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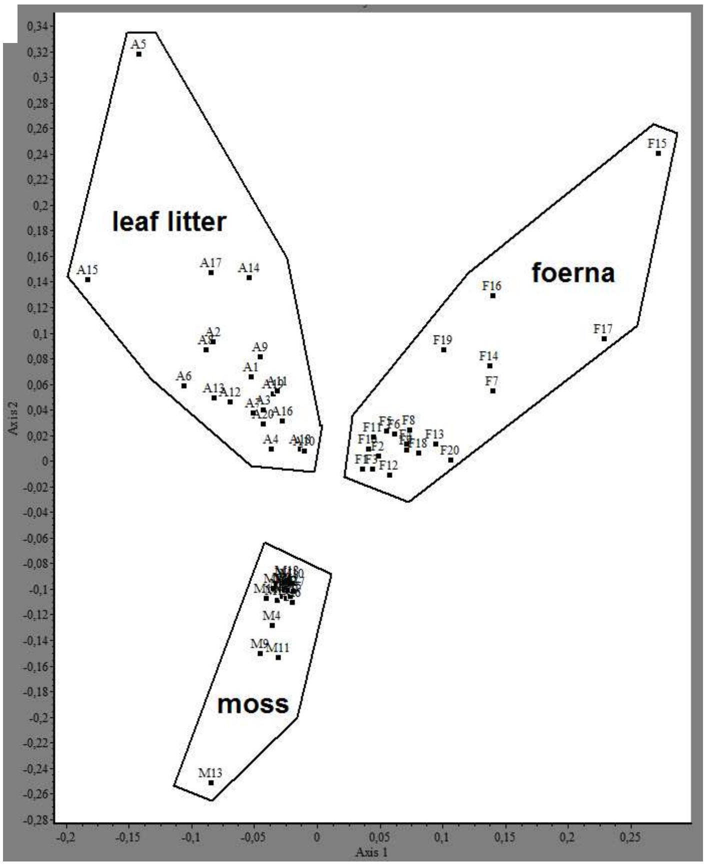

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SIMULATING THE EFFECTS OF ANTHROPOGENIC DISTURBANCES IN LANDSCAPE PATCHES: AN APPLICATION IN NORTH PORTUGAL

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Abstract. A simple methodology was applied with the purpose of understanding the effects of anthropogenic disturbances in the biodiversity of landscape patches, namely by using Soil Surface Dwelling Arthropods as relevant indicators in changing scenarios. The goal of the present paper is to apply and extend the above academic concepts to landscape management, and to demonstrate the potential of a Stochastic Dynamic Methodology in implementing the respective actions. Since many of the ecosystem phenomenological aspects are the result of whole-system properties, the main purpose of the Stochastic Dynamic Methodology is to promote a mechanistic understanding of the holistic ecological processes, based on statistical parameter estimation methods. In this perspective, the proposed protocol is compatible with most activities undertaken by conventional ecological science, i.e., pattern seeking, the ability to explain past and present states, and the ability to predict future states. Additionally, in contexts relating to landscape management, the results of the Stochastic Dynamic Methodology applied to monitoring and restoration activities are intuitive and can be easily communicated to non-experts (ranging from students to resource users and senior policy makers).

Keywords: *Soil Surface Dwelling Arthropods; Stochastic Dynamic Methodology; Biodiversity Indicators; Landscape patches; Landscape management*

Introduction

The world's landscapes are influenced by a set of anthropogenic pressures that put at risk their sustainability and weaken their ecological functions and societal services (Palmer et al., 2005; Tianhong et al., 2009). This problem has lead to an emphasis for developing accurate assessments of ecosystems status (Rapport et al., 1998; Rapport and Singh, 2006; Giordani et al., 2009). In this scope, the need for rapid, standardized and cost-saving assessment methodologies is crucial, namely to predict how anthropogenic environmental changes will affect the abundance of species, guilds and communities (Andreasen et al., 2001; Gibbons et al., 2009). For conservation and management purposes, the use of appropriate biodiversity indicators may reveal the effect of changes in environmental factors (Jackson et al., 2000; Niemeijer and de Groot, 2008) and provide useful data to characterize composition, structure, and function of complex systems (Müller et al., 2000; Jørgensen, 2008; Doren et al., 2009). Since most environmental impacts are phenomena occurring after a time-lag, early indications of change need to be identified (Bässler et al., 2009).

Arthropods are reported, in many situations, as relevant biodiversity indicators (e.g. Ueahara-Prado et al., 2009). Despite their inconspicuous nature, Soil Surface Dwelling Arthropods (SSDA) are one of the most important components of ecological communities in terms of both abundance and diversity (Nakamura et al., 2007). In fact, SSDA are small, diverse, usually sensitive to environmental variability, rapidly respond to disturbance, provide cheap and easy sampling measurements and, therefore, may be assumed to reflect habitat heterogeneity, patch diversity and environmental stress (e.g. Negro et al., 2009; Hoffmam, 2010). If we are able to determine, in a reliable approach, the effects of patch changes in the SSDA abundance and/or composition and their characteristic values in reference areas then we will be able to follow their trends and use then as robust biodiversity indicators (Nakamura et al., 2007).

In many occasions, the indicators trajectory can be followed a priori by using dynamic models for a better comprehension of the stress-response relationships (e.g. Lancelot et al., 2009). In fact, ecological studies have been improved by creating dynamic models that simultaneously attempt to capture the structure and the composition in systems shaped by environmental disturbances (e.g. Chaloupka, 2002; Santos and Cabral, 2004; Santos et al., 2010). The comprehensive use of ecological models has shown a constant and remarkable growth in the last three decades (Williams and Poff, 2006; Jørgensen, 2008). When properly developed and tested, the ecological models must be applied with insight and with regard to their underlying assumptions. These requirements could result in models capable of simulating conditions that are difficult or impossible to understand otherwise. Moreover, the application of ecological models can synthesize the pieces of ecological knowledge, emphasizing the need for a holistic view of a certain environmental problem (Forrester, 1973; Jørgensen, 2001; Schizas and Stamou, 2007; Jørgensen, 2008).

Since many of the ecosystem phenomenological aspects are holistic, whole-system properties, the main vocation of the Stochastic Dynamic Methodology (StDM) is a mechanistic understanding of ecological processes, based on statistical parameter estimation methods (Santos and Cabral, 2004; Cabral et al., 2008; Santos, 2009). The StDM is a sequential modelling process developed in order to predict the ecological status of systems, from which management strategies can be designed. This recent research is based on the premise that the general patterns of ecological phenomena are emergent indicia of complex ecological processes that do indeed reflect the operation of universal law-like mechanisms (Cabral et al., 2008). In this scope, the StDM was successfully applied in several types of scenarios, such as agro-ecosystems (Santos and Cabral, 2004; Cabral et al., 2007), mountain running waters and reservoirs (Cabecinha et al., 2004; Cabecinha et al., 2007; Cabecinha et al., 2009), estuaries (Silva-Santos et al., 2006; Silva-Santos et al., 2008), wildlife conservation (Santos et al., 2007; Silva et al., 2010), bird survey testing (Santos et al. 2009) fire effects in forest ecosystems (Silva-Santos et al., 2010) and wind farm impacts in mountain ecosystems (Santos et al., 2010).

Therefore, the ultimate objective of the paper was to show, in contexts relating to landscape management, the possibility of using a StDM protocol for predicting changes in the biodiversity associated to patch dynamics, which will be valuable to those engaged in research and teaching landscape ecology.

Materials and methods

Study site

The methodology was applied in the campus of the University of Trás-os-Montes e Alto Douro. The campus is situated near the town of Vila Real (41º 30’ N, 7º 10’ W), north Portugal (*Fig. 1a*), in a plateau with an average altitude of 500 meters above the sea-level. The bio-climatologists classify the study area as “mesomediterranean” with precipitation values of 1200 mm/year and an average annual temperature of 12 ºC. The campus mosaic is representative of the region’s landscape (*Fig. 1b*).

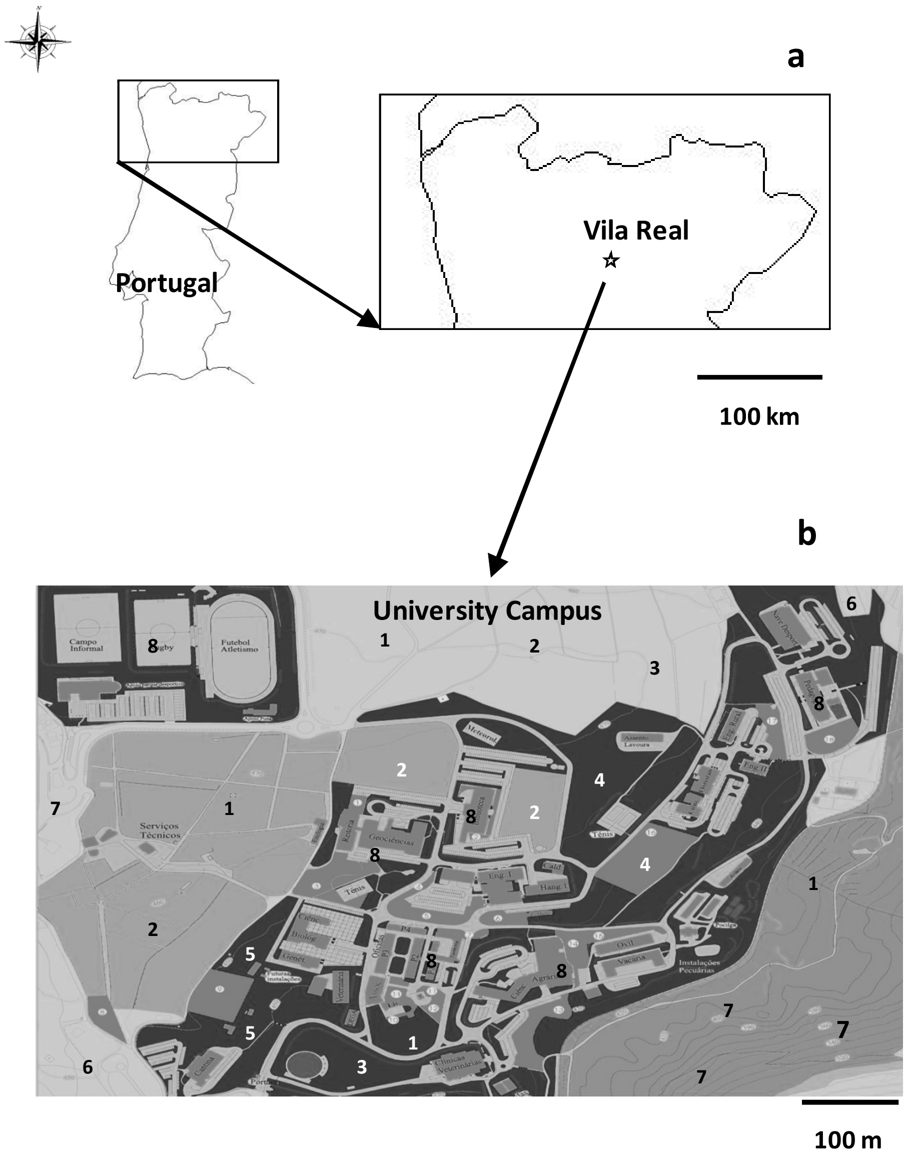


***Figure 1**. Location of the of study site in (a) North Portugal and (b) a detailed map of the University of “Trás-os-Montes e Alto Douro” Campus: (1) vineyards, (2) cereal fields, (3) olive orchards, (4) woodlands, (5) chestnut plantations, (6) pastures, (7) shrublands (8) social areas not considered for the implementation of the protocol*

Data collection

One hundred and twenty seven circular plots, each one with a radius of 25 meters (to eliminate the factor size and shape of patch), were surveyed during one month (May 2009). Overall, the plots represent the structural heterogeneity of the study area, in terms of the different local characteristics of the main habitats (mosaic), an important requirement for the unbiased comprehension of the indicators response in a hypothetical patch. Each plot description and surveys were made in its centre, sited by using a Global Position System device (Magellan GPS 320®) and the respective slope, aspect and topography were recorded.

The vegetation cover was estimated by using visual estimates and reference frames (using crown densitometer) for the trees and shrubs layer and direct measurements (using frame quadrats) for the herbaceous and mosses layer. The vegetation height was measured using a clinometer for the trees and shrubs layer (Suunto 802575) and direct measurements for the herbaceous and mosses layer (using a metric diameter tape).

Soil Surface Dwelling Arthropods (SSDA) were sampled using two pitfall traps of 500 cm^3 in each plot (depth 10 cm, mouth diameter 8 cm, bottom diameter 6 cm) filled to one third with polyethylene glycol. Trap locations were separated by 5m intervals and were removed after seven days. All SSDA specimens were isolated from the debris in the laboratory, stored in 80% ethyl alcohol, and identified to the Order level using taxonomic keys (Borror et al., 1992) and a binocular microscope (Leica Zoom 2000). All of the specimens were deposited in an insect storage room in the laboratory.

Data analysis

Determining the taxa responses

The statistical procedure selected to test for relationships between the biodiversity indicators (SSDA Orders) and the vegetation characteristics was a stepwise multiple regression analysis (Zar, 1996). A step down procedure was used so that the effect of each variable in the presence of all other related variables could be examined first with the least significant variable being removed at every step. The analysis stopped when all the remaining variables had a significant level $P < 0.05$ (Zar, 1996). Although the lack of normality distribution of the dependent variables was not solved by any transformation (Kolmogorov-Smirnov test), the linearity and the homoscedasticity of the residuals were achieved by using logarithmic transformations ($X' = \log[X + 1]$) in each side of the equation, i.e., on both the dependent and independent variables (Zar, 1996). The lack of substantial intercorrelation among independent variables was confirmed by the inspection of the respective tolerance values. The statistical analysis was carried out using the software SYSTAT 8.0®.

Conceptualisation of the model

Since the previous statistical procedure was supported on a database which included gradients between the different habitats, over space, the significant partial regression coefficients were assumed as relevant ecological parameters in the dynamic model construction. This is the heart of the philosophy of the StDM. In a holistic perspective, the partial regression coefficients represent the global influence of the environmental variables selected, which are of significant importance on the indicators, namely on several complex ecological processes associated with arthropod taxa abundance and composition (Santos and Cabral, 2004; Santos, 2009). These processes were not included explicitly in the model, but were implicitly related to the state variables (or indicators) under consideration. STELLA 9.0.3.® software was used for the development of the dynamic model. Thereafter, the SSDA were assigned to feeding guilds, based on literature review, and aggregated into trophic groups (predators, omnivorous, herbivorous and detritivorous) with the intention of facilitating the perception of the structural and functional changes occurring in the studied landscape.

Model simulations

The guilds' trends were then simulated using realistic scenarios of plant succession and plant retro-regressive succession associated with possible man-made disturbances (through 20 years, using the year as unit of time) in selected patches of the landscape. The scenarios considered, for academic demonstration and discussion, were: a) a forested patch, affected by a wild fire scenario and the subsequent secondary succession process and b) a patch where cereal crop is substituted by chestnut (*Castanea sativa*) orchards.

Results

Effects of the vegetation variables on the selected indicators

A total of fifteen dependent variables (our arthropod indicators) such as bugs (L Heteroptera, *Table 1*) and eight independent variables, regarding the habitat characterization (e.g. L Vegetation Height, *Table 1*) were considered in the multiple-regression analysis to search for significant relationships between these components of the study system. Depending on the indicator group, the significant associations with the habitat characteristics were variable. The regression equations and their significance for all the combinations performed are listed in *Table 2*.

Table 1. *Specification of the key variables considered in this study*

Variables	*Specification*	**Model Codes for logarithms (L)**
Vegetation variables		
Vegetation height	Meters	L Vegetation Height
Tree stratum height	Meters	L Tree Height
Shrub stratum height	Meters	L Shrubland Height
Herb stratum height	Meters	L Herbaceous Height
Tree stratum cover	Percentage	L Tree Cover
Shrub stratum cover	Percentage	L Shrubland Cover
Herb stratum cover	Percentage	L Herbaceous Cover
Moss stratum cover	Percentage	L Mosses Cover
Arthropod groups		
Acari	Abundance	L Acari
Araneida	Abundance	L Araneida
Chilopoda	Abundance	L Chilopoda
Coleoptera	Abundance	L Coleoptera
Collembola	Abundance	L Collembola
Diplopoda	Abundance	L Diplopoda
Diplura	Abundance	L Diplura
Diptera	Abundance	L Diptera
Heteroptera	Abundance	L Heteroptera
Hymnoptera	Abundance	L Hymnoptera
Isoptera	Abundance	L Isoptera
Lepidoptera	Abundance	L Lepidoptera
Opiliones	Abundance	L Opiliones
Orthoptera	Abundance	L Orthoptera
Thysanoptera	Abundance	L Thysanoptera

Table 2. *The regression equations, degrees of freedom (DF), coefficient of determination (R^2), F-value and their significance level (* P< 0.05, ** P< 0.01 and *** P<0.001) for all the combinations selected as significant by the stepwise multiple regression (n.a., not applicable). The specification of all variables' codes is available in Table 1.*

Equations	**DF**	**R^2**	**F**
Log Araneida = 0.750 – 0.186 Log Vegetation Height	125	0.040	5.21*
Log Acari = - 0.0844 + 0.619 Log Shrubland Height + 0,548 Log Herbaceous Cover	125	0.225	17.88***
Log Opiliones = 0,0298 – 0.0336 Log Vegetation Height	125	0.038	4.93*
Log Chilopoda= 0.0048	125	n.a.	n.a.
Log Diplopoda = 0.0062	125	n.a.	n.a.
Log Lepidoptera = 0.0125 + 0.155 Log Herbaceous Cover – 0.236 Log Mosses Cover	125	0.089	6.01**
Log Thysanoptera = 0.0072	125	n.a.	n.a.
Log Hymenoptera = 0.203 + 0.407 Log Herbaceous Cover – 0.572 Log Mosses Cover + 0.175 Log Tree Height	125	0.108	4.92**
Log Diptera = 0.5339	125	n.a.	n.a.
Log Collembola = 0.936 – 0.760 Log Tree Height + 0.011 Log Shrubland Height + 0.836 log Tree Cover – 0.766 Log Shrubland Cover	125	0.212	8.16***
Log Orthoptera = - 0.0120 + 0,0825 Herbaceous Height	125	0.035	4.5*
Log Coleoptera = 0.601 – 0.416 Log Vegetation Height	125	0.193	29.59***
Log Heteroptera = 0.166 + 0.239 Log Shrubland Height – 0.579 Shrubland Cover + 0.673 Mosses Cover	125	0.071	3.12*
Log Diplura = 0.0048	125	n.a.	n.a.
Log Isoptera = 0.0859 – 0.0801 Log Vegetation Height – 0.250 Log Shrubland Cover + 0.443 Log Mosses Cover	125	0.106	4.83**

Construction of the model and equations

The diagrams of the sub-models presented in the *Fig. 2* are based on (a) the relationships detected in the multiple regression analysis (*Table 2*), (b) the expected succession of the vegetation in this type of patches of north Iberia, highly influenced by anthropogenic disturbances (e.g. Santos et al., 2010), and (c) the SSDA orders aggregation into guilds. The description of the model, equations and source code are shown in *Appendixes 1* and *2*.

Model simulations

The scenarios considered, for demonstration purposes, were based on a possible succession of the habitat structure facing the temporal drifts that could occur in a patch of the studied area. The temporal unit chosen was the year, considered acceptable to simulate the changes and the consequent ecological trends of SSDA throughout a period of 20 years (*Fig. 2c* and *2d*, total taxa abundance and composition, guilds abundance and composition).

The *Fig. 3a* and *3b* show a scenario where a forest patch is affected by fire in the third year, starting a new succession process: (*3a*) the vegetation cover (dominated by the tree cover) is expected to decrease sharply, being afterwards dominated by the herbaceous cover and in the last years by the shrubland cover; (*3b*) the vegetation height has the same pattern of the vegetation cover, changing from a tree dominated area to a herbaceous dominated area which allows a gradual shrubland recovery.

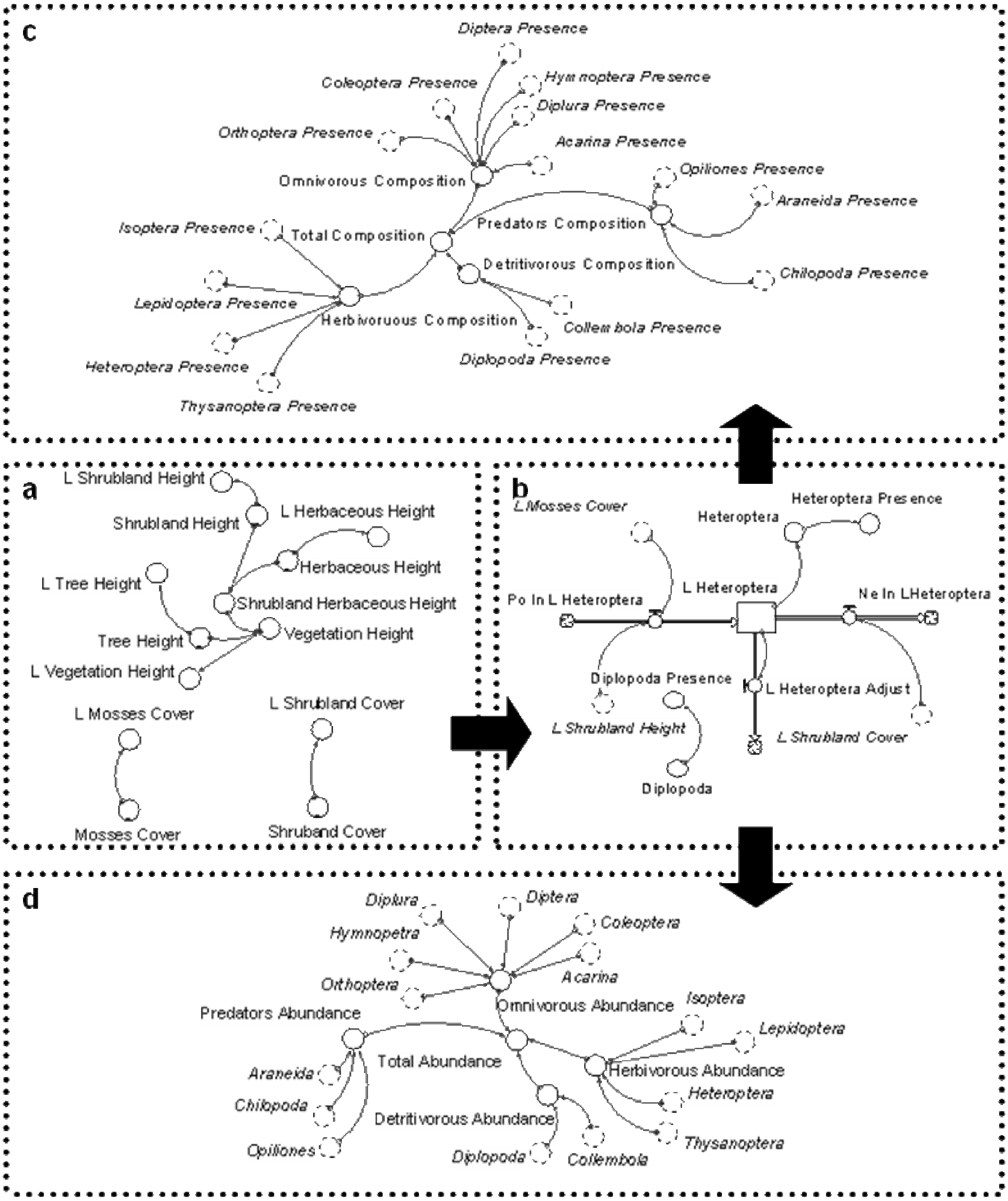


Figure 2. *Conceptual diagram of the sub-models used (a) to simulate the vegetation dynamics, (b) to predict arthropods' dynamics in response to changing vegetation and (c and d) to estimate the guilds' dynamics. Rectangles represent state variables; other variables, parameters or constants are small circles; sinks and sources are cloudlike symbols; flows are thick arrows; all the relations between state variables and other variables are fine arrows. As an example, only the variables associated to the Heteroptera and Diplopoda are represented in the sub-models a and b to illustrate the respective link with the guilds' sub-models c and d. The specification of all variable codes is expressed in Table 1 and in the Appendix 1 and Appendix 2*

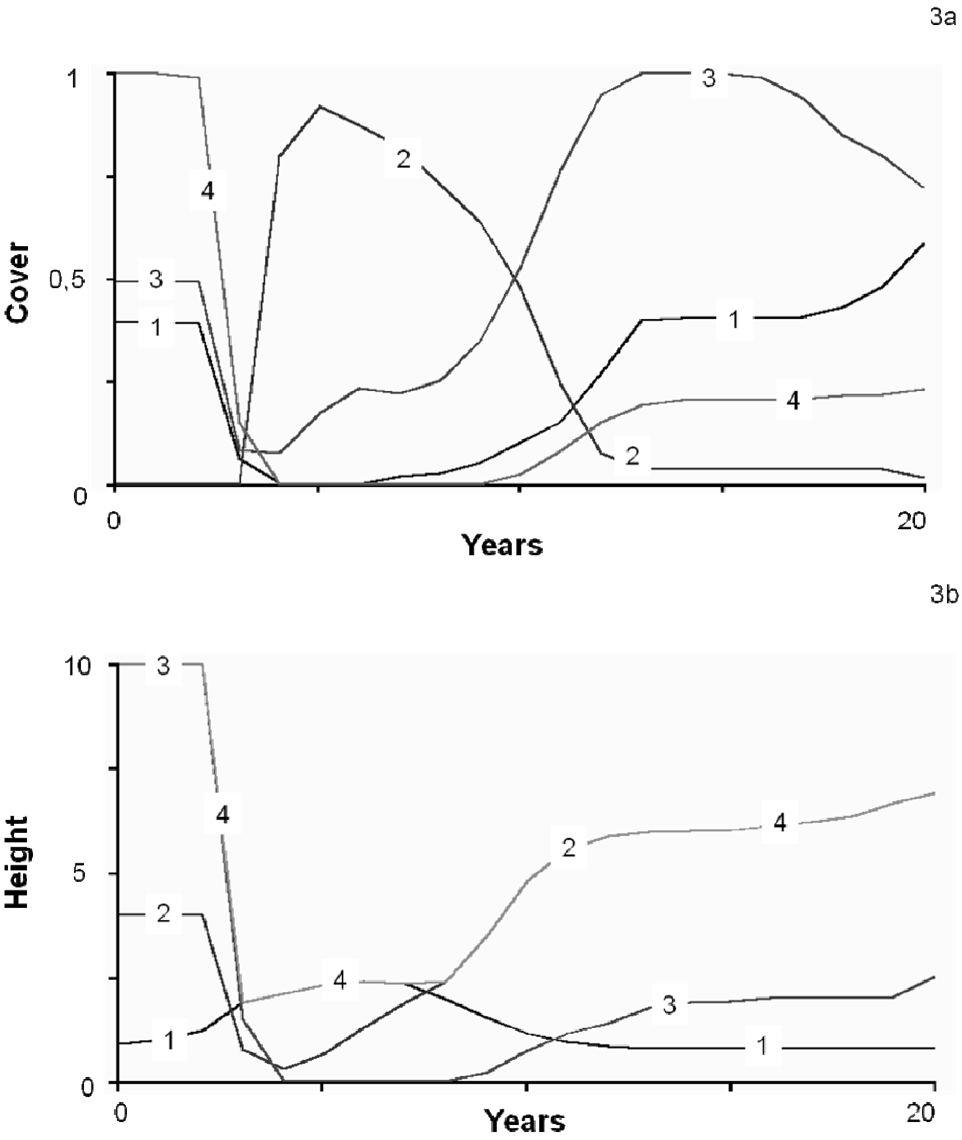


Figure 3. *Simulation of a forest plot is affected by a fire in the third year and the vegetation re-establishes to a shrubland in the subsequent years. Figure and lines explanation: Fig. 3a illustrates the changes in cover by stratum (in proportion), (1) Mosses, (2) Herbaceous, (3) Shrub and (4) Tree; Fig. 3b illustrates the changes in height by stratum (in meters), (1) Herbaceous, (2) Shrub, (3) Tree and (4) Vegetation*

In response to this scenario the simulations of the SSDA guilds are shown in *Fig. 4a and 4b*. In the *Fig. 4a* the abundance of SSDA (guilds and total taxa) is influenced (the variations in abundance are dissimilar) by the vegetation structural changes. Even though total taxa and most guilds respond by increasing their abundance in the post-fire (100%, 500%, 40% and 35%, for Total taxa, Detritivorous, Omnivorous and Predators, respectively) their numbers tend to the pre-fire values as the vegetation structure becomes more complex (approximating pre-fire conditions). The only exception to this increasing trend in post-fire conditions is the Herbivorous guild that clearly reacts with a decline in 35 % in numbers, although shifting to the initial values as the conditions become similar to pre-fire.

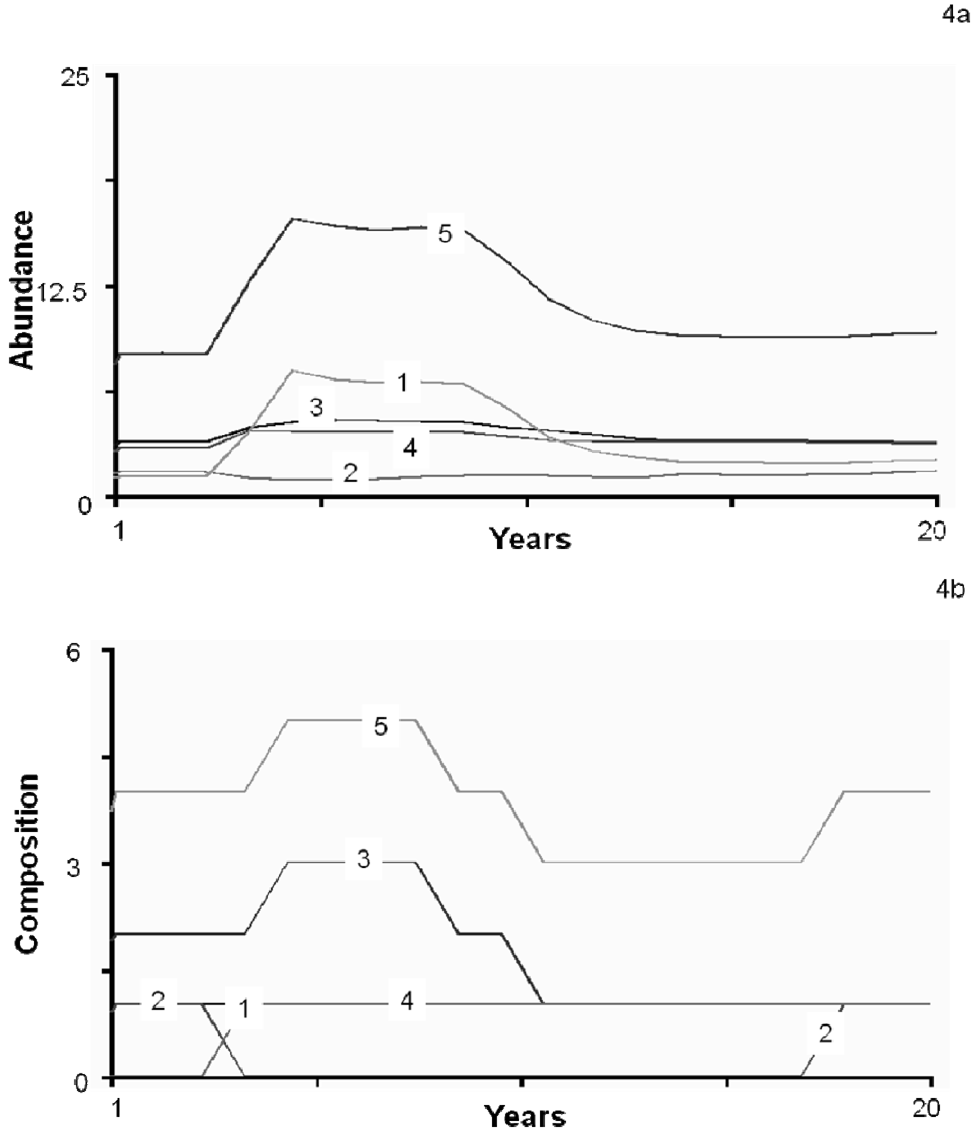


Figure 4. *The response of the indicators (arthropods' guilds abundance and composition and total taxa abundance and composition) to the scenario of figure 3. Figures and lines explanation: Fig. 4a illustrates the changes in abundance of the indicators and Fig. 4b shows the changes in the respective composition, (1) Detritivorous, (2) Herbivorous, (3) Omnivorous, (4) Predators and (5) Total taxa*

The SSDA guilds' composition (or taxa diversity) varies, namely to post-fire conditions and vegetation recover through divergent and alternate trends (*Fig. 4b*). In fact, there is an initial increase for Total taxa and Omnivorous (25% and 50%, respectively) followed by a regular decline (25% and 50%, respectively) (*Fig. 4b*). Other taxa have different responses: the Herbivorous react with an initial decrease (100%) and a subsequent increase and stabilization, the Predators do not change while the Detritivorous increase (0 to 1) (*Fig. 4b*).

