COLONIZATION PATTERNS OF VASCULAR PLANT SPECIES ON DECAYING LOGS OF FAGUS SYLVATICA L. IN A LOWER MOUNTAIN FOREST BELT: A CASE STUDY OF THE SUDETEN MOUNTAINS, (SOUTHERN POLAND)

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Abstract. Little is known about the vascular plant flora that grows on dead wood or about the ecological conditions of its development. We focused on montane beech forests in the Sudeten Mts. in southern Poland and chose *Fagus sylvatica.* The studies were conducted in the years 2003-2006 between 460 and 890 m a.s.l. Among others, the following parameters of dead logs were recorded: the type of forest community, the status of protection (protected vs. managed), altitude, shade, the humidity of the log, the area of the log [m^2] and the degree of the decomposition class. In total, 165 dead logs were analyzed. In total, 25 vascular plant species were encountered. *Oxalis acetosella, Impatiens parviflora* and *Calamagrostis arundinacea* were the most frequently found. The species composition of the colonized fallen logs differed significantly between the two forest communities in terms of DCA plot scores and also between the managed vs. protected forests. Canonical Constrained Analysis (CCA) revealed that decomposition degree, altitude and shade are significant factors among the five environmental variables that were studied. The study showed that the mean decomposition degree of beech logs as well as the cover of vascular plants overgrowing the logs was higher in the managed forests.

Keywords: *coarse woody debris, fallen trees, beechwood, montane forests*

Introduction

One of the features of natural and primeval forests is the amount of decaying dead wood. If dead wood is present in a semi-natural and managed forest, it can permit the maintenance of forest ecosystems that function properly. In Poland, the amount of dead wood is small, even in forest reserves (Pasierbek et al. 2007). Generally, in European montane regions, the percentage of dead wood is higher than in submontane regions and lowlands. On average, fallen dead wood contributes more to the total dead wood volume than standing dead wood, especially in beech forests (Christensen et al. 2005). Dead wood is important because it forms refuges for many living organisms, such as fungi, lichens, liverworts, mosses, invertebrates and vertebrates including amphibians, birds and small mammals (Ódor and van Hees 2004; Jonsson et al. 2005; Caruso et al. 2008; Ols et al. 2013). Vascular plants also inhabit dead wood; however, their habitats are mainly on the forest floor, i.e. mineral soils. Little is known about dead wood vascular plant flora and the ecological conditions of its development and its structure.

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Hopefully, this study will fill this gap to some extent. We focused on montane forests in the Karkonosze Mts. within Sudeten Mts range in southern Poland and chose one tree species – beech *Fagus sylvatica*. Due to intensive management and the exploitation of the temperate and boreal forests in Europe in the past, dead wood was relatively scarce or entirely absent over prolonged periods (Christensen et al. 2005). In the European beech forest zone, the degree of decomposition in available coarse dead wood is not known with certainty, but it is estimated to be comparable to the degree found in Fennoscandia which was estimated at 90-98% (Christensen et al. 2005; Ódor et al. 2006). Generally, in the mountain region of Central Europe, human activity is weakened; therefore, remnants of semi-natural beech stands are more widespread and less disturbed than in the Atlantic lowlands of north-western Europe (Peterken 1996; Standovár and Kenderes 2003; Ódor et al. 2006). As a result, it can be expected that the flora that colonize the dead wood of *Fagus sylvatica* would be richer and more natural than in beechwoods located in lowlands.

The main goal was to examine whether dead wood serve as a important habitat for some vascular plants. The particular objectives of this study were to characterize the species composition of the vascular plant flora that colonize dead logs, to examine how the degree of decomposition, humidity and size of dead logs can impact the inhabiting plants as well as to check whether there are any differences between forest communities.

