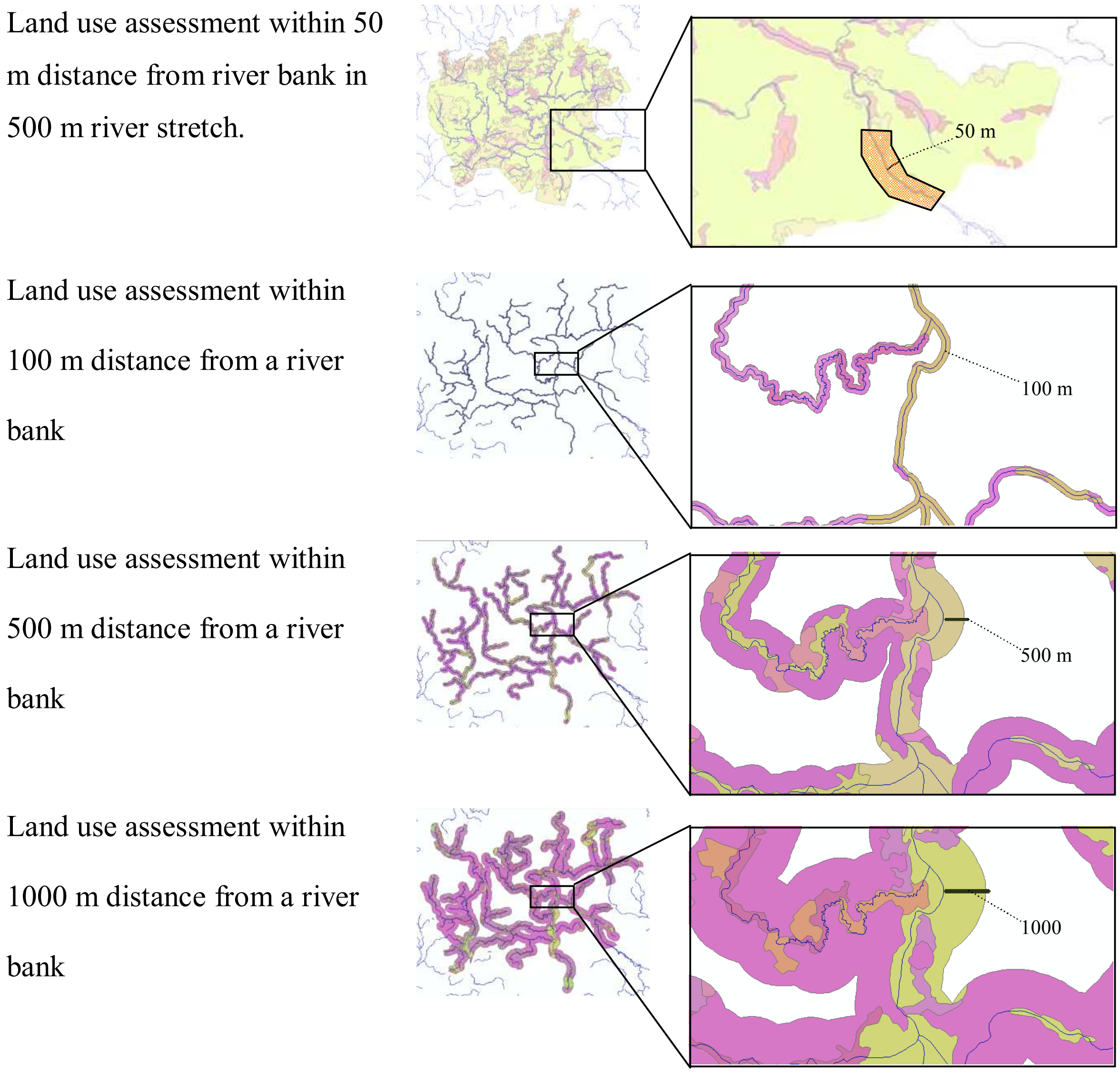
Land use assessment along river corridors

Catchment land use assessment was conducted using a digital hydrographical map of Poland (MPHP 2005), which included the layer of abiotic typology of rivers. Also the Polish database of land use, CORINE Land Cover, was used. It includes grids of minimal size of 25 ha. For the purpose of the analyses, land use types were merged into four main categories: built-up areas (which included housing, industry, railroads, roads, etc.), agricultural land (tilled lands, grasslands, pastures, etc.), forest and other semi-natural area, and the last category – surface waters and wetlands. The analyses were conducted using Arc Gis software ver. 9.2.

The applied analyses covered the land use structure in different spatial scales. They were conducted across the whole distance of the river (from the source to the survey site), considering four different distances from the river bank: 100 m, 500 m, 1000 m and in the whole catchment. These data were supplemented by information on the structure of land use along the river on a distance of 500 m and a width of 100m (50m to the left and right side of the river) collected in accordance with the methodology of River Habitat Survey (*Fig. 2*).

Analyses

To assess the strength of relations between macrophytes and different spatial scales of land use, the Monte-Carlo multivariate test on the species assemblage (full model, 500 permutations) was conducted. It was applied with the use of CANOCO software. The scale which was statistically most important for macrophyte development was utilized in further analysis.



Land use assessment of the whole catchment from the sources to the survey site.

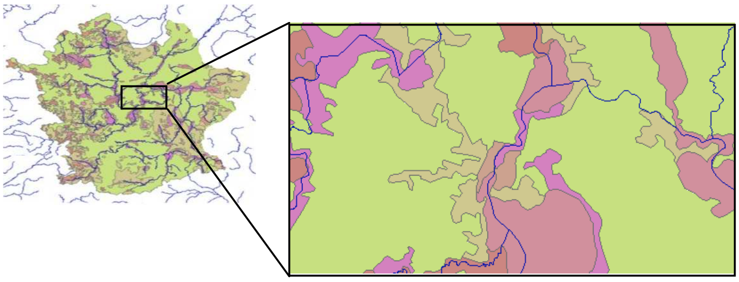


***Figure 2**. Land use assessment within 100 m, 500 m and 1000 m distance from the river for the whole catchment from the source to the survey site.*

Functional groups of macrophytes were estimated in the gradient of agricultural land percentage. The purpose was to reveal the relation between the land use and the development of river macrophyte differences. Analyses considered six functional groups of macrophytes: filamentous algae, bryophytes, submerged, floating-leafed (rooted), emergent, and terrestrial and ecotonal weeds. Moreover, the relations between the land use and the macrophyte species were considered utilising CCA analysis. CANOCO software and STATISTICA ver. 8 were used for statistical analyses.

Results

Macrophyte reaction against different spatial scales of land use analysis

The undertaken Monte-Carlo permutation test revealed that the development of aquatic plants depends on the catchment land use (Table 2). The analysis based on the comparison of five distinct parameters (catchment area, percentage share of: (1) agricultural lands, (2) forest, (3) surface water and (4) built-up areas) with the data on percentage share of macrophytes observed in the surveyed river stretches. The results included such parameters as explained variance, statistical significance and F-statistics value. The highest values of explained variance were observed in case of stony rivers (explained cumulative variance = 1,16 for 100 m river corridor). In other analysed river types the value of explained cumulative variance was also relatively high. The highest values were observed in 500 m and 1000 m river corridors. In small sandy rivers and large siliceous ones a little bit higher values of explained variance are observed in case of 500 m (0,75 and 0,81 respectively) and 1000 m (0,76 and 0,8 respectively). In stony rivers a little bit more important appears to be 100 m corridor (explained cumulative variance = 1,16).

In the majority of the analysed cases, the land use types which explain the highest share of variance, are agricultural lands and forests. The difference is observed in case of surface waters and built-up areas. The former appears to be more important in case of small sandy and large siliceous rivers. The latter is more important in case of stony rivers. The catchment area was statistically not important as a parameter to explain macrophytes composition in almost all analysed cases.

The analyses conducted for all rivers without dividing them into separate types revealed complete lack of any statistical significance. Based on the results of Monte Carlo test, further Canonical Correspondence Analyses (CCA) were performed taking into account the comparison of 500 m river corridor land use with macrophytes structure. The 500 m corridor was chosen due to the fact that this category appeares to be statistically most important in all analysed river types.

***Table 2**. Monte-Carlo permutation test (full model) of macrophyte reaction to different spatial scales of land use structure.*

Spatial category	Land use category	Smal sandy rivers					Stony rivers					Large siliceous rivers				
		p value	Stat. signif.	F-stat.	Var. expl.	Cumulat. Var. Expl.	p value	Stat. signif.	F-stat.	Var. expl.	Cumulat. Var. Expl.	p value	Stat. signif.	F-stat.	Var. expl.	Cumulat. Var. Expl.
50 m (RHS survey site)	Heavily modified	0,02	*	1,59	0,15	0,54	0,35	-	1,06	0,16	0,65	0,92	-	0,64	0,09	0,48
	Agricultural land	0,052	-	1,37	0,13		0,318	-	1,06	0,16		0,84	-	0,7	0,1	
	Forests	0,32	-	1,38	0,13		0,164	-	1,17	0,17		0,66	-	0,86	0,12	
	Surface waters	0,114	-	1,32	0,13		0,386	-	1,09	0,16		0,13	-	1,32	0,17	
	Catchment area	-	-	-	-		-	-	-	-		-	-	-	-	
100 m	Heavily modified	0,09	-	1,53	0,15	0,7	0,008	**	1,49	0,22	1,16	-	-	-	-	0,72
	Agricultural land	0,042	*	1,32	0,13		0,002	***	1,9	0,28		0,54	-	1,54	0,2	
	Forests	0,002	***	1,72	0,16		0,002	***	1,77	0,26		0,38	-	1,07	0,14	
	Surface waters	0,036	*	1,43	0,14		0,114		1,27	0,19		0,008	**	1,87	0,24	
	Catchment area	0,154	-	1,22	0,12		0,032	*	1,4	0,21		0,39	-	1,01	0,14	
500 m	Heavily modified	0,078	-	1,63	0,16	0,75	0,044	*	1,42	0,21	1,09	-	-	-	-	0,81
	Agricultural land	0,012	*	1,44	0,14		0,002	***	1,85	0,25		0,014	*	1,73	0,22	
	Forests	0,002	***	1,88	0,18		0,002	***	1,74	0,27		0,002	***	1,76	0,23	
	Surface waters	0,034	*	1,56	0,15		0,276	-	1,15	0,17		0,022	*	1,7	0,22	
	Catchment area	0,152	-	1,22	0,12		0,064	-	1,31	0,19		0,4	-	1,7	0,14	
1000 m	Heavily modified	0,054	-	1,69	0,16	0,76	0,022	*	1,51	0,22	1,1	-	-	-	-	0,8
	Agricultural land	0,006	**	1,59	0,15		0,002	***	1,91	0,28		0,004	***	1,85	0,23	
	Forests	0,002	***	1,9	0,18		0,002	***	1,73	0,25		0,01	**	1,78	0,23	
	Surface waters	0,02	*	1,61	0,15		0,364		1,1	0,16		0,05	*	1,5	0,2	
	Catchment area	0,136	-	1,26	0,12		0,078		1,3	0,19		0,42	-	1	0,14	
Whole catchment	Heavily modified	0,756	-	0,77	0,08	0,58	0,304		1,2	0,18	0,84	0,78	-	0,72	0,1	0,71
	Agricultural land	0,034	*	1,44	0,14		0,15		1,17	0,17		0,1	-	1,34	0,18	
	Forests	0,022	*	1,44	0,14		0,1		1,23	0,18		0,12	-	1,31	0,17	
	Surface waters	0,624	-	0,9	0,09		0,774		0,79	0,12		0,51	-	0,93	0,13	
	Catchment area	0,46	-	1,4	0,13		0,102		1,27	0,19		0,45	-	0,97	0,13	

Explanation: ** - p value < 0.005, * - p value 0.005-0.05.

Macrophyte development in small sandy rivers

Basing on the results of Monte Carlo test, three vectors of land use categories were chosen in Canonical Correspondence Analysis. These were: agricultural lands, forests and surface waters. These three land use categories presented the highest statistical significance measured by the variance explained (respectively: 0,13, 0,16 and 0,14). They also presented relatively high values of F-statistic (respectively: 1,32, 1,72 and 1,43).

The conducted Canonical Correspondence Analysis revealed a relationship between different macrophyte species and forms of land use of the catchment (Fig. 3). Sites located in catchments dominated by forests were overgrown by the following bryophytes: *Brachythecium rivulare*, *Pellia endiviifolia* and *Conocephalum conicum*. They were accompanied by several vascular plants: *Carex paniculata*, *Caltha palustris*, *Berula erecta*, *Lysimachia thyrsiflora, Lysimachia nummularia, Epilobium palustre and carex acutiformis*. The presence of agricultural lands was connected with the following species: *Potamogeton compressus, Callitriche sp., Rorippa amphibia, Agrostis stolonifera, Scutellaria galericulata and Ranunculus sceleratus*.

The third vector revealed another direction of variability related to the abundance of surface waters and wetlands in the catchments. The following macrophyte species indicated the strongest relationship with this gradient: *Potamogeton natans*, *Phragmites australis, Lysimachia vulgaris* and *Juncus articulatus*.

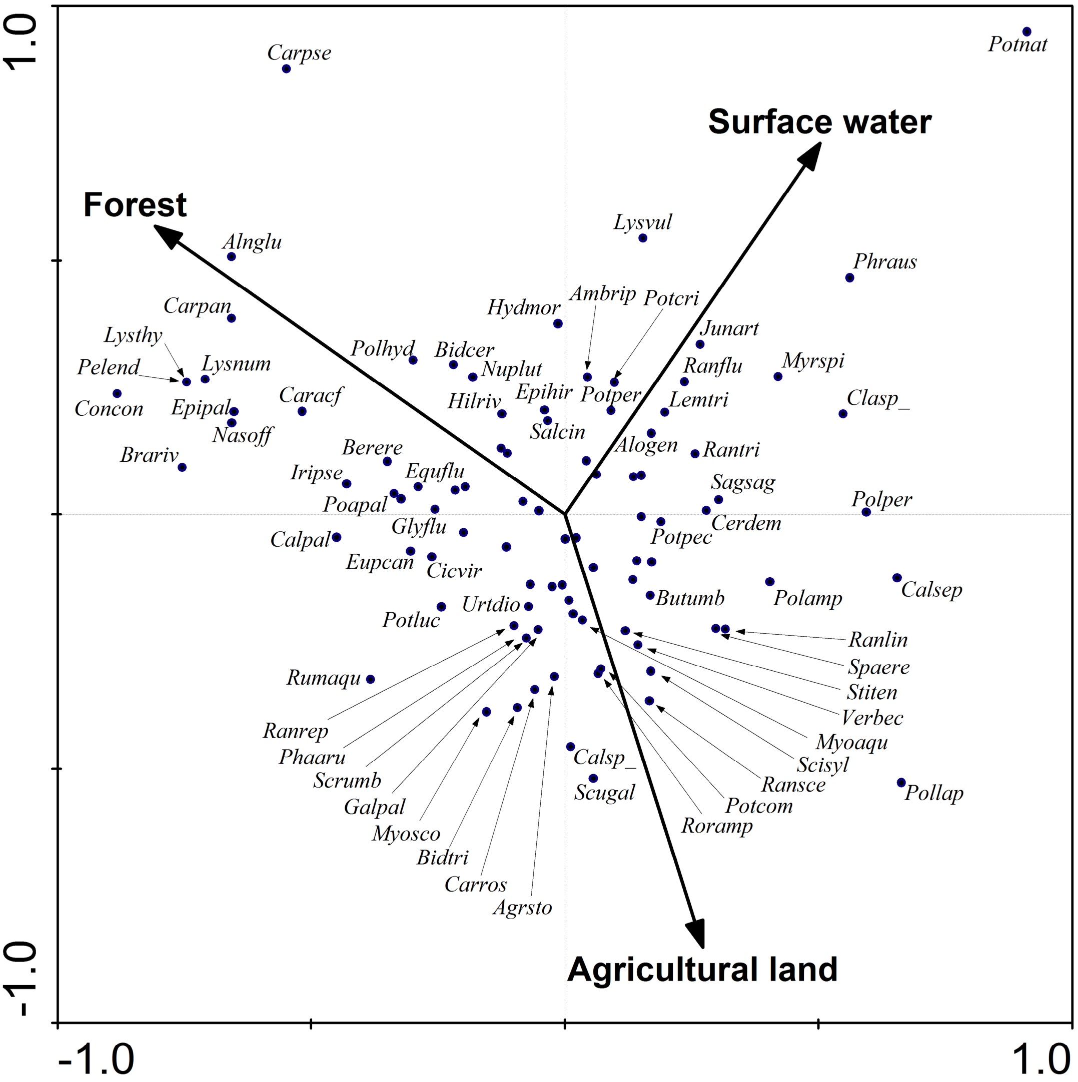


***Figure 3**. CCA ordination of macrophyte taxa and catchment land use types in small sandy rivers. Explanation: species names in Appendix 1; the diagram of canonical values for first and second axis. Implemented shortcuts of macrophyte names represent the first three letters of the Latin genus name and the second three are the letters of the Latin species name. Arrows represent analysed land use forms: surface waters and wetlands, forests and other semi-natural areas, built-up areas and agricultural lands.*

Differences of the proportion of functional growth forms of macrophytes were observed in the gradient of variously utilised land in the catchment in small sandy rivers (*Fig. 4*). Analyses were limited to the amount of agricultural land since the former results had detected the strongest importance of this type of land utilisation on macrophytes. A low percentage of agricultural land was reflected strongly by high abundance of mosses. On the other hand, a high percentage of agricultural area was strongly related to high abundance of free-floating plants. Increased area of agricultural land was reflected by a decreased proportion of emergent plants.

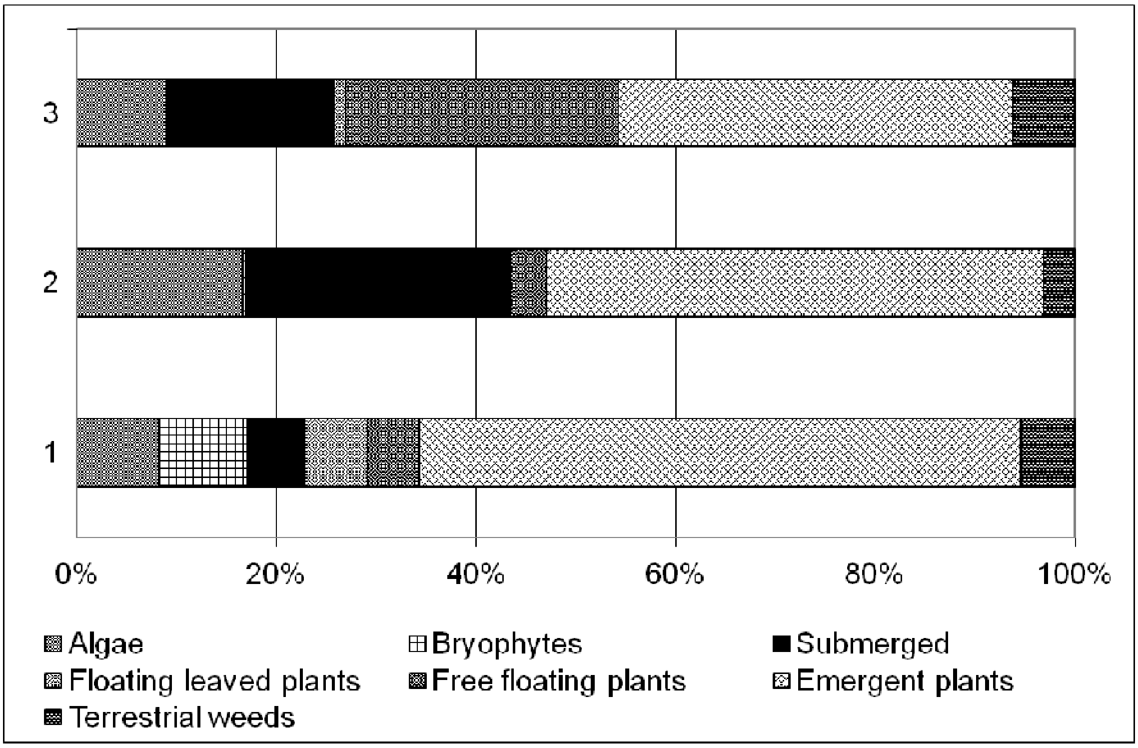


***Figure 4**. Proportions of functional growth forms of macrophytes developing in the gradient of agricultural land percentage in catchments of small sandy rivers. Explanation: percentage limits of agricultural land amount: 1: 0-33% (number of sites: 21), 2: 34-66% (number of sites: 38), 3: 67-100% (number of sites: 29).*

Macrophyte development in stony rivers

Basing on the results of Monte Carlo test, three vectors of land use categories were chosen in Canonical Correspondence Analysis. These were: agricultural lands, forests and built-up areas. These three land use categories presented the highest statistical significance measured by variance explained (respectively: 0,22, 0,28 and 0,26). They also presented relatively high values of F-statistic (respectively: 1,49, 1,9 and 1,77).

Significant correlations between various macrophyte species and the analysed forms of land use in stony rivers were observed (*Fig. 5*). Conducted canonical analysis revealed that the sites located in catchments characterized by a major proportion of forests were inhabited by some bryophyte species, such as *Brachythecium rivulare* and *Scapania sp.*. Such vascular plants as *Lysimachia nummularia*, *Caltha palustris, Solanum dulcamara Sagittaria sagitifolia* and *Potamogeton alpinus* were also observed.

Agricultural lands were mainly connected with the presence of such vascular plants as: *Lemna gibba, Rorippa amphibia, Bidens frondosa* and *Callitriche sp.* Build-up areas were connected with the presence of such plants as: *Cladophora sp., Bidens triparita, Polygonum hydropiper and Impatiens glandulifera* (*Fig. 5*).

The proportion of functional growth forms of macrophytes in the gradient of variously utilised land of the stony river catchment was different than in case of sandy rivers (*Fig. 6*). Bryophytes were generally much more abundant and most extensively developed when the percentage of agricultural land was medium (34-66%). The development of algae was relatively very intense except for rivers with limited agriculture in the catchment (lower than 33%). A low percentage of agricultural land was reflected in a large proportion of emergent plants. In catchments mostly covered by such category of land use, also mosses are disappearing.

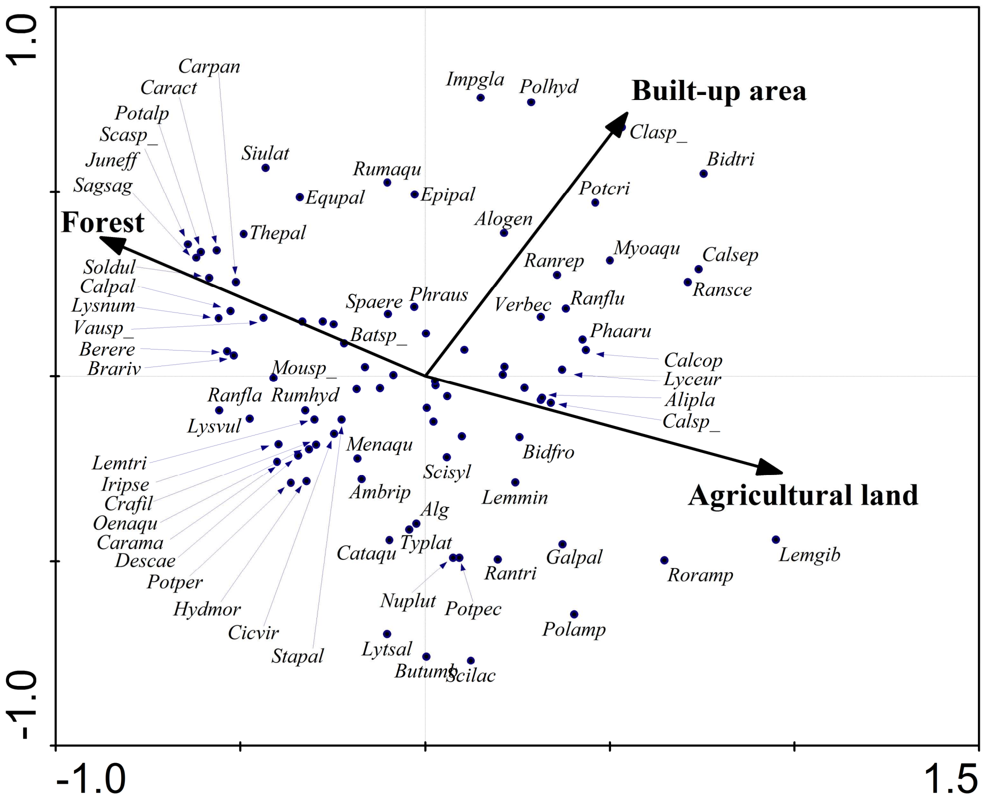


__Figure 5.__ CCA ordination of macrophyte taxa and catchment land use types in stony rivers. Explanation: species names in Appendix 1; arrows represent analysed land use forms: surface waters and wetlands, forests and other semi-natural areas, built-up areas and agricultural lands.

Macrophyte development in large siliceous rivers

Basing on the results of Monte Carlo test, three vectors of land use categories were chosen in Canonical Correspondence analysis. These were: agricultural lands, forests and surface waters. These three land use categories presented highest statistical significance measured by variance explained (respectively: 0,22, 0,23 and 0,22). They also presented relatively high values of F-statistic (respectively: 1,73, 1,76 and 1,7).

A significant percentage of agricultural land in catchments of large siliceous rivers was reflected by the presence of such vascular plants as *Sparganium emersum, Polygonum amphibium, Calystegia sepium, Sparganium erectum, Bidens frondosa* and *Sparganium erectum* (*Fig. 7*). The gradient of forests in a catchment was correlated with several other species: *Ranunculus trihophyllus, Stigeoclonium tenue, Nuphar lutea Epilobium hirsutum* and *Fontinalis antypyretica*. There are also some species which closely correspond with surface waters and wetlands in catchments. These are *Bidens cernua*, *Polygonum hydropiper*, *Phragmites australis* and *Lysimachia vulgaris.*

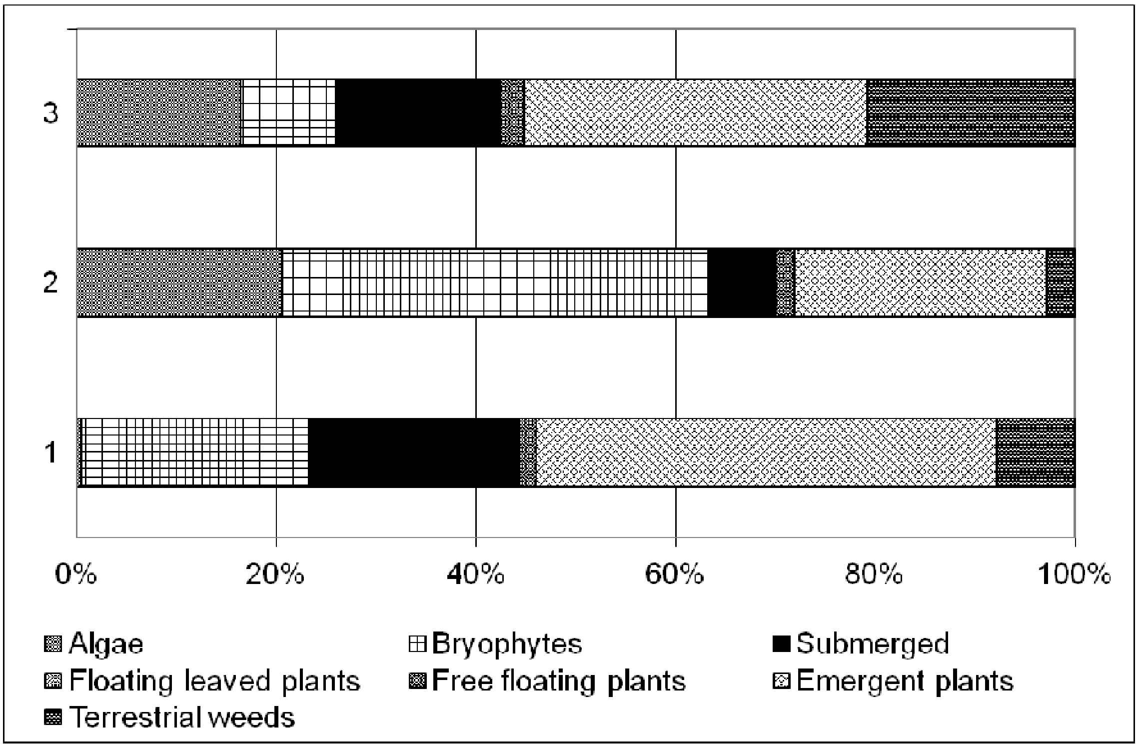


Figure 6. *Proportions of functional growth forms of macrophytes developing in the gradient of agricultural land percentage in catchments of stony rivers. Explanation: the following percentage limits of agricultural land amount were implemented (y axis): 1: 0-33% (number of sites: 21), 2: 34-66% (number of sites: 38), 3: 67-100% (number of sites: 29).*

Large siliceous rivers showed a unique pattern of the proportions of macrophte functional groups (*Fig. 8*). Firstly, the proportion of submerged vascular plants was much higher in comparison to other river types, when the catchment had a small to medium percentage of agricultural land (lower than 66%). The presence of mosses was very limited. Increasing area of agricultural land was reflected in increasing cover of plants with floating leaves (rooted and fee-floating).

Discussion

The presented results show the pattern of diverse development of aquatic plants under the variable use of catchment land. The principal pattern discovered was the change of vegetation across the gradient indicating the relation with the proportion of agricultural land and forests in catchments. A growing relative amount of farmlands causes bryophyte decline and increases abundance of filamentous algae and free floating species. Such a pattern indicates deterioration of ecological status, which was presented in the literature and explained as a result of replacement of forests by agricultural land in a catchment (Allan & Flecker 1993, Robach et al. 1996, Baattrup-Pedersen et al. 2006, Szoszkiewicz et al. 2006, Utz et al. 2009). Several long-term analyses that were conducted by Harding et al. (1998) found that land-cover patterns at the catchment scale from 1950, compared with land-cover patterns from 1970 and 1990, were the single strongest predictor of current biodiversity in North Carolina streams. According to existing scientific analyses, the degradation of catchment land use leads to lower biodiversity of freshwater ecosystems (Bastian & Bernhardt 1993, Harrison, 1987; Kornas, 1983; Kronert et al. 1999).

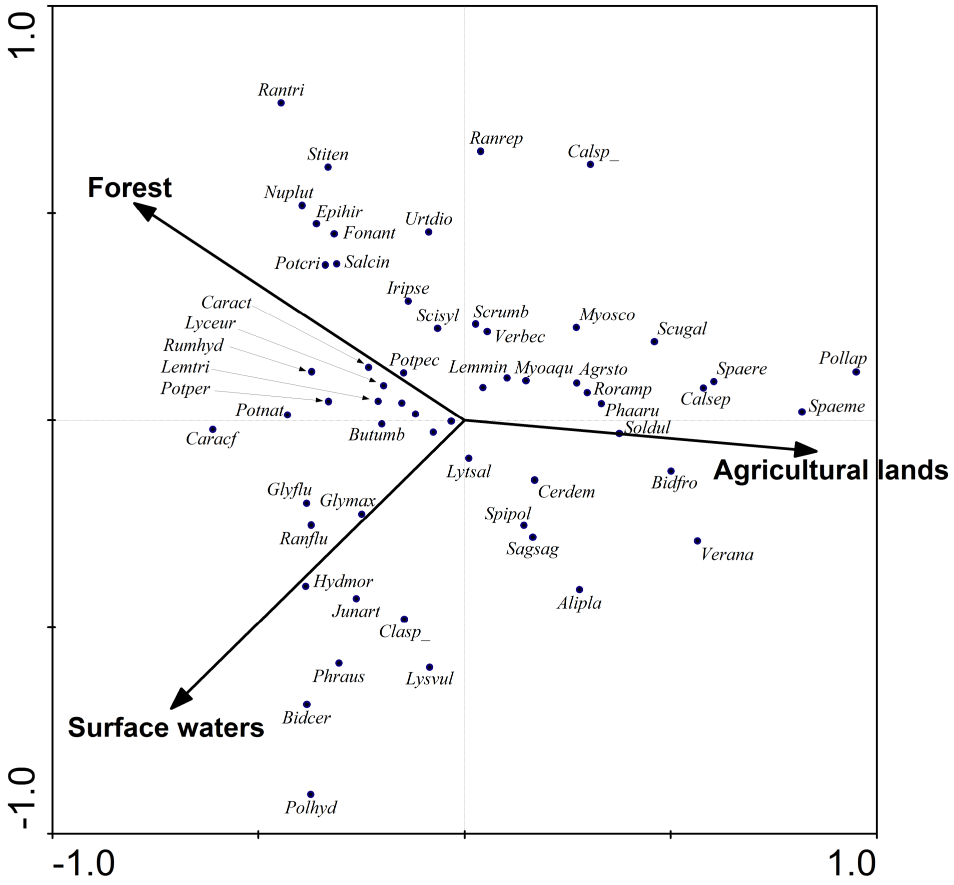


__Figure__ 7. CCA ordination of macrophyte taxa and catchment land use types in large siliceous rivers. Explanation: species names in Appendix 1; arrows represent analysed land use forms: surface waters and wetlands, forests and other semi-natural areas, built-up areas and agricultural lands.

The surveys which also included analyses of relations between land use and ecological status of rivers were conducted by Streyer et al. (2003). He was analysing land use impact in different corridors on ecological status of rivers. He took into consideration several factors, which define ecological status, although the investigation of relations between the land use and macrophytes was less detailed. He did not analyse macrophyte composition, specifically, he did not analyse individual species preferences in different land use types. Although, similarly to the results in this article, he found the positive relation between the presence of cultivated lands and plant species abundance. He also observed that various land use types have different significance in different spatial scales.

Many surveys have confirmed that catchment deforestation resulting in an increased proportion of agricultural lands is the main factor influencing the quality of running waters (Allan & Flecker 1993, Hering et al. 2006 Robach et al. 1996, Baattrup-Pedersen et al. 2006, Szoszkiewicz et al. 2006a). This process causes such effects as increased human population density in the river surroundings or increased erosion. Catchment modifications also often result in reinforcement or resection of the river channel (Baattrup-Pedersen & Riis 1999, Bunn et al. 1999, Sponseller et al. 2001, Szoszkiewicz et al. 2007). Also Smith et al. (1997) and Clark (1998) observed that catchment land use is an important factor influencing the ecological status of running waters independently of morphological modifications present in the river channel.

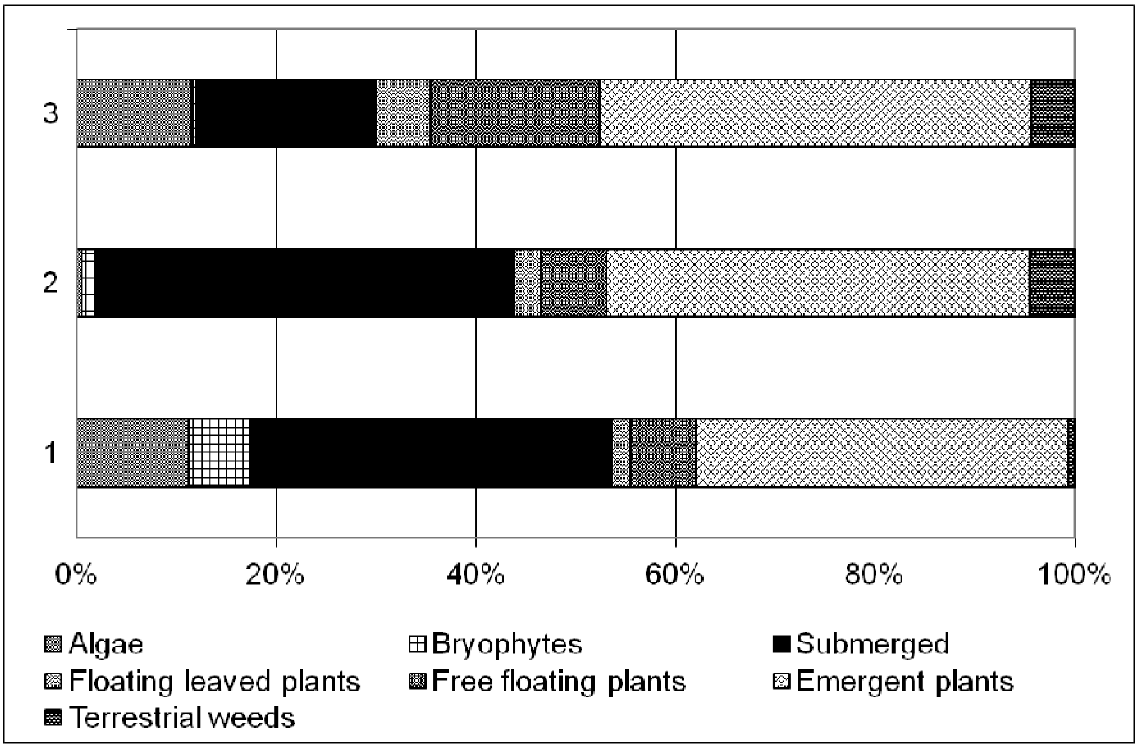


__Figure 8.__ Proportions of functional growth forms of macrophytes developing in the gradient of agricultural land percentage in catchments of large siliceous rivers. Explanation: the following percentage limits of agricultural land amount were implemented (y axis): 1: 0-33% (number of sites: 21), 2: 34-66% (number of sites: 38), 3: 67-100% (number of sites: 29).

Within the revealed relation between vegetation development and land use, bryophyte decline seems to be the most significant indicator of catchment deterioration. Mosses and liverworts are regarded as sensitive indicators of any human modifications in rivers, because they are overgrown by other macrophytes in nutrient-rich ecosystems and prefer natural substrates such as stones or wood. Such preferences of this group of macrophytes are used in many biological methods of ecological status assessment to indicate natural conditions (Dawson et al. 1999, Haury et al. 2006, Meilinger et al. 2005, Szoszkiewicz et al. 2006a).

Analyses have shown that the proportion of built-up areas is less important than the presence of forests or agricultural lands. This observation is in contrast to conclusions presented by several other authors. Osborne and Wiley (1988) and Silva and Wiliams (2001) indicate urban land use as the most important predictor of water quality variability.

Applied statistical tools revealed the importance of spatial scale of the catchment structure analysis. The lowest variance explained is observed for investigations conducted for the whole catchments and in the closest, local scale of 50 m corridors along 500 m long river stretch. The highest statistical significance is observed in case of 100 meters - 1000 meters from a river channel. Macrophytes in stony rivers are a little bit more related to closer surroundings (100 m – 500 m) and plants composition in small sandy and large siliceous rivers are more related to further land-use structure (500 m – 1000 m).

The analysis of the functional growth forms distribution along the gradient of percentage of agricultural land was supported by the results of CCA analysis. Conducted surveys revealed that the plants which most strongly indicate the presence of agricultural land and built-up areas in a catchment are algae (*Cladophora sp.*, *Spirogyra sp.*, *Oedogonium sp*, *Enteromorpha sp.*) and the following vascular species: *Potamogeton nodosus*, *Potamogeton lucens*, *Lemna gibba* and *Spirodela polyrhiza.* Most of these species are already used in biological monitoring as indicators of poor ecological quality (Haury et al. 2006, Holmes et al. 1999). Moreover, such typically ruderal species as *Agrostis stolonifera*, *Calystegia sepium*, *Epilobium hirsutum*, *Ranunculus sceleratus* and *Urtica dioica* are highly correlated with such areas. Although they are not included in any widely used European biological method due to their broad scale of tolerance to ecological conditions (Holmes et al. 1999, Haury et al. 2006, Szoszkiewicz et al. 2006a), they may provide some clues indicating that the surveyed site is somehow impacted by human activity.

The CCA analysis of species distribution along the analysed gradients of land use types suggests that these are bryophytes which are most connected to the presence of forests and other semi-natural areas. Regarding liverworts, the most characteristic for such conditions were *Chiloscyphus polyanthos*, *Marchantia polymorpha*, *Marsupella emarginata*, *Pellia epiphylla* and *Scapania sp.*; and among bryophytes they were *Brachythecium rivulare* and *Brachythecium plumosum*. Also several vascular plants can be found as good indicators of natural conditions: *Carex pseudocyperus*, *Carex paniculata*, *Glyceria plicata* and *Juncus bulbosus*. Based on the results of CCA analysis, several species typical for catchments rich in surface waters and wetlands can also be identified. Among them there are bryophyte species (*Drepanocladus aduncus*), *Chara* species and several vascular plants (*Carex rostrata*, *Carex vesicaria*, *Hottonia palustris*, *Potamogeton obtusifolius*, *Ranunculus lingua*). Most of these species were identified as indicators of high quality conditions (Holmes et al. 1999, Haury et al. 2006).

Catchments dominated by built-up areas or agricultural lands were often under degradation according to hydromorphological and biological parameters, which results in a higher proportion of river stretches completely exposed to the sun. This may result in higher growth of macrophytes (Abernethy et al. 1996, Vermaat & Debruyne 2003). Moreover, sites characterized by lack of forests in the catchment are often nutrient-rich due to nutrient inflow through surface and ground waters. This results in intensive growth of macrophytes, mainly free floating and submerged. Sites characterized by a highly modified catchment are often exposed to intensive erosion of unnaturally high banks (Hellsten & Riihimäki 1996).

The conducted surveys showed that different river types are characterised by unique reaction of macrophyte development to catchment deterioration. Large siliceous rivers, which were generally the richest in submerged plants, indicated decline of this group of macrophytes with a growing percentage of agricultural land. Small sandy rivers which were especially rich in emergent plants indicated decline of this group of macrophytes along with a growing percentage of agricultural land. Stony rivers located in a relatively natural catchments were rich in a variety of bryophytes. The result of the degradation of river catchments of this type was a significant increase in the share of common macrophytes.

Conclusions

1. The land use structure was a significant factor influencing ecological status of lowland rivers measured by a composition of macrophytes.
2. In all analysed river types statistically the most significant relation was the one between macrophytes and land use in 500 m corridors along the rivers. Macrophytes in stony rivers are also vulnerable to land use in 100 m corridors, whereas small sandy and large siliceous – to land use in 1000 m corridors.
3. Regardless of the river type, a significant area of agricultural land in the catchment resulted in a decline of bryophytes, whereas the proportion of filamentous algae and free floating species increased.
4. Several types of specific reactions of macrophyte development as a reaction to catchment deterioration were also revealed: a decline of submerged plants (large siliceous rivers); a decline of emergent plants (small sandy rivers); growing abundance and significant presence of terrestrial and ecotonal weeds (stony rivers).
5. The land use pattern influenced development of individual species. Agricultural land and built-up areas in a catchment stimulate development of some algae (*Cladophora sp.*, *Spirogyra sp.*) and several vascular plants (*Potamogeton lucens*, *Lemna gibba*, *Spirodela polyrhiza*). The dominance of forests and other semi-natural areas stimulates growth of liverworts (*Chiloscyphus polyanthos*, *Marchantia polymorpha*, *Marsupella emarginata*, *Pellia epiphylla* and *Scapania sp.*) and mosses (*Brachythecium rivulare*, *Brachythecium plumosum*) as well as several vascular plants (*Carex pseudocyperus*, *Carex paniculata*, *Glyceria plicata* and *Juncus bulbosus*).

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Appendices

Appendix 1*. List of identified macrophytes and their abbreviations*

Species name	Abbrev.	Species name	Abbrev.
Acorus calamus L.	Acocal	*Mentha aquatica* L.	Menaqu
Agrostis stolonifera L.	Agrsto	*Menyanthes trifoliata* L.	Mentri
Alisma plantago-aquatica L.	Alipla	*Microspora sp.*	Micsp_
Alopecurus geniculatus L.	Alogen	*Mnium hornum* Hedw.	Mnihor
Amblystegium riparium Hedw.	Ambrip	*Mnium undulatum*	Mniund
Batrachospermum sp. Roth	Batsp_	*Mougeotia sp.*	Mousp_
Berula erecta Huds.	Berere	*Myosotis scorpioides* L.	Myosco
Bidens cernua L.	Bidcer	*Myosotis sp.* L.	Myosp_
Bidens frondosa L.	Bidfro	*Myosoton aquaticum* Hedw.	Myoaqu
Bidens tripartita L.	Bidtri	*Myriophyllum spicatum* L.	Myrspi
Brachythecium plumosum Hedw.	Braplu	*Myriophyllum verticillatum* L.	Myrvet
Brachythecium rivulare Shimp.	Brariv	*Nasturtium officinale* W. T. Aiton	Nasoff
Brachythecium rutabalum Hedw.	Brarut	*Nuphar lutea* (L.) Sm.	Nuplut
Butomus umbellatus L.	Butumb	*Nymphaea alba* L.	Nymalb
Calla palustris L.	Calpas	*Oedogonium sp.*	Oedsp_
Callitriche cophocarpa Sendt.	Calcop	*Oenanthe aquatica* (L.) Poir.	Oenaqu
Callitriche palustris L.	Calpau	*Pellia endiviifolia* (Dicks.) Dumort.	Pelend
Callitriche sp. L.	Calsp_	*Petasites hybridus* L.	Pethyb
Caltha palustris L.	Calpal	*Peucedanum palustre* (L.) Moench	Peupal
Calystegia sepium L.	Calsep	*Phalaris arundinacea* L.	Phaaru

Cardamine amara L.	Carama	*Phragmites australis* (Cav.) Trin. ex Steud.	Phraus
Carex acuta L.	Caract	*Plantago major* L.	Plamaj
Carex acutiformis L.	Caracf	*Poa palustris* L.	Poapal
Carex elata All.	Carela	*Polygonum amphibium* L.	Polamp
Carex hirta L.	Carhir	*Polygonum hydropiper* L.	Polhyd
Carex paniculata L.	Carpan	*Polygonum lapathifolium* L.	Pollap
Carex pseudocyperus L.	Carpse	*Polygonum persicaria* L.	Polper
Carex riparia Curtis	Carrip	*Potamogeton alpinus* Balb.	Potalp
Carex rostrata Stokes	Carros	*Potamogeton berchtoldii* Fieber	Potber
Carex sp. L.	Caresp	*Potamogeton compressus* L.	Potcom
Carex vesicaria L.	Carves	*Potamogeton crispus* L.	Potcri
Catabrosa aquatica (L.) P.Beauv.	Cataqu	*Potamogeton lucens* L.	Potluc
Ceratophyllum demersum L.	Cerdem	*Potamogeton natans* L.	Potnat
Ceratophyllum submersum L.	Cersub	*Potamogeton nodosus* Poir.	Potnod
Chara sp. L.	Chasp_	*Potamogeton obtusifolius* Mert.	Potobt
Chara vulgaris L.	Chavul	*Potamogeton pectinatus* L.	Potpec
Chiloscyphus pallescens (Ehrh. Ex Hoffm.) Dum.	Chipal	*Potamogeton perfoliatus* L.	Potper
Chiloscyphus polyanthus (L.) Corda	Chipol	*Potamogeton sp.* L.	Potsp_
Cicuta virosa L.	Cicvir	*Potamogeton trichoides* Cham.	Pottri
Cladophora sp. Kutz.	Clasp_	*Potamogeton x sparganiifolius*	Potxfl
Conocephalum conicum L. (Lindb.)	Concon	*Potentilla anserina* L.	Potans
Cratoneuron filicinum Spruce	Crafil	*Ranunculus acris*	Ranacr
Deschampsia caespitosa (L.) Beauv.	Descae	*Ranunculus aquatilis*	Racaqu
Drepanocladus aduncus Warnstorf	Dreadu	*Ranunculus circinatus*	Rancir
Eleocharis palustris L.	Elepal	*Ranunculus flammula*	Ranfla
Elodea canadensis Michx.	Elocan	*Ranunculus fluitans*	Ranflu
Enteromorpha sp.	Entsp_	*Ranunculus lingua*	Ranlin
Epilobium hirsutum L.	Epihir	*Ranunculus repens*	Ranrep
Epilobium palustre L.	Epipal	*Ranunculus sceleratus*	Ransce
Epilobium sp. L.	Episp_	*Ranunculus trichophyllus*	Rantri
Equisetum fluviatile L.	Equflu	*Rhynchostegium riparioides* (Hedw.) C.Jens.	Rhyrip

Equisetum palustre L.	Equpal	*Rorippa amphibia* (L.) Besser	Roramp
Eupatorium cannabinum L.	Eupcan	*Rumex aquaticus* L.	Rumaqu
Filipendula ulmaria (L.) Maxim.	Filulm	*Rumex hydrolapathum* Huds.	Rumhyd
Fontinalis antipyretica Hedw.	Fonant	*Sagittaria sagittifolia* L.	Sagsag
Galeopsis tetrahit Lindtley	Galtet	*Salix alba* L.	Salalb
Galium palustre L.	Galpal	*Salix aurita* L.	Salaur
Galium uliginosum Pall. Ex M. Bieb.	Galuli	*Salix cinerea* L.	Salcin
Geranium palustre L.	Gerpal	*Salix fragilis* L.	Salfra
Glechoma hederacea L.	Glehed	*Scapania sp.*	Scasp_
Glyceria fluitans (L.) R. Br.	Glyflu	*Scapania undulata* (L.) Dum.	Scaund
Glyceria maxima (Hartman) Holmberg	Glymax	*Scirpus lacustris* L.	Scilac
Glyceria plicata	Glypli	*Scirpus sylvaticus* L.	Scisyl
Hildenbrandia rivularis (Fries) Fries	Hilriv	*Scrophularia umbrosa* Dumort.	Scrumb
Hottonia palustris L.	Hotpal	*Scutellaria galericulata* L.	Scugal
Hydrocharis morsus-ranae L.	Hydmor	*Sium latifolium* L.	Siulat
Hygroamblystegium fluviatile Loeske	Hygflu	*Solanum dulcamara* L.	Soldul
Hygroamblystegium tenax Jennings	Hygten	*Sparganium emersum* Rehmann	Spaeme
Impatiens glandulifera Royle	Impgla	*Sparganium erectum* L.	Spaere
Iris pseudacorus L.	Iripse	*Spirodela polyrhiza* (L.) Schleid.	Spipol
Juncus articulatus L.	Junart	*Spirogyra sp.*	Spisp_
Juncus bulbosus L.	Junbul	*Stachys palustris* L.	Stapal
Juncus effusus L.	Juneff	*Stellaria palustris* Retz.	Stepal
Lemanea fluviatilis L.	Lemflu	*Stigeoclonium tenue* Kutzing	Stiten
Lemna gibba L.	Lemgib	*Stratiotes aloides* L.	Stralo
Lemna minor L.	Lemmin	*Symphytum officinale* L.	Symoff
Lemna trisulca L.	Lemtri	*Taraxacum officinale* Wigg.	Taroff
Lolium perenne L.	Lolper	*Thelypteris palustris* Schott	Thepal
Lycopus europaeus L.	Lyceur	*Typha angustifolia* L.	Typang
Lysimachia nummularia L.	Lysnum	*Typha latifolia* L.	Typlat
Lysimachia thyrsiflora L.	Lysthy	*Urtica dioica* L.	Urtdio
Lysimachia vulgaris L.	Lysvul	*Vaucheria sp.*	Vausp_
Lythrum salicaria L.	Lytsal	*Veronica anagallis-aquatica* L.	Verana

Marchantia polymorpha L.	Marpol	*Veronica beccabunga* L.	Verbec
Marsupella emarginata Lindenb.	Marema		

USING SPATIO-TEMPORAL MODELLING AS A DECISION SUPPORT TOOL FOR MANAGEMENT OF A NATIVE PEST HERBIVORE

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Abstract. Landscape modification can alter the distribution and abundance of wildlife, which can result in irruptions of native species causing significant impacts on economically and ecologically valuable systems. This study investigated the applications of the Spatio-Temporal Animal Reduction (STAR) model, originally designed for the management of feral ungulates, by adapting it for the management of a native pest herbivore (the Tasmanian pademelon, *Thylogale billardierii*) within an agricultural-forest mosaic, typical of Tasmanian (Australian) agricultural landscapes. Empirical data of habitat and demographic features of a pest population were inputted into STAR to test the cost-effectiveness of three simulated density reduction models. Compared with the projected population growth under no management, simulations demonstrated that low, medium and high density reduction all reduced population abundance over 10 years. Cost increased with the level of population reduction due to increasing difficulty with locating individuals. The revenue gained from a simulated harvest was greatest for medium-intensity density reduction. We propose STAR can be used as a decision support tool to guide situations considering resource availability, browsing intensity and site-specific management objectives. The application of STAR highlights the model's adaptability across diverse pest populations, landscape features and where there is competition for resources between domestic and native populations.
Keywords: *decision support tool, native herbivore, pest species, spatio-temporal model, wildlife management*

Introduction

Human population growth necessarily results in an increasing demand for resources (e.g. mineral, agricultural and residential), and this causes global conflict between humans and wildlife (Vitousek *et al.,* 1997; Chapin *et al.,* 2000; Weinbaum *et al.,* 2012). Given the high demand for food, this conflict is often most obvious and vigorous at the agricultural-wild land interface (Edwards *et al.,* 1996; Viggers and Hearn, 2005). The modification and development of landscapes for economically valuable systems inadvertently alters the distribution and abundance of native species (Rounsevell *et al.,* 1991; Edwards *et al.,* 1996). Importantly, such agricultural intensification often results in irruptions of pest species, through the reduction in natural enemies, an increase in the scale of cultivation, and a decline of host plant defences (Soroush, 2010). In some situations, agricultural development may cause increases in the abundance of some native herbivores species. This often occurs where fragments of native vegetation provide shelter adjacent to improved pasture and water points (Calaby and Grigg, 1989; Rounsevell *et al.,* 1991). The browsing and grazing pressures exerted by native herbivores on managed land can result in significant costs associated with economic productivity and environmental management (e.g. Coleman *et al.,* 1997; Bulinski and McArthur, 1999; Torstenson *et al.,* 2002).