The *Fig. 5a* and *5b* illustrate a scenario where a patch of pasture is converted to a Chestnut orchard in the third year: (*5a*) the vegetation cover (dominated by the herbaceous cover) is expected to disappear, being afterwards dominated by the cover of the planted small trees; (*5b*) the vegetation height has the same pattern as the vegetation cover, increasing as a result of the substitution of the herbaceous plants by the trees in a gradient from a herbaceous dominated area to a tree dominated area.

In response to this scenario the simulations of the SSDA guilds are illustrated in *Fig. 6a and 6b*. The *Fig. 6a* shows that the abundance of most guilds decreases gradually, responding to structural changes in the vegetation (76%, 27%, 17%, 28% and 50% for Detritivorous, Predators, Herbivorous, Omnivorous and Total taxa) although with a small increase in the Predators (15%) and Omnivorous (2%) in the planting period. In this scenario, the taxa composition only changes by a decline in the Omnivorous (33%) and Total taxa (20%).

Discussion

The obtained results are in conformity with several studies that tried to identify the disturbance effects on the local communities by using indicator taxa approaches (e.g. Billeter et al., 2008; Gibbons et al., 2009; Sattler et al., 2010). In fact, the response of arthropods to vegetation changes is variable, depending on the type of change, ecosystem and group studied (e.g. Antunes et al., 2008; Hoffmam et al., 2010). The apparent resilience of some guilds may show taxa adaptation to disturbances and low diversity, in agreement to the results obtained in other studies in similar conditions (e.g. Bengtsson, 2002; Moretti et al., 2006; Slocum and Mendelssohn, 2008). On the other hand the idea that the diversity increases as succession proceeds (Martinko et al., 2006) is not corroborated by our simulation results. The obtained results show that the initial phases of succession (dominated by herbaceous layer) seem to contain the most abundant and diverse SSDA communities. Previous studies have demonstrated that coexisting taxa partition their resources and/or that the compositional similarity between communities is determined by environmental factors, lending support to the niche-assembly model. However, no attempt has been made to investigate whether the amount of resources that reflects relative niche space controls relative taxa abundance and composition in communities (Schweiger et al., 2005). Here, we demonstrate that the relative abundance and composition in patches is significantly related to the relative structure of the vegetation (biomass). Since the abundance and composition of the SSDA guilds is determined by the habitat structure, the simulations allow a better perception of the biodiversity consequences related to vegetation structural changes. Therefore, the proposed methodology should be considered as a complementary tool in landscape management, by using indicators within the “data space” of the environmental gradients monitored in particular systems, such as our case-study in anthropogenic habitats of North Portugal.

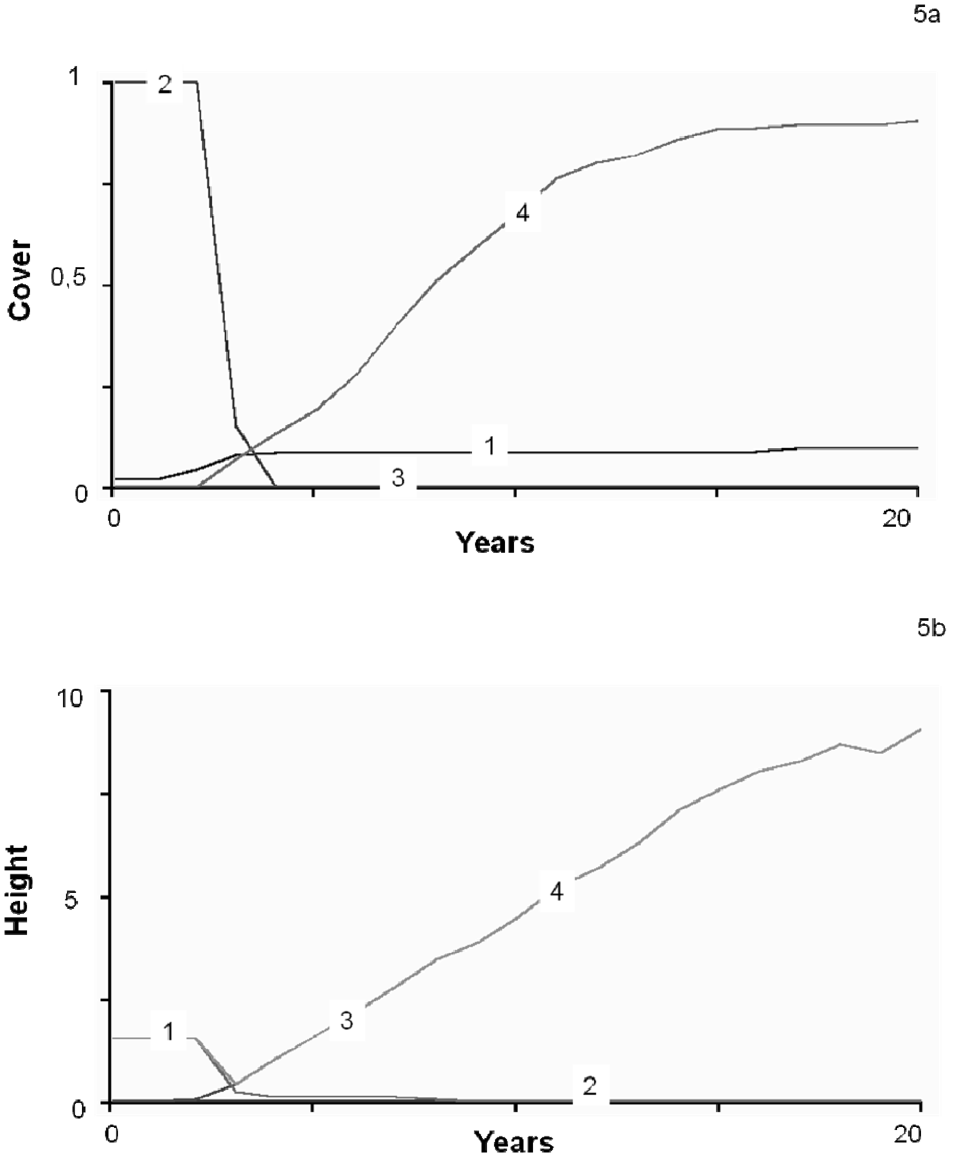
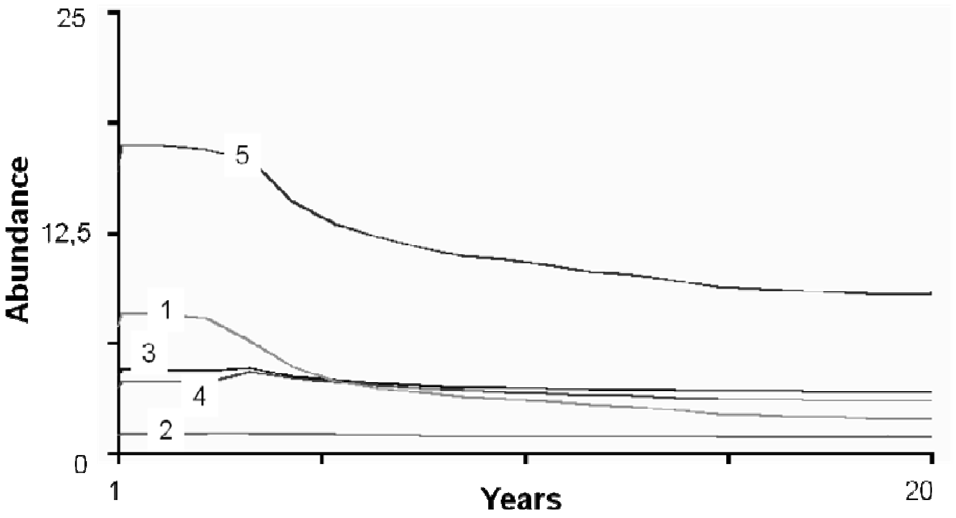


***Figure 5**. Simulation of a scenario where a pasture is substituted in the third year by a Chestnut orchard that evolves in the subsequent years. Figure and lines explanation: Fig. 5a illustrates the changes in cover by stratum (in proportion), (1) Mosses, (2) Herbaceous, (3) Shrub and (4) Tree; Fig. 5b illustrates the changes in height by stratum (in meters), (1) Herbaceous, (2) Shrub, (3) Tree and (4) Vegetation*

6a



6b

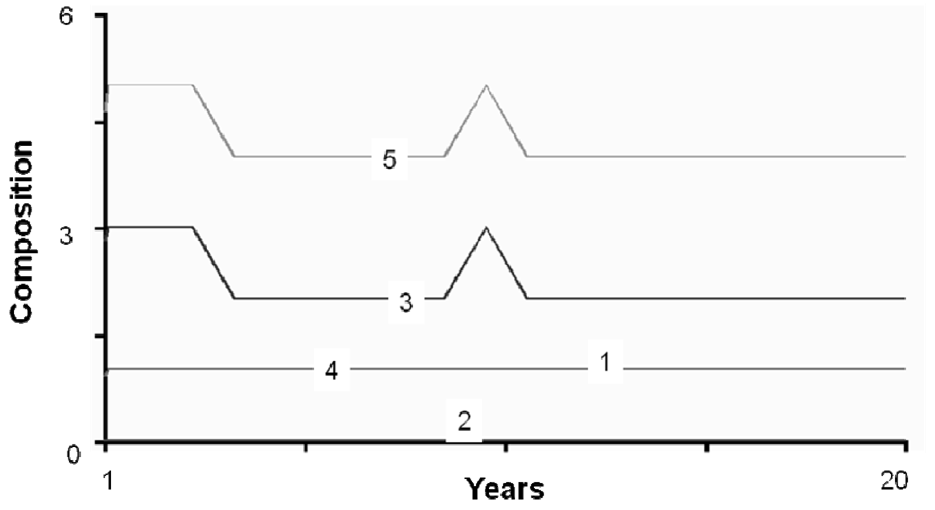


Figure 6. *The response of the indicators (arthropods' guilds abundance and composition and total taxa abundance and composition) to the scenario of figure 5. Figures and lines explanation: Fig. 6a illustrates the changes in abundance of the indicators and Fig. 6b shows the changes in the respective composition, (1) Detritivorous, (2) Herbivorous, (3) Omnivorous, (4) Predators and (5) Total taxa*

As with any ecological modelling procedure (Jørgensen, 2001), the complexity of a StDM model is determined by the problem, the choice of the key-components in the studied ecosystem and the available data. One of the most important requirements identified in the StDM is linked to the quality of the data-base, crucial for the performance of the model outputs. The central requirements of the StDM data set recorded includes: (1) a large amount of data, because in the regression analysis the slope is chosen so that the sum-of-squares distance between each data point and the fitted line is minimised (Sokal and Rolf, 1995) and (2) the pertinent spatial and temporal gradients of changes, in order to represent the relevant attributes of the scenarios selected and to allow realistic simulations of the indicator's responses, given that in regression analysis the limits of the Y prediction are imposed by the extreme points of the fitted line (Waite, 2000; Steele et al., 2005). Other criteria from stepwise regression, such as Akaike selection criteria (AIC) (Akaike, 1974) and Mallows Cp (Mallows, 1973) could be used for choosing the most parsimony model to be incorporated in a StDM approach.

Models produced in the form of rules, based on machine learning approaches, are transparent and can be easily understood (Mendonza and Prabhu, 2005). The structure of such models should be straightforwardly interpretable in order to allow the incorporation of pertinent qualitative data before the simulations (Dale and Beyler, 2001; Lancelot et al., 2009). The StDM exhibits these structural qualities but provides also simple, suitable and intuitive outputs, easily interpreted by non-experts (ranging from students to senior policy makers). Another goal when developing methodologies for assessing changes in the ecological integrity of systems is the feasibility of application and extent to which the results can be applied in other areas, taxa and problems (Andreasen et al., 2001; Duelli and Obrist, 2003). In fact, despite the limitations inherent to an academic demonstration, the protocol proposed is expeditious and easily applicable to other types of databases dealing with ecosystems affected by gradients of changes (Santos, 2009). Although conceptually simple, our StDM protocol captures the complexity of some holistic ecological trends, including realistic temporal and spatial gradients of environmental characteristics, which allowed the simulation of structural changes when habitat and environmental conditions are substantially changing due to anthropogenic-induced alterations (Cabral et al., 2008). Since the biodiversity of the patch communities can be only partly assessed by SSDA indicators diversity and composition, this approach also provides a useful starting point, allowing the precise development of more complicated models, with introduction of other indicators, interactions and interferences (such as the trophic relationships) with precise applicability conditions.

The ultimate goal is to produce simulation models that allow the creation of landscape dynamics from changes is patches, basis of spatially explicit biodiversity assessments (Muzy et al., 2005). Therefore, we believe that our approach will provide the development of techniques in the scope of landscape ecology by creating expeditious interfaces with Geographic Information Systems, which will make the methodology more instructive and credible to decision makers and environmental managers (Costanza, 1992; Santos and Cabral, 2004).

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Appendix 1

The *Fig. 2a* shows part of the sub-model diagram that intends to predict the vegetation succession in different deterministic ecological circumstances (only the vegetation characteristics related to *Fig. 2b* taxon are exposed). Considering that the forecast of vegetation succession highly depends on local characteristics (Santos et al 2010) and for an easier construction of the model, the dynamics of vegetation cover and height (e.g. Shrubland Cover, Shrubland Height) were introduced in the model as table functions (*Fig. 2a, Appendix 2* –Table functions), based on the average values for each type of vegetation of the database. The vegetation variables were logarithm transformed for a compatible integration in the balances of the state variables (*Fig. 2a* and *Fig. 2b*, *Appendix 2* – Associated variables). This transformation (e.g. L Shrubland Cover) was incorporated because the data required for the state variables balances should have the same units used to obtain the partial regression coefficients, assumed as holistic ecological parameters (see Methods). Therefore, only logarithms of the environmental variables are acceptable in the inflows and outflows of the state variables (*Fig. 2b* and *Appendix 2* – Difference equations and Process equations). In *Fig. 2b*, the sub-model diagram attempts to predict the response of each taxon to the changes that take place in the selected plot (the figure only shows Heteroptera as an example, for other taxon details see *Appendix 2*). The independent variables were the logarithms of the vegetation characteristics considered (e.g. L Shrubland Cover) (*Fig. 2a*, *Appendix 2* – Associated variables) and the selected state variables were the logarithms of the arthropod dependent variables (e.g. L Heteroptera) (*Fig. 2b, Table 1*). The initial values for these state variables were assumed to be zero (our initial situation in t0) (*Appendix 2,* Process equations). Later, for simulations representation, the initial value was discarded, since only in t1 (the first point of the simulation) it was possible to take into account the influences of the vegetation on the estimates. The processes that affect the state variables are described by difference equations (*Appendix 2*, Difference equations). The inflows (e.g. Po In L Heteroptera) affecting the state variables (e.g. L Heteroptera) were based on positive constants and all positive partial coefficients resulting from the previous multiple regression analysis (*Table 1*, *Fig. 2b, Appendix 2* – Difference and Process equations). On the other hand all the state variables were affected also by outflows (e.g. Ne In L Heteroptera) related to the negative constants and partial regression coefficients influences (*Table 1, Fig. 2, Appendix 2* – Difference and Process equations). Although the output for each taxon simulated is composed of a given value per time unit, the respective state variable could have a cumulating behaviour over time in response to changes in the vegetation conditions. Thus, to avoid this, an additional outflow adjustment was incorporated in each state variable (e.g. L Heteroptera Adjust). These outflow adjustments aimed to empty the state variables in each time step, by a “flushing cistern” mechanism, before beginning the next step with new environmental influences (*Fig. 2b* and *Appendix 2* – Difference and Process equations). For process compatibilities and a more realistic comprehension of the model simulations, some conversions were introduced, denominated associated variables (*Fig. 2b* and *Appendix 2* – Associated variables). Regarding the selected taxon, these conversions were obtained through an inverse transformation (anti-logarithmic), which transforms logarithms into the original measurement units (e.g. Heteroptera). Therefore, the model is prepared to accept and transform real data from the variables and to convert logarithmic outputs from specific estimation back into the original units. All taxa whose results were indifferent to the independent variables used in the multiple regression analysis (*Table 2*) were introduced in the model in averaged values (e.g. Diplopoda) and the limit values for their variation are determined in accordance with realistic ranges, such as the respective standard error (*Fig. 2b*). These limits imposed maximum and minimum values for each stochastic variable, included in the model as random functions (*Fig. 2* and *Appendix 2* – Stochastic variables). In order to transform taxon abundance into a binomial presence/absence register (e.g. Heteroptera Presence), a logical equation was inserted (*Fig. 2* and *Appendix 2* – Other variables). In *Figs. 2c* and *2d* the sub-model diagrams aims are to forecast the response of the macro indicators, the SSDA guilds, to the structural changes occurring in the vegetation. The indicators selected were: the SSDA guilds composition (*Fig. 2c*, number of taxa) (e.g. Herbivorous Composition) and the SSDA guilds abundance (*Fig. 2d*, number of individuals) (e.g. Herbivorous Abundance). Additionally, the contributions of these groups were considered for the total SSDA abundance and composition estimates (Total Abundance and Total Composition). These variables result from simple mathematical operations between the previous explained associated, stochastic and other variables, called composed variables (*Figs. 2c* and *2d*, *Appendix 2* – Composed variables).

Appendix 2

Mathematical equations used in Stella for the relationships between indicator estimates and the vegetation characteristics. The specification of variable codes is expressed in Table 1.

EQUATIONS
State variable equations
L_Acarina(t) = L_Acarina(t - dt) + (Po_In_L_Acarina - Ne_In_L_Acarina - L_Acarina_Adjust) * dt
L_Araneida(t) = L_Araneida(t - dt) + (Po_In_L_Araneida - Ne_In_L_Araneida - L_Araneida_Adjust) * dt
L_Coleoptera(t) = L_Coleoptera(t - dt) + (Po_In_L_Coleoptera - Ne_In_L_Coleoptera - L_Coleoptera_Adjust) * dt
L_Collembola(t) = L_Collembola(t - dt) + (Po_In_L_Collembola - Ne_In_L_Collembola - L_Collembola_Adjust) * dt
L_Heteroptera(t) = L_Heteroptera(t - dt) + (Po_In_L_Heteroptera - Ne_In_LHeteroptera - L_Heteroptera_Adjust) * dt
L_Hymnoptera(t) = L_Hymnoptera(t - dt) + (Po_In_L_Hymnoptera - Ne_In_L_Hymnoptera - L_Hymnoptera_Adjust) * dt
L_Isoptera(t) = L_Isoptera(t - dt) + (Po_In_L_Isoptera - Ne_In_L_Isoptera - L_Isoptera_Adjust) * dt
L_Lepidoptera(t) = L_Lepidoptera(t - dt) + (Po_In_L_Lepidoptera - Ne_In_L_Lepidoptera - L_Lepidoptera_Adjust) * dt
L_Opiliones(t) = L_Opiliones(t - dt) + (Po_In_L_Opiliones - Ne_In_L_Opiliones - L_Opiliones_Adjust) * dt
L_Orthoptera(t) = L_Orthoptera(t - dt) + (Po_In_L_Orthoptera - Ne_In_L_Orthoptera - L_Orthoptera_Adjust) * dt
Process equations
(a) INIT L_Acarina = 0 Po_In_L_Acarina = 0.619* L_Shrubland_Cover + 0.548* L_Herbaceous_Cover Ne_In_L_Acarina = 0.085 L_Acarina_Adjust = L_Acarina
(b) INIT L_Araneida = 0 Po_In_L_Araneida = 0.749 Ne_In_L_Araneida = 0.185*L_Vegetation_Height L_Araneida_Adjust = L_Araneida
(c) INIT L_Coleoptera = 0 Po_In_L_Coleoptera = 0.601 Ne_In_L_Coleoptera = 0.416*L_Vegetation_Height L_Coleoptera_Adjust = L_Coleoptera
(d) INIT L_Collembola = 0 Po_In_L_Collembola = 0.936+ 0.011*L_Shrubland_Height + 0.836*L_Tree_Cover Ne_In_L_Collembola = 0.760*L_Tree_Height+0.766*L_Shrubland_Cover L_Collembola_Adjust = L_Collembola
(e) INIT L_Heteroptera = 0 Po_In_L_Heteroptera = 0.166+0.239 *L_Shrubland_Height+0.673*L_Mosses_Cover Ne_In_LHeteroptera = 0.579*L_Shrubland_Cover L_Heteroptera_Adjust = L_Heteroptera
(f) INIT L_Hymnoptera = 0 Po_In_L_Hymnoptera = 0.203 + 0.407* L_Herbaceous_Cover + 0.175 *L_Tree_Height Ne_In_L_Hymnoptera = 0.572*L_Mosses_Cover L_Hymnoptera_Adjust = L_Hymnoptera
(g) INIT L_Isoptera = 0 Po_In_L_Isoptera = 0.0859+0.443*L_Mosses_Cover Ne_In_L_Isoptera = 0.0801*L_Vegetation_Height+0.250*L_Shrubland_Cover L_Isoptera_Adjust = L_Isoptera
(h) INIT L_Lepidoptera = 0

Po_In_L_Lepidoptera = 0.0125 + 0.155 *L_Herbaceous_Cover
Ne_In_L_Lepidoptera = 0.236*L_Mosses_Cover
L_Lepidoptera_Adjust = L_Lepidoptera

(i) INIT L_Opiliones = 0
Po_In_L_Opiliones = 0.0298
Ne_In_L_Opiliones = 0.0336*L_Vegetation_Height
L_Opiliones_Adjust = L_Opiliones

(j) INIT L_Orthoptera = 0
Po_In_L_Orthoptera = 0.0825*L_Herbaceous_Height
Ne_In_L_Orthoptera = 0.012
L_Orthoptera_Adjust = L_Orthoptera

Associated variables

Acarina = 10^(L_Acarina)
Araneida = 10^(L_Araneida)-1
Coleoptera = 10^(L_Coleoptera)-1
Collembola = 10^(L_Collembola)-1
Heteroptera = 10^(L_Heteroptera)-1
Hymnopetra = 10^(L_Hymnoptera)-1
Isoptera = 10^(L_Isoptera)-1
Lepidoptera = 10^(L_Lepidoptera)-1
Opiliones = 10^(L_Opiliones)-1
Orthoptera = 10^(L_Orthoptera)-1
L_Herbaceous_Cover = LOG10(Herbaceous_Cover+1)
L_Herbaceous_Height = LOG10(Herbaceous_Height+1)
L_Mosses_Cover = LOG10(Mosses_Cover+1)
L_Shrubland_Cover = Log10(Shruband_Cover+1)
L_Shrubland_Height = LOG10(Shrubland_Height+1)
L_Tree_Cover = LOG10(Tree_Cover+1)
L_Tree_Height = LOG10(Tree_Height+1)
L_Vegetation_Height = LOG10(Corrected_Vegetation_Height+1)

Stochastic variables

Chilopoda = normal(0.2,0.01)
Diplopoda = normal(0.2,0.01)
Diplura = normal(0.2,0.01)
Diptera = normal(1.37,0.01)
Thysanoptera = normal(0.2,0.01)

Other variables

Acarina_Presence = If Acarina >= 1 then 1 else 0
Araneida_Presence = If Araneida >= 1 then 1 else 0
Chilopoda_Presence = If Chilopoda >= 1 then 1 else 0
Coleoptera_Presence = If Coleoptera >= 1 then 1 else 0
Collembola_Presemce = If Collembola >= 1 then 1 else 0
Diplopoda_Presence = If Diplopoda >= 1 then 1 else 0
Diplura_Presence = If Diplura >= 1 then 1 else 0
Diptera_Presence = If Diptera >= 1 then 1 else 0
Heteroptera_Presence = If Heteroptera >= 1 then 1 else 0
Hymnoptera_Presence = If Hymnopetra >= 1 then 1 else 0
Isoptera_Presence = If Isoptera >= 1 then 1 else 0
Lepidoptera_Presence = If Lepidoptera >= 1 then 1 else 0
Opiliones_Presence = If Opiliones >= 1 then 1 else 0
Orthoptera_Presence = If Orthoptera >= 1 then 1 else 0
Thysanoptera_Presence = If Thysanoptera >= 1 then 1 else 0

Composed variables
Corrected_Vegetation_Height = If Tree_Height < Vegetation_Height then Vegetation_Height else Tree_Height
Detritivorous_Abundance = Collembola+Diplopoda
Detritivorous_Composition = Collembola_Presemce+Diplopoda_Presence
Herbivorous_Abundance = Heteroptera+Isoptera+Lepidoptera+Thysanoptera
Herbivoruous_Composition = Heteroptera_Presence+Isoptera_Presence+Lepidoptera_Presence+Thysanoptera_Presence
Omnivorous_Abundance = Acarina+Coleoptera+Diplura+Diptera+Hymnopetra+Orthoptera
Omnivorous_Composition = Acarina_Presence+Coleoptera_Presence+Diplura_Presence+Diptera_Presence+Hymnoptera_Presence+Orthoptera_Presence
Predators_Abundance = Araneida+Chilopoda+Opiliones
Predators_Composition = Araneida_Presence+Chilopoda_Presence+Opiliones_Presence
Total_Abundance = Herbivorous_Abundance+Omnivorous_Abundance+Predators_Abundance+Detritivorous_Abundance
Total_Composition = Herbivoruous_Composition+Omnivorous_Composition+Predators_Composition+Detritivorous_Composition
Vegetation_Height = If Herbaceous_Height > Shrubland_Height then Herbaceous_Height else Shrubland_Height
Table functions
Herbaceous_Cover = GRAPH(time) (0.00, 1.00), (1.05, 1.00), (2.11, 1.00), (3.16, 0.00), (4.21, 0.00), (5.26, 0.00), (6.32, 0.00), (7.37, 0.005), (8.42, 0.00), (9.47, 0.00), (10.5, 0.00), (11.6, 0.00), (12.6, 0.00), (13.7, 0.00), (14.7, 0.00), (15.8, 0.00), (16.8, 0.00), (17.9, 0.00), (18.9, 0.00), (20.0, 0.00)
Herbaceous_Height = GRAPH(time) (0.00, 1.50), (1.05, 1.50), (2.11, 1.50), (3.16, 0.00), (4.21, 0.1), (5.26, 0.1), (6.32, 0.1), (7.37, 0.1), (8.42, 0.00), (9.47, 0.00), (10.5, 0.00), (11.6, 0.00), (12.6, 0.00), (13.7, 0.00), (14.7, 0.00), (15.8, 0.00), (16.8, 0.00), (17.9, 0.00), (18.9, 0.00), (20.0, 0.00)
Mosses_Cover = GRAPH(time) (0.00, 0.02), (1.05, 0.02), (2.11, 0.045), (3.16, 0.085), (4.21, 0.085), (5.26, 0.085), (6.32, 0.085), (7.37, 0.085), (8.42, 0.085), (9.47, 0.085), (10.5, 0.085), (11.6, 0.085), (12.6, 0.085), (13.7, 0.085), (14.7, 0.085), (15.8, 0.085), (16.8, 0.095), (17.9, 0.095), (18.9, 0.095), (20.0, 0.095)
Shruband_Cover = GRAPH(time) (0.00, 0.00), (1.05, 0.00), (2.11, 0.00), (3.16, 0.00), (4.21, 0.00), (5.26, 0.00), (6.32, 0.00), (7.37, 0.00), (8.42, 0.00), (9.47, 0.00), (10.5, 0.00), (11.6, 0.00), (12.6, 0.00), (13.7, 0.00), (14.7, 0.00), (15.8, 0.00), (16.8, 0.00), (17.9, 0.00), (18.9, 0.00), (20.0, 0.00)
Shrubland_Height = GRAPH(time) (0.00, 0.00), (1.05, 0.00), (2.11, 0.00), (3.16, 0.00), (4.21, 0.00), (5.26, 0.00), (6.32, 0.00), (7.37, 0.00), (8.42, 0.00), (9.47, 0.00), (10.5, 0.00), (11.6, 0.00), (12.6, 0.00), (13.7, 0.00), (14.7, 0.00), (15.8, 0.00), (16.8, 0.00), (17.9, 0.00), (18.9, 0.00), (20.0, 0.00)
Tree_Cover = GRAPH(time) (0.00, 0.00), (1.05, 0.00), (2.11, 0.00), (3.16, 0.08), (4.21, 0.145), (5.26, 0.205), (6.32, 0.31), (7.37, 0.45), (8.42, 0.55), (9.47, 0.625), (10.5, 0.73), (11.6, 0.8), (12.6, 0.8), (13.7, 0.8), (14.7, 0.8), (15.8, 0.8), (16.8, 0.8), (17.9, 0.8), (18.9, 0.8), (20.0, 0.805)

IMPLICATIONS FOR SHALLOW LAKE MANIPULATION: RESULTS OF AQUARIA AND ENCLOSURE EXPERIMENTS MANIPULATING MACROPHYTES, ZOOPLANKTON AND FISH

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Abstract. The Massaciuccoli Lake, a 700 ha shallow lake, located in the Migliarino San Rossore Massaciuccoli Natural Park, along the Tuscan coast (Italy), has undergone a dramatic reduction of the macroalgal and submerged macrophytic communities over the last fifty years. This reduction was both in terms of the number of species and abundance, leading in recent years to a turbid water-phytoplankton dominated state and to the disappearance of submerged macrophytes. The aim of this study was to investigate, in aquaria experiments, the minimum threshold of light, necessary for the survival of a macrophyte, *Myriophyllum verticillatum* L., caused by sediment resuspension. Subsequently enclosure experiments were carried out with biomanipulation techniques (such as the removal of the fish community, the inoculation of zooplankton, *Daphnia magna* Strauss and forced reintroduction of the macrophyte) in enclosures. The results showed that, currently, the PAR intensity at the bottom of the lake is not sufficient to allow spontaneous recovery of the submerged vegetation. The positive effect of biomanipulation, due to fish removing plankton and phytoplankton-grazing, may lead to a significant clarification of the water column in the treated enclosures, allowing radication and growth of submerged vegetation. Nevertheless, an effective recovery of Massaciuccoli Lake is not likely to be achieved until an integrated approach (ranging from the reduction of the pollutant sources to partial dredging of the sediment, because of the high nutrient levels), is performed.

Keywords: *restoration, shallow lakes, aquatic macrophytes, biomanipulation, enclosures*

Introduction

The number of aquatic ecosystems contaminated by anthropogenic-derived pollutants is at present increasing. Human activity adds excessive amounts of plant nutrients (primarily phosphorus, nitrogen, and carbon) to streams and lakes in various ways. Runoff from agricultural fields and breeding, industrial chemicals, urban lawns and domestic sewage, are the principal sources of these nutrients. This enrichment (notably in N and P) above natural levels is traditionally defined as eutrophication. This "nutrient pollution" problem represents the most important water quality problem worldwide (Harper, 1992; Lau et al., 2001). The enrichment causes an increase in the net primary productivity of ecosystems and an associated decline of water quality. Compared with deep-water lakes, shallow lakes tend to be more prone to eutrophication as they have a smaller dilution capacity (Lennox, 1984).