Material and methods

The studies were conducted in the years 2003-2006 in the area of the Karkonosze National Park (KNP) and its surroundings, in patches of the beech forests *Dentario enneaphylli-Fagetum* and *Luzulo luzuloidis-Fagetum* and a managed fir forest *Picea abies* between 460 and 890 m a.s.l. The Karkonosze Mt. range forms the border between Poland and the Czech Republic. It lies within the range of the Sudeten Mts. whose highest peak is Śnieżka (1602 m a.s.l.) This area is characterized by a severe high mountainous climate (*Fig. 1*). The montane vegetation is distributed along an altitude gradient: foothills (up to 500 m a.s.l.), lower forest montane zone (500-1000 m), upper forest montane zone (1000-1250 m), subalpine zone (1250-1450 m) and alpine zone (1450-1602 m). Because of human interference, profound changes have taken place. Anthropogenic Norway spruce tree stands have replaced the natural vegetation, which occur only fragmentarily nowadays. Norway spruce trees were introduced by foresters using seeds originating from the Alp Mts. Therefore, spruce mono-cultures were weakly resistant to both local biotic and abiotic conditions. Moreover, air pollution (sulphur dioxide, NOx and dusts, acid rains, etc.), which took place in the years 1960-1980, caused damage to the spruce leaves. As a consequence, the weakened trees of *Picea abies* were more vulnerable to fungi infections (Fabiszewski and Wojtuń1994; Stachurski et al. 1994). At present, spruce-cultures are in the decline in the Sudeten Mts. (Jadczyk 2009). For these reasons, forests in the Sudeten Mts. are rich in the dead wood of *Picea abies*. Because of the die-back of spruce, forest management practices are currently aimed at the reconstruction of tree stands. Therefore, beech *Fagus sylvatica* is being planted. In contradistinction to Norway spruce, the condition of beech trees is much better; however, it is less distributed than Norway spruce. Beech tree stands are mainly situated in the northern part of the KNP and in two enclaves of the KNP near Szklarska-Poręba and Jelenia Góra. Other trees stands of *Fagus sylvatica* are situated in the vicinity of the KNP near Kowary (*Fig. 1*). Almost all of the beechwoods were investigated in the study area. Amongst the various forms of dead wood, we chose selected forms of coarse dead wood, i.e. dead logs. Fine coarse dead wood and snags, stumps and tree fall disturbances were not taken into account in this study. All of the dead logs that were found were inventoried and analyzed in terms of selected biometric and habitat features. The following parameters of dead logs were recorded: GPS coordinates, the type of forest community, the status of protection (protected vs. managed), altitude, light availability (shading) in 5 degree scale (1 – full light, 5 – deep shade), the humidity of the log (1 – dry, 2 – intermediate, 3 – humid), the length of the log [m], upper diameter [cm], lower diameter [cm], the area of the log [m^2] and the class of decomposition (stage of decay). The area of the log was calculated using the formula for a truncated cone (Zielonka and Piątek 2004). The criterion of division into eight decomposition classes, which was originally used by McCullogh (1948) and with modifications by Dynesius and Johnsson (1991) and Holeksa (2001), was adapted after Zielonka and Piątek (2004). The percentage cover of vascular plants and the cover data of particular vascular plant species were noted. Moreover, total cover of moss species was recorded. The frequency of colonized dead logs vs. non-colonized dead logs in relation to the status protection of the forest was analyzed using the chi-squared test. The Spearman rank correlation test was used to examine the relationships between the analyzed variables. Because multiple tests were used, both the Holm adjusted p-values and in some cases p-values without correction were used following the recommendations by Moran (2003). The mean cover of vascular plant layer and the mean decomposition degree between the two phytocoenoses, *Luzulo luzuloidis-Fagetum* and *Dentario enneaphylli-Fagetum*, as well as between the managed and protected forests were compared using the Wilcoxon sum rank test. Detrended Correspondence Analysis (DCA) was used to study the variation of species turnover between the types of forest. The similarities of vegetation between the types of forest were compared by comparing the means of the coordinates of the plots along the two first axes of the DCA. The comparison of mean values permitted the identification of any significant differences in species composition between the groups of logs. The classification of indicator species for vegetation of groups of logs in particular forest communities was performed using the indicator value, i.e., the IndVal method (Cáceres et al. 2010). Constrained Correspondence Analysis (CCA) was used to analyze any differentiation of the species that colonize dead wood and the five selected environmental factors were used as the constrained variables. Strongly correlated variables with a high inflation factor were excluded from the analysis. The full model with 999 permutations of the Monte Carlo test was applied. All data analyses and calculations were done with R 2.15 software (R Core Team 2012).

Results

In total, 165 dead beech logs were analyzed including 128 in *Luzulo luzuloidis-Fagetum*, 27 in *Dentario enneaphylli-Fagetum* and 10 in the *Picea abies* community. When the presence/absence of plants encountered between the two former forest communities were compared, there was no significant difference (*Fig. 2*). Colonized dead logs in *Luzulo luzuloidis-Fagetum* and *Dentario enneaphylli-Fagetum* constituted 42.2% and 48.1%, respectively. Of the total 25 vascular plant species, 14 vascular plant species were found in *Dentario enneaphylli-Fagetum* and 19 in *Luzulo luzuloidis-*

Fagetum. The species composition of colonized fallen logs differs significantly between the two forest communities in terms of the mean values of the coordinates of the logs along the second DCA axis ($W = 170.5$, $p<0.01$) and between the managed vs. protected forests along both axes ($W = 249$, $p<0.01$, $W = 202$, $p< 0.01$)(*Fig 3*).