The management of pest species generally attempts to decrease population numbers below a critical damage threshold level (Shea *et al.,* 1998; McMahon *et al.,* 2010). In order to achieve this, determination of an acceptable level of damage and its relationship to pest density across the landscape is required (Buckley *et al.,* 2004; Edwards *et al.,* 2004), in combination with the selection of the most cost effective management strategies available (Buckley *et al.,* 2004).The suppression of population densities of native pest herbivores remains difficult across the Australian landscape, and the critical damage threshold may shift in response to localised features, as (*i*) native herbivore species have large population densities at the interface of managed landscapes, necessitating extensive, large-scale and highly coordinated management approaches to keep population numbers low (McMahon *et al.,* 2010); (*ii*) the cost of management increases with decreasing animal densities (Bayliss and Yeomans, 1989); (*iii*) pest herbivore species may display cryptic and/or nocturnal behaviour, which can make access and control strategies challenging and costly (Coleman *et al.,* 1997; le Mar and McArthur, 2001); (*iv*) management practices attract socio-political scrutiny and regulation, including modified approaches to lethal density reduction strategies traditionally adopted for invasive species management (Coleman *et al.,* 1997; Buckley *et al.,* 2004) and the implementation of alternative non-lethal control strategies (Coleman *et al.,* 2006; Day and MacGibbon, 2007; Wiggins *et al.,* 2010); and (*v*) follow-up monitoring of population densities and the effectiveness of the management strategy is often not always performed or practical to do so (McMahon *et al.,* 2010).

An improved approach to the control of population densities of native pest herbivores may be aided by the development of decision support tools using spatio-temporal explicit models which can identify near optimal cost-effective removal strategies of pest species. Such tools enable improved management planning by combining information on pest population dynamics, associated financial costs and landscape/habitat structure. Moreover, spatio-temporal models have the capacity to inform managers on the likely behavioural movement of animals into (and out of) control areas and population densities relative to preferred habitat type. By taking immigration (and emigration) movements into account, management can be target-specific to problematic areas. Additionally, simulation models allow for updates to control strategies as new knowledge is acquired (Haule *et al*., 2002; Bradshaw and Brook, 2007; McMahon *et al.,* 2010). It is desirable, and often essential, for pest management strategies to minimise the cost-benefit ratio of the control strategy. Modelling important ecological and economic factors allows researchers and managers to explore a range of cost-benefit outcomes as a result of contrasting management approaches.

The Spatio-Temporal Animal Reduction (STAR) model provides a practical decision support tool that explores the biological, logistical and financial consequences of density reduction management scenarios in a virtual landscape (McMahon *et al.,* 2010). STAR provides a virtual framework for making informed comparisons of different management scenarios, thereby identifying the minimum proportion of the pest population to be removed for effective population control; identifying habitat-specific problem areas and optimal control techniques to be used; and incorporating information on the spatial and temporal costs of pest species density reduction. It is a user-friendly tool that allows managers to update new data and changing economic conditions (McMahon *et al.* 2010). The flexibility of STAR may therefore enhance the use of adaptive management principles in which the results of management actions (e.g. experiments) inform future planning and decision-making (e.g. Bradshaw *et al.,* 2007; McCarthy and Possingham, 2007).

A concrete example of the need to trial new management approaches concerns the management of native pest herbivores in Tasmanian agricultural landscapes. The use of 1080 poison to control pest populations is currently being phased out across the Tasmanian landscape, in response to social concerns over the environmental impact of this poison on non-target species. Alternative wildlife management strategies are currently being sought and tested (e.g. Coleman *et al.,* 2006). While alternative management strategies may provide socially preferred means for mitigating browsing damage across the landscape, their cost-effectiveness and execution is generally not considered to offer the same level of cost-effectiveness as density reduction strategies through broad-scale lethal (*i.e.* 1080 poison) control (Coleman *et al.,* 2006).

The aim of this research was to investigate the utility of the Spatio-Temporal Animal Reduction (STAR) model (McMahon *et al.,* 2010) in the management of a native pest herbivore within an agricultural-forest mosaic. We investigated a browsing macropod, the endemic Tasmanian pademelon (*Thylogale billardierii*), which is commonly distributed across the Tasmanian landscape as a case study to adapt and test the applications of STAR. A combination of factors likely contribute to the widespread distribution and abundance of pademelons in Tasmanian, including a shift in landscape structure toward a more extensive agricultural-forest mosaic post-European settlement (e.g. Calaby and Grigg, 1989, Rounsevell *et al.,* 1991) and the recent removal or declines in densities of top-order predators from the system (Figueirido and Janis, 2011, Hamede *et al.,* 2013). Specifically, we aimed to (*i*) input habitat and demographic data collected from of a native pest population into the STAR model; and (*ii*) test the cost-effectiveness of a range of density reduction management strategies targeting the control of a native pest population. We provide a range of simulated models based on a wildlife management strategy (culling) adopted across the Tasmanian landscape relevant to macropod herbivore management, and provide a case study which contributes to testing the adaptability and flexibility of the STAR model across a diversity of pest populations and landscape features.

Materials and Methods

Target native pest species

The Tasmanian pademelon (*Thylogale billardierii*), at high densities, can cause extensive damage to managed landscapes including agricultural and forestry industries (Coleman *et al.,* 1997; le Mar and McArthur, 2001). Pademelons are small, sexually dimorphic macropods that display cryptic behaviour and are predominately crepuscular (most active at dawn and dusk) (le Mar and McArthur, 2001; Wiggins *et al.,* 2010). The distribution of the Tasmanian pademelon is widespread and common throughout the Tasmanian landscape, with population densities reported as highest in areas where agricultural land is adjacent to native forest (Rounsevell *et al.,* 1991). The total area of agricultural land within 1 km of eucalypt forest in Tasmania is 925,080 ha, which occurs across 14 % of the total landscape area (TASVEG, 2005; *Fig. 1*).

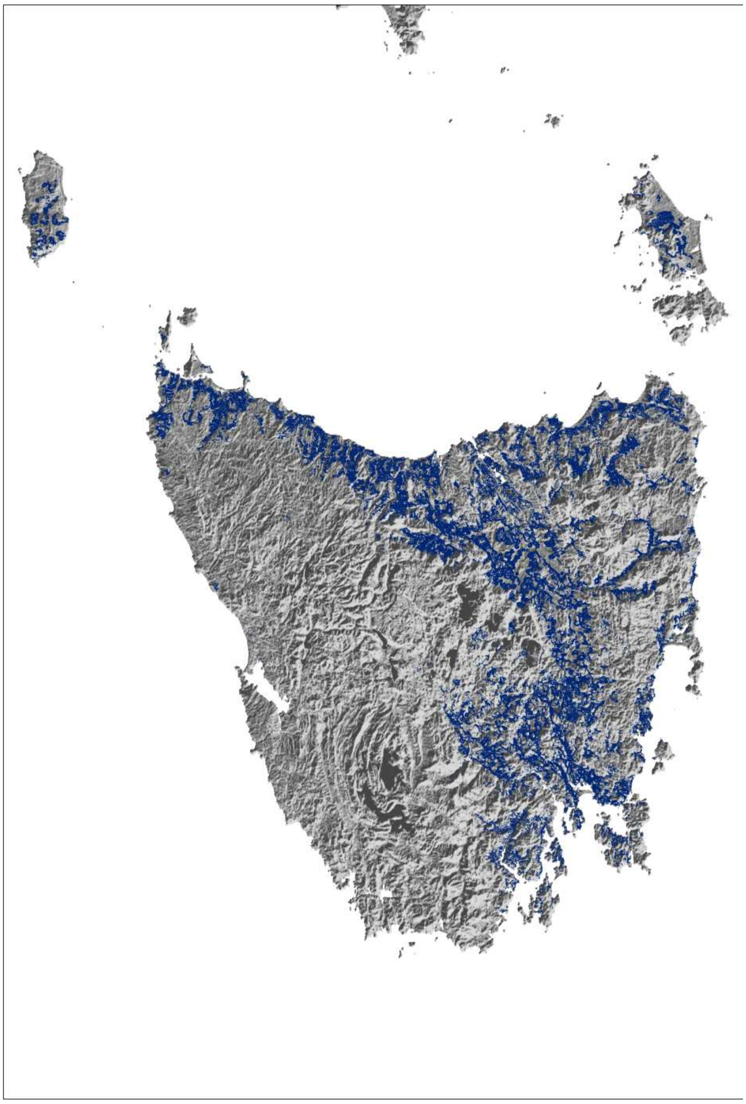

Figure 1. *The total area of agricultural land within 1 km of eucalypt forest in Tasmania, including the Bass Straight Islands (calculations derived from TASVEG, 2005).*

Habitat structure

A 2 x 2 km cell grid of the study site was constructed, using habitat features derived from habitat surveys and TASVEG (2005) (see Wiggins and Bowman, 2011 for additional detail), to create a vector grid for use in the STAR model (*Appendix*). This grid features in output models created using STAR, which is used to display animal responses to proposed management scenarios. In total, 187 cells comprised the study site, giving an overall area of 748 km^2.

Model structure

STAR was originally written as an interactive Microsoft Excel® spreadsheet-based model (using Visual Basic for Applications (VBA) language) designed to be a user friendly, ecologically realistic tool for pest management applications. Specific details of model structure, coding and user manual access are described in McMahon *et al.* (2010). The model presented here has been modified to incorporate the landscape configuration of our study site in Scottsdale, north-eastern Tasmania, Australia (41°06'S, 147 35'E), based on a cellular lattice framework, and is thus spatially explicit with respect to habitat type, elevation and landscape terrain relevant to this, and similar agricultural-forest mosaic areas across the Tasmanian landscape (Wiggins and Bowman, 2011). These features can have important implications on management approaches (and therefore success) as heterogeneous landscapes can alter access to, and costs associated with, animal management in both targeted and adjacent cells. The model structure uses density-feedback and economic cost functions to calculate the cost-benefit ratios of proposed management scenarios (detailed in McMahon *et al.,* 2010) and has been updated to incorporate cost-benefit functions relative to the proposed management scenarios currently practiced in Tasmania (Wiggins *et al.,* 2010). Briefly, animal density is represented as a numeric response between the following parameters in the STAR model: habitat carrying capacity, rate of recruitment, rate of immigration and emigration, culling rate, spatial and temporal variation in landscape features and management-specific costs, location, time-frame, frequency and benefits (i.e. commercial harvesting). A comprehensive explanation of specific cell mechanics is presented in McMahon *et al.,* (2010), where each cell in the grid acts as a specific unit within the overall dynamics of the study area and can be influenced by surrounding cell parameters. Thus, the model is habitat driven in that animal densities are ultimately linked to the habitat quality of the region of interest. The change in animal numbers in each individual cell is governed by the following equation (*Eq. 1*):

$$N_{i,j,t+1} = N_{i,j,t}\, e^{r_m\left[1-\left(\frac{N_{i,j,t}}{K_{i,j}}\right)^{\grave{e}}\right]} - C_{i,j} - \left(E_{i,j} - I_{i,j}\right) \qquad (Eq.\ 1)$$

where i is the cell row number in the study site cell grid, j is the cell column number, t is the time interval, $N_{i,j,t+1}$ = number of animals in cell i,j at the next time interval (t+1), $N_{i,j,t}$ = number of animals in cell i,j at time interval t, r_m = maximum rate of population increase when resources are not limiting, $K_{i,j}$ = habitat-specific maximum carrying capacity, è is a shape parameter that modifies the relationship between r and population size (N), $C_{i,j}$ = total number of animals culled in cell i,j, $E_{i,j}$ = total number of animals emigrating from cell i,j, and $I_{i,j}$ = total number of animals immigrating into cell i,j (McMahon *et al.,* 2010).

Management scenarios

Within the suite of pre-specified management scenarios designed to simulate pest population management options in STAR, we tested four density targets that aim to reduce densities to specified targets over large areas (also tested in McMahon *et al.,* 2010 for invasive species management). The culling technique, lethal control by a trained marksman using a spotlight, is based on current practices routinely used across the Tasmanian landscape (Coleman *et al.,* 2006; Wiggins *et al.,* 2010). The density targets included (*i*) zero density reduction to examine how populations are expected to change over time under no management intervention and provide a comparative (control) simulation (0 animals removed); (*ii*) low-intensity density reduction (17 % initial cull rate and 9 % follow-up cull rate *per* year); (*iii*) medium-intensity density reduction (30 % initial cull rate and 19 % follow-up cull rate *per* year); and (*iv*) high-intensity density reduction (50 % initial cull rate and 31 % follow-up rate *per* year). These scenarios enable comparisons of the cost-effectiveness of low- to high-intensity density reduction operations across the agricultural study site over a 10-year management period, selected to represent a timeframe relevant to an individual land manager. Follow-up cull rates were derived from McMahon *et al.,* (2010) to achieve the pre-defined density-reduction targets over the specified management timeframe, but can be modified to best suits individual user interests or needs.

Under natural population conditions (*i.e.* no management regime), populations that exist below ecological carrying capacity (as predicted for pademelons at our study site) would be expected to reach high densities across the majority of the landscape (Coulson *et al.,* 2004). Following on from this prediction, low-intensity management would be expected to create high density areas which may have a greater potential to fill any ‘gaps’ in population distribution, as animals in areas of high densities will naturally filter into areas of lower densities, given similar levels of habitat suitability (; Porter *et al.,* 1991; Efford *et al.,* 2000). How long any created gaps will persist in the environment is a direct function of the intrinsic growth potential of the population and the scale of the management intervention (Efford *et al.,* 2000, Wiggins *et al.,* 2010). There is the potential that if density reduction is performed at too low a level, the management strategy may generate a steady increase in population numbers across the landscape, thus having little or no net effect on population density (Caughley, 1985). Medium- and high-intensity management strategies would be expected to achieve a greater reduction in high density areas across the landscape.

Within each scenario, we compared (*a*) the total number of animals removed and remaining after culling; (*b*) the associated culling cost *per* animal and total management costs; and (*c*) the offset to costs, which aimed to calculate the revenue obtained from a commercial-based culling operation.

Model parameters

The following model parameters were standardised across each of the scenarios: The carrying capacity *K* for each habitat type was set at 177 animals *per* square km in agricultural habitat, 44 animals *per* square km in native forest habitat and 89 animals *per* square km for all other habitat types (DPIPWE Density Indices, 2009). The relative suitability of each habitat type was set at 57 % for agricultural habitat, 15 % for native forest habitat and 28 % for all other habitat types (Wiggins *et al.,* 2010). It was assumed that the habitat mosaic remained unchanged through the study period. The initial population size - the proportion (fraction) of *K* - was set to 0.5, which set the habitat-specific carrying capacities in the cells occupied by pademelons to 50 % of the maximum, giving initial population densities of 88 animals *per* square km in agricultural habitat, 22 animals *per* square km in native forest habitat and 44 animals *per* square km for all other habitat types. This value was guided by knowledge of previous and current density reduction strategies used in the area (Wiggins *et al.,* 2010, Wiggins and Bowman, 2011) and population demographic features (McMahon *et al.,* 2011), indicating the population was not at carrying capacity. An average density of 51 animals *per* square km was calculated from the above parameters and the total population number, current *N*, was 39,000 animals across the entire 748 km^2 management zone. The maximum annual growth rate of the population was estimated at 0.17 (McMahon *et al.,* 2011) and the maximum dispersal rate of animals was set at 0.50 (with a maximum dispersal rate of 1 possible) to account for varying levels of immigration and emigration rates (specific to each cell) that a natural population may display. Dispersal is limited to adjacent cells per season and enables the user to test the effect of dispersal rate, particularly if it is not known, by incorporating different rates as part of the model building phase.

The cost (quoted in Australian dollars) of culling was set at $150 *per* hour with overheads estimated at 50 %, incorporating equipment hire/use, consumables and additional labour costs. A revenue value of $2 was assigned as the market value *per* individual to test the offset to costs, to examine the viability of a revenue-based trade in wallaby products such as meat (e.g. Wilson and Edwards, 2008).

Results

STAR density reduction outputs

This study tested four simulated models chosen to replicate a range of management strategies currently applied across the Tasmanian landscape:

Zero density reduction

Average population size was predicted as 53,740 individuals within the specified study area over 10 years in response to no management (*Table 1*, *Fig. 2*), taking into account population growth estimates (McMahon *et al.,* 2011). This represents a 25 % increase in population size from the initial projected population. Animal abundance was predicted as high across 60 % of the mapped landscape (*Fig. 3*).

Table 1. *STAR model output for simulated management scenarios of zero-, low-, medium- and high-intensity animal removal strategies.*

Management scenario	**Animals removed / Animals remaining**	**Cost *per* animal[1] AU$**	**Total management cost AU$**	**Offset to costs[2] AU$**
(i) Zero density reduction	0 / 53,740	0.00	0	0
(ii) Low-intensity reduction	39,430 / 43,390	0.29	11,376	67,312
(iii) Medium-intensity reduction	63,960 / 32,860	0.41	25,996	101,921
(iv) High-intensity reduction	71,230 / 20,880	0.73	52,126	90,334

[1]average cost per animal culled; [2]offset to costs of the density-reduction (culling) strategy provided from commercial-based culling operations.

Low-intensity density reduction

Average population size was predicted to reach 43,390 individuals within the specified study area over 10 years in response to low-intensity management (*Table 1, Fig. 2*). This represents a 10 % increase in population size from the initial projected population. A total of 39,430 individuals were culled during the 10 year timeframe at a total cost of $11,376 (*Table 1, Fig. 2*).

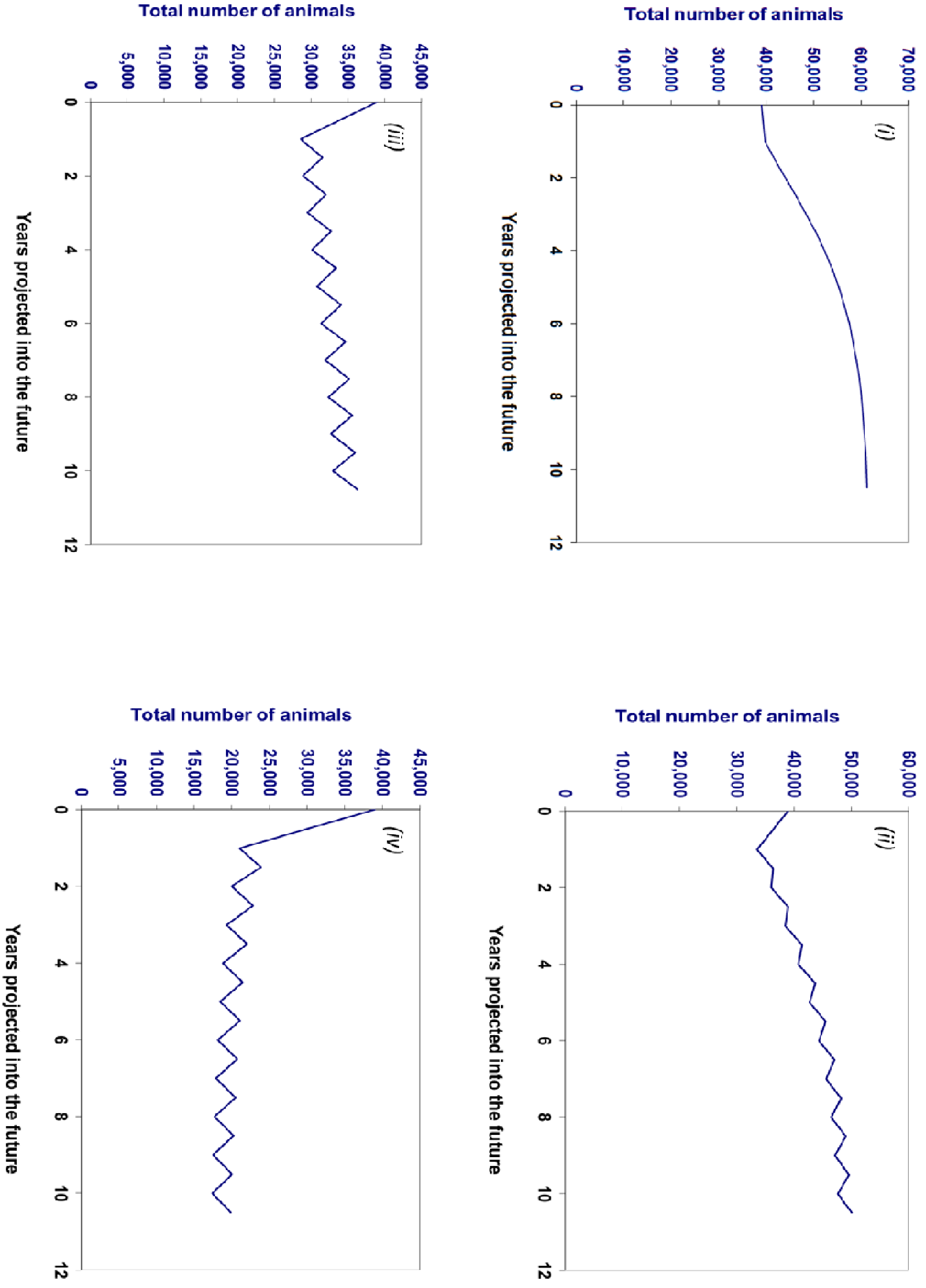

***Figure 2**. Population reduction strategies of (i) zero density reduction; (ii) low-intensity density reduction; (iii) medium-intensity density reduction; and (iv) high-intensity density reduction strategies for the management of a pest herbivore population (the Tasmanian pademelon Thylogale billardierii) across a heterogeneous landscape over a 10 year management period*

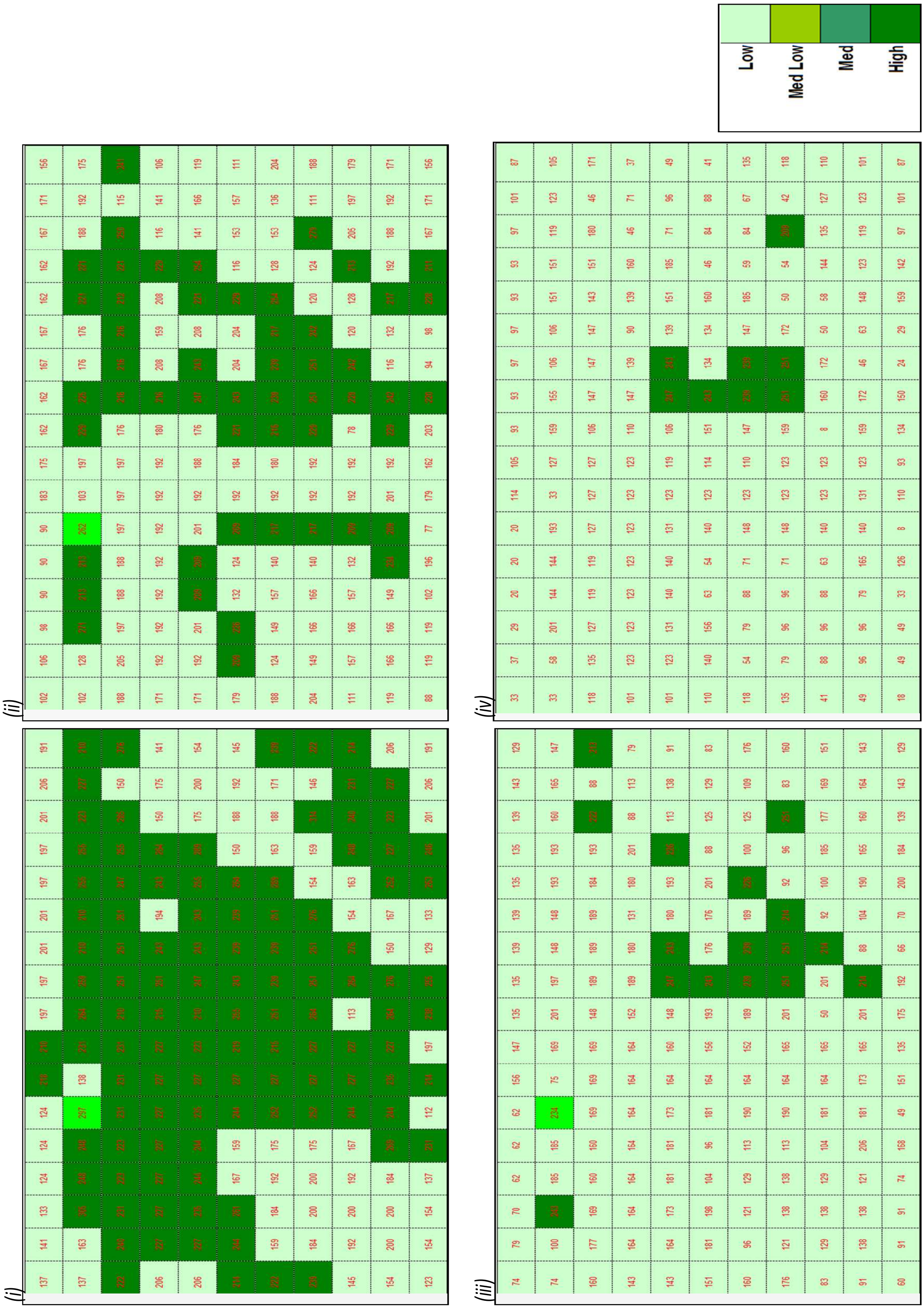

***Figure 3**. Population abundance of a pest herbivore (the Tasmanian pademelon Thylogale billardierii) in response to (i) zero density reduction; (ii) low-intensity density reduction; (iii) medium-intensity density reduction; and (iv) high-intensity density reduction scenarios in the study site over a 10 year management period. Note: density level categories indicated by multiple shades of green represent low, medium-low, medium and high density levels.*

Animal abundance was predicted as high across 28 % of the landscape, which equates to a 53 % reduction in high abundance cells compared with zero density reduction (*Fig. 3*). The culling cost *per* individual ($ individual culled^{-1}) increased to a peak value of $0.36 individual^{-1} in year 2, then gradually decreased to $0.25 individual^{-1} from year 7 (average cost $0.29 individual^{-1}; *Fig. 4*). The total revenue gained (offset of culling costs) from a market value of $2 *per* individual was $67,312 over 10 years.

Medium-intensity density reduction

Average population size was predicted to reach 32,860 individuals within the specified study area over 10 years in response to medium-intensity management (*Table 1, Fig. 2*). This represents a 16 % decrease in population size from the initial projected population. A total of 63,960 individuals were culled during this timeframe at a total cost of $25,996 (*Table 1, Fig. 2*). Animal abundance was predicted as high across 9 % of the landscape, which equates to a 69 % reduction in high density cells compared with low-intensity reduction, and an 86 % reduction compared with zero intensity reduction (*Fig. 3*). The culling cost *per* individual increased to a peak value of $0.45 individual^{-1} in years 2 and 3, which decreased to $0.40 individual^{-1} from year 9 (average cost $0.41 individual^{-1}; *Fig. 4*). The total revenue gained was $101,921 over 10 years.

High-intensity density reduction

Average population size was predicted to reach 20,880 individuals within the specified study area over 10 years in response to high-intensity management (*Table 1, Fig. 2*). This represents a 54 % decrease in population size from the initial projected population. A total of 71,230 individuals were culled within this timeframe at a total cost of $52,126 (*Table 1, Fig. 2*). Animal abundance was predicted as high across 4 % of the landscape, which equates to a 50 % reduction in high density cells compared with medium-intensity reduction, and a 93 % reduction compared with zero intensity reduction (*Fig. 3*). The culling cost *per* individual started at the lowest value of $0.32 individual^{-1} in year 1, and increased annually to a peak value of $1.01 individual^{-1} in year 10 (average cost $0.73 individual^{-1}; *Fig. 4*). The total revenue gained was $90,334 over 10 years.

Discussion

The Spatio-Temporal Animal Reduction (STAR) model provides a practical decision support tool to explore the biological, logistical and financial consequences of density reduction management in a virtual landscape (McMahon *et al.,* 2010). While STAR was originally designed for the management of feral ungulates in the World Heritage Kakadu National Park (Australia), this study examined an applied case study of the model to test its applicability across a different set of pest population dynamics and landscape features. Density-reduction applications of STAR were tested to aid in the strategic management of a native marsupial pest herbivore, the Tasmanian pademelon *Thylogale billardierii*, in an agricultural-forest mosaic that has resulted in rapid increases in the abundance of macropod herbivores (Rounsevell *et al.,* 1991; Viggers and Hearn, 2005; Coulson, 2009).

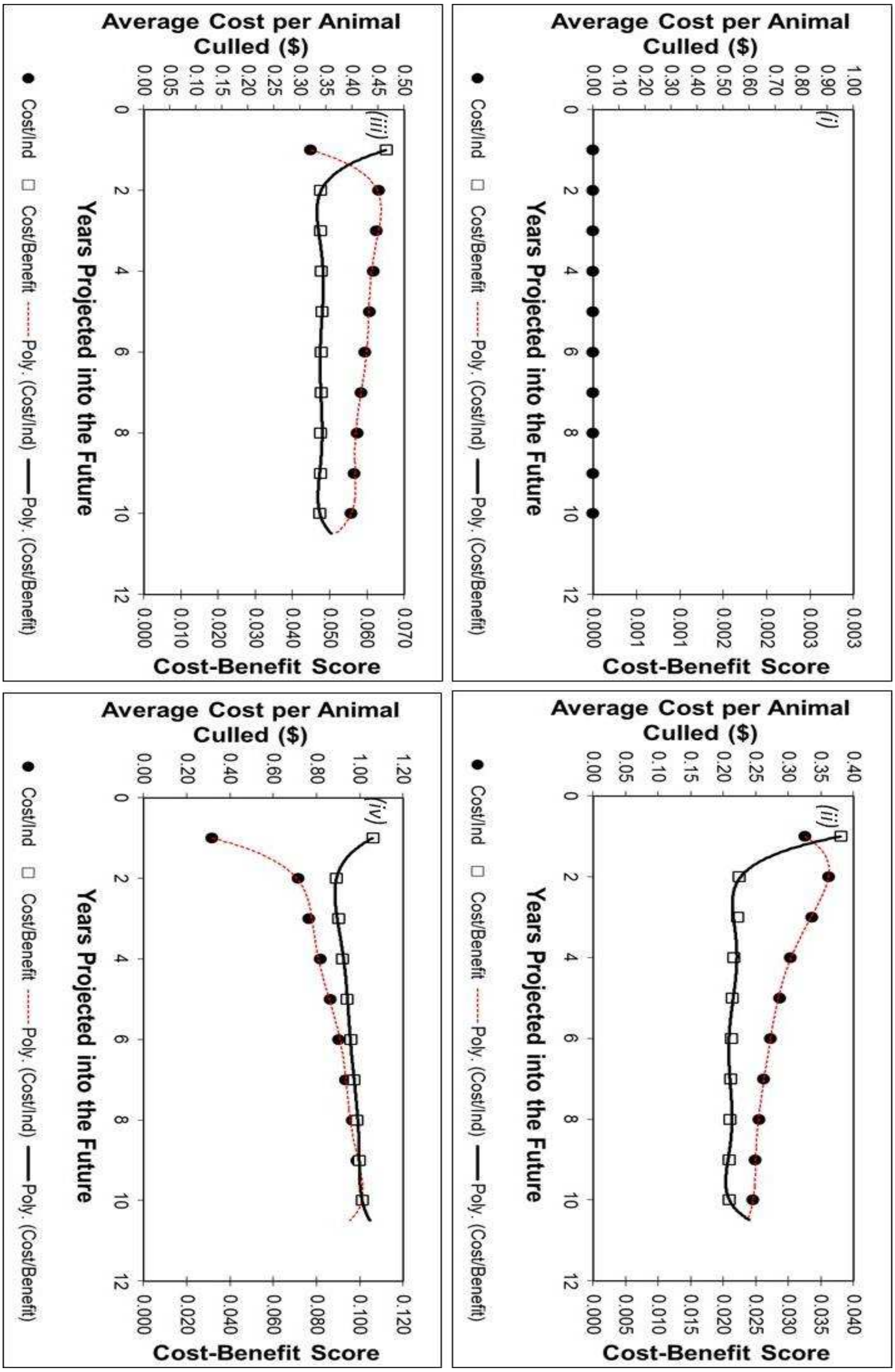

Figure 4. *Average cost of culling animals (AU$individual culled-1) and predicted cost-benefit score for the population reduction strategies of (i) zero density reduction; (ii) low-intensity density reduction; (iii) medium-intensity density reduction; and (iv) high-intensity density reduction scenarios for the management of a pest herbivore population (the Tasmanian pademelon Thylogale billardierii) across a heterogeneous landscape over a 10 year management period.*

The low, medium and high density reduction simulations were all effective at reducing population abundances compared to no management, while comparison to the initial population parameter (current *N*) yielded a population increase for low density reduction. With increasing density reduction targets (low- to high-intensity), greater density reductions and cost-benefit scores were achieved, but there were increasing associated costs driven by the higher-intensity management objectives. The average *per capita* cost *per* individual culled increased with increasing density reduction targets, resulting in the offset to culling costs (revenue gained) being greatest for medium density reduction. The lower population size achieved under high density reduction is likely to correlate with increasing difficulty (and therefore cost) associated with locating (and culling) individuals at low densities (e.g. Lurz *et al.,* 2008; Fitzpatrick *et al.,* 2009).

Selection of an appropriate density reduction management strategy

Model predictions indicated that under natural population conditions under no management regime, pademelons reached high densities across the majority of the landscape, indicating the population exists below ecological carrying capacity (Coulson *et al.,* 2004). Low-intensity management predicted pademelons would reach high densities across one third of the landscape. While low-intensity management may be an appealing strategy in situations of limited resource availability (i.e. time or budgetary constraints), medium- and high-intensity management strategies achieved a greater reduction in high density areas across the landscape. The medium-intensity density reduction strategy offered the most cost-effective scenario for the management of Tasmanian pademelons in an agricultural-forest mosaic, a habitat interface which is representative of managed landscapes across the Tasmanian environment (*Fig. 1*).

There are, however, a variety of factors which need to be considered when developing a wildlife management plan for the control or conservation of a species. Selection of an appropriate management strategy commonly includes an integrated approach of planning, implementation and adaptive management features (e.g. Gibbs *et al.,* 1999). Cultural and socio-political values imposed on wildlife can additionally shape management strategies and the management timeframe adopted. In this current study, the revenue gained from the medium-intensity density reduction strategy was predicted as \$136 *per* km^2 over a 10 year timeframe. Considering the total area of agricultural-forest interface occurring within Tasmania (*Fig. 1*), upon extrapolation, these predictions demonstrate the potential for \$AU 1 million in revenue to be gained from harvesting operations across one Australian state, over a 10 year management period. However, the management of native and endemic pest species within Tasmania has attracted considerable socio-political opposition as these species concurrently have high conservation and biodiversity value (e.g. Coleman *et al.,* 2006; Wiggins *et al.,* 2010).

In situations where density reduction is being considered for the management of a pest species, or where the operational harvest of a species occurs, we propose that initial planning stages be guided by the use of spatially explicit decision support tools such as STAR, which can act as a predictive tool for the selection of an appropriate density reduction target, taking into account situation- and site-specific features including resource availability (*i.e.* time, budget), logistical constraints (*i.e.* whole- versus partial-site access), habitat features and management objectives (*i.e.* damage mitigation, integrated management approaches). The use of demographic models of population growth has been identified as one of the most commonly used measures for assessing the sustainable management of wildlife populations (Weinbaum *et al.,* 2012). An additional factor that offers to strengthen the selection of simulated density reduction targets is the estimation or quantification of damage intensity resulting from various densities of the target pest population. While the measurement of this factor was outside the original scope of our empirical data collection (see Wiggins *et al.,* 2010; McMahon *et al.,* 2011), upon collection this information can be inputted into STAR for the continued updating of study-specific features and the resulting improvement of model output interpretation (McMahon *et al.,* 2010).

Spatially explicit models such as those used in STAR can provide comparative density reduction outcomes and revenue-based harvesting potential to guide management planning efforts. It should be noted, however, that as a modelling-based decision support tool, outcomes will be influenced by input values which are variable in nature and may be influenced at a highly-localised scale. While the use of statistical approaches (*i.e.* Bayesian inference) can aide in improving model performance, the objective of this research was to provide a case study to demonstrate the applications of an accessible, user-friendly decision support tool for which data collection and input techniques are easy to perform, model output is easy to understand and highly adaptable to specific situations.

Conclusions

Human-wildlife conflicts can arise within landscapes modified and developed for management purposes. While the management of native or endemic pest species may attract considerable socio-political opposition (e.g. see Clarke and Ng, 2006, Richardson, 2012), density reduction approaches to wildlife management are likely to remain important control options in the quest for maintaining species' abundances, given their broad-scale accessibility by land managers and their cost-effectiveness (Porter *et al.,* 1991; McNulty *et al.,* 1997; Wiggins *et al.,* 2010). Decision support tools such as STAR offer an approach in which management programmes can be developed to maximise the agricultural/utilitarian outcome by reducing the minimum number of animals for maximum exclusion, thus reducing the impact on the broader endemic population (see also McMahon *et al.,* 2010). When developing a wildlife management strategy, we recommend that initial planning stages be guided by the use of a spatially explicit model such as STAR, which can act as a predictive tool in strategic management planning and contribute to synergising modelling, monitoring and management efforts (Chee and Wintle, 2010). STAR can be accessed by researchers and land managers alike to facilitate the adoption of strategic management planning with relative ease and transparency. A key feature of STAR is the facility to input and update information easily, making it a truly adaptive management tool where site-specific information can be fed back into the model for situation-based projections and improved land-use decisions can be made (McCarthy and Possingham, 2007).

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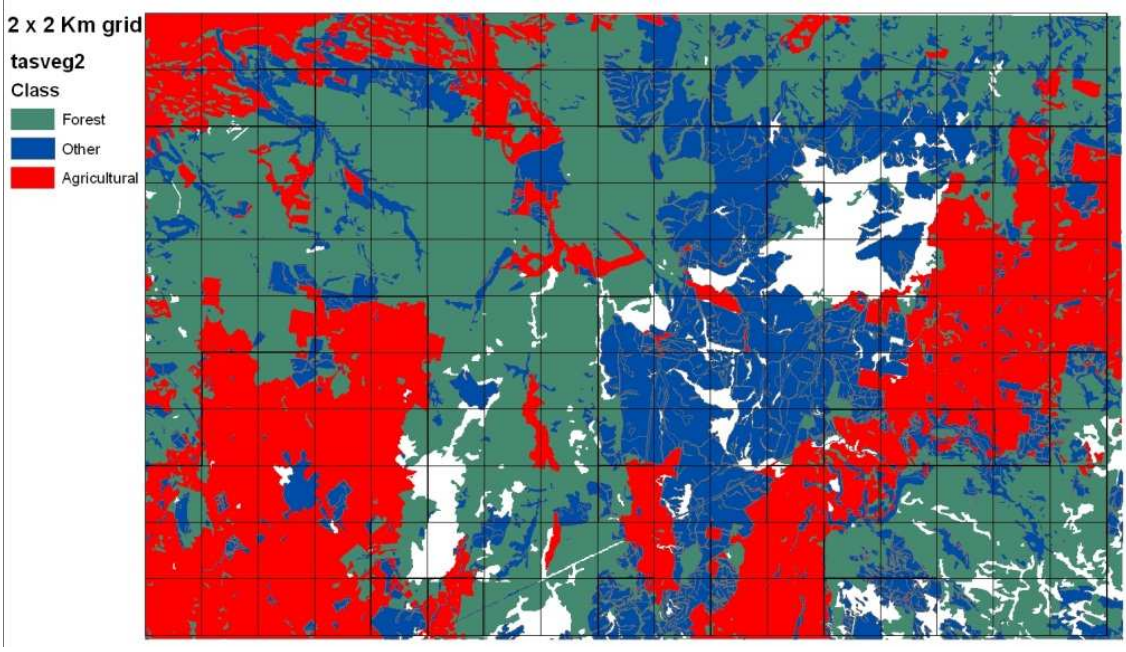
Appendix

Appendix: *A 2 x 2 km cell grid of the study site, using habitat features derived from habitat surveys (Wiggins et al., 2010) and TASVEG (2005). This grid formed the basis of a vector grid which was created for use in the STAR model.*



CORRELATION BETWEEN ARTHROPODS AND PHYSICAL AND CHEMICAL CHARACTERISTICS OF WATER AND SOIL RETAINED IN TILLANDSIA VIOLACEA (BROMELIACEAE) IN AN ABIES- QUERCUS FOREST IN CENTRAL MEXICO

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Abstract. The effect of the support tree species and the seasonal and altitudinal variations on the physical-chemical characteristics of the water and soil accumulated in plants of the epiphytic bromeliad *Tillandsia violacea* were studied in "El Chico" National Park, a temperate forest from Hidalgo, in Central Mexico. The relationship of these factors with arthropods living in theme was also studied. It was found that the tree support species influenced the ions concentration in water and soil. The highest values of ions were found in plants from *Abies religiosa.* Altitude and the season of sampling affected calcium concentrations, as well as pH of soil and water accumulated on epiphytic *Tillandsia*. Electrical conductivity, calcium and dissolved organic carbon (DOC) in epiphytic water accounted for more than 60% of the variation in the density of arthropods. In the accumulated soil pH (among 3.4-8.3), organic carbon content (17.16-30.07 mg g^{-1}), sodium and potassium concentrations (0.26-1.43, 0.49-34.87 cmol $^{+}$ kg^{-1}, respectively) were the most important factors correlated with the total fauna abundance. These results showed the role of epiphytic plants as refuges and nutrient source for arthropod communities and their influence on energy flow in this ecosystem.

Keywords: *Altitude, dissolved organic carbon, ions contents, nutrients, suspended soils.*

Introduction

Epiphytic plants are very frequent in tropical ecosystems, representing about 10% of all the vascular plants species in different kinds of forests (Kress, 1986; Nieder, et al., 2001). In such ecosystems, their biomass can represent up to 30% of the total leaf biomass (Nadkarni, 1984; 1992); thus significantly contributing to the diversity of animal and plant species in Ecuador, Peru and Venezuela (Gentry and Dodson, 1987; Ibisch et al., 1996; Barthlott et al., 2001). Their bearing on the nutrient cycle is of great importance because they sequester atmospheric nitrogen and make it available to other levels of the ecosystem (Edwards and Grubb, 1977; Nadkarni, 1984, 1992; Clark et al., 1998).

These characteristics suggest that epiphytic plants are a key resource for their function (Nadkarni, 1994), in specific environments where competition by light, nutrients and space are factors that limited the development of the understory layer. Due to their morphology, most of these plants give shelter and supply food to a variety of arthropods and other animals (Benzing, 1990; Gutiérrez et al., 1993; Richardson, 1999). They accumulate large amounts of organic matter and litter inside, as well as water, which are vital as an energy source for the different animal communities and the maintenance of general moisture of the whole ecosystem (De Buen, 1953; Bohlman et al., 1995; Zotz and Thomas, 1999). In fact, the epiphytic environment represents one of the most nutrient-poor habitats for vascular plants, thus the bromeliads have consequently developed mechanisms to accumulate mineral salts dissolved in rainwater and related canopy fluids, and leach significant amounts from the host plants (Tukey, 1970; Benzing, 1980). An input of nutrients in the canopy of the tropical forest comes from the forest leaching and atmospheric aerosols. Soil ions become available to epiphytic plants microbial mineralization, nitrogen fixation, canopy roots and the plant capacity to accumulate plant and animal remains and litter (Benzing, 1990; Vence and Nadkarni, 1990, 1992).

Many studies have demonstrated that the so-called ’suspended soils’ (Delamare-Devouteville, 1948), nevertheless its scarce, have many nutrients and they constitute an important pool of organic matter, sometimes functioning as “buffers” of mineral nutrients from the atmosphere for their further incorporation to the ecosystem (Nadkarni and Matelson, 1991). Despite their importance for the fauna they shelter, few studies have been carried out in these peculiar microcosms concerning their physical and chemical properties, considered as arboreal Histosol by some authors (Bohlman et al., 1995) or arboreal soil (Nadkarni et al., 2002). Recent studies propose that the arboreal soils can be considered as true soils (Enloe et al., 2006).

Most of the studies about the mineral epiphytic nutrition have been conducted in tropical forests (Clarkson et al., 1986; Hietz et al., 1999; Richardson et al., 2000b). Nevertheless, little information is available about temperate forests. Recent studies show that the presence and abundance of vascular epiphytes in temperate forests are more common that has been considered previously, and in some places they are similar to those of tropical forest (Zotz, 2005). An only few studies are focused in the origin and characteristics of the arboreal soils (Enloe et al., 2006, 2009)

In the present study we evaluated the influence of support trees (oak vs. spruce) of *Tillandsia violacea* Baker on the nutrient concentration in the accumulated water and soil in epiphytes, and its relation with arthropods inhabiting on the plants in temperate forests in Central Mexico.

Materials and methods

Study site

"El Chico" National Park (2793 ha) is located on the north-east slope of the Valley of Mexico (Vargas-Márquez, 1984), approximately 24 km north-west of Pachuca City, in the highest part of the Pachuca Range (20°13'45"N, 98°47'23"W). Elevation ranges from 2320 m asl to 3090 m asl (SEDUE, 1988). The soils of the area have very friable consistency, with contents of N, P and S, they has been classified as Andosols, derived from volcanic ash mainly of basaltic composition, with very high organic matter content in the A horizon, which gives them dark colour (Melo and López, 1994). The climate according to the Köppen classification system, modified by García (1981) in the area, is temperate and humid, with a cold and rainy winter. The annual average temperature ranges between 10^o to 14^oC, with a minimum of -6^o to -9^oC; annual rainfall varies between 600 and 1500 mm.

The dominant vegetation is *Abies religiosa* (Kunth.) Schltdl. & Cham. forest (Rzedowski, 1988). This kind of forest grows at elevations between 2700 and 3000 m asl, mainly on slopes, where trees are protected from strong winds and solar radiation. The oak forest varies in architecture and composition. *Quercus* spp. trees reach heights from 20 to 30 m and they often have many epiphytic plants. Dominant species are *Q. laurina* Humb. & Bonpl., *Q. rugosa* Née, *Q. laeta* Liebm., *Q. mexicana* Humb. & Bonpl. and *Q. crassifolia* Humb. & Bonpl. This community is located between 2300 and 3000 m asl, although it occurs as an ecotone with the cloud montane forest at 2600 and 2800 m asl with *Abies religiosa* at its upper limit (Rzedowski, 1988).

The average density (± S.E.) of *Tillandsia violacea* Baker on *Quercus* spp. in the study area is about 5.9 ± 4 ind. per tree and in *Abies religiosa* is 7.8 ± 4 ind. per tree (Castaño-Meneses et al., 2003)

Sample collection

One randomly stratified spatio-temporal sampling was done. Sampling units were specimens of *Tillandsia violacea* of two different sizes (plant diameter at the base of the rosette: 25-40 cm and > 40 cm). They were carefully collected to avoid damage and loss of materials such as water, soil and fauna, stored into polyethylene bags and transported to the laboratory. Plants were dissected leaf by leaf, in order to obtain the suspended soil and fauna accumulated. Ten plants of each size were collected in two kinds of support tree (*Quercus* spp. and *Abies religiosa*) at two different altitudes where both tree species were present (2765 and 2900 m asl) during the rainy (September 1998) and dry (April 1999) seasons. The water and soil accumulated on the plants were isolated for chemical analysis. Water was collected when the plants were separated from the support tree, storage in separated plastic jars for their latter quantified and analyzed. That water was filtered to obtain the solid particles, those particles were homogenized and analyzed separately. The litter accumulated in the epiphytes was processed by Berlese-Tullgren funnels to extract the arthropods. To check that the collected material was suspended soil, the presence of minerals was studied by mineralogical qualitative analysis of semipermanent slides mounted in cedar oil using a petrographic microscope.