Changes in nutrient loading are probably a major reason why the stability properties of shallow lakes have changed. At low nutrient levels the system has one globally stable equilibrium, a clear-water state. If the nutrient level is raised further the stability of the clear state decreases, and slight perturbations are enough to cause a switch to the turbid equilibrium. These nutrients also proved to be powerful stimulants to an excessive algal growth, or “blooms” of algae. The algal blooms led to competitive interactions with submerged plants that are largely disappearing (Moss et al., 1996a). Submerged aquatic macrophytes are important because they stabilize the macrophytes-dominated state by increasing water transparency and the environmental quality of shallow lakes (Carpenter and Lodge, 1986; Jeppesen et al., 1998; Scheffer, 1998). Macrophytes reduce nutrient levels and sediment resuspension, they provide shelter for zooplankton and young fish and a habitat for macro-invertebrates. Macrophytes also permit the physical stabilization of the bottom and can excrete allelopathic substances that inhibit the growth of other photoautotrophs, such as epiphyton and phytoplankton (Scheffer et al., 1993; Lauridsen et al., 1996; Petrini et al., 1996; Scheffer, 1998; Van Donk and Van de Bund, 2002; Mulderij et al., 2003; Mulderij et al., 2005; Mulderij et al., 2007). Submerged plants affect their environment, but obviously the environment also has an effect on the plants. This positive feedback is thought to cause alternative stable states in shallow lakes. So a lake can be in one state or another within a certain range of nutrient concentrations. This phenomenon is important for lake management as the shift from turbid to clear water is expected to occur at much lower nutrient levels than the opposite shift, a process called “hysteresis” (Scheffer et al., 1993).

Restoration of eutrophic lakes in their turbid water-phytoplankton dominated state, presents several difficulties and the most important is associated with the long term-phosphorus release from the sediments (Jeppesen et al., 1990; Pitt et al., 1997; Søndergaard et al., 2002). Due to this phenomenon, many attempts at restoring eutrophic lakes by means of reduction of the nutrient loading, have given unsatisfactory results, since high values of phosphorus concentration in the water column have been observed for long periods following the intervention (Scheffer et al., 1993; Phillips et al., 1994). Moreover, in many cases, nutrient removal from sources such as very large lakes can be too expensive, and nutrient loading control from agricultural areas can be difficult since there is not usually a restricted source.

These difficulties in restoration caused the efforts of researchers to focus on the use of biomanipulation techniques as an alternative and more controllable tool (Drenner and Hambright, 1999). Biomanipulation was introduced as a lake-restoration technique that focused on trophic-cascade top-down effects (Shapiro et al., 1975). Many articles confirm the positive effects of biomanipulation (Jeppesen et al., 1990; DeMelo et al., 1992; Meijer et al., 1994; Hansson et al., 1999; Drenner and Hambright, 1999; Meijer et al., 1999). The idea is to control fish and zooplankton communities (substantial reduction of the overwintering planktivorous and benthivorous fish stock that causes large filter-feeding zooplankton). This action exerts a higher grazing pressure on phytoplankton, thus forcing a spring clear water phase. Reduction of benthivorous fish further supports the clearing of the lake, because it reduces the resuspension of the sediment and the release of nutrients in the water due to these bottomfeeders (Breukelaar et al., 1994; Havens, 1993). Clear water, during spring, allows the submerged vegetation to grow and creates a stable clear water state (Hosper and Meijer, 1993; Hosper, 1997; Hosper et al., 2005). The achievement of a total macrophyte recovery actions would be required on several fronts, ranging from the drainage of the sediment to the reduction of the nutrient charge (Berklioglu et al., 1999; Meijer et al., 1999).

In this paper we relate to Massaciuccoli Lake, an eutrophic shallow lake in Central Italy where a constant and progressive reduction of the macroalgal and submerged macrophytic community (such as *Chara spp*., *Potamogeton pectinatus* L., *Potamogeton* *crispus* L., *Ceratophyllum demersum* L., *Utricularia vulgaris* L., *Myriophyllum verticillatum* L., *Elodea canadensis* Michaux) has been shown to occur over the last 50 years (Brunelli and Cannicci, 1942; Pedreschi, 1956). Over the last 10 years a continuous reduction of submerged macrophytes has occurred (Tomei et al., 1997) which resulted in only two species remaining (*M. verticillatum* and *E. canadensis*) which have now recently disappeared (Ciurli et al., 2008). Extensive research was carried out in this lake to identify the turbid water state and eutrophication conditions (Ceccarelli et al., 1997; Cuppen et al., 1997). The turbidity of water does not provide sufficient irradiation for plants at the lower levels of the water column (Ciurli and Alpi, 1999). Turbidity is due to several factors: 1) suspension of minute particles brought about by the erosion of agricultural soils located all around the lake (Pensabene et al., 1997), 2) the phytoplankton-dominated state due to the excess of nutrients, brought about by emissaries (Mason, 1997), 3), sediment re-suspension by crayfish, Procambarus clarckii Girard (Gherardi, 1999; Barbaresi and Gherardi, 2001), 4) loss of cohesion of the bottom due to the absence of radicated plants (Schutten et al., 2005).

In the current paper we investigated the macrophytes recovery potential, using the model species *M. verticillatum* L., through in vitro tests (tests in aquaria) and in vivo tests, practicing biomanipulation techniques in enclosures/exclosure experiments in a lake. We have chosen this plant because, together with *E. canadensis*, it has been the last to disappear. *M. verticillatum*, shows the best capacity to colonise the aquatic environment due to its tendency toward lengthening, and generally showed more remarkable results than *E. canadensis* in reducing nutrient content in water, particularly regarding nitrogen, showing phytodepuration capacity (Ciurli et al., 2008).

The final aim of the research was to obtain evidence for the possibility of the recovery of aquatic macrophytes, and for their important role in the equilibrium of the lake.

Considering that the ultimate goal is the reintroduction of macrophytes in the lake, the intermediate objective is to understand how such plants could grow in lake water. The working hypotheses are: 1) to assess the feasibility of the growing macrophyte *M. verticillatum*, in aquaria filled with water and sediment lake. 2) characterize, in aquaria, the minimum threshold of light necessary for the survival of the macrophyte. 3) try to carry out the biomanipulation, in enclosures, not accompanied by P load reduction. The results obtained by testing of the above-mentioned hypotheses could be important for the forced reinsertion of submerged vegetation in the whole lake after the causes of degradation have been removed.

Materials and methods

The model species was *M. verticillatum* L., collected in a small stream flowing into the lake in the “Piaggetta” zone, where submerged vegetation is still present. Scions were collected by cutting the apical part of the plant, and were carefully washed to remove mud and algae before placing them into the propagation aquaria.

In vitro test

Aquaria preparation

Two aquaria systems were prepared: as plant propagation (to obtain more working material), as investigation about the minimum survival light threshold of *M.*

verticillatum, caused by resuspension of sediment (to perform turbidity tests). Five propagation aquaria of 200 l capacity (with dimensions 40 x 100 x 50 cm) were prepared, with a layer (10 cm) of aqualite (Aqualine®) and an upper layer (5 cm) of fine gravel filled with tap water. The filtration system consisted of a pump with a submerged filter, with a 400 l flow per hour. The lighting plant was composed, for each aquarium, of two fluorescent Dennerle lamps (Trocal 3085, 30 W) with reflecting parabola, connected with a timer set on 10 hours of light per day. The temperature was kept at 18-20°C with a 150 W immersion heater. The plant scions did not show transplant shock and were periodically trimmed to keep them at a constant length of about 30 cm.

Aquaria experiment

Each aquarium had three replicates and the experiments were repeated many times in one year of experimentation in the laboratory. This was possible because the experiments were carried out in growth chamber in controlled conditions. The aquaria used for the turbidity tests were smaller (25 × 30 × 33 cm) with a 30 l capacity, totally covered with black film to stop natural light. They were filled with water and sediment collected in three different parts of the lake: Central Lake (CL), Foce Barra (FB) and Centro Chiaro (CC) whose sediment chemistry is reported in *Table 1*. The samples of water and sediment were put into opaque plastic containers to protect them from the natural light, and directly brought to the laboratory for the preparation of the aquaria. Chemical analyses (ammoniacal, nitrous, nitric and total nitrogen, orthophosphate and total phosphorus) of these waters and sediments were performed according to the procedures indicated in (APHA, 1998) in order to put the results in correlation with different plant growth responses.

10 plant scions per aquarium (corresponding to the density of 80 unit m^{-2}, data obtained from experimental preliminary tests) of 10 cm length (obtained from the pruning of propagation aquaria plants) were placed at the bottom of the aquaria. The lighting system for each aquarium consisted of one single fluorescent Dennerle lamp, connected to a timer set on 10 hours of light per day. Aquaria were totally covered with black film to stop natural light. The irradiation in each aquarium was measured daily at 15 cm depth using an aquatic Quantometer Radiation Sensor (Li-Cor) in order to determinate the PAR (Photosynthetically Active Radiation). Since our intention was to simulate the natural lake conditions, different light intensities, in aquaria, were artificially reproduced through constant lighting (except for the control in the dark) and through sediment re-suspension (instead of artificial shading) using pumps which raised the sediment. The pump was placed with the rotor near the bottom in order to prevent disturbance of the water-air interface and so avoid uncontrolled extra-oxygenation of the water column, and detached sufficiently to prevent shading from silt on the leaves. The five cases were: 1) aquarium completely dark (dark control, corresponding to PAR of the lake bottom 3 m), 2) maximum turbidity (corresponding to 60-50 cm depth), 3) medium turbidity (50-30 cm depth), 4) minimum turbidity (30-15 cm) and 5) no turbidity (light control, with the pump switched off) (*Table 2*). Extra care was taken in placing the pumps to prevent the water flux from causing mechanical stress to the plants. Height and fresh and dry weight were measured for every plant at every treatment, at the beginning and at the end of the trial, which lasted 4 weeks.

Table 1. *Water and sediment chemistry of the three different sampling sites of Massaciuccoli Lake (* the values were under the lower limit of determinability)*

		Centro Lago	**Foce Barra**	**Centro Chiaro**
NO_2^--N (µg/l)	**Water**	10.36	11.48	2.94
	Sediment	5.07	7.12	4.96
NO_3^--N (µg/l)	**Water**	26.18	37.24	21.42
	Sediment	30.27	71.80	40.05
NH_3^-N (µg/l)	**Water**	129.92	173.46	37.80
	Sediment	119.00	75.40	120.23
Total N (µg/l)	**Water**	3647.00	2262.26	696.08
	Sediment	7.68	7.93	20.39
PO_4^--P (µg/l)	**Water**	<6.2*	<6.2*	<6.2*
	Sediment	51.73	48.61	142.42
Total P (µg/l)	**Water**	52.39	81.84	41.85
	Sediment	780.35	819.68	1161.20

Description of site studied

The Massaciuccoli Lake, with an extension of 700 ha, is located in the northern part of the Migliarino San Rossore Massaciuccoli Natural Park, 10 km north of Pisa (Italy), lat. 43° 50’ 14”, long. 10° 21’ 39” (*Fig. 1*). The lake and the surrounding wetlands cover about 2700 ha of the territory of the Natural Park. They represent the widest wetland area along the Tuscan Coast and they have obtained national and international importance for their flora and fauna communities. The lake is located 4 km from the sea, 0 meters above sea level, and it is classified as a shallow lake as its average depth is 2.5 – 3 m (Scheffer, 1998).

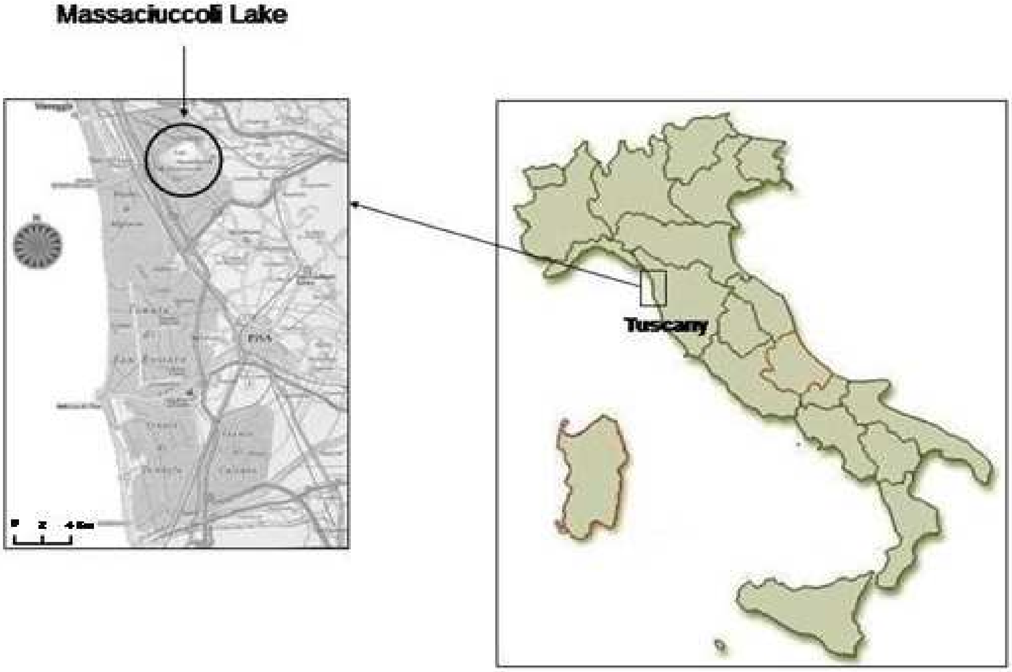

Figure 1. *Location of the experimental area*

In vivo tests:enclosure/exclosure experiments

It was not possible to carry out a project involving biomanipulation, whether or not accompanied by P load reduction, in the whole of the shallow lake due to poor financing, thus an attempt at biomanipulation was made in enclosures. Nine cylindrical enclosures, 3 m high and with a diameter of 6m, indicated as A1, 2, 3, B1, 2, 3, C1, 2, 3, were placed in an area of the lake where the water depth is about 2 m, called Punta Grande (PG). The enclosures were open cylinders of small-meshed plastic net (350 μm diameter), so as to prevent zooplankton from escaping to the outside, but at the same time enable water to flow freely. Their upper part was fixed to a floating plastic ring, protruding 50 cm out of the water in order to prevent the entrance of fish and crayfish. The lower parts of the enclosure were fixed to a heavy chain, sunk into the sediment for more then one meter, so that the mesocosmos were totally isolated. Fish were removed in winter from all the enclosures with an electrostunner. A trap was placed in every enclosure and controlled weekly to capture the possible fish or crayfish. Afterwards, in Spring, the zooplankton inoculum (about 5000 individuals of *D. magna*) was added to the enclosure A replicates; the inoculum had been raised in a rain-water basin in the Department of Crop Biology. The pre-existing zooplankton community in the water of Centro Chiaro was considered not influential because of its scarcity (Baldaccini et al., 1997). Zooplankton was periodically monitored through collection with a tube (180 × 10 cm diameter) by filtering 14 l of water through a plankton net (mesh size 100 μm); samples were preserved in a solution of 40 g l^{-1} sucrose and 4% formaldehyde (Haney and Hall, 1973) until counting. The experimental scheme consisted in the application of biomanipulation in the three A replications, no experiments in the three B replications, which represented enclosure controls, and reinsertions of plants without biomanipulation in C replications, which represented the biomanipulation controls.

Zooplancton communities in the A enclosure replicates showed marked changes in water transparency compared to the open lake water (exclosure) and to B and C replicate enclosures, even if the lake bottom view in enclosures was not achieved. Plants were placed in A enclosure and C enclosure replicates in June, when in the three A enclosure replicates (where biomanipulation had been made) the value of Secchi depth was 70 cm, light intensity was 600 μmol s^{-1} m^{-2} and chlorophyill-a was 3 μg l^{-1} (see results). 15 cm scions of M. verticillatum, taken from the above-mentioned propagation aquaria, were tied to 0.5 m^2 nets with 1 cm mesh. The nets with the plants were maintained floating in water at three different depth levels: water surface, 30 cm and 1.5 m of depth. To keep the depth the nets were tied to a floating cork on top and to a lead weight at the bottom. Different light levels, the same as in the aquaria were obtained by the placement of the macrophyte scions at different depths in the water column in enclosures where biomanipulation techniques had been applied (*Table 3*). The plant scions at each level were 30. Height and dry weight of the macrophytes were measured at the beginning and at the end of the trial as in the laboratory experiment.

During the experimental period from May to August the following elements were measured: pH (portable membrane pH-meter, model HI 8314, Hanna Instruments), electrical conductivity (conducimeter model HI 8733, Hanna Instruments), dissolved oxygen and temperature (OX 22, Aqualytic), transparency (Secchi disc); PAR (Photosynthetically Active Radiation) irradiation (Quantometer Radiation Sensor, Li-Cor), chlorophyll-a (measured through spectrophotometric way). Samples were taken from the central part of Massaciuccoli Lake (three replicates for each sample), inside each enclosure and exclosure (APHA, 1998).

Measurements were performed around 12:00 h, at the hour of the day associated with maximum irradiation. The aquaria test lasted two years and started one year before experiments in the lake. The preparation before plants were planted was about 1 month, and the enclosure/exclosure was replicated for three years.

Statistical analyses

Two-way Anova with interaction was used to test for differences between water-sediment combinations and between light regimes in the turbidity experiment, in which the average of the ten plants of each aquarium was considered as one replicate; a test for heteroschedasticity was run to confirm the homogeneity of variance among the different aquaria. One-way Anova was used to test for differences between different depth levels in the enclosures experiment, followed by Tukey’s Honestly Significant Difference test. Significance in the differences between the trends of chemical-physical parameters was investigated by comparing 95% C.I. error bars referred to the repeated measurements.

Statistical analyses were run with R© software (R Development Core Team, 2005), graphical elaboration was made with Graph Pad©.

Table 2. *Five cases of different turbidity levels in the aquaria, obtained by changing the pump flow. The light intensity equivalent to 0 was obtained maintaining the aquarium completely dark without lamp, the other values of light intensity were obtained using one lamp per aquarium but with different turbidity levels*

Turbidity level	Light intensity (µmol s^{-1} m^{-2})
Completely dark	0
Maximum turbidity	20
Medium turbidity	30
Minimum turbidity	36
No turbidity	54

Results

Aquaria experiments

The comparison between the growth of *M. verticillatum* in the three different water-sediment systems shows very highly significant differences in growth responses depending on the sampling site ($p < 0.001$, one-way ANOVA) (*Fig. 2*). Plants grown on sediment and water from Centro Chiaro (CC) reached the greatest heights even at the lowest light levels (20 µmol s^{-1} m^{-2}), because of lengthening. If we consider the accumulation of dry mass, the best results were obtained by plants grown on the Centro Lago (CL) system (water and sediment), mainly at the highest light levels. Plants grown on the Foce Barra (FB) system represent an intermediate situation between the two former ones, showing generally lower growth levels.

Different light levels are also associated with highly significant different growth levels ($p < 0.01$). In general, plants show the best growth results not only at maximum, but also at medium light levels (30 – 36 µmol s^{-1} m^{-2}), giving reason to hope for a good recovery potential also in field experiments. The effect of the combination of light regime and type of water and sediment is highly significant too in causing different growth responses ($p < 0.01$).

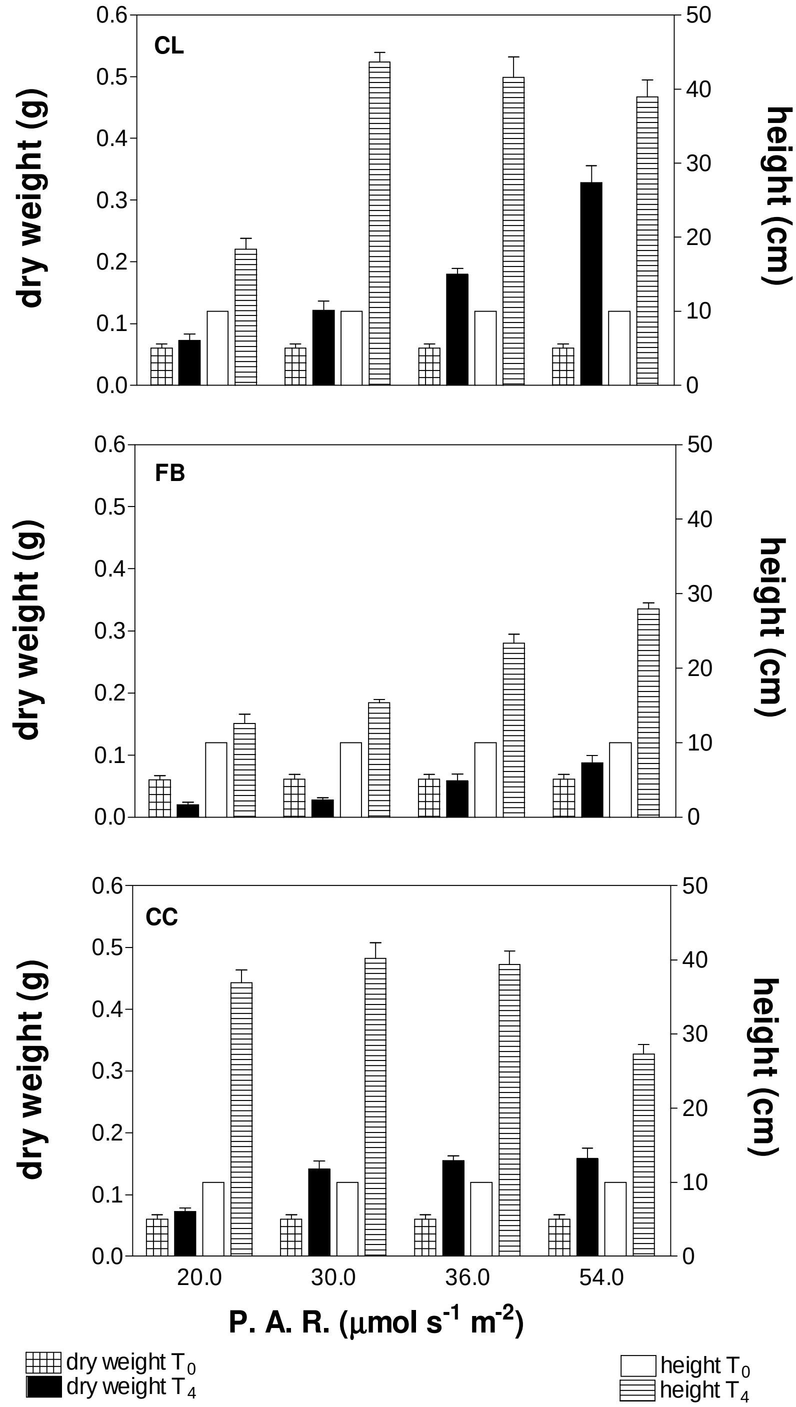

Figure 2. *Growth performances (dry weight and height) of Myriophyllum verticillatum L. in water and sediment systems from three different sites of the Lake (named Centro Lago, CL; Foce Barra, FB; Centro Chiaro, CC) at different irradiation levels, at the beginning (T_0) and at the end (T_4) of the experiment, after four weeks. In T0 the height of all the plants was 10 cm and the dry weight 0,06 g as it is possible to observe in the figure. The bars represent the mean and standard error out of three replicates of ten plants each*

Enclosure experiments

At the end of the experimentation, plants from three A enclosure replicates showed an average 50 % rate of survival at 1.5 m depth, 100 % at 30 cm and 100 % above the surface (*Fig. 3*); plants from enclosures C (without biomanipulation) had all disappeared.

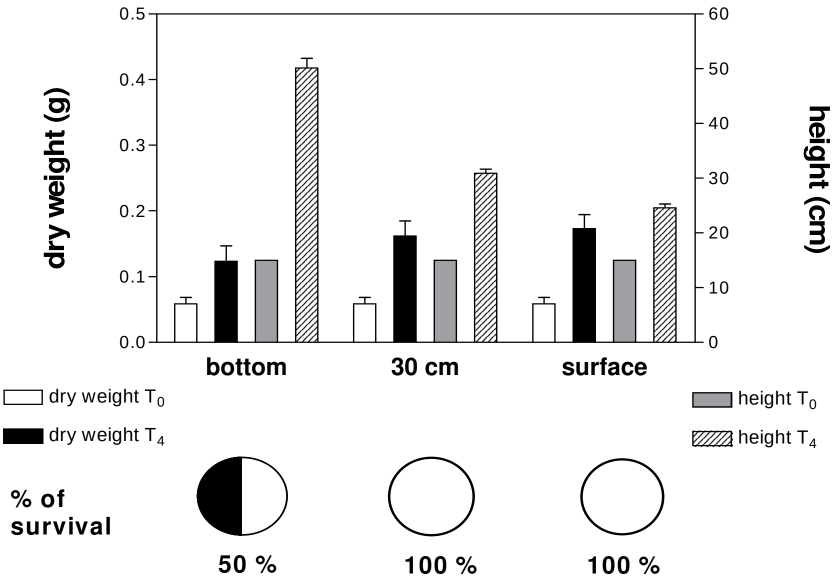

Figure 3. *Average growth and % survival of plants grown in three enclosure A replicates at different depth levels: bottom of the lake (1,5 m of depth, 30 cm of depth and surface of the water). The bars represent the mean and standard error out of thirty plants replicated twice*

In A enclosure replicates, plant scions at 1.5 m reached the highest values of height, while the minimum height was shown by the scions above the surface. Very highly significant differences ($p < 0.001$, one-way ANOVA) of height increases were related to the different depth levels; Tukey's test indicated the pair surface-1.5 m as the source of maximum diversity (p=1,0*10-7). On the contrary, plants grown at 1.5 m showed minimum dry biomass production, with significant differences ($p < 0.05$) compared to plants at the surface.

Secchi depth and PAR irradiation (measured at 30 cm depth) remarkably differed among the three sets of enclosures and the two open water sampling sites (Punta Grande and Centro Lago, exclosure) (*Fig. 4*). In A replicates, in particular, Secchi depth and PAR irradiation increased sharply during the first two months, and decreased in the second part of the experiment. The maximum clarification of water was reached in A replicates at the end of June, when Secchi depth was about 70 cm and PAR irradiation was 600 µmol m^{-2} s^{-1}; these values successively dropped down to 35 cm and 300 µmol m^{-2} s^{-1}, respectively, at the end of August. In B and C enclosure replicates the values of Secchi depth and PAR irradiation were significantly lower than in A replicates ($p < 0.001$), the transparency fluctuating between 30 and 50 cm in B and between 40 and 55 cm in C; the light intensity was between 250 and 350 µmol m^{-2} s^{-1} in B and between 250 and 400 µmol m^{-2} s^{-1} in C. In Punta Grande and Centro Lago these parameters showed even lower values.

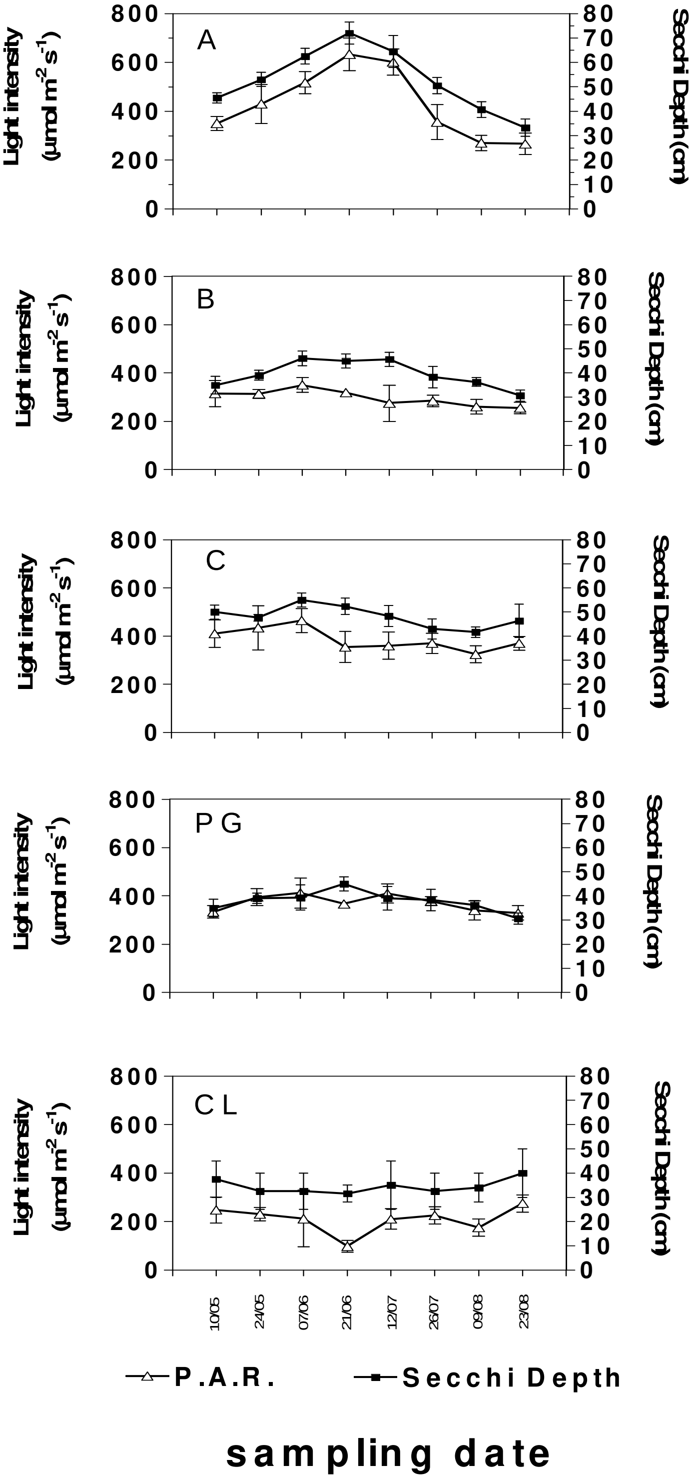

Figure 4. *Trends of Photosynthetic Active Radiation (PAR) and Secchi depth in the three replicated enclosures of A, B, C and two open water sampling sites: "Punta Grande" (PG), "Centro Lago" (CL)*

The chlorophyll-a content followed the opposite tendency regarding Secchi depth and PAR irradiation. At all the sampling sites chlorophyll decreased during the first two months, to rise again starting from the end of June (*Fig. 5*). A enclosure replicates showed chlorophyll-a values which were constantly lower than the other stations. The differences of the chlorophyll-a values in A replicates as to B replicates and A replicates as to C replicates were very highly significant ($p < 0.001$, Tukey's test), and significant between A replicates and Centro Lago (CL) ($p < 0.01$) and A replicates and Punta Grande ($p < 0.05$).

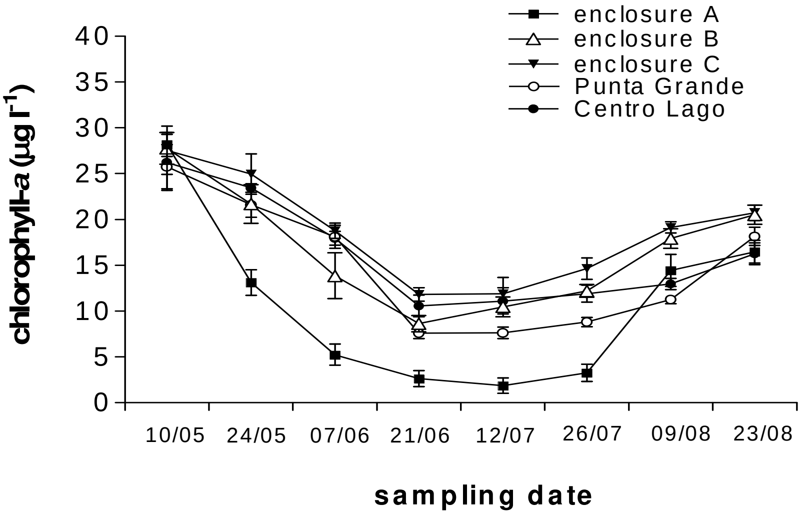

Figure 5. *Trends of clorophyll-a content in enclosure replicates water of the five sampling sites; the water analyses were carried out twice a month from May to August. Data are the averages out of three replicates*

The state of PAR radiation in A replicates, shown in *Fig. 6*, was measured at different depths along the water column. PAR intensity on the surface was constant during the whole trial. The values at 30 cm depth showed a similar trend to those of Secchi depth and opposite to those of chlorophyll-a, starting from 300 µmol m^{-2} s^{-1}, reaching a maximum of 600 µmol m^{-2} s^{-1} at the end of June and dropping at the end of the experiment to values lower than at the beginning. The PAR radiation at 50 cm was less variable, and at the lowest levels it remained almost flat, showing no correlation with the clarification of the upper layer.