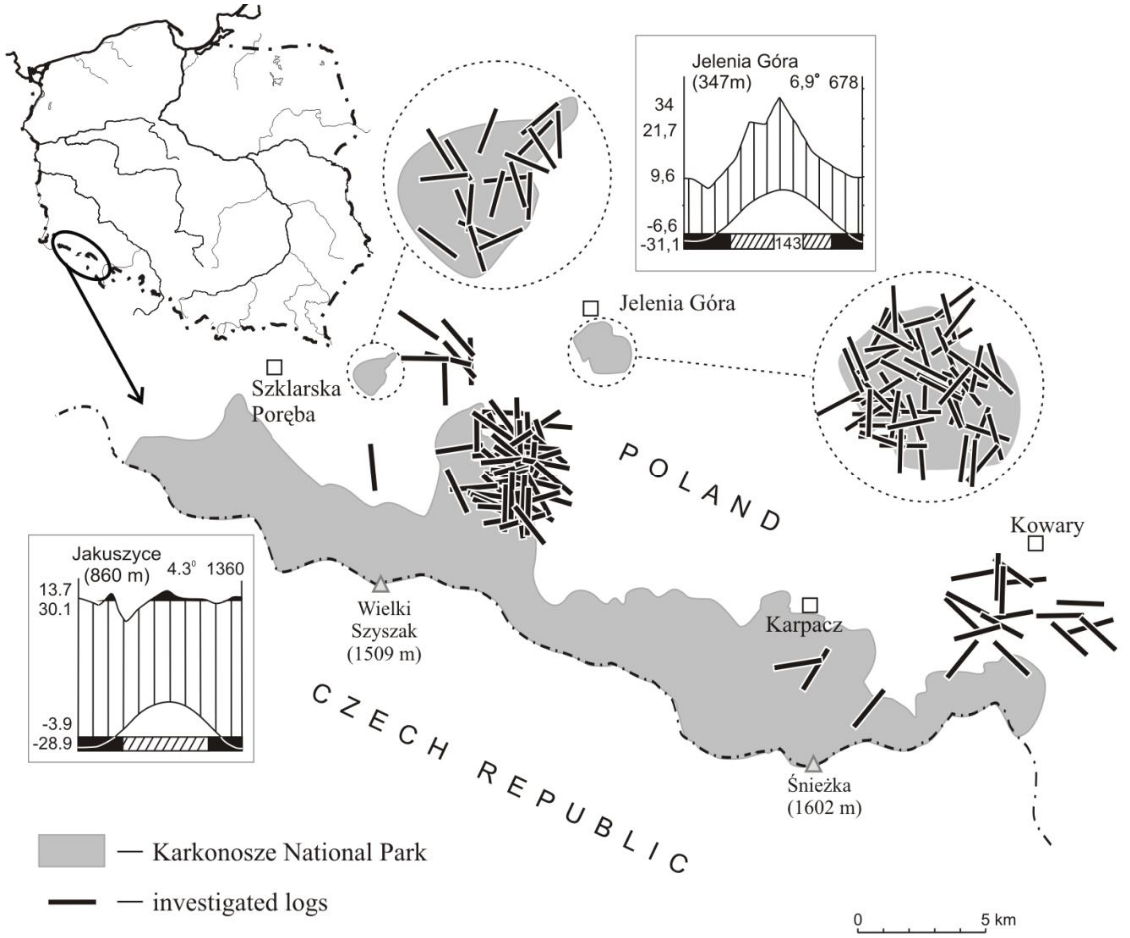

***Figure 1**. Distribution of the investigated logs of Fagus sylvatica and the climatic conditions of the study area.*

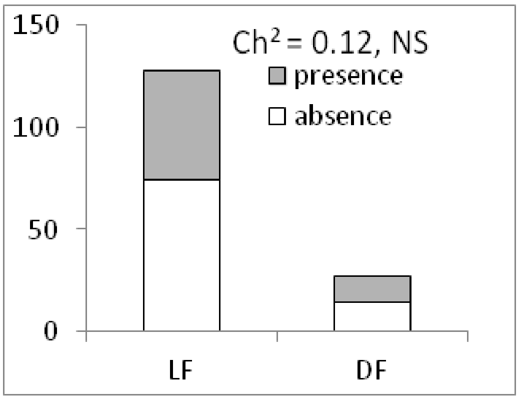

***Figure 2**. Comparison of the presence/absence of plants on dead logs between the two types of forest community. LF – Luzulo luzuloidis-Fagetum, DF – Dentario enneaphylli-Fagetum.*