Water analyses

The water obtained was filtered through Whatman No. 1 paper in order to separate and obtain liquid and solid fractions. Dry solid fraction was quantified and also homogenized by grinding and sieving through 10 mm. Water analyses were expressed on a volume basis, and included: pH, electrical conductivity (EC), soluble cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) and chloride (Cl^-). Dissolved organic carbon (DOC) was evaluated by Walkley and Black Method (Jackson, 1982).

Soil analyses

Solid fractions (soil and litter) from the plants processed by Berlese funnels were analyzed for pH in H_2O (1:10), total organic carbon (TOC), by Walkley and Black Method (Jackson, 1982); and exchangeable cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+), Ca^{2+} and

Mg^{2+} were extracted by 1M ammonium acetate pH 7 and quantified by EDTA, whereas K^+ and Na^+ were evaluated using a flame photometer (Page et al., 1982).

Statistical analysis

In order to evaluate the normal distribution of the data, the Kolmogorov-Smirnov test was used, and the variables with percentage values were arcsine-square-root transformed before the analysis (Sokal and Rohlf, 1973). To evaluate the differences between water physical and chemical parameters at the sites of sampling (altitudes), Mann-Whitney U-test was used because not enough water was obtained from all the epiphytic plants to allow the analysis. The effect of (1) the supporting tree type, (2) the size of the epiphytic plants and (3) the altitude and season of sampling on chemical and physical data of the water and also of the soil were evaluated with Multiple Variance Analysis (MANOVA). Season is a qualitative variable that was coded as 0 and 1 for the model. Duncan's test was used to detect differences (Zar, 1984). To compare the value of the parameters of the water and soil during the rainy season, a t-test was run. A multiple regression analysis was used to evaluate the relationship between the physical and chemical parameters and the arthropod densities. Analyses were performed with the program STATISTICA ver. 6.0 (StatSoft, 1995).

A multivariate technique, a Canonical Correspondence Analysis (CCA) was used to evaluate the influence of edaphic variables on faunal composition at the dry and rainy seasons. Explanatory variables include pH, Ca^{2+}, Mg^{2+}, K^+, Na^+ and TOC. The analysis was performed in CANOCO 4.0 for Windows (ter Braak and Smilauer, 1998). We use 500 permutations under reduced model by Monte Carlo test.

Results

Water analyses

Enough water was obtained to measure chemical properties only during the rainy season. At 2765 m asl, mean diameter of the epiphytic plants was 40.10 ± 11.01 cm (range 26-76 cm). From them, a total of 2743 ml of water was obtained (N = 23). At 2900 m asl, the size of the plants was 45.21 ± 10.95 cm of diameter (range 28.0-64.5 cm), with a total of 3321 ml of accumulated water in plants (N = 32). Average results for the parameters evaluated at the two altitudes are shown in *Table 1.*

Mean values in comparison between the supporting tree and the size of the epiphytic plant are shown in *Tables 2* and *3*. In *Table 4* the results of the MANOVA are shown.

Calcium concentration was affected by altitude (*Table 4*), the highest concentrations occurring at 2765 m asl. For the supporting tree there was a significant statistical value in the amount of calcium, magnesium, sodium and potassium, however calcium was the only element affected by the size of the epiphytic plants (*Table 4*). Significant interactions between the tree species and altitude, and the tree and the size for the pH, and on the amount of calcium, and the altitude were found.

The result of the multiple regression analysis was significant when fauna of the epiphytic plants was correlated with the physical and chemical parameters, with a correlation coefficient of 0.61 ($F_{5,49} = 5.69$; $P = 0.0003$). The parameters better correlated with fauna abundance were the electric conductivity ($r_{49} = 0.94$; $P = 0.006$), DOC ($r_{49} = 0.48$; $P = 0.008$) and the amount of calcium ($r_{49} = 0.37$; $P = 0.03$).

***Table 1**. Average ± standard error (range) and U values for physical and chemical parameters for water accumulated in Tillandsia violacea from two altitudes in "El Chico" national Park, Hidalgo State.*

Parameter	**Altitude 2765 m** **N = 23**	**Altitude 2900 m** **N=32**	**U**	**P**
pH	5.61 ± 0.21 (4.10-8.60)	5.22 ± 0.12 (4.20-6.70)	358	0.17
EC (dS/m)	0.73 ± 0.31 (0.19-7.60)	0.37 ± 0.04 (0.1-0.76)	273	0.08
Ca^{2+} (cmol$^+$ L^{-1})	1.72 ± 0.30 (0.20-6.50)	1.20 ± 0.12 (0.25-3.50)	303	0.30
Mg^{2+} (cmol$^+$ L^{-1})	1.27 ± 0.18 (0.50-4.25)	2.19 ± 0.57 (0.25-15.00)	355	0.88
Na$^+$ (cmol$^+$ L^{-1})	4.12 ± 1.90 (0.24-42.80)	1.37 ± 0.24 (0.17-4.57)	341	0.70
K$^+$ (cmol$^+$ L^{-1})	2.78 ± 1.34 (0.04-30.77)	1.09 ± 0.25 (0.10-6.31)	283	0.17
Cl$^-$ (cmol$^+$ L^{-1})	7.67 ± 0.54 (5.00-14.00)	7.60 ± 0.44 (4.00-13.00)	355	0.89
C (g L^{-1})	17.71 ± 2.08 (1.96-45.24)	16.02 ± 1.90 (2.78-41.33)	315	0.41
C (soil) (g kg^{-1})	19.42 ± 0.57 (10.43-22.72)	19.80 ± 0.34 (12.99-22.77)	337	0.65

***Table 2.** Average ± SE (range) for physical and chemical parameters for water accumulated in T. violacea from two species of support trees at 2765 m asl (N=23). Different letters in the same row indicated significant differences (Duncan's test).*

Parameter	***Abies religiosa***		***Quercus* spp.**	
	Medium	**Large**	**Medium**	**Large**
pH	5.97±0.66^a	5.98±1.56^a	5.76±0-99^a	4.55±0.48^a
EC dS/m	0.43±0.17^a	1.95±3.16^a	0.29±0.08^a	0.38±0.27^a
Ca^{2+} cmol$^+$ L^{-1}	1.60±1.34^a	3.35±1.95^b	1.04±0.58^a	1.05±0.82^a
Mg^{2+} cmol$^+$ L^{-1}	2±1.37^a	1.15±0.72^a	1.12±0.83^a	0.81±0.24^a
Na$^+$ cmol$^+$ L^{-1}	3.63±3.08^a	11.66±17.7^a	0.36±0.11^a	1.88±3.15^a
K$^+$ cmol$^+$ L^{-1}	2.07±2.74^a	8.28±12.76^a	0.48±0.21^a	0.77±0.3^a
Cl$^-$ cmol$^+$ L^{-1}	8.33±3.58^a	7.80±3.11^a	6.67±2.11^a	8.0±2.58^a
C g L^{-1}	19.63±4.34^a	16.68±18.61^a	19.18±8.44^a	17.47±6.7^a
C (soil) g kg^{-1}	19.86±0.76^a	19.00±2.13^a	18.07±4.85^a	20.59±0.9^a

When each taxon of arthropods was analyzed separately, comparatively more significant correlations were found for the percentage of DOC and the EC. The correlation coefficient for most abundant groups is shown in *Table 5*, except for Oribatid and Prostigmatid mites, which were not correlated with any parameter.

Groups which are less abundant but significantly correlated with some of the parameters were the Hexapoda larvae (r_{49} = -0.34; $P < 0.05$) and Hymenoptera (r_{49} = -0.50; $P < 0.05$), with DOC; Diplopoda with sodium (r_{49} = -0.96; $P < 0.05$), ants with pH ($r_{4\ 9}$= -0.37; $P < 0.05$) and Crustacea with calcium (r_{49} = 0.37; P = 0.05).

It is necessary to point out the fact that electric conductivity was significantly related with abundance of total arthropods, illustrating the fact that accumulated water in the epiphytic plants is an important storage of available nutrients for the fauna.

Suspended soil analyses

Suspended soils were obtained from 49 epiphytic plants (1164 g of soil in 4.96 m^2) during the rainy season and from other 80 epiphytic plants (2082 g of soil in 7.46 m^2)

during the dry season. Between the sampling sites there were differences in the amount of sodium, but for the other seasons there were significant differences in the calcium concentration for the rainy season and for the dry season with pH (*Table 6*).

***Table 3**. Average ± standard error (range) for physical and chemical parameters for water accumulated in T. violacea from two species of support trees at 2900 m asl (N=32). Different letters in the same row indicated significant differences (Duncan's test).*

Parameter	***Abies religiosa***		***Quercus* spp.**	
	Medium	**Large**	**Medium**	**Large**
pH	4.77 ± 0.20 ^{a}	5.41 ± 0.83 ^{a}	5.33 ± 0.46 ^{a}	5.34 ± 0.85 ^{a}
EC dS m^{-1}	0.57±0.15 ^{a}	0.47±0.24 ^{ab}	0.29±0.21 ^{b}	0.22±0.10 ^{b}
Ca^{2+} $cmol^{+}$ L^{-1}	0.92±0.03 ^{ab}	1.66±0.88 ^{a}	1.03±0.64 ^{ab}	1.05±0.56 ^{b}
Mg^{2+} $cmol^{+}$ L^{-1}	3.96±5.51 ^{a}	2.87±2.31 ^{a}	1.89±2.85 ^{a}	0.57±0.20 ^{a}
Na^{+} $cmol^{+}$ L^{-1}	1.95±1.48 ^{a}	1.71±1.67 ^{ab}	1.07±1.45 ^{ab}	0.81±0.58 ^{b}
K^{+} $cmol^{+}$ L^{-1}	1.85±1.63 ^{a}	0.90±0.81 ^{a}	0.61±0.53 ^{a}	1.06±1.80 ^{a}
Cl^{-} g L^{-1}	7.0±1.26 ^{a}	6.87±1.70 ^{a}	7.43±3.10 ^{a}	8.80±2.60 ^{a}
C g L^{-1}	20.20±14.0 ^{a}	15.51±22 ^{a}	16.71±11 ^{a}	14.09±80 ^{a}
C (soil) g kg^{-1}	19.85±5.8 ^{a}	20.55±40 ^{a}	18.47±80 ^{a}	20.04±2.70 ^{a}

***Table 4.** Values of F from analysis of variance for the support tree (T), altitude (Al), and size (S) effects on physical and chemical parameters from water accumulated in T. violacea. * = significant differences to P < 0.05; df =1, 45*

Parameter	**T**	**Al**	**S**	**T x Al**	**T x S**	**Al x S**	**T x Al x S**
pH	1.12	1.81	0.19	4.36*	4.36*	3.30	0.60
EC dS m^{-1}	3.92	1.78	1.74	1.66	1.06	2.69	1.79
Ca^{2+} $cmol^{+}$ L^{-1}	9.35*	6.06*	4.63*	5.85*	4.58*	1.08*	1.11
Mg^{2+} $cmol^{+}$ L^{-1}	3.87*	1.52	2.54	0.002	1.46	0.26	0.05
Na^{+} $cmol^{+}$ L^{-1}	4.98*	2.25	3.16	1.23	2.78	2.70	1.18
K^{+} $cmol^{+}$ L^{-1}	4.48*	1.78	2.18	1.03	2.73	2.39	2.61
Cl^{-} $cmol^{+}$ L^{-1}	0.09	0.49	0.06	1.33	1.72	0.02	0.02
C g L^{-1}	0.03	0.60	0.11	0.01	0.37	0.16	0.07
C (soil) g kg^{-1}	0.01	0.82	0.08	1.78	2.07	1.48	0.28

***Table 5.** Correlation coefficients between density of main arthropod groups and physical and chemical parameters from water accumulated in T. violacea. *P < 0.05, N = 55*

Parameter	**Mesostigmata**	**Collembola**	**Psocoptera**	**Homoptera**
pH	0.11	0.13	-0.02	0.008
EC	**0.96***	**0.96***	0.50	0.46
Ca^{2+}	0.30	0.12	0.17	**0.30***
Mg^{2+}	-0.08	-0.05	0.004	0.01
Na^{+}	-0.37	-0.11	-0.77	0.74
K^{+}	-0.68	-0.48	0.56	-0.71
Cl^{-}	0.05	-0.08	-0.17	0.04
C dissolved	**-0.36***	-0.21	**-0.47***	**-0.31***
C (solid fraction)	0.07	0.19	0.09	0.10

The results of the MANOVA, to evaluate the effect of the altitude, supporting tree and the size of the plant and the interactions between them, are shown in *Table 7*. Supporting tree had a significant effect on the amount of magnesium and organic carbon, whereas the size has effect on the pH. Season and tree species have an interactive effect on the pH, while interaction of tree and the size of the epiphytic plant influenced significantly the amount of total organic carbon (TOC).

Multiple correlations of the chemical parameters and the fauna, including the weight of soil obtained, altitude, season, tree and size of epiphytic were significant for the total abundance and also for the most abundant groups of arthropods (*Table 8*).

Most correlations of the fauna groups were negative with the pH and TOC, whereas with soluble base contents, such as sodium and potassium were positive. There are strong differences between the parameters that affect playing a role on the abundance of arthropods during the seasons that were compared. Percentage of TOC has a significant correlation with (r = -0.17, $P < 0.05$) and the season of sampling (r = -0.33, $P < 0.05$); we have found higher amount at lower altitude and during the rainy season.

A *t*-test (*Table 9*) was done to evaluate the differences among soil and water parameters. Significant differences were observed in all the parameters. Values were higher in soil samples than in water samples, except for sodium. The pH was more basic in water than in the soil.

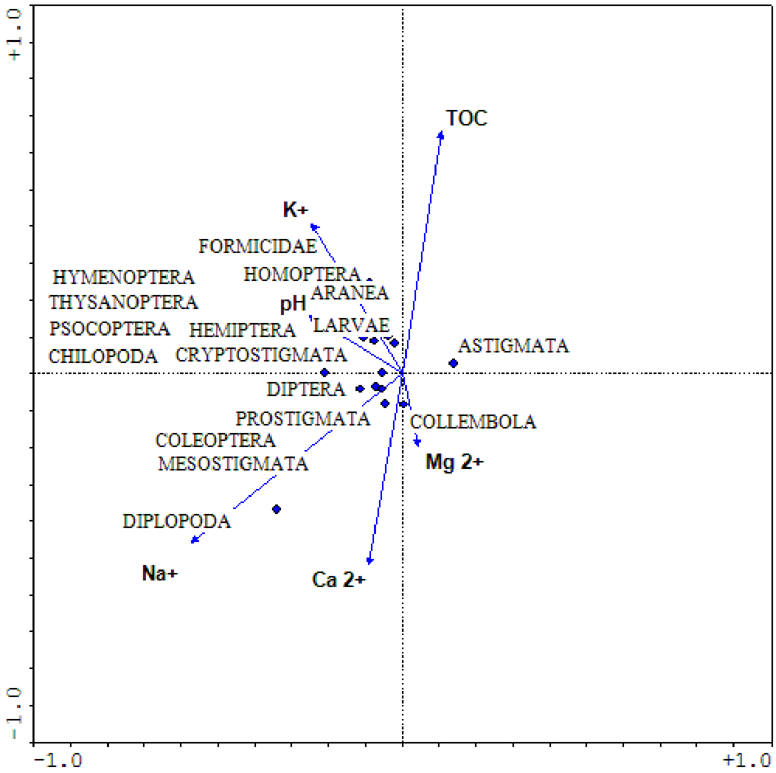

Figure 1. *CCA analysis for the edaphic parameters and composition of arthropods in T. violacea for the dry season.*

The CCA analysis for the dry season show that canonical axes explain 93.75% of observed variance in arthropods composition, with Na^+ best explaining axis 1 (r = 0.56) and TOC best correlated to axis 2 (r = 0.66; overall Monte Carlo test of significance P

= 0.05 with 500 permutations; *Figure 1*). The two axes explain 65.1% of total variance observed. In general, most of arthropod groups are related with axes 1, while Astigmata and Collembola are better related with axes 2.

For the rainy season, according with CCA analysis, canonical axes explain together 95.86% of the variance in composition. The Na^+ (r = 0.75) and TOC (r = 0.64) are the best correlated with axis 1, while the axes 2 is better correlated with pH (r = 0.71) and Mg^{2+} (r = 0.55). The overall Monte Carlo test was significant (P = 0.03 with 500 permutations; *Figure 2*). The two axes explain 78.7% of total variance. Collembola, Isopoda, Neuroptera and Thysanoptera, were the groups better related with axes 2.

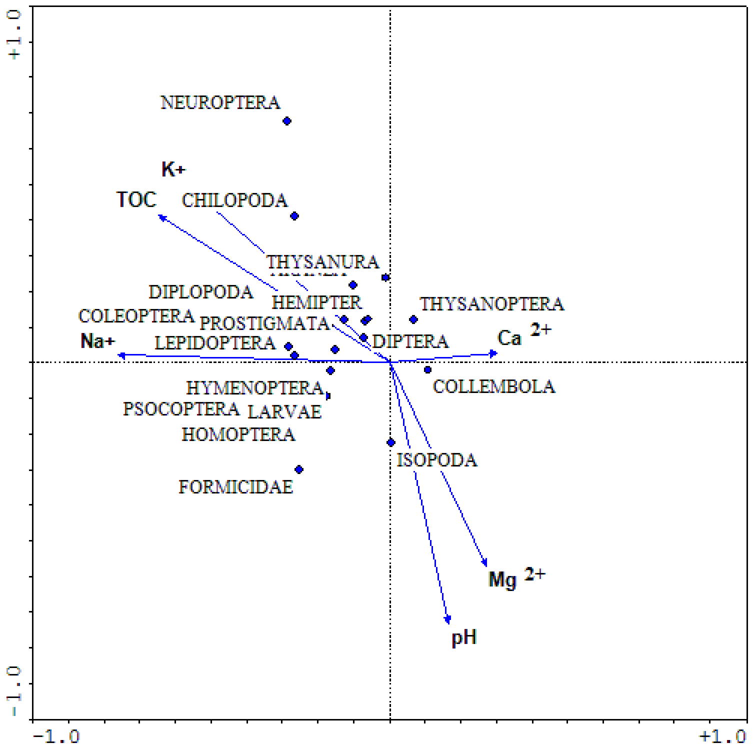

Figure 2. *CCA analysis for the edaphic parameters and composition of arthropods in T. violacea during rainy season.*

Table 6*. Average. standard error, range and U values for physical and chemical parameters from accumulated soil in T. violacea at two altitudes in "El Chico" National Park, Hidalgo State, during rainy (September 1998) and dry (April 1999) seasons. ns= no significant; *P<0.05, **P<0.005*

Parameter	**Altitude 2, 765**		**Altitude 2,900**		***U***	***P***
	Rainy Season N=28	**Dry Season N=50**	**Rainy Season N=21**	**Dry Season N=50**		
pH	4.42 ±0.43 (3.7-5.1)	4.48 ±0.5 (3.7-6.9)	4.49 ±0.43 (3.8-5.3)	4.5 ±0.77 (3.4-8.3)	2320	ns
Ca^{2+} $cmol^+$ kg^{-1}	22.5 ±7.51 (10.5-42)	30.53 ±14.02 (13.8-30.5)	27.54 ±8.93 (14.95-43.05)	29.81 ±17.91 (9.2-97.75)	2690	ns
Mg^{2+} $cmol^+$ kg^{-1}	9.92 ±6.56 (1.15-22.05)	19.05 ±21.86 (1.15-97.75)	10.89 ±5.25 (1.15-20.7)	19.26 ±17.76 (2.3-103.5)	2336	ns
Na^+ $cmol^+$ kg^{-1}	0.58 ±0.25 (0.26-1)	0.47 ±0.07 (0.35-0.7)	0.54 ±0.21 (0.26-1)	0.66 ±0.24 (0.3-1.43)	1964	**
K^+ $cmol^+$ kg^{-1}	4.16 ±2.09 (1.54-9.74)	4.54 ±3.06 (1.59-19.23)	3.66 ±2.14 (0.49-7.95)	5.13 ±5.42 (0.66-34.87)	2634	ns
C g kg^{-1}	29.33 ±3.81 (22.18-36.08)	27.68 ±3.27 (21.04-35.75)	29.52 ±2.98 (22.08-35.1)	26.47 ±4.04 (17.16-30.07)	2275	ns

***Table** 7. F values form MANOVA to evaluated the support tree species (T), size (S) of T. violacea (excluding small plants), collect season (E), altitude (Al), and their interactions effects on physical and chemical parameters for accumulates soils in "El Chico" National Park, Hidalgo State. *P<0.05 ; df= 1,102.*

Source	pH	Ca^{2+} cmol^{+} kg^{-1}	Mg^{2+} cmol^{+} kg^{-1}	Na^{+} cmol^{+} kg^{-1}	K^{+} cmol^{+} kg^{-1}	C mg g^{-1}
T	0.65	0.07	0.21	1.71	2.35	0.23
E	0.17	2.93	**6.06***	0.42	0.75	**17.42***
S	**5.22***	1.85	1.34	1.61	1.36	0.16
Al	0.30	0.90	0.61	0.95	0.0002	1.28
T*E	**3.99***	1.13	0.81	0.42	0.60	1.71
T*S	0.09	0.52	1.14	0.09	0.11	**8.12***
T*Al	0.28	0.22	1.13	0.21	0.34	0.70
E*S	0.09	0.001	1.51	0.06	0.07	2.26
E*Al	0.06	0.77	0.39	**15.48***	0.61	1.70
S*Al	0.02	0.28	0.33	0.22	**4.34***	1.81
T*E*S	0.06	0.42	1.43	1.55	0.02	0.58
T*E*Al	0.41	0.15	1.42	0.10	0.01	0.01
T*S*Al	0.08	0.50	2.83	0.07	0.59	0.13
E*S*Al	0.15	0.39	0.63	2.99	0.08	0.27
T*E*S*Al	0.27	**3.4***	**4.01***	0.004	1.55	0.003

***Table 8.** Multiple regression coefficients (r) between fauna and physical and chemical parameter from soil accumulated in T. violacea. T= Tree; S= Size; Al= Altitude; E= Collect season; W= recuperated soil weight; %C= Carbon percentage; Ca= Calcium; Mg= Magnesium; Na= Sodium; K= Potassium; df= 11,113,*= P<0.05*

Taxa	T	S	Al	E	W	%C	Ca	Mg	Na	K	pH	r	*F*	*P*
Cryptostigmata	0.16*	0.35*	0.18*	0.17*	0.005	-0.13	-0.26*	-0.15	0.13	0.09	-0.54*	**0.68**	9.63	0.00001
Prostigmata	0.13	0.31*	0.16	0.17	-0.03	-0.08	0.20	-0.15	0.14	-0.02	-0.41*	**0.57**	4.89	0.00004
Astigmata	-0.01	0.05	-0.01	0.31*	0.29*	-0.12	-0.20	0.04	0.07	0.28*	-0.08	**0.55**	4.52	0.00001
Collembola	0.12	0.28*	0.17*	-0.14	0.23*	-0.20*	0.13	-0.05	-0.01	0.03	-0.29*	**0.62**	6.39	0.00001
Larvae	0.11	0.34*	0.03	-0.20*	0.14	0.03	0.003	0.03	0.01	-0.001	0.06	**0.51**	3.66	0.0002
Diptera	0.03	0.49*	0.03	-0.28*	-0.16	0.01	-0.06	0.05	-0.09	0.05	0.02	**0.54**	4.16	0.00004
Homoptera	0.16	0.04	0.002	-0.33*	-0.001	-0.04	-0.16	0.10	-0.13	-0.19*	0.14	**0.42**	2.26	0.02
Thysanoptera	0.02	-0.14	0.28*	-0.08	0.44*	-0.04	-0.23	0.02	-0.15	-0.11	0.30*	**0.52**	3.74	0.0001
Araneae	-0.07	0.20*	-0.17*	0.45*	0.08	0.12	0.06	-0.09	-0.07	-0.22*	0.36*	**0.61**	6.20	0.00001
Coleoptera	0.15	0.40*	-0.10	-0.36*	-0.12	0.05	-0.07	0.07	0.03	0.02	0.03	**0.58**	5.19	0.00001
Total abundance	0.09	0.24*	0.02	0.15*	0.32*	-0.21*	-0.03	-0.02	0.14	0.22*	-0.10*	**0.69**	9.63	0.00001

Mineralogical analysis

Mineral content of the soil samples from epiphytic plants varied from 5 to 20 g kg^{-1} dry weight in all the samples. Feldspars of the plagioclase series and orthoclase; quartz, phytoliths and volcanic glass were the most abundant light minerals with low amounts of apatite and calcium carbonate crystals. Among heavy minerals, we identified pyroxenes of enstatite-hyperstene series and augite, as well as amphiboles like basaltic, green and brown hornblends. These results confirm that this substrate is a real soil, by the presence of minerals.

***Table 9**. Values of t test between the chemical parameters of water and soil from T. violacea*

Parameter	t	df	P
pH	7.26	106	0.001
Calcium	20.4	103	0.001
Magnesium	9.92	103	0.001
Sodium	2.04	103	0.04
Potassium	3.24	103	0.001
Carbon	125	103	0.001

Discussion

The importance of the epiphytic plants in the uptake, storage and release of nutrients, which represent main processes for the functioning of the ecosystems, has been observed in different vegetation types (Coxson and Nadkarni, 1995). The presence of different rock-derived minerals in the solid fraction confirms that this material is in fact a suspended soil and not just an accumulation of organic matter and detritus.

Our results showed that the ions contents in the water and soil accumulated in *T. violacea* are strongly affected by the supporting tree species, because the storage and soluble nutrients depend on the quality of litter and its decomposing rate. For instance, it has been found that concentrations of phosphorus and potassium are usually larger in *Abies* forest than in other conifers (Kavvadias et al., 2001). Also, the rate of decomposition of oak litter is 50% higher than those of pine as has been documented in central Himalaya (Upandhyay et al., 1989; Usman et al., 2000). The concentration of potassium in water and soil was higher in the epiphytic plants sampled from *Abies religiosa* than those from *Quercus* spp., as found by other authors in localities from Canada and France (Lavigne et al., 2001; Ponette et al., 2001; Elliot et al., 2002).

The presence of different ions and their availability are necessary for various processes of the plants and ecosystems. For example, the potassium gathered with nitrogen and phosphorus have been related to an increase of growth of *Tillandsia guatemalensis* in greenhouses; whereas when they occur separately, they have little effect (Castro-Hernández et al., 1999).

Many studies on nutrient cycling show that, in the tropical forest, primary production could be limited by the availability of potassium (Vitousek, 1984; Tanner et al., 1998; Bedford et al., 1999). The Ca/Mg ratio has been used as an index of the influence of the vegetation in the biogeochemical cycles of the ecosystem (Quideau et al., 1999). The presence of calcium is related to vegetation and it is essential for the development of plant roots.

The concentration of ions in the water is lower than that found in the phytotelm of the Bromeliaceae in tropical forest in Puerto Rico (Richardson et al., 2000a). Nevertheless, in our study in the suspended soil, the calcium, potassium and magnesium concentrations were higher than those reported in plants studied in Puerto Rico. Our explanation of these differences can be related to the rain pattern and the period of time that the plants lack of water. It has been found that the tank bromeliads store large amounts of water together with organic residues (mainly leaves of the host tree) and suspended soils. There is a high concentration of nitrogen, mainly due to the action of the rain, which carries this nutrient from the canopy (Hietz et al., 1999; Zotz and Hietz, 2001). On the other hand, in our work we found a contrasting effect depending on the season of collection which was very important in determining the accumulation of nutrients. During the dry season, ion concentration and TOC were higher than in the rainy season. Also, we recorded a higher abundance of the arthropods from epiphytic plants during this season. On the other hand, the concentration of the salts was positively related with the abundance of the fauna as it happens in ephiphytes at the tropical rain forests (Richardson et al., 2000a).

Epiphytic plant size is a very important factor, as it has been directly related with the water and suspended soil storage capacity (Zotz and Thomas, 1999), as well as with the abundance of the inhabiting fauna. A higher accumulation of detritus and water influences the rate processes for aerial soil formation, and it promotes biological activity to increase the nutrient fluxes in the system and higher amounts of nutrients can be assimilated at shorter or longer periods.

It has been found that the amount of TOC is correlated with the occurrence of some of the microbial heterotrophic processes, such as respiration, denitrification and also with leaching of organic carbon (Andersson et al., 1994).

The value of the quantified parameters is different from those that have been measured in tank bromeliads in tropical forest. Those differences include the water and soil accumulated in *T. violacea* in El Chico; our results (*Table 1*) shown lower values than those recorded by Richardson et al. (2000b) for *Guzmania* spp. and *Vriesea sintenisii* in a tropical rain forest in Puerto Rico. An explanation to this, is the relationship between pH and DOC. Richardson et al. (2000b) found a pH of 5.6, while ours were around 4 (*Tables 1* and *6*). It has been observed that an increase in the pH, increases microbial activity, rising the deposition of DOC and dissolved organic nitrogen (DON), due to the production of a higher amount of negative charge colloids in the humus, and also the increase of solubility (Andersson et al. 2000). In this study the pH and the organic carbon were the most significantly correlated factors and it was also found that the highest abundance of arthropods was at pH levels between 4 and 5.

The CCA analysis of both rainy and dry seasons, show that the pH, TOC, and Na^+ and Mg^+ contents are the main factor to explain the variation in the composition of arthropod community associated to *T. violacea*.

Taking into account of this, the function of *T. violacea* in the temperate forest could play an important role in the storage of nutrients, mainly during the dry season, when it is used as a shelter by different groups of insects. However, during the rainy season, many nutrients become available when they are dissolved into the water, in that form where they can still be used by the larvae of several groups as Diptera. The accumulation of litter in *T. violacea* is a very important factor, because litter production and its fall is the largest pathway for nutrient and organic mater flux into the soils, that is fundamental to understand the ecosystem function (Meentemeyer et al., 1982; Barbosa and Fearniside, 1996).

Studies done in a tropical low forest in Puerto Rico have demonstrated that in this environment the highest accumulation of nutrients and fauna is done by other plants with phytothelm, but not the bromeliads (Richardson et al., 2000a). Recent studies about the origin and formation processes of arboreal soils in Northern California, shown than the climate affects the formation of soil in canopies, by their influence on the OM translocation and decomposition (Enloe et al., 2006, 2009). In an environment with strong seasonal changes, such as the forest from "El Chico", the function of the bromeliads, as shelter and supply of nutrients is of great importance for the arthropods communities mainly for the maintenance of the biological diversity.

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PLANT INVASIONS ACROSS DIFFERENT HABITAT TYPES AT FLORISTIC SURVEY

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Abstract. On the basis of detailed floristic survey the level of invasion in various EUNIS habitat types identified in NW Poland was assessed. In a data set of 2131 floristic lists the mean number and mean proportion of native species, archaeophytes and neophytes was calculated for each of 25 habitat types. Relationships between this three groups of species were analysed using Pearson correlation. A total of 840 vascular plant species, including 77 archaeophytes and 114 neophytes were recorded. The most invaded habitats were: arable land, fallows and field margins, trampled areas, gardens and parks, lines of trees, anthropogenic tall-forb stands (they contained on average 20-67% alien plants). Most of mean numbers and mean percentage numbers of both alien plants groups in particular habitat types were higher compared to the results obtained from phytosociological databases, therefore the level of invasion assessed on the basis of phytosociological data can be underestimated.

Keywords: *alien species, archaeopytes, EUNIS, level of invasion, neophytes*

Introduction

Enriching the existing vegetation of an area with geographically alien species is the most conspicuous anthropogenic effect on the flora. A particularly rapid increase in the intensity of alien plants expansion was observed in the recent centuries (Lambdon et al., 2008). It is widely recognized that the invasion of alien plants (*sensu* Pyšek et al., 2004) threats natural ecosystems, as well as human health and economy (Wade et al., 1997; Pimentel et al., 2005; Tokarska-Guzik et al., 2006; Hejda et al., 2009; Follak et al., 2013), therefore investigating the causes and mechanisms of biological invasions is one of the most urgent task of modern geobotany. A very important line of research tackles the extent of resistance of various habitats and plant communities to penetration of adventive species. Individual habitats vary considerably in their susceptibility to invasion (invasibility *sensu* Lonsdale, 1999; Richardson and Pyšek, 2006), as well as actual level of invasion (*sensu* Hierro et al., 2005), so reliable quantitative information is crucial for effective management and planning of invasive plant control, but studies providing solid quantitative data are still rather few (Pyšek et al., 1998; Chytrý et al., 2005; Vilà et al., 2007; Botta-Dukát, 2008; Sîrbu et al., 2012).

The most of analyses of the level of invasion in different habitats are based on phytosociological relevés, which were made with the purpose to study particular vegetation, not to study particular habitat. Examples using a systematic floristic study of the area are extremely rare (Pyšek et al., 2002; Jauni and Hyvönen, 2010). It would be interesting to compare the results obtained from systematic detailed floristic survey through the habitats with those from phytosociological databases. Studies of this type, carried out in an area under legal protection, with very few habitats altered by human activities, identify which less-disturbed, (semi-)natural habitats can be vulnerable to the spread of alien species. That is the reason why the present study was based on floristic research conducted in Barlinek-Gorzów Landscape Park (NW Poland).

The high quality assessment of particular species in the local and regional floras investigated with respect to their taxonomic identity, time of immigration and invasion status is crucial for comparative analyses (Pyšek et al., 2004). In recent floras alien plant species are classified according to their invasion status as casual or naturalized and according to their residence time into archaeophytes (species that arrived before AD 1500) and neophytes (species introduced after that date). The establishment and spread of naturalized neophytes in Poland is well documented (Tokarska-Guzik, 2005) and the list of archaeophytes is available (Zając, 1979), but a precise assignation of some species is still doubtful (Mirek et al., 2002).

The major aims of this work was (1) to assess the level of invasion in various habitat types of the studied area, expressed as the number of alien species they harbour and the proportion of aliens to the total number of species, and to compare it with other authors results obtained from phytosociological databases, (2) to provide the lists of archaeophytes and neophytes which occur in the broadest range of habitats, (3) to determine relationships between alien and native species across and within habitats.

Material and methods

Study area

Barlinek-Gorzów Landscape Park (BGLP) is located in the North-West of Poland, at 52°48'N-53°05'N and 15°08'E-15°26'E. It encompasses a valuable, about 24 000 ha, fragment of Pomeranian Lakelands. The greater part of this area is a sandy sandr varied with postglacial channels and peat basins, while its very northern part presents glacial forms connected with the southern range of the Pomerania phase of the Vistula glaciation (Kondracki, 2000). The frontal moraine features humus-rich podsols overlaying till clay as well as brown forest soils lined by heavy clay. The glacial outwash plain is covered mainly by podsols devoid of any clay lining (Mikołajski, 1966). This area is in the zone of influences of the oceanic climate. The mean annual air temperature is 8.1° C. The mean annual precipitation sum is 500-600 mm, with distinct decrease during summer months. The duration of the vegetation season ranges from 200 to 220 days (Koźmiński and Michalska, 2001).

Forests, covering about 80% of the Park's surface area, are the most important component of its natural world. The forests are mainly broadleaved deciduous forests (in particular acidophilous beech-oak forest, lowland forb-rich and acidophilous beech forests) as well as mixed broadleaved deciduous and coniferous forests. Coniferous forest, riparian woodlands and alder carrs are less common. Due to the human activity the tree stands are often dominated by the artificially introduced *Pinus sylvestris*. Important are also *Fagus sylvatica* subsp. *sylvatica*, *Quercus robur* and *Q. petraea*, followed by *Carpinus betulus*, *Betula pendula*, and the artificially introduced *Picea abies*. The study area is also characteristic for chains of post-glacial lakes with small rivers flowing through them. Compared to adjacent areas, the Park's natural environment is relatively weakly transformed due to the low intensity of urbanisation and industrialisation – the area lacks large urban centres, major roads and railway tracks. Anthropogenic pressure is basically limited to forest management, extensive agriculture on the northern part, and tourism.

Data collecting and statistical analyses

The present study is based on the research on distribution and anthropogenic transformations of the BGLP vascular flora, carried out in 1998-2002 (Myśliwy, 2003). The Park area was divided into 271 quadrants (side length of 1 km), which constitute a decimal resolution of the cartographic grid used in the “Distribution Atlas of Vascular Plants in Poland” (Zając, 1978); each quadrant was equivalent to a site. On average 7-8 floristic lists were obtained in each quadrant, with a due consideration to the full variety of habitats (plots 40-200 m^2). Only vascular plants were recorded. On account of the application of a cartogram grid to systematic field surveys, the difference in the number of floristic lists in individual habitat types is more a result of habitat availability in an area than of inhomogeneity of survey coverage. The total number of 2131 floristic lists were analyzed in this study.

Classification of habitat types was based on the European Nature Information System (EUNIS) (Davies et al., 2004). Level 2 and Level 3 of the EUNIS hierarchy were used, from the version of classification available online from January 2013 (in one case Level 1 was accepted and in one case two habitat types at Level 3 were merged). Generally, the area was found to support 25 habitat types encompassing a full range from those little altered by anthropogenic influences to the strikingly anthropogenic ones, the latter being rather rare (*Table 1*). Number of lists assigned to particular habitats ranged from one to 479 (*Table 2*).

Each plant species from analysed floristic lists were classified into native, archaeophyte or neophyte, using studies of Zając (1979), Zając et al. (1998), Zając and Zając (2001), Mirek et al. (2002), Tokarska-Guzik (2005) and Tokarska-Guzik et al. (2012). The non-native plants were classified with respect to Western Pomerania as the area of reference, so species native to Poland but alien to NW Poland were treated as aliens. In case of species with doubtful status in the Polish or Pomeranian flora the following assignations were adopted: native species – *Berteroa incana*, *Cirsium vulgare*, *Erysimum cheiranthoides*, *Geranium columbinum*, *Rumex thyrsiflorus*, *Teucrium scorodonia*; neophytes – *Oenothera biennis*, *Primula elatior*, *Viola odorata*; archaeophytes – *Anchusa officinalis*, *Geranium molle*, *Malva alcea*, *Silene latifolia* subsp. *alba*, *Misopates orontium*, *Pastinaca sativa*. For each floristic list the number of species within each of above three categories was counted, as well as proportion of archaeophytes and neophytes from total number of species was calculated. The mean number and mean proportion of native species, archaeophytes and neophytes was then calculated for each habitat type. To check if the differences were statistical significant the non-parametric Kruskall-Wallis test of ranks was used. The proportion of floristic lists with at least one alien species and at least one neophyte was also calculated for each habitat type.

Relationships between native species, archaeophytes and neophytes across and within habitat types were analysed using Pearson correlation. The number of species within each species group and each floristic list was square-root transformed after adding 0.5 to each value. For the analyses across habitats averages of these transformed values were taken. All statistical analyses were performed using the STATISTICA 10 program (www.statsoft.com).

Names of vascular plant species follow Flora Europaea (Tutin et al., 1964-1980).

Table 1. *The list of EUNIS habitat types identified in the study area (NW Poland)*

EUNIS habitat type			**Habitats sampled**
Level 1	**Level 2**	**Level 3**	
C: Inland surface waters	C1: Surface standing waters		lakes, fish ponds, midfield and mid-forest waterbodies, drainage ditches
	C2: Surface running waters		small lowland rivers
	C3: Littoral zone of inland surface waterbodies		waterfringing vegetation by rivers, lakes, ponds, ditches and waterbodies
D: Mires, bogs and fens	D1: Raised bogs & D2: Valley and transition mires & D4: Base-rich fens		mires, bogs, fens
E: Grasslands and lands dominated by forbs, mosses or lichens	E1: Dry grasslands		sand and xerothermic grasslands
	E2: Mesic grasslands		*Arrhenatheretalia* meadows and pastures, usually managed
	E3: Wet grasslands		*Molinietalia* meadows usually unmown
	E5: Woodland fringes and clearings and tall forb stands	E5.1: Anthropogenic herb stands	semi-natural vegetation of roadsides and neighborhoods of rural cottages
		E5.2: Thermophile fringes of woodland & E5.4: Moist or wet tall-herb fringes	vegetation at sunny and shaded edges of woodlands, along waterbodies and watercourses, on forest-dividing lines
F: Heathland, scrub and tundra	F3: Temperate and mediterranean scrub		temperate thickets and scrub
	F9: Riverine and fen scrubs		riverside, lakeside and fen scrub of *Salix* and/or *Alnus* ssp.
	FA: Hedgerows		strips of shrubs within cultivated land or along roads
G: Woodland, forest and	G1: Broadleaved deciduous woodland		woodland, forest, plantation dominated by broadleaved deciduous trees

other wooded land	G3: Coniferous woodland		woodland, forest and plantations dominated by coniferous trees
	G4: Mixed deciduous and coniferous woodland		woodland, forest of mixed broadleaved deciduous and coniferous trees
	G5: Lines of trees, small anthropogenic woodlands, recently felled woodland, early-stage woodland and coppice	G5.1: Lines of trees	lines of trees along paths and roads
		G5.6: Early-stage natural and semi-natural woodlands and regrowth	woodland regrowth, including raised bog pre-woods
		G5.8: Recently felled areas	clearings
H: Inland unvegetated or sparsely vegetated habitats	H5: Miscellaneous inland habitats with very sparse or no vegetation	H5.6: Trampled areas	unsurfaced pathways
I: Regularly or recently cultivated agricultural, horticultural and domestic habitats	I1: Arable land and market gardens	I1.3: Arable land with unmixed crops grown by low-intensity agricultural methods	cereal, rape and root crops
		I1.5: Bare tilled, fallow or recently abandoned arable land	fallows, arable field margins, forest plots tended by hunters
J: Constructed, industrial and other artificial habitats	I2: Cultivated areas of gardens and parks		small village parks, old cemeteries
	J2: Low density buildings	J2.5: Constructed boundary	fences and walls
	J4: Transport networks and other constructed hard-surfaced areas	J4.1: Disused road, rail and other constructed hard-surfaced areas	disused railway track
		J4.2: Road networks	gravel and stone paved roads

***Table 2.** Mean numbers of species in floristic lists assigned to particular habitat types*

EUNICE[1] habitat type	**n[2]**	**Mean No. of species**			**Mean % No. of species**		
		Nat[3]	**Arch[4]**	**Neo[5]**	**Nat[3]**	**Arch[4]**	**Neo[5]**
C1 Standing waters	80	4,5	0	0,1	97,4	0	2,6
C2 Running waters	13	2,5	0	0,1	98,9	0	1,1
C3 Littoral zone	119	11,3	0,1	0,2	98,6	0,3	1,2
D Mires, bogs, fens	66	23	0,1	0,3	98,5	0,3	1,2
E1 Dry grasslands	39	45,5	6,9	2,8	82,7	12,2	5,1
E2 Mesic grasslands	35	37,9	4,6	1,4	86,9	9,9	3,1
E3 Wet grasslands	85	46,9	0,7	0,5	97,6	1,3	1,1
E5.1 Anthropogenic tall-forb stands	179	26,7	3,2	2,9	79,5	9,7	10,8
E5.2 & E5.4 Woodland fringes	479	18,8	0,7	1,1	91,1	2,9	6,1
F3: Temperate scrub	9	29,3	0,8	2,7	88,8	2,2	9
F9: Riverine and fen scrubs	22	24	0,2	0,6	96,7	0,6	2,7
FA: Hedgerows	21	36,1	4,5	4,3	80,6	9,9	9,5
G1: Broadleaved woodland	380	29,4	0,3	1,3	95,5	1	3,6
G3: Coniferous woodland	189	21,1	0,2	1,1	93,7	0,8	5,5
G4: Mixed woodland	220	23,4	0,2	1	95,2	0,8	4
G5.1: Lines of trees	16	33,3	3,8	4	77,8	9,7	12,5
G5.6: Early-stage woodlands	34	29,1	0,8	1,5	92,1	2,4	5,5
G5.8: Recently felled areas	12	25,4	1,4	1,3	82,7	3,2	14,1
H5.6: Trampled areas	26	10,7	2	1,3	76	13,2	10,8
I1.3: Arable land	31	5,7	9,6	1	32,9	59,2	7,8
I1.5: Fallows, fields margins	50	25,6	8,7	2,6	66,1	26,4	7,5
I2: Gardens and parks	6	35	0,7	9	77,5	1,8	20,7
J2.5: Constructed boundaries	1	-	-	-	-	-	-
J4.1: Disused rail	5	31,4	1,2	1,2	93,1	3,8	3,1
J4.2: Road networks	14	11,8	1,9	0,7	83,3	12,2	4,5

[1] *EUNIS habitat names are abbreviated: for full names see Table 1;* [2] *n: number of floristic lists;* [3] *Nat: native species;* [4] *Arch: archaeophytes;* [5] *Neo: neophytes*

Results

The data set of 2131 floristic lists contained 649 (77.3%) native species, 77 (9.2%) archaeophytes and 114 (13.6%) neophytes (including 88 naturalized and 26 casual). The average proportion (± standard deviation) of this three species groups in individual floristic list was 90.6 ± 13.2%, 4.2 ± 10.1% and 5.2 ± 7.6 % respectively. The total number (species pool) of archaeophytes and neophytes in particular habitats ranged in turn: 0-61 and 1-71.

The list of archaeophytes and kenophytes occurring in the highest number of habitats were compiled (*Table 3*). Among archaeophytes 19 species (24.7%) may be considered as generalists (they occupied more then ten habitat types), while 38 (49.4%) – as specialists (occurring in 1-5 habitats). In case of neophytes the group of specialists was composed of 77 (67.4%) species, while only 15 neophytes (13.2%) were generalists (all of them are naturalized species in Poland). The group of species, occurring in 6-10 habitat types were similar for archaeophytes and neophytes – 20 (26.0%) and 22 (19.3%) species respectively.

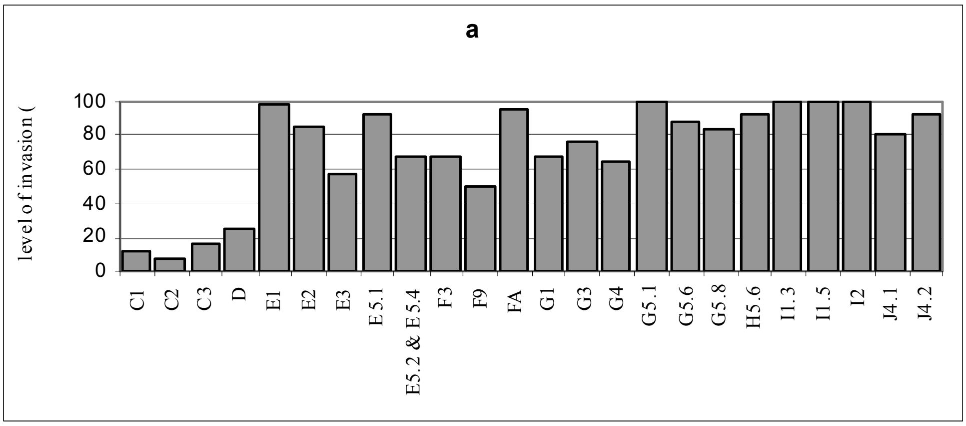
***Table 3**. Fifteen archaeophytes and neophytes with the broadest habitat range (n = 25). Species are ranked in decreasing order according to the number of EUNIS habitat types in which they were recorded*

Archaeophytes	No. of habitats	Neophytes	No. of habitats
Myosotis arvensis	18	*Conyza canadensis*	18
Fallopia convolvulus	17	*Impatiens parviflora*	18
Silene latifolia subsp. *alba*	16	*Quercus rubra*	15
Vicia tetrasperma	16	*Epilobium adenocaulon*	14
Capsella bursa-pastoris	15	*Oxalis europaea*	14
Matricaria perforata	15	*Picea abies*	14
Bromus sterilis	14	*Robinia pseudacacia*	13
Geranium pusillum	14	*Senecio vernalis*	13
Senecio vulgaris	14	*Violoa odorata*	13
Vicia hirsuta	14	*Erigeron annuus*	13
Anagallis arvensis	13	*Aesculus hippocastanum*	12
Lactuca serriola	13	*Juncus tenuis*	12
Lamium purpureum	13	*Prunus serotina*	12
Viola arvensis	13	*Pyrus communis*	12
Ballota nigra	12	*Solidago canadensis*	11

The proportion of floristic lists containing at least one alien species was very high for most of habitat types, the same was stated in case of neophytes (*Fig. 1a, 1b*). Considering the total number (species pool) of both alien groups in each habitat type greater differentiation between habitats was obtained (*Fig. 2*). When the habitat comparison was based on total number of occurrences (records) of alien species instead of total number of species – few habitats remained among the most invaded (*Fig. 3*).

The mean number of species per floristic list was the highest in grasslands, especially in dry (E1) and wet grasslands (E3), while the lowest – in waters (C1, C2), followed by littoral zone (C3), trampled areas (H5.6) and road networks (J4.2). There were significant differences in number of native species, archaeophytes and neophytes per floristic list among habitats (Kruskall-Wallis test of rank, H = 699.7872 for native species, H = 877.8600 for archaeophytes, H = 465.2251 for neophytes, $P < 0.001$). Arable land (I1.3), fallows and field margins (I1.5), dry grasslands (E1), followed by mesic grasslands (E2) and hedgerows (FA) harbored the highest mean numbers of archaeophytes (*Table 2*). The highest mean numbers of neophytes were found in gardens and parks (I2), followed by hedgerows (FA), lines of trees (G5.1) and anthropogenic tall-forb stands (E5.1). Considering mean percentage number of alien species trampled areas (H5.6) were among five the most invaded habitats (*Table 2*). The lowest mean numbers and the lowest mean percentage numbers of aliens, both archaeophytes and neophytes, were detected in standing waters (C1), running waters (C2), littoral zone (C3), mires, bogs and fens (D), riverine and fen scrubs (F9), and wet grasslands (E3). Archaeophytes were rare also in woodland habitats: in broadleaved

(G1), coniferous (G3) and mixed woodland (G4), as well as in woodland fringes (E5.2 & E.5.4). An unexpected result was that in road networks archaeophytes were approximately three times more often recorded then neophytes (*Table 2*).