The physical and chemical parameters of water followed trends that were quite similar among the five stations (*Fig. 7*), with the exception of water pH in Centro Lago (CL), which was constantly higher than the other sites during the whole year.

Discussion

The in vitro experiment showed different growth responses at the same light levels according to the different medium used. These results are meaningful if we explain them on the basis of the chemical analyses of water and sediments from the different locations: in fact the water and sediment chemistry of Centro Chiaro (CC) and Centro Lago (CL) are contrasting (*Table 1*). Water from CC shows the lowest nutrient levels, while its sediment is the richest one, especially for total N (20.39 µg l-1 in water against

696.08 μg l^{-1} in sediment). Since CC is a closed shallow area surrounded by close emerged vegetation, the low nutrient levels of its water are in accordance with the action of phytodepuration performed by botanical associations which are typical of peaty soils (Farahbakhshazad et al., 2000; Farahbakhshazad and Morrison, 2003), e.g. *Phragmites australis* (Cav.) Trin., *Cladium mariscus* L., *Typha angustifolia* L.

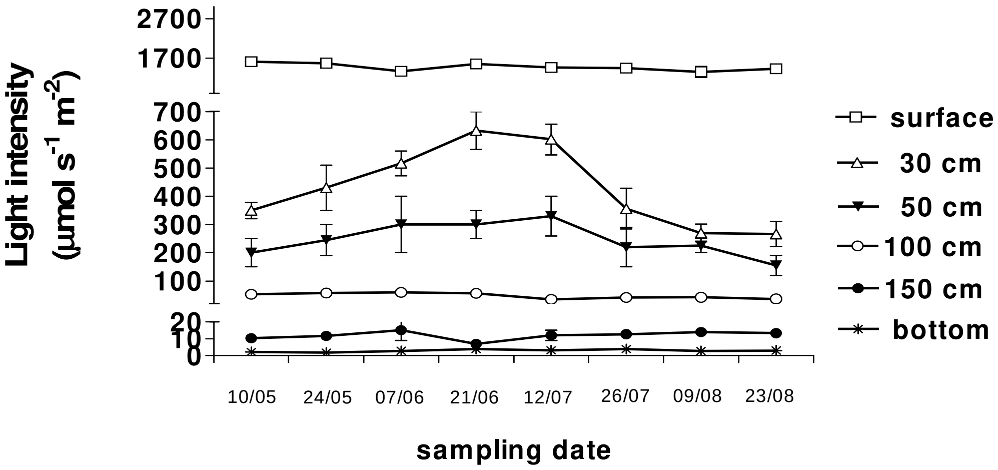

Figure 6. *Intensity of PAR radiation at different depths in A enclosure replicates; the analyses were carried out twice a month from May to August*

On the other hand, the high nutrient levels of the Centro Chiaro sediment are due to the accumulation of organic matter deriving from the degradation of plant residues which naturally sink to the bottom, as well as the organic matter deriving from the submerged vegetation which was present until some years ago. This shows that the different chemistry causes different growth responses at the same turbidity levels (Bachmann et al. 2002).

The higher dry matter content in plants grown in waters with higher nutrient levels Centro Lago can be evidence of a preferential foliar absorption acted by submerged plants (Brinx, 1997; Wen-Yuan et al., 2004); in fact, the lengthening of plants in the Centro Chiaro, water and sediment, at low light intensities is a symptom of stress. Moreover, since the availability of inorganic carbon has been shown to have a major effect on the interrelationship between light intensity and photosynthesis of submerged macrophytes (Van den Berg et al., 2002), further studies should be carried out to correlate information about pH, alkalinity, calcium content and bicarbonate concentrations of water and sediments with the substantial differences in macrophyte growth when incubated in sediments taken from different parts of a lake.

Considering jointly the results of the two in vitro experiments provides evidence that, at present, the PAR intensity along the water column of the lake is not sufficient to allow the existence of submerged vegetation, particularly near the bottom, where rooting of macrophytes occurs. This phenomenon is due to the turbidity of the lake, caused by a resuspension phenomenon due to the absence of vegetation and by an excessive amount of phytoplankton consequent to eutrophication (Pensabene et al., 1997; Mason, 1997).

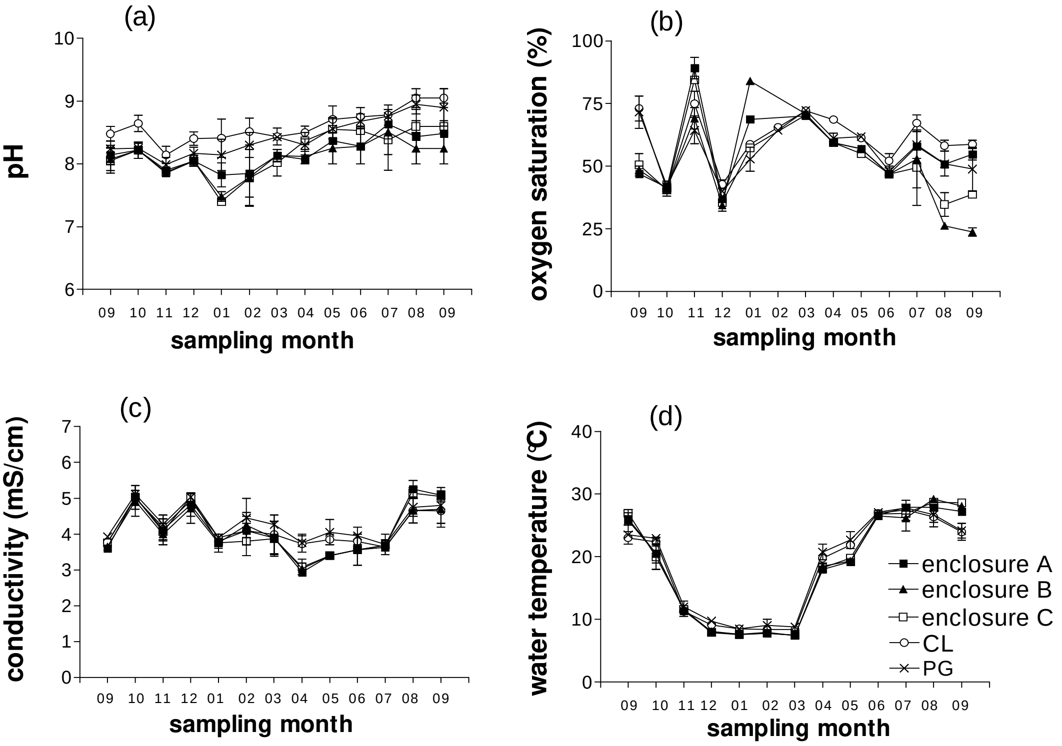

Figure 7. *Trends of pH (a), oxygen saturation (b), conductivity (c) and water temperature (d) at the investigates sites of the Lake, during one year from September to September The months are indicated in abscissa*

In the aquaria experiments the different levels of irradiation were simulated at known chemical-physical conditions of water and sediment; the results indicated that the optimal light conditions for growth are, at present, available at about 30 cm of depth in the lake (*Table 2*). Since the average depth is 2.5 – 3 m, this means that the present conditions cannot allow spontaneous recovery of submerged vegetation. According to this evidence it can be seen in what way these values could be reached at the bottom, allowing plant recovery, if higher penetration of light through the water column could be achieved.

The in vivo experiments, showed the positive effect of biomanipulation on the growth of our model species, which was able to grow even at 1.5 m depth, although the scarceness of light present caused abnormal lengthening. The increase in length combined with a low weight increase at low light conditions indicates shoot elongation to be a valid strategy activated by *M. verticillatum* to compensate conditions of light scarcity. This indicates *M. verticillatum* as being particularly fit for the primary colonization of the bottom of turbid lakes (Ciurli et al., 2008). This species provides a first clarification of the water column and allows the subsequent setting of plants such as *Elodea canadensis*, which in virtue of their tendency to form dense carpets could provide a stabilization of the bottom in the medium-long term (Ciurli et al., 2008).

We retain that the grazing effect carried out by *D. magna* (data not shown) together with fish removal caused the phytoplankton population to drop, leading to a remarkable clarification of water in the treated enclosures; evidence of such an effect of clarification carried out by zooplankton has been provided also by other authors

(Perrow et al., 1994, 1997; Darchambeau et al., 2005). The decline over time of the effect of *D. magna*, in terms of water clarification, can find an explanation in its seasonal dynamics, especially in the contest of a forced predator-prey system (Scheffer et al., 1997). The disappearance of plants from C enclosure replicates, even on the surface and at 30 cm depth, provides evidence of an actual difference between in vitro an in vivo conditions, and suggests the concomitant effect in the lake of an additional factor contrasting plant growth, such as an excessive development of epiphyton due to the lack of organisms capable of grazing it in the space of the enclosure (Brönmark, 1985). This factor appears to be fully counterbalanced by D inoculation. Nevertheless, an effective recovery of Massaciuccoli Lake cannot be obtained until an integrated approach, ranging from the reduction of pollutant sources to partial dredging of the sediment, is adopted. The constant nutrient charge, and the long term phosphorus release from the sediment, are in fact a serious obstacle in returning the lake to a clear water state.

Several authors (Van der Does et al., 1992; Kleeberg and Kohl, 1999; Meijer et al., 1999; Søndergaard et al., 2001) have shown the benefits of (partial) dredging the sediment and biomanipulation projects accompanied by P load reduction to allow the recovery of submerged vegetation. Further long term experiments will be conducted, in particular by removing part of the sediment from inside the enclosures, in order to demonstrate the negative effect of long term nutrient release. Plastic enclosures will be used to prevent the surrounding water from affecting the phosphorus concentration in the above standing water.

Table 3. *Mean light intensities (over the year) at different depths in the water column of Massaciuccoli*

Water depth (cm)	**Light intensity (µmol s^{-1} m^{-2})**
Surface	120
15	54
30	30
50-60	20
100	2
150	0
250-300	0

Conclusions

The aquaria experiments, with water and sediment coming from three different parts of the lake, show the feasibility of macrophyte *M verticillatum* to grow in controlled conditions.

The minimum threshold of light necessary for the survival of the macrophyte has been characterized in aquaria with sediment resuspension to simulate the natural lake conditions. This device permitted us to reproduce the turbidity at different depth levels, using water and sediment lake.

The mesocosms represented by the enclosures placed in the lake give a realistic, small scale simulation of what can happen on a larger scale if systematic intervention of macrophytes reintroduction were performed. Being *M. verticillatum* the last one to disappear, its partial recovery in the experimental context can represent, on a whole-lake scale, the first step to provide adequate conditions for the recovery of the other macrophytic species, in order to restore the equilibrium in the lake ecosystem.

The present situation can not allow spontaneous recovery of aquatic macrophytes because of the insufficient amount of light reaching the bottom; nevertheless, conditions compatible with plant life can be achieved on the medium period if combined interventions aimed at the clarification of the water column are put into action.

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THE STUDY OF *NODULARIA SPUMIGENA* BLOOM EVENT IN THE SOUTHERN CASPIAN SEA

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Abstract. Despite the increase in phytoplankton population in the Caspian Sea, there is few detail study regarding bloom of some species in the recent years. Previous studies have announced the bloom-forming Cyanophyta *Nodularia spumigena* Mertens to the Caspian Sea. In this study, we attempt to understand the bloom events that are involved *Nodularia spumigena* population and environmental parameters in the Iranian coastal water in 2009. The preliminary results suggested that *Nodularia spumigena* observed in the Caspian Sea in some seasons at different depths but bloom formation starting from middle of summer and reach a maximum in the early of autumn at the surface. Result of this study also showed that phytoplankton assemblage comprised of 46 species after bloom. Bacillariophyta had the highest number of species (17) follow by Pyrrophyta (14), Cyanophyta and Chlorophyta (6) and Euglenophyta (3). But, phytoplankton species of bloom sample is classified in three groups: Cyanophyta, Pyrrophyta and Bacillariophyta which Cyanophyta dominated over the other groups of algae and formed more than 98% and 96% of phytoplankton abundance and biomass, respectively. As of now, the pattern of dominant and frequent species in the Caspian Sea indicated that the health of the water body and aquatic organisms are at risk.

Keywords: *Phytoplankton, Cyanophyta, bloom, Caspian Sea, Iran*

Introduction

Algal blooms that adversely affect environmental, plant, or animal health are referred to as harmful algal blooms (HABs) (Backer, 2002). HABs occur in freshwater, marine, and estuary systems and are quickly becoming a public health issue. CyanoHABs are primarily associated with surface scums from blue-green algae or Cyanophyta. There are about 150 known genera of Cyanophyta, 40 of which are known to be toxic (Saker et al., 1999). The primary toxin-producing Cyanophyta include *Anabaena, Aphanizomenon, Cylindrospermopsis, Microcystis, Nodularia* and *Planktothrix (Oscillatoria).*

The first report of toxic Cyanophyta bloom in the world was recorded in lake Alexandrina, Australia as early as in the 1800s (Francis, 1878). The Cyanophyta *Nodularia spumigena* Mertens (*N. spumigena*) is a filamentous, heterocystous, nitrogen-fixing species known to thrive in brackish and saline waters. There are several reports related to *Nodularia* bloom in fresh water lake of Turkey (Akcaalan et al., 2009), estuaries of Australia (Blackburn et al., 1996), brackish lakes of Australia and New Zealand (Heresztyn and Nicholson, 1997; Woodward and Shulmeister, 2005), saline lakes and lagoons of USA (Beutel et al., 2001; Galat et al., 1990), Mexico (Falcon et al., 2002), Uruguay (Perez et al., 1999) and Baltic Sea (Sivonen et al., 1989; Kahru et al., 1994; Stal et al., 2003; Mazur-Marzec et al., 2006). In September 2005, researchers of the Caspian Environment Programme were informed by the Department of Environment of the Gilan Province (I.R. Iran) of the occurrence of Anomalous Algal Bloom (AAB) in the southern of Caspian Sea with affected area as wide as 20,000 km^2 (Soloviev, 2005). This phenomenon repeated within a short period of time in coastal waters of the southwestern of Caspian Sea in 2009 as reported by the Ecological Aquatic Center of the Caspian Sea (EACCS). In 2010, *N. spumigena* was again bloomed in early August in the southern of Caspian Sea (unpublished data).In the southern of Caspian Sea, phytoplankton composition during bloom and the early hours after bloom as well as factors favor the bloom of *N. spumigena* have not been fully established yet. In this paper, we attempt to study phytoplankton structure (abundance and composition) and environmental parameters at few hours after bloom. In addition, phytoplankton composition during bloom and meteorological parameter (wind) adopted from satellite which effected on spread of bloom was investigated.

Table 1. *Longitude and latitude of Tonekabon station in the southern of Caspian Sea*

Stations	Longitude	Latitude
A (5m)	50° 54’ E	36° 49’ N
B (10m)	50° 54’ E	36° 49’ N
C (20m)	50° 55’ E	36° 50’ N
D (50m)	50° 57’ E	36° 53’ N
E (100m)	50° 59’ E	36° 56’ N

Materials and methods

Sampling and environmental parameters

The Caspian Sea Investigation of hydrology and hydrobiology cruise was planned in 2009 in the southern of Caspian Sea. This cruise was carried out aboard the vessel, R/V *Gilan*. Water samples (at few hours after bloom) were collected at five sampling stations (A to E) within the 0-100 m depth along a transect perpendicular from the Tonekabon coast at 5 m (Station A), 10 m (Station B), 20 m (Station C), 50 m (Station D) and 100 m (Station E) interval (*Table 1* and *Fig. 1*). At each sampling station, physico-chemical parameters were determined within the 0-100 m depth: A(0), B(0), B(10), C(0), C(10), C(20), D(0), D(10), D(20), D(50) and E(0), E(10), E(20), E(50), E(100) with figure in bracket showing the sampling depth in meter at each station. Temperature was determined using an inverse thermometer and a salinometer (Model GM65, Russian) was used to measure the salinity levels and pH meter (WTW 320, Germany) used to measure the pH of water sample. Water samples were analyzed for nutrients concentration manually with spectrophotometer in the laboratory. Nitrate and nitrite were determined by reduction column and the standard pink azo dye method and ammonia by the hypo-phenol oxidation-blue dye method (DIN=NO_2^-, NO_3^- and NH_4^+); phosphate (DIP) and silicate (DSi) were determined by the standard molybdenum blue and yellow method as suggested by APHA (2005). Digestion of samples for the determination of total nitrogen (TN) and total phosphorus (TP) were done following the persulphate digestion procedure of Valderrama (1981). DON was calculated as the difference between total nitrogen and DIN concentrations and DOP was calculated as the difference between total phosphorus minus phosphate concentration (Yurkovskis, 2004).

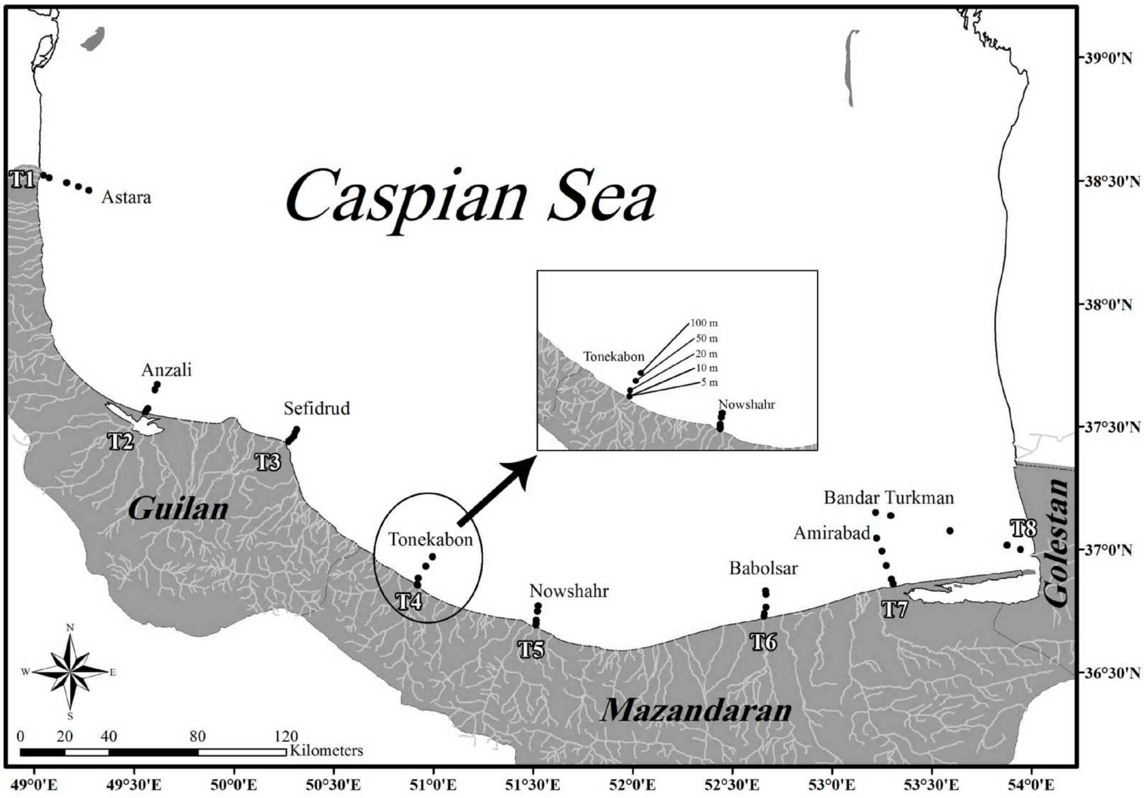

Figure 1. *Map of the southern Caspian Sea showing locations and depths of sampling points along the Tonekabon transects*

Phytoplankton analysis

Phytoplankton samples (at few hours after bloom) were collected using a 1.6 liters Ruttner sampler at five sampling stations (A to E) (Vollenweider, 1974). Totally, 15 water samples were taken as same as environmental samples. The samples were kept in 0.5 litter bottles and preserved using buffered formaldehyde to yield a final concentration of 2%. The samples were left to settle for at least 10 days following which they were concentrated to about 30 ml by sedimentation and centrifugation. The quality and quantity analysis of phytoplankton for this study was reported in detail by Nasrollahzadeh et al. 2008a. Abundance of filamentous species from the bloom sample was expressed as filamentous per ml (mean length and width of 20 filaments were considered as a filament unit).

Statistical analyses

Principal component Analysis (PCA) is used to understand the correlation structure of collected data and identify the most important factors contributing to the data structure (Schoer, 1985; Buckley and Winters, 1992; Padro et al., 1993; Moncheva et al., 2001). All tests were performed at 5% significance level. Principle Component Analysis (PCA) was applied to score and narrow down the selection of parameters (Moncheva et al., 2001). The environmental data obtained from the sampling period was used for statistical analyses, which were performed using SPSS 11.0 software. Eight environmental variables were reduced into three variable factors (PC1, PC2 and PC3) using the principal component analysis. The eigenvalues, which give the variance of the factor components, were used as criteria for determining significant changes (eigenvalues > 1). For easier interpretation, factor axes were modified by 'factor rotation' using varimax (Lau and Lana, 2002).

Species richness, Shannon-Weaver diversity index and evenness were done using the software Multivariate Statistical Package (MVSP) version 3.13d.

Results

Composition of phytoplankton during bloom

Phytoplankton species of bloom sample (2009) is classified in three groups: Cyanophyta, Pyrrophyta and Bacillariophyta. Cyanophyta dominated over the other groups of algae and formed more than 98 and 96% of phytoplankton abundance and biomass, respectively. Pyrrophyta and Bacillariophyta shared almost 2 to 4% of the phytoplankton community in term of abundance and biomass. Species richness was not very low (17), but Shannon-Weaver diversity index and evenness were very low (0.01 bits/individual and 0.04 respectively). Cyanophyta contained 2 species: *Oscillatoria* sp. and *N. spumigena*. *N. spumigena* had highest abundance and biomass (> 99%) (*Table 2*). Concentration of *N. spumigena* was as high as 5830 filaments/ml with 0.05 mg/ml biomass. While the concentration of the other present species was 83 cells/ml with 0.002 mg/ml biomass. *Prorocentrum proximum* from Pyrrophyta division was dominant among the eleven species. Bacillariophyta composition was consisted of four species. Maximum abundance of Bacillariophyta was found belonging to *Nitzschia acicularis* but the maximum biomass documented was *Rhizosolenia calcar-avis*.

Table 2. *Species composition, abundance and biomass percentage of phytoplankton in the Caspian Sea during bloom in summer 2009*

Species	Abundance percentage of corresponded division	Biomass percentage of corresponded division
Cyanophyta		
Nodularia spumigena	99.96	99.99
Oscillatoria sp.	0.04	0.01
Pyrrophyta		
Prorocentrum proximum	44.58	73.99
Exuviaella cordata	20.74	1.38
Peridinium latum	11.15	11.10
Peridinium achromaticum	6.81	6.78
Goniaulax polyedra	6.50	2.59
Peridinium sp.	3.41	0.88
Peridinium trochoideum	2.79	0.74
Prorocentrum scutellum	1.55	1.70
Glenodinium lenticula	0.93	0.41
Goniaulax sp.	0.93	0.37
Glenodinium behningii	0.62	0.08
Bacillariophyta		
Nitzschia acicularis	83.33	15.80
Nitzschia longissma	8.33	2.69
Cyclotella meneghiniana	6.25	11.85
Rhizosolenia calcar-avis	2.08	69.66

The microscopic observation of *N. spumigena* showed straight, branchless and olive-green to yellowish color filaments with cylinder or barrel shape cells (length less than width). The cell length:width ratio was 1:1.8. Each filament contained 12-200 cells. Heterocyst usually observed with every 10-12 vegetative cells. *Table 3* summarizes the results of microscopy measurement of *N. spumigena*.

Table 3. *Microscopic measurements of Nodularia spumigena in the Caspian Sea collected during bloom in summer 2009*

	Minimum (µm)	**Maximum (µm)**	**Mean ± S.D. (µm)**
Length of filaments	44	732	297±163
Length of vegetative cells	3.0	4.4	3.7±0.5
Width of vegetative cells	6.0	9.0	6.7±1.0

Composition of Phytoplankton after bloom

Phytoplankton assemblage comprised of 46 species after the 2009 bloom. Bacillariophyta had the highest number of species (17) follow by Pyrrophyta (14), Cyanophyta and Chlorophyta (6) and Euglenophyta (3). The number of species represented at each station is shown in *Fig. 2*. This figure shows that the species richness increased from station A to station C and then decreased toward station E. Species richness decreased from surface to bottom at stations B and E while it increased in water column at stations C and D.

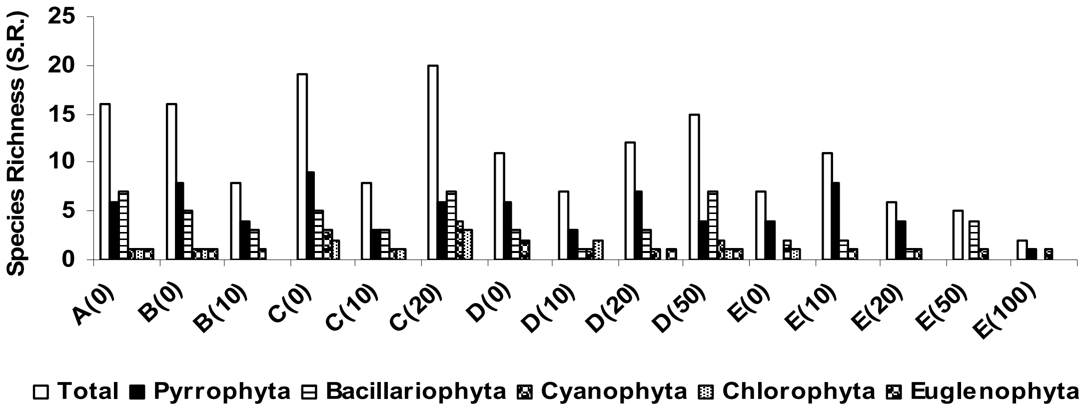

Figure 2. *The number of Phytoplankton species represented at different depth (sampling depth in meter in bracket) of the Tonekabon transect (5 sampling station, A-E) in the southern of Caspian Sea after the 2009 bloom*

The highest and lowest value of total phytoplankton biomass recorded at station E at 10 m depth (413 mg/m3) and 100 m (1.4 mg/m3) depth, respectively. Pyrrophyta showed the highest biomass (78 mg/m3) while the Chlorophyta and Euglenophyta recorded a minimum biomass (0.4 mg/m3). Biomass of each phytoplankton division showed decreasing trend between the two depths (surface to bottom). However, there were some incongruities in these biomass patterns fluctuating from surface to bottom. Where biomass of Pyrrophyta increased from surface to 10 m and then decreased toward bottom while biomass of Bacillariophyta showed sharp decrease from surface to 10 m, then slightly increased at 20 and 50 m depths and reached thereafter to zero biomass at the bottom. Bacillariophyta played the main role in the formation of the phytoplankton biomass at the surface and 10 m depth while at the deeper depths Pyrrophyta dominated the phytoplankton biomass. As the *Fig. 3* shows, Chlorophyta and Euglenophyta were only recorded in low biomass at some depths of the offshore water.

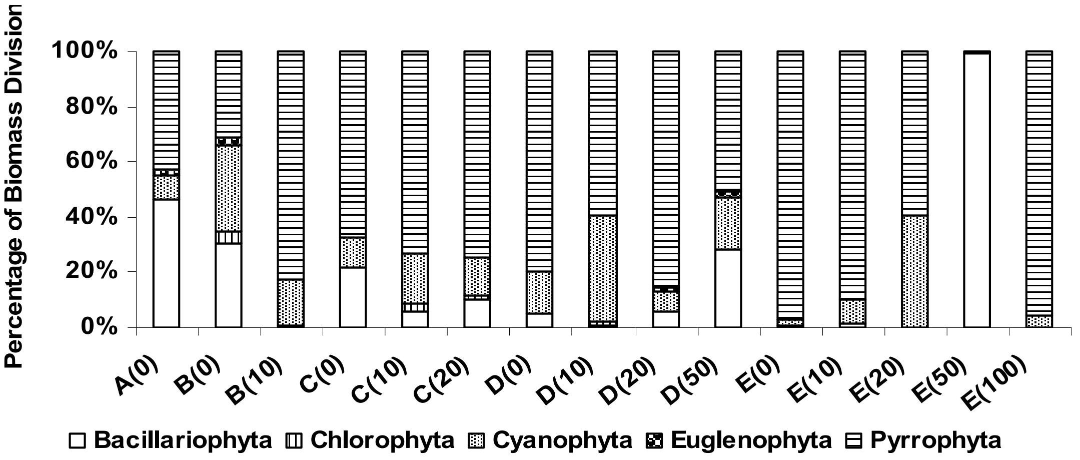

Figure 3. *The percentage of biomass of each division at different depth of the five sampling stations (A-E) in the Tonekabon transect in the southern Caspian Sea (2009). Bracket shows the station depth in meter at each sampling station*

Fig. 4 shows the phytoplankton species that contributed more than 70% of biomass. Biomass of the major Pyrrophyta species was more evenly distributed with *Prorocentrum proximum* and *Exuviaella cordata*. Whereas *Glenodinium lenticula* and *Peridinium achromaticum* only contributed slightly in terms of biomass. *Cyclotella meneghiniana*, *Cerataulina pelagica*, *Nitzschia acicularis*, *Thalassionema nitzschioides* and *Thalassiosira caspica* formed more biomass than the others species of Bacillariophyta. Cyanophyta biomass was represented mainly by *Oscillatoria* sp. and rarely *Nodularia spumigena*.

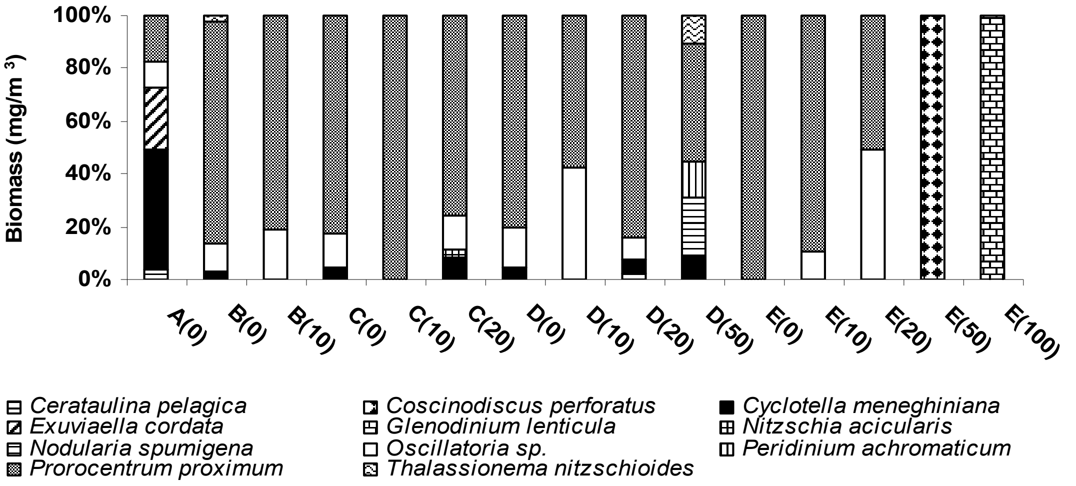

Figure 4. *The percentage of biomass of phytoplankton species at different depth of Tonekabon transect in the southern of Caspian Sea (2009)*

Fig. 5 shows the difference of the contribution of the phytoplankton abundance divisions at each station. Cyanophyta was the dominant division from surface to bottom in inshore and offshore stations. Pyrrophyta was present at all depths (except at some depth at offshore station). Bacillariophyta was dense at surface of inshore and certain depths of the offshore water.