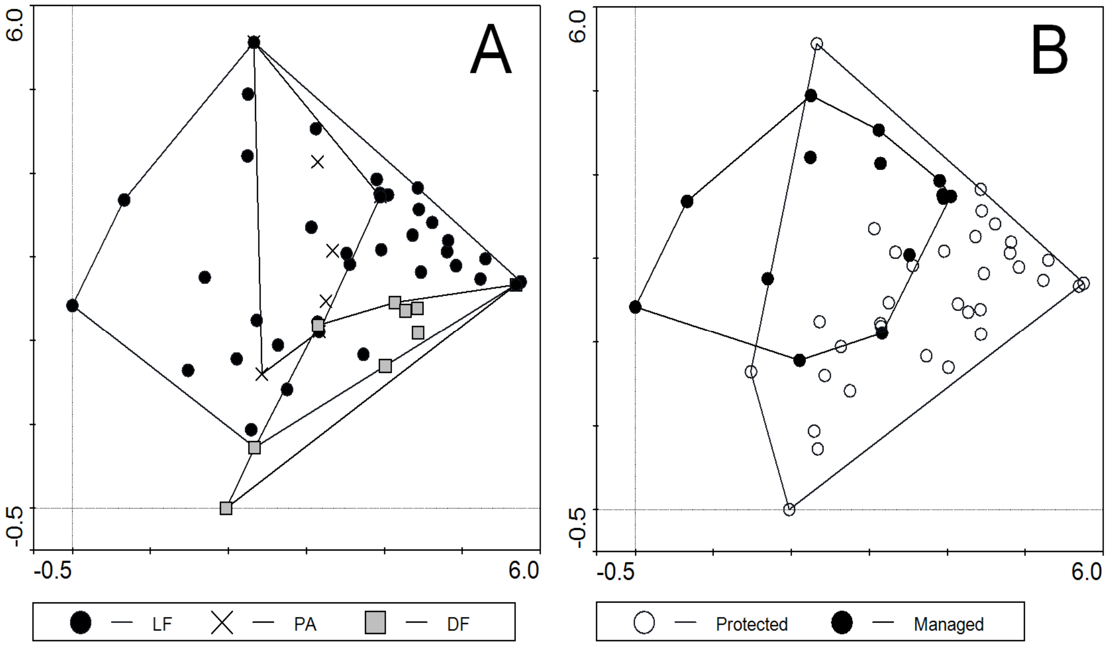

Figure 3*. The ordination of the fallen logs of Fagus sylvatica along the two first DCA axes (λ1 = 0.803, λ2 = 0.635) based on the cover of colonizing vascular plant species in relation to the type of forest community and the protection status of the forests. LF – Luzulo luzuloidis-Fagetum, DF – Dentario enneaphylli-Fagetum PA - Picea abies community.*

Based on IndVal method two plants *Picea abies* (seedlings) and *Dryopteris carthusiana* are significantly indicator species for *Luzulo luzuloidis-Fagetum* scoring IndVal=0.517, p= 0.008 and 0.411, p=0.028 respectively. For two communities combined *Dentario enneaphylli-Fagetum* and *Luzulo luzuloidis-Fagetum* beech *Fagus sylvatica* as seedling is significant indicator (IndVal=0.465, p=0.043).

Canonical Constrained Analysis (CCA) revealed that, among the environmental factors, the degree of decomposition, altitude and shade were significant factors accounting for p = 0.001, p = 0.001 and p = 0.029, respectively (*Fig. 4*). These three variables are arranged along the first axis of CCA. The most frequent species are *Oxalis acetosella* and *Impatiens parviflora* (*Table 1*). Species such as *Vaccinium myrtillus, Convallaria majalis, Picea abies* (seedlings), *Urtica dioica and Luzula luzuloides* are mainly associated with higher altitudes. Other species such as *Fagus sylvatica* (seedlings), *Oxalis acetosella and Phegopteris connectilis* are equally sensitive to the degree of decomposition, shading and altitude. Some species including *Acer pseudoplatanus* (seedlings), *Dryopteris* sp., *Galeobdolon luteum or Poa nemoralis* are confined to dead logs with a higher humidity; however, humidity was a non-significant factor in relation to species composition in general (*Fig. 4*).

The total cover of vascular plants was negatively correlated with altitude but positively with the degree of decomposition and humidity, as well as with the area of dead log coverage. Species richness was negatively correlated with altitude and positively correlated with the degree of the decomposition of dead logs. The number of species also increased significantly with an increasing area of dead log coverage. Vascular plants increase, in terms of their total cover and species richness, along with the increase of moss cover (*Table 2*).