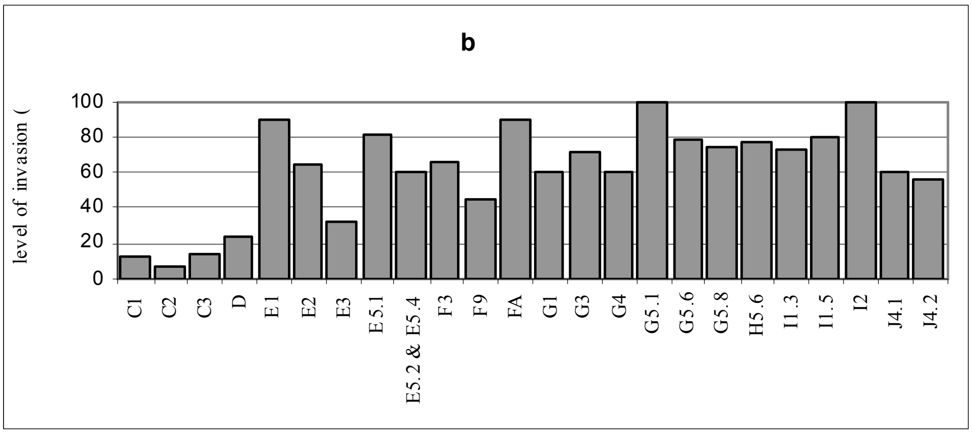


Figure 1. *Level of invasion expressed by the proportion of floristic lists containing at least one alien species (a) or at least one neophyte (b). EUNIS habitat names are abbreviated: for full names see Table 1*

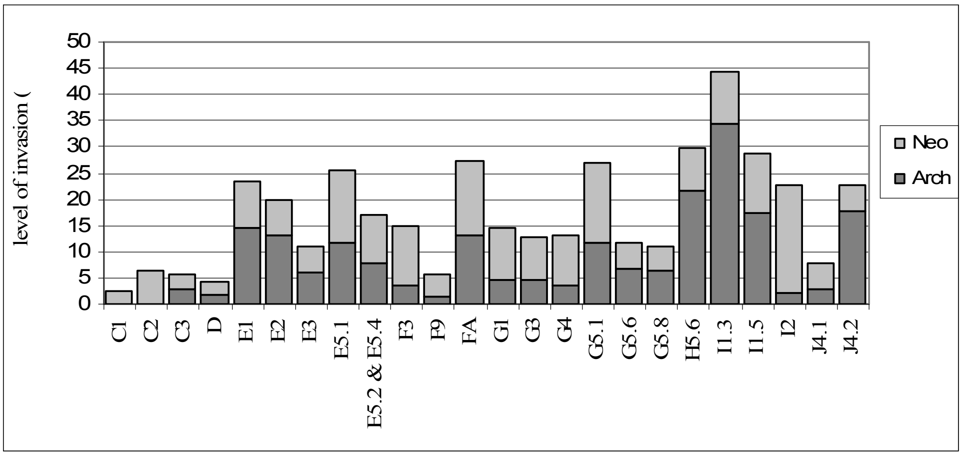


Figure 2*. Level of invasion expressed by the proportion of alien species to the total number of species occurring in the habitat. EUNIS habitat names are abbreviated: for full names see Table 1. Arch: archaeophytes, Neo: neophytes*

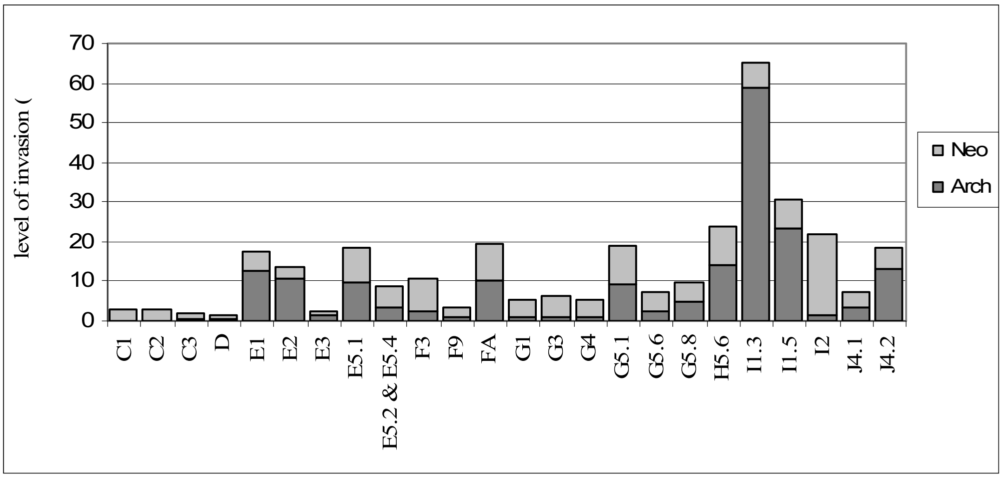


Figure 3*. Level of invasion expressed by the proportion of records of alien species to the total number of records of species occurring in the habitat. EUNIS habitat names are abbreviated: for full names see Table 1. Arch: archaeophytes, Neo: neophytes*

Table 1*. Pearson correlation coefficients between the numbers of archaeophytes (Arch), neophytes (Neo) and native species (Nat) calculated within habitats. Square-root transformed (after adding 0.5) species numbers were used for calculation*

EUNICE[1] habitat type	n[2]	Arch vs. Nat		Neo vs. Nat		Arch vs. Neo	
C1: Standing waters	80	no Arch		n.s.		no Arch	
C2 Running waters	13	no Arch		n.s.		no Arch	
C3 Littoral zone	119	0.483	***	0.318	***	0.325	***
D Mires, bogs, fens	66	n.s.		n.s.		0.311	*
E1 Dry grasslands	39	0.630	***	0.473	**	0.606	***
E2 Mesic grasslands	35	n.s.		n.s.		0.756	***
E3 Wet grasslands	85	0.294	**	n.s.		n.s.	
E5.1 Anthropogenic tall-forb stands	179	0.627	***	0.534	***	0.594	***
E5.2 & E5.4 Woodland fringes	479	0.410	***	0.393	***	0.397	***
F3: Temperate scrub	9	n.s.		n.s.		n.s.	
F9: Riverine and fen scrubs	22	n.s.		n.s.		n.s.	
FA: Hedgerows	21	0.520	*	0.545	*	n.s.	
G1: Broadleaved woodland	380	0.319	***	0.549	***	0.306	***
G3: Coniferous woodland	189	0.512	***	0.418	***	0.327	***
G4: Mixed woodland	220	0.384	***	0.489	***	0.294	***
G5.1: Lines of trees	16	0.627	**	0.689	**	0.574	*
G5.6: Early-stage woodlands	34	0.472	**	0.585	***	n.s.	
G5.8: Recently felled areas	12	0.624	*	n.s.		0.675	*
H5.6: Trampled areas	26	n.s.		n.s.		n.s.	
I1.3: Arable land	31	0.623	***	0.510	**	n.s.	

I1.5: Fallows, fields margins	50	0.599	***	0.557	***	0.590	***
I2: Gardens and parks	6	n.s.		0.885	*	n.s.	
J2.5: Constructed boundaries	1	-		-		-	
J4.1: Disused rail	5	n.s.		n.s.		n.s.	
J4.2: Road networks	14	0.708	**	n.s.		n.s.	
No. of positive significant correlations		15		13		12	
No. of non significant correlations		7		11		10	

[1] *EUNIS habitat names are abbreviated: for full names see Table 1;* [2] *n: number of floristic lists; n.s.: non significant; no Arch: no occurrence of archaeophytes in the habitat; significance levels: ***P < 0.001; **P < 0.01; *P < 0.05*

The analyses of relationship between the number of archaeophytes and native species performed within habitats revealed positive significant relationship in 15 habitats and non significant in seven (*Table 4*). No relationship between the number of species in both above groups was found in the analysis across different habitats (r = 0.162, P = 0.450). In case of relationship between the number of neophytes and native species the within-habitat analyses revealed 13 positive and 11 non significant correlations and significant but not very strong positive correlation in between-habitat analysis (r = 0.503, P < 0.05; Fig. 4). The number of archaeophytes and neophytes is positively correlated in 12 habitats and non significant in ten (*Table 4*). Between-habitat analysis detected no relationship between the number of two latter species groups (r = 0.378, P = 0.069), but the significance was quite close to threshold value.

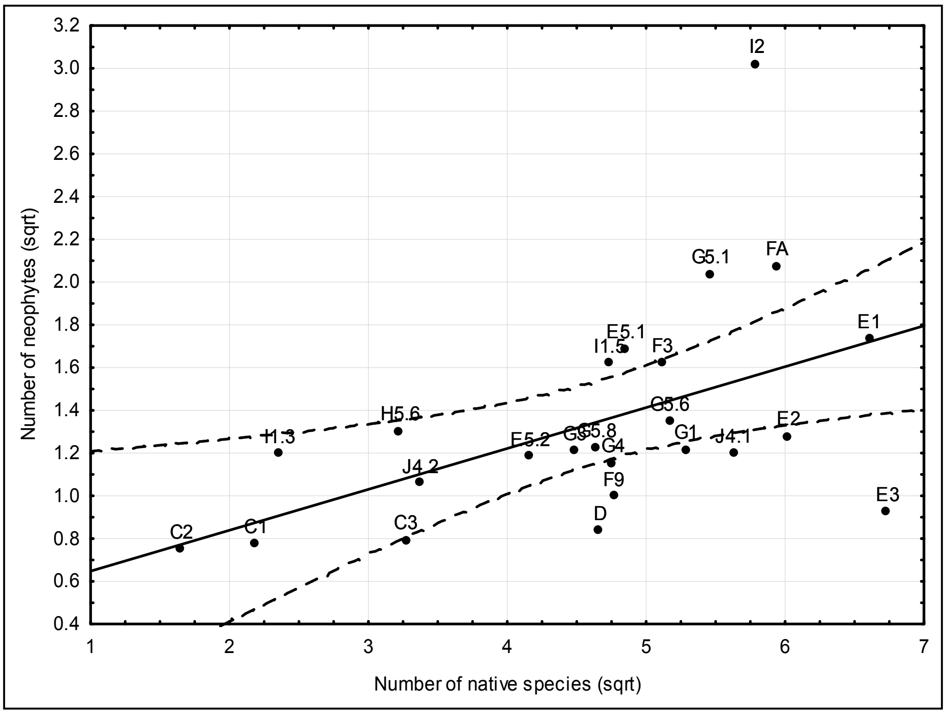


Figure 1. *The relationship between the number of neophytes and native species. Averages from square-root transformed values were used for habitats, 95% confidence interval is shown (r = 0.5, P < 0.05). EUNIS habitat names are abbreviated: for full names see Table 1*

Discussion

The vascular flora of Poland is estimated to contain 3476 species, including 2537 native species (73.0%) and 939 aliens (157 archaeophytes – 4.5%, 764 neophytes – 22.0%, further divided into two groups: established – 370 species, and casual – 394 species, and 18 species of uncertain status in Poland – 0.5%) (Tokarska-Guzik et al., 2012). In the area of BGLP 24.2% species of the entire Polish vascular flora were present (the proportion would be slightly higher if extinct and probably extinct species in the study area were included; compare Myśliwy, 2008a, 2010; Myśliwy and Bosiacka, 2009). Corresponding proportion for alien plants is 20.3%, which shows that obtained data is representative and makes it possible to analyze the local pattern of plant invasions. The fraction of archaeophytes invading BGLP (49.0%) is much higher compared to neophytes (14.9%). The same was detected also in Czech nature reserves (Pyšek et al., 2002).

Although the group of neophytes makes up 20.0% of the flora of Poland, it amounts 5.2% of the species found in an average floristic list (plot), exceeds 10% in anthropogenic tall-forb stands, trampled areas, lines of trees and recently felled areas, and only in case of gardens and parks reaches 20.7%. This contrast is mainly due to rare casual species, what pointed out e.g. Chytrý et al. (2005). Indeed in the data set used in this study neophytes were over-represented among very rare (1-3 sites of a total 271) and rare (4-8 sites) species (together they constitute 61.4% of all neophytes recorded). Very common (136-202 sites) and common (68-135 sites) neophytes were as follow: *Picea abies*, *Juncus tenuis*, *Impatiens parviflora*, *Conyza canadensis*, *Oxalis europaea* and *Prunus serotina*. The archaeophyte most common in the study area was *Fallopia convolvulus*, followed by *Capsella bursa-pastoris*, *Silene latifolia* subsp. *alba*, *Matricaria perforata* and *Lamium purpureum*. However very rare and rare archaeophytes constitute only 35.1% of all archaeophytes recorded in BGLP, so the rest of them are more frequent in the study area. This is in line with the proportion of archaeophytes in floristic lists (4.2%), which is similar to their proportion in the total flora of the country (4.5%). The same pattern of alien species frequency was detected in other neighboring landscape parks in Poland (Stępień, 2008, 2009), and it can be explained with the duration of their spreading over the territory and colonizing different habitats, which is clearly longer for archaeophytes (Chytrý at al., 2005; Pyšek et al., 2005; Richardson and Pyšek, 2006; Jauni and Hyvönen, 2010).

It must be underlined that most of mean numbers of archaeophytes and neophytes, as well as their mean percentage numbers obtained in this study were clearly higher compared to the results obtained from phytosociological databases (e.g. Chytrý et al., 2005, 2008; Vilà et al., 2007). This might be caused by the tendency to place phytosociological plots in sites with a high probability of including presumed diagnostic species (Chytrý, 2001) and to omit ecotones and disturbed, untypical phytocoenoses by phytosociologists. Such homogeneous stands of vegetation are probably less invaded, while ecotonal sites can be important habitats of some alien species. Therefore species lists obtained from heterogeneous plots are more complete then phytosociological relevés and give more reliable picture of pattern of plant invasions. The difference in the results would probably be higher if the number of analyzed floristic lists would be larger, comparable to phytosocjological databases, because as it was shown by Sîrbu et al. (2012), the number of relevés per habitat type significantly influences the probability to detect neophytes in a given habitat. In the data set used in this study there were also higher proportion of alien species, both archaeophytes and neophytes, with the broadest habitat range (compare Chytrý et al., 2005), and the higher proportion of lists containing at least one alien (neophyte) species (compare Sîrbu et al., 2012), probably as a consequence of sampling method. The level of invasion assessed on the basis of phytosociological data can be underestimated.

It was confirmed in this study that the most invaded habitats are those nutrient-rich and with frequent disturbances, both anthropogenic and natural, while by contrast nutrient-poor habitats, not affected by man are usually invaded to a lesser degree (Rejmánek, 1989; Deutschewitz et al., 2003; Chytrý et al., 2005, 2008, 2009; Tokarska-Guzik, 2005; Vilà et al., 2007; Sîrbu et al., 2012). Moreover archaeophytes and neophytes have different habitat affinities, which reflects their history of invasion and their ecology in the native range (Pyšek et al., 2005; Richardson and Pyšek, 2006). Archaeophytes tend to be over-represented in arable land, because their spread was caused by agricultural activities (Zając, 1979). Their affinity to dry and mesic grasslands can be explained by their origin from open grasslands and therophytic habitats of southern Europe and Near East – about 60% of archaeophytes recorded in BGLP are of Mediterranean, Mediterraneo-Irano-Turanian and Irano-Turanian origin (Myśliwy, 2008a). Some of them are even diagnostic species of xerothermic communities in Poland (Myśliwy, 2010). On the other hand some dry grasslands have been ploughed in the past and nowadays they often neighbor arable fields, which influence their species composition (Botta-Dukát, 2008). The invasion of neophytes is connected with urban and transport development, and human population density play a significant role in recent alien invasions (McKinney, 2002; Pyšek et al., 2002; Pino et al., 2005; Tokarska-Guzik, 2005), so neophytes are over-represented in ruderal vegetation associated with human settlements. Due to their origin from temperate forests of North America and Eastern Asia in their secondary range neophytes are often components of woodlands and wet habitats (Chytrý et al., 2005, 2008; Botta-Dukát, 2008; Sîrbu et al., 2012). Fragmentation of forest complexes by many kilometers of roads and paths facilitates non-intentional introduction of alien species (Trombulak and Frissel, 2000; Watkins et al., 2003). Fortunately the studied area is crossed by very few paved roads which could act as corridors for aliens to spread into natural communities. Instead, in commercially managed forests, the network of forest dividing-lines have been added, but their influence on forest interior’s flora is moderate, in contrast to typical forest roads (Myśliwy, 2008b). Planting of trees originating both from other continents (particularly North-American) and from elsewhere in Poland (larch and spruce beyond their natural ranges) is an example of intentional introduction. Inappropriate forest management conducted with the direct introduction of alien species shouldn’t be permitted and the principles of environmentally-friendly forestry practices should be observed.

On the example of *Asteraceae* family Jackowiak (1999) analysed the possibilities and limitations in prognosis of further expansion of alien plants and came to the conclusion that the exchange of the flora between various regions of the world has not been completed yet. All alien plants introduced to new areas should be assessed for their potential to escape, naturalize and cause damage. Some of them have the capacity to become invasive and these deserve very close attention (Kowarik, 1995; Wade, 1997; Starfinger, 1998; Pyšek et al., 2004).

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EFFECTS OF ENVIRONMENTAL HETEROGENEITY ON THE COMPOSITION OF INSECT TROPHIC GUILDS

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Abstract. Distribution of the most diverse groups of insects is highly related to the structural complexity of the plant community, and increasing complexity, in turn, correlates with increasing diversity, richness and abundance of insects. As such, this study aims to examine the relationship between environmental heterogeneity and the richness, abundance and trophic guilds of insects. The study was conducted in the Serra da Bodoquena, Mato Grosso do Sul, Brazil. Samples were collected using an entomological net, and analysis of the distribution of functional groups along the environmental gradient was performed by direct ordering. The richness and abundance of insect species differ among the different vegetation types, such as clean pasture or cerrado. Using a functional Heterogeneity Index (HI), generalist groups showed a wide distribution, while more specialized groups were restricted to more structurally complex environments.

Keywords: *structural complexity, environmental gradient, functional groups*

Introduction

The correlation between species diversity and environmental heterogeneity may vary according to the species group and can depend on both biotic and abiotic factors, according to the group being studied (Tews et al., 2004). However, distribution of the most diverse groups of insects is highly related to the structural complexity of vegetation, and, generally, the richness, diversity and abundance of insects increase as environmental heterogeneity increases. This was verified in Coleoptera (Halffter and Arellano, 2002; Almeida and Louzada, 2009; Janssen et al., 2009), Hymenoptera (Fonseca and Diehl, 2004) and Orthoptera (Azevedo et al., 2011).

Through environmental heterogeneity, studies have shown that disturbances in vegetation structure resulting from human or animal activity are harmful to insect fauna and cause a reduction in both diversity and abundance (Fonseca and Diehl, 2004; Vasconcellos et al., 2010). In addition, replacing native vegetation with exotic species, often montypic, e.g., areas with *Pinus* spp., can directly interfere with the richness and abundance of insects (Romero-Alcaraz and Ávila, 2000; Ganho and Marinoni, 2006; Almeida et al., 2011).

Heterogeneity also directly influences the availability of resources and favorable conditions for soil organisms (Warren and Zou, 2002). For example, Orthoptera

(Gryllidae) has increased its richness in more closed, i.e., heterogeneous environments because these sites have high humidity and a high quantity of litter and organic matter (Azevedo et al., 2011).

Forest structure has been identified as a key determinant of biodiversity (Spies, 1998). Schäffers et al. (2008), through predictive analysis of co-correlation and comparisons between the composition of arthropods and plant species, demonstrated that flora is the most effective indicator of arthropod composition. Therefore, using several variables, many researchers have recently employed a heterogeneity index, or HI, to represent such composition, e.g., Morato (2004), McElhinny et al. (2006) and Janssen et al. (2009).

The relationship between species and environment can be analyzed in several ways, in particular by the formation of guilds. Guilds, as defined by Root (1967), consist of a group of species that exploit the same class of environmental resources and with similar patterns of exploitation. Using this concept, the guild becomes an interesting unit for both research related to interspecific interactions and analysis of communities, since it is a functional unit, making it unnecessary to consider each species as a separate entity (Odum, 1985; Fauth et al., 1996).

The present study aimed to determine the effects of environmental heterogeneity on species richness, abundance and trophic guilds of insects. Furthermore, the study is based on the hypothesis that increasing environmental heterogeneity results in greater richness, abundance and frequency of occurrence of trophic guilds.

Materials and Methods

Study Area

The study was conducted at Estancia Mimosa Ecotourism (EME) (20º 58'57.70"S and 56º 30'58.40"W), about 400 hectares within the municipality of Bonito, Mato Grosso do Sul. The southernmost boundary of the farm is the Mimoso River, which constitutes about 15-20 km of the National Park of Bodoquena Range (PNSB) (*Fig.1*).

The Bodoquena Range is underlain by limestone rocks, Corumbá Group (Neoproterozoic III), with altitudes ranging from 450 to 650 m, mostly devoid of soil development. The rocky substrate is covered by one of the last remnants of vegetation of the plateau and is characterized by the predominance of seasonal deciduous and semi-deciduous forest (Boggiani et al., 1999). Savanna and gallery forest physiognomies are also found (Faria and Araújo, 2010).

Sampling

Samples were collected between September 2011 and June 2012 in 164 randomized samples units (5 subplots 16m^2) totaling 80m² each and spaced at least 200m apart to avoid possible overlap between the samples. These units were distributed in six distinct physiognomies sampled according to their representation in the study area (Table 1).

The collection method used was an insect net adapted from Waquil (1997). From 15 to 20 plots were sampled monthly in the morning between 8:00 and 10:00 A.M. In each sampling unit, insects' understory was captured with a handnet, and a sampling time of ten minutes per plot was maintained. The captured insects were placed in Falcon tubes containing 70% alcohol, properly labeled, and taken to the Laboratory of Zoology at the Universidade Federal de Mato Grosso do Sul for triage and identification of specimens.

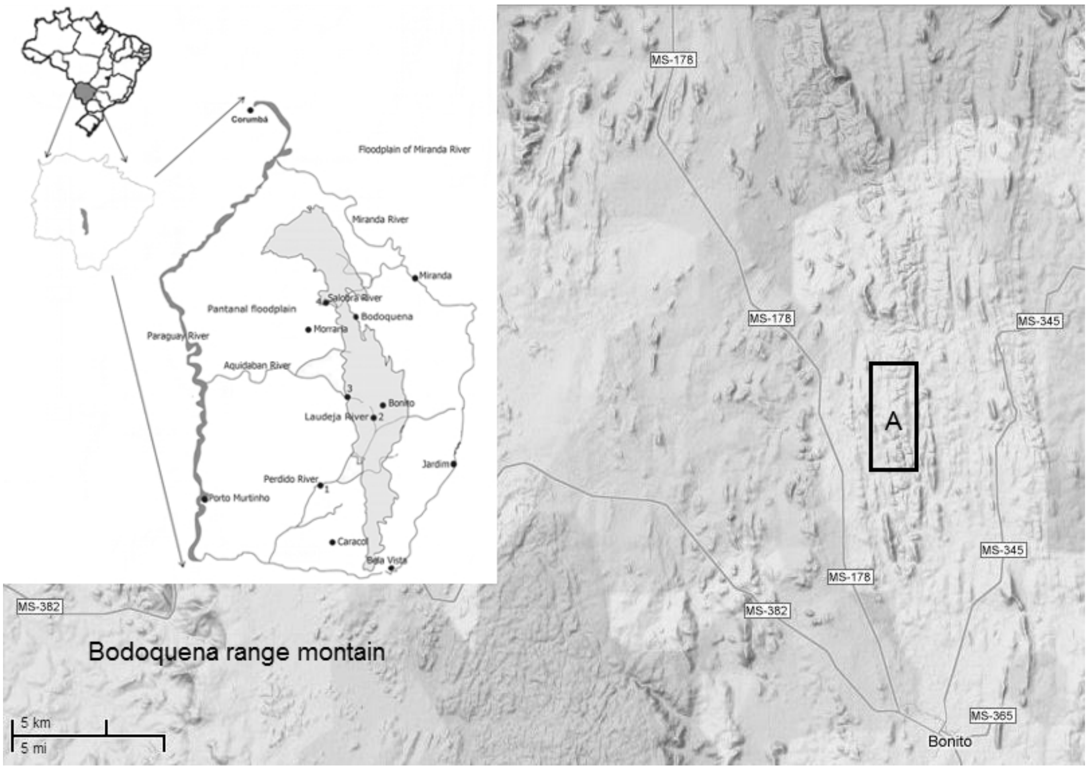

Figure 1. *Location of Bodoquena Mountains and Estância Mimosa Ecoturismo (A) in the municipality of Bonito, Mato Grosso do Sul, Brazil. Adapted from Dalzochio et al. (2011).*

Table 1. *Description of vegetation types found in the study area of Bodoquena Mountains, State of Mato Grosso do Sul. Number of samples in each vegetation type (n). Adapted from Ribeiro and Walter (1998); Cirelli and Penteado-Dias (2003).*

Phytophysiognomy	Description
Seasonal Forest (44)	Predominance of large woody individuals, 10 to 25 meters, with a dense canopy and understory continuous and well developed.
Riparian Forest (9)	Forest vegetation accompanying rivers of medium and large size of the Cerrado region, where woody vegetation does not form galleries. Overall, this forest is relatively narrow, rarely exceeding 100 meters in width on each side. This formation commonly occurs on rough terrain and may not always be an obvious transition to other forest formations, such as the dry forest and the Cerradão.
Cerradão (14)	Forest formation with xeromorphic aspect. Characterized by the presence of species that occur in the Cerrado and also by wood species. While it is a forest, it is more floristically similar to the Cerrado. It predominantly provides a continuous canopy and tree cover which can oscillate 50 to 90%. The average height of the tree stratum varies from 8 to 15 meters, providing light conditions that favor the formation of differentiated shrub and herbaceous plants. Epiphytes are reduced.
Cerrado sensu stricto (34)	Characterized by the presence of low, very rigid trees with such characteristics as sloping and winding, with branches twisted and irregular, often with evidence of burnt leaves. Subshrubs and shrubs are scattered, with some species presenting

	underground organs of resistance (xylopodes), allowing regrowth. In the rainy season, shrubs and herbaceous plants grow rapidly. Woody species in general have trunks with thick cork, chipped or grooved, and apical buds of many species are protected by dense hairiness.
Dirty pastures (28)	Brachiaria pastures and other exotic grasses with high coverage of bushes and trees.
Clean pastures (35)	Brachiaria pastures and other exotic grasses with low coverage of bushes and trees.

All insects were morphotyped and subsequently identified up to the family level. Identification keys used were based on Costa et al. (2006), Triplehorn and Johnson (2011) and Raphael et al. (2012). To classify insects, feeding guilds were based on Price et al. (1987), Buzzi (2003), Triplehorn and Johnson (2011), and Rafael et al. (2012) (Table 2). The identified specimens were deposited in the Zoological Collection of the Universidade Federal de Mato Grosso do Sul (ZUFMS).

***Table 2**. Definitions of guilds presented in the study and their references.*

Trophic Guild	Definition	Reference
Herbivorous	Feed on solid plant tissue.	Triplehorn and Jonnson (2011)
Phytophagous	Feed on living liquid tissues of plants.	Rafael et al. (2012)
Nectarivorous	Feed on nectar, such as bees, butterflies and some flies.	Buzzi (2003)
Polyphagous	Feed on pollen.	Rafael et al. (2012)
Gall[1]	Induce changes in plant tissue in order to protect and feed their larvae.	Price et al. (1987)
Predator	Attack and eat other animals, usually small animals or those less fit.	Triplehorn and Jonnson (2011)
Hematophagous [2]	Feed on blood.	Rafael et al. (2012)
Parasitoid [3]	In their larval stage, feed on other animal tissues for a relatively long time. The host usually dies at the end.	Rafael et al. (2012)
Detritivore; Saprophagous	Feed on plant waste, dead trees, or dead animals, such as carcasses and dung.	Triplehorn and Jonnson (2011)
Mycetophagous	Feed on fungi.	Rafael et al. (2012)
Omnivorous	Feed on both animals and plants.	Buzzi (2003)

[1] *Guild composed entirely of individuals of the order Diptera, where it was considered the only food of the larval stage;*

[2] *Diptera representatives of this guild were just female individuals, given the importance of this feature (meal) for the maintenance of its life cycle (Culicidae, Ceratopogonidae and Psychodidae);*

[3] *Guild composed mostly of individuals of the order Hymenoptera, considering only the the feeding the larval stage, as adults often do not feed.*

To obtain environmental variables, each plot was divided into five sub-plots, one being central with four cardinal positions (north, south, east and west) spaced at least ten meters apart. Each subplot was 16m², and the following variables were recorded: number of trees (dbh ≥ 10cm), number of dead trees, number of shrubs (dbh < 10cm), canopy height (m), canopy cover (%), herbaceous plant height (cm), herbaceous plant cover (%), and litter cover (%), obtaining an average for each point.

Data Analysis

Descriptive analyses were emphasized, including communities and their taxonomic levels, number of families, number of trophic guilds and their respective percentages. For each plot sampled, the richness and abundance of insects, as well as the composition of trophic guilds, were noted. For eusocial insects, the abundance not was considered, just the frequency in the samples.

To detect differences between richness and abundance of insects between the environments, Analysis of variance (ANOVA) and the Kruskal-Wallis test were used, as well as non-metric Multidimensional Scaling (NMDS) for the composition of the community.

Principal Component Analysis (PCA) was used to rescue the chageover in relation to the structural complexity of assessing the applicability of the functional Heterogeneity Index.

A functional Heterogeneity Index (HI), as modified by Morato (2004), was generated from the sampled environmental data sampled. The environmental data were standardized, and the frequency of each variable was calculated for each plot. Finally, we calculated the sum of all frequencies per plot, thus giving weight to the magnitude of the variables in each plot, resulting in the following equation:

$$HI= \Sigma_{f_i}^{n} \qquad \text{(Eq. 1)}$$

where n = number of variables, f = frequency of the variable and i = variable.

With this HI, no loss of variation of the sampled data occurs, since it regains the average values of distribution, and thus, any variation in vegetation structure is rescued. With the values of HI, a simple regression analysis was performed to relate this index to the richness and abundance of insects. An analysis based on the observation of the distribution of trophic guilds along the environmental gradient was performed by direct ordering using HI as the gradient.

Results

A total of 1,177 insects were collected. They were distributed among 109 families and 16 orders, of which the most representative were Orthoptera (303), Hemiptera (288), Hymenoptera (237) and Coleoptera (190). The most abundant families were Formicidae (201) (Hymenoptera); Acrididae (134) and Tettigoniidae (124) (Orthoptera); Pentatomidae (70) (Hemiptera) and Chrysomelidae (54) (Coleoptera). Of these 109 families, 80 had equal or less than to five individuals.

Differences were only found in 1) richness between the clean pasture and other vegetative formations and 2) abundance between clean pasture and Cerrado. The other formations showed no difference between the two community metrics evaluated (Table 3). The guilds with the highest representation were herbivores (36.97%), phytophagous species (20.20%), omnivores (18.86%) and predators (8.51%), which showed high frequency throughout the sampling period. Of the seven guilds remaining, six were more specialized, thus representing 11.40% of the total. The remaining 4.06% represents the saprophage guild.

Table 3. *Comparison of Richness (gray) and Abundance (white) in relation to forest types (ANOVA (F) or Kruskal-Wallis (H) test).*

	Clean Pasture		**Dirty Pasture**		**Cerrado**		**Cerradão**		**Riparian Forest**		**Seasonal Forest**	
	F	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
Clean Pasture	--	--	3.631	0.061**	9.356	0.003*	3.591	0.065**	4.204	0.048*	6.248	0.015*
Dirty Pasture	2.109^k	0.034^k	--	--	0.934	0.337	0.00202	0.964	0.351	0.557	0.0476	0.828
Cerrado	3.804	0.05*	0.32^k	0.572^k	--	--	0.692	0.41	0.000027	0.996	0.834	0.364
Cerradão	0.972^k	0.324^k	1.388^k	0.239^k	1.568	0.217	--	--	0.461	0.504	0.0197	0.889
Riparian Forest	1.137	0.294	0.218^k	0.641^k	0.05*	0.816	0.694	0.414	--	--	0.331	0.567
Seasonal Forest	1.974	0.164	1.061^k	0.303^k	0.828	0.366	0.422	0.519	0.112	0.739	--	--

* significant difference (P = 0.05)
** significant difference (P = 0.1)
^{k}=Kruskal-Wallis test

When HI is related to the six vegetation types generated by the grouping of structurally similar environments, we note that three groups were formed, with the corresponding changes in HI, even with large overlap of values: low heterogeneity (clean pasture (HI = 0.025 ± 0.009) and dirty pasture (HI = 0.043 ± 0.013)), intermediate heterogeneity (Cerrado sensu stricto (HI = 0.050 ± 0.014) and Cerradão (HI = 0.051 ± 0.011)), and high heterogeneity (riparian forest (HI = 0.071 ± 0.018) and lowland forest (HI = 0.063 ± 0.017)). Only two types of vegetation showed no overlapping HI values, indicating structural complexities at two extremes, i.e., clean pasture *vs*. riparian forest (Fig. 2). In regression analysis, no significant relationship was observed between HI and either the number of species or abundance of insects (P> 0.05) (Fig. 3). Comparing the structure of insect community (NMDS) in relation to environmental variables by PCA, it was found that only 18% of community structure is explained by environmental factors (Fig. 4).

In direct ordering analysis (Fig. 5), there was a low proportion of groups in relation to increased IH. Frequencies of only the most highly specialized groups, such as parasitoids, hematophagous species and gallers, had increased along with greater values of HI. The four less specialized groups occurred throughout the gradient of heterogeneity. The insects in these groups are largely leaf-hoppers (phytophagous), grasshoppers and beetles (herbivores), praying mantis, lacewings, bug killers (predators), and ants and cockroaches (omnivores).

Discussion

Areas with less heterogeneity showed lower species richness and abundance of individuals. Other studies have demonstrated this relationship for different groups of insects (Romero-Alcaraz and Ávila, 2000).

Comparing HI and the physiognomies studied, we note the grouping of similarly structured environments. This same model where vegetation types are grouped according to structural complexity, i.e., less complex (fields) to more complex (forests), has been employed in other studies (Almeida and Louzada, 2009; Silva et al., 2010).

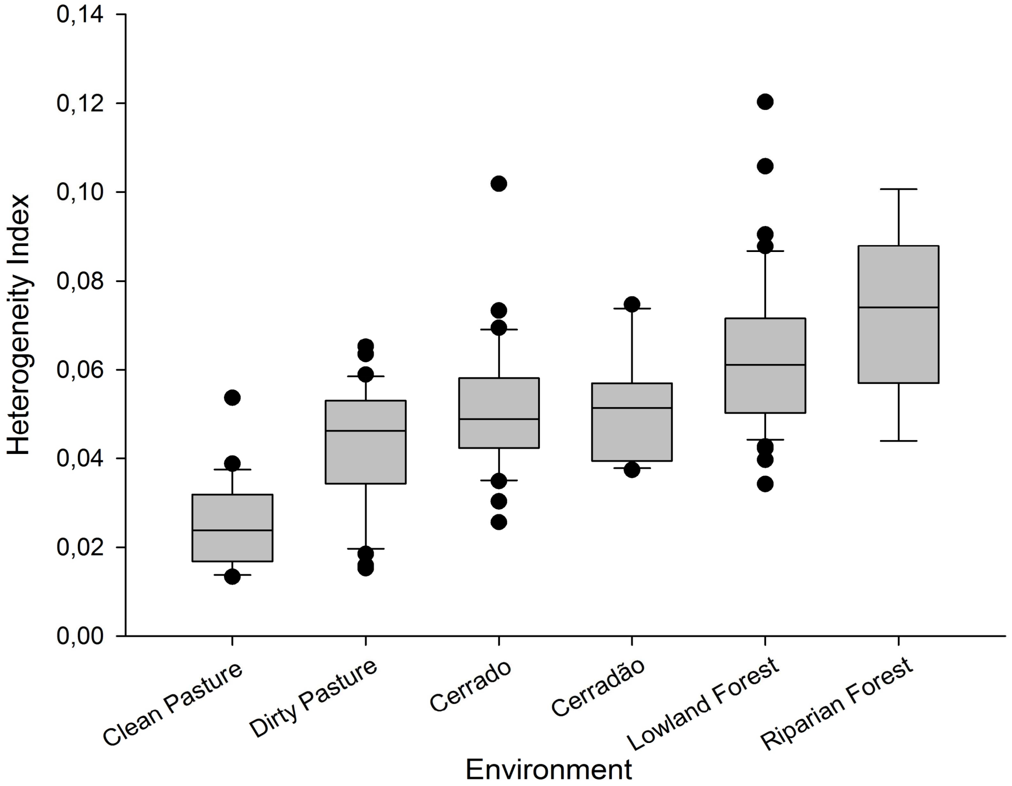

***Figure 2**. Heterogeneity Index in each of the six vegetation types located in Estancia Mimosa Ecotourism, Bodoquena Mountains, Brazil, from 09/2011 to 06/2012.*

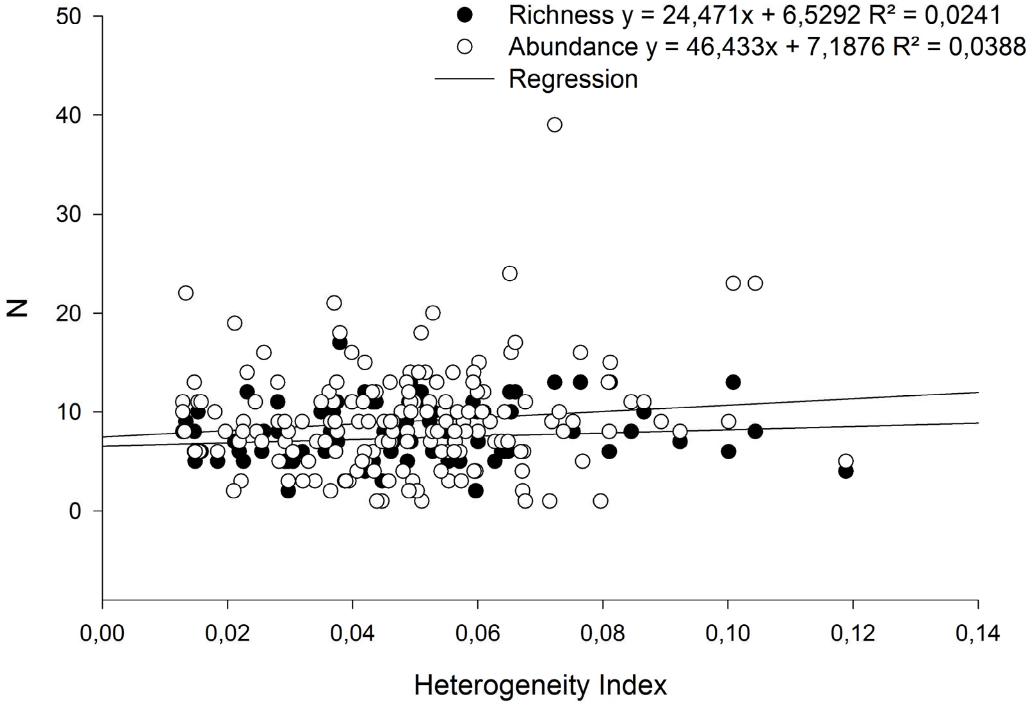

***Figure 3**. Linear regression between richness and abundance of insects and Heterogeneity Index at Estancia Mimosa Ecotourism, Bodoquena Range Mountains, Brazil from 09/2011 to 06/2012.*

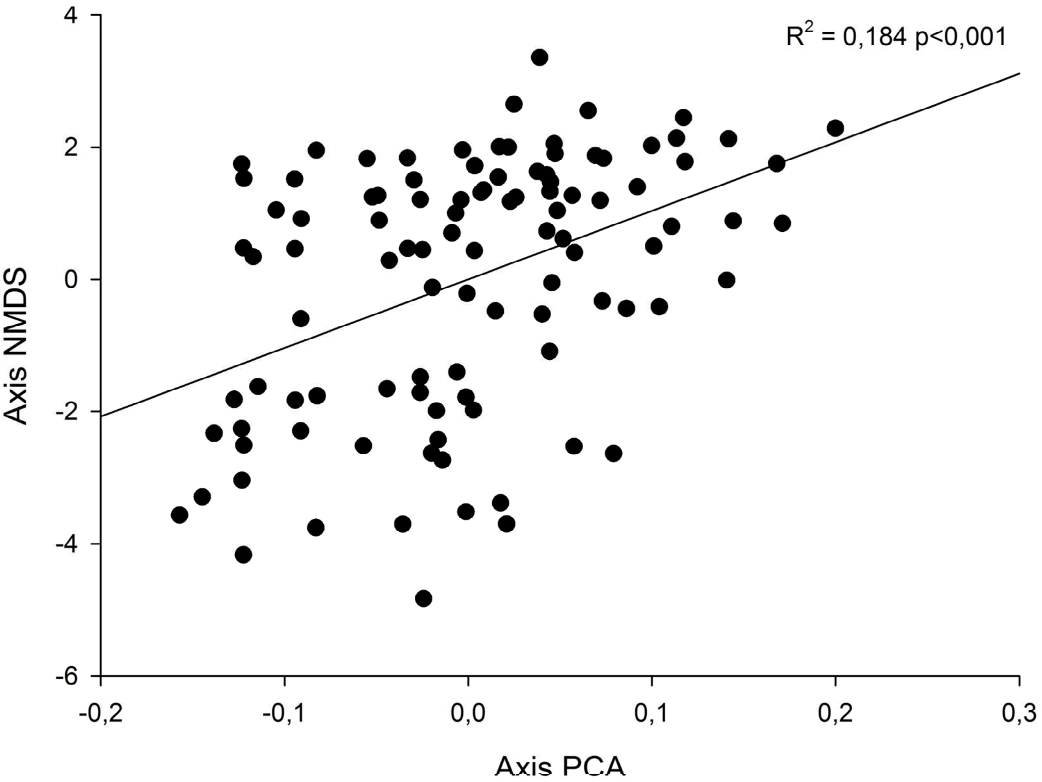

***Figure 4**. Relationship between insect community (NMDS) and Environmental Variables by PCA.*

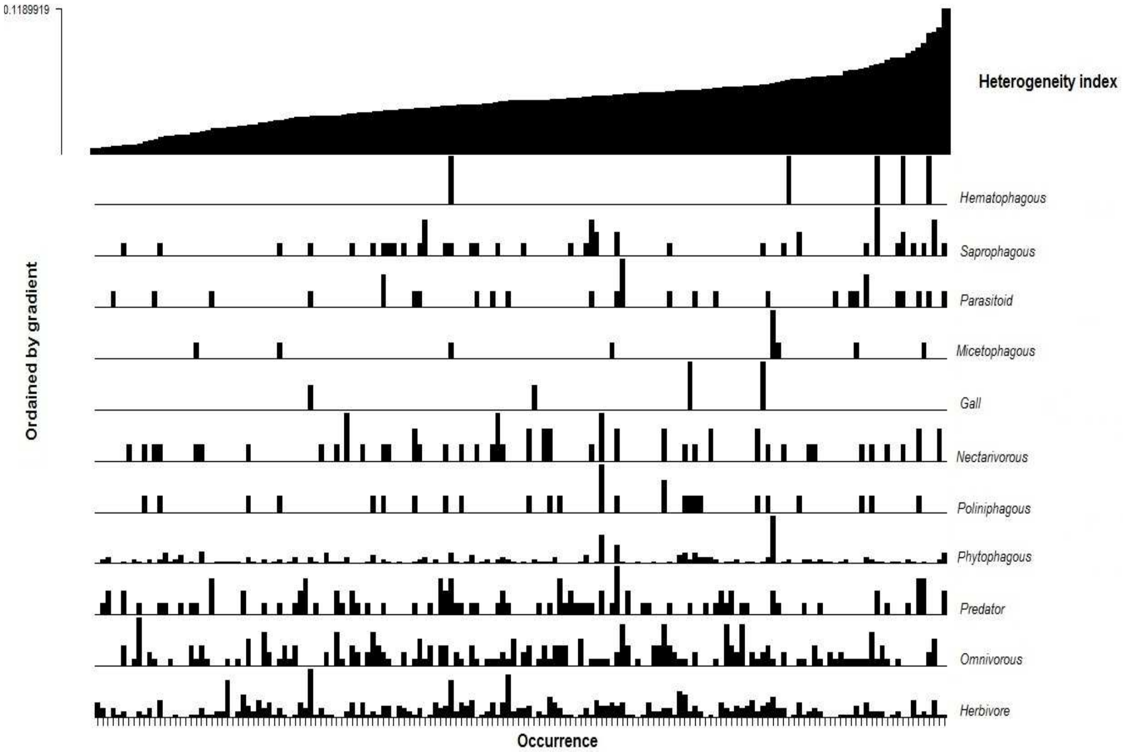

***Figure 5**. Direct Ordering Analysis of the frequency of trophic guilds in relation to the values of environmental heterogeneity at Estancia Mimosa Ecotourism, Bodoquena Range Mountains, Brazil from 09/2011 to 06/2012.*

Through linear regression analysis, we saw that the HI did not significantly influence the variables of richness and abundance. Similarly, Loyola and Martins (2008), working with solitary wasps and bees, demonstrated that the structural complexity of vegetation was not a predictor of richness and abundance. However, we can still see that a few individuals occupied environments with HI ≥ 0.08, a fact that may be related to a high degree of specialization in eating habits, such as representatives of the Culicidae family, where females are hematophagous. Lourenço-de-Oliveira (1984) compared the frequency of Culicidae (Diptera) in different environments. The largest proportion of Culcidae (63.2%) was found in secondary forest, most likely because this environment was better preserved and, hence, more heterogeneous with the additional benefit of favorable climatic conditions for these insects when compared to the less heterogeneous, more degraded environments.

The functional composition of the ecosystem can respond more efficiently to the community structure than metrics such as richness and abundance. Neves et al. (2010) show how community composition reflects the successional stages when compared to using only richness.

In the direct ordering analysis, the group of parasitoids had a peak in frequency in an environment with an intermediate level of HI, a phenomenon possibly resulting from the sampling methodology used, i.e., an insect net, when the Malaise trap, as employed by Cirelli and Penteado-Dias (2003) and Azevedo et al. (2006), would have been the ideal technique for capturing insects belonging to this group. This group also showed a gradually increasing frequency distribution, according to environmental heterogeneity, which resulted in a higher frequency in environments with high HI. Similarly, Azevedo et al. (2006), working with Bethylidae fauna (Hymenoptera) and conducting studies of community structure and faunal similarity among eight different localities, proposed that environmental heterogeneity could be an important factor explaining the relative abundance and composition of these parasitoids, since geographical distance is not a factor for this group.

The hematophagous species clearly showed a higher frequency in environments with high HI, thus demonstrating a preference for local and structurally more complex environments that provide a favorable microclimate. This was similar to the result obtained by Marques and Forattinni (2008) who identified a greater abundance of hematophagous insects (Culicidae) in preserved heterogeneous environments (forest), when compared to areas with less heterogeneity and higher degree of disorder, such as found in peri-urban habitats.

Galling insects, such as the Cecidomyiidae (Diptera), were found most frequently in environments with intermediate HI, as represented by Cerrado and cerradão areas. Notably, xeric, hygrothermally-challenged environments were preferred for their nutritional value with typical sclerophyllous vegetation, confirming the results of Fernandes and Price (1991), Fernandes et al. (1995) and Gonçalves-Alvim and Fernandes (2001).

Groups having a direct relationship with the reproductive structures of plants (polyphagous and nectarivorous) occurred in "pulses", during periods when the flowers fulfill different needs of insects, such as mating, feeding and ovi-position (Villiers, 1972). Seasonality can also interfere with this distribution, since the insects commonly exhibit a seasonal pattern of distribution throughout the year (Silva et al., 2011).

With respect to saprophagous species, we obtained gradually increasing frequency from intermediate heterogeneity values. Clearly, this group maintains a close relationship with other animals or plants which provide food. Almeida and Louzada (2009) hypothesized that increased heterogeneity would attract a greater number of animals and therefore generate a higher number of detritus in more complex environments. In a complementary way, Hülle (2006) noted that large- and medium-sized mammals prefer areas with moderate complexity, a fact that allows us to understand the presence of insects' decompositor in environments with this characteristic physiognomy.

The four less specialized groups showed similar distribution, low frequency in environments with low HI, high frequency in intermediate levels of HI and small decline in frequency in environments with high HI. This low frequency in environments with high complexity was also verified by Menezes et al. (2009) who found that the soil macrofauna of forest fragments in the dry season is dominated by social insects, especially ants. Therefore, interspecific competition may be one explanation for this small decline in these locations.

This distribution pattern in which the frequency varies along the gradient, with modest increase in frequency in environments with intermediate values of HI, was also evaluated for Hemiptera and Hymenoptera predators (Uehara-Prado et al., 2010).

Using the gradient of structural complexity of vegetation, as determined through the use of a functional index of heterogeneity, three structurally distinct environments were delineated, while, at the same time, preventing the loss of variation of environmental data. Thus we obtained a gradient that reflects exactly the variation of heterogeneity.

The diversity and abundance of insects showed an unsatisfactory relationship with the HI. The eleven feeding guilds showed different distribution patterns, and only highly specialized feeding guilds showed gradual distribution, with the frequency increasing with increasing structural complexity of vegetation.

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EFFECT OF WATER LEVEL FLUCTUATIONS ON FISHERY AND ANGLERS' CATCH DATA OF ECONOMICALLY UTILISED FISH SPECIES OF LAKE BALATON BETWEEN 1901-2011

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Abstract. Surveys aiming at analysing spatial and temporal changes of the fish stock of Lake Balaton have an almost 100 year history. Drastically low water levels which could be observed in the past years and which were most probably caused by global climate change provide a good reason to study population dynamic changes induced by water level fluctuations. The present study attempts to give a historical description of changes in the catch data of economically utilised fish species in relationship with water level fluctuations, based on catch data of the Balaton Fish Management Non-Profit Ltd. (predecessor: Lake Balaton Fishing Ltd.) and its predecessors, covering a 110-year period.

Keywords: *Lake Balaton, fisheries and anglers catch data, water level fluctuations, non-indigenous species*

Introduction

Shallow lakes are more vulnerable to human activities and natural changes than deep ones. Shallowness can be measured through the dynamic ratio of a lake and lakes of similar shallowness can be compared in terms of sensitivity and vulnerability to various stresses. Several shallow lakes and communities in their basins experience disastrous changes due to lake and watershed mismanagement as well as climate change. Due to water management interventions, several natural lakes apply water conveyance from another watershed to improve water balance and manage level drop and/or large fluctuation. This problem exists in most shallow lakes from Europe and North-America (Jeppesen et al., 2005, Lyche-Solheim et al., 2013).