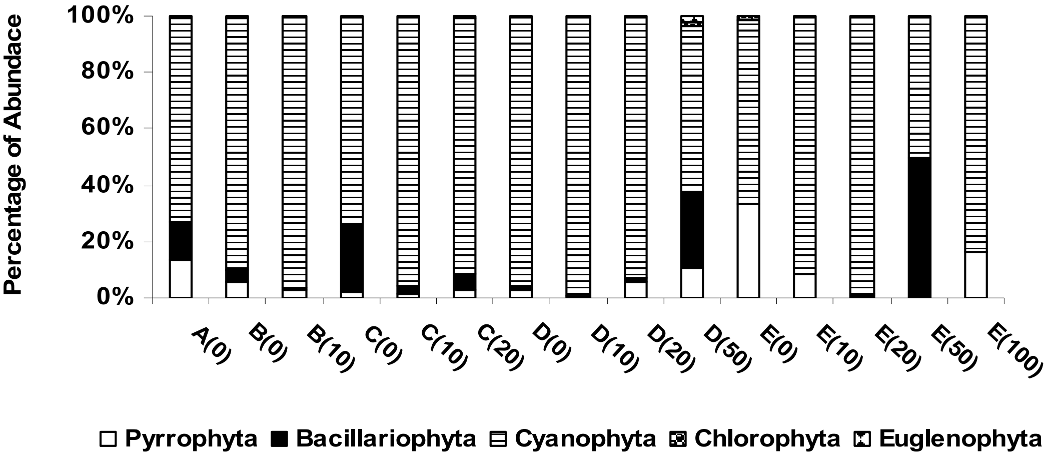

Figure 5. *Contribution of phytoplankton divisions at different depth of the five sampling stations (A-E) at the Tonekabon transect in the southern of Caspian Sea. Bracket shows the station depth in meter at each sampling station after the 2009 bloom*

22 of the most frequently found phytoplankton species contributed more than 70% of abundance at each station as shown in *Table 4*. Cyanophyta was represented mainly by *Oscillatoria* sp. and it was the dominant species at all stations. Nine species out of the twenty-two most common phytoplankton were from Pyrrophyta. Pyrrophyta abundance was formed mostly by *Prorocentrum proximum* and *Exuviaella cordata.* The rest of the Pyrrophyta species were found at certain stations especially station D and station E. Bacillariophyta hold the maximum diversity (11 species) but with lower density as compared to Pyrrophyta. Among the dominant Bacillariophyta species, 4 species were more substantial: *Thalassionema nitzschioides, Cyclotella meneghiniana, Nitzschia acicularis* and *Chaetoceros peruvianus*. The Bacillariophyta species at 10 meter depth (both inshore and offshore) played a minimum role in phytoplankton abundance while more contribution was observed at 50 m depth of stations D and E. The presence of *N. spumigena* occurred at surface water C (0), D (0), E (0) and at 50 m (station D).

Environmental factors

In the brackish Caspian Sea, weather temperature varied from 24.0 to 26.0°C. The surface water temperature was 24.0-26.2°C in summer 2009, after bloom of *N. spumigena* (*Fig. 6*).

In summer 2009, the pH values for the surface layer of the southern of Caspian Sea come to 8.10-8.66 (*Fig. 7*). This value gradually dropped with depth especially in the inshore water and at the bottom, which came to less than 8.30.

Table 4. *Abundance percentage of dominant species (>70%) of phytoplankton in the southern of Caspian Sea (Tonekabon transect) after bloom in summer 2009*

Station Species	**A (0)**	**B (0)**	**B (10)**	**C (0)**	**C (10)**	**C (20)**	**D (0)**	**D (10)**	**D (20)**	**D (50)**	**E (0)**	**E (10)**	**E (20)**	**E (50)**	**E (100)**
Cyanophyta (%)															
Oscillatoria sp.	72	90	97	96	95	90	95	99	93	50	63	91	99	50	83
Nodularia spumigena										9	2				
Pyrrophyta (%)															
Exuviaella cordata	12	2	1			1	1		2	4	6	6			
Glenodinium lenticula															16
Goniaulax digitale															
Goniaulax polyedra											5				
Goniaulax sp.															
Gymnodinium sp.															
Peridinium achromaticum										2	2				
Peridinium trochoideum										2					
Prorocentrum proximum		2	1	1	1	1	1		2	4	21	2			
Bacillariophyta (%)															
Cerataulina pelagica		1								7					
Chaetoceros peruvianus	2				2	1									
Chaetoceros convolutus		1													
Coscinodiscus perforatus														10	
Cyclotella meneghiniana	4					1			1	4					
Navicula sp.										2				10	
Nitzschia acicularis	1	1				3			1						
Nitzschia sp.										2					
Pseudo-nitzschia seriata														20	
Thalassionema nitzschioides										7					
Thalassiosira caspica										4					

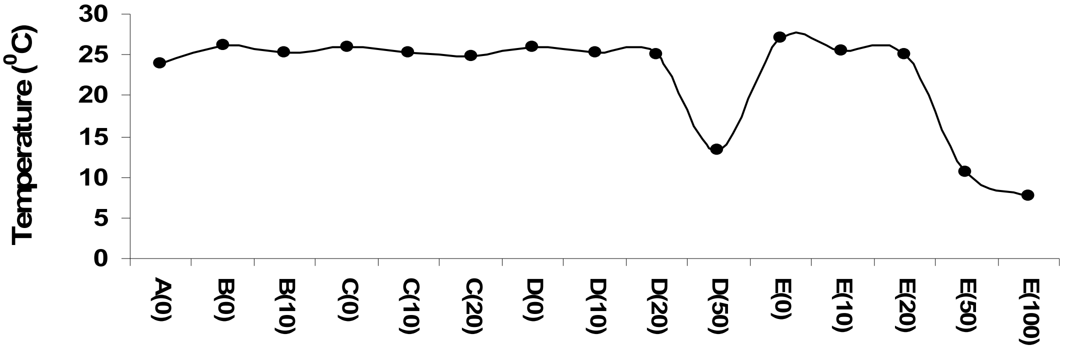

Figure 6. *Vertical distribution of temperature at different depth of Tonekabon transect [Five sampling stations, A-E with sampling depth in bracket (meter)] in the southern of Caspian Sea, summer 2009*

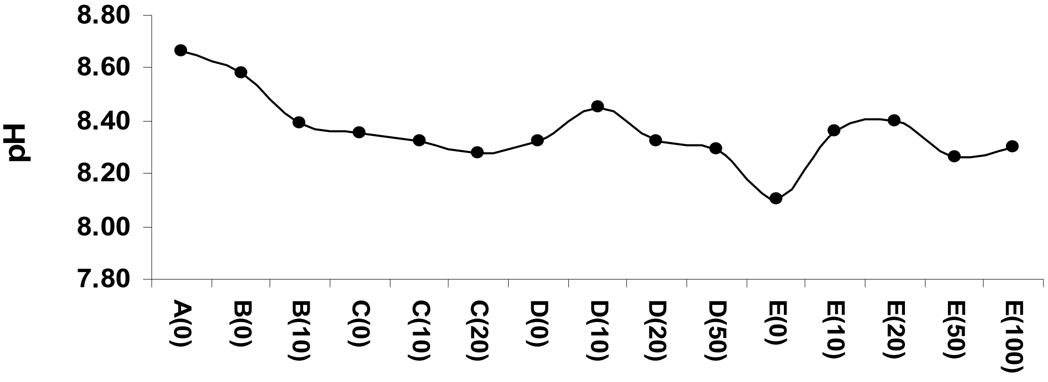

Figure 7. *Vertical distribution of pH at different depth of Tonekabon transect Five sampling stations, A-E with sampling depth in bracket (meter) in the southern of Caspian Sea, summer 2009*

We applied PCA to the eight variables collected during cruises of the year 2009 (NO_2^-, NO_3^-, NH_4^+, DIN, DON, DIP, DOP, DSi). In summer 2009 (after bloom of *N. spumigena*), the PC1 accounted for 51.94% of total variance comprised of DIP, DOP, TP, TN, DON and DSi, representing variations in inorganic and organic compounds (P and Si) in the water. PC2, consisted of 20.03% of total variance, represents the nutrient enrichment, including the dissolved inorganic nitrogen. PC3 with 14.04% of the total variance represents the NH_4+.

In summer 2009, molar ratio of dissolved inorganic nitrogen/dissolved inorganic phosphorus (DIN/DIP) was less than 10 (*Fig. 8*).

In 2009, wind speed for August 15 ranged from 2 to 4 m/s and did not register significant scattering**.** However, in 16 August 2009, (with wind speed ranged from 0 to 2 m/s) wide area with strong scattering was observed (QUIKSCAT). Overall, maximum concentrations of chlorophyll (Soloviev, 2005) as estimated from wind speed were observed in August 16, 18, and 19 (wind speed ranged 0 to < 2 m/s). This trend was also happened during development of the bloom process in 2005 (*Fig. 9*).

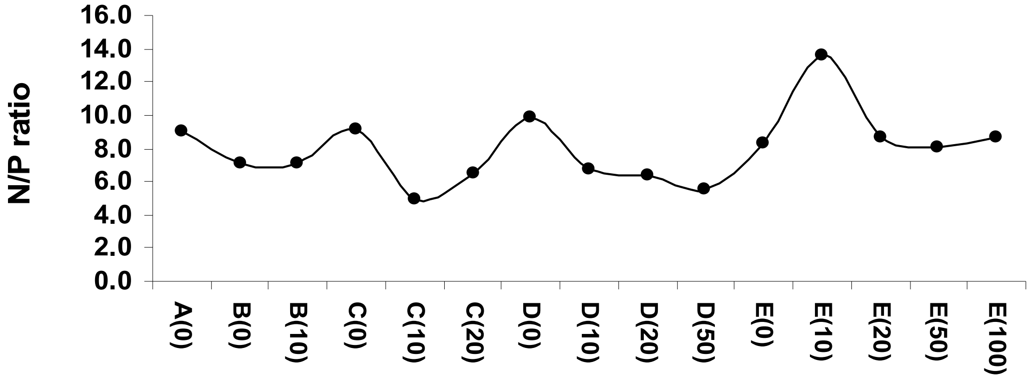

Figure 8. *Vertical distribution of N/P ratio at different depth of Tonekabon transect Five sampling stations, A-E with sampling depth in bracket (meter) in the southern of Caspian Sea, summer 2009*

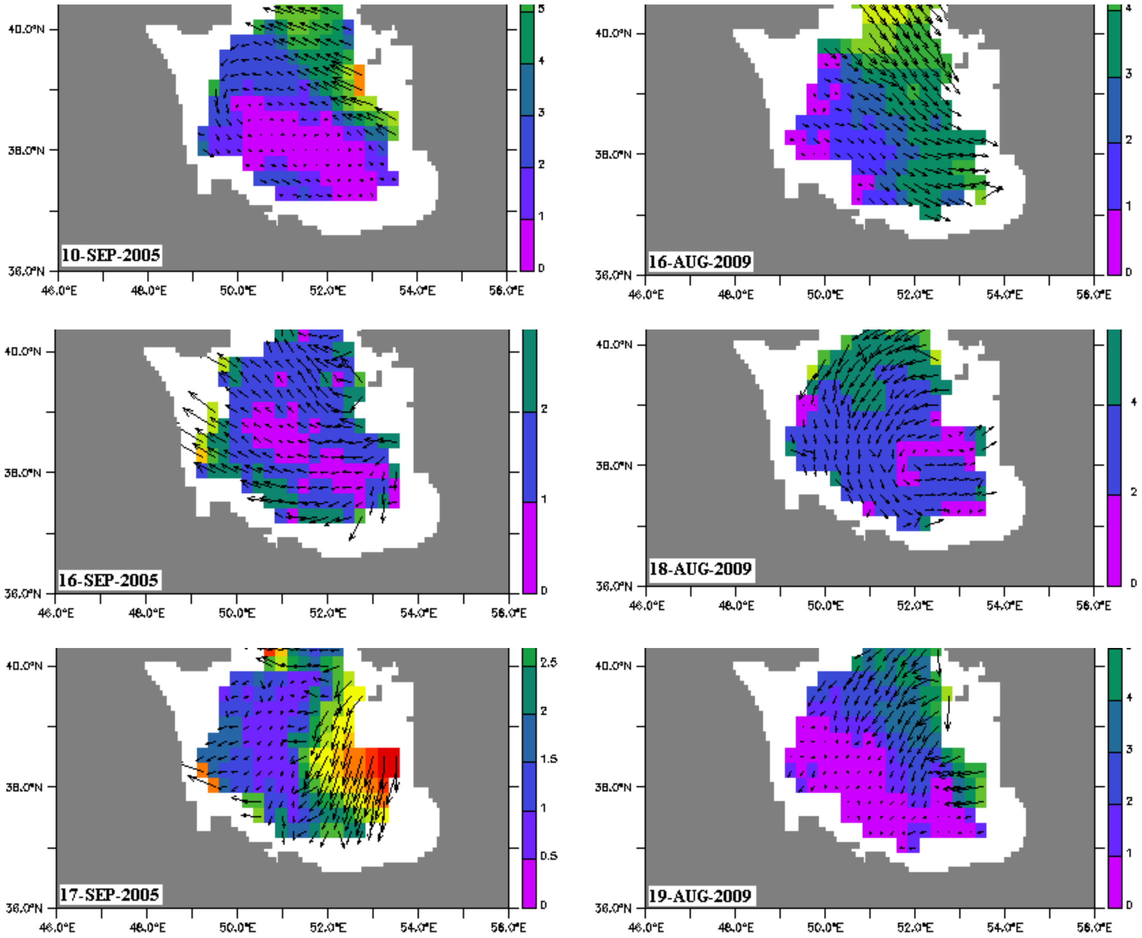

Figure 9. *Wind speed (m/s) during peak phytoplankton bloom in September 2005 and August 2009 in the southern of Caspian Sea*

Discussion

During eutrophication, when phytoplankton biomass increases, there are concurrent changes in taxonomic structure. Most notably is the relative biomass of Cyanophyta that increases with eutrophication. This relationship has been noted in boreal, temperate, subtropical and tropical ecosystems (Auer et al., 2004). Nasrollahzadeh et al. (2008a) reported that the southern of Caspian Sea's (as a subtropical system) status shifted from oligotrophy to meso-eutrophy in the last decade (after the alien introduction of *Mnemiopsis leidyi*). According to their study, the summer abundance of phytoplankton was 10,664 (cells/l), where Cyanophyta, Pyrrophyta and Bacillariophyta abundance were 9, 23 and 22 percent respectively before the invasion of *Mnemiopsis leidi*. Whereas after the invasion, phytoplankton abundance reached 22,433 (cells/l) in summer and the abundance percentage of Cyanophyta, Pyrrophyta and Bacillariophyta were 52, 7 and 32, respectively. They also mentioned that the number of species and abundance of Cyanophyta increased after *Mnemiopsis leidyi* invasion in 1999.

In 2000s, we found blooms occurrences that coincided with changes in trophic status in the southern of Caspian Sea. Cyanophyta blooms displayed a range of temporal dynamics. The southern of Caspian Sea had Cyanophyta (*N. spumigena*) blooms that started in summer and last into autumn in 2005. Then again in 2009, we observed blooms that occurred as extreme peaks but crashed after lasting just for days or weeks In our current study, *N. spumigena* abundance was more than 98% of the of phytoplankton abundance in the 2009 bloom sample.

In the last decades, dramatic and intensive summer blooms of Cyanophyta *(N. spumigena* and *Aphanizomenon flos-aquae*) were also observed in several regions of the Baltic Sea (Gorokhova, 2009). In this study, *N. spumigena* blooms recorded a stunning 112,000 filaments/ml and 5830 filaments/ml in 2005 and 2009 respectively, while in Iznik Lake (freshwater), the *N. spumigena* bloom recorded a 130 filaments/ml that consisted 53% of the total abundance (Akcaalan et al., 2009). In Finland Gulf, *N. spumigena* and *Aphanizomenon flos-aquae* made 35.8% of the total phytoplankton biomass in August 2000-2005 (Raateoja et al., 2010). The results showed bloom of this species in the Caspian Sea was many more fold in terms of number of filament /ml than recorded in other seas and lakes. This also showed that the bloom of this species during 2005 was many fold more than what it recorded in 2009.

The vertical distribution of the coexisting nitrogen-fixing *N. spumigena* in the southern of Caspian Sea was distinctive as compare to systems elsewhere. *N. spumigena* usually prefers near-surface water at the top 5 meter of the water mass as observed in the Baltic Sea. In the Caspian Sea, *N. spumigena* existed at water surface during the bloom but was also found in water column as deep as 50 m after the bloom.

The results showed that species richness, abundance and biomass of Bacillariophyta declined during the bloom. Pyrrophyta and Cyanophyta are able to out-compete other groups to build massive abundance and formed mono/low species structure of phytoplankton during the bloom. This is due to the physiological and toxicity features of these species. For instance, Cyanophyta has the ability to fix atmospheric nitrogen whereas Pyrrophyta can tap into heterotrophic nutrition. These phenomena during the bloom not only will impact the water quality but also cause the quality of food for consumers to declines, for the reason that a diverse phytoplankton population is of importance to support a healthy ecosystem (Karlson, 2010). When comparing the current result with the long term study of Nasrollahzadeh et al. (2008a), the shift of phytoplankton functional group from Bacillariophyta and Pyrrophyta to Cyanophyta and Pyrrophyta is an evidence of the up-ward alteration in trophic state of the Caspian Sea. Some dominant species (*Oscillatoria* sp., *Prorocentrum proximum* and *Nitzschia acicularis*) in the Caspian Sea are indicators of high trophic state, organic pollution and with the potential to form bloom and adversely affect the water quality (Hutchinson, 1957; Palmer, 1980; Hernandez et al., 2000; Wei et al., 2008; Cabecinha et al., 2009).

High pH and scarcity of free CO_2 theoretically favor the growth of Cyanophyta that have a low Ks for CO_2 uptake. In return, Cyanophyta can and use bicarbonate as a C source (Dokulil and Teubner, 2000). In the Caspian Sea, carbonate alkalinity and pH values were significantly greater than in the water of other region by the actions of river discharge that changed the chemical composition of the sea and also peak of photosynthesis in summer (Kosarev and Yablonskaya, 1994; Kostinave and Kosarev, 2005). Our results confirmed that water pH of slight alkaline is a favorable condition for Cyanophyta growth in summertime.

There is an ongoing debate on whether nitrogen or phosphorous is the limiting factor for eutrophication in the Caspian Sea. Studies have shown that the limiting factor is area-specific and changes over time. In the Caspian Sea can be phosphorous-limiting near the river mouth, but at all seasons are nitrogen-limited in the offshore area (Kosarev and Yablonskya, 1994). During all seasons (1994-2005) phytoplankton productivity was nitrogen limited (4.47 to 5.78) while the levels of P and Si always remained high (Nasrollahzadeh et al., 2008b). In the summer 2009, the low ratio of dissolved inorganic nitrogen/dissolved inorganic phosphorus DIN to DIP (less than 10) of the surface water, in combination with a period of calm and sunny weather that increase the stability of the water column, lead to nutrient-depletion euphotic zone that is isolated from nutrient-rich water mass below. These conditions favor the nitrogen-fixing Cyanophyta.

Recent research has pointed to the role of dissolved organic compounds in algal nutrition, competitive interactions and determination of community structure (Antia et al. 1991; Seitzinger and Sanders, 1997). In particular, certain harmful (*i.e.* toxic, hypoxia/anoxia-inducing, food web-altering) algal bloom groups, including dinoflagellates and blue-green algae (Cyanophyta), are known to contain species capable of growing in either autotrophic or heterotrophic modes (Antia et al., 1991), enabling them to exploit both inorganic and organic nutrient enrichment. In the present study, the percentage of DON was observed to be more than 90% of the total nitrogen of which is the compound available for Cyanophyta and dinoflagellates to grow. In addition, PCA analysis showed that organic nutrients such as DON and DOP were represented at PC1 with total variance more than 30%, which confirmed our aforementioned discussion.

The direction and strength of wind blowing over the Caspian Sea are determined by three factors: the distinctive general circulation of the atmosphere, the temperature field that is created by the sea itself and the relief of its coastline. The average wind speed over the sea is 5.7 m/s. The greatest average speeds are observed in the middle part of the sea and are on the average of 6 - 7 m/s per year. The average wind speeds are significantly lower in the southern of Caspian Sea: 4 - 5 m/s, 3.5 – 4.0 m/s on the eastern coast, and 2.5 – 3.0 m/s in the southeast. Low wind speeds (2.2 – 3.0 m/sec) are observed on the southwestern coast, in Iranian waters and on the southern coast of the Caspian Sea (Caspian Scientific Network, 2003). Strong Cyanophyta bloom occurred in the southern of Caspian Sea at the beginning of the second decade. These blooms developed in August and existed till the end of September in 2005 (Soloviev, 2005). Soranno (1997) also documented short–lived surface blooms throughout the summer and fall, and linked them with periods when there was a combination of low wind velocity, absence of rainfall and higher than average solar radiation. Surface blooms collapsed when wind velocities increased with cloudy weather or rainfall occurred. Result of our study showed that surface blooms collapsed when wind speed increased to more than 2.0 m/s.

As a conclusion, with considering increased load of nutrients, a slightly alkaline pH, a low N:P ratio and global warming may favor the growth of Cyanophyta, the role of light wind (calm weather) is most important to the summer bloom in the Caspian Sea. Research also must continue to focus on defining the underlying conditions that promote the blooms, to ascertain if controllable variables including P and N loading rates, N:P loading ratios, flushing rate, etc. can be manipulated to reduce the overall risk of blooms, even when atmospheric conditions are favorable for their occurrence.

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THE RELATIONSHIP BETWEEN PLANT DIVERSITY AND PRODUCTIVITY IN NATURAL AND IN MANAGED GRASSLANDS

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Abstract. Despite the fact that several experiments have been conducted to explore the biodiversity-productivity relationship in synthesized and natural plant communities, the results obtained were contradictory and no clear consensus has been reached. Recent experiments that surveyed mature natural plant communities have investigated this relationship across environmental gradients, where biotic and/or abiotic factors are correlated with the observed diversity and productivity levels. We studied the effect of plant diversity on ecosystem productivity in agriculturally managed (managed at a low intensity with moderate level of disturbance) and natural (no history of management) grasslands at a within-site scale in order to minimize the confounding effect of environmental factors. We tested the effects of two diversity measures (species richness and species evenness) on productivity within- and across-sites scale. Our results indicated that this relationship was different between the natural and the managed grasslands and varied according to the diversity measure. Species richness only poorly explained the variation in productivity for the managed grasslands, while the variations in system productivity were better explained by species evenness. Interestingly, our results from the natural low productive, species poor grassland are in agreement with the results obtained from the recent experiments that artificially manipulated diversity levels and found an asymptotic increase in productivity along with increasing species richness. Our results provide additional evidence of the complex behavior that measures of species diversity that combine several aspects of diversity such as species evenness, species identity as well as the interactions among the species may be better determinants of the response of the ecosystem to biodiversity.
Keywords: *biodiversity, ecosystem productivity, evenness, dry acidic grasslands, managed grasslands, species richness*

Introduction

In the past decade, the effect of species loss on ecosystem functioning such as primary productivity, nutrient relations, ecosystem stability and vulnerability to invasion, as well as vegetation dynamics has received priority in ecological research (Huston, 1997; Tilman et al., 1997; Hector et al., 1999; Loreau, 2000; Kahmen et al., 2005). In spite of the fact that a large number of experiments have been conducted to investigate this relationship, a common theory was not developed so far (Schmid, 2002; Hector et al., 2007).

Generally, two approaches have been used to study the relationship between species richness and ecosystem functioning; the experimental and the observational approach. In the first approach, plant diversity was artificially manipulated (Symstad and Tilman, 2001), while in the observational approach, plant diversity and composition were not manipulated; instead comparative studies were conducted in natural plant communities to relate the differences in diversity to the observed productivity along environmental gradients (Kahmen et al., 2005; Grace et al. 2007).

Using the experimental approach, it was concluded that the productivity of the ecosystem increases asymptotically with increasing diversity (Tilman et al., 1997; Hector et al., 1999). This result has been attributed mainly to niche complementarity and facilitation among species (Hooper, 1998; Loreau and Hector, 2001), as well as to the “sampling effect”, i.e. an increased probability of including a highly productive or highly competitive species at the higher diversity levels (Huston et al., 2000).

Experiments based on the observational approach show that biodiversity reaches a peak at intermediate productivity levels, which is described by the “hump-backed” model (Grime, 1973). This model indicates that species richness declines in more productive sites due to higher rates of extinction of less competitive species as a result of higher dominance of strong competitors (Kahmen et al., 2005; Grace et al., 2007). The observational approach studies the effect of species diversity on productivity across environmental gradients, where other factors such as soil fertility, climate, disturbance regime, or herbivory are correlated with the observed diversity and productivity levels (Kahmen et al., 2005). Few experiments have distinguished between environmental and biodiversity effects by employing a multivariate statistical analysis (Kahmen et al., 2005; Grace et al., 2007). Generally, a strong correlation between species richness and productivity was not evident in these experiments. This might be explained by the overwhelming effects of environmental factors which might have masked the diversity effect (Diaz and Cabido, 2001; Schmid, 2002; Hector et al., 2007).

New approaches based on natural vegetations are needed to overcome the above mentioned limitations (Chapin et al., 2000; Loreau et al., 2001; Kahmen et al., 2005). In this kind of approach, biotic and/or abiotic factors should not be correlated with the diversity and productivity levels. In the present study, the relationship between plant diversity and productivity was analyzed at two levels; within-site level (i.e. among plots within a site) in order to minimize the influence of environmental factors, and across-site level to investigate this relationship across environmental gradients to be compared with the hump-shaped model. We have chosen two different grasslands, species rich, agriculturally managed grasslands (thereafter managed grasslands) which were managed through cutting and/or aftermath grazing and have 9-17 species m^{-2}, and dry acidic nutrient poor not managed grasslands (thereafter natural grasslands) and have a species richness range of 1-5 species 0.25m^{-2}).

We studied the effect of two components of biodiversity, species number and evenness, on biomass production (thereafter productivity) in order to test whether different diversity components would have different relationships with productivity. Our hypothesis was that at within-site scale there will be a general positive relationship between diversity and productivity, while this relationship will follow the hump-shaped model when the relationship is investigated across environmental gradients.

Materials and methods

Study sites

The study sites were chosen from two different grassland types, agriculturally managed and natural grasslands. Chemical characteristics of the managed grassland soil (0-10cm depth) were: pH 6.2, 0.06 mg P g^{-1} soil and 0.15 mg K g^{-1} soil; while for the dry acidic natural grassland were: pH 5.3, 0.022 × 10^{-3} $NH4^+$ kg^{-1} soil and 1.22 × 10^{-3} g NO_3^- kg^{-1} soil. Data from the managed grasslands were collected in 2005, while the data collection from the natural grasslands took place in 2006. Data from these grasslands were analyzed separately to eliminate the confounding effect of management history and inter-annual variation in environmental conditions.

In 2005, seven sites (M1–M7), each measuring 30 × 30 m^2 and differing in plant community composition and productivity level (*Table 1*) were established in agriculturally managed grasslands near Goettingen, central Germany (51° 31′ N, 9° 55′ E). The sites had not received any mineral fertilizer application, tillage or re-sowing for at least the last decade. The management consisted of one or two hay cuts per year with occasional aftermath grazing.

In 2006, another three sites (D1–D3) each measuring 10x10 m^2 were chosen from a natural (with no management history) dry acidic nutrient poor grassland area located near Fuerth, southern Germany (49° 26′ N, 10° 50′ E), representing early stages of succession and dominated by two pioneer species: *Corynephorus canescens* and *Rumex acetosella* (*Table 1*).

Table 1. *Dominant species, species number and average biomass production of the managed and the natural grasslands*

Site	Dominant species	Avg. and range of species number	Avg. and range of above-ground biomass (g DM m^{-2})
M1	*Arrhenatherum elatius* *Festuca rubra*	9.7 m^{-2} (8-13)	536 (420-651)
M2	*Festuca rubra* *Agrostis stolonifera*	17.9 m^{-2} (14-23)	576 (377-749)
M3	*Arrhenatherum elatius* *Rhinantus angustifolia*	16.6 m^{-2} (11-22)	409 (284-507)
M4	*Alepocurus pratensis* *Dactylis glomerata*	12 m^{-2} (6-16)	392 (328-506)
M5	*Elymus repens* *Ranunculus repens*	11 m^{-2} (7-15)	562 (415-687)
M6	*Festuca rubra* *Holcus lanatus*	10.6 m^{-2} (6-16)	406 (296-621)
M7	*Lolium perenne* *Trifolium repens*	13.4 m^{-2} (10-19)	445 (303-582)
D1	*Corynephorus canescens* *Rumex acetosella*	2.5 0.25m^{-2} (1-4)	49 (14-154)
D2	*Corynephorus canescens* *Rumex acetosella*	2.0 0.25m^{-2} (1-4)	61 (9-142)
D3	*Corynephorus canescens* *Rumex acetosella*	3.8 0.25m^{-2} (2-5)	91.2 (41-141)

Data collection

In 2005, 18 one square meter quadrats were established within each site (M1–M7) and used as the experimental units for analysis of the diversity- productivity relationship. Each site was divided into three blocks, in each block six quadrats were established, the quadrats were fixed systematically along the block (beginning, middle and the end of the block). For each quadrat, all occurring plant species were recorded and the proportion of each species from the total dry weight biomass was visually estimated following a method of percentage ranking (Whalley and Hardy, 2000). Above-ground biomass (used as surrogate for the above-ground productivity) was measured for each quadrat by harvesting all plant material (>2 cm). The harvest took place when the swards reached the maximum yield (at peak standing crop point). The sampling period lasted from beginning of June to mid July. Before harvesting, the yield was recorded periodically using the rising plate meter method to determine peak standing crop (Correll et al., 2003). This is a nondestructive method which can be used repeatedly on the same plot to estimate the herbage yield. Below-ground biomass (thereafter below-ground productivity) was measured for three sites (because of time and labor limitations): M1, M2 and M3 by taking a soil cylinder (10cm diameter x 20cm depth) at the center of each quadrat. Root samples were then washed with running tap water over a sieve (1 mm mesh size), cleaned manually with forceps and collected on aluminum trays. All harvested plant material was oven dried (103 °C) until constant mass and weighed.

In the natural grassland, 15 quadrats of 0.25 m^2 were established randomly at each of the three sites. The same measurements as described for the managed grassland were carried out in these quadrats, except that Braun-Blanquet classes were used to estimate species canopy cover instead of the estimation of dry biomass proportion for each species and that the root biomass for each quadrat was harvested totally (by excavating all root materials in the upper 20 cm) rather than taking soil cylinders as done in the managed grassland plots.

Data analysis

The diversity–productivity relationship was analyzed at two levels; within-site level (data from one site) and across-sites level (data from all sites within each grassland type).

Diversity analysis

Diversity measures included species richness (S) and species evenness (J') (Ma, 2005). Species richness was determined as the total number of species present in a quadrat. Species evenness was obtained using the following equation:

$$J' = D'/D_{max} \qquad \text{(Eq.1)}$$

where $D_{max} = S$
and $D' = 1 / \sum (P_s^2)$, where P_s is species proportion.