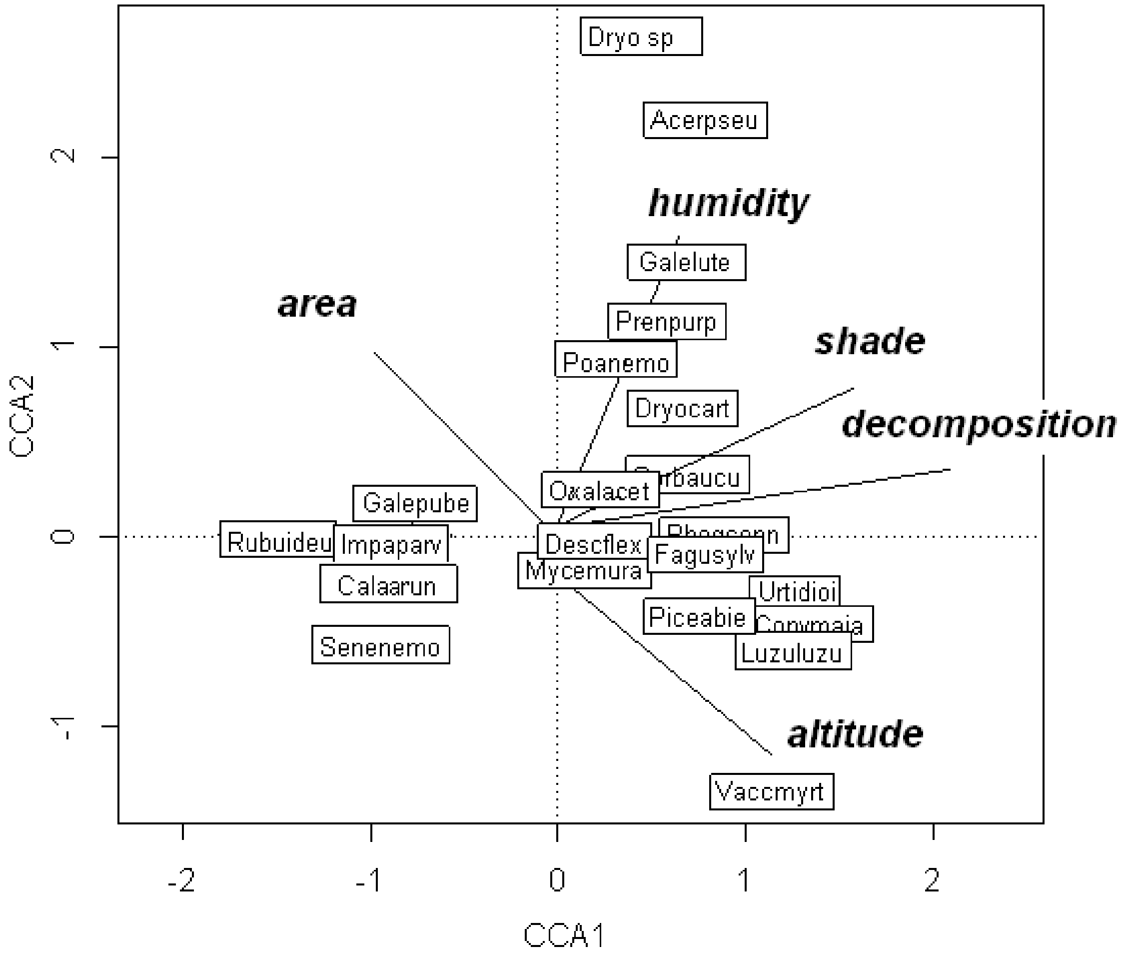

Figure 4. *Biplot of plant species that colonize the dead logs of Fagus sylvatica along the two first axes of Constrained Correspondence Analysis (CCA)(λ1 = 0.58, λ2 = 0.27). The diagram accounts for 10.6% and 66.8% of the variance of species cover data and species-environment relation, respectively. Only the degree of decomposition, altitude and shade are significant environmental factors. Abbreviations: Dryo sp – Dryopteris sp, Acerpseu – Acer pseudoplatanus, Calaarun – Calamagrostis arundinacea, Galelute – Galeobdolon lueum, Prenpurp – Prenanthes purpurea, Poanemo – Poa nemoralis, Dryocart – Dryopteris carthusiana, Oxalacet – Oxalis acetosella, Galeopube – Galeopsis pubescens, Rubuideu – Rubus idaeus, Impaparv – Impatiens parviflora, Descflex – Deschampsia flexuosa, Sorbaucu – Sorbus aucuparia, Fagusylv – Fagus sylvatica, Urtidioi – Urtica dioica, Piceabie – Picea abies, Luzuluzu – Luzula luzuloides, Convmaja – Convallaria majalis, Senenemo – Senecio nemorensis, Vaccmyrt – Vaccinium myrtillus, Phegconn – Phegopteris connectilis.*

The cover of vascular plants on decaying logs and the degree of decomposition did not differ significantly between the types of forest community. However, the status of protected forests did have an effect. In managed forests, independent of the type of community cover of plants, overgrown laying logs and the mean degree of decomposition were significantly higher (*Fig. 5*).

***Table 1**. The number of occurrences of vascular plant species that colonize the fallen logs of Fagus sylvatica in Sudeten Mts*

Species	*Luzulo luzuloidis-Fagetum*	*Dentario enneaphylli-Fagetum*	*Picea abies* community
Acer pseudoplatanus	2	0	0
Alliaria petiolata	0	1	0
Calamagrostis arundinacea	12	1	0
Convallaria majalis	1	0	0
Deschampsia flexuosa	4	0	0
Dryopteris carthusiana	4	0	2
Dryopteris sp.	1	0	0
Fagus sylvatica	10	9	1
Galeopsis pubescens	1	0	0
Hedera helix	0	1	0
Impatiens parviflora	15	7	0
Lamiastrum galeobdolon	1	0	0
Luzula luzuloides	2	0	0
Mercurialis perennis	0	1	0
Mycelis muralis	0	1	0
Oxalis acetosella	30	3	4
Phegopteris connectilis	0	1	0
Picea abies	15	2	4
Poa nemoralis	0	1	0
Polygonatum verticillatum	1	0	0
Prenanthes purpurea	2	0	0
Rubus ideus	2	0	0
Senecio nemorensis	3	2	0
Sorbus aucuparia	1	1	1
Urtica dioica	0	1	0
Vaccinium myrtillus	6	0	3
Total	113	32	15

***Table 2.** Intercorrelations (Spearman rank correlation) between the parameters associated with dead logs of Fagus sylvatica and the species that inhabit them*