Several scientific studies have found that Lake Balaton does not show extreme characteristics as compared to other lakes in Central Europe, but the drainage area of Lake Balaton is very sensitive to both climate changes and human impacts (Padisák, 1998, Padisák et al., 2006, Dokuil et al., 2010). Its surroundings have been inhabited since the Stone Age; however, heavy human impact can be recognized during the past 6000 years (Korponai et al., 2009). Eutrophication was a common problem of Lake Balaton in 1960-1990, though the level of seriousness varies from very serious to acceptable (Specziár and Vörös, 2001, Istvánovics et al., 2007). The backwaters and Lake Balaton have water level problems due to water overuse or climate change. Dropping water level and shrinking of surface area always result in deterioration of water quality and loss of aquatic habitats (Scheffer et al., 2006). Water quality and level improvement measure applied include stopping or restricting agricultural activities, sewage treatment, dredging, biomanipulation, wetland restoration with filtration functions, direct runoff control, soil treatment to immobilize phosphorus and pesticides, etc. (Tátrai and Istvánovics, 1986, Mátyás et al., 2004, Sipkay et al., 2007, Korponai et al., 2009, Erős et al., 2012). Magnitude of measures (spending) is neither proportional to the seriousness of the problem nor to the population affected but it is more a function of the economic power of the countries (Várkuti et al., 2008). Most European shallow lakes and watersheds have a management plan developed but these plans do not entirely reflect the total economic value of the lakes and these tendencies are observed on Lake Balaton and the drainage area (Bíró et al., 2002, Sály et al., 2011).

The majority of ecological studies review the issues of short-term (maximum one season-long) observation but long-term research is becomes more important these days (Specziár and Bíró, 1998) because the exact trends of the physical and chemical processes within the water body and population dynamics of the species which live in them as well as changes in field use on drainage area are based on these studies (Smith, 2003, Erős et al., 2012). Assessing the results of the long-term fisheries catch data we draw not only the conclusion about the population of fish species and processes in community ecology but can important elements of the ecological condition of water bodies, too. Using this knowledge, we can determine the ecological and biological framework of conservation and restoration (Hobbs and Norton, 1996, Daskalov, 2002, Van De Bund et al., 2002, Mátyás et al., 2004, Søndergaard et al., 2008).

Based on long-term analysis we can receive a better picture of the changes in the quality of the environment and thereby the past and current trends and changes will be easier to interpret (Sipkay et al., 2009, Verasztó et al., 2010, Vadadi-Fülöp et al., 2012).

Literature of history of fisheries research on Lake Balaton

There are detailed, historical data available on the size and composition of the fish fauna of Lake Balaton, including mosaic-like pattern, temporal and spatial changes (Vutskits, 1897, Lukács, 1932, Bíró, 1978, 1983, 1997, 2000, Paulovits et al., 1991, 1994, Ponyi 2001, Szabó and Héri, 1998, Specziár et al., 1998, Tölg et al., 1998, Tátrai et al., 2000. Harka and Sallai, 2004, Weiperth et al., 2008, 2009, Specziár, 2010, Takács et al., 2011**,** Ferincz et al., 2011, 2012, Specziár et al., 2013.). Drastically low water levels which appeared several times during the past years and which are considered as a consequence of global climate change (Lake et al., 2001**,** Somlyódy and Honti, 2005, Istvánovics et al., 2007) might have significantly influenced the dynamics of economically utilised fish stocks, including exotic species. These changes support the necessity of research started quite recently which seek relationships between water level and population dynamic changes of fish stocks (Bíró, 1978, 2000b, Paulovits et al., 2007, Korponai et al., 2010, Weiperth et al., 2008, 2009, Specziár, 2010).

The present study, following this line, attempts to give a historical overview of the changes in the catch of some economically important fish species in relation to water level fluctuations based on long-term fisheries and anglers' catch data, covering a 110-year period. Also, some careful assessment is given on the relationship between catch data and magnitude of the water level fluctuations. In addition to catch data, data on stocking and outfishing on fisheries ponds and lakes are used to describe the temporal changes in the stock of eel and other, economically utilised non-native fish species. There have been attempts to stock non-native fish into Lake Balaton since the 19th century, however, large-scale stocking was started in the 1960's, when species which were considered economically and biologically important (gibel carp, eel, Chinese carps: grass carp, silver carp, bighead carp) were released into Lake Balaton and the surrounding fish ponds in relatively high quantity (Pintér, 1980, 2002, Bíró 2000a,b, 2001, Paulovits et al., 2007, Weiperth et al., 2009, Specziár, 2010, Ferincz, 2011).

Material and methods

Study area

Lake Balaton is the largest shallow lake in Central Europe, and it is also a characteristic element of the hydrology of Hungary. Its watershed covers 5774 km^2, the area of its water surface is exactly 594 km^2. Length of the lake is 77 km, the largest width is 12 km, the smallest width is 1.5 km (*Fig. 1.*). In comparison to the size of the water basin, the water depth is rather shallow, with an average of 3-4 ms, the deepest being 11 m in the so-called Tihany-well.

Its bed was artificially modified in the 1800's, afterwards the Kis-Balaton was dried out. In the 19th century a sluice was built at Siófok, on the South of the lake, which connected Lake Balaton to the River Danube. Through the sluice, the water level of the lake was controlled and lowered, which resulted in the partial drying up of the Kis-Balaton. The Kis-Balaton Wetland functioned as a natural filter zone, retaining nutrients carried by River Zala. Disappearance of the natural filter zone resulted in the increase of nutrient load in Lake Balaton which in turn was one of the main factors causing the serious eutrophication of the lake by the 1960's. The Kis-Balaton Water Protection System was built to protect the water quality of the lake via retaining nutrients (Pomogyi, 1993, Korponai et al., 2009).

The natural outflow of the lake is provided by the Sió-Canal. Water transport of the Canal, as well as its water-tapping and the water level are controlled by the sluices at Siófok, the barrage at Balatonkiliti, as well as the works in the mouth of Sió-Canal. The maxiumum capacity of the sluices are 80 m^3/s (in practice 50 m^3/s) (Zákonyi 2004).

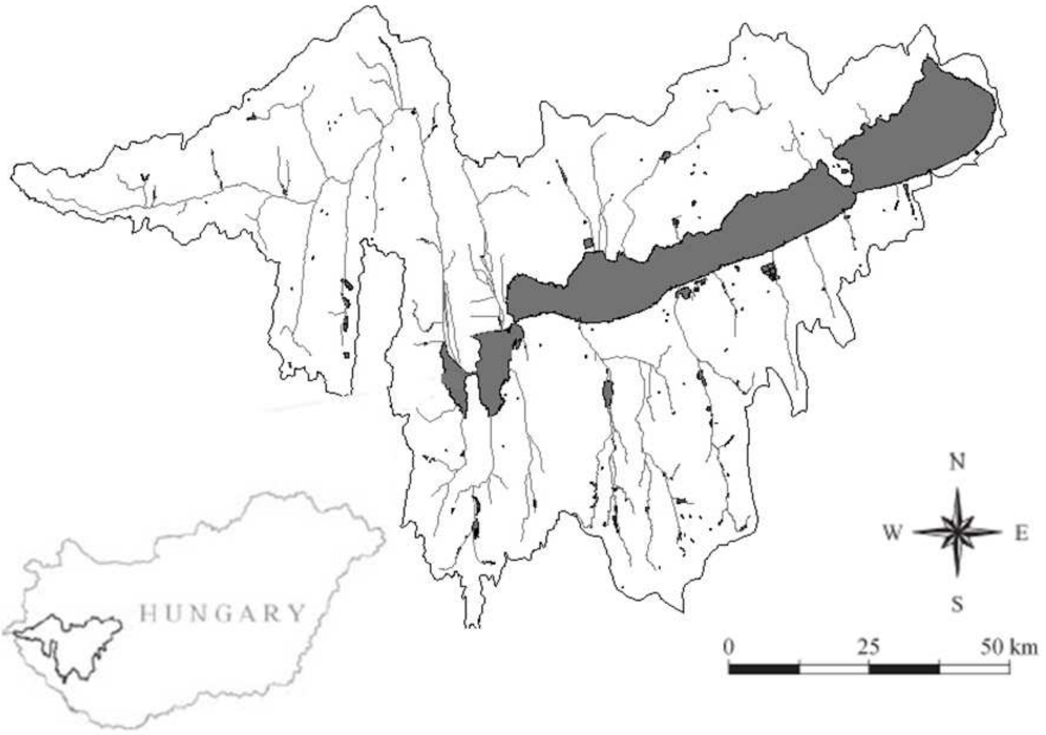

Figure 1. *Overlooking map of the Balaton catchment*

The water balance of the lake is characterised by an average of in the last 110 years: 621 mm precipitation, 908 mm inflow, 800 mm evaporation, 610 mm water-tapping on the Sió-Canal. Water usage (drinking water, industrial, agricultural) is the smallest in absolute value among all the elements. It describes the difference between the quantities of water that were removed from the lake for any type of use and those that were pumped back after use. The average change of water-level within a year was 30 cm but in 1921 the annual water-level fluctuation was 104 cm (*Fig. 2.*).

Until the mid 1980s (for more than 60 years) inflow accounted for 60%, precipitation for 40% of the input in the water budget. In the years after this period the ratio began to change – primarily due to the significant decrease in the inflow to the lake. The proportion of the inflow gradually receded and the proportion of precipitation increased. In the first half of the 2000's the proportion of inflow dropped under 50% and precipitation accounted for more than 50% of all the input – this was the reversal of the previous period. After this, as more years with more precipitating followed, the proportions of inflow and precipitation moved back towards the values which traditionally characterized an average water budget. This phenomenon directs our attention to the following: prolonged and unidirectional meteorological – and the resulting hydrological – extremes can temporarily cause drastic changes in the historically accepted proportions between the elements of the input side of the water budget (EuLakes, 2012a,b).

Used data bases and statistical analyses

Our surveys were based on the long-term catch data of the Balaton Fish Management Non-Profit Ltd. (predecessor: Lake Balaton Fishing Ltd.) and of the National Federation of Hungarian Anglers (MOHOSZ). In order to gain more detailed information, data provided by the War Historical Archives of Department of Defence and National Agricultural Library and Documentation Centre were also processed. 100-year water level data were obtained from the database of the General Director of Water Management (www.hydroinfo.hu).

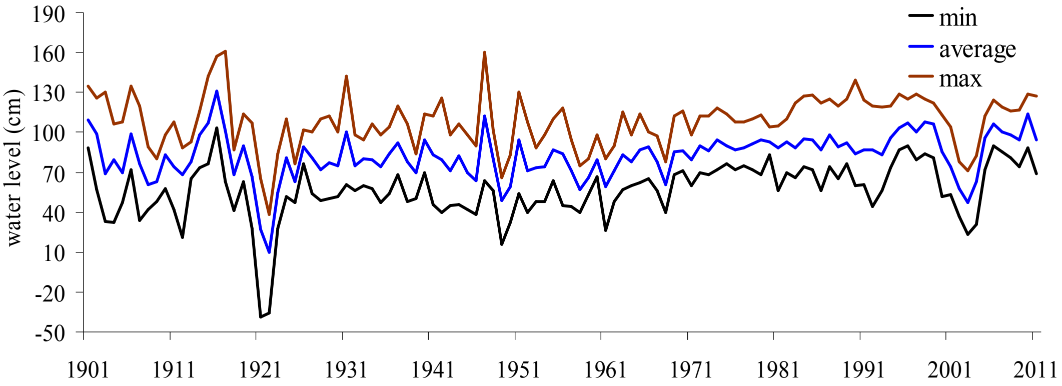

Figure 2. *Changes in water level between 1901 and 2011*

Databases were analysed using PAST software (Hammer et al., 2001). Of statistical methods, in order to establish relationship between water levels and catch data of fish species reproducing in Lake Balaton cross-correlation was applied, population growth of non-indigenous species was analysed by polynomial regression, finally water levels and general catch data were compared using Pearson's correlation (Olden and Neff, 2001, Reizigel et al., 2007, Bíró, 2011, Probst et al., 2011). In case of species where correlation was found between catch data and water level changes, correlation coefficients and correspondence values are indicated in the axes of the figures. In order to avoid possible data loss, correlation coefficients have not been normalised.

Investigated fish species

Our surveys covered those 11 species which are presently economically important: eel (*Anguilla anguilla* Linnaeus, 1758), asp (*Aspius aspius* Linnaeus, 1758), catfish (*Silurus gleanis* Linnaeus, 1758), pike (*Esox lucius* Linnaeus, 1758), pikeperch (*Sander lucioperca* Linnaeus, 1758), Volga pikeperch (*Sander volgensis* Glemin, 1788), carp (*Cyprinus carpio* Linnaeus, 1758), sabre carp (*Pelecus cultratus* Linnaeus, 1758), including the non-indigenous grass carp (*Ctenopharyngodon idella* Valenciennes, 1844), bighead carp species (*Hypopthalmichthys molitrix* Valenciennes, 1844, *H. nobilis* Richardson, 1845) and their hybrids, as well as Gibel carp (*Carassius gibelio* Bloch, 1782). Relatively low number of species involved might be explained by the fact that although there are altogether 32 species occurring in the lake (Takács et al., 2011), of which 20-24 can be regarded as common, only 15-17 species can be found in the catch (Pintér, 1980, 2002, Weiperth et al., 2008, 2009, Specziár, 2010). Analysing catch data of introduced alien species (Chinese carps: grass carp, bighead, silver carp and their hybrids), which are utilised economically but do not reproduce in the lake, temporal changes in their stock size can be discussed. The recruitment in these species does not happen in a natural way but only via stocking, in contradiction to gibel carp, which has appeared in the statistics with fluctuating catch data since it was first recorded in 1959 in the southern fish ponds and in Kis-Balaton. Catch data of Lake Balaton include species utilised only for a shorter period. Data of these species were not analysed statistically (*Table 1.*).

***Table 1**. Exploited fish species of Lake Balaton*

Fish species	Origin of Balaton catchment area	Reproductive strategy	Year range of data for fishery's period	Year and mass in ton of maximum catch
Anguilla anguilla	native	does not reproduce (catadromous)	1961-today	1996: 509 t
Ctenopharyngodon idella	non-native (Far East)	escaped from fish farm	1975-today	1994: 10.7 t
Aspius aspius	native	lithophil	1901-today	1929: 29.9 t
Alburnus alburnus	native	phyto-lithophil	1952-1960, 1967, 1975	1952: 49.9 t
Pelecus cultratus	native	pelagophil	1901-2011	1965: 354.4
Tinca tinca	native	phytophil	1950-1989	1967: 1.06 t
Carassius gibelio	non-native (Far East)	phytophil	1959-today	1996: 29.9 t
Cyprinus carpio	native	phytophil	1901-today	1929: 83 t
Hypopthalmichthys molitrix, H. nobilis, H. molitrix x H. nobilis hybrid	non-native (Far East)	escaped from fish farm	1972-today	2004: 437.9 t
Silurus glanis	native	phytophil (substratum choosers)	1901-today	1953: 27.8 t
Esox lucius	native	phytophil	1901-today	1949: 40.8 t
Onchorhynhus mykiss	non-native (North America)	introduced for aquaculture utilization	1963-1991 (drainage area)	1986: 0.7 t
Perca fluviatlis	native	phyto-lithophil	1924-55	1947: 0.3 t
Lota lota	native	lito-pelagophil	1950-1959, 1996-1998 (Zala River)	1950: 0.075, 1996: 0.063
Sander lucioperca	native	phytophil (nest sawners)	1901-today	1943: 236 t
Sander volgensis	native	phytophil (nest sawners)	1950-today	1953: 11 t

Results

Native fish species

The origin of the eel still raises some questions, but it has become accepted that a native fauna element in Lake Balaton, although there are no reliable data on its early population size (Specziár, 2010). There were plans completed to stock eel for economic purposes by the 1950s', however the results of the so called 'eel project' which was started in 1961 are somewhat contradictory. The quantity of stocked juvenile eels already decreased in the middle 1970s' (*Table 2.*) (Gönczy and Tölg, 1997), but it showed an increase in trend till the fish kill in 1991, having its maximum quantity (509 t) in 1996, after the second big fish kill of 1995, then a continuous decrease started (*Fig. 3.*). The two most important methods for fishing were the electric trail-net, and eel traps placed at the Sió-sluice. Electric fishing was terminated in 1996, afterwards disparities between anglers' and fisheries catches disappeared. When correlating aggregated catch data and water-tapping quantity via the Sió-sluice, significant positive correlation was found (R^2=0.791; P= 0.018). Analysing relationship between anglers' catch data and annual average water levels, R^2=0.718 (P=0.0036) value was obtained.

Table 2. *Introduction and catch of eel in five years cumulation value between 1961-2011*

Years	*locating stock (million individuals)*	*total catch (t)*
1961-1965	*10*	*3.6*
1966- 1970	*15.7*	*78.7*
1971-1975	*15.8*	*121.4*
1976-1980	*15.0*	*184.8*
1981-1985	*14.6*	*581.9*
1986-1990	*8.0*	*642.9*
1991-1995	*4.0*	*1519*
1996-2000	-	*925*
2001-2005	-	*59.7*
2006-2011	-	*106.5*

In the most intensive fishing period of asp the highest average catch was 24 t/year, which was achieved twice (1928, 1954). Afterwards, the catch was showed a decreasing trend till the 1990s'. From the 2000s', total catch has been showing again a decreasing tendency, with 0.3-5.6 t/year, nowadays being under 0.4 t (*Fig. 4.*). Negative correlation was found between catch data of asp and water level changes for years 4 and 8 (*Fig 5.A.*).

In case of catfish also a negative correlation was found for the periods between years 2-5, and 12-16 (*Fig. 5.B.*). Temporal pattern of the catch showed relatively low amplitudes. Total catch has never exceeded 28 t, but nowadays it hardly reaches 3 t. In addition, significant fish kills contributed to mortality (*Fig. 4.*).

Negative correlation is given between catch data of pike and water level changes for years 1-6 and 13-21 (*Fig. 5.C.*). Catch of pike in Lake Balaton is characterised by extreme years. From 1901-50, the average catch was 18 t/year, than after the 1960's a slow decrease could be observed. However, in certain years very high catches were recorded (*Fig. 4.*).

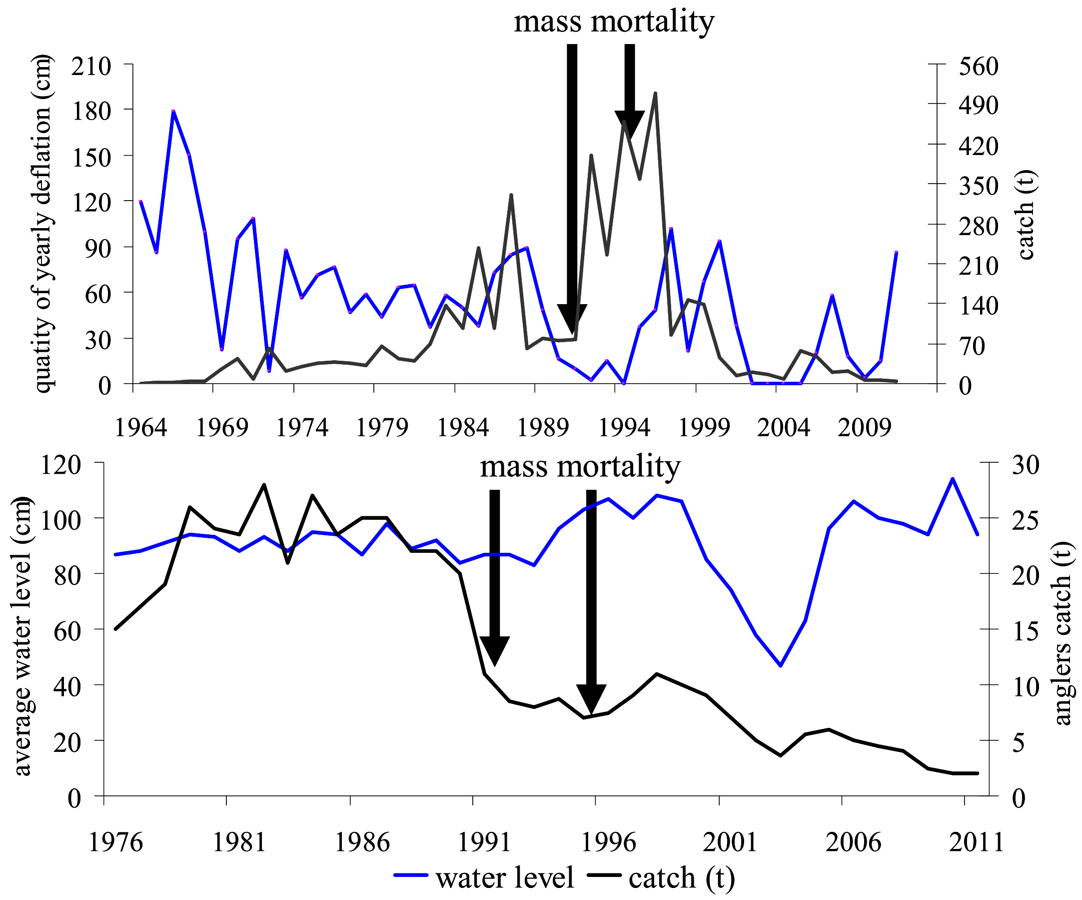

Figure 3. *Eel fisherman and anglers catches in Lake Balaton between 1964-2011 (yearly totals (t))*

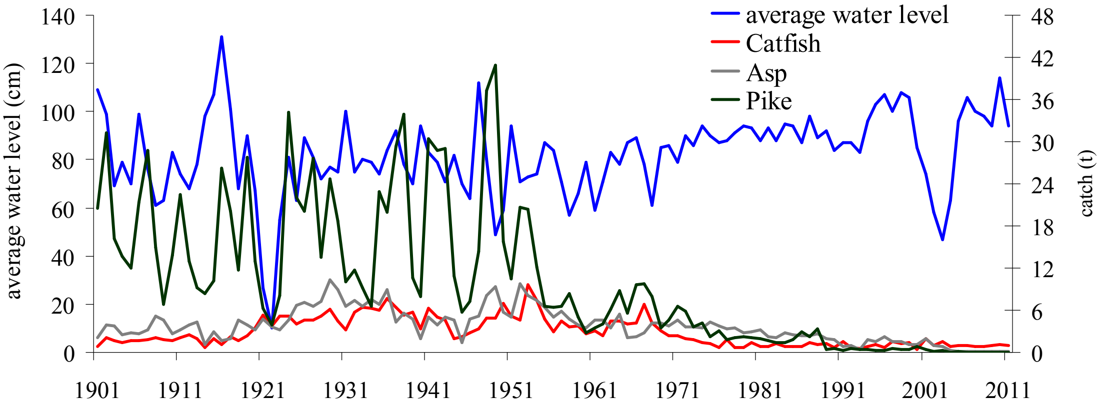

Figure 4. *Catch of asp, catfish, pike in Lake Balaton between 1901-2011 (yearly totals (t))*

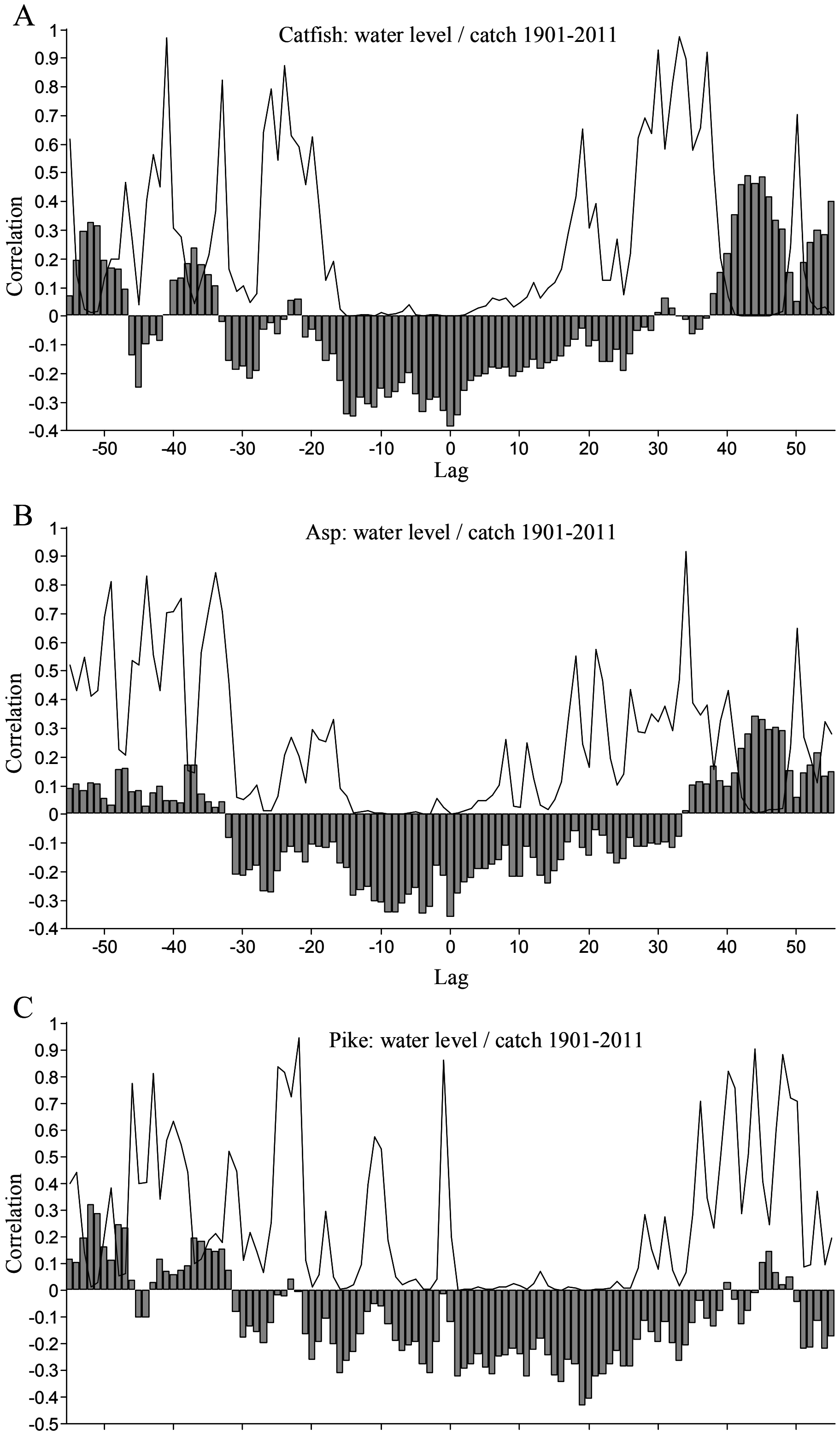

Figure 5. *Cross-correlation between average annual water level and annual catches by commercial fisherman and anglers of catfish, asp and pike in Lake Balaton between 1901-2011 The lines mark the confidence limit. Lag number correspond to years.*

Pikeperch showed a maximum catch between 1930-1965 (average 150 t). In the past 10 years, the average has moved between 15.3-1.6 tons annually (*Fig. 6.*). Negative correlation was given for years 2-4, and 10-14 (*Fig. 8.A.*).

Volga pikeperch holds a special position, as already the first references mention its occurrence in Lake Balaton (Herman, 1887, Pintér, 2002), but catch data has included this species only from 1950. Catch data showed negative correlation from the second year (*Fig. 8.B*). Catch of the species has drastically decreased from the 1990s', nowadays only anglers' catch data include it, in an almost negligible ratio (0.4%) (*Fig. 7.*).

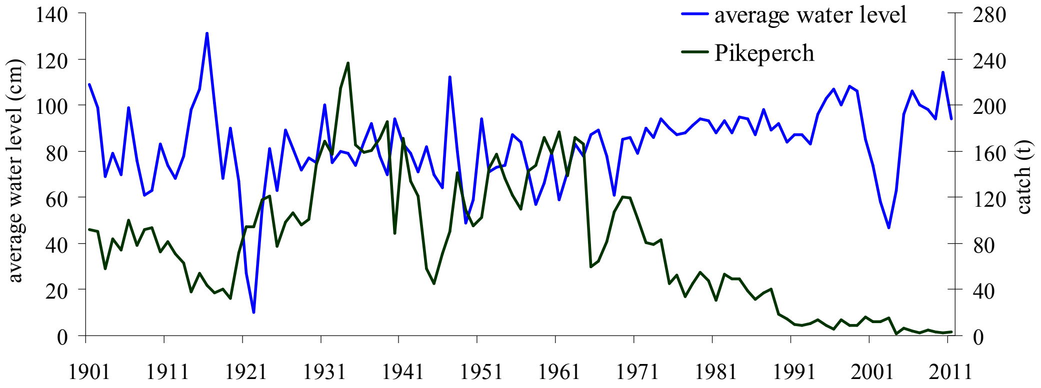

Figure 6. *Catch of pikeperch in Lake Balaton between 1901-2011 (yearly totals (t))*

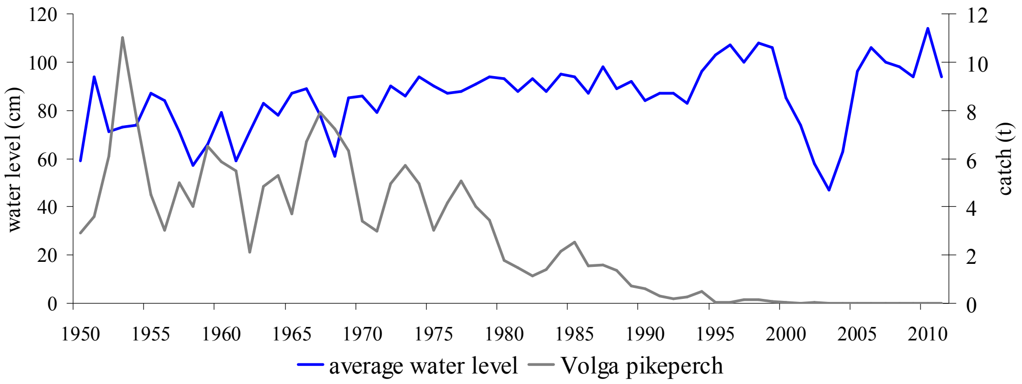

Figure 7. *Catch of Volga pikeperch in Lake Balaton between 1950-2011 (yearly totals (t))*

Carp has been intensely utilised from the beginning of large-scale fishery in Lake Balaton, due to its economic value. Also, this species is under heavy pressure by anglers as well. Its catch data showed high extremities between 1917-1975, than started to show a decreasing tendency, despite intense stocking (*Fig. 9*). Negative correlation is given between catch data of carp and water levels for years 6-18 (*Fig. 11.A.*).

When analysing catch data of sabre carp, it might be seen that from the late 1970s' the quantity of the catch has significantly decreased (*Fig. 10*). Negative correlation is given for the first 5 years and for years 10-15 (*Fig. 11.B.*)

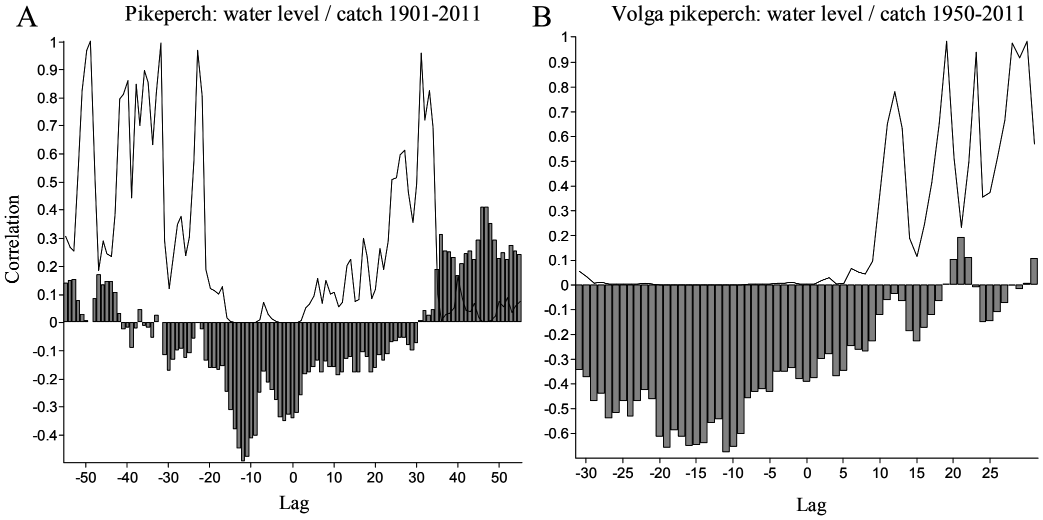

Figure 8. *Cross-correlation between average annual water level and annual catches by commercial fisherman and anglers of pikeperch and Volga pikeperch in Lake Balaton between 1901-2011. The lines mark the confidence limit. Lag number correspond to years.*

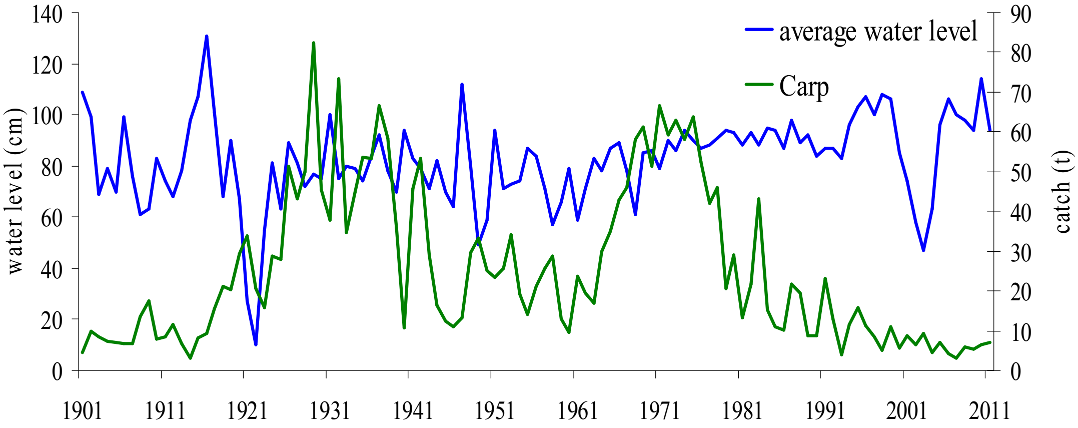

Figure 9. *Catch of carp in Lake Balaton between 1950-2011 (yearly totals (t))*

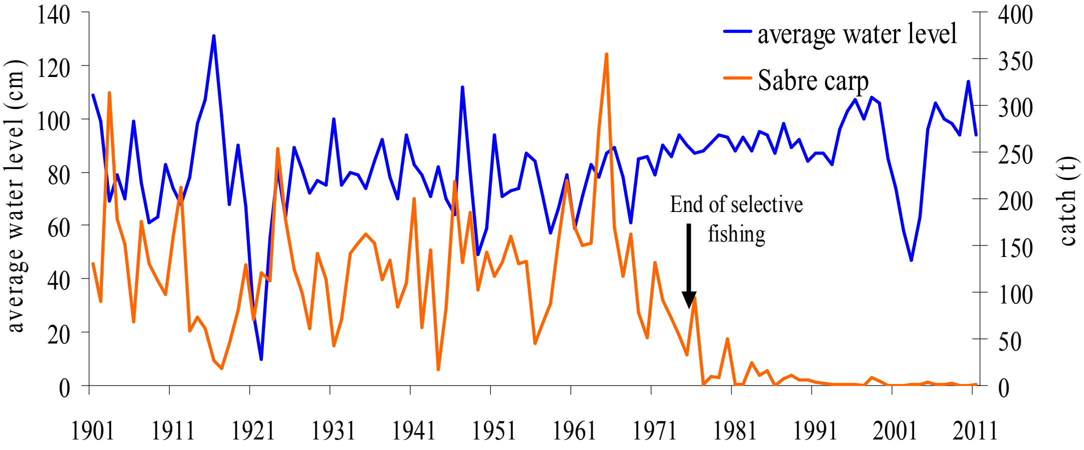

Figure 10. *Catch of sabre carp in Lake Balaton between 1950-2011 (yearly totals (t))*

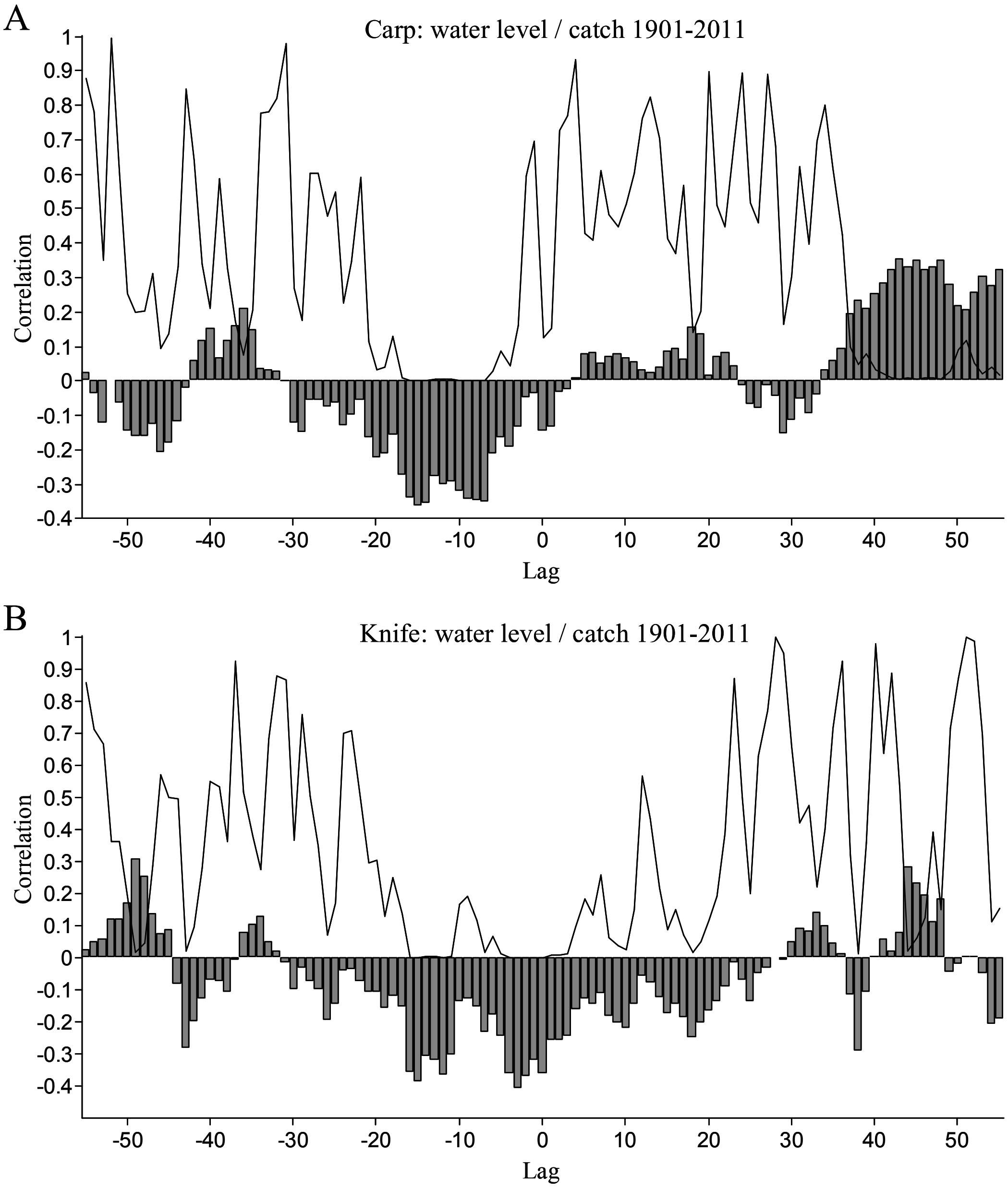

Figure 11. *Cross-correlation between the average annual water level and annual catches of carp and sabre carp by commercial fisherman and anglers in Lake Balaton between 1901-2011. The lines mark the confidence limit. Lag number correspond to years.*

Non-native fish species

Grass carp holds a specific status, as it has never been intentionally introduced to Lake Balaton. Still, catch of fisheries and anglers amounts to an annual 5 tons. Based on polynomial regression, catch data show an increasing trend ($R^2_{fisheries}$= 0.671, $p_{fisheries}$< 0.05; $R^2_{anglers}$= 0.551, $p_{anglers}$< 0.05) (*Fig. 12., 13.*). Correlation between catch of grass carp and average water levels gave a 0,674 value, (p= 0.0012), meaning that there is a positive correlation: when the water level is higher, the catch of grass carp is also higher (*Fig. 14.*).

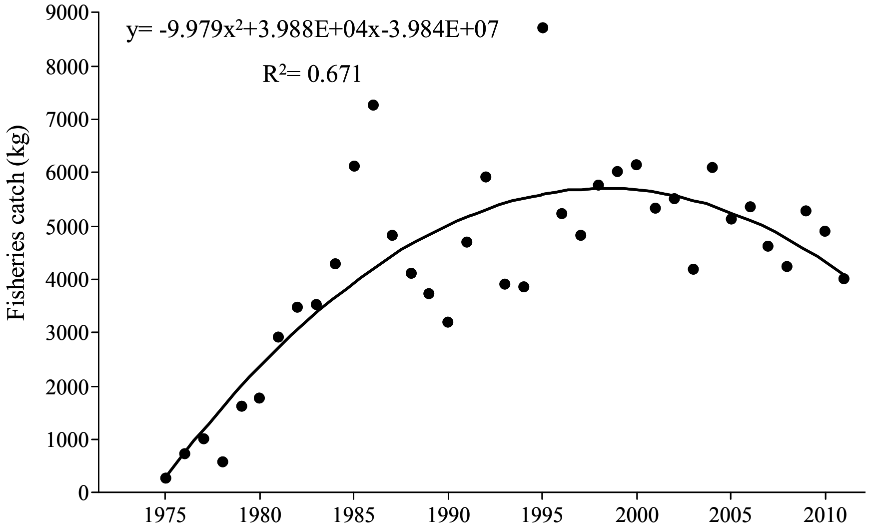

Figure 12. *Fisheries catch of Grass carp in Lake Balaton between 1975-2011*

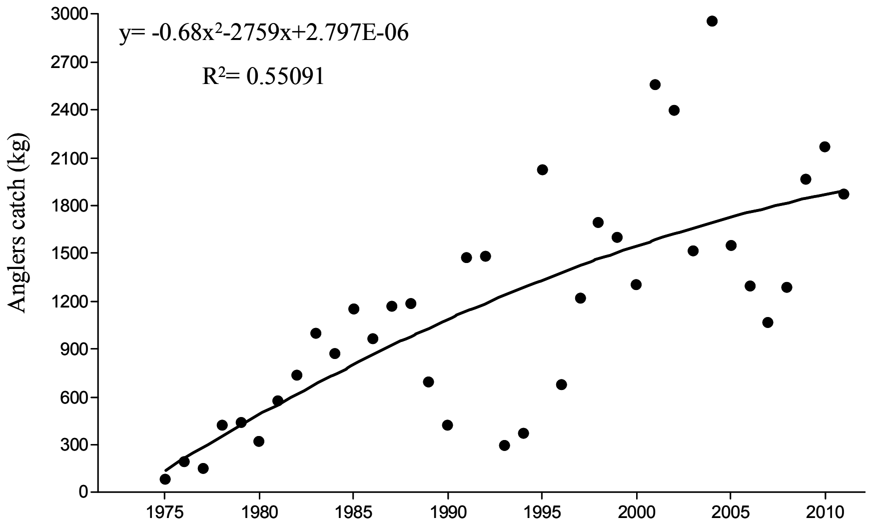

Figure 13. *Anglers catch of Grass carp in Lake Balaton between 1975-2011*

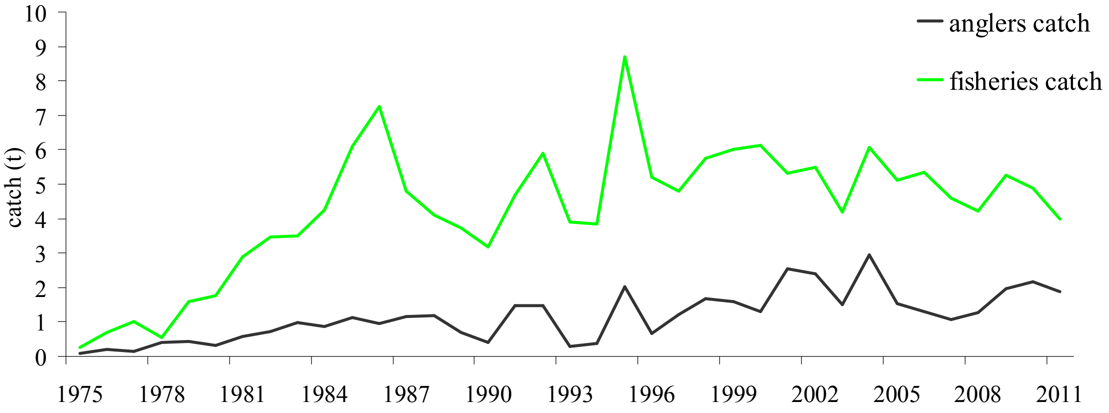

Figure 14. *Grass carp catch in Lake Balaton between 1976-2011*

Bighead carp species were stocked to Lake Balaton between 1972 and 1983 (except in 1974-75). Altogether 1 764 580 two-year old specimens were released (with a biomass of 350 098 kg). After 1984 stocking was stopped (Specziár, 2010). The fishing of silver carp has become intensive in the lake by the 1990s' and still shows an increasing tendency ($R^2_{\text{fisheries}} = 0.833$, $p_{\text{fisheries}} < 0.05$ (*Fig. 15.*) due to the application of new fishing techniques and selective fishing. Both species, as well as their hybrids have become one of the most important species of fisheries. The maximum catch was recorded in 2004 (437 tons). Since 2005, the average catch has been 293 tons (*Fig. 16.*).

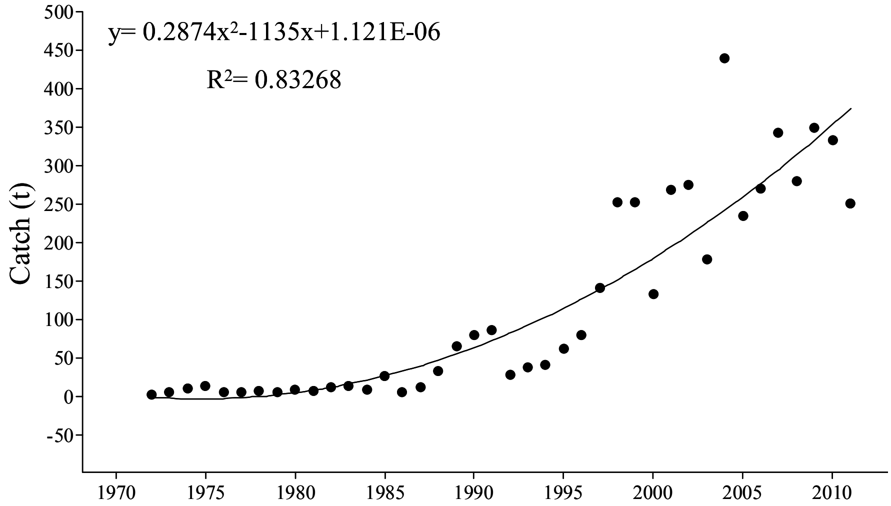

Figure 15. *Polynomial regression of the catch of silver carp, bighead carp and their hybrids in Lake Balaton between 1972-2011*

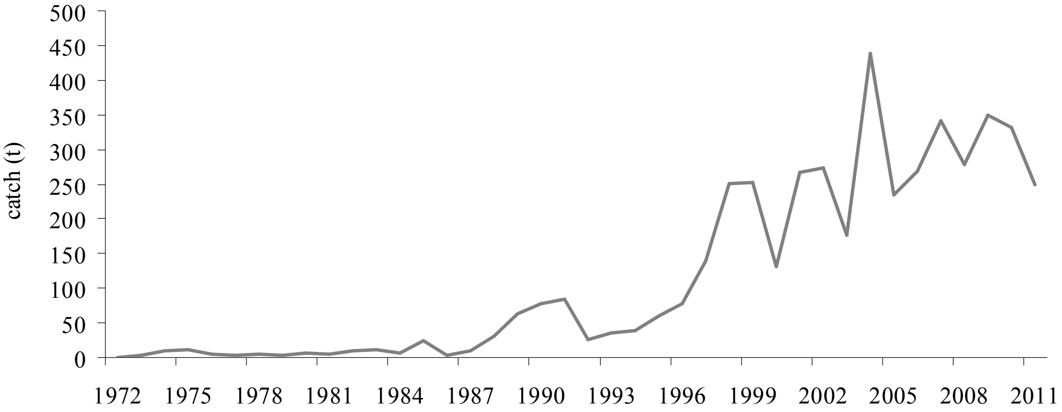

Figure 16. *Catch of silver carp, bighead carp and their hybrids in Balaton between 1972-2011*

In the catch data of the gibel carp, two outstanding figures appear: 7.7 tons in 1959 and 9.6 tons in 1960. There was a rapid increase in the second half of the 1980s' (1992: 23 t), than a fall to 4.4 tons in 1993. There was an app. 30 t catch maximum in 1996, followed by a decrease. Between 1997 - 2011 the average annual catch was 4.6 tons (*Fig. 17.*).

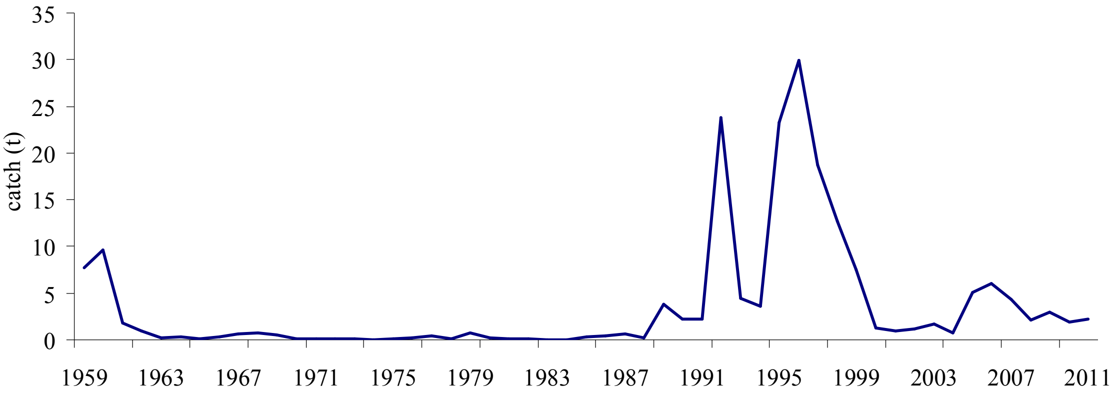

Figure 17. *Catch of gibel carp in Lake Balaton catchment between 1959-2011*

Discussion

Changes in fisheries catch in Lake Balaton between 1901-2011

The summarised fisheries catch data showed, that in the last 20 years the eel and the other non-native fish species (mainly the three Chinese carp species) gave 93% of total fish catches in Lake Balaton (*Fig. 18.*). The 'A' fish category includes the fish species of high economical value: asp, carp, catfish, Chinese carp species, eel, pike, pikeperch and Volga pikeperch. The 'B' fish category includes the common fish species e.g.: bream, bullhead species, gibel carp, perch, roach, white bream, and sabre carp since 1977. The analysis of the categories due to the diversity and quantity of fish species is not resoluble. The total yearly catches of two fish categories of the fisheries company showed comparable tendency (*Fig. 19.*) (Weiperth et al., 2008, 2009).