Statistical analysis

Least squares simple linear regressions were used to analyze the relationship between plant diversity (species richness and evenness) and productivity (above- and below-ground biomass). Assumptions of the regression models were tested by graphical and numerical methods as Q-Q plot and Kolmogorov-Smirnov test for normality of residuals and scatter diagrams of residuals and predicted values for the constancy of variance. Statistical analyses were performed using SPSS version 12.0 (SPSS 2001).

Results

Within-site level

In the managed grasslands, the relationship between species richness and above-ground productivity was significant for two sites (M5 and M6) with a negative correlation. Evenness was positively correlated with above-ground productivity for two sites (M1, M2). The relationship between diversity and below-ground productivity was rather weak as only one site (M1) showed a significant correlation ($R^2= 0.23$, $P<0.1$) between evenness and below-ground biomass (*Table 2*). In the natural grasslands, no significant relationship between species richness and evenness and productivity was found.

Table 2. *R^2 of the relationship between plant diversity and productivity in the managed and natural grasslands, within-site level (NS = not significant; + $p<0.1$; * $p<0.05$; ** $p<0.01$.)*

	Above-ground		**Below-ground**	
	Species number	**Evenness**	**Species number**	**Evennes**
Managed				
M1	0.00^{ns}	0.19^{+}	0.0^{ns}	0.23^{+}
M2	0.07^{ns}	0.25^{*}	0.06^{ns}	0.12^{ns}
M3	0.00^{ns}	0.04^{ns}	0.0^{ns}	0.0^{ns}
M4	0.01^{ns}	0.11^{ns}	-	-
M5	0.37^{**}	0.04^{ns}	-	-
M6	0.19^{+}	0.00^{ns}	-	-
M7	0.00^{ns}	0.00^{ns}	-	-
Natural				
D1	0.05^{ns}	0.04^{ns}	0.18^{ns}	0.11^{ns}
D2	0.10^{ns}	0.05^{ns}	0.10^{ns}	0.04^{ns}
D3	0.02^{ns}	0.11^{ns}	0.00^{ns}	0.06^{ns}

Across-sites level

Evenness seems to be more important in explaining the variation in above- and below-ground productivity than species richness. Across all managed sites, productivity increased linearly with increasing evenness ($R^2 = 0.14$ and 0.11, $P < 0.001$ and $P < 0.016$ for above- and below-ground productivity, respectively). However, there was no significant relationship between species richness and productivity (*Fig. 1*).

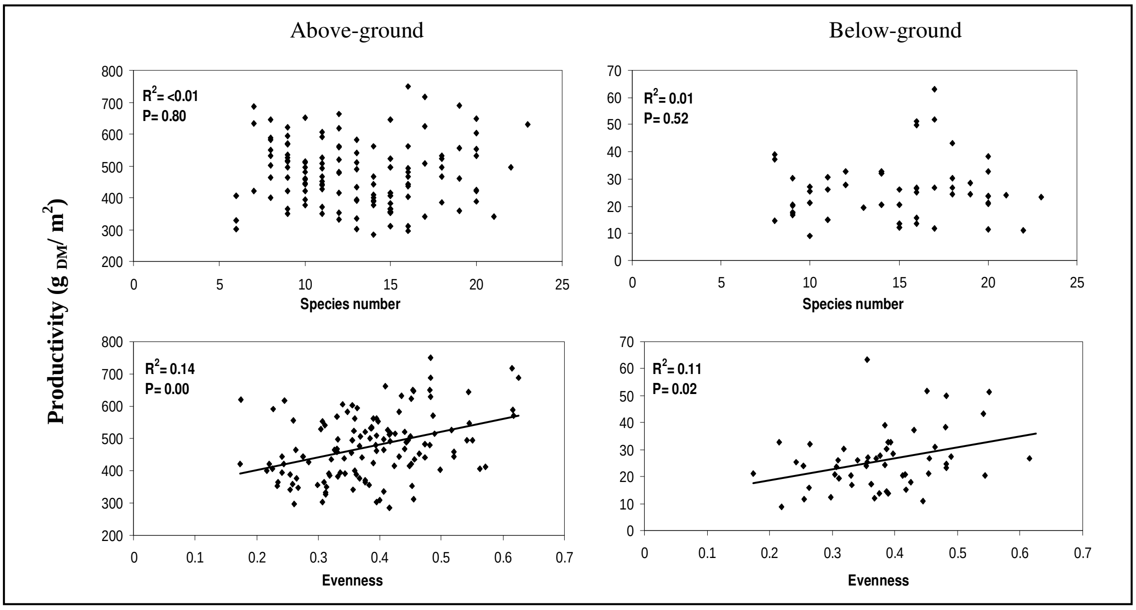

***Figure 1.** The relationship between diversity (species richness and evenness) and productivity (above- and below-ground) in the managed grasslands across sites*

In the natural sites (*Fig. 2*), productivity (both above- and below-ground) increased linearly with increasing species richness. No such effect was found for the relationship between productivity and evenness.

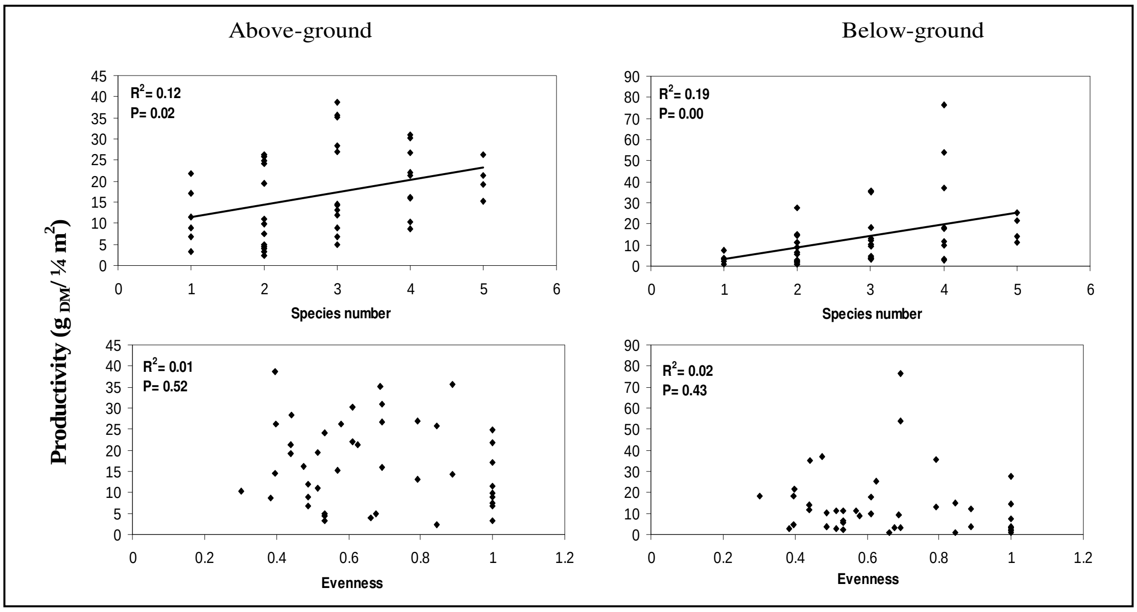

***Figure 2.** The relationship between plant diversity (species richness and evenness) and productivity (above- and below-ground) of the natural grasslands across sites*

Discussion

Most of the controversies regarding the diversity-productivity relationship are raised from two points: (i) the effect of various diversity components (species richness, evenness, community composition and functional diversity) on ecosystem processes, and (ii) the inconsistency between results obtained from the experimental and the observational approach (Huston, 1997; Grime, 1997; Diaz and Cabido, 2001; Loreau et al., 2002; Hector et al., 2007). In the present experiment, the relationship between diversity and productivity in the managed grasslands varied according to the diversity measure used (i.e. species richness or evenness). The positive relationship between evenness and productivity at within-site as well as across-sites scale in the managed grasslands indicates that evenness may be more related to ecosystem functions than species richness. It has previously been hypothesized that evenness might be more related to productivity and nutrient cycling of the ecosystems than number of species (Wilsey and Potvin, 2000). Furthermore, ecosystems with evenly distributed species might use resources more efficiently, both spatially and temporally. Compared to evenness, species richness is greatly influenced by sub-ordinate species that have low abundance, while those species would have negligible effects on productivity or other ecosystem functions (Tilman et al., 1997; Spehn et al., 2002; Laird et al., 2003; Kahmen et al., 2005). Previous studies also indicated that species richness showed only weak relationships with productivity (Tilman et al., 1997; Kahmen et al., 2005).

Contrary to the managed sites, none of the natural sites showed a significant relationship between diversity and above-ground productivity at within-site scale. Previous studies (eg. Laird et al., 2003) have shown a significant relationship between diversity and productivity in sites characterized as early successional. Our results could be explained by the fact that the variations in diversity and productivity levels within each site of the dry acidic grassland were too low to produce statistically significant relationship (*Table 1*).

Overall, our analysis at within-site scale indicates that there is no general relationship between plant diversity and productivity that is consistent for all sites and that the type of the diversity-productivity relationship could be influenced by the existing environmental conditions. Therefore, our results are not in agreement with the results obtained from experimental grasslands where an asymptotic increase in productivity along with increasing species richness was found (Hector et al., 1999).

Our analysis at across-sites level allows comparing our results with the observational approach as both of them investigate the diversity-productivity relationship across environmental gradients. The results obtained from the managed grasslands (*Fig. 1*) showed a general positive diversity-productivity relationship when the diversity measure was evenness. However, species richness showed no significant relationship with productivity nor did it behave according to the hypothesized hump-backed model (Grime, 1973). The explanation for this might be that obtaining the hump-shaped relationship requires the extreme environmental conditions to be included into the surveyed plots (stress factors at one end and conditions that favor high productivity at the other end). A lack of these extreme conditions which cause low and high productivity levels would produce a diversity productivity relationship that is located at the center of the hump-shaped curve (*Fig. 1*). Another explanation could also be that the complementarity between species does not increase with increasing number of species (redundancy) in the relatively species rich managed grasslands (Laird et al., 2003). This is confirmed by the positive species richness-productivity relationship in the natural grasslands (*Fig. 2*), which is characterized by a lower number of species compared to managed grasslands.

Interestingly, our results from the natural grasslands at across-site scale are in agreement with the results obtained from the experimental approach (e.g. Naeem et al., 1996; Hector et al., 1999). In those experiments, the positive relationship has been mainly attributed to niche complementarity and sampling effect, where it has been argued that the diversity effect could also be driven by the plots with low species richness (Kahmen et al., 2005). In our natural grasslands, it seems that those factors might also play an important role in producing such positive relationships in early successional sites because of the low species number and of ecological processes which have not yet reached equilibrium (Wardle, 1999; Laird et al., 2003).

Our results indicate that the relationship between diversity and productivity is different for the managed, species rich, productive grasslands in one hand and the nutrient poor, low productive natural grasslands in another hand. Compared to the managed grasslands, natural dry acidic grasslands are characterized by lower fertility and lower water availability during the growing season due to the low water holding capacity of the sandy soils. Diversity (species richness) effects might be stronger in low productive nutrient and species poor grasslands than in the agriculturally managed grasslands, where plant species could be functionally more redundant. This might explain why increasing species number was accompanied by higher productivity in the natural, but not in the managed grasslands, at across-site scale. On the other hand, it may also be that there is no causal relationship between the higher biomass values and the higher species richness values in our natural grasslands and that the observed positive relationship might just be due to the fact that the higher species numbers in the more productive plots results from a better species establishment in these plots after disturbance (Laird et al., 2003).

Conclusions

A reconciliation of the results obtained from the experimental and the observational approaches might enable us to understand the factors that regulate the relationship between biodiversity and ecosystem functioning as well as expand our current knowledge of conservation and ecosystem processes in natural ecosystems. We think that a single general diversity-productivity relationship does not properly characterize the natural vegetations, thus biotic and/or abiotic factors might affect this relationship significantly. We suggest that future experiments should be continued in natural vegetations and that the investigated sites should be pre-classified according to their differences in ecological, compositional, or environmental characteristics. There is a need to include environmental factors in the future studies (rather than to control them) in order to investigate their effects on the biodiversity-productivity relationship.

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RURAL ROADS AS BARRIERS TO THE MOVEMENTS OF SMALL MAMMALS

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Abstract. Over the last 50 years increasing fragmentation of habitats has occurred due to road building. Fragmenting a large population into smaller ones can have a negative effect on population demography. In England much small mammal habitat occurs in suburban and rural areas where the majority of roads are either single track or two lanes, and traffic density is light. This study investigated the effect of minor roads on the movements of two small mammals, the wood mouse (*Apodemus sylvaticus*) and bank vole (*Myodes glareolus*). Capture-Mark-Recapture was used to study movements beside and across rural roads of two different widths. The results suggest that even small roads less than 6metres wide, with relatively little traffic, do act as partial barriers for these small mammals, reducing their movement between habitats on either side of the road. It is evident that island populations are being created as a result of road construction, even access roads within nature reserves.

Keywords*: habitat fragmentation, barrier, wood mouse, bank vole, animal movements*

Introduction

Fragmenting a large population into smaller units has a negative effect on population demography (Fischer and Lindenmayer, 2000). The degree to which this fragmentation affects animal survival depends upon both the size of the fragments and the degree of isolation imposed on their inhabitants. Habitat fragmentation may deplete a large and varied gene pool, increasing the chances of deleterious mutations becoming fixed within the populations and reducing fecundity due to inbreeding depression. Balanced immigration and emigration are likely to be compromised, increasing the risk of local extinctions. Vulnerability to extinction for a given population is negatively correlated to the size of that population (Goodman, 1987; Lande, 1993), therefore with smaller habitat fragments and hence smaller populations, the degree of isolation is crucial. The concept of metapopulations suggests that, if there are no barriers between population fragments, the risk of extinction is much reduced (Hanski, 1999). If however the intervening space between fragments acts as a barrier to movement the risk of extinction is enhanced.

Over the last 50 years increasing fragmentation of habitats has occurred due to development and road building (Gerlach and Musolf, 2000). In many parts of Europe roads have created a fine network of “islands”, averaging less than 3.2km^2 in size (Mader, 1984). In 1985 the UK had 365000km of roads, by 2009 this had risen to approximately 394000km, of which 87% are classed as ‘minor’ (B and C) roads (Dept. for Transport, 2011). As more roads are constructed, so more habitats become fragmented into smaller patches, many of which consist entirely of roadside verge. Although verges offer wildlife habitats (Adams and Geiss, 1983; Way, 1977), they are potentially isolated islands if the road itself forms a barrier to movement. For small mammals any movement across roads potentially places an individual in danger not only from traffic, but also from predators. Sikorski and Bernstein (1984) showed that parts of a population inhabiting territories on either side of a road differed in certain non-metric dental and cranial features. Since then a number of studies of genetic differentiation have found that roads have a negative effect on genetic diversity in many animal species (Holderegger and Di Giulio, 2010). This seems to indicate that roads act as total barriers for some small mammals, creating island populations and increased extinction risk.

Oxley et al. (1974) suggested that small forest mammals were reluctant to venture on to road surfaces where the distance between forest margins exceeded 20 metres. Mader (1984) found that none of the 121 yellow necked mice (*Apodemus flavicollis*) he had marked crossed a highway 6m wide. However Bakowski and Koziakiewicz (1988) showed that a 5m wide gravel forest road, whilst not a barrier to *A. flavicollis*, did act as a barrier for *Clethrionomys glareolus*. Whatever the reason for not crossing a road, reduced interaction between populations may have far-reaching effects, such as reduction or even loss of gene flow. Roads may also disrupt social organisation: Mansergh and Scotts (1989) demonstrated that survival rates of the mountain pygmy-possum (*Burramys parvus*) had been reduced by road construction. The road was found to act as a barrier to dispersal of males; reducing the over-winter survival rate of females. Over time this could have led to the extinction of this rare Australian mammal, which had previously only been known from fossils. Two tunnels were built under the road at strategic points, allowing the social structure to resume its normal pattern and the over winter survival rate of the females recovered.

In England many of the habitats suitable for small mammals are found in suburban and rural areas where the majority of roads are either single track or two lanes with a hard surface, and traffic density for most of the time is light. It is important for reasons mentioned above that we should know whether we are increasingly creating isolated island populations by building more roads. This applies not only to these public roads but also access roads within forestry areas and nature reserves. It is therefore important to understand the degree of isolation that roads impose upon the inhabitants of habitat fragments. The present study therefore investigated the extent to which even small roads (DoT classification B & C) compromise animal movements, implying that wider roads (and those with a physical barrier along their centre or margins) act as increasingly severe obstructions.

Methods

The wood mouse, *Apodemus sylvaticus,* and the bank vole, *Myodes glareolus,* were chosen as study species due to their frequent occurrence in roadside verges, and therefore their potential vulnerability to isolation effects from roads. Two study sites were chosen with road widths of 6m and 2m, both with verges of equal or greater width than the road itself and with both study species at similar population densities indicated by approximately 10 captures per 100 trap nights effort. In addition both sites had similar amounts and types of vegetation on either side of the road, and had minimal disturbance by pedestrians and vehicles

Site A was in Denham, Buckinghamshire (51.560620N, -0.502567W). This was a 6m wide road with a tarmacadam surface. The verges either side were approximately eight metres in width and bordered by pasture land. The fields on one side were separated from the verge by a continuous wire fence and on the other by a dense hawthorn hedgerow. The vegetation on both sides was similar, consisting mainly of *Arrhenatherum elatus* and *Rubus fruticosa*. Traffic was mainly between 08.00-10.00 and 17.00-19.00, with approximately 20 vehicles per hour during these periods. A total of 120 Longworth traps were set in six transect lines, three on each side of the road parallel to it and 3m apart. Each trap line consisted of 20 traps at 5m intervals.

Site B was a 2-2.5m wide road with a similar hard surface in Langley, Berkshire (51.523933N, -0.516744W). The road had verges of 3m width either side, bordered by pasture land. The verges on both sides were separated from the agricultural land by a continuous wire fence. Again the vegetation either side was similar with a predominance of *Hedera helix* and *Rubus fruticosa,* but, unlike site A, both sides of the road were lined by tall trees which created a continuous canopy over the road in some places. Traffic was very light with the busiest times being 07.00-09.00and 17.00-19.00 with an average of 5 vehicles per hour during those periods. A total of 100 Longworth traps were set in four transect lines 3m apart, two either side of the road and parallel with it. Each line consisted of 25 traps placed 3m apart.

Trapping was carried out during June, July and August. The traps were supplied with hay as bedding material, adequate food and baited with fried bread. They were checked twice daily at 06.30 and 18.30. The Capture-Mark-Release (CMR) method was employed. Each animal captured was marked by fur clipping (Gurnell and Flowerdew 1995) and species, sex, breeding condition, weight and place of capture were all recorded. A total of 6600 trap nights effort was employed, 3600 at site A, and 3000 at site B. For the first 3240 trap nights at site A and 2700 at site B, animals were released at the point of capture, whilst for the remaining period animals captured (other than pregnant or lactating females) were translocated to the opposite side of the road to see whether they would cross the road to return to their point of capture. The null hypothesis was that there would be no significant difference between the frequency of movements across the road, compared with movements over similar distances within the verge habitats.

Results

The numbers of animals captured, marked and re-captured per 100 trap nights effort for both sites are shown in *Table 1.* Animals captured at both sites were predominantly adults in breeding condition (71% at site A and 69% at site B).

Table 1. *Number of marked animals, captures on side 1 and 2 and catch per unit effort at Site A (3600 trap nights effort) and B (3000) trap nights effort*

	site	**sex**	**No. of marked individuals**	**No. of captures**	**Catch per 100 TNE**
A. sylvaticus	A	Male	33	238	6.611
	A	Female	23	113	3.138
	B	Male	23	197	6.566
	B	Female	19	109	3.633
M. glareolus	A	Male	32	194	5.388
	A	female	36	329	9.138
	B	Male	28	181	6.033
	B	female	26	142	4.730

At site A this generated a total of 608 recorded movements of which 377 (62%) were equivalent to or greater than the width of the road. Of these, 158 involved *A. sylvaticus* and 219 *M. glareolus*, with the number of recorded road crossings being 4 and 8 respectively. At site B a total of 419 movements were recorded, of which 395 (94%) were greater than the width of the road were recorded, but there were only 12 for each species involving road crossings. Two hundred and five movements involved *A sylvaticus* and 214 *M. glareolus*, with 12 recorded road crossings for each species. All classes of movement are presented in figure 1. Thus of all the 772 movements recorded, equivalent to the road width or greater, only 36 (4.7 %) involved actual road crossings.

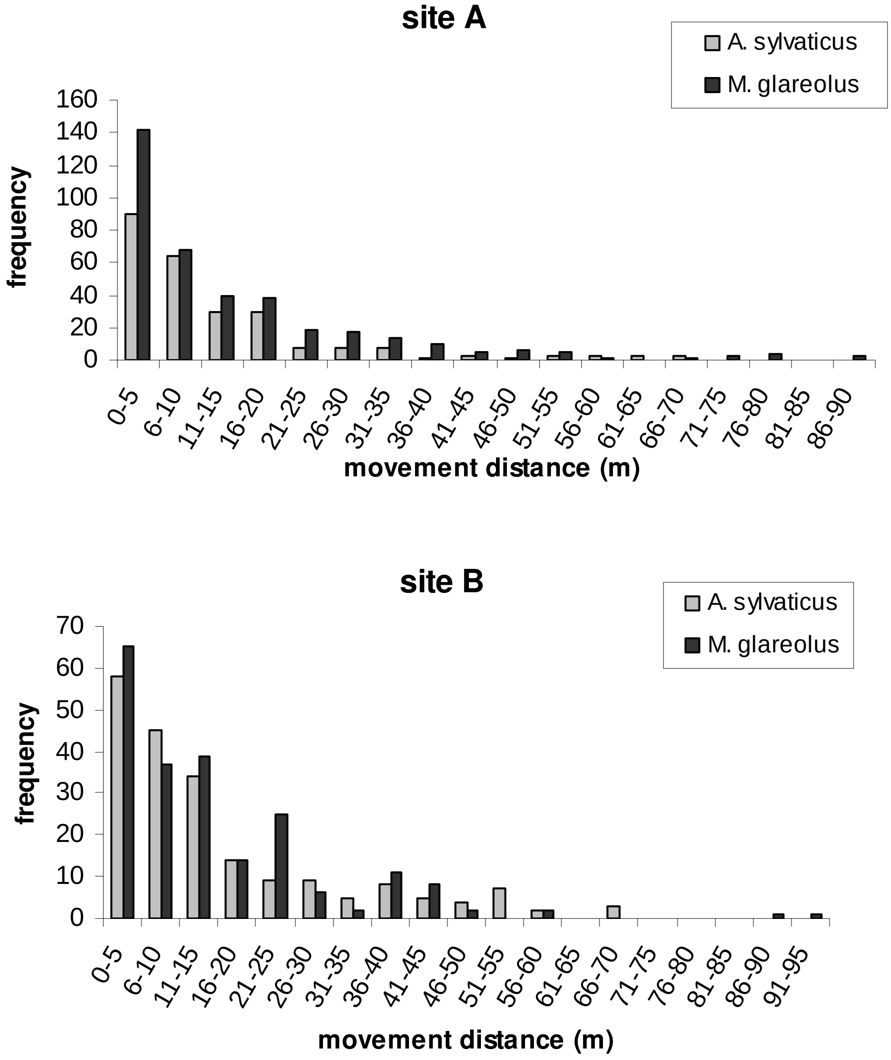

Figure 1. *Frequency of movements of different distances for A. sylvaticus and M .glareolus at site A (top) and site B (bottom)*

A binomial test was used to determine whether there was a significant difference between the number of movements equal to or greater than the width of the road within the verge and the number of movements across the road. In all cases there was a very highly significant difference and the null hypothesis was rejected (*Table 2*).

Table 2. *Movement data and results of binomial tests comparing numbers of recorded movements within verge and across roads*

Site	Species	Number of movements within verge ≥ width of road	Number of movements across road	Z statistic	P (2 tailed)
A	*A. sylvaticus*	154	4	11.85	<0.001
	M. glareolus	211	8	13.65	<0.001
B	*A. sylvaticus*	193	12	12.57	<0.001
	M. glareolus	202	12	12.91	<0.001

A binomial test was then used to investigate whether the verges on either side of the roads had similar numbers of marked animals of each species. For both *A. sylvaticus* and *M. glareolus* at site B there was no significant difference. However for site A the results indicate that there was a highly significant difference between the numbers of *A. sylvaticus* marked in side 1 and side 2 (*Table 3*) suggesting that the population on side 1 was smaller than that on side 2.

Table 3. *Numbers of marked animals and results of binomial test on differences in the numbers marked on each side of the roads*

Site	Species	Number of animals marked side 1	Number of animals marked side 2	Z statistic	P (2 tailed)
A	*A. sylvaticus*	18	38	-2.54	0.0111
	M. glareolus	22	34	-1.47	0.1416
B	*A. sylvaticus*	17	21	-0.49	0.6265
	M. glareolus	32	23	1.08	0.2807

We then tested the movement data to determine if there were any significant differences between species in the number of recorded movements equal to or greater than the width of the road. For site A a highly significant difference was found (z=-2.93; 2 tailed p=0.003). This result, plus the highly significant difference found in the numbers of *A. sylvaticus* marked (*Table 2*) suggests that, for *A. sylvaticus* at site A, the verge on side 1 was unable to support a population similar in size to that of side 2.

At site B the number of recorded movements equal to or greater than the width of the road was not significantly different between species (Z= -0.40; 2 tailed p=0.687) neither was there any difference in the numbers of marked animals found (*Table 2*) and, unlike site A, 50% of recorded crossings were made by *A. sylvaticus*. We also tested the hypothesis that there was no significant difference in the distances travelled between male and female animals within a species. The only significant difference was between male and female *A. sylvaticus* at site A (U=135,n_1=19, n_2=23, p<0.05). There was no significant difference between distances travelled by *M.glareolus* and *A. sylvaticus* at site A or site B. Movements across the road by both species are shown in *Figures 2* and *3*.

It can be seen from these plots that, whilst crossings by *M. glareolus* cover the length of the trap grid at site A, those of *A. sylvaticus* (although only two animals were recorded crossing) are concentrated at one end, yet this species occurred in all areas of both trapping grids. In addition 75% of crossings recorded for *M. glareolus* were from side 2 to side 1, whilst the opposite was true for *A. sylvaticus* with 75% from side 1 to side 2. Only 28.5 % of animals recorded crossing the road were males (all males recorded crossing being *M. glareolus*), but these accounted for 41% of the crossings recorded this being due to only one female being recorded making multiple crossings. Of all animals recorded crossing at site A only one was sub-adult, all other animals involved were adults in breeding condition weighing >20g

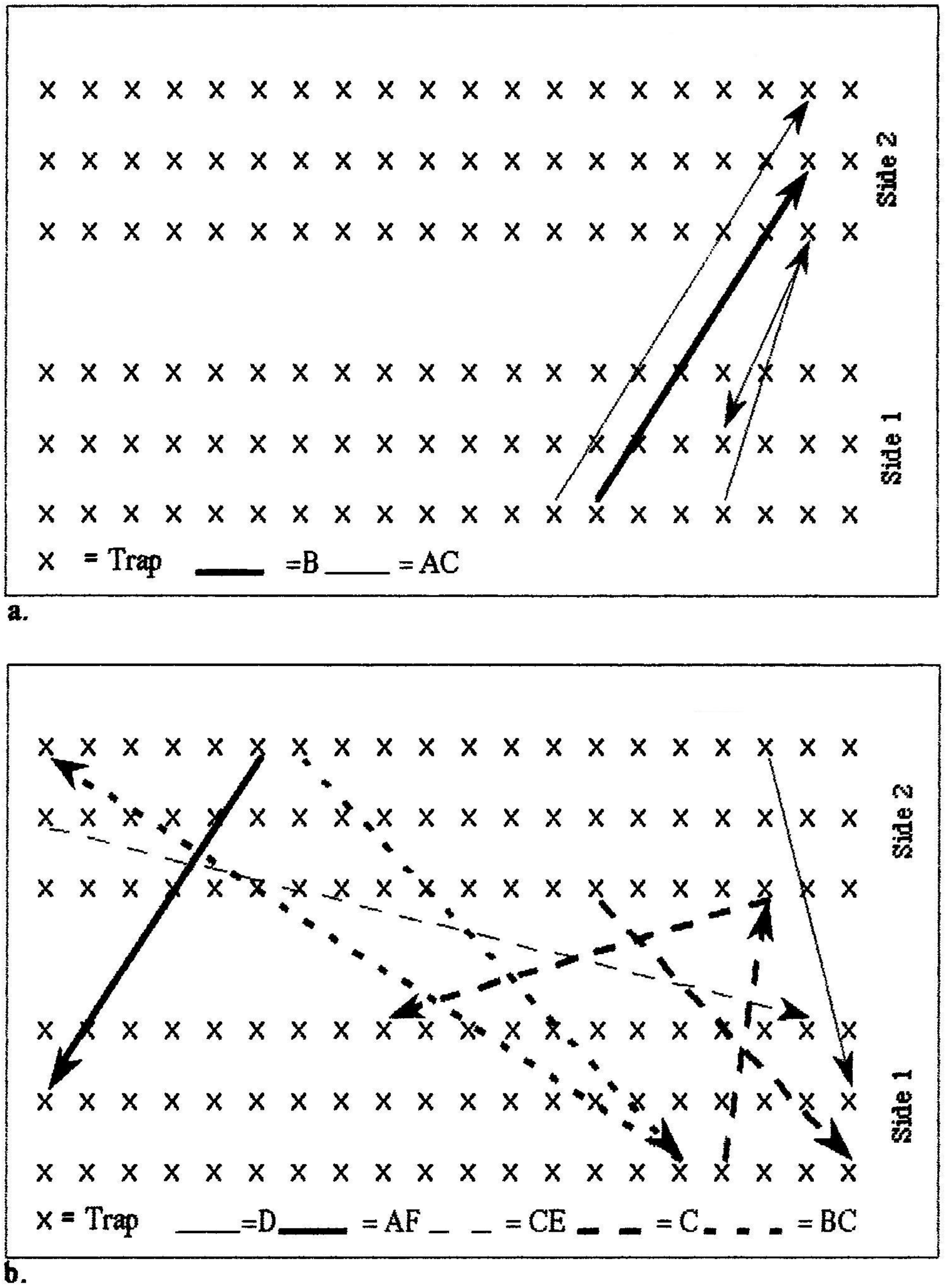

Figure 2. *Diagrammatic representation of recorded movements across road at site A by A. sylvaticus (top) and M. glareolus (bottom).*

Unlike site A, crossings at site B were recorded along the length of the trap grids (*Fig. 3*). Crossings were approximately equal in both directions for both species. *M. glareolus* accounted for 60% of the animals recorded crossing but only 50% of the crossings. Males accounted for 80% of the animals involved in crossing with one animal (*A. sylvaticus*) accounting for 29% of the crossings. Two animals crossing at site B were sub-adults (one male and one female *M. glareolus*).