	Altitude	Mosses cover	Vascular plants cover	Decomposition degree	Humidity	Shadow	Area
Mosses cover	-0.35^{4}						
Vascular plants cover	-0.23^{2}	0.51^{4}					
Decomposition degree	ns	0.41^{4}	0.27^{4}				
Humidity	ns	0.23^{3}	0.19^{1}	0.34^{4}			
Shadow	ns	ns	ns	0.29^{4}	0.18^{1}		
Area of log	ns	ns	0.21^{1}	-0.32^{4}	ns	ns	
Species richness of vascular plants	-0.19^{1}	0.48^{4}	0.95^{4}	0.25^{3}	ns	ns	0.22^{1}

1-p<0.05 without Holm's adjusted p-values, Holm's adjusted p-values: 2-p<0.05, 3-p<0.01, 4-p<0.001

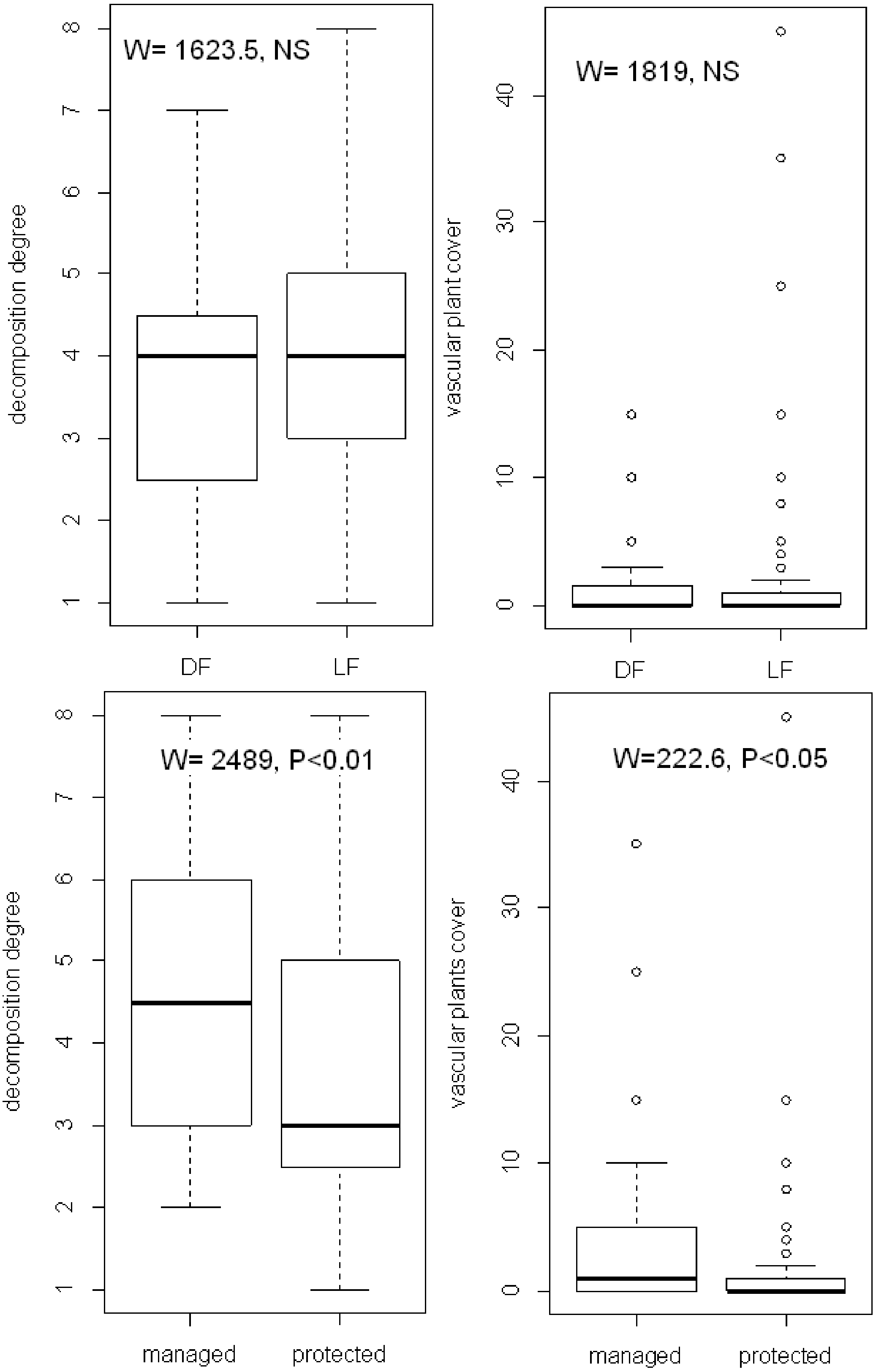

Figure 5. *Comparison of the cover of vascular plant species and the degree of decomposition between the types of forest community LF Luzulo luzuloidis-Fagetum, DF Dentario enneaphylli-Fagetum and between managed and protected forests.*

Discussion

Vascular plant species in contradistinction to epixylic species such as lichens, mosses and liverworts are "true" colonizers of dead wood. These plants colonize dead wood from substrate as bare soil or litter in the course of dead wood decomposition. They are usually not encountered on living trees in temperate regions, whereas cryptogamous plants and mosses can be found on the bark of living trees very frequently. Thus, lichens and bryophytes growing on dead wood are most frequently relics of epiphytic flora, but only in some stages of decay. In the literature, there is almost no information about the species richness and species composition of vascular plant taxa that inhabit the dead wood of *Fagus sylvatica*.