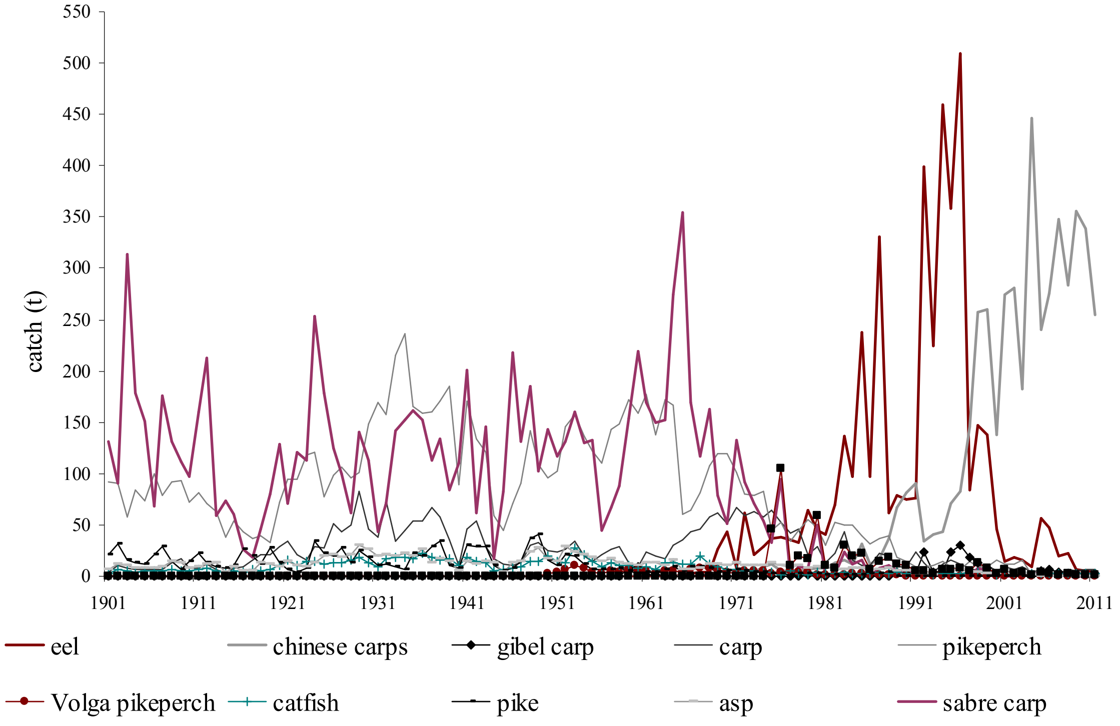

Figure 18. *The economical important fish catches in Lake Balaton between 1901-2011*

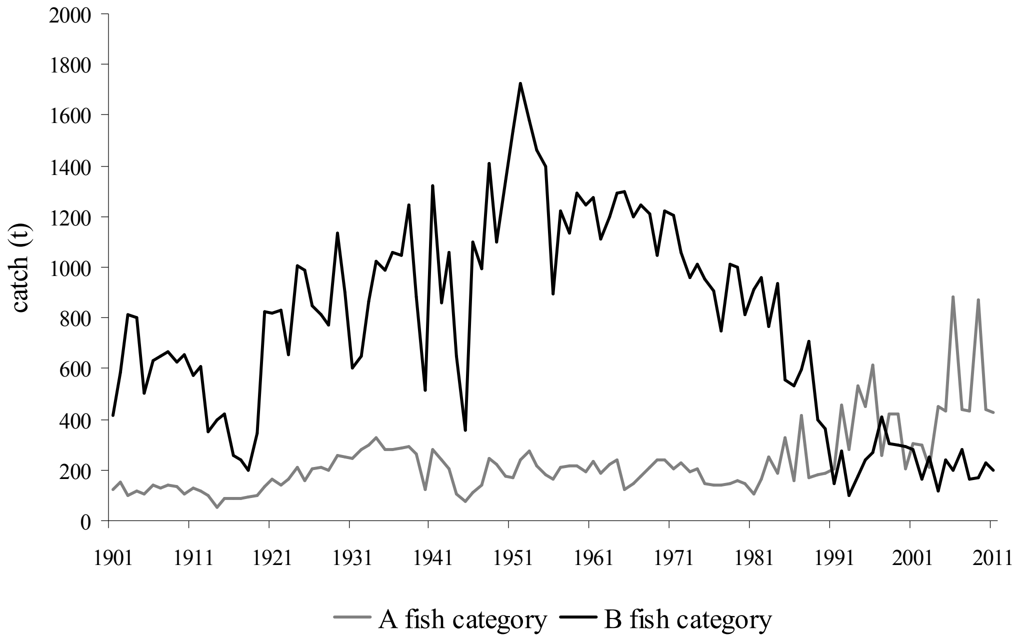

Figure 19. *The catches of the two categories of fisheries in Lake Balaton between 1901-2011*

The decreasing tendencies of both categories were observed since the middle of the 1980s', when the intensive selective fishing of eel and the other non-native fish species began. The catch of all native fish species have gradually reduced since 1977 (Weiperth et al., 2008, 2009, Specziár, 2010).

Native fish species

According to the special literature stock size changes of certain species correlates with water level fluctuations, while the catch of others does not. Changes in correlation coefficients show a specific dynamics for most species in Lake Balaton. Based on fisheries and anglers' catch data, the rise in eel catches till the fish kill in 1991 resulted from intensification of fishery. The enhanced selective eel fishing was followed by a second fish kill, eel catch reached its maximum in 1996 (509t), than a continuous decrease was experienced, due natural mortality and selective fishing (*Fig. 3., Fig. 18.*). After electric fishing was stopped, the eel traps near the Sió sluice have been the most important catch methods in Lake Balaton. These fixed traps utilise the catadromous nature of the adult eel, when the water-tapping is moving. Efficiency of catch of the traps significantly depends on the quantity of water-tapping. Analyses have revealed that in case of positive water balance, eel catch of traps increase in parallel with the quantity of regulated outflow. Efficiency of traps is significantly affected by the fact that in case of negative water balance, sluices are kept closed even for years. When analysing anglers' catch, water level changes should also be taken into consideration, as eel has the highest abundance in the littoral, finding appropriate food and shelter in the reed and along the rip-raps (Gönczy and Tölg, 1997). The decrease of anglers' catch data were observed in many years, when the water level was drawn back from the littoral zone. It is very difficult to give a quantitative estimate on the actual stock size of eel in the lake. Since stocking was stopped, the stock has been continuously decreasing and ageing. Actually, there have been no studies initiated to follow these changes.

It should be noted, that other background variables (e.g.: number of active fisheries days pro year, number of anglers pro year, long-term data sets of environmental variables) might play also important role in the system and affected the result of cross correlations. These datasets were omitted from our study, due their unreliability. As a result only the first year period can properly be discussed in the view of cross-correlation analysis of each fish species. Exact assessment of our results, detected several decades later, needs much more reliable data not only about stocks of fish species, but also other background variables.

Lower water level had a positive influence on the share of predator species reproducing in lake. Asp and pikeperch quantity indicated that hard bottom plays a key factor in the reproduction strategy of both species (Balon, 1975, 1990, Bíró, 1985, Weiperth et al., 2008, 2009). Here, when the water level is low, the water body warms up sooner, hatching time gets shorter and – supposing that other environmental parameters are also favourable – most possibly survival rate of the eggs is higher. The reproduction and feeding of catfish are greatly connected to the littoral, therefore its catch data significantly correlated with higher water levels. Negative correlation given for pike might be explained by the fact that it requires shallow areas for spawning, which warm up faster, and the ratio of these habitats is higher when the water level is lower. In anglers' catch, after the 1980s', good year/bad year phenomenon appeared, that is, maximum catch occurs 3-4 years after a successful spawning (1984; 1987-1988). In the 2000s', the catch of pike shows a high fluctuation, however, app. 90% is provided by anglers. Negative correlation given for pikeperch has already been analysed in detail asp was discussed. It is the temporal pattern of this correlation can be explained by other biotic and abiotic factors, such as stockings or reproduction success of prey fish. However, further studies are needed to clarify the role of these factors. Volga pikeperch showed the highest sensitivity to low water levels, as the maximum catch was given following lowest water levels. This tendency could be experienced till 1987, afterwards a drastic decrease of the catch has started. It raises several questions, as though several studies have been initiated on the biology of this species, the population has been decreasing since 1980 (*Fig. 7.*) and the stock seems to be unable to grow strong despite low water levels.

When analysing carp catch data, it should be taken into consideration that the recent carp stock can only be maintained by artificial stocking, as the quantity of offspring from natural spawning is negligible (Specziár, 2010). The maintenance of the common carp population depends on the dispersion of the stocks in the water body and its distribution of age-groups. At the evaluation of sabre carp catches the special reproduction strategy of species has to be taken into consideration, and also that the selective fishery of sabre carp was stopped in 1975-76, moreover, in 1982 there was a local sabre carp kill (Staszny and Paulovits, 2007). At present, selective fishery methods are not used to catch sabre carp in Lake Balaton, but every year in some areas it is presented on the fisheries catch (*Fig. 10.*). The population of the sabre carp in Balaton can be considered stable after the foregoing results of the scientific studies (Staszny and Paulovits, 2007, Specziár, 2010). After the correlation value of sabre carp it could be stated that the spawning of sabre carp in lower water-level is more successful and the maximum of fisheries catch of species can be achieved in three or four years.

Non-native fish species

The appearance of grass carp in Lake Balaton, as well as the development of its stock raises numerous questions, as this species has never been legally stocked, moreover, its reproduction has not been proven so far (Pintér, 1980, 1989, 2002; Bíró, 2000 a,b, 2001; Weiperth et al., 2008, 2009; Specziár, 2010, Ferincz et al., 2012, Ferincz, 2013, Kováts et al., 2013). The first specimens were most probably introduced with carp stocks, nowadays this species might reach the lake from fishponds operated on the southern tributaries. Apart from bighead, silver carp and their hybrid species, grass carp is the only species which shows an increasing tendency of the catch (*Fig. 15., 16.*). Unfortunately, there has been no comprehensive study available on the function of this species in Lake Balaton, nevertheless, there is an increasing need for such a study, because in case the stock size is overgrown the carrying capacity of the lake, it might threaten reeds, which have a constantly worsening condition (Specziár, 2010). In parallel, the terms of fishpond operation in the catchment should be checked and revised, in order to stop recruitment from them.

In the past decades, along with the more and more intensive fishing of bighead and silver carp and their hybrid, catches have shown an increasing trend (*Fig. 16.*). All these can be the result of new, selective fishing methods (Varga, 2011). In the statistical analyses, however, it might have a masking effect on the possible influence of water level changes. Several studies have been completed addressing the size and structure of the stock as well as the feeding habit of this species (Herodek et al., 1989; Tátrai et al., 2007; Boros et al., 2012), however, data are contradictory. Its reproduction has not been proven, the recruitment of the stock most possibly occurs from the catchment (Tátrai et al., 2009; Boros et al., 2012).

When analysing the fishery data of the gibel carp, it should be taken into consideration that 83% of the catch is given by the Kis-Balaton Water Protection System (KBWPS), 16% by the southern fishponds and only 1% comes from Lake Balaton. The increase of the population started in the 1980s' (Bíró and Paulovits, 1994, Ferincz et al., 2011, 2012). This species hardly appeared in the catch data after it had been introduced to the lake, it reached its maximum catch after the flooding of the first and second stage of the KBWPS. The KBWPS and the density of juvenile coming from the inflows played a main role in the appearance and spread of the gibel carp in Lake Balaton. These factors determined also the population in Balaton until the 2000' years. Since then, the species has been forced back in Lake Balaton. In the fisheries' statistics only the data of fishponds and KBWPS are separately recorded, the catches in Balaton are recorded to the so called B fish category and also anglers do not have to separate them from the 'other' fish category (*Fig. 19.*). As a consequence, a detailed evaluation cannot be given about the catches of the species.

Finally, we can conclude that apart from natural factors, utilisation by fishery and anglers might have a significant influence on the size and composition of fish stock in natural waters. There are several papers available discussing this problem (Cane, 1980; Paulovits et al., 1994, 2007; Brämick et al., 2008, Pascual et al., 2007, Humpl et al., 2009; Móreh et al., 2009). When analysing the different fisheries and anglers catches it should not be ignored that the different fisheries-anglers methods applied in Lake Balaton in the different periods have continuously changed (size and time of utilization of the net, time interval of utilisation of knife-net, change of close seasons and size limits), which in some cases decreases the comparability of data. In addition, water level changes affect catches indirectly, influencing applicability of different fishing and angling methods via exerting effect on the water depth, quantity and composition of phyto- zooplankton and aquatic macrophytes, macroinvertebrates communities (e.g.: Bíró, 2000a,b, Specziár and Vörös, 2001, Sipkay et al., 2007). It should also be taken into consideration that changes in the size and composition of the fish stock are influenced by both anthropogenic and natural factors. The major shift in the trophic status of the lake which has started in the 1990's (eutrophic/hypertrophic basins have become mesotrophic) initiated such ecological processes which resulted in the drastic reduction of the size of natural fish stock of the lake, as well as lowering of its sustainability.

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Appendices

Appendix 1. *The fisheries catches of 9 economical important fish species and the cumulate data of silver carp, bighead carp and their hybrids in Lake Balaton between 1901-2011*

Year	Fish species / catch in tons				
	asp (*Aspius aspius*)	carp (*Cyprinus carpio*)	catfish (*Silurus glanis*)	bighead carp, silver carp and their hybrid *(Hypopthalmichthys molitrix, H. nobilis)*	eel (*Anguila anguila*)
1901	5.92	4.5	2.34	-	-
1902	11.33	10.0	6.30	-	-
1903	11.06	8.5	4.93	-	-
1904	7.53	7.2	4.15	-	-
1905	7.96	7.0	4.73	-	-
1906	7.76	6.7	5.08	-	-
1907	9.26	6.9	5.31	-	-
1908	15.00	13.6	6.12	-	-
1909	13.38	17.5	5.45	-	-
1910	7.73	8.0	4.72	-	-
1911	9.36	8.5	6.17	-	-
1912	11.39	11.4	7.46	-	-
1913	12.73	6.6	5.68	-	-
1914	3.35	3.0	2.12	-	-
1915	8.67	8.2	5.64	-	-
1916	4.87	9.5	3.44	-	-
1917	5.53	15.6	6.42	-	-
1918	13.24	21.2	4.81	-	-
1919	11.24	20.4	6.72	-	-
1920	9.22	29.3	10.21	-	-
1921	13.78	33.9	15.43	-	-
1922	10.76	20.7	11.14	-	-
1923	9.31	15.9	15.04	-	-
1924	13.51	28.8	14.98	-	-
1925	19.58	28.0	11.84	-	-
1926	20.64	51.3	13.37	-	-
1927	19.00	43.1	13.30	-	-
1928	21.10	50.0	15.20	-	-
1929	29.92	82.4	17.87	-	-
1930	25.89	45.4	13.20	-	-
1931	19.24	37.8	9.52	-	-
1932	21.65	73.4	16.84	-	-
1933	19.07	34.6	18.66	-	-
1934	22.02	44.7	18.48	-	-
1935	19.87	53.7	17.49	-	-
1936	25.99	53.4	22.25	-	-
1937	12.73	66.7	18.81	-	-
1938	16.41	58.3	15.50	-	-
1939	13.92	36.0	16.88	-	-
1940	5.60	10.7	9.95	-	-
1941	14.52	45.7	18.34	-	-
1942	11.59	53.2	14.71	-	-
1943	14.48	29.0	13.56	-	-
1944	13.39	16.5	5.80	-	-
1945	4.11	12.4	6.54	-	-
1946	13.68	11.1	8.12	-	-
1947	14.91	13.2	9.68	-	-
1948	23.56	29.7	14.42	-	-
1949	27.35	33.3	14.39	-	-
1950	16.62	25.1	20.30	-	-

Year	Fish species / catch in tons				
	asp (*Aspius aspius*)	carp (*Cyprinus carpio*)	catfish (*Silurus glanis*)	bighead carp, silver carp and their hybrid *(Hypopthalmichthys molitrix, H. nobilis)*	eel (*Anguila anguila*)
1951	14.73	23.6	14.87	-	-
1952	28.48	25.7	13.38	-	-
1953	23.58	34.1	27.90	-	-
1954	21.39	19.2	21.60	-	-
1955	18.92	14.2	13.88	-	-
1956	14.77	21.3	8.59	-	-
1957	17.00	25.6	12.84	-	-
1958	13.74	28.9	10.47	-	-
1959	11.43	12.8	10.82	-	-
1960	10.11	9.7	7.86	-	-
1961	13.31	23.6	9.09	-	-
1962	13.50	19.6	7.02	-	-
1963	10.19	17.0	12.92	-	-
1964	15.73	29.9	12.91	-	0.2
1965	6.20	34.9	11.75	-	1.1
1966	6.68	42.8	12.08	-	2.3
1967	8.29	46.0	19.91	-	3.5
1968	12.32	58.0	12.38	-	4.4
1969	12.23	61.2	9.06	-	25.6
1970	10.99	51.5	6.89	-	42.8
1971	13.34	66.6	7.01	-	8.1
1972	10.56	59.3	5.88	0.1	61.4
1973	10.39	63.0	5.36	3.7	21.0
1974	10.30	58.2	4.11	9.0	29.4
1975	12.53	63.7	3.70	11.6	36.6
1976	10.97	52.6	1.91	5.2	38.1
1977	9.69	42.1	5.20	5.1	35.8
1978	10.05	46.0	2.03	5.8	32.2
1979	8.06	20.7	1.89	6.0	64.4
1980	8.57	29.0	4.15	8.1	44.3
1981	9.53	13.3	2.57	8.0	40.5
1982	6.53	21.7	2.31	13.5	70.0
1983	6.16	43.2	3.81	16.0	135.9
1984	8.15	15.4	2.61	11.9	97.4
1985	7.51	11.0	2.27	31.9	238.1
1986	6.96	10.1	2.30	11.2	97.7
1987	7.11	21.7	4.19	16.4	331.3
1988	7.68	19.5	3.06	36.6	61.1
1989	5.74	8.8	3.75	67.2	78.4
1990	5.25	8.8	2.18	82.0	74.3
1991	2.43	23.1	4.43	91.0	76.8
1992	2.91	13.0	2.01	33.5	399.2
1993	1.12	4.0	1.44	40.3	225.1
1994	5.31	11.5	2.55	43.8	459.9
1995	4.65	15.8	3.25	70.4	357.9
1996	6.70	11.4	2.17	83.1	509.4
1997	4.33	8.3	4.42	146.0	84.6
1998	4.34	5.2	3.53	257.7	147.0
1999	3.24	11.0	4.18	259.3	138.4

Year	Fish species / catch in tons				
	asp (*Aspius aspius*)	carp (*Cyprinus carpio*)	catfish (*Silurus glanis*)	bighead carp, silver carp and their hybrid *(Hypopthalmichthys molitrix, H. nobilis)*	eel (*Anguila anguila*)
2000	3.13	5.7	1.28	137.9	45.8
2001	5.74	8.9	5.73	274.7	14.0
2002	2.75	6.5	2.96	281.1	19.0
2003	2.45	9.4	4.39	182.6	16.0
2004	0.84	4.6	2.31	446.9	8.6
2005	0.41	7.0	2.86	240.5	56.7
2006	0.37	4.2	3.01	275.0	47.6
2007	0.31	3.0	2.56	347.8	19.7
2008	0.33	6.1	2.64	284.0	22.7
2009	0.30	5.2	2.65	356.0	6.6
2010	0.31	6.4	3.09	338.1	5.6
2011	0.30	7.1	2.73	254.4	4.4

Year	Fish species / catch in tons					
	gibel carp (*Carassius gibelio*)	pike (*Esox lucius*)	pikeperch (*Sander lucioperca*)	sabre carp (*Pelecus cultratus*)	Volga pikeperch (*Sander volgensis*)	grass carp (*Ctenopharyngodon idella*)
1901	-	20.51	92.30	130.951	-	-
1902	-	31.24	90.56	90.225	-	-
1903	-	16.20	58.19	313.657	-	-
1904	-	13.71	84.23	178.309	-	-
1905	-	11.93	74.02	150.85	-	-
1906	-	21.31	100.18	68.051	-	-
1907	-	28.81	78.24	176.001	-	-
1908	-	14.93	91.74	130.837	-	-
1909	-	6.82	93.40	112.003	-	-
1910	-	13.88	72.24	97.01	-	-
1911	-	22.52	81.78	159.524	-	-
1912	-	12.91	71.23	212.035	-	-
1913	-	9.20	62.89	59.101	-	-
1914	-	8.38	38.20	73.405	-	-
1915	-	10.26	53.71	60.849	-	-
1916	-	26.18	43.52	26.348	-	-
1917	-	20.11	37.37	18.187	-	-
1918	-	11.71	39.97	45.379	-	-
1919	-	27.81	32.32	80.102	-	-
1920	-	12.91	71.54	129.215	-	-
1921	-	6.29	94.35	70.313	-	-
1922	-	3.95	94.62	120.419	-	-
1923	-	8.04	117.53	112.792	-	-
1924	-	34.21	120.86	253.798	-	-
1925	-	22.08	77.21	178.338	-	-
1926	-	20.06	98.58	124.282	-	-
1927	-	27.60	106.40	99.5	-	-
1928	-	13.60	96.20	61.2	-	-
1929	-	24.67	101.16	140.867	-	-
1930	-	18.67	148.53	112.808	-	-
1931	-	10.00	169.01	42.998	-	-
1932	-	11.73	157.69	70.433	-	-
1933	-	9.21	214.70	141.535	-	-
1934	-	6.54	236.77	152.034	-	-
1935	-	22.84	165.67	161.613	-	-
1936	-	19.96	159.32	152.775	-	-
1937	-	29.33	160.65	112.816	-	-
1938	-	33.85	171.10	134.187	-	-
1939	-	10.59	185.30	83.712	-	-
1940	-	7.93	88.73	109.734	-	-
1941	-	30.47	171.17	200.186	-	-
1942	-	28.74	134.31	62.312	-	-
1943	-	29.02	121.11	145.075	-	-
1944	-	10.86	58.45	16.81	-	-
1945	-	5.79	45.25	83.008	-	-
1946	-	7.28	71.12	218.399	-	-
1947	-	14.43	90.77	131.138	-	-
1948	-	37.23	141.58	185.305	-	-
1949	-	40.89	108.61	102.727	-	-
1950	-	15.83	95.31	143.101	2.91	-

Year	Fish species / catch in tons					
	gibel carp (*Carassius gibelio*)	pike (*Esox lucius*)	pikeperch (*Sander lucioperca*)	sabre carp (*Pelecus cultratus*)	Volga pikeperch (*Sander volgensis*)	grass carp (*Ctenopharyngodon idella*)
1951	-	10.48	102.60	116.563	3.62	-
1952	-	20.63	145.57	131.17	6.10	-
1953	-	20.32	157.23	160.149	11.01	-
1954	-	12.16	136.73	130.064	7.73	-
1955	-	6.62	121.65	132.928	4.49	-
1956	-	6.49	109.70	44.646	3.03	-
1957	-	6.50	143.08	66.922	5.00	-
1958	-	8.31	148.02	87.784	4.03	-
1959	7.7	5.05	172.16	153.871	6.50	-
1960	9.6	2.73	158.42	219.553	5.88	-
1961	1.8	3.49	176.62	169.836	5.47	-
1962	1.0	4.00	138.47	149.9	2.12	-
1963	0.2	6.13	171.73	151.838	4.83	-
1964	0.4	8.79	166.30	274.924	5.30	-
1965	0.1	5.65	60.02	354.582	3.70	-
1966	0.4	9.59	64.92	169.647	6.71	-
1967	0.7	9.82	81.68	117.464	7.90	-
1968	0.7	7.93	107.09	162.568	7.24	-
1969	0.5	3.44	119.90	78.512	6.33	-
1970	0.1	4.66	119.48	50.987	3.41	-
1971	0.1	6.60	101.30	132.082	3.00	-
1972	0.1	5.90	80.72	91.816	4.95	-
1973	0.1	3.62	79.08	71.475	5.71	-
1974	0.0	4.15	82.97	53.448	4.95	-
1975	0.1	2.29	44.92	33.035	3.01	36.045
1976	0.3	3.10	52.50	94.274	4.18	98.449
1977	0.4	1.88	33.93	0.622	5.08	5.702
1978	0.1	2.11	45.26	9.365	4.03	13.395
1979	0.7	2.19	54.53	8.467	3.43	11.9
1980	0.2	2.16	47.64	50.03	1.78	51.805
1981	0.1	1.88	30.49	1.563	1.49	3.049
1982	0.1	1.61	53.05	1	1.12	2.118
1983	0.0	1.40	49.46	23.846	1.40	25.249
1984	0.0	1.36	49.47	11.333	2.15	13.478
1985	0.3	1.80	39.06	15.377	2.54	17.914
1986	0.4	2.94	31.55	0.01	1.54	1.551
1987	0.7	2.25	36.85	7.57	1.60	9.167
1988	0.3	3.32	40.00	10.853	1.35	12.198
1989	3.8	0.43	18.34	5.795	0.72	6.513
1990	2.2	0.57	14.39	5.517	0.60	6.12
1991	2.2	0.28	9.41	3.127	0.29	3.415
1992	23.8	0.50	8.66	2.777	0.20	2.976
1993	4.4	0.35	10.23	1.629	0.28	1.906
1994	3.6	0.39	13.94	0.741	0.48	1.22
1995	23.2	0.31	9.26	1.687	0.04	1.722
1996	29.9	0.32	6.05	0.698	0.03	0.725
1997	18.7	0.49	13.92	0.592	0.17	0.759
1998	12.7	0.41	9.16	8.218	0.15	8.364
1999	7.5	0.47	8.60	4.987	0.06	5.051

Year	Fish species / catch in tons					
	gibel carp (*Carassius gibelio*)	pike (*Esox lucius*)	pikeperch (*Sander lucioperca*)	sabre carp (*Pelecus cultratus*)	Volga pikeperch (*Sander volgensis*)	grass carp (*Ctenopharyngodon idella*)
2000	1.3	0.84	15.88	0.032	0.04	0.074
2001	1.0	0.42	11.79	0.26	0.01	0.269
2002	1.2	0.19	11.82	0.268	0.02	0.29
2003	1.7	0.32	15.33	1.495	0.00	1.499
2004	0.7	0.06	1.62	0.934	0.01	0.939
2005	5.1	0.08	6.50	4.001	0.01	4.007
2006	6.0	0.05	4.12	1.788	0.04	1.828
2007	4.4	0.04	2.67	1.134	0.02	1.154
2008	2.1	0.04	4.60	2.109	0.03	2.139
2009	3.0	0.05	3.33	0.385	0.01	0.395
2010	1.9	0.04	2.11	0.379	0.02	0.399
2011	2.3	0.04	2.89	1.009	0.01	1.019

THE ROLE OF SURROUNDING LANDSCAPE IN DETERMINING SPECIES RICHNESS OF MESIC GRASSLANDS IN PANNONIAN BASIN AND CARPATHIAN MOUNTAINS

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Abstract. Alpha diversity of mesic grasslands of the *Arrhenatheretalia* order was related to the surrounding landscape structure. The following questions were addressed: i) Does structure of surrounding landscape affect local species richness? How important is the influence of surrounding landscape in comparison with influence of climatic-topographical and soil-geological variables? ii) Do the landscape effects differ in climatically distinct regions (Pannonian, Carpathian colline-submontane and Carpathian montane-subalpine)? iii) Which of the surrounding habitats contribute to species richness and which species immigrate to grasslands most frequently? Species data were extracted from Slovak vegetation plot database. Surrounding land-use (in terms of cover and diversity of various habitat types in plot neighbourhood of 4 km^2) was derived from CORINE land cover maps and National Grassland Inventory. Both, percentage cover and diversity of different habitat types in plot neighbourhood affected local species richness. In the Pannonian Basin, mesic grasslands had the lowest species richness but the landscape factors explained the highest proportion of its variance. In the montane-subalpine Carpathian region, the effect of landscape factors was least pronounced. The surrounding landscape affected number, proportion and cover of satellite species in plots. Mesic grasslands of the Pannonian Basin hosted the largest number, proportion and cover of archaeophytes and neophytes.

Keywords: *geology, land use, natural habitats, target species, satellite species*

Introduction

Mesic grasslands of the *Arrhenatheretalia* order are widespread in the whole temperate Europe over all types of geological bedrock (Ellenberg, 1996; Dierschke and Briemle, 2002). The order includes semi-natural grasslands on fresh, regularly or occasionally improved habitats, distributed from the lowlands to the subalpine mountain zones (Dierschke, 1997; Janišová et al., 2007; Chytrý, 2012). As their habitats were created by man, along with the suitable climatic and edaphic conditions their maintenance is conditioned by traditional management both in the present and in the past (Klimek et al., 2007; Hájková et al. 2011). Due to their intermediate position along the moisture and nutrient gradients mesic habitat conditions are suitable not only for most grassland species but also for plenty of species diagnostic for other vegetation types and thus intensive immigration of species from surrounding habitats can be expected. This makes the *Arrhenatheretalia* communities especially suitable for a study how the surrounding landscape affects the grassland composition and species richness.

It is supposed that the habitats surrounding certain plant communities constitute sources of species which are not regular components of the given community, as their ecological optima lie in another vegetation type, but which can survive under sub-optimal conditions (the so called satellite species). In such a way, all habitat types, being natural or non-natural, can constitute sources of additional plant species and thus contribute to increasing local grassland diversity (Hanski, 1982; Schmida and Wilson, 1985; Pärtel et al., 2001; Öster et al., 2007). The influence of the surrounding landscape, in terms of heterogeneity and habitat cover, has already been studied at different scales and on different taxa and species groups (Shmida and Wilson, 1985; Sönderström et al., 2001; Reitalu et al., 2012; Schmucki et al., 2012; Janišová et al., 2013). In this contribution, landscape structure was related to local species richness measured at very small scale within the single georeferenced phytosociological plots. A large vegetation database was used comprising several thousand of plots. The following questions were addressed: i) Does structure of surrounding landscape affect local species richness of mesic grasslands in the Carpathian-Pannonian regions of Slovakia? How important is the influence of surrounding landscape in comparison with influence of climatic-topographical and soil-geological variables? ii) Do landscape effects differ in climatically distinct regions (Pannonian, Carpathian colline-submontane and Carpathian montane-subalpine)? iii) Which of the surrounding habitats contribute to species richness in mesic grasslands and which species immigrate to grasslands most frequently?

Material and methods

Relevé selection

A dataset of 1 705 vegetation plots ordered to the phytosociological order *Arrhenatheretalia elatioris* by an electronic expert system (Janišová et al., 2007), containing 785 species of vascular plants, was used for the analyses. This dataset was extracted from the Slovak vegetation database (code EU-SK-001, Šibík, 2012), originally containing 51 180 plots of all vegetation types (as of 1 January 2011). Only plots with accurate geographical location and plot size between 15 and 25 m^2 were included (as there was no positive relation indicated between plot size and number of species, the whole interval of plot sizes was used to ensure enough replicates for all studied factor levels). Only plots from altitudes of up to 1600 m were included, which corresponds to the natural timberline in the Western Carpathian Mts. Only plots with cover of shrub and/or tree layers lower than 25% were included. To reduce the effect of oversampling in certain areas, this dataset was geographically stratified (Knollová et al., 2005). One plot of each syntaxon (determined in most cases at the level of associations by the original author) was randomly selected from a grid square of 0.5′ longitude and 0.3′ latitude (approximately 0.6 x 0.56 km) (*Figure1.*). The resulting dataset included plots recorded between the years 1933 and 2010. Multiple records of species in different layers within one plot were combined so that each species appeared in each relevé only once. Bryophytes and lichens were deleted as they were not recorded in all relevés. Juvenile trees and shrubs were deleted, too.

Landscape characteristics

The effects of land use were studied in plot neighbourhoods of 4 km^2 (a circle with a radius of 1.128 km centred at the plot). According to the CORINE land cover maps (Bossard et al., 2000), 26 habitat classes were distinguished in the neighbourhood of the studied plots. For the purpose of this study, they were combined into two main (natural and semi- natural vs. non-natural) and four subordinate habitat categories (non-forest, forest, agricultural, artificial) as summarized in *Table 1*.

***Table 1**. Definition of habitat types used in this study based on habitat classes of CORINE land cover maps*

Habitat types		**CORINE habitat class**
Natural and semi-natural habitats	Non-forest habitats	2.3.1 Pastures
		2.4.3 Land principally occupied by agriculture with significant areas of natural vegetation
		3.2.1 Natural grasslands
		3.2.2 Moors and heathland
		3.2.4 Transitional woodland-scrub
		3.3.2 Bare rocks
		3.3.3 Sparsely vegetated areas
		4.1.1 Inland marshes
		4.1.2 Peat bogs
	Forest habitats	3.1.1 Broad-leaved forest
		3.1.2 Coniferous forest
		3.1.3 Mixed forest
Non-natural habitats	Agricultural habitats	2.1.1 Non-irrigated arable land
		2.2.1 Vineyards
		2.2.2 Fruit trees and berry plantations
		2.4.2 Complex cultivation patterns
	Artificial habitats	1.1.1 Continuous urban fabric
		1.1.2 Discontinuous urban fabric
		1.2.1 Industrial or commercial units
		1.2.2 Road and rail networks and associated land
		1.2.4 Airports
		1.3.1 Mineral extraction sites
		1.3.2 Dump sites
		1.3.3 Construction sites
		1.4.1 Green urban areas
		1.4.2 Sport and leisure facilities

In addition to landscape parameters calculated from CORINE land cover maps, percentage cover of ecologically valuable grasslands was estimated according to the National Grassland Inventory based on field surveys performed during 1998–2010 (Šeffer et al., 2002). For the purposes of this study, valuable grasslands were defined as biodiverse grasslands of higher nature value. They included 88% of the mapped grasslands (intensively used, fertilized and ruderal grasslands were excluded from this category directly during the field survey). The percentage cover values of six habitat categories based on the CORINE land cover maps and one category based on the National Grassland Inventory were related to local species richness of vascular plants calculated for individual vegetation plots. The Shannon diversity index of habitat diversity was calculated for each plot based on the cover of habitat classes in the plot neighbourhood of 4 km^2 as $H' = -\sum p_i \ln p_i$, where p_i is the proportion of each of the habitat class. The index was calculated for the diversity of (i) all habitats, (ii) natural and semi-natural habitats, (iii) natural and semi-natural non-forest habitats, and (iv) non-natural habitats.

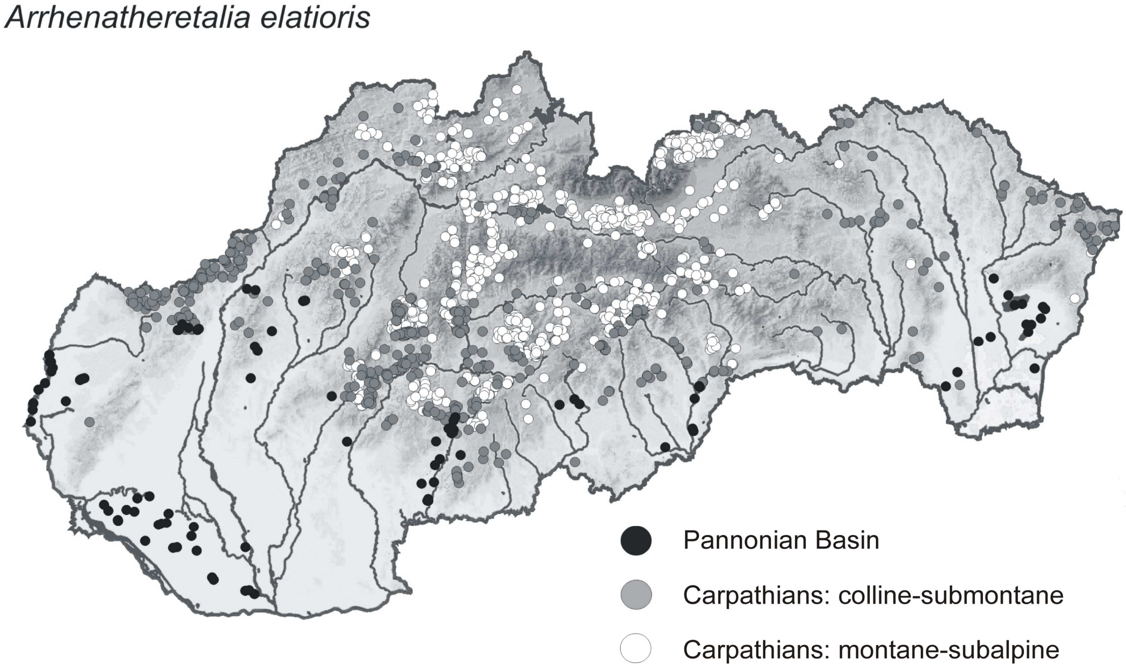


Figure 1. *Distribution of the Arrhenatheretalia plots in three climatic regions of Slovakia*

Environmental variables

Along with the landscape factors the following environmental factors were related to local species richness: altitude; annual precipitation and mean annual temperature (both calculated in a GIS environment using long-term measurements from period 1951–1980 and topographic data); Climatic Water Balance (index calculated as difference between precipitation and evapotranspiration used as indicator of landscape humidity during the growing season April–September; modelled in a GIS environment using the FAO Penman-Monteith equation according to Hlásny and Baláž, 2008); moisture (unweighted means of Ellenberg indicator values, according to Ellenberg et al. 2002, were used as a fine-scale surrogate for water availability at the plot location); soil reaction and soil nutrients (both expressed as unweighted means of Ellenberg indicator values). Digitalized geological map of Slovakia (Biely et al., 2002) was used to set geological bedrock of the sampled sites. For the purpose of this study, the great variety of geological substrates was converted into 6 categories: 1 - acid plutonic, volcanic or metamorphosed rocks (granites, dacites, granodiorites, ryolites, phyllites, schists and gneisses), 2 - intermediate and basic plutonic, volcanic or metamorphosed rocks (andesites, diorites, basalts, gabros, amphibolites), 3 - mesozoic sedimentary rocks (limestones and dolomites), 4 - sandstones and claystones of the flysch belt, 5 - sandstones, claystones and shales of other than flysch origin, 6 - neogene and paleogene sediments (mainly claystones and sandstones).

Climatic regions

For the purpose of this paper, three climatic regions were distinguished based on the climate classification of Slovakia (based on long-term averages of temperature and precipitation mainly during the growing season) by Džatko at al. (1989): i) the Pannonian Basin including lowland to colline regions with warm to very warm, very dry and continental climate; ii) the colline-submontane Carpathians including regions with moderately warm to moderately cold and dry to moderately humid climate; and iii) the montane-subalpine Carpathians including regions with cold to very cold and humid climate.

Categorisation of species groups

Along with the analyses of the whole dataset (including all vascular plant species recorded in the plots) we analyzed a data sub-sets containing the following species groups: target species (typical of mesic grasslands of the *Arrhenatheretalia* order; 108 species), forest species (typical of forest habitats), alien species (including archaeophytes and neophytes according to Medvecká et al., 2012) and native species of non-natural habitats (typical of agricultural and urban habitats). Species typical of individual habitat types were determined on the whole Slovak Vegetation Database (geographically stratified dataset including plots of all syntaxa) by means of the *phi*-coefficient (only species with *phi* > 0.10 were selected as typical) calculated in JUICE (Tichý 2002) with standardisation of relevé groups to equal size and using Fisher's exact test at P<0.001 (Tichý, 2002).

Data analysis

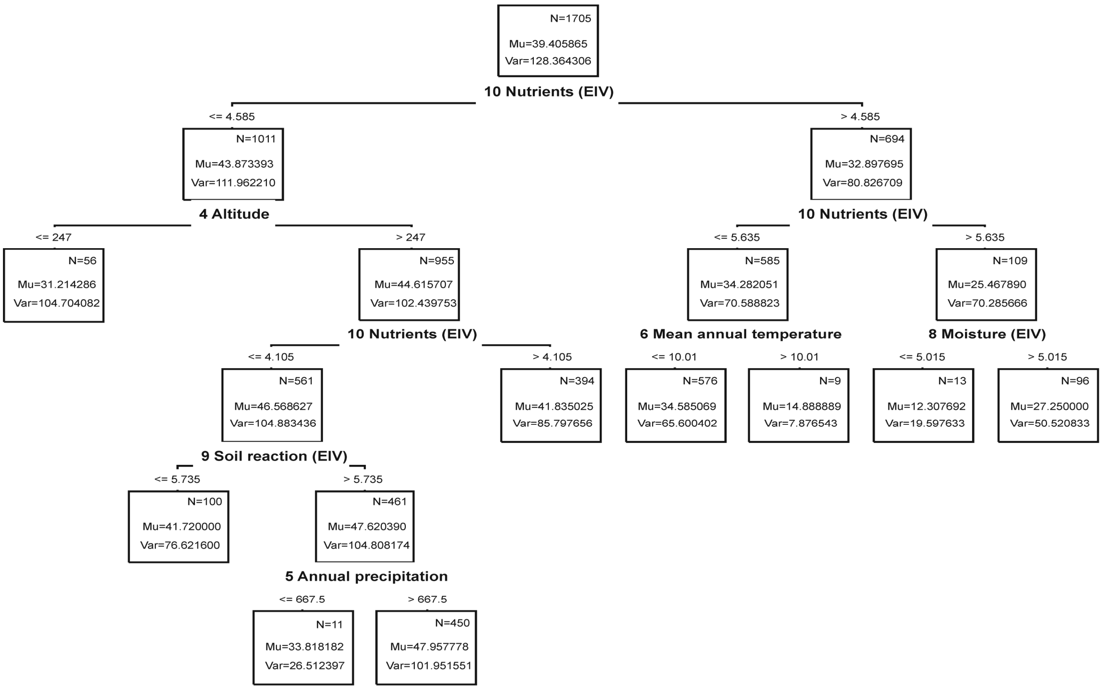
Complex relationships between number of vascular plants and environmental variables were assessed using regression trees (Breiman et al., 1984), calculated in the STATISTICA software (StatSoft Inc., 2006). Optimal tree size was determined by tenfold cross-validation procedure with a standard error rule set to 0.1. Each decision tree was pruned (prune of variance) after the data were split, with a minimum of 100 cases per branch and a maximum of 1000 nodes per tree. The importance plots ranking the predictors on a 0–100 scale were used to determine which variable is the most significant predictor.

Differences in number of species belonging to given species groups between the studied regions and in the region characteristics were tested by using the analysis of variance and Fisher LSD multiple comparison test in the STATISTICA program (StatSoft Inc., 2006). Series of simple linear and quadratic regressions were performed for local species richness as the dependent variable and 18 environmental factors as continuous predictors. Bonferroni correction was used setting critical values of α as α /18. The Akaike information criterion (AIC) was used to select the most appropriate model.

Results

Landscape effects on local species richness of mesic grasslands in the studied regions

Mesic grasslands in the Carpathian Mts (*Fig. 1*) were more species-rich (with 40 vascular plants in a plot on average) than mesic grasslands in the Pannonian Basin (with 30 vascular plants in a plot on average). This conclusion was valid also if only target *Arrhenatheretalia* species were considered (*Fig. 2*).



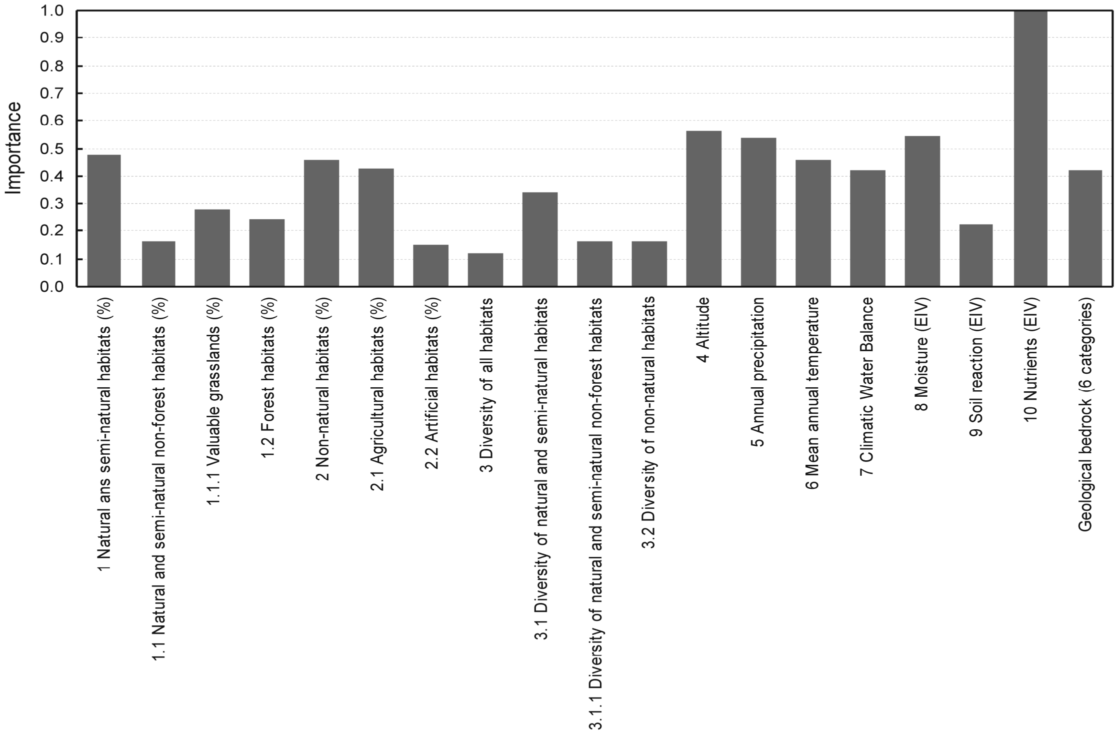


***Figure 2.** Average number of species in different species groups in mesic grasslands of the Pannonian Basin (P), colline-submontane Carpathians (C1) and montane-subalpine Carpathians (C2). T – target species of mesic Arrhenatheretalia meadows, F – forest species, A – archaeophytes, N – neophytes, NN – native species of non-natural habitats, others – other species not ordered to the former groups. Differences among the regions were tested by ANOVA at alpha 0.05 and the results of multiple comparison tests are indicated by letters (values that do not differ are depicted by the same letter).*

The overall diversity of mesic grassland vegetation as reflected in the number of recorded *Arrhenatheretalia* phytosociological associations was also higher in the Carpathians (10 and 11 association in C1 and C2, respectively) than in the Pannonic Basin (7 associations, *Table 2*).

Table 2. *Characteristics of the whole teritory and the three distinct climatic regions are shown. Differences among the regions were tested by ANOVA at alpha 0.05 and the results of multiple comparison tests (Fisher LSD test) are indicated by capital letters (values that do not differ are depicted by the same letter).*

Characteristics (mean ± St. dev.)	**All climatic regions**	**Pannonian Basin**	**Carpathians (colline-submontane)**	**Carpathians (montane-subalpine)**
Number of analyzed plots	1705	98	506	1101
Local species richness (number of all vascular plants)	39 ± 11	30 ± 12^{A}	40 ± 12^{B}	40 ± 11^{B}
1 Proportion of natural and semi-natural habitats	79 ± 26	36 ± 33^{A}	71 ± 26^{B}	86 ± 20^{C}
1.1 Proportion of natural and semi-natural non-forest habitats	37 ± 22	17 ± 18^{A}	33 ± 20^{B}	41 ± 22^{C}
1.1.1 Proportion of valuable grasslands	18 ± 17	7 ± 11^{A}	13 ± 13^{B}	22 ± 18^{C}
1.2 Proportion of forest habitats	42 ± 28	20 ± 26^{A}	38 ± 25^{B}	45 ± 28^{C}
2 Proportion of non-natural habitats	21 ± 26	62 ± 33^{C}	29 ± 26^{B}	14 ± 20^{A}
2.1 Proportion of agricultural habitats	16 ± 23	54 ± 30^{C}	22 ± 23^{B}	10 ± 17^{A}
2.2 Proportion of artificial habitats	4 ± 8	8 ± 11^{B}	6 ± 9^{B}	3 ± 7^{A}
3 Diversity of all habitats	1.2 ± 0.3	0.9 ± 0.4^{A}	1.3 ± 0.3^{C}	1.2 ± 0.3^{B}
3.1 Diversity of natural and semi-natural habitats	0.9 ± 0.3	0.4 ± 0.3^{A}	0.9 ± 0.3^{B}	1.0 ± 0.3^{C}
3.2 Diversity of natural and semi-natural non-forest habitats	0.5 ± 0.2	0.3 ± 0.2^{A}	0.5 ± 0.2^{B}	0.5 ± 0.2^{C}
3.3 Diversity of non-natural habitats	0.3 ± 0.2	0.4 ± 0.2^{C}	0.4 ± 0.3^{B}	0.2 ± 0.2^{A}
4 Altitude (m a.s.l.)	620 ± 237	177 ± 72^{A}	416 ± 98^{B}	754 ± 166^{C}
5 Annual precipitation (mm)	857 ± 137	624 ± 55^{A}	793 ± 81^{B}	907 ± 129^{C}
6 Mean annual temperature (°C)	5.9 ± 1.4	9.1 ± 0.9^{C}	7.0 ± 0.9^{B}	5.2 ± 0.9^{A}
7 Climatic Water Balance (index)	-17 ± 124	-242 ± 57^{A}	-93 ± 80^{B}	39 ± 101^{C}
8 Moisture (unweighted means of Ellenberg indicator values)	4.8 ± 0.5	4.7 ± 0.6^{A}	4.6 ± 0.5^{A}	4.8 ± 0.5^{B}
9 Soil reaction (unweighted means of Ellenberg indicator values)	6.3 ± 0.6	6.7 ± 0.5^{B}	6.6 ± 0.5^{B}	6.2 ± 0.6^{A}
10 Soil nutrients (unweighted means of Ellenberg indicator values)	4.5 ± 0.7	4.5 ± 0.9^{A}	4.5 ± 0.7^{A}	4.4 ± 0.7^{A}
Arrhenatherion elatioris (number of associations)	5	4	5	5
Cynosurion cristati (number of associations)	3	3	3	2
Polygono bistorti-Trisetion flavescentis (number of associations)	4	0	2	4
Total number of the *Arrhenatheretalia* associatios	12	7	10	11

The studied regions differed in many characteristics of the surrounding landscape. In the Pannonian Basin the average proportion of non-natural habitats was 2-3 times higher and the proportion of natural and semi-natural habitats was 2 times lower than in the

Carpathians. Similarly, diversity of natural and semi-natural habitats increased towards high altitudes and harsh montane climate. Diversity of non-natural habitats was lowest in the montane-subalpine Carpathians while the Carpathian colline-submontane regions and the Pannonian Basin did not differ in this characteristic (*Table 2*).