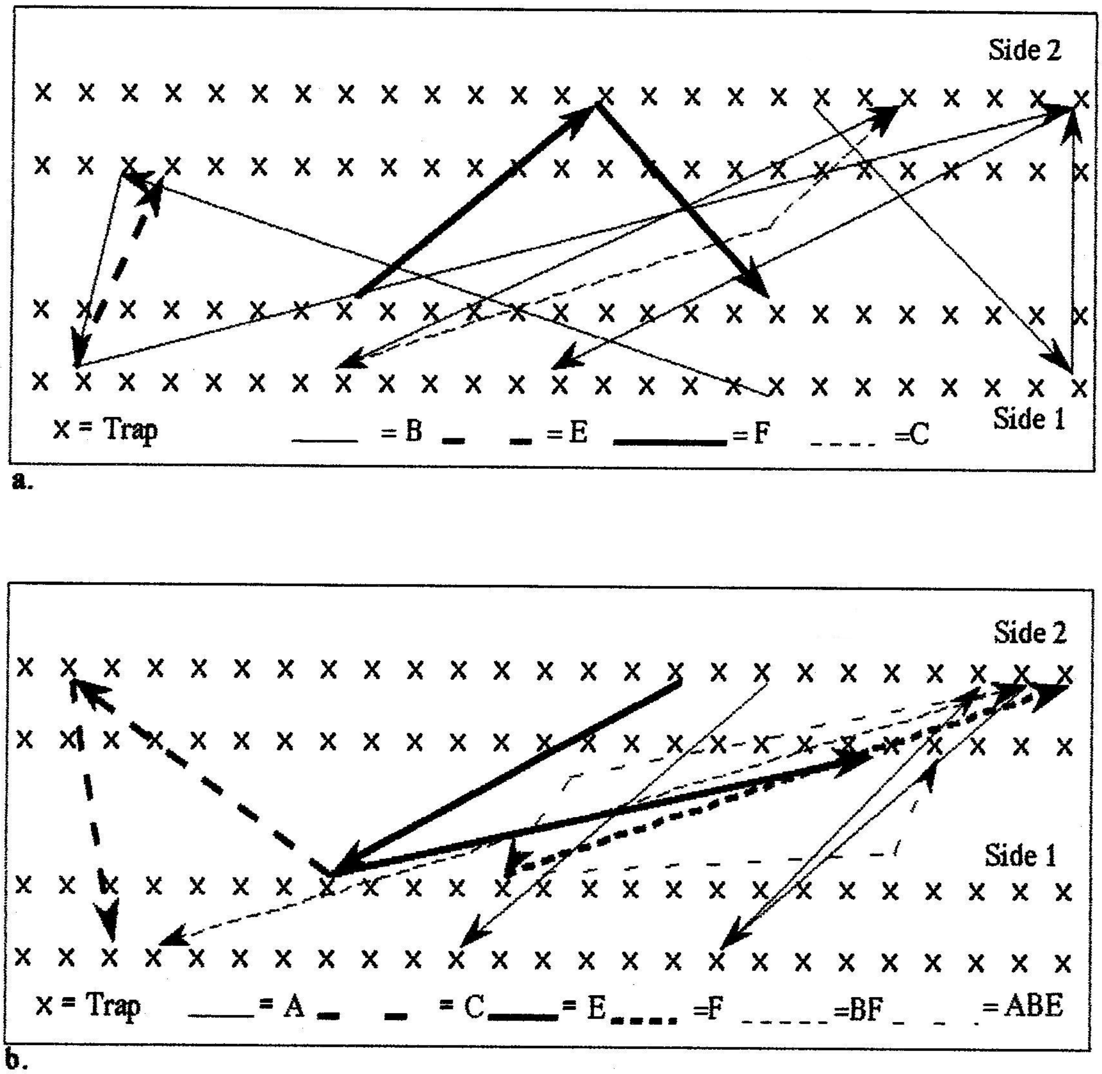

Figure 3. *Diagrammatic representation of recorded movements across road at site B by A.sylvaticus (top) and M.glareolus (bottom)*

Only a small proportion of the total population made road crossings, 7% of marked *A. sylvaticus* and 12% of marked *M. glareolus* at site A, and at site B comparable figures were 28 % for *A. sylvaticus* and 22% for *M. glareolus*.

Table 4. *Details of all animals involved in recorded road crossings at sites A and B*

site	Species	mark	sex	Weight (g)	Breeding condition	Number of crossings recorded	Total no. of captures
A	*A. Sylvaticus*	B	F	29	Perforate	1	11
		AC	F	>30	Pregnant	3	5
	M. glareolus	D	F	25	Perforate	1	3
		AF	F	25	Perforate	1	6
		CE	F	25	Perforate	1	8
		C	M	28	Scrotal	3	13
		BC	M	16	Abdominal testes	2	6
B	*A. Sylvaticus*	C	F	25	Pregnant	2	9
		B	M	27	Scrotal	7	21
		E	M	22	Scrotal	1	6
		F	M	23	Scrotal	2	26
	M. glareolus	ABE	F	15	Imperforate	2	6
		A	M	28	Scrotal	3	18
		C	M	25	Scrotal	2	11
		E	M	14	Abdominal testes	2	10
		F	M	24	Scrotal	1	5
		BF	M	24	scrotal	2	4

To investigate whether the breeding condition of the animals affected the type of animal crossing the roads, the number of breeding (scrotal, perforate and pregnant) and non-breeding (abdominal and imperforate) animals recorded (*Table 4*) as crossing the road was compared to the number of breeding and non-breeding marked animals within the verges, which had been recorded as having travelled distances equal to or greater than the widths of the roads but had not been recorded crossing the road (*Fig. 4*).

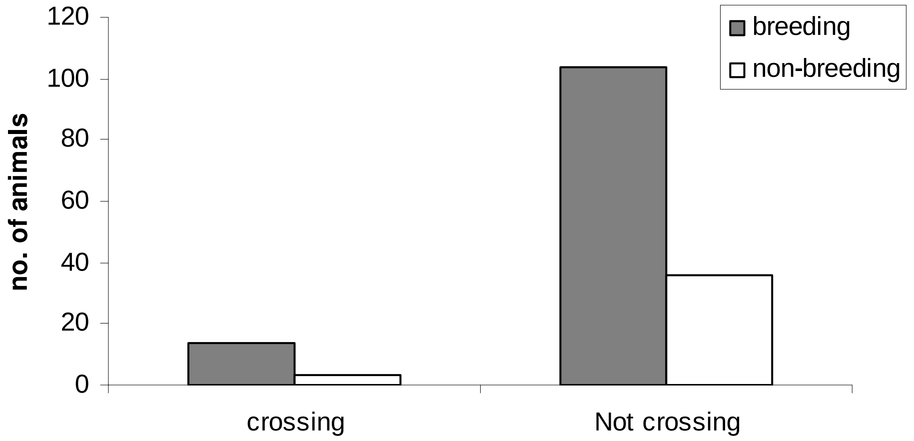

Figure 4. *Comparing the numbers and breeding condition of animals recorded crossing the road with those recorded as having travelled distances ≥road width within the verge but not crossing the road*

There was no significant difference between the numbers of breeding and non-breeding animals travelling distances greater than or equal to the width of the road within the verge, and those crossing the road (χ^2= - 0.53, d.f.=1, right tail *p*=0.46). This suggests that breeding condition is not a significant factor affecting whether animals cross the road or not.

Forty nine animals were translocated from their capture site to the opposite side of the road, 33 at site A and 16 at site B, of which 11 (33%) and 5 (31%) respectively were recorded as returning to the side of the road where they were originally captured (*Fig. 5).*

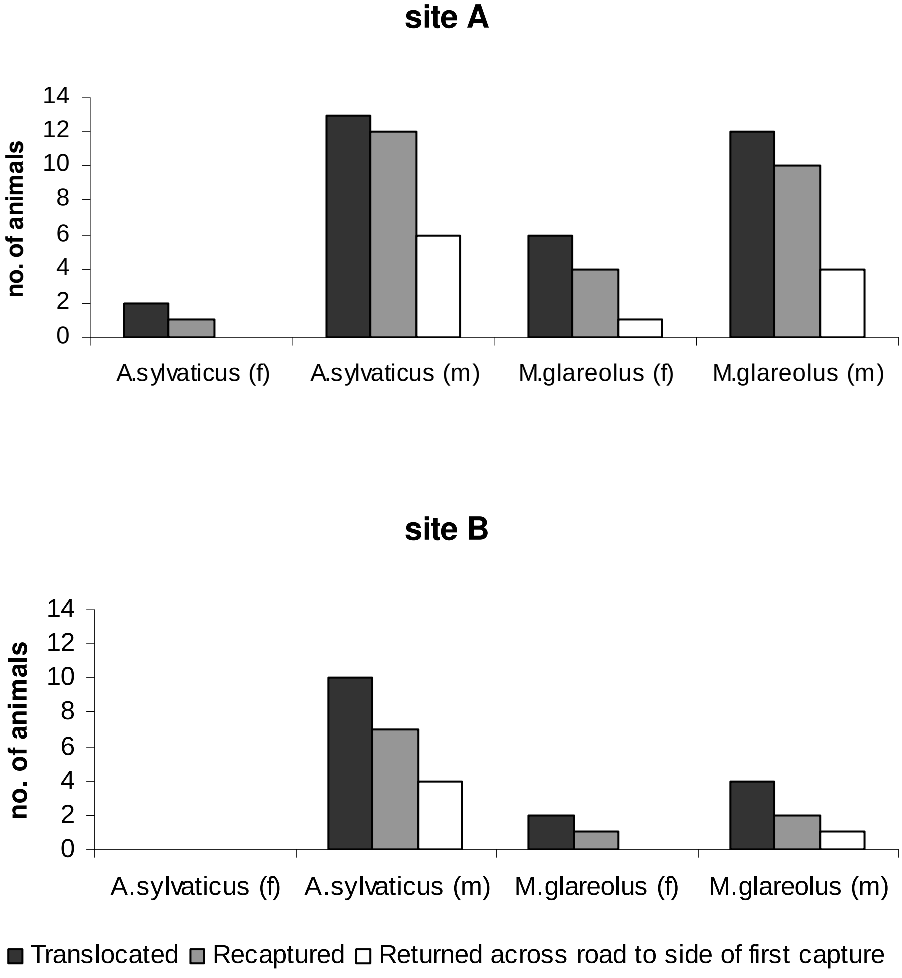

Figure 5. *Numbers of animals translocated, recaptured, and crossing the road to the side from which they originated, broken down by species and sex*

Overall 37 (75.5 %) translocated animals were recaptured, but only 16 (43.2%) of those recaptured had crossed the road to return to the side on which they had been captured.

At site A the difference between the numbers of *A. sylvaticus* and *M. glareolus* crossing the road to their original side was significant (χ^2=5.25, df=1, *p*=0.022), but no such difference between the two species was found at site B (χ^2=0.476, df=1, *p*=0.49). Similarly, when the data for both species were pooled there was no significant difference between sites A and B in the total number of animals crossing back (χ^2=0.10, df=1, *p*=0.92), suggesting that greater road width at site A was not a disincentive to crossing.

Discussion

This study was designed to investigate whether small rural roads act as a barrier to the free movement of small mammals. Therefore as far as possible factors that may have influenced movements were minimised. By having similar and adequate vegetation on both sides of the road, and choosing roads with low volumes of traffic, little pedestrian use and avoiding periods of verge management, it was possible to minimise the effects of territory, food supply and vehicle disturbance.

A significant difference in the number of *A. sylvaticus* found either side of the road at site A suggested that differences existed between these verges. This might have been due to the vegetation, but the only statistically significant vegetational difference was found to be at site B (possibly because side 2 had a raised bank with a slope of 1:2). However, the plant data only allowed for species abundance and did not take into account the amount of each species that was present, or the three dimensional area they produced. Observations at site A suggest that, especially for *Rubus fruticosa*, this three dimensional area was greater (c40%) on side 2 than side 1. The verge on side 1 also differed in that a small (15x6m) but densely wooded area created space with little ground cover. These differences between the verges may account for the small number of *A. sylvaticus* marked in this verge (side 1) and the fact that only one observed crossing for this species was towards this side.

No other significant differences in the numbers of animals marked on either side of the road were found, indicating that for *M. glareolus* at site A and for both species at site B, both verges at each site were capable of supplying the needs of the resident small mammal populations. Therefore unless the population density reached levels that one side of the road could no longer support, any crossing movements recorded were likely to be normal and possibly regular events. These are probably more important in terms of continuous gene flow than the passing through of migrating sub-adults.

All animals involved in the study were capable of travelling distances equivalent to or greater than the width of the roads, yet there was a very highly significant difference between the number of animals travelling these distances within the verge and the number recorded crossing the roads, indicating that the road is to some extent an obstacle. As no significant difference in movements was found between *A. sylvaticus* and *M. glareolus* their behaviour in respect of the road is evidently similar and the species will only be considered separately where necessary.

The number of animals that crossed the road was very small (5.6 % of all marked animals at site A and 10.4% at site B). As the study was carried out during the breeding season the majority of road crossing was expected to involve sub-adult animals dispersing in search of territory and would probably be one-way movements, but this was not supported by the data. At site A the majority (57%) of animals crossing the road were perforate females making a single crossing, and the remaining 43% consisted of one pregnant female and two males, only one of which was in breeding condition. All three made multiple crossings. No perforate females were recorded crossing at site B although 80% were adults and the remaining 20% were non-breeding sub-adults. With the exception of two scrotal males, all those that crossed the road were recorded making multiple crossings, yet no significant difference in the distance travelled by adults and sub adults for either species was found. The trapping dominance of adult animals may have been due to the inherent problems of live trapping with single capture traps such as the Longworth, which have been shown to be biased towards adults (Montgomery, 1979). Approximately 80% of trapped animals at both sites, and of both species were adults conforming to other studies such as Gliwicz (1970) and Jensen (1975) which have shown that trappability is highest in mature animals. Additionally the majority of the crossings recorded were made by *M. glareolus*, which is surprising since *Apodemus* is generally considered to be the more wide-ranging species. Our results show that individuals of both species were able to cross the roads at both sites, yet few were recorded actually doing so. The fact that at both sites, and for both species, crossings did occur confirms previous findings that road surface is not a critical factor, (Oxley et al., 1974). Traffic may also be discounted due to low volume and the high occurrence of animals in traps close to the road edge at site A, where the highest volume of traffic was recorded. “Road clearance”, an area devoid of refuges and cover, is suggested as the most important inhibiting factor (Bider, 1968; Oxley et al., 1974). Data from the present study also suggest that it is the lack of cover that inhibits crossings, in that both species seem to prefer dense cover. This is indicated by the difference in numbers of animals captured in the inner and outer lines at site B, where cover in the outer line was light and patchy, compared to site A where cover was dense for all trap lines.

The crossing routes shown in figures 2 and 3 are probably unrealistic, as it is more likely that animals would use the shortest route possible. However the fact that crossings originate from both sides of the road, and the animals involved do not appear to head for one specific area, indicates that crossings are not for a single specific resource and similar results would be likely to occur at any point along the road.

A. sylvaticus are territorial during the breeding season (Flowerdew, 1991) and have a magnetic sense of direction which allows them to find their home territory (Mather and Baker, 1981). Adult *M. glareolus* are described as living within fixed home ranges to which they return when displaced (Karlsson, 1984). Therefore if translocated it might be expected that the majority, if not all the animals, would return. However, although three-quarters of our translocated animals were recaptured, only 43.2% were returnees, with *M. glareolus* producing fewer returnees than *A. sylvaticus*. This could, indicate that the road is a total barrier to part of the population as proposed by Bakowski and Kozakiewicz (1988), even when the instinct to return is strong. Mazurciewicz characterised *M. glareolus* as being highly sedentary, the reason suggested by Bakowski and Kozakiewicz (1988) for the low occurrence of recorded road crossings. However in tests with no barrier both Bovet (1978) (with *A. sylvaticus*) and Karlsson (1984) (with *M. glareolus*) found that about 30% of animals failed to return “home”.

The time of day when animals were translocated may also have affected their behaviour. Both Karlsson (1984) and Bovet (1960) suggest that sunlight disorientates bank voles, although this conflicts with the findings of another study (Mather and Baker, 1980) which showed that vision combined with olfaction were the major senses involved in navigation of up to 80m distance for both *A. sylvaticus* and *M. glareolus*. In the present study 71% of animals were translocated between 06.00 and 07.00 and the remaining 29% between 18.00 and 19.00. The time allowed for animals to return is also important. Durup et al. (1973) showed that homing from a distance of 25-30m was accomplished in about 36 hours by *M. glareolus*, and in about 50 hours by *A. sylvaticus*. Therefore the length of time allowed in the present study for the animals to return may have been insufficient, as 27% of our animals were translocated only 36 hours before the end of the study. Long distance movements and occasional excursions have been explained as being undertaken by dispersing animals and exploratory ventures out of home ranges. Such movements occur mainly in the reproductive season (Maza et al., 1973), which is when the present study was conducted. This could explain both the single (dispersal) and double (exploratory) crossings, but not, for example, the adult *A. sylvaticus* at site B recorded crossing seven times. This number of crossings by a single male may represent searching for suitable females, but, out of 19 adult males, this was the only one recorded making multiple road crossings.

Andrzejewski and Babinskawerka (1986) proposed that for *M. glareolus* territories are much larger than many suggest. If so, it may be that some territories included parts of the opposite verge. This could explain multiple crossings by adult animals and indicate that the road is not perceived as a barrier. This has been discussed in the context of home ranges by other authors (Szacki et al., 1993; Vasilev et al. 1996) and may explain why only part of a population is involved in crossing roads.

It is important to remember that studies such as the present one are limited and that there will be many gaps in the information gained. For example the distances travelled were calculated from two consecutive captures, but this may not be a true representation. The journey may have been cut short by the trap and the actual route taken by the animal is unknown. Many consecutive trappings were 48 hours apart or longer, and it is not known where the animal travelled during this time. If the animal crossed the road other than in search of food there is little incentive for it to enter a trap. Crossings may also be more frequent than indicated by trapping. During a three-hour study at site B starting from dusk, four crossings were registered by use of an infra-red night scope. It was not possible to identify how many individuals were involved by this method, but it does suggest that crossings may be more common than shown by trapping.

The use of single-entry traps has many limitations and the data generated from their use suffers from pseudo-replication. However this study has shown that rural roads of 6m or less in width are not a total barrier, but are perhaps treated more as a boundary, which is crossed by members of all categories of the two species studied. Although a difference in the number of crossings was recorded between site A and site B suggesting that the width of the road made a difference, it is thought that this is more likely to result from the difference in population levels of *A. sylvaticus* found between side 1 and side 2 at site A. Nevertheless it is clear that small mammals appear reluctant to cross roads, even narrow ones like these.

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SEASONAL CHANGE OF ORIBATID MITE COMMUNITIES (ACARI, ORIBATIDA) IN THREE DIFFERENT TYPES OF MICROHABITATS IN AN OAK FOREST

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Abstract. Oribatid mites are one of the most abundant groups of the ground-dwelling mesofauna. They can be found in almost every terrestrial habitat all over the world and they are characterized by great species richness and great number of individuals. In spite of that not enough is known about their behaviour on community level and their spatial and temporal pattern in different habitats of the world. In our present study the seasonal behaviour of oribatid mite communities was analysed in three types of microhabitats in a temperate deciduous forest: in leaf litter, soil and moss. Samples were collected at a given site in a year and a half and the oribatid mite communities living there were studied on genus level along with the changes of meteorological factors characteristic of the area. The results show that corresponding to similar previous researches, the communities in our study do not have a seasonally changing, returning pattern either. Based on this, we can conclude that climatic differences and differences in other seasonally changing factors between the seasons do not have a significant role in the annual change of communities. Besides that we discovered that the communities of the three microhabitats are not completely the same. It is the oribatid mite community of the moss which differs mostly from communities in the leaf litter and in the soil. Our study calls attention among others to the fact that compositional changes of the oribatid mite communities living all over the world and their causes are unclear to date.

Keywords: *oribatid mites, communities, microhabitats, seasonality, temperate deciduous forest*

Introduction

In most habitats oribatid mites account for the biggest part of microarthropods (e.g. Schenker, 1986; Johnston and Crossley, 2002). They can be found in most terrestrial microhabitats: in soil, leaf litter, moss, underwood, foliage and in aquatic habitats as well (Behan-Pelletier, 1999). They can be found mostly in great species richness and abundance in their habitats (Behan-Pelletier, 1999). They play a significant role in decomposition processes because they fragment the organic matter and influence the biomass and species composition of fungi and bacteria (Wallwork, 1983; Seastedt, 1984; Yoshida and Hijii, 2005). As this group plays a significant role in soil processes, it is necessary to get to know its spatial pattern and the causes of pattern generation, which can be used later for indication (Behan-Pelletier, 1999).

Several researches have already been done to detect the pattern and composition of oribatid mite communities and their exact causes. However, it is hidden to date by which mechanisms the structure and functioning of the individual communities are affected.

Climatic factors belong to the most determinant ones. The above statement is reflected by the number of researches as well, because most of the studies on oribatid mite communities investigate how the communities react to the meteorological factors changing naturally or artificially, especially to temperature and the amount of precipitation (Gergócs and Hufnagel, 2009). First it is worth investigating the effect of the natural changes of the climatic factors, and the most appropriate way to do this is to study the seasonal changes of the communities. The mistake in most of these studies is that the research on seasonality is conducted over a maximum of a year (e.g. Schenker, 1984), so it cannot be determined whether the observed phenomena occur similarly each year. The most significant research on this topic was conducted by Irmler (2006) in a beech forest in Germany. He investigated monthly changes of ground-dwelling oribatid mite communities over a seven-year period and found that there are no important changes among the communities.

Our research is similar to his one, however, it differs as well because we collected samples every two weeks and not once a month, and we investigated not one but three microhabitats over a one and a half year period in an oak forest in Hungary.

The other difference is that data on oribatid mites were recorded on genus level and not on species level. The decision on the genus level can be justified by several factors. The analysis of Caruso and Migliorini (2006) showed that there were not any important changes in the data when studying human disturbance on oribatid mites and changing from species level to genus level. Podani (1989) found a similar result in case of plants, namely, changing to genus level does not mean a significant difference when comparing the studied habitats. Osler and Beattie (1999), Hammer and Wallwork (1979) and Norton et al. (1993) concluded according to their studies that the many widespread genera and families indicate that the similarity between oribatid mite communities should be studied above species level. In their meta-analyses their assumption was confirmed that taxonomical levels above the species level are more suitable to compare habitats. According to this we also compared the data series recorded with time difference on genus level.

The aim of our study was to explore which seasonal changes occur in oribatid mite communities living in three types of microhabitats in an oak forest and what role the most important climatic factors that is the amount of precipitation and temperature have in these changes.

Materials and methods

Samples for our study were collected in 2005 and 2006 in a given quadrate of 100 m^2 in a dry oak forest near Törökbálint, Hungary (47°25’38” N, 18°54’16” E). In the sampling quadrate the most common tree species is turkey oak (*Quercus cerris*), common trees are field maple (*Acer campestre*), common ash (*Fraxinus excelsior*) and wild service tree (*Sorbus torminalis*). The most important herbaceous plants are broad-leafed Solomon's seal (*Polygonatum latifolium*) and garlic mustard (*Alliaria petiolata*). Samples were collected every two weeks from 15th March till 1st December 2005 and from 26th March till 30th July 2006. Every time samples were collected randomly from three types of substrate within the quadrate: from 500 cm^3 leaf litter, 300 cm^3 foerna (from under the leaf litter) and 0.5 dm^2 hypnum moss (*Hypnum cupressiforme*) living on tree trunks. So we obtained altogether 19 moss samples and 18 leaf litter and 18 foerna samples in 2005 (the first time no leaf litter and foerna samples were collected); in 2006 we collected 10 leaf litter, 10 moss and 9 foerna samples (the tenth sample could not be analyzed for technical reasons). Components of the mesofauna were extracted with the help of a modified Berlese-Tullgren funnel (Coleman et al., 2004) and conserved in isopropyl alcohol, then they were sorted into larger groups, and finally oribatid mites were identified on genus level. For the identification the works of Balogh (1965), Balogh and Balogh (1992, 1972), Balogh and Mahunka (1980), Olsanowski (1996) and Hunt et al. (1998) were used.

The samples were analyzed based on oribatid mite communities with the help of multivariate computer aided methods using the software Syn-tax 2000 (Podani, 2001). The size difference between the samples from the three types of substrate was compensated by comparing the ratio and presence-absence data of the oribatid mite genera with the help of non-metric multidimensional scaling and classification method using several distances. Monte Carlo method was used to check these analyses. The means of the genus proportion of the substrate types were calculated, each mean was multiplied by a number created by a random generator twenty times, then these new values were divided by the sum of the random numbers. This way twenty data series were generated per substrate. These were compared using classification and ordination. Correspondence analysis and PCA analysis were used to identify the genera which are responsible for possible differentiations. The difference of the genera in specimen number and proportion was checked using Mann-Whitney test between the substrate types. The average diversity and the genus number were calculated in case of each substrate type.

In order to recognize seasonality patterns, each season was marked (in winter no sampling was conducted), and the changes in genus diversity and total abundance during a period of the study were displayed separately in case of the three substrate types. The relationship between the pattern changes of the communities and meteorological factors was analyzed with the help of data series from the meteorological station in Pestszentlőrinc (47°25’53” N, 19°10’57” E). This station can be found 21 air km eastwards from our sampling point. In the case of precipitation the total precipitation amount of the sampling day and that of the preceding 5 and 10 days, and the standard deviation of the precipitation amount of the preceding 5, 10, 15, 20, 25 and 30 days were considered. In the case of temperature minimum and maximum values of the given days were available in our database, so in our analyses the means of these two values were considered. As for temperature data, the mean temperature of the sampling day and that of the preceding 5 and 10 days, and the standard deviation of temperature of the preceding 5, 10, 15, 20, 25 and 30 days were involved in our analyses. A redundancy analysis was conducted for all substrates first and then separately. In order to make the relationships more exact, the correlation between certain genera and meteorological factors was studied based on Spearman’s analysis.

Results

52 genera were found in the three types of substrate altogether, in leaf litter and foerna 48-48 genera and in moss only 30 occurred. According to the ordination and

classification (*Fig. 1*) it can be stated that oribatid mite communities of the leaf litter and foerna do not differ from each other but they differ from moss samples. In these figures it can be seen as well that the samples collected in different times are completely mixed, the different markings of the three seasons are also mixed without a pattern in the figures, which confirms our assumption that no seasonal effects can be found in the case of oribatid mite genera.

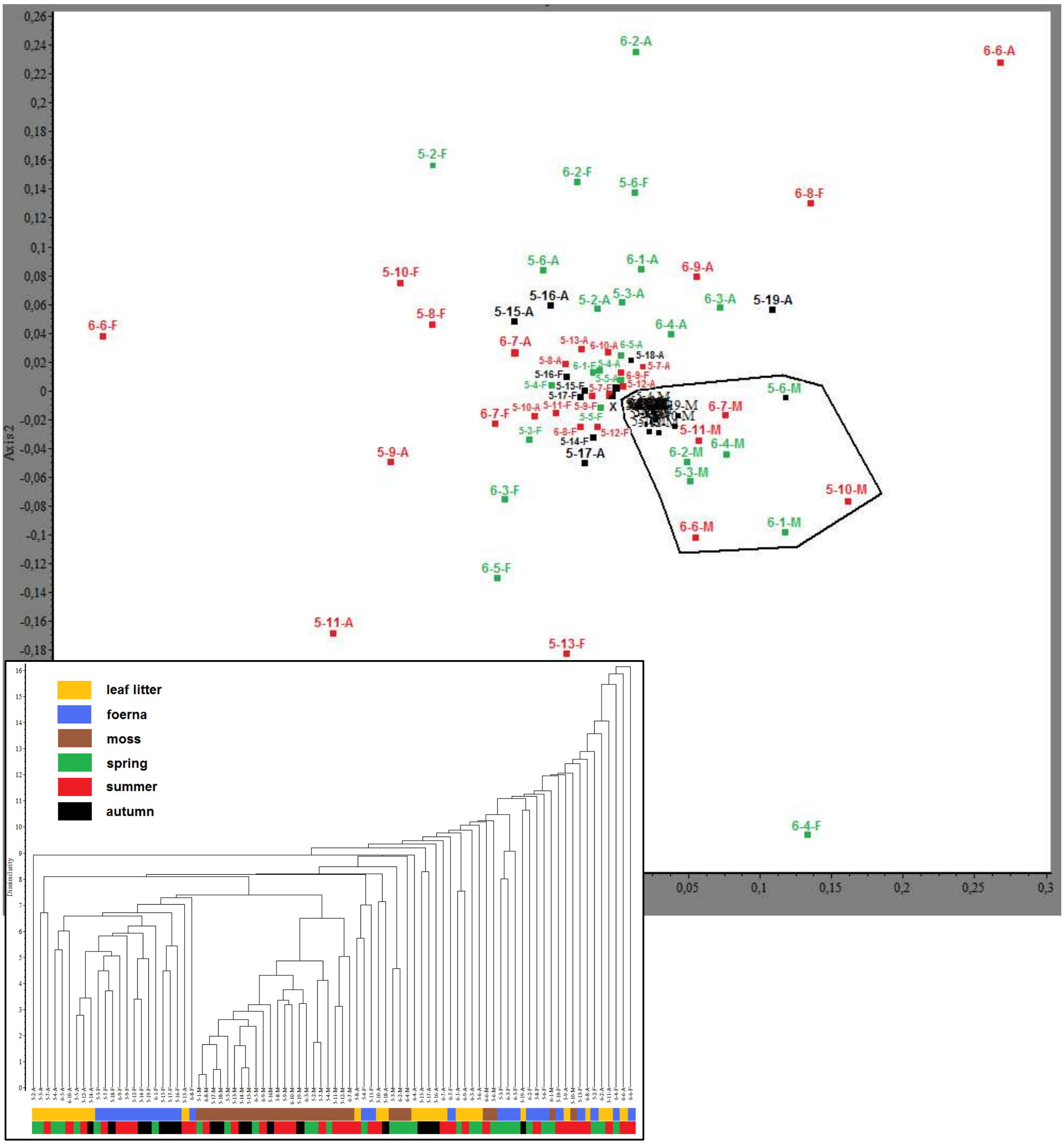

***Figure 1. Large figure**: Non-metric multidimensional scaling of the ratio of the genera and juvenile groups in the three substrates using Euclidean distance. The first number in the codes represents 2005 (5) or 2006 (6), the second one is the chronologic serial number of the samples and the third symbol in the code means the substrate. M=moss, F=foerna, A=leaf litter. Spring samples were marked with green, summer samples with red and autumn-winter samples with black colour. Moss samples are framed. X=5-14-A, 5-18-F, 5-19-F. **Small figure**: Classification of the same series using group-average method with Euclidean distance. Considered groupings were marked with a colour code based on substrate types and seasons*

Monte Carlo experiments conducted in order to check the above analyses can be seen in *Fig. 2*. It shows that leaf litter and foerna substrates also differ from each other, however, not to the same degree as from the moss substrate.