The exception is a study by Chmura (2008), who found 12 vascular plant species on 29 decaying logs in a much smaller area in one nature reserve in lowlands in the *Dentario glandulosae-Fagetum* community. However, such data is available for cryptogams. Żarnowiec and Staniaszek-Kik (2009) found 19 lichens, 3 liverworts and 19 mosses on dead logs of beech in a *Dentario enneaphylli-Fagetum* community in the same region. The number of species of vascular plant flora in this study was slightly lower – 14. Other deciduous trees whose fallen logs were studied in terms of the species composition of colonizing flora had a similar species richness of the 26 vascular plant species found on the logs of all of the tree species studied including pine *Pinus sylvestris*: birch *Betula pendula* and aspen *Populus tremula* (16), hornbeam *Carpinus betulus* (12) and oaks *Quercus* sp. (22) (Nowińska et al. 2009).

On decaying logs of the spruce *Picea abies* in a subalpine forest *Plagiothecio-Piceetum* in the Tatra Mountains, the species richness of vascular plants was higher when compared to this study (20); however, a larger number of logs were observed (Zielonka and Piątek 2004). Similar species richness (23) on spruce logs was found by Kushnevskaya et al. (2007) in the late-successional boreal forests of north-western Russia. In contrast, in the Primeval Bialowieża Forest, 108 species were observed on the wood and 138 on the bark of the laying logs of 303 vascular plant species that were reported from six forest communities growing on different substrates (Chlebicki et al. 1996). Zielonka and Piątek (2004), Chmura (2008) and Nowińska et al. (2009) demonstrated that, with a more advanced decomposition stage, the number of species increases; this was confirmed by our observations. In addition, a positive relationship was also found for the total cover of all plants (*Table 2*).

The previous authors claim that the 6th stage has the most suitable conditions for vascular plants. With an increasing coverage area of logs, the number and cover of vascular plant species increases; however, this is a weak correlation (*Table 2*). It is interesting that such a positive relationship was found for the coverage area of log and the degree of decomposition. In the first case, it is a well-known phenomenon called the species-area relationship (Rosenzweig 1995), which is also true for small objects in the microscale.

The total number of individuals increases with area, leading to an increased probability of encountering more species within larger areas (Scheiner et al. 2000). Moreover, the larger logs are likely to be more heterogeneous as to types of microsites. Those which can be found on logs are: bark, wood at different decomposition stages, moss mats, cracks in stems filled with mineral soil or humus (Nowińska et al. 2009). The pattern that was observed is in contrast to the colonization of *Picea abies* logs in the Tatra Mts, where thick logs had a lower cover of herbs than thin logs. Medium logs were mostly covered by herbaceous plants (Zielonka and Piątek 2004). The second case is more intriguing. Perhaps it is a coincidence or it may be connected with the age of the trees and the time that had passed since their fall which enabled colonization.

We found that the degree of decomposition increases when light availability is lower. This may be associated with other findings, i.e. a positive correlation between shade and humidity. Moisture enhances the process of decomposition, but light can lead to an increase in temperature and the drying out of the bark and wood of decaying logs. Generally, moisture is believed to increase significantly during wood decay (Bütler et al. 2007). Vascular plants responded similarly as mosses what was manifested by positive correlation (*Table 2*). Mosses are known to keep humidity what explain why vascular plant are associated with them on decaying logs.

In this study, both species richness and the cover of vascular plants decreased with increasing altitude. Two species were found to be frequent colonizers: *Oxalis acetosella* and *Impatiens parviflora*. The former is a native plant with a rather wide synecological amplitude. In contradistinction to the latter, it seems to be more confined to the dead wood of *Pinus sylvestris* (Nowińska et al. 2009) than to deciduous trees. Furthermore, as the study by Zielonka and Piątek (2004) showed, this species had a positive response to the degree of decomposition of *Pinus sylvestris* logs. In the case of beech, the species was frequent enough that no environmental factors influenced its behavior (*Fig. 4*). Its common or even massive presence in the Sudeten Mts. is caused by the encroachment of many lowland species into afforested areas due to the decline of Norway spruce stands. *Oxalis acetosella* was one of the few species that did not disappear because of spruce regeneration. Its further spread into other types of woods was possible owing to its wide ecological tolerance (Jadczyk 2009). Small balsam *Impatiens parviflora* is one of the most invasive alien neophytes in Polish and European flora (Tokarska-Guzik 2005; Hejda 2012) and has successfully penetrated natural forest communities including nature reserves (Chmura and Gucwa-Przepióra 2012; Schmidt 2012). Previously, it was reported as the most common colonizing species on beech dead wood (Chmura 2008) and hornbeam *Carpinus betulus,* pine *Pinus sylvestris* (Piskorz and Klimko 2001) and oaks (*Quercus* sp)(Nowińska et al. 2009). Amongst the almost 1,600 various structural elements of dead wood (logs, snags, stumps, tree-fall disturbances) of *Fagus sylvatica* and *Picea abies* in the Sudeten Mts., Staniaszek-Kik and Żarnowiec (2012) found the species on 44 elements, mainly on the logs and stumps of *Fagus sylvatica*. The present detailed study demonstrated that *Impatiens parviflora* does not show any species preferences for significant environmental factors, such as shading, altitude and the degree of decomposition. *Vaccinium myrtillus* or *Luzula luzuloides* are associated with a higher altitude, especially the former, and occur more frequently in subalpine spruce forests. Analyzing most frequent, indicator species for particular plant communities where decaying logs were studied it can be inferred that there are no exclusively vascular plants inhabiting dead wood. All of them are common forest floor species both herbaceous and seedlings of trees. One of them, indicated in the study, is *Picea abies* which commonly is reported to regenerate on dead wood (Holeksa 2001) or *Fagus sylvatica* which obviously is common in beechwoods. In disturbed forest i.e. *Picea abies* community no significant indicator plant species were noted. The data that showed a higher mean decomposition stage and a higher mean cover of vascular plants on logs in managed forests than protected ones could be a result of forest management practices and the disturbances that are associated with them. The quantities of dead wood are normally much lower in managed forests than in unmanaged old-growth forests (Ódor and Standovar 2001). However, forest management treatments can lead to a more abundant presence of dead wood because of artificial tree stand thinning and the abandonment of logs. As a consequence, according to the intermediate disturbance hypothesis, species richness and species diversity is higher (Wilkison 1999). The results suggest that a greater number of disturbances favors the development of vascular plants on decaying logs (*Fig. 3*). Unknown role of plants in acceleration of decomposition of dead wood due to mechanical action of roots should be further studied in the future. Perhaps vascular plants enhances to nutrient cycling of dead wood in forest ecosystems.

To sum up, it can be concluded that the species diversity of vascular flora that colonize the logs of *Fagus sylvatica* does not differ significantly on floras inhabiting the dead wood of other deciduous trees and even conifers. It is rather species-poor, with a single common species. Contrary to epiphytic and epixylic mosses and lichens vascular plants present on decaying logs are common species, thus dead wood does not contribute to species richness and diversity of this group of taxa.

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KINETICS OF BIODEGRADATION OF PHENOL AND A POLYPHENOLIC COMPOUND BY A MIXED CULTURE CONTAINING *PSEUDOMONAS AERUGINOSA* AND *BACILLUS SUBTILIS*

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Abstract. Biological degradation of phenolic compounds by a mixed bacterial culture was investigated. *Pseudomonas aeruginosa* and *Bacillus subtilis* were the two strains used in the study. pH and the temperature for the growth of bacteria was found to be 7±0.2 and 37±2°C, respectively. The mixed microbial culture was able to degrade 250 mgl^{-1} of phenol and wattle completely within 36 and 48 hours, respectively. The growth kinetics of the mixed culture was also investigated over a wide range of initial concentrations of phenol and wattle by using Haldane's model. The Haldane growth kinetic model adequately described the degradation of phenolic compounds by the mixed microbial culture. Both phenol and wattle were found to inhibitory compounds. The decay coefficient for the growth of microbial culture on phenol and wattle was found to be 0.0069 and 0.0082 h^{-1}, respectively.

Keywords: *phenol, wattle, synergism, biodegradation, growth kinetics*

Introduction

Due to their widespread use and subsequent introduction into the environment, phenolic compounds have been included to the list of pollutants by the environmental protection agencies. When discharged, phenolic wastes impart a carbolic odour to the river and are toxic to aquatic animals (Dean-Ross, 1995). The toxicity of phenol and phenolic compounds often result in the reduction of bio-treatment efficiency of wastewaters even at relatively low concentrations (Ahmed, 2001). Although physio-chemical methods have been employed for removal of phenol and its derivatives, biological methods are preferred as the former is costly and often produce other undesirable products which are toxic, requiring further processing steps.

Treatment of phenolic wastes can be done using microbes or chemicals or by physical means. Bacteria play a major role in the degradation of phenol in soil, sediment and water. Using bacteria to digest waste and environmentally harmful compounds has several advantages over conventional treatment systems (Ojumu, 2005). The cost of remediation is less than that for conventional treatments. The diversity of microbes available makes the treatment of a wide variety of waste possible. Bacterial degradation of compounds is usually complete under appropriate conditions; toxic compounds are often completely converted into harmless compounds. Benzoate, catechol, *cis-cis-*

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muconate, β-ketoadipate, succinate and acetate have all been identified as intermediates in the biodegradation of phenol (Baradarajan, 1995).

For the cleanup to be achieved within a reasonable time frame without the threat of persistent, undesirable by-products, the entire system must be optimized. The need for optimization stems from the fact that the effect of microbes acting together is even better than their individual effects. This phenomenon is termed as 'synergism' (Zache, 1988). Biological degradation of organic substrates using pure microbial cultures occasionally produce toxic intermediates, which can be overcome by the use of mixed cultures that have a wider spectrum of metabolic properties.

In order to describe the substrate