Irrespectively from the region, local species richness of the studied grasslands increased with increasing diversity or percentage cover of different natural and semi-natural habitats in the plot neighbourhood. Conversely, local species richness decreased with higher proportion or diversity of non-natural habitats in the surroundings. According to simple regression models (*Table 3*), proportion of all natural and semi-natural habitats explained the highest percentage variance in local species richness in all studied regions. Among the landscape factors, it was the best predictor of high local species richness in mesic grasslands, while proportion of all non-natural habitats was the best predictor of low local species richness. Diversity of natural and semi-natural habitats including the diversity of non-forest habitats was important mainly in the Pannonian Basin. Percentage of variance explained by single regression models was much higher for the Pannonian Basin than for the Carpathian regions in all studied landscape factors.

Landscape effects in comparison with effects of other environmental factors

In the whole dataset without regard to climatic regions, soil nutrients and moisture (both expressed by Ellenberg indicator values) explained the highest percentage variance in single regression models for local species richness (*Table 3*). The landscape factors and the other environmental factors studied explained lower, but similar percentage variance. Similar results were obtained by the regression tree analysis (*Fig. 3*). The optimal regression tree for local species richness had nine terminal nodes. The first split was based on nutrients (EIV), with lower species richness associated with nutrient-rich grasslands (694 of 1705 plots). The other group of nutrient-poor grasslands was further split by altitude, which separated species-poorer sites at lowest altitudes from other sites. Plots at higher altitudes were further divided into a species-poorer group in nitrogen-richer sites, and the remaining group of plots was further split by soil reaction (EIV). The more species-rich group in more basiphilous habitats was finally divided according to annual precipitation where a small group of 11 plots with lower precipitation was species poorer than the rest of plots. For nutrient-rich group, the next division was also based on nutrients (EIV), with higher richness at the nutrient-poorer sites, this group of plots further split according to the mean annual temperature. The most nutrient-rich plots were further divided by moisture and the drier group reached the absolutely lowest species richness in the dataset.

The effect of geological bedrock as the only categorical variable was studied for the whole dataset (*Fig. 4*). Number of target species was the highest in mesic grasslands on sandstones, claystones, intermediate (mainly andesites) and basic rocks (mainly basalts) and the lowest on neogene and paleogene sediments. Calcareous and acid substrates had intermediate richness of target species. There were no differences in number of satellite species (including forest species and all species of non-natural habitats) among plots on different geological bedrock. The highest number of other species (including mainly generalist species and species of semi-natural non-forest habitats other than mesic grasslands) was found on mesosoic sedimentary rocks and sandstones, claystones and shales of other than flysh origin.

***Table 3.** Summary of simple regression models for local species richness as dependent variable and environmental factors as predictors Linear and quadratic relationships were compared and the model with lower AIC is presented by arrows ↑ or ↓ for linear relationships, ∩ for hump-back and U for U-shape quadratic relationships. Percentage variance of dependent variable explained by the model is shown in the parentheses. Significant relationships were indicated after using Bonferroni correction. Regression models for moisture, soil reaction and soil nutrients based on Ellenberg indicator values were corrected using the modified permutation test (Zelený & Schaffers 2012).* P < 0.05, ** P < 0.01 and *** P < 0.001, n.s. – non significant.*

Environmental factors	**All climatic regions**	**Pannonian Basin**	**Carpathians (colline-submontane)**	**Carpathians (montane-subalpine)**
Landscape factors				
1 Natural and semi-natural habitats (%)	∩*** (7.9)	∩*** (42.9)	U*** (6.6)	∩** (1.7)
1.1 Natural and semi-natural non-forest habitats (%)	∩*** (1.2)	∩*** (27.5)	↑** (2.9)	n.s.
1.1.1 Valuable grasslands (%)	∩*** (3.1)	∩*** (19.8)	↑** (3.5)	n.s.
1.2 Forest habitats (%)	∩*** (3.0)	∩*** (29.7)	n.s.	↑** (1.2)
2 Non-natural habitats (%)	∩*** (3.0)	∩*** (37.9)	U*** (6.5)	∩** (1.6)
2.1 Agricultural habitats (%)	∩*** (6.6)	∩*** (22.1)	↓*** (5.5)	∩* (1.3)
2.2 Artificial habitats (%)	U*** (1.7)	U*** (20.3)	U* (2.7)	n.s.
3 Diversity of all habitats	∩*** (1.3)	↑** (15.0)	n.s.	n.s.
3.1 Diversity of natural and semi-natural habitats	∩*** (6.0)	↑*** (37.8)	n.s.	∩** (1.5)
3.1.1 Diversity of natural and semi-natural non-forest habitats	∩*** (1.3)	∩*** (27.3)	n.s.	n.s.
3.2 Diversity of non-natural habitats	↓*** (2.1)	n.s.	U*** (5.2)	n.s.
Climatic-topographical factors				
4 Altitude (m a.s.l.)	∩*** (5.8)	∩*** (39.8)	∩** (3.2)	∩** (1.5)
5 Annual precipitation (mm)	∩*** (5.9)	∩*** (23.8)	↑*** (4.4)	∩* (1.1)
6 Mean annual temperature (°C)	∩*** (5.6)	∩*** (37.1)	n.s.	n.s.
7 Climatic Water Balance (index)	∩*** (4.6)	∩*** (28.9)	n.s.	∩* (1.2)
Soil-geological factors				
8 Moisture (unweighted means of Ellenberg indicator values)	U*** (10.4)	n.s.	U*** (12.5)	U*** (1.5)
9 Soil reaction (unweighted means of Ellenberg indicator values)	∩* (0.6)	n.s.	n.s.	↑*** (2.2)
10 Soil nutrients (unweighted means of Ellenberg indicator values)	∩*** (28.8)	∩** (14.8)	∩*** (29.9)	↓*** (31.4)

Satellite species provided by surrounding habitats

Out of 785 species in the whole dataset, 108 were identified as target species of the *Arrhenatheretalia* grasslands, the rest of species were either the generalist species occurring in various types of habitats or the satellite species, colonizing the studied grasslands from the neighbouring communities (satellite species). Among the satellite species, 78 species were typical of forest habitats, 49 were archaeophytes, 15 neophytes and 38 species were native species typical of non-natural habitats. In comparison with the Carpathians, mesic grasslands in the Pannonian Basin contained less target species in the target species pool and individual plots and less forest species in the forest species pool and individual plots (*Table 4*). Contrastingly, they contained more archaeophytes, neophytes and native species of non-natural habitats in individual plots, while number of these species in the regional species pools did not differ from the Carpathian regions.

Table 4. *The size of regional species pools for individual species groups and average number of species belonging to particular species groups in plots (in parentheses)*

	Pannonian Basin	**Carpathians colline-submontane**	**Carpathians montane-subalpine**
Target species	94 (18)	107 (30)	108 (30)
Forest species	16 (0.4)	51 (0.9)	71 (1.0)
Archaeophytes	35 (1.6)	35 (0.8)	32 (0.4)
Neophytes	10 (0.3)	10 (0.2)	10 (0.1)
Native species of non-natural habitats	26 (1.2)	27 (0.9)	29 (0.8)

Satellite species recorded in mesic grassland plots are listed in Appendix 1.

Discussion

As shown in previous studies (Steiner & Köhler, 2003; Schmucki et al., 2012; Janišová et al., 2013), grassland species richness is affected by the surrounding landscape and mesic grasslands belong to the most affected communities due to their central position on the moisture and nutrient gradients and suitable habitat conditions for most of the generalist species. The results of this study emphasize the role of land-use history and landscape structure in shaping the composition and diversity of grassland ecosystems. Lower local species richness in the Pannonian Basin may not only be the result of lowland more continental climate in this region which is less suitable for maintenance of mesophilous grassland communities, but also the result of long-lasting intensive human influence. Due to the transformation of grasslands to agricultural fields, reduction in size and diversity of natural and semi-natural habitats and resulting course and uniform landscape structure, the remaining grassland patches resemble islands isolated in the intensive agricultural landscape. Grassland species pool in this region has been gradually reduced due to insufficient connectivity and size of grassland complexes. This may be the reason why average mesic grassland plot in the Pannonian Basin hosts by one quarter less vascular plant species than a comparable plot in the Carpathians. Although the diversity of non-natural habitats is much higher in the Pannonian Basin than in the Carpathians, the enrichment by archaeophytes and native species of non-natural habitats is not sufficient to increase significantly the overall local species richness of these meadows.

Among the abiotic habitat conditions, nutrient status of habitat seems to play the most important role for determination of local species richness in the studied grasslands. Negative influence of excess nutrients (mainly phosphorus and potassium) on vascular plant species richness was found in many studies dealing with various types of grassland vegetation (e.g. Janssens et al., 1998; Critchley et al., 2002; Hejcman et al., 2007; Merunková & Chytrý, 2012) and it is attributed mainly to change of dominant species and an increase of sward height. Moisture availability was another important predictor of local species richness in the studied grasslands. Moisture gradient is considered to be the main determinant of compositional change in most European grassland communities (e.g. Merunková & Chytrý, 2012; Moeslund et al., 2013). Our study confirmed the importance of moisture for grassland diversity and suggests that both, topographically controlled soil moisture expressed by average Ellenberg indicator values and precipitation as a broad-scale surrogate for water availability are good predictors of species richness in mesic grasslands (*Table 3, Fig. 3*).

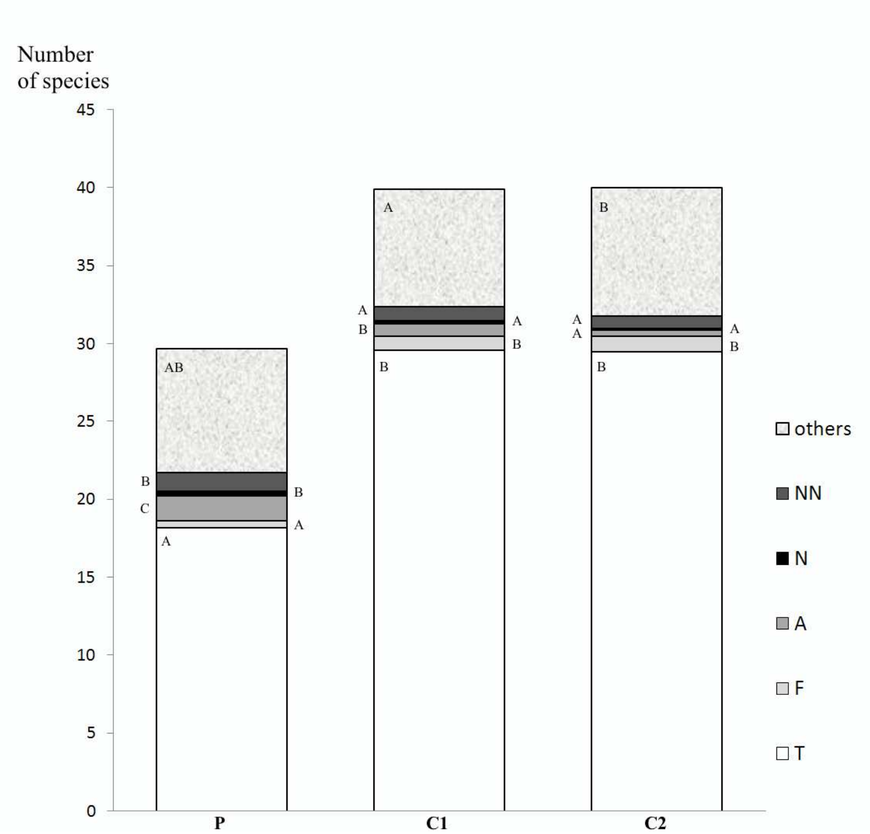


Figure 3. *Regression tree for local species richness (15-25 m²) and relative importance of environmental factors for prediction of local species richness. Mean number of vascular plant species (Mu), variance (Var) and number of plots (N) belonging to a node are given at each node. EIV: plot means of unweighted Ellenberg indicator values.*

Most of the studied climatic-topographical and soil-geological factors had stronger influence on local species richness than the studied landscape factors if all grassland plots were taken into consideration (*Fig. 2, Table 3*). However, in the Pannonian Basin, landscape factors had comparable importance and one of the landscape factors (proportion of natural and semi-natural habitats) explained the largest proportion of variance in local species richness. This suggests special importance of landscape context in the regions strongly modified by human activities. The obtained results emphasize the importance of careful landscape planning for maintaining diverse grassland communities and avoiding spread of invasive alien species.

Mesic grasslands differed in their species composition and diversity also as a result of different geological bedrock. Sandstones, claystones, andesites and basalts seem to provide optimal habitat condition for development of species-rich mesic grassland communities as they support the highest number of target grassland species (*Fig. 4a*).

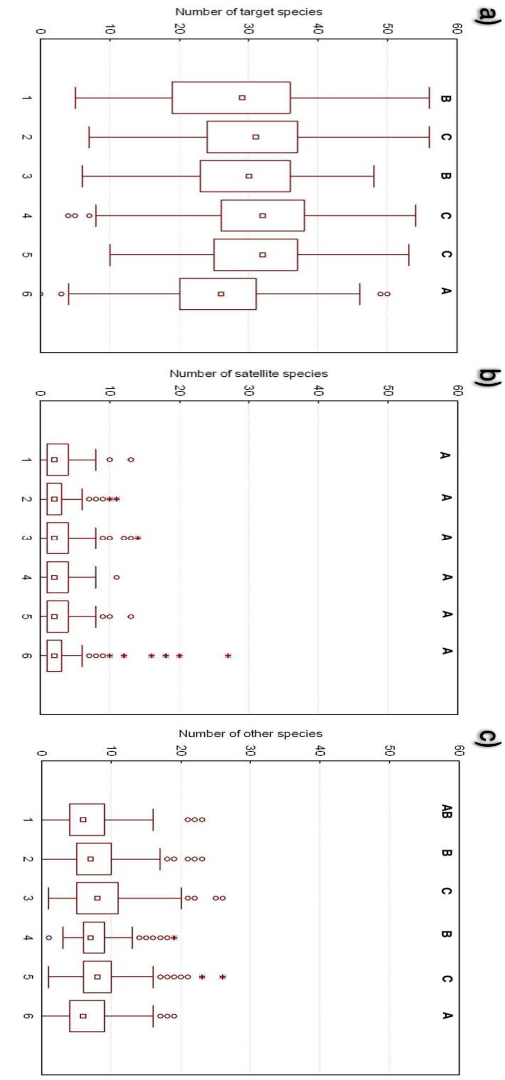


Figure 4. *Effect of geological bedrock on species richness of a) target, b) satellite and c) other species in mesic grasslands of Slovakia. Geological bedrock: 1 - acid plutonic, volcanic or metamorphosed rocks, 2 - intermediate and basic plutonic, volcanic or metamorphosed rocks, 3 - mesozoic sedimentary rocks, 4 - sandstones and claystones of the flysch belt, 5 - sandstones, claystones and shales of other than flysch origin, 6 - neogene and paleogene sediments. Differences among regions were tested by ANOVA at alpha 0.05 and the results of multiple comparison tests are indicated by letters (values that do not differ are depicted by the same letter).*

As sandstones and claystones also can support high number of generalist species and species of other natural and semi-natural non-forest habitats (*Fig. 4c*), the most species-rich mesic grasslands can be found especially on this kind of geological bedrock. High number of “other” species indicated in mesic grasslands on calcareous bedrock (*Fig. 4c*) supports the idea of large species pool of calcareous species in Europe as suggested by Ewald (2003). On the other hand, occurrence of satellite species (both from natural and non-natural habitats) seems not to be dependent on the bedrock type and is rather influenced by the structure of the surrounding landscape.

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Appendix

Appendix 1. *List of satellite species recorded in mesic grassland plots (for species present only in certain region, the abbreviation P, C1 or C2 is given in parentheses)*

Forest species: *Ajuga reptans*, *Anemone nemorosa* (C1, C2), *Anemone ranunculoides* (C2), *Asarum europaeum* (C1, C2), *Astragalus glycyphyllos*, *Brachypodium sylvaticum* (C1, C2), *Calamagrostis arundinacea* (C2), *Campanula persicifolia* (C1, C2), *Campanula rapunculoides* (C1, C2), *Campanula trachelium* (C1, C2), *Carex alba* (C1, C2), *Carex digitata* (C1, C2), *Carex montana* (C1, C2), *Carex muricata* agg., *Carex pilosa* (C1), *Carex sylvatica*, *Cirsium erisithales* (C1, C2), *Clinopodium vulgare*, *Convallaria majalis* (C2), *Daphne mezereum* (C2), *Dentaria bulbifera* (C1, C2), *Digitalis grandiflora* (C1, C2), *Dryopteris filix-mas* (C1, C2), *Epilobium montanum* (C2), *Festuca altissima* (C1), *Festuca heterophylla* (C1), *Fragaria moschata* (C1, C2), *Fragaria vesca*, *Galium odoratum* (C2), *Galium schultes*ii (P, C2), *Genista tinctoria* (C1, C2), *Gentiana asclepiadea* (C1, C2), *Geranium robertianum* (C1), *Geum urbanum*, *Hacquetia epipactis* (C1), *Hieracium* l*achenalii* (C2), *Hieracium murorum* (P, C2), *Hieracium racemosum* (P), *Hieracium sabaudum*, *Hypericum montanum* (C2), *Isopyrum thalictroides* (C1), *Lathyrus niger* (C2), *Lathyrus vernus* (C1, C2), *Lilium martagon* (C2), *Luzula luzulina* (C2), *Luzula* l*uzuloides* (C1, C2), Luzula sylvatica (C2), Maianthemum bifolium (C1, C2), Melampyrum sylvaticum (C2), Melica nutans (C1, C2), Mercurialis perennis (C1, C2), *Oxalis acetosella* (C1, C2), *Paris quadrifolia* (C1), *Petasites albus* (C2), *Platanthera bifolia* (C1, C2), *Poa nemoralis*, *Polygonatum multiflorum* (C1, C2), *Polygonatum odoratum* (C1, C2), *Polygonatum verticillatum* (C2), *Pulmonaria mollis* (C1, C2), *Pulmonaria officinalis* agg., *Pyrethrum corymbosum* (C1, C2), *Ranunculus lanuginosus* (C2), *Rubus idaeus* (C2), *Rubus saxatilis* (C2), *Sanicula europaea* (C2), *Scrophularia nodosa* (C1, C2), *Senecio ovatus* (C2), *Soldanella hungarica* (C2), *Solidago virgaurea* (C1, C2), *Stachys sylvatica* (C2), *Stellaria holostea* (C1, C2), *Symphytum tuberosum*, *Tithymalus amygdaloides* (C2), *Vaccinium myrtillus* (C2), *Vicia cassubica* (C2), *Viola reichenbachiana*, *Viola riviniana* (C1, C2).

Archaeophytes: *Adonis aestivalis* (P), *Anchusa officinalis* (P, C1), *Anthemis arvensis*, *Apera spica-venti* (C1), *Arctium lappa* (C1, C2), *Arctium tomentosum* (C1, C2), *Ballota nigra* (C1), *Berteroa incana* (P, C2), *Bromus arvensis* (C1), *Bromus tectorum* (P), *Capsella bursa-pastoris*, *Cardaria draba* (P, C1), *Carduus acanthoides*, *Chelidonium majus* (P), *Cichorium intybus*, *Convolvulus arvensis*, *Cynodon dactylon* (P), *Fallopia convolvulus* (P, C1), *Geranium dissectum* (C1, C2), *Geranium pusillum*, *Lactuca serriola*, *Lamium amplexicaule* (P), *Lamium purpureum* (P), *Lathyrus tuberosus*, *Lepidium campestre* (C1, C2), *Marrubium vulgare* (P), *Melampyrum arvense* (C1, C2), *Melilotus albus* (C1, C2), *Melilotus officinalis*, *Myosotis arvensis*, *Raphanus* *raphanistrum* (C1, C2), *Reseda lutea* (P), *Scleranthus annuus*, *Senecio vulgaris* (C1), *Silene latifolia* ssp. *alba* (P), *Sinapis arvensis* (C1, C2), *Sonchus arvensis* (C2), *Thlaspi arvense* (P, C2), *Tithymalus platyphyllos* (P), *Tripleurospermum perforatum*, *Valerianella locusta*, *Verbena officinalis* (P, C2), *Veronica agrestis* (C1, C2), *Veronica arvensis*, *Vicia angustifolia*, *Vicia hirsuta*, *Vicia sativa*, *Viola arvensis*, *Viola odorata*.

Neophytes: *Conyza canadensis* (P, C2), *Erigeron annuus*, *Juncus tenuis* (C1, C2), *Malva moschata* (P, C1), *Matricaria discoidea*, *Medicago sativa*, *Medicago* x *varia* (C1), *Oenothera biennis* agg. (P), *Onobrychis viciifolia* agg., *Oxalis dillenii* (P), *Rumex thyrsiflorus* (P), *Solidago gigantea* (P), *Trifolium hybridum*, *Veronica peregrina* (C2), *Veronica persica* (C1).

Abbreviations: EIV: Ellenberg indicator values, P: Pannonian Basin, C1: colline-submontane Carpathians, C2: montane-subalpine Carpathians

Plant nomenclature: Marhold and Hindák (1998)

GRASSLAND FIRES IN HUNGARY – EXPERIENCES OF NATURE CONSERVATIONISTS ON THE EFFECTS OF FIRE ON BIODIVERSITY

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Abstract. Fire as a natural disturbance has been present in most European grasslands. Controlled burning was also an important component of the traditional landscape management for millennia. It was mainly used to reduce litter and woody vegetation and to maintain open landscapes suitable for farming. Due to socio-economical changes traditional and sustainable use of fire was ceased and replaced by arsons and technical fires in Europe. Despite its wide application in the past and the considerable extension and frequency of current grassland fires, the impact of fire on the grassland biodiversity is still scarcely documented in Europe. The aim of this study is to offer a perspective on the issue of fire impact on grasslands, by overviewing published information and practical experiences from Hungary. Our results suggest that fire can be detrimental for several taxa (e.g. insects or ground-dwelling birds), but can also promote population growth of several endangered species by reducing litter or by creating and maintaining open habitats. We also found that fire may be effective in controlling invasive plant species. The effect of fire on grassland biodiversity may be rather context-dependent. There is a critical need for developing robust evidences on the context-dependence of fire effect on biodiversity. For this, well designed prescribed burning experiments are crucial.

Keywords: *wildfire, prescribed burning; nature conservation management, invasive species, grazing*

Introduction

Fire is a natural disturbance which can occur in all terrestrial ecosystems (Sousa, 1984). Paleoecological evidences show that fire was present in many parts of Europe even before the human colonization of this continent (Feurdean et al., 2012). Burning became a frequently applied management practice during Neolithic ages. Fire was used for a wide range of purposes like maintaining pastures suitable for animal husbandry, preparing arable fields for farming and also for increasing the productivity of farmlands (Vale, 2002; Anonymous, 2010; Papanastasis, et al. 1990). Societies adapting burning as management tool were well aware about the negative effects of uncontrolled fire on the capacity of ecosystems to provide goods and services. Therefore, as every other management intervention, traditional burning was under strict informal regulation at the level of local communities (Anonymous, 2010). The sharp socio-economic changes occurring in Europe in the past two centuries resulted in the erosion of the traditional ecological knowledge regarding burning as land management tool (Bruce and Goldammer, 2004). However, fire still has a key importance in the maintenance of many European landscapes (Anonymous, 2010). The relative importance of natural fires to human made fires decreased significantly with the increasing human domination on the landscapes, and the traditional land management with fire was recently replaced with arsons (illegal burnings for grassland management, fires set for fun and/or by vandalism) and technical fires (Deák et al., 2012; Vázquez and Moreno, 1998; Young et al., 2004). However, arsons are performed with ignoring the traditional knowledge (which would put the fire management into a landscape historical context). Consequently, the application of fire is “out of context” (i.e. its value to maintain specific ecosystem goods and services decreased), and the proportion of large uncontrolled fires increased.

Human activities related to land management influence the frequency and extension of fires in several ways. First the abandonment of the grasslands results in the accumulation of vegetation biomass (Bakker and Berendse, 1999; Valkó et al., 2012a; Kiss et al., 2011; Házi et al. 2011, 2012) which in turn increases the risk of ignition (Brockway et al., 2006; Ónodi et al., 2008). Second, according to climate change scenarios, large parts of Europe will be affected by drier and warmer summers, making these landscapes prone to fires (Garamvölgyi and Hufnagel, 2013; Pautasso et al., 2010). Third the fragmentation of native vegetation (e.g. by urbanisation, infrastructural development and expansion of croplands) will reduce the incidence of large scale burnings (Anonymous, 2010). Thus, it is crucial to understand the ecological processes connected to grassland fires, just as responses of species, functional groups and ecosystems. This knowledge can be applied in designing fire suppression and prevention strategies in the future.

Although fire was and still is a significant factor in many European grasslands, the effects of fire on flora, fauna and habitat structure is poorly studied; only 11 publications were available for international readers (ISI Web of Knowledge, International Forest Fire News) about the effects of prescribed fires in European grasslands (Valkó et al., 2012b). Other source of information includes a few studies on wildfires and the non-published field experiences of land users, managers and scientists.

Aims of the study

Here we overview the field experiences by Hungarian conservation practitioners to understand fire effects on Hungarian grasslands and their biodiversity. The experience of managers can be considered as evidence, besides the published (scholarly or non-scholarly) literature (Sutherland et al., 2004). We present our findings in the context of the European available informations.

Materials and methods

To gain knowledge on the effects of burning on grasslands we compiled a questionnaire including 12 questions (*Appendix 1*). The questions focused on the main attributes of fire events and their effects on plant and animal species together with habitat structures (*Appendix 1*). We distributed the questionnaires among experts of all the ten national park directorates in Hungary. We interviewed them since our goal was to gain experiences from those people who are dealing with the monitoring and management of the majority of nature-close grasslands in Hungary. In this paper we focused only on fire effects on grassland, and did not consider experiences about marsh, heathland, shrubland and forest fires. We simplified the categorization of grassland types from which we gained information as listed in *Table 1*. We always indicated the source of information (from which national park directorate was the data obtained). We used the official abbreviations of the national park directorates (NPDs) in the text; the full name of these parks is presented at *Table 1*. We were aware that the information provided by various experts of the national park directorates, does not always represent the official viewpoint of the institutes. When presenting our results, we combine the informations gathered from the interviews with the available scholarly literature at European level. After these, we will discuss the conservation implications of our findings.

Table 1. *Official names, abbreviations and total area of the Hungarian National Parks and grassland types affected by burning based on the questionnaire survey.*

National Park Directorate										
Abbreviations	**ANPD**	**BFNPD**	**BNPD**	**DDNPD**	**DINPD**	**FHNPD**	**HNPD**	**KMNPD**	**KNPD**	**ŐNPD**
Official names	Aggtelek NPD	Balaton-felvidék NPD	Bükk NPD	Duna-Dráva NPD	Duna-Ipoly NPD	Fertő-Hanság NPD	Hortobágy NPD	Körös-Maros NPD	Kiskunság NPD	Őrség NPD
Total area (ha)	19.892	56.793	39.063	50.105	60.314	23.488	74.222	51.066	48.198	43.950
Characteristic grasslands										
Steppic grasslands										
Alkali steppes			*		*	*	*	*	*	
Loess steppes	*	*	*		*		*	*	*	
Steppes on sandy soils					*	*	*		*	
Steppes on rocky outcrops	*		*							
Mesophilous and wet meadows										
Lowland mesophilous meadows			*				*		*	
Mountain mesophilous meadows			*							*
Wet meadows (fen meadows and marshes)	*		*		*	*	*	*	*	*
Degraded grasslands										
Degraded dry grasslands							*		*	
Old-fields and abandoned vineyards	*				*		*		*	
Grasslands with woody species										
Wooded pastures			*							
Grasslands threatned by woody encroachment	*		*							
Grassland-shrubland mosaic habitats	*	*							*	
Not specified				*						

Results of the questionnaire survey

We received completed questionnaires from all ten national park directorates indicating the importance of the topic and the awareness of grassland burning. Here we summarise and evaluate the experiences on grassland fires based on the information derived from the questionnaires (see *Table 2*).

Grasslands affected by burning

Fire types – Human-induced and wildfires

According to the questionnaire survey, grassland fires are typical in all Hungarian national parks in several grassland types. The ANPD, BNPD, HNPD and KNPD are those national parks which have to face with most extent fires (*Table 2*). Majority of the reported fires are human-induced ones, mainly arsons. The general aim of the arsons is presumably illegal grassland and rangeland management. Illegal burning is generally applied for (i) improving the quality of the grasslands (pastures and hay-meadows), (ii) reducing the amount of accumulated litter, (iii) rolling back weeds and shrubs and (iv) enhancing grassland productivity. (v) Arsons are usually applied for maintaining road verges because of its cost effectiveness (*Table 2*). These activities affect several 10,000 hectares per year in total. Fires set for 'fun' should be mentioned as well, as in many regions they affect extent areas. Some of the grassland fires are originated from the spreading of fires initiated to eliminate by-products of forestry (forestry waste: branches, leaves, sawdust) and reed management (leaves, flowers, broken stems). This phenomenon can also have reverse effects: grassland fires often spread over reed beds, forests and even settlements. Technical fires are often initiated by heavy machinery used in grassland management. One of the most frequent igniters are the sparks induced by the friction of overheated metal moving parts of mowing machines. Wildfires are less typical than human-induced fires in every national park except for FHNPD, where all the reported fire events were considered as wildfires. Fires set for nature conservation purposes are so rare that we did not include them in the table. The reason for the infrequent application of fire in nature conservation is that Hungarian environmental authorities – like in many other countries in Europe – generally do not issue permits to apply prescribed burning. The decision is generally justified with the protection of air quality.

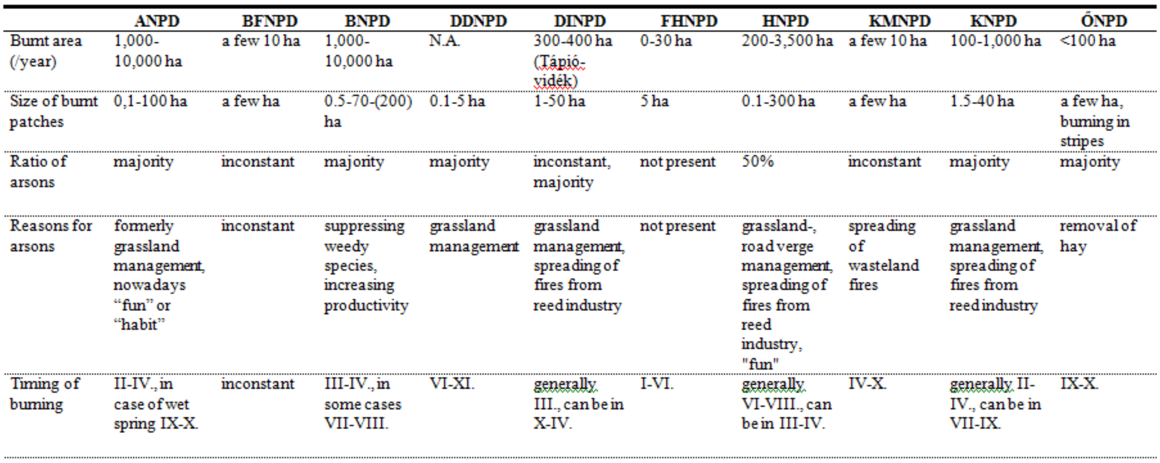
Fire season

Fire events occur in all seasons of the year (*Table 2*). The occurrence of fire was most typical in spring (Végvári et al., 2011). Regular spring fires were reported from five national parks (ANPD, BNPD, DINPD, HNPD and KNPD).

Land use

Fires were most typical in unmanaged grasslands, but they were reported also in mown and grazed stands. Cessation of management increased the probability of fire events, mainly due to litter accumulation. A high number of fire events were reported from hay-meadows (ANPD, BNPD, DINPD, FHNPD, HNPD, KMNPD, KNPD, ŐNPD). The most common reason reported was that technological fires ignited by mowing machinery are generally applied in hay-meadows. The other reason for more frequent fire events in meadows is that the biomass removal has an uneven temporal pattern: till the day of mowing there is a high, even biomass accumulation. This pattern is in contrast with the grazed sites, where due to the continuous management there is a lower, uneven biomass accumulation.

Table 2. *Occurrence of grassland burning in Hungarian national park directorates based on the answers for the questionnaires.*



	ANPD	BFNPD	BNPD	DDNPD	DINPD	FHNPD	HNPD	KMNPD	KNPD	ŐNPD
Burnt area (/year)	1,000-10,000 ha	a few 10 ha	1,000-10,000 ha	N.A.	300-400 ha (Tápió-vidék)	0-30 ha	200-3,500 ha	a few 10 ha	100-1,000 ha	<100 ha
Size of burnt patches	0,1-100 ha	a few ha	0.5-70-(200) ha	0.1-5 ha	1-50 ha	5 ha	0.1-300 ha	a few ha	1.5-40 ha	a few ha, burning in stripes
Ratio of arsons	majority	inconstant	majority	majority	inconstant, majority	not present	50%	inconstant	majority	majority
Reasons for arsons	formerly grassland management, nowadays “fun” or “habit”	inconstant	suppressing weedy species, increasing productivity	grassland management	grassland management, spreading of fires from reed industry	not present	grassland-, road verge management, spreading of fires from reed industry, "fun"	spreading of wasteland fires	grassland management, spreading of fires from reed industry	removal of hay
Timing of burning	II-IV., in case of wet spring IX-X.	inconstant	III-IV., in some cases VII-VIII.	VI-XI.	generally III., can be in X-IV.	I-VI.	generally VI-VIII., can be in III-IV.	IV-X.	generally II-IV., can be in VII-IX.	IX-X.

Grassland types

Fire is present in almost every grassland types of Hungary (see *Table 1*). The most affected grassland types are wet meadows (in 8 national parks) and steppic grasslands (loess steppes in 7, alkali steppes in 6 and steppes on sandy soils in 4 national parks). Regular fires are also typical in old-fields, abandoned vineyards and grassland-shrubland mosaic habitats.

Effects of fire on the structure and biodiversity of grasslands

Structural effects

One of the most characteristic effects of fire is that it reduces the amount of accumulated litter (BNPD, DINPD, DDNPD, HNPD, KMNPD, KNPD, Altbäcker, 2005; Ónodi et al., 2008; Ónodi 2011; Ryser et al., 1995) and in some cases it creates open soil surfaces (KMNPD, Antonsen and Olsson, 2005; Hansson and Fogelfors, 2000). Fire temperature and severity increases with the amount of accumulated litter; that is why fire causes less damage in short-grass grasslands like alkali steppes compared to grasslands characterised by tall grasses like alkali meadows. In abandoned loess steppes and alkali meadows accumulated biomass results in extremely high-temperature fires especially after a long dry period. In this case even roots and grass tussocks can be damaged and disintegrated (HNPD; Miller, 2000). In some cases the original state recovers in 3-5 years (in case of good water supply and proper management by grazing; HNPI), but in other cases the encroachment of competitor grasses (mainly *Calamagrostis epigeios*) is typical (HNPD; Végvári et al., 2011).

Invasive and weedy species

The effects of fire on invasive species vary depending on burning conditions and grassland type (Keeley, 2006). Overall, invasive species with a hard seed-coat (e.g. Fabaceae species) and/or a good re-sprouting ability seems to be favoured by fire. One invasive plant species (*Robinia pseudo-acacia*) was found to benefit from fire: fire enhanced its germination and the spreading of the sprouts (BNPD, DINPD and KNPD; see also Maringer et al., 2007). In case of some other invasive species, contradictory experiences were reported even for the same species. Regular fires can facilitate the spreading of *Solidago* species in lowland meadows (DINPD and KNPD; see also Simmons et al., 2007), probably because these species have an effective re-sprouting ability from rhizomes. In contrast, according to the experiences in the DDNPD regular fire events combined with grazing can be a proper tool for controlling the population of the invasive *Solidago* species in lowland meadows (see also Johnson and Knapp, 1995). It suggests that the suppression of *Solidago* species by livestock grazing is more feasible after burning, than with grazing alone. On one hand, fire makes infested grassland stands more adequate for the (re)introduction of grazing by removing the accumulated litter and shrubs. On the other hand, *Solidago* species allocate most resources to re-sprouting after fire, therefore the production of secondary metabolites is lower. Similarly, Cummings at al. (2007) found that the poisonous invasive species *Lespedeza cuneata* can be rolled back effectively by the combination of prescribed burning and grazing in North-American prairies. The cover of weeds increased after fire events in several grassland types (DDNPD, DINPD and HNPD). In alkali grasslands the cover of certain short lived weeds like *Chenopodium album* and *Amaranthus albus* increased in the year after fire, but their proportion decreased to the original level for the second year (HNPD; Blumenthal et al., 2005; Végvári et al., 2011).

Woody species

Hungary harbours several grassland habitat types (like forest steppes on sandy soils and wooded pastures) which are characterised by the co-occurrence of woody species. In such habitats fire has serious detrimental effects on *Juniperus communis*. The reason for this is that *Juniperus* ignites easily, and its high content of volatile oils makes it highly susceptible to burning. In most cases fire extirpates shrubs locally (ANPD, BNPD, KNPD; Ónodi et al., 2008, Thomas et al., 2007). Fire is also an important natural determinant of the dynamic of ecosystems dominated by *J. communis*. For example, fire events with a *ca* 10-20 year frequency are important in forming and sustaining the mosaic structure of the habitats dominated by *Juniperus* (Altbäcker, 1998). In mixed stands of *J. communis* and *Populus alba*, burning increase the area of open grasslands. However, *Populus alba* and sometimes *Robinia pseudo-acacia* can invade the opened areas after fire, finally resulting in the decrease of grassland cover and the encroachment of woody vegetation (KNPD; Ónodi et al., 2008), including shrubs like *Prunus spinosa* and *Crataegus monogyna* (BNPD, DINPD, KNPD). Similarly to *J. communis*, fire also suppresses resinous pine species, such as *Pinus nigra* (BNPD) and *Pinus sylvestris* (ANPD; Carlisle and Brown 1968) which are common invaders of unmanaged grasslands. Fire can also damage single trees in wood-pastures (BNPD, DINPD; Kenéz, 2007; Szabó, 2007). In the traditional wood-pastures from Southern Transylvania (Romania), the uncontrolled pasture fires damages especially the large, ancient trees (oaks), every year (Hartel et al., 2013). As the large ancient trees are keystone structures for biodiversity (Manning et al., 2006), their disappearance may have serious consequences on the local and regional biodiversity of entire landscapes.

Perennial grasses

Frequent fires generally cause an increased abundance of competitor grasses with tillers, like *Brachypodium pinnatum* (ANPD; Kahmen et al., 2002; Köhler et al., 2005; Ryser et al., 1995) or *Calamagrostis epigeios* (HNPD, KNPD; Hille and Goldammer, 2007; Marozas et al., 2007). In some cases burnt grasslands are invaded by the terrestrial form of reed (*Phragmites australis*, KMNPD). In foothill steppic grasslands formed in abandoned vineyards single fires resulted in the decreased abundance of *Stipa tirsa* (BNPD). Consequently due to lowered competition and reduced amount of litter, fire resulted in the population growth of two rare species (*Echium russicum* and *Thlaspi jankae*), both listed in the Annex II of the Habitats Directive. In parallel, the cover of *S. tirsa* increased considerably (BNPD) after frequent fires in the same habitat type. Similar to this pattern in the Tardonai-hills the abundance of the *Stipa pulcherrima* increased after regular burning and parallel several subordinate species were rolled back (Garadnai, 2007). In loess steppes the abundance of grasses decreased while that of forbs increased after fire (KMNPD).

Rare and protected forbs

The effects of the fire on protected and rare forb species depends strongly on burning season. Spring and summer fire can have different effects even on the same species depending on the phenological state and specific traits of the species. A recent study has demonstrated that the major traits determining the response of plants to fire are their (i) life form, (ii) presence/absence of perennating buds, (iii) density, spatial orientation and some other characteristics of the seeds (Pyke et al., 2010). Perennial species are least sensitive to fire, as they can more easily recover in the years following fire. Annual species are most sensitive before seed set and seed dispersal. Species with long-term persistent seeds or underground storage organs are better adapted to fire than species with transient seed banks or aboveground perennial buds (Pyke et al., 2010). The precipitation patterns considerably influence the effects of fire on vegetation. Whereas the vegetation can recover easily after fire in a year with a normal or high precipitation, fire often leads to degradation in dry years (BNPD).

Occasional fire events before the flowering and ripening period (April-May) can cause a considerable increase in the population of *Pulsatilla* and *Adonis* spp. In the BNPD fire had a positive effect on the population of *A. vernalis*. Occasional spring fires occurring after snowmelt initiated the germination of the *Adonis volgensis* (KMNPD; Illyés et al., 2007). On the populations of *Pulsatilla pratensis* ssp. *hungarica* former military fires and further occasional arsons had a positive effect by reducing the litter and creating open gaps favouring germination and seedling establishment (HNPD). Occasional fires had a positive effect on the populations of the protected *Thlaspi jankae, Phlomis tuberosa, Prunella grandiflora, Ranunculus illyricus* in mesophilous meadows originated from clear-cuts (BNPD) and on *Chamaecytisus supinus* in lowland hay-meadows (ŐNPD). Increase in abundance of certain species can be related to induced germination and seedling emergence which is facilitated by (i) temporal increases of soil nutrients after fire (Blodgett et al., 2000), (ii) decreased competition of living neighbours (Maret and Wilson, 2005) and (iii) new open soil surfaces which favour the germination of several species (Rebollo et al., 2001). (iv) The smoke and its aqueous solution (smoke-water) promote the germination of several species (Mojzes and Kalapos, 2012). Geophytes regenerate well after fire from their underground storage organs (Pyke et al., 2010). Fire can increase their populations by providing more favourable microsites by the removal of accumulated litter. These findings were confirmed by the experts opinions. In KNPD a considerable population growth of *Bulbocodium vernum* and *Crocus reticulatus* was observed after late winter fires. However, later fires in the flowering period (early spring) are detrimental for the populations of *Pulsatilla grandis, A. vernalis* (BNPD) and *P. nigricans* (FHNPD). In frequently burnt steppic grasslands, the flowering shoots of *P. grandis* decreased to 5-10% of that was found in the previous years (BNPD). Due to frequent fires the population of the *T. jankae* was reduced to 10% in 6 years while even in the neighbouring non-burnt patches the population grew (ANPD).

Animal species

Fire has significant effects on the elements of fauna. The most important reasons for animal injury or death caused by fire are (i) oxygen deficiency, (ii) exposure to lethal heat and (iii) toxic compounds of smoke (Engstrom, 2010). The most vulnerable animal taxa are those with limited mobility. Negative effects of fire on invertebrates, was reported from almost all national parks. Especially the rare and endangered species are vulnerable which live on the ground surface or lay their eggs on the surface of short herbs or inside their shoots (ANPD, BNPD, DINPD, FHNPD, HNPD, KNPD and ŐNPD). Autumn fire negatively affects ant populations as a result of overheating the nest (HNPD). The vulnerability of a species is higher in sensitive life stages, like in nesting season or moulting period (e.g. snakes in ecdysis; Russel et al., 1999). In the nesting season fire has detrimental effects on ground-dwelling birds like *Otis tarda*, *Asio flammeus*, *Vanellus vanellus, Limosa limosa* and *Tringa totanus* (HNPD, KMNPD, KNPD; Lyon et al., 2000; Swengel, 2001; Végvári et al., 2011). Populations of *Ablepharus kitaibelii* are threatened by early spring fires, while populations of *Lacerta viridis, Sorex* spp. and *Carabus* spp. are endangered by late spring and summer fires (BNPD).

Fire has some sort of secondary effects on animals, mainly through changes in habitat structure and food availability (Engstrom, 2010). Fire can have a positive effect on the population of the *Euphydryas aurinia,* by favouring the population growth of the host plant of the butterfly (*Chamaecytisus supinus*; ŐNPD). Fire often increases food availability and quality; recently burnt sites are preferentially selected by grazing ungulates (Fuhlendorf and Engle, 2001) because foliage of re-growing herbs and shrubs is more palatable, richer in nutrients and crude protein (Tracy and McNaughton, 1997). Predators and scavengers are also attracted to burnt sites because of the more abundant and more exposed food source compared with non-burnt sites (Lyon et al., 2000). This especially applies to small mammals which are preyed upon by avian predators in large densities as a result of vegetation cover loss. As a behavioural adaptation to fire-exposed prey storks, buzzards, falcons, harriers, eagles and large-bodied gulls (e.g. *Larus cachinnans*) are generally attracted to burning vegetation, possibly using smoke as visual and its smell as olfactory clues (Conner et al., 2011; Lyon et al., 2000). While raptors specialize on small mammals which are trying to escape from fire, storks and gulls can find large quantities of burnt or escaping flocks of orthopterans. Post-burning sites attract sizeable flocks of migrating waders – mostly plovers (*Charadrius* spp.) – especially after rainy periods. Winter wildfires seem to be an effective tool for establishing open lek areas for *Otis tarda* in unmanaged grasslands (HNPD; Végvári et al. 2011). Bustards need open grassland patches for lekking, where females are attracted to loose groups of displaying males. In this case, areas burnt in the previous year with short green vegetation seem to have multiple benefits for lekking bustards: (i) the grass is short which increases visibility and manoeuvrability of males' movements (ii) short green grass and white balls of displaying males create larger visual contrasts than for unburned, tall and yellow vegetation thus possibly attracting more females to lek sites (iii) burned sites provide larger amounts of prey for lekking males which can enhance breeding success (HNPD).

Grassland recovery

The original (pre-fire) vegetation usually recovers well after non-regular fires, but in some cases active post-fire management is needed (Pyke et al., 2010; Robichaud, 2000). Mesophilous and wet meadows in a good nature conservation state regenerate well in a few years after burning (HNPD, KNPD). Active post-fire management is generally applied for reducing the possible negative effects of the fire in meadows of the DDNPD and alkali steppes and alkali meadows of the HNPD, which usually includes grazing and/or brushcutting. Moderate grazing applied after the fire event helps in decreasing the abundance of weeds and recovering the habitat diversity. Alkali grasslands of the KNPD and HNPD generally regenerate quickly after fire and post-fire management is not essential for their recovery. In alkali landscapes, fire does not have permanent negative effects on the diversity and species composition of the grasslands (HNPD).

Effects of fires on land use

As a side effect of burning, grasslands become more adequate for grazing and mowing after fire: the amount of litter decreases, standing dead biomass disappears and the quality of the forage increases (ANPD, HNPD, KNPD, Tracy and McNaughton, 1997). This phenomenon motivates many land users to apply unauthorised fire management on their land. An additional reason for this practice is that - as in case of arable lands - farmers use fire to decrease the population of some pathogens and pest species (Lyon et al., 2000).

Effects associated with fire-fighting actions

Disturbance of grasslands during fire-fighting actions can also be detrimental for wildlife. In case of uncontrolled wildfires and arsons there is no opportunity to ensure safety measures in advance. In some cases drastic measures must be taken to protect human life and private property. These measures are (i) establishing firebreaks with ploughing (HNPD, KNPD) or (ii) fire-fighting with earthmoving equipment (KNPD). Fire-engines often produce deep tracks in grasslands, especially in moist soils (e.g. in wet meadows), which results in the degradation of the grassland structure and in creating soil erosion patterns disrupting local hydrology. These linearly disturbed soil surfaces provide proper sites for the germination, establishment and spreading of invasive species. In the ANPD a whole population of *Iris aphylla* ssp. *hungarica* had to be destroyed while establishing a firebreak to protect a forest from the spreading-over of a grassland fire. Besides the negative nature conservation consequences of fire-fighting with earthmoving equipment, the method is not always sufficient in the terms of fire protection, as the semi-buried embers can glow for several days and can cause re-ignition (HNPD).

Discussion

Our results show that the effect of fire is context-dependent and burning can have both positive and negative effects on grassland biodiversity. Uncontrolled fires may have serious detrimental effects on rare species, habitats, personal safety and private property. Generally uncontrolled fires have the following negative effects: (i) the homogenisation of habitats mainly by facilitating competitor grass species, (ii) burning in flowering or ripening period causes serious damages in the populations of protected species, (iii) fire in the nesting season seriously damage the populations of ground-dwelling birds, (iv) fire has a considerable negative effect on invertebrates regardless to the season, (v) it can promote the spreading of invasive species, (vi) damages natural woody vegetation and (vii) fire-fighting actions can lead to the degradation of grasslands.

According to our study, fire can also have positive effects on grassland habitats and grassland species. These results are in accordance with other findings from Europe. (i) Burning decreases the amount of accumulated litter. (ii) Fire can restore or establish habitats and suitable micro-sites for rare plant or animal species. (iii) Combination of fire and grazing was reported to be successful in suppressing the population of invasive plant species.

Implications for nature conservation

Monitoring fires in the landscape

An important component of understanding the effects of arsons and wildfires on wildlife is to create a database which contains records on all fire events and their relevant parameters and sources. These informations will be useful in understanding the origins (e.g. its controlled, or accidental, uncontrolled nature) and spatio-temporal dynamic of fire and ultimately could be useful in prioritizing fire prevention strategies. These observations should be complemented with obvious natural casualties such are the extension of the burnt patch, changes in the abundance of some plant and animal species (e.g. the keystone, or very obvious species). These informations could be gathered e.g. by training rangers in the park.

The need for prescribed burning experiments

According to our results fire does not have definite positive or negative effects from the nature conservation viewpoint. As experimentally robust evidences on the fire effects on biodiversity are lacking in Hungary, we urge the establishment of well designed species- and habitat specific experiments to address the context dependence of fire effects on biodiversity.