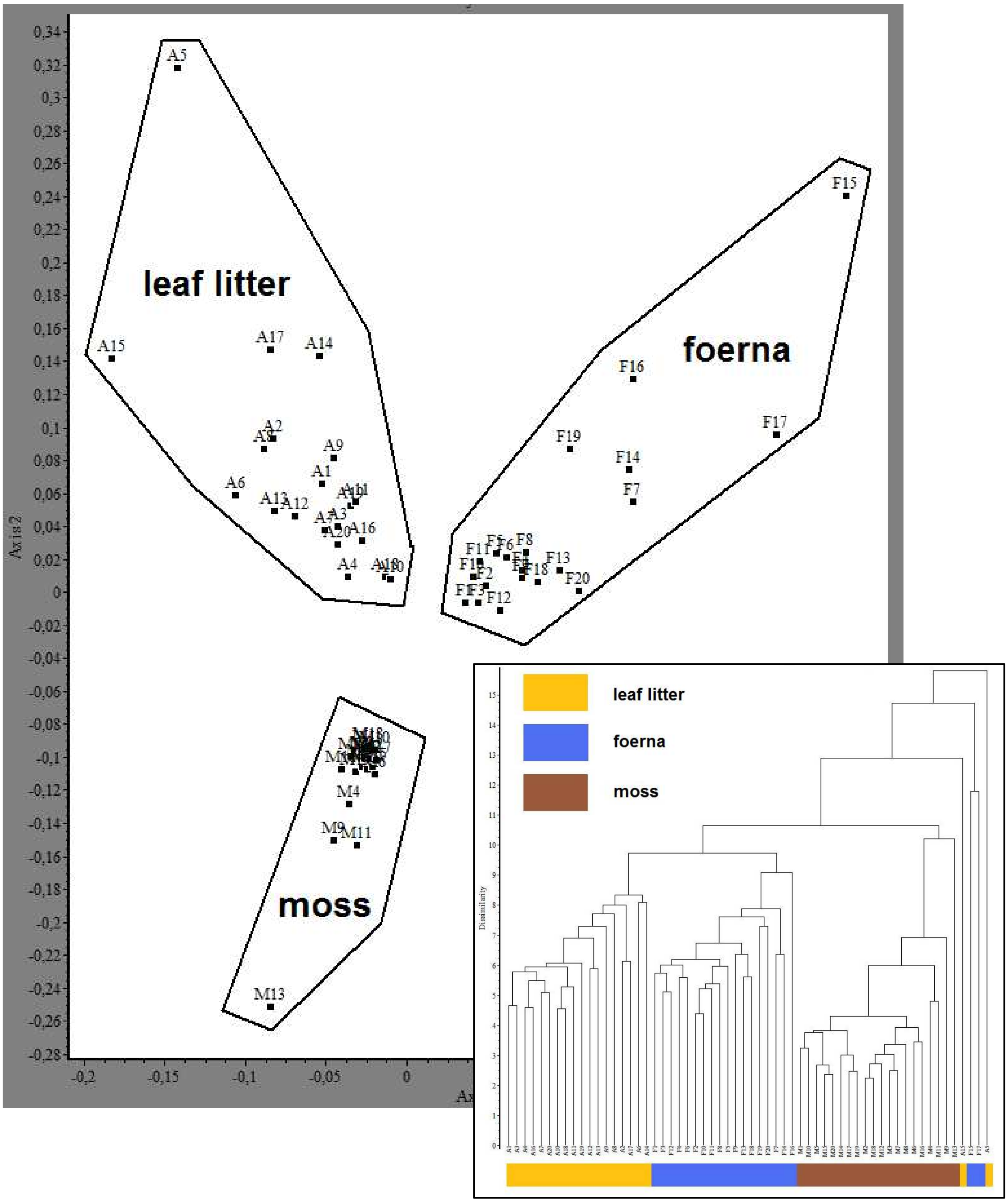

Figure 2. ***Large figure****: Non-metric multidimesional scaling of the samples created with Monte Carlo method from the ratios of the three substrates: M1-20; A1-20; F1-20 (standardised Euclidean distance).* ***Small figure****: Classification of the same series using group-average method with standardised Euclidean distance. Separation of the three substrate types was marked with a colour code*

Separation of moss samples is mainly caused by their smaller genus diversity and smaller genus richness and by the fact that the following genera: *Tectocepheus*, *Eremaeus*, *Zygoribatula* and the juvenile group, to which larvae and nymphs belong, are present in a larger number here (*Fig. 3*). In moss samples 7, in leaf litter 19 and in foerna samples 22 genera were present on average during a period of one and a half years. Genus diversity of the samples from all three substrates differ from each other significantly ($p<<0.01$). Moss samples have the lowest diversity and foerna samples have the highest one (*Table 1*).

Table 1. *Average diversity along with standard deviation in the three substrates during a period of one and a half years*

	Foerna	Leaf litter	Moss
Shannon mean (±standard deviation)	2.20 (±0.21)	1.871 (±0.34)	0.98(±0.26)
Simpson mean (±standard deviation)	0.82 (±0.07)	0.73 (±0.11)	0.52 (±0.10)
Average number of genera	21.7 (±4.5)	19.3 (±4.3)	6.6 (±2.7)

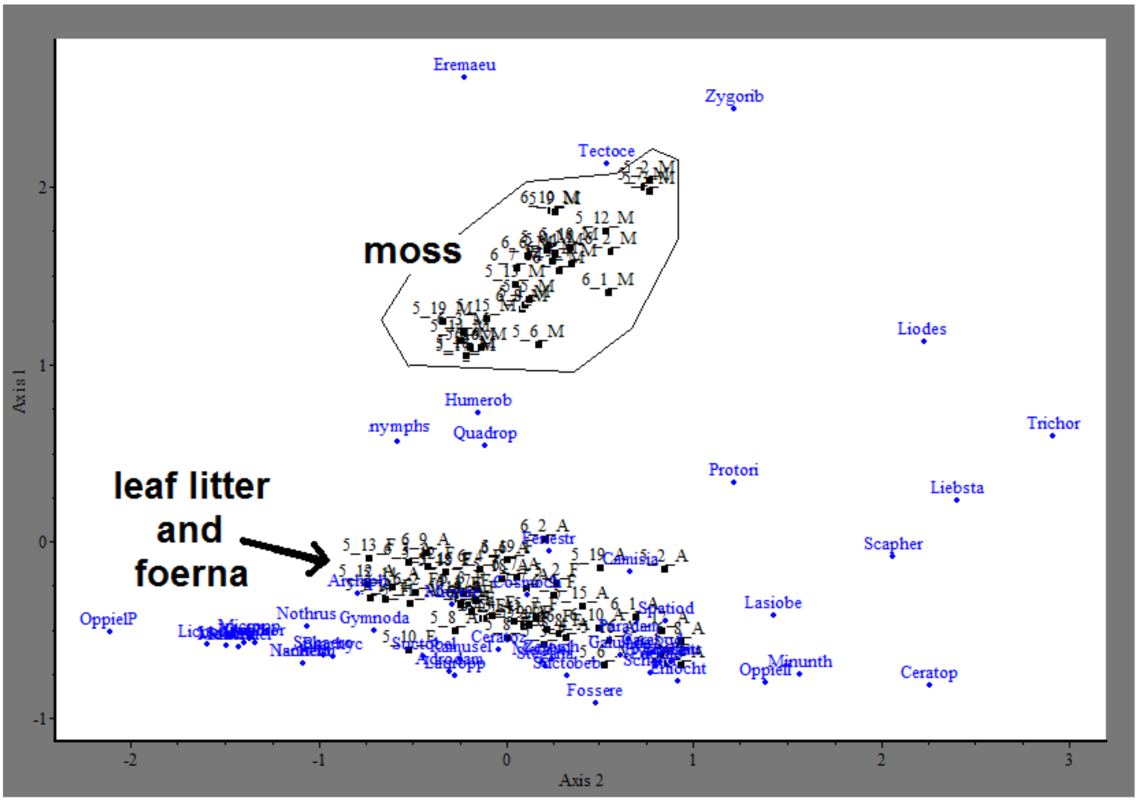

Figure 3. *Correspondence analysis on the ratios of the genera and nymphs (juvenile group) in the three substrates*

The proportions of the genera *Tectocepheus* and *Zygoribatula* and the juveniles in the three substrates can be seen in *Table 2*. The difference of these values in the three substrates was significant in all cases ($p<<0.01$). The genus *Eremaeus* occurred only in moss samples except for only one foerna sample, with 10.9 specimens on average, 15 times out of 29, but with rather varying abundance and proportion in time. Besides the above, two other genera near the moss group: *Humerobates* and *Quadroppia* can be seen in *Fig. 3*. *Humerobates* is a rare component, and in the case of *Quadroppia* no difference was found between the proportions compared to the other two substrates.

Table 2. *Proportions of Tectocepheus, Zygoribatula and the juveniles in the three substrates. The values in moss samples are highlighted because these values are significantly different from the values of the two other substrates.*

	Leaf litter	**Foerna**	**Moss**
juvenile group / total number of specimens	0.188±0.106	0.201±0.104	0.397±0.206
juvenile group / adults	0.249±0.174	0.280±0.169	0.919±0.775
proportion of *Tectocepheus*	0.020±0.037	0.017±0.020	0.261±0.170
proportion of *Zygoribatula*	0.017±0.023	0.005±0.004	0.230±0.187

The reason of the difference between foerna and leaf litter can be seen in the PCA analyses *(Fig. 4)*. *Table 3* represents in which genera the numbers of specimens and the ratios significantly differ between the two substrates. In all cases it can be shown that the genera displayed in *Table 3* are present with larger abundance and relative abundance in foerna samples.

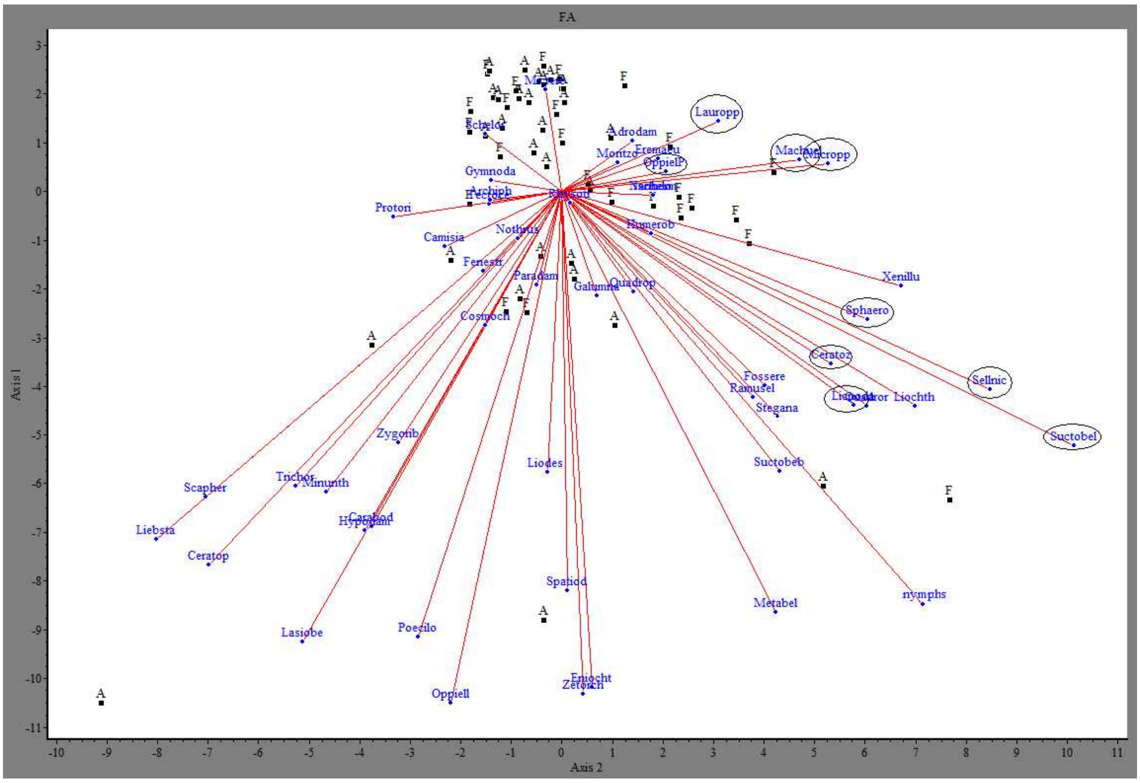

Figure 4. *PCA analysis with the ratios of the juvenile group ("nymphs" in the figure) and the genera of the leaf litter and foerna samples (standardised). A=leaf litter, F=foerna; the first 7 letters of the genera are displayed and the genera, the abundance and proportion of which significantly differ from each other in the two types of substrate, are circled*

Table 3. *Abundance and proportion of the genera causing the difference between leaf litter and foerna samples*

	with the numbers of specimens			**with proportions**		
genera	**leaf litter**	**foerna**	**p(same)**	**leaf litter**	**foerna**	**p(same)**
Sellnickochthonius sp.	0.8	2.3	0.028	0.0014	0.0043	0.025
Ceratozetes sp.	1.9	3.0	0.055	0.0048	0.0092	0.049
Lauroppia sp.	0.3	8.1	9.76E-08	0.0012	0.0250	3.06E-07
Licnodamaeus sp.	60.1	68.7	0.04	0.1323	0.1577	0.0604
Machuella sp.	0.1	2.4	1.05E-05	0.0002	0.0058	9.19E-06
Microppia sp	0.0	2.0	2.32E-05	0.0002	0.0048	0.000034
Oppiella (Perspicuoppia) sp.	0.0	1.2	0.017	0.0001	0.0030	0.018
Sphaerochthonius sp.	7.2	11.4	0.002	0.0200	0.0274	0.0006
Suctobelbella sp.	11.8	29.5	0.0002	0.0298	0.0750	4.95E-05

Seasonal changes could not be detected in the communities of the three substrates. These results can be seen on *Fig. 1* and *5-6.* The second number in the codes of the samples in *Fig. 1* (the large one) means the progress of time during the year. It can be observed well that the numbers do not form groups according to the date of sampling. This proves that the progress of time that is seasonality does not generate patterns in oribatid mite communities. In *Fig. 5* change in the Shannon diversity of the genera in the case of the three substrates and in *Fig. 6* change in abundance of the oribatid mites can be observed, however, a seasonal pattern recurring the following year cannot be detected.

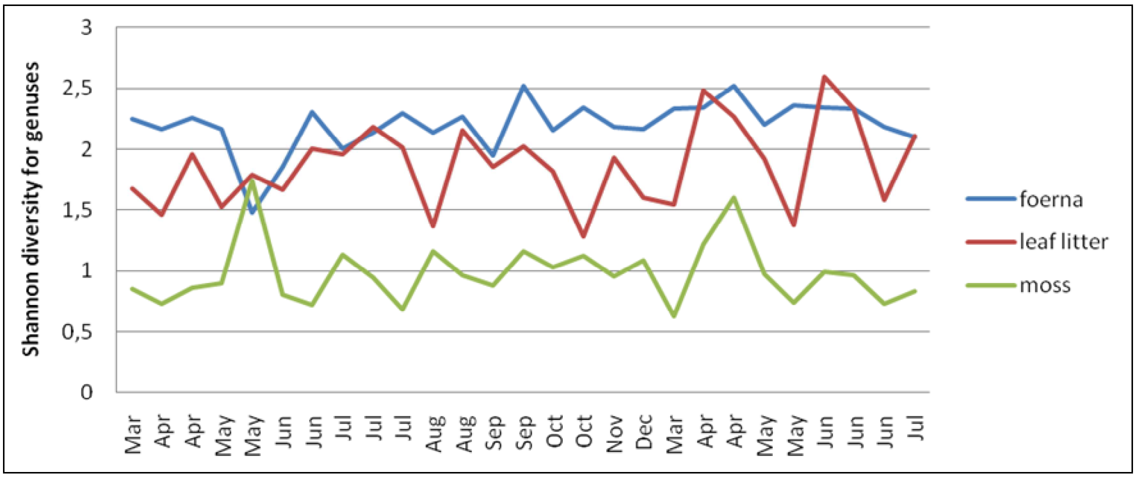

Figure 5. *Change in the Shannon diversity of the genera in the case of the three substrates (2005-2006)*

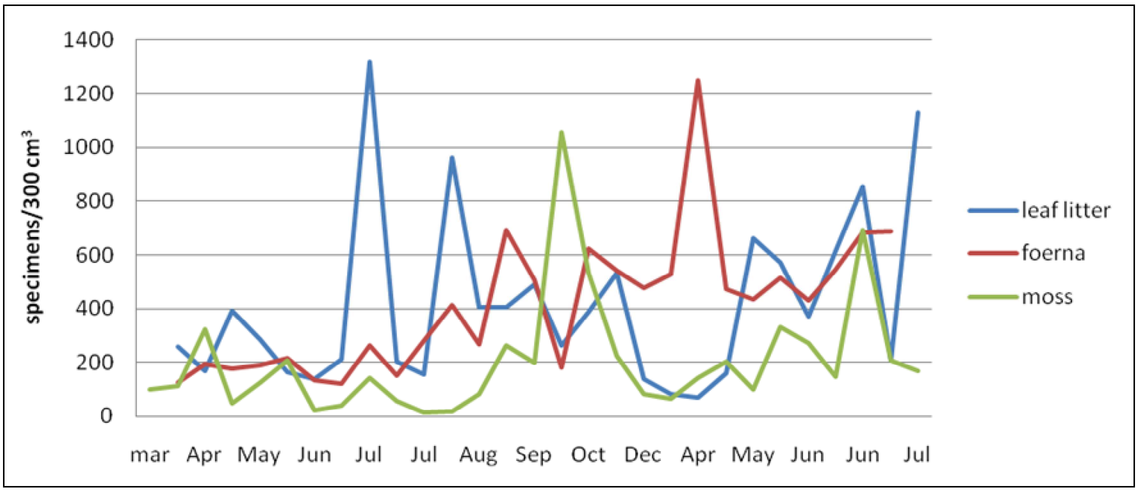

Figure 6. *Change in abundance of the oribatid mites (2005-2006)*

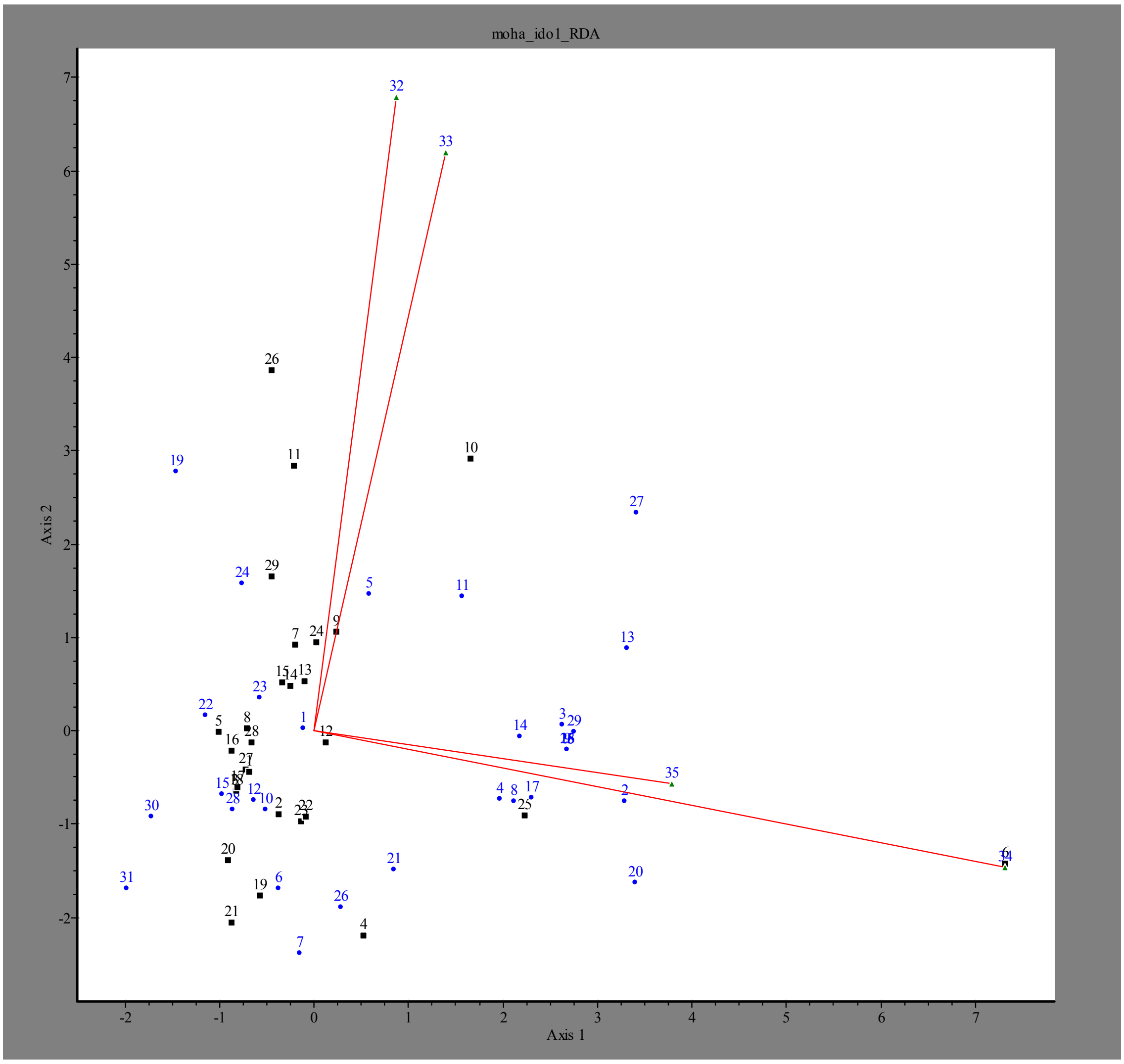

Figure 7. *Redundancy analysis of moss samples collected during one and a half year regarding the precipitation amount and mean temperatures. (32: mean temperature (of 5 days); 33: mean temperature (of 10 days); 34: total amount of precipitation (in 5 days); 35: total amount of precipitation (in 10 days). The first axis is for 46% and the second one is for 31%.)*

We have not found any relationships between the annual changes in precipitation amount and temperature and the composition and structure of oribatid mite communities in leaf litter and foerna, however, in the case of moss we have. The result of the redundancy analysis is displayed in *Fig. 7*. The location of the samples is affected by mean temperature to a larger degree than by the precipitation amount. This can be caused by the genus *Tectocepheus*, which is one of the most frequent genera in moss samples (43% of the adult specimens on average), and its correlation is r=0.38 regarding the five-day mean temperature (Spearman's correlation; p=0.043); and regarding the ten-day mean temperature its correlation is r=0.45 (Spearman's correlation; p=0.014) (*Fig. 8)*.

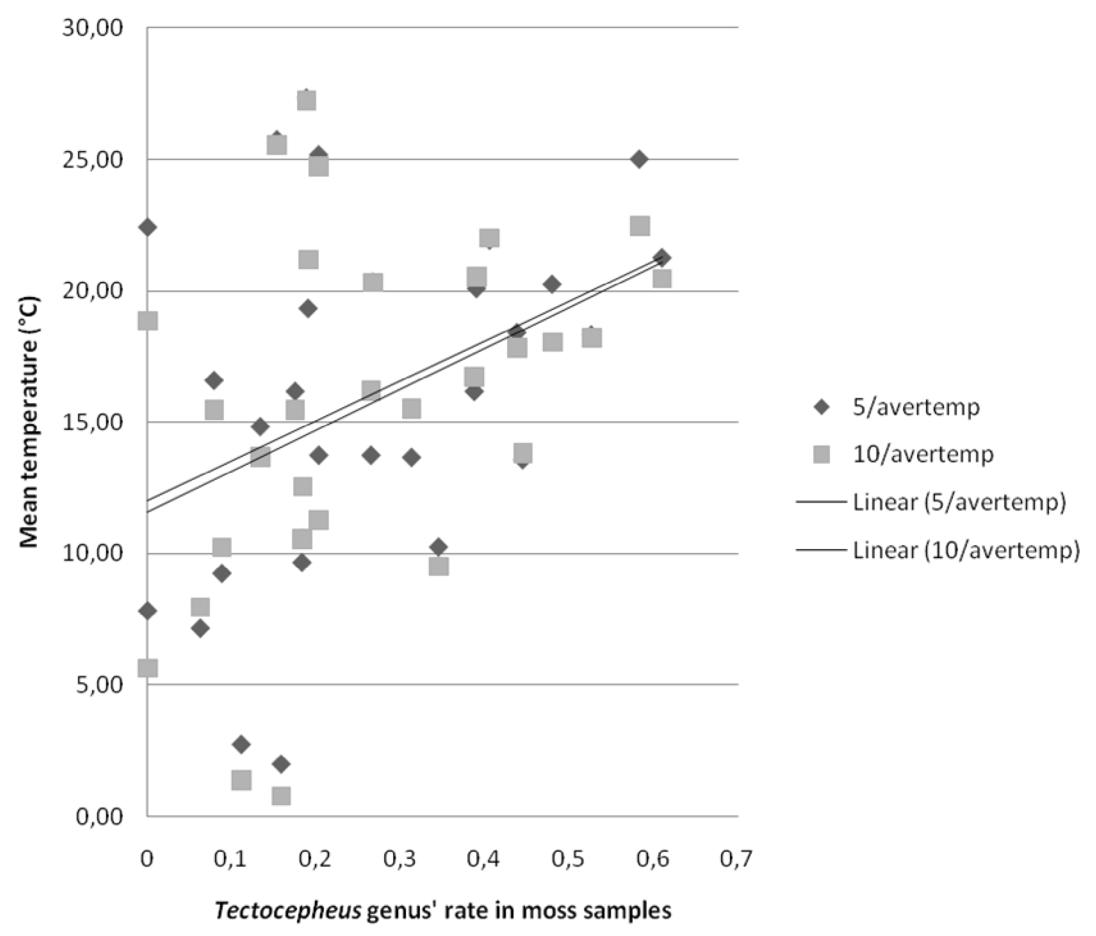

Figure 8. *Correlation between the genus Tectocepheus' rate and the five-day and ten-day mean temperature*

The most frequent groups within the moss are the genera *Tectocepheus* and *Zygoribatula* and the juvenile group. *Table 4* shows that the ratio of the two most frequent genera is inversely proportional to the juvenile group that is the proportion of the juvenile group is only larger if the proportion of the two genera is smaller. The proportion of juveniles and adults changes during the one and a half year (*Fig. 9*), however, this pattern does not recur the following year, and it is not in connection either with the precipitation amount or with mean temperatures or with the values of standard deviation.

Table 4. *Correlation and its observed p-values in the case of two genera (Tectocepheus and Zygoribatula) with the juvenile group in moss samples*

	correlation \ p-values		
	juveniles	***Tectocepheus***	***Zygoribatula***
juveniles		0.001398	0.010454
Tectocepheus	-0.56524		0.058951
Zygoribatula	-0.46803	-0.3548	

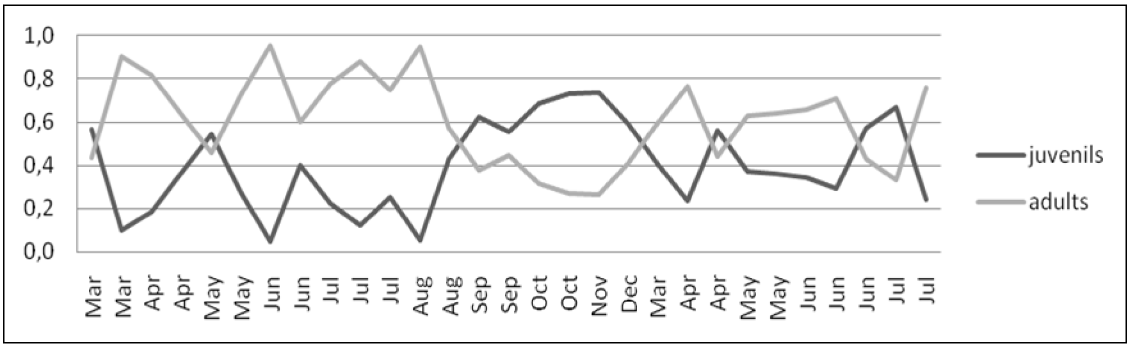

Figure 9. *The proportion of juveniles and adults changes during the one and a half year*

Discussion

According to the research in seasonality during one and a half year we conclude that if any change occurs in the community during the year, it is not seasonal, i.e. neither genus-level diversity, nor abundance, nor the structure of the community have a recurring pattern in leaf litter, foerna and moss microhabitats. These results correspond with several previous data from the literature. Irmler (2004, 2006) observed oribatid mite communities in two different habitats over several years and he did not find any seasonal regularity either, except for some species. Those who interpreted their results in a way that seasonal change was found in oribatid mite communities drew a conclusion based on only one studied year or on the difference between two sampling months (Schenker, 1984; Stamou and Sgardelis, 1989; Askidis and Stamou, 1991; Clapperton et al., 2002). Such a hypothesis was, among others that important changes may occur between two temperate seasons in oribatid mite communities (Stamou and Sgardelis, 1989; Askidis and Stamou, 1991; Clapperton et al., 2002). Although some of these studies were conducted in other types of habitats, i.e. not in temperate deciduous forests, a study covering a shorter period than a year is considered to be insufficient in the case of all habitats when observing regularities in seasonality. Schenker (1984) carried out a one year study around a turkey oak (*Quercus cerris*), a beech (*Fagus sylvatica*) and a Scots pine (*Pinus sylvestris*) in a moist deciduous forest in Switzerland. He found that annual change in abundance can be observed mostly around the beech and the pine, whereas it can hardly be observed around the oak, and species composition is not affected by seasonality, either. Oribatid mites occurred approximately in the same abundance further away from the oak trunks, than in the soil around it. For our study, data were collected in an oak forest. This may be the reason for the fact that we have not found any seasonal changes or changes in the genus composition, either. However, since Irmler (2006) conducted his studies in a beech forest and could not observe any seasonality either, and Schenker (1984) collected samples for only one year, it cannot be stated that the lack of seasonality is characteristic of oak forests.

If changes in communities do not occur seasonally, this can be brought into connection with micro- and macroclimatic effects. According to the results of Stamou and Sgardelis (1989) it could be concluded that the density of oribatid mites is largely influenced by temperature, although several later studies showed that temperature does not have the power to shape communities (Haimi et al., 2005). Irmler (2006) found that the structure of the community was in connection with the annual mean temperature only, and only some species showed significant correlation with some climatic factors. Webb et al. (1998) showed in the case of oribatid mite species living in polar areas that these species do not depend on seasonal changes, life cycle of the studied oribatid mite species is mainly influenced by temperature fluctuation. In our study proportional change correlating with temperature could be observed in the case of the genus *Tectocepheus* only and only in moss, however, no such connection can be found in the study of Irmler (2006), for example. Based on our results – just like based on those of Irmler (2006) – it can be concluded that the structure of oribatid mite communities is not affected by climatic factors in leaf litter and foerna substrates. In moss samples the connection with temperature was due to the genus *Tectocepheus*.

Seasonality can also be observed in the decomposition of plant material. Quantity and quality of the decomposing plant material change seasonally in the leaf litter and in the soil so it can be assumed that oribatid mite communities may change correspondingly during the year. However, the exact role of oribatid mites in the decomposition of the leaf litter is not completely clear till this day (Lindo and Winchster, 2007). The most important role of oribatid mites in the decomposition is the spreading of microbiota as they feed mainly on fungi and bacteria, and they are not in direct connection with the leaf litter input, accordingly (Maraun et al., 2001). This corresponds with the phenomenon observed by us, that the quantity of leaf litter may not have influenced the compositional changes of the communities.

Our result that leaf litter and foerna substrates differ from moss was not interpreted by other literature yet, however, there are observations regarding other types of substrates. A common result is for example that the oribatid mite community living in the foliage of the trees differs significantly from the one living in the soil under the trees (e.g. Yoshida and Hijji, 2005; Karasawa and Hijii, 2008). Karasawa and Hijii (2004) showed that the substrate of oribatid mite communities living in the soil, in the foliage, on the bark of the tree trunks and on the remnants of algae accumulating on soil significantly differ from each other in seaside forests. In our study the community living in hypnum moss was simpler than the one living in the soil in the forest. Communities of moss and lichen are always relatively simple (Gjelstrup, 1979; Gjelstrup and Søchtig, 1979; Smrz, 1992; Smrz and Kocourková, 1999; Smrz, 2006). The three frequent genera found in moss turned up in the observations of others as well. *Zygoribatula exilis* is assumed to be a species living in moss (Gjelstrup, 1979), however, the *Zygoribatula* species found by us could be found on the forest ground as well. The genus *Tectocepheus* occurs everywhere from drier and more disturbed habitats to intact forests, accordingly it can also be found in moss in great quantities, especially because climatic fluctuation is larger in moss, what only some species can tolerate (Gjelstrup, 1979). A common epiphyte is *Eremaeus oblongus* (Smrz and Kocourková, 1999), in our study the genus *Eremaeus* could be found only in moss (except for only one foerna sample).

Therefore our research showed that oribatid mite communities living in soil, leaf litter and hypnum moss, in Hungarian oak forests – similarly to those living in German beech forests – did not show seasonal changes. This result is important on the one hand because according to this, we are not bound to a season regarding sampling. However, besides that it would be important to detect the cause of the still occurring changes and patterns exactly. Furthermore it is unclear as well whether non-woody biocoenoses in the temperate zone or oribatid mite communities living in other climatic zones show seasonal changes.

Communities living in the soil differ from the oribatid mite community of moss living on tree trunks more significantly than from the community of the leaf litter. Oribatid mites in moss, especially the genus *Tectocepheus*, may be influenced by climatic factors to a large degree. In future it would be necessary to study oribatid mite communities of various microhabitats in order to detect exactly by which factors and to what degree their composition, changes and patterns are affected.

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