Prescribed burning experiments offer the possibility for the quantitative analysis of fire effects and their results can offer solutions for several nature conservation problems: (i) the removal of accumulated biomass from abandoned pastures and meadows, where traditional management is not sustainable any more, (ii) the prevention or suppression of woody encroachment in abandoned pastures and meadows, (iii) the control of invasive species (by burning or by the combination of grazing and burning). Prescribed burning might be a proper tool for preventing extent and uncontrolled wildfires (Baeza et al., 2002) and accordingly it can contribute to the protection of personal safety and private property. Furthermore, small-scale, controlled prescribed burning experiments should be designed for the quantitative analysis of fire effects on grassland habitats and species. Based on these results, prescribed burning could be integrated in conservation plans of rare and protected species which found to be promoted by burning.

Application circumstances of prescribed burning in practice

Prescribed burning experiments require a careful application of fire under specified fuel and weather conditions to reach specific goals (Castellnou et al., 2010). Thus, in the planning and implementation phase of prescribed burning experiments, several important details should be considered which we listed here.

Permissions

It is essential to have the permissions of competent governmental bodies and stakeholders: the Environmental Protection Inspectorate, the nature conservation manager (generally the National Park Directorates), land owner/user and the Fire Service. However, the strict regulation of burning by law in most European countries (including Hungary) limits the possibility of implementing even small-scale prescribed burning experiments (Goldammer and Montiel, 2010). The main reasons why prescribed burning is prohibited by law are to mitigate air pollution and/or to protect human life and property. However the emission of air-pollutant compounds from controlled, small-scale prescribed burning experiments would be significantly smaller than that of regular, uncontrolled fires with several 10,000 hectares in each year. Additionally, these small-scale burning experiments could effectively contribute to developing new strategies in nature conservation and even fire suppression.

Defining management targets

As the first step of the planning phase, objectives of the management should be defined (e. g. the removal of litter, facilitating the germination of protected species, suppressing invasive species). Application circumstances like patch size, location of the burnt areas, timing and frequency of burning should be harmonised with these aims.

Survey of the pre-burn state

Prior to burning, species composition of the grasslands should be surveyed, in order to have an overview on that which plant and animal taxa can be potentially affected. Sacrificing one protected species for advancing another one should be avoided. If this dilemma would emerge, burning should not be used. To decrease the risk of damaging the populations of rare species, experimental burnings can be implemented in disturbed areas where no unique nature conservation values are present.

Extension of burning

In general, burning in a mosaic pattern is the most favourable for most species (Parr and Andersen, 2006). The size of the patches should be approximately 1-3 ha, which enables a fast grassland recovery by resettling of plants and animal taxa from the neighbouring non-burnt patches. It is very important to designate burnt and non-burnt control plots for the better understanding of fire effects.

Frequency of burning

Both European studies and the experiences from Hungary point out that annual burning is not favourable as it results in the degradation of grasslands in the long run (Kahmen et al., 2002; Wahlman and Milberg, 2000). The practice of annual burning allows no time for grassland regeneration, and can lead to untargeted states of succession. When the aim of management is to maintain open landscapes and preserve species-rich grasslands, least frequent burning is recommended. Proper burning frequency significantly varies across grassland types, but at least 3-5 years may be appropriate to avoid degradation.

Burning season

Burning season depends on the grassland type, plant and animal species present and the aim of burning. For example, for the reduction of litter layer late winter or early spring fires (Towne and Owensby, 1984), for invasion control, growing-season fires are the most effective (MacDougall and Turkington, 2005). Some hardly foreseen parameters such as the precipitation in the previous weeks or days also influence the timing of the burning. A general rule is that burning in flowering season of the natural vegetation and in nesting period should be avoided, as it has a high potential for damaging the target communities.

Implementation phase

In the implementation phase well-equipped and experienced teams are needed. Necessary preparations (like creating firebreaks by mowing) should be arranged in every case. Contrary to wildfires if a well planned prescribed burning is applied it is possible to arrange those preparations which are necessary for preventing or minimising the potential negative effects of burning (and even fire-fighting) on natural values, private property and air quality. These precautional measures include precise selection of the area and also taking into account the weather circumstances (direction and strength of the wind). Surrounding areas can be protected by designating the burnt area between natural borders (channels, dirt roads, overgrazed areas or open water surfaces) which can act as natural firebreaks.

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Appendices

Appendix 1. *Questionnaire sent to expert from Hungarian national park directorates.*

1. Are spontaneous fires (wildfires, technical fires) and/or arsons present in the NPD's range of action?
2. If yes, which grassland types are affected? (e.g. steppic grasslands, mesophilous and wet meadows, degraded grasslands or grasslands with woody species)
3. What kind of land-use or nature conservation management is present in the burnt grasslands? (e.g. grazing, mowing, no management; intensity: low, adequate, high)

4. What percent of the fires affects protected or Natura 2000 sites?
5. How far are the affected grasslands from settlements and roads? (e.g. in a close vicinity to settlements; far from roads and settlements)
6. Approximately how many hectares of grasslands are burnt in a year?
7. What is the average extension of the burnt patches?
8. What is the typical period for grassland fires? (season or month)
9. What is the ratio of the arsons and spontaneous fires?
10. What might be the aim of the arsons?
11. Which are the most detrimental effects of the arsons and wildfires?
12. According to the field experiences what are the main effects of fire on natural grasslands? (e.g. changes in the amount of litter; altering the structure of the grassland; effects of burning on woody species, invasives, populations of protected plants- and animal species; regeneration of the vegetation after fire)

EFFECTS OF ABANDONMENT ON THE FUNCTIONAL COMPOSITION AND FORAGE NUTRITIVE VALUE OF A NORTH ADRIATIC DRY GRASSLAND COMMUNITY (ĆIĆARIJA, CROATIA)

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Abstract. *Carici humilis-Centaureetum rupestris* association is one of the most widespread types of pasture in Istria and of great environmental and economic interest. The research aims were to test if forage quantity, nutritive value and functional composition (grasses and grass-like, legumes, and forbs percentage) of these pastures change significantly in relation to: time of the growing season, management type (low use intensity grazing/grazing abandonment) and topographic variables. During 2008, phytomass samples were collected in grazed and ungrazed pastures of Ćićarija (Croatia), in May, June, and September. Their composition in dry matter, ash, crude protein (CP), neutral and acid detergent fiber (NDF, ADF), acid detergent lignin (ADL) and in-vitro dry matter digestibility (IVDMD) were determined and net energy for lactation (NEL) and yield NEL were calculated. These variables followed a double trend, linked to the phenological phases of grasslands and to a land use/topographic gradient. Yield, yield NEL, dry matter, NDF, ADF and ADL increased during the growing season, while CP, NEL and IVDMD decreased. Yield, yield NEL, grasses and forbs, ash, and IVDMD were tied to the ungrazed pastures spread on the slopes, while ADF, ADL and dry matter had stronger correlation with grazed grasslands spread on the flat lands.

Keywords: *Carici humilis-Centauretum rupestris Horvat 1931, Brachypodium rupestre, productivity, functional groups, forage quality.*

Introduction

Throughout Europe, owing to their low agricultural productivity (Willems, 1990; van Dijk, 1991), semi-natural calcareous grasslands are in strong decline in extension and threatened by abandonment (Luick, 1998; Zervas, 1998; Dullinger et al., 2003; Sebastià et al., 2008; Penksza et al. 2010, 2013; Házi et al. 2011, 2012; Szentes et al., 2011;

Zimmermann et al., 2011, Kiss et al., 2011). This trend has been also observed in the North Adriatic pastures (Kaligarič et al., 2006) and on the Ćićarija mountainous plateau (Vitasović Kosić, 2011; Vitasović Kosić et al., 2011), where the low intensity use or the abandonment of grassland management practices led to the triggering of vegetation dynamic processes and to a consequent change in floristic, coenological and functional composition (Vitasović Kosić et al., 2012). Indeed, the variability of species assemblage in semi-natural grasslands depends on environmental factors (Belesky et al., 2002a; Catorci et al., 2012b, Zimmerrmann et al., 2011,Szentes et al., 2009a, 2009b, 2012) and on grazing management and history (Holechek et al., 1995; Belesky et al., 2002b). These interacting factors create a mosaic of plant communities, which results in variations in palatability and nutritive value for grazing ruminants (Roukos et al., 2011). For instance, the decrease in herbage use due to pasture abandonment or improper management causes the spread of taller and less digestible vegetation (Louault et al., 2002).

The ability of a grassland to sustain animal growth depends on its phytomass production and herbage nutritive value (Synman, 2002). Hence, the determination of forage quality is important for both nutritional and economic reasons (Rotar et al., 2010). The nutritive value of forage depends on its chemical composition (dry matter; crude ash, crude protein; fraction of fibers, i.e. neutral detergent fiber and, acid detergent fiber; acid detergent lignin; net energy for lactation; *in-vitro* dry matter digestibility), and is influenced by several factors, including climatic conditions, soil, land form, floristic and functional composition (e.g., the proportion of grasses to legumes), selective ability of grazing animals, stage of maturity of plants when harvested, and forage preservation methods (Sheaffer et al., 1990; Van Soest, 1994; Buxton, 1996; Pérez-Corona et al., 1998; Tallowin and Jefferson, 1999; Vásquez de Aldana et al., 2000; Bruinenberg et al. 2002; Arzani et al., 2006; Dale et al., 2013).

The species composition of a pasture influences animal products, particularly milk and cheese produced in the Mediterranean environments (Licitra et al., 1997). Feed values from several types of Mediterranean pastures were investigated throughout the Mediterranean countries (Tsiovaras et al., 1993; Zervas et al., 1996; Licitra et al., 1997: Ammar et al., 1999, 2004; Skapetas et al., 2004). However, there is no previous report about the forage nutritive value of Ćićarija grasslands and, more specifically, of grasslands referred to as the *Carici humilis-Centaureetum rupestris* Horvat 1931 association, which is one of the most widespread types of pasture in the Istrian Peninsula (Poldini, 1989), and thus of great potential interest, not only for its biodiversity (Ćićarija grasslands were proposed as Special Protection Area of Natura 2000 network as an important site for habitat 62A0 and bird species conservation), but also from an economic viewpoint.

Our hypothesis was that the changes in management of pastures underlie variations in quantity and nutritive value (chemical composition and *in-vitro* digestibility) of forage. More specifically, our aims were to test if these variables: i) change significantly during the growing season; ii) show significant variations between low intensity use and abandoned pastures; iii) are affected by topographic variables (altitude, aspect, slope angle, and land form).

Material and methods

Study area

The study area (about 1000 ha) is located at the north of the Istrian Peninsula, on the Ćićarija mountainous plateau (45° 29’ 56’’- 45° 30’ 00’’N, 13° 59’ 54’’- 14° 00’ 29’’E), ranging from 550 to 650 m a.s.l.

The climate is transitional between Mediterranean and continental pre-Alpine, with cool, rainy winters and long, dry summer periods (Poldini, 1989). Precipitation is about 1400 mm/yr, most of which falls in autumn; a less pronounced secondary peak occurs as spring turns to summer. From a bioclimatic viewpoint, the study area belongs to the submediterranean belt (Kaligarič, 1997) and the epimediterranean zone of the mediterranean-mountain vegetation belt (Čarni, 2003).

The bedrock consists of limestone; soils are generally brown, shallow and clast-rich.

Pastures, formerly grazed by 160 000 sheep, are for the most part undergrazed because of low density of grazers (nowadays there are less than 200 sheep, few cattle heads and horses) or completely abandoned, while meadows are not regularly mown or, in some cases, originate from seeded meadows.

The pastoral landscape is characterized by pastures and meadows, referred to as *Scorzonero villosae-Chrysopogonetalia grylli* Horvatić & Horvat in Horvatić 1963 order (*Festuco-Brometea* Br.-Bl. & Tx. ex Br.-Bl. 1949 class) and *Arrhenatheretalia elatioris* Tüxen 1931 order (*Molinio-Arrhenatheretea* Tüxen 1937 class), respectively.

Experimental design and data collection

Within homogeneous macro-environmental conditions (submediterranean bioclimatic belt and limestone bedrock), we selected two localities characterised by different land use histories: Slum pastures (hereafter named “Pasture 1”, 550 m a.s.l) subjected to low intensity use (0.5-1 sheep per ha) referred to as the *Carici humilis-Centaureetum rupestris* association, and Vodice pastures (hereafter named “Pasture 2”; 650 m a.s.l.) abandoned since 30-40 years ago, referred to as the *Brachypodium rupestre* variant of the above-mentioned association.

During the 2008 growing season, samples of phytomass (fresh green matter) were mown with the quadrat method (Whalley and Hardy, 2000) in five 1 x 1 m fenced plots, placed, about every 100 m, along a transect in each locality, three times, i.e. in spring (May, 26th-27th; up to early May it snowed), summer (June 23rd-24th, optimum of vegetation), and autumn (September, 22nd-23rd, after the summer drought period). During dormancy of vegetation (winter period) samples were not collected. Sampling was done by cutting all herbaceous vegetation at 5 cm above soil level, and immediately manually separated into the botanical functional groups: grasses and grass-like species (*incl. Poaceae, Cyperaceae* and *Juncaceae*), legumes (*Fabaceae*) and forbs (all other dicotyledonous families) and weighed. Then the grassland samples, placed in individual paper bags and transported to the laboratory, were dried for 48 h in a 50 ºC oven, ground with a Cyclotec 1093 mill (FOSS Tecator, Höganäs, Sweden), through a 1 mm sieve and stored until further analysis.

Analytical methods

Each sample was analysed with regard to: aboveground fresh phytomass (g/m^2) of fresh matter; forage chemical composition in dry matter (g/kg DM); crude ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL); *in-vitro* dry matter digestibility (IVDMD).

The percentage contribution in terms of grasses and grass-like species, legumes and forbs to the total fresh matter (g/m^2) and productivity was calculated.

The N content of each sample was determined using a Kjeltec 2200 digestion unit (Foss Tecator, Häganäs, Sweden), using AOAC (1990) method. NDF, ADF and ADL were determined using a Fibertec System 2021 Fiber Cap (Foss Tecator, Höganäs, Sweden) according to methods of Van Soest et al. (1991), and ash was determined as the gravimetric residue after heating to 550 °C for 4 h, according to AOAC (1990) method. IVDMD was measured according to Holden (1999) using an ANKOM Daisy^{II} Incubator (ANKOM Technology Coorporation, Macedon, NY, USA). Rumen fluid was obtained from two Istrian sheep (slaughtered at sales for meat) fed with forage from the study area. Net energy for lactation (NEL) and yield NEL were calculated according to NRC (2013).

Statistical analysis

We assessed distribution normality and variance homogeneity of data using the Kolmogorov-Smirnov and Levene tests, respectively. We performed ANOVA and t-tests when data met the assumptions required for parametric tests and Kruskal-Wallis and Mann-Whitney U-tests when data did not, to understand which variables were significantly different ($p < 0.05$) between the two relevé groups (low intensity use pastures and abandoned pastures) at each time (spring, summer, and autumn) and among times inside each group. In the latter case pairwise comparisons (t-tests and Mann-Whitney U-tests) between times were run when ANOVA or Kruskal-Wallis test, respectively, detected significant differences ($p < 0.05$) among times; the Holm correction for multiple comparisons was used to avoid Type I error.

To identify the main gradients of variation, the relations among the collected data on yield, botanical functional groups (fresh matter) and forage feed value (response variables), and between response variables and explanatory variables, we performed Canonical redundancy analysis (RDA) of the "plots-by-response variables" matrix, constrained by the type of management (abandoned and low use intensity pasture), the time of growing season (May, June, and September), altitude, slope angle, slope aspect, and land form (flat land, slope with concavity). Prior to RDA, the response variable data were standardized.

We used the STATISTICA software (Statsoft Inc. 2005) to perform the statistical tests, and R, version 2.15.0. (R Core Team, 2012), and *vegan* R-package, version 2.0-4, to perform RDA.

Results

Productivity of Carici humilis-Centaureetum rupestris

Content of dry matter, phytomass yield and yield net energy for lactation (NEL) of pastures referred to as *Carici humilis-Centaureetum rupestris* are presented in *Table 1.* The results indicate that dry matter of forage increased from spring to autumn; significant differences ($p < 0.05$) were recorded: at each time between grazed and ungrazed pastures, and in both the land use types between September and the other phases of the growing season. Abandoned pastures had higher average yield and yield

NEL. Yield peaked in June in both types of management, but differences were significant only in autumn. Yield NEL did not show significant differences between the two types of land use in any considered phase of the growing season.

Table 1. *Average dry matter content (DM), green mass yield and yield net energy for lactation (yield NEL) ± Standard deviation in May, June and September in the pastures of Carici humilis-Centaureetum rupestris association with different types of management (P1: Slum, low use intensity pasture P2: Vodice, abandoned pasture). Significance values from ANOVA or Kruskal-Wallis test (p) (comparisons among times during the growing season in each locality) and from t-test or Mann-Whitney U-test ($p_{may,\ june,\ sept}$) (comparisons between localities at each time) are indicated. Values within the same column with the same superscripts do not differ significantly.*

Locality	Season	DM (g/kg)	Yield (g/m^2)	Yield NEL (GJ/ha)
P1	May	386.4^a ± 23.24	403.6^a ± 31.71	6.11^a ± 0.50
	June	435.4^a ± 21.83	573.8^b ± 97.25	8.79^a ± 1.87
	September	604.92^b ± 55.67	410.0^a ± 101.25	8.55^a ± 2.37
	Average	475.57 ± 101.80	462.47 ± 112.10	7.82 ± 2.06
	p	< 0.001	0.011	0.003
P2	May	339.26^a ± 19.49	407.0^a ± 59.15	5.81^a ± 0.98
	June	348.36^a ± 17.27	685.6^b ± 191.10	9.77^b ± 2.81
	September	517.42^b ± 22.59	606.0^{ab} ± 55.65	10.53^b ± 0.83
	Average	401.68 ± 86.78	566.20 ± 164.40	8.70 ± 2.71
	p	0.001	0.021	0.003
P1 vs. P2	p_{may}	0.008	0.913	0.5580
	p_{june}	<0.001	0.229	0.4260
	p_{sept}	0.017	0.005	0.1170

Proportion of grasslands botanical functional groups

The contribution of functional groups varied in the two types of management (*Fig. 1*). During the growing season, in the grazed pastures (P1) average mass fraction of grass and grass-like plants slightly increased, forbs slightly decreased, and legumes remained substantially unchanged. In the abandoned pastures (P2) between summer and autumn the sudden decrease of forbs group caused high increase of grass and grass-like group.

The two pastures did not show significant differences in the amount of legumes and forbs fresh matter during the whole growing season. The share of grass/grass-like plants and forbs diverged significantly ($p < 0.01$) in September (*Fig. 1*).

Relation among phytomass production, forage nutritive value and environmental variables

The first axis of RDA, performed using altitude, aspect, slope angle, land form, type of land use and time of the growing season as explanatory variables, explained the 51.9% of the constrained variance and was linked to the time of the growing season; The second axis explained the 27.0% of the variance and was mainly linked to a land use gradient (*Fig. 2*) which, in turn, was correlated to topographic factors (particularly altitude). More specifically, abandoned pastures were positively correlated to higher altitudes, greater slope angles and southerly aspects (negative values), while grazed pastures were positively correlated with flat lands.

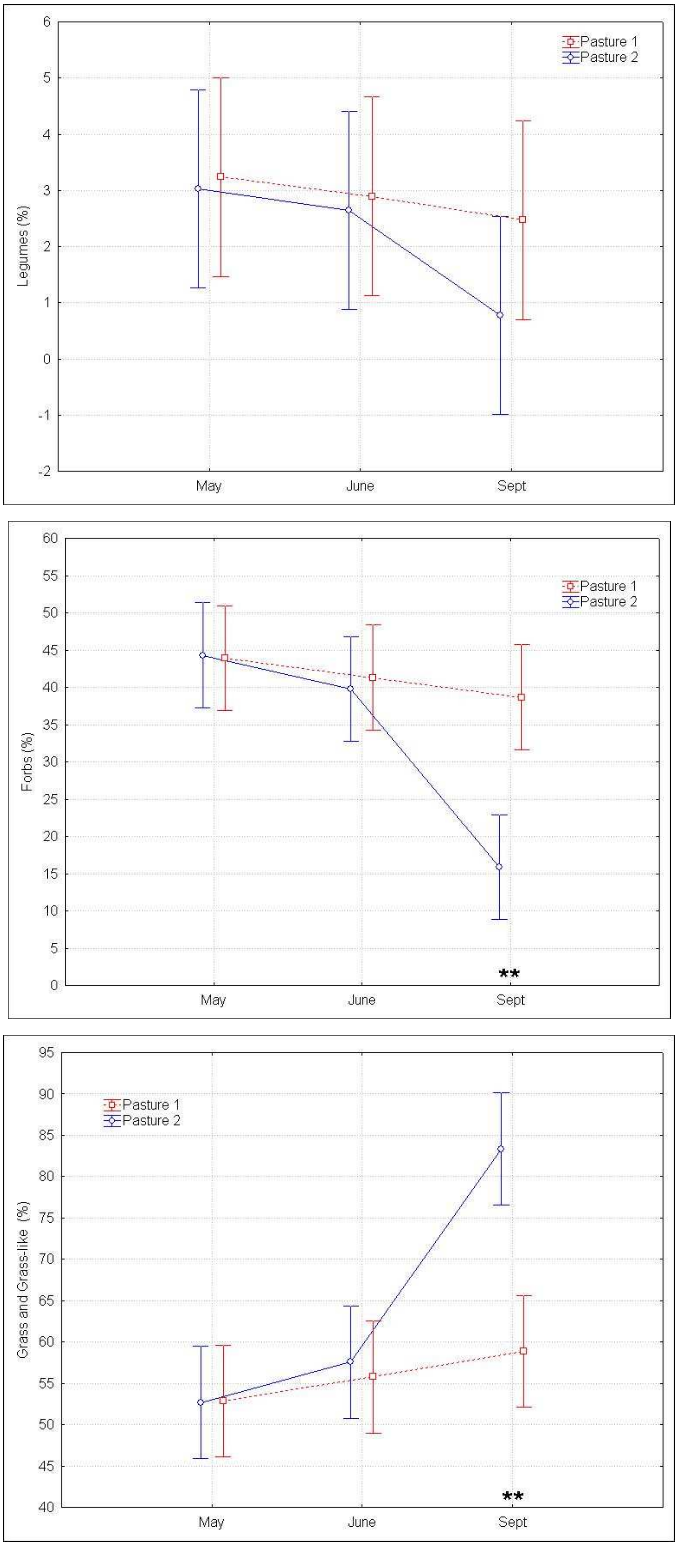


Figure 1. *Trends of the average percent values in fresh matter of botanical functional groups (legumes, forbs, grass and grass-like species) in the North Adriatic pastures referred to the Carici humilis-Centaureetum rupestris association. Bars indicate Standard deviation (Pasture 1: Slum, low use intensity pasture; Pasture 2: Vodice, abandoned pasture;* ** *p < 0.01).*

Yield, dry matter and the variables linked to forage feed value, followed a double trend, linked to the phenological phases of grasslands and to the land use/topographic gradient: higher yield, yield NEL, DM, ADF and ADL were related to the second half of the growing season, while greater CP, NEL and IVDMD to the first phenological phases. Yield, yield NEL, ash and IVDMD were tied to the ungrazed pastures spread on the slopes, while ADF, ADL and DM had stronger correlation with grazed grasslands of flat lands. DM, NDF, ADF, and ADL were negatively correlated to CP content, NEL and IVDMD. The greater amount of grasses/grass-like plants and forbs (fresh matter) was found in abandoned pastures.

Forage nutritive value of North Adriatic pastures

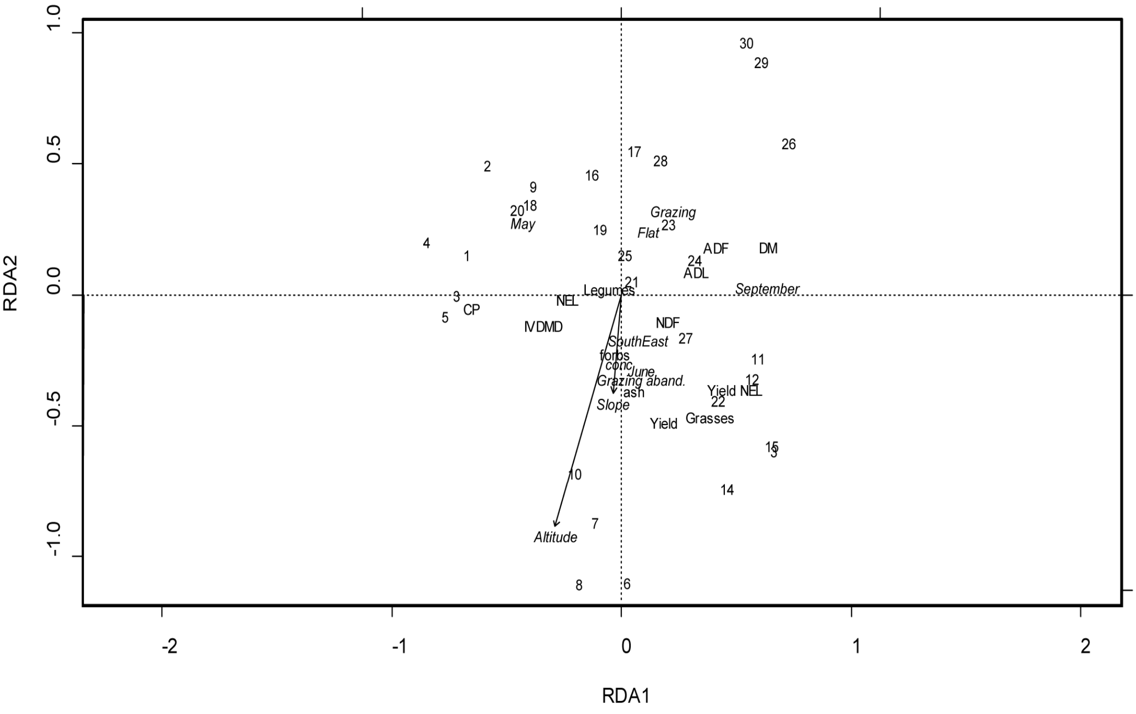
The pastures of the study area have an average low CP content (90.39 g/kg DM) and a high content of fiber fractions: NDF (601.34 g/kg DM), ADF (368.87 g/kg DM), low IVDMD (49.52 %), and low NEL (4.57 MJ/kg). CP content ranges from 62.62 to 129.08 g/kg DM, NDF from 557.87 to 674.24 g/kg DM, IVDMD from 459.26 to 536.78 g/kg DM, and NEL from 4.1 to 5.4 MJ/kg DM.

In May, grazed and ungrazed pastures differed significantly in CP (greater in P2) and ADF (greater in P1). In June additional differences were recorded also in ash content and in IVDMD (both greater in P2), while the only significant difference in September was highlighted for ash (*Table 2*).

Table 2. *Average values ± Standard deviation of crude ash (Ash), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) content, net energy for lactation (NEL), and in-vitro dry matter digestibility (IVDMD), measured during the growing season in the pastures of Carici humilis-Centaureetum rupestris association with different types of management (P1: Slum, low use intensity pasture; P2: Vodice, abandoned pasture). Significance values from ANOVA or Kruskal-Wallis test (p) (comparisons among times during the growing season in each locality) and from t-test or Mann-Whitney U-test ($p_{may,\ june,\ sept}$) (comparisons between localities at each time) are indicated. Values within the same column with the same superscripts do not differ significantly between times in each locality. Pairwise comparisons were not performed when ANOVA or Kruskal-Wallis test did not highlight significant differences ($p \geq 0.05$).*

Locality	Season	Ash (g/kg DM)	CP (g/kg DM)	NDF (g/kg DM)	ADF (g/kg DM)	ADL (g/kg DM)	NEL (MJ/kg)	IVDMD (g/kg DM)
	May	55.34±7.86	102.08^{a}±9.28	596.02±31.88	378.24±19.00	86.18^{ab}±31.96	4.65±0.81	514.24±51.28
	June	50.71±3.19	81.03^{b}±6.76	600.97±51.57	392.01±20.44	79.13^{a}±12.78	4.81±0.34	467.08±21.98
	Sept.	46.61±4.12	62.62^{c}±3.53	576.08±77.25	381.44±28.93	116.20^{bc}±16.26	4.10±0.55	459.96±31.06
	Aver.	50.89±6.25	81.90±17.88	591.02±53.66	383.90±22.33	93.84±26.29	4.52±0.64	480.43±42.27
P1	*p*	0.141	< 0.001	0.953	0.629	0.045	0.141	0.077
	May	54.31^{a}±3.20	129.08^{a}±4.12	557.87^{a}±38.97	328.84^{a}±13.44	62.93±21.00	5.39^{a}±0.39	536.78^{a}±35.71
	June	66.16^{ab}±8.71	102.45^{b}±5.05	602.84^{ab}±38.70	362.94^{b}±7.88	70.48±11.15	4.85^{ab}±0.36	534.12^{a}±32.26
	Sept.	$70.79^{.b}$±8.61	65.12^{c}±1.75	674.24^{b}±58.57	369.75^{b}±10.34	108.94±49.09	3.63^{b}±1.07	459.26^{b}±23.89
	Aver.	63.75±9.87	98.88±27.39	611.65±65.59	353.84±21.05	80.79±35.85	4.63±1.00	510.05±46.99
P2	*p*	0.011	<0.001	0.006	<0.001	0.122	0.005	0.003
	p_{may}	0.840	<0.001	0.129	0.001	0.211	0.225	0.443
	p_{june}	0.006	<0.001	0.841	0.018	0.287	0.855	0.005
P1 vs. P2	p_{sept}	<0.001	0.193	0.053	0.411	0.762	0.406	0.969

Figure 2*. Redundancy analysis ordination graph for yield, forage feed value variables and botanical functional groups, using altitude, aspect, slope angle, land form (conc - slightly concave slope; flat - flat land), management type (grazing - low intensity use pasture; grazing aband.: abandoned pasture), and time (May, June, and September) as constraining variables. Numbers indicate sites (DM - dry matter; ash - crude ash; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; ADL - acid detergent lignin; NEL - net energy for lactation; IVDMD - in-vitro dry matter digestibility).*



Discussion

The research outcomes highlighted that the variables which define the quantity and quality of forage, follow a main gradient related to the phenology of the grassland community. In fact, we observed a general trend during the growing season, characterised by the increase in dry matter, fiber (NDF and ADF) and lignin (ADL) content, and the decrease in NEL, IVDMD and CP. Thus, pastures provide more digestible and high quality value forage in spring, when plants are still young. The forage quality declined during the growing season because of the increase in fibers and lignin content, which is responsible for the relative increase in dry matter, and reducing the energy available per mass unit. The increase in fiber is in accordance with Pérez Corona et al. (1994) and Heitschmidt et al. (1995) who related the stage of maturity of plants and age of tissue to the NDF content, which increases in older tissue. We detected also a significant decrease in crude protein content from May to September. This is consistent with Kirby et al. (1989) and Vázquez de Aldana et al. (2000) who observed that the crude protein content declines with stage of maturation. Considering that 6-8% of crude protein is considered as a threshold, below which either the intake or digestibility of forage falls off, and animal maintenance requirements are not met (Milford and Minson, 1965; Tamminga et al., 1979; Nocek and Russell, 1988), pastures of the study area have not good nutritional value (6-6.5% of crude protein) only at the end of the growing season.

The obtained results confirm also that the dry matter content is a good indicator of nutritional value, which agrees with Miraglia et al. (2008) who stated that the higher dry matter content is indicator of lower crude protein and higher share of lignin in semi-natural pastures.

The RDA highlighted a second gradient linked to land use. More specifically, abandonment of North Adriatic grasslands is related to the higher altitudes and steeper south-east-facing slopes, while grazing, although with low intensity, persists on flat lands. A similar spatial trend of abandonment was recorded also in the Apennine ridge (Bracchetti et al., 2012; Catorci et al., 2012a).

The ungrazed pastures analysed were particularly related to grasses and grass-like plants. This observation is consistent with the results of previous research carried out in North Adriatic grasslands which highlighted a species change in the community, due to the triggering of dynamic successional processes as a consequence of management abandonment (Vitasović Kosić et al., 2011). In particular, it was acknowledged that this change was caused mainly by the spread of *Brachypodium rupestre*, a competitive stress-tolerant graminoid which quickly spreads by means of rhizomes (Grime et al., 1988) and tends to form extensive patches (Catorci et al., 2013a). The invasion of *Brachypodium* species in abandoned grasslands is widely documented throughout Europe (e.g. Buckland et al., 2001; Bonanomi and Allegrezza, 2004), where it is causing threat to biodiversity (During and Willems, 1984; Bobbink and Willems, 1987; Wilson et al., 1995; Bonanomi et al., 2006, 2009; Catorci et al., 2011), and was recorded in the North Adriatic pastures as well (Vitasović Kosić et al., 2012). The affirmation of grasses (including also other graminoids such as *Bromus erectus* Huds., *Festuca* sp. pl., and *Koeleria* sp. pl.) with tolerance strategies and erect leaves in the abandoned condition is consistent with the findings of some authors (e.g. Skarpe, 2001; Pykälä, 2004; Catorci et al., 2013b) who argued that exclusion of grazers causes the increase in abundance of plants with erect leaves to the detriment of prostrate species.

It is known that abandonment may change the main parameters affecting the forage quantity/quality (Pavlů et al., 2006). In fact, in the study case, yield differed between grazed and ungrazed pastures (average values of 4.62 and 5.66 g/m^2, respectively), diverging significantly in the later stage of the growing season (*Table 1*). The results indicate that in ungrazed pastures, yield and yield NEL increased significantly from May to June, when *Brachypodium rupestre* is in full growth, reaching its peak in phytomass and the optimal period for reproduction before the summer drought period. This may account for the greater phytomass values of ungrazed pastures than in grazed ones. Indeed in the study area, the cover of *Brachypodium rupestre* may be greater than 50% (Vitasović Kosić et al., 2012). Moreover, in more productive undisturbed grasslands species do not allocate resources to produce antiherbivore defence structures, but devote them to new photosynthetic tissue (Coley et al., 1985). This may account for the lower fiber and lignin content, and the related higher values of IVDMD, recorded in abandoned pastures.

The higher forage digestibility and CP content may be substantially ascribed to the forb and legume functional groups; instead grass and grass-like species are more related to fibers and lignin content (*Fig. 2*). Indeed, it is known that most of the non-grass species have lower fiber content than the grasses and are highly digestible (Mojo et al., 1993), and leguminous plants have a higher nutritional value than grass species (Peeters and Janssens, 1998).

Also forbs showed a greater amount of fresh matter in summer *(Fig. 2)*. This is consistent with Ramirez and Nunez-Gonzalez (2006), who observed that relatively high proportion of forbs in summer helps to maintain nutrients, moisture and yield of grasslands during the summer drought, contributing to maintain the stability of nutritive value of semi natural grassland throughout the growing season.

Moreover, the abandoned condition and the amount of grass, was mainly linked to south-east facing slopes. This is in accordance with Peco et al. (2012) and Catorci et al. (2013b) who argued that, in the most stressing submediterranean habitats such as southerly aspects, which experience a summer drought stress period, the spread of grasses is promoted by the absence of herbivory.

Grazing is positively related to lignin and fiber content and negatively related to NEL, yield, yield NEL, and IVDMD. Indeed plants respond to herbivory reducing the probability and severity of grazing by changing tissue chemistry, growth rate, morphology and resource allocation (Belsky, 1986; Bryant et al., 1992; Rooke and Bergström, 2007), producing toxic secondary metabolites and involving mechanical defences like spines, tough leaves and protective coatings (Alba et al. 2011, Vitasović Kosić et al., 2011).

Conclusions

Pastures of Ćićarija are very valuable for their high potential production, their good nutritional value (especially for the spring protein content and digestibility), and their high plant diversity; therefore, special attention should be paid to their protection and conservation through management measures, such as maintaining a general low pressure of grazing by means of grazing rotation, to prevent the process of secondary succession and the spread of unpalatable competitive tall grasses at a landscape level.

As factors which affect plant diversity affect yield and nutritive value of grassland as well (White, 1983), knowing the phytomass production and the forage nutrient content of pastures is the first step in predicting the minimum number of herbivores required to maintain the optimal plant diversity of Ćićarija.

The results of this research could allow the draft of the guidelines for a comprehensive and optimal management of grasslands, not only in the study area, but also in other submediterranean areas where *Carici humilis-Centauretum rupestris* association spreads. This can contribute to the improvement of the sheep production and its high-value products (milk, meat and cheese) and, at the meantime, to the preservation of the diversity of flora and grassland vegetation in Croatia and Europe.

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IMPROVING NUTRITIONAL QUALITY OF THE GOAT MILK BY GRAZING

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Abstract. The aim of study was to investigate the effect of grazing on the nutritional quality, such as composition, vitamins and fatty acids content of dairy goat milk. Before vegetation period, all goats were kept indoors and nutrition based on hay diet. After turn-out to pasture, all goats were grazing. Bulk milk samples were collected on 12 consecutive days at two sampling periods: in indoor and in grazing months and analysed for milk compositions and vitamin A, E and D_3, as well as for fatty acid contents. The milk from grazing goats had significantly higher fat, protein and total solids non-fat than goats kept indoors. Grazing caused higher concentrations of vitamin A (0.026 vs. 0.036 mg/100ml; $P<0.01$) and D_3 (0.075 vs. 0.089 mg/100ml; $P<0.05$) compared to feeding hay. During the grass diet the rumenic acid (0.56 vs. 0.66 g/100g fatty acids; $P<0.05$) and n-3 fatty acids (0.36 vs. 1.19 g/100g fatty acids; $P<0.001$) contents in milk significantly increased. In this study, n-6/n-3 ratio of 10.17 and 1.82 were found in milk samples of goats that fed indoor and grass, respectively. It can be concluded that the milk from grazing goats is more advantageous for human nutrition, than the milk produced by animals fed hay based diet.

Keywords: *goat, grazing, milk, vitamins, fatty acids*

Introduction

Pasture is one of the most natural feeding sources for animals. The digestibility, protein and energy content of the grass are notable, the crude fat content relatively low, 2-3% of dry matter, nevertheless the grass is rich in α-linolenic acid (Cabiddu et al. 2005), accordingly pastures and other green forages diet have been associated with high content of n-3 fatty acids of milk fat. Feeding green forages to dairy animals increased the concentrations of long chain fatty acids, such as α-linolenic acid and reduced linoleic acid and n-6/n-3 ratio in milk and in cheese (Tsiplakou et al., 2006; Pajor et al. 2012). It is well known, that the high ratio of n-6/n-3 fatty acids is a risk factor in coronary heart disease (CHD). The recommend value for the n-6/n-3 ratio is less 4:1 (Simopoulos, 2004).

In addition, the milk possesses other favourable components, such as rumenic acid and fat-soluble vitamins. The importance of goat milk consumption is recently increasing, because the milk compounds are beneficial effect in human diet.

Numerous studies on effect of pasture on milk fatty acids, especially on rumenic acid composition were carried out in cattle and sheep (e.g. Frelich et al. 2012 and Tsiplakou et al., 2006). The rumenic acid (c9t11CLA isomer) has a range of positive health properties such as anticarcinogenic (Ip et al., 1991) and antiatherogenic effects (Nicolosi al., 1997). In contrast, literature reports are limited about goat milk and cheese

(El-Salam and El-Shibiny, 2012). The fat-soluble vitamins are knowingly favourable in human diet for their antioxidant potential and positive impact on health (Bergamo et al., 2003).

Moreover, there is little available information about the content of vitamins in goat milk, especially about pasture kept goats compared to other ruminants (e.g. Kondyli et al. 2007).

Thus, the aim of this study was to investigate the effect of the grazing on certain nutritional quality compounds (vitamin A, E and D_3, n-3 fatty acids and rumenic acid) of goat milk.

Materials and method

Experimental animals and diet

The study was carried out in a goat farm in Borsod-Abauj-Zemplen County (Northeast Hungary). 54 Hungarian Native goats on different parities but in the same stage of lactation were involved in this study. Before grazing months (May), all goats were kept indoors and their nutrition was based on *ad libitum* alfalfa hay and also 350 g/day grain mix.

After turn-out to pasture the grazing group stayed all day long on the pasture; however, they were also fed with 350 g/day grain mix. Both groups had same composition of the grain mix, which was given twice a day in equal amounts at milking time. A commercial vitamin (A, D3, E) and trace-mineralized salt block was provided free choice to all goats. The diets were adjusted to the NRC (2007) recommendations of energy and protein requirements for dairy goats (body weight: 60 kg; 2.5 kg of milk/day).

Utilization of native pasture was extensive in order to avoid over-grazing. The stocking density of the pastures grazed by the goats was about 0.5 AU/ha. The main grass and legume species were *Festuca pseudovina* and *Trifolium pratense*. Other species were *Elymus repens*, *Elymus hispidus, Bromus inermis, Calamagrostis epigeios* and *Arrhenatherum elatius.* The average annual rainfall of the area is approx. 695 mm. The annual green grass yield was 5.2 t/ha. During the daily routine the grazing goats were driven to pasture after the morning milking, and collected in the afternoon to be milked and confined for the entire night.

Milk samples were taken at two sampling times: in mid-April and in mid-July when the goats were on average among the 30 and 120 days in lactation. Samples of pooled milk were collected twice a day during 12 consecutive days of the experimental months at 6.00 a.m. and 6.00 p.m., all milk samples were frozen and stored at -20 ^{0}C until further analysis. Before laboratory analysis, twice a day gathered milk samples were combined to one sample for the analysis of chemical composition.

Chemical analysis

Fat, protein, lactose and total solids without fat contents of milk were determined using a Bentley device (Bentley Combi apparatus, Bentley Instruments Inc, Chaska, MN, USA).

The milk fat was dissolved in sodium hydroxide-methanol solution and re-esterified to methyl-esters according to the AOAC (1990) method using boron trifluoride (BF_3). Methyl esters of fatty acids were determined by gas chromatography using a Shimadzu

GC 2010 apparatus (Japan) with a flame ionization detector (FID) and column (CP-SIL-88, 100 m × 0.25 mm × 0.2 µm).The split injection ratio was 50:1. The column oven temperature was held at 80°C for 0 min, then programmed at a rate of 2.5°C/min up to 205°C and held for 20 min and then increased again to 225°C at 10°C/min, and held for 5 min. The injector and detector temperatures were 270°C and 300°C, respectively. Helium was used as the carrier gas, applying a flow rate 28 cm/s. Peaks were identified on the basis of the retention times of standard methyl esters of individual fatty acids (Mixture Me 100, Larodan Fine Chemicals AB, Sweden). The proportions of the individual acids were calculated by the ratio of their peak area to the total area of all observed acids.

Fat-soluble antioxidants (vitamin A, E and D_3) were analysed using the high-performance liquid chromatographic method described by Kerti and Bardos (2006). The vitamin detection was performed at 325 nm for vitamin A, 290 nm for vitamin E and 265 nm for vitamin D_3. The peak areas were integrated and quantified by using the Chrompass software (Jasco, Japan).

Statistical analysis

Effects of feeding method was analysed as independent variable. Statistical analysis was processed by the SPSS 21.0 softwer package (Shapiro-Wilk test for normality distribution, F test for equality of Variances, t-test and Welch's corrected t-test). Significance was taken at an alpha level of 0.05.

Results and discussion

There was no significant difference in the milk production of the examined groups; daily milk yield during the investigation was 2.5 kg in control goats and 2.6 kg in experimental goats.

Milk composition was significantly affected by diet. Hay diet caused lower ($P<0.05$) fat (3.11 g/100g), protein (2.94 g/100g) and total solids non-fat (7.87 g/100g) composition compared to milk from pasture kept goats (3.70, 3.22 and 8.15 g/100g, no data in Table). Thus, the grazing significantly improved the milk composition during experiment. It is well known, that the goat milk fat and protein content is affected by many factors, such as breed, parity, stage of lactation and diet (Kuchtik et al., 2008; Novotna et al., 2009) and it has great effect on cheese composition. Soryal et al. (2004) in Alpine goat and Pajor et al. (2009) in Hungarian Native goat found that the grazing slightly increased the milk compositions.

The contents of the selected vitamins in the goat milk are presented in *Table 1.*

Table 1. *Effect of grazing on vitamin content of goat milk (mg/100 ml)*

Items	**Control diet**	**Experimental diet**	**Total**	**SEM**	**P**
Vitamin A	0.025	0.036	0.031	0.002	0.001
Vitamin E	0.122	0.140	0.131	0.011	0.454
Vitamin D_3	0.075	0.089	0.082	0.003	0.017

P: level of significance

The average contents of vitamins A and D_3 were 0.031 mg and 0.082 mg/100 ml. In goats reared indoor lower contents of A and D_3 vitamins (0.025 mg and 0.075 mg/100 ml of milk) were found compared to goats with experimental diets (0.036 mg and 0.089 mg/100 ml of milk)

Mean of vitamin E concentration was 0.131 mg/100 ml, however vitamin E contents of milk did not show any significant differences between the experimental periods. The mean values of vitamin A and E as well as vitamin D_3 in present study were slightly higher compared to values which published by Kondyli et al. (2007) and Raynal-Ljutovac et al. (2008).

Our results showed that milk from grazing goats is a good dietary source of vitamins A, E and D_3 whereas these are also well known for their antioxidant potential (Bergamo et al., 2003).

The results of the fatty acid analysis of milk samples are presented in *Table 2*.

***Table 2.** Effect of grazing on fatty acid profile of goat milk (g/100g total fatty acids)*

Fatty acids	**Control diet**	**Experimental diet**	**Total**	**SEM**	**P**
C4:0	0.58	0.71	0.64	0.03	0.037
C6:0	1.32	1.73	1.52	0.06	<0.001
C8:0	1.68	2.20	1.94	0.08	<0.001
C10:0	7.59	9.28	8.43	0.29	0.001
C12:0	3.40	3.39	3.40	0.07	0.971
C14:0	11.27	9.91	10.59	0.21	<0.001
C14:1c9	0.16	0.08	0.12	0.01	<0.001
C16:0	28.76	27.83	28.29	0.26	0.072
C16:1c9	0.96	0.73	0.84	0.03	<0.001
C18:0	10.71	17.97	14.34	0.81	<0.001
C18:1n-9c	17.89	14.43	16.16	0.58	0.001
C18:1*t11*TVA	0.77	2.15	1.46	0.19	<0.001
C18:2n-6	2.89	2.00	2.45	0.10	<0.001
*c9t11*CLA	0.56	0.66	0.61	0.02	0.021
C18:3n-3	0.35	1.19	0.77	0.09	<0.001
C20:4n-6	0.24	0.14	0.19	0.01	<0.001
SMCFA	11.17	13.91	12.54	0.44	<0.001
C:12+C:14+C:16	43.43	41.13	42.20	0.49	0.008
SFA	75.65	78.30	76.98	0.60	0.024
MUFA	20.25	17.71	18.98	0.55	0.016
PUFA	4.09	3.99	4.04	0.07	0.477
n-6	3.18	2.15	2.66	0.12	<0.001
n-3	0.36	1.19	0.77	0.09	<0.001
n-6/n-3	10.17	1.82	6.00	1.04	<0.001

P: level of significance; SMCFA: Short and medium chain fatty acids (C_4-C_{10}); SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; CLA: conjugated linoleic acid

Grazing significantly increased the contents of short chain fatty acids [butyric (C4:0), caproic (C6:0), caprylic (C8:0) and capric acids (C10:0)], stearic (C18:0), α-linolenic (C18:3), total saturated fatty acids (SFA) and as well as significantly decreased the contents of the miristic (C14:0), miristoleic (C14:1), palmitoleic (C16:1), oleic acid (C18:1), linoleic (C18:2), arachidonic (C20:4) and total monounsaturated fatty acids (MUFA) in milk samples. On the contrary, the concentrations of lauric (C12:0), palmitic acid (C16:0) and total polyunsaturated fatty acids (PUFA) in milk samples did not show any significant differences between experimental periods.

Grazing significantly increased the concentrations of short and medium chain fatty acids (MCFA) in milk. These fatty acids are hydrolyzed rapidly and are absorbed directly to the liver via portal vein (Papamandjaris et al. 1998). Therefore the medium chain fatty acids have been used for patients that have malabsorption syndrome. Recently, the relations between the medium chain fatty acids and certain metabolic syndromes are summarized in a review report by Nagao and Yanagita (2010).

Concentrations of lauric, myristic and palmitic acids were lower in milk samples of the grazing group than in the control group. These acids are known to be hypercholesterolemic, whilst the other major saturated fatty acid (SFA), e.g. stearic acid, does not. Ulbricht and Southgate (1991) reported that these fatty acids to be responsible for increase the level of total and LDL cholesterol concentrations in blood serum**.**

The grazing positively affected the concentration of rumenic and vaccenic acids in the milk. The rumenic and vaccenic acids concentrations in the milk were 0.56 and 0.77 vs. 0.66 and 2.15% for hay based and experimental diet, respectively. The polyunsaturated fatty acids, such as linoleic and linolenic acid, are partly saturating in the rumen by biohydrogenation. Throughout this process the rumenic acid is formed from linoleic acid in the rumen by anaerobic bacteria (such as *B. fibrisolvens*), with vaccenic acid (t11C18:1)(TVA) as intermediates. TVA is converted to CLA by Δ^9-desaturase in mammary gland (Bauman et al. 2001). Because of biohydrogenation in rumen, the relative percentage of C18:0 was significantly increased in milk from grazing goats (17.97%) compared to control group (10.71%).

In present study, grazing significantly decreased the n-6 fatty acids and increased the n-3 fatty acids contents, however the n-6/n-3 ratios were favourable, 1.82 instead of 10.17. The n-6/n-3 ratio is generally used to assess the nutritional value of fats. According to Simopoulos (2004), recommend value for the n-6/n-3 ratio is less 4:1. The low ratio of n-6/n-3 in the milk of grazing goats is meeting with the new recommendations for human nutrition. It is well known, that grass is rich in α-linolenic acid, authors reported that 50-60% of the total fat is n-3 fatty acids in grass (Cabiddu et al. 2005; Tsvetkova and Angelow, 2010). The relatively unfavourable n-6/n-3 ratio in control group was probably due to lower n-3 fatty acids concentrations in hay which is affected by oxidative and leaf losses during hay making (Doreau and Poncet, 2000; Dewhurst et al., 2006). However, the relatively high PUFA content of grass is inhibiting de novo fatty acid synthesis in mammary gland (Couvrer et al., 2006). This confirmed by decrease of miristic and miristoleic fatty acids content of grazed goats' milk samples**.**

Conclusions

In conclusion, significant difference was found in milk composition, vitamin as well as fatty acid profile between two treatments. Grazing significantly increased the concentrations of vitamin A and D_3, rumenic acid, short and medium chain fatty acids, as well as n-3 fatty acids in milk. These results show that grazing can enhance the nutrition value and quality of goat milk, consequently, consumers have nutraceutical benefits from consumption of milk from grazing goats due to higher concentrations of health promoting compounds.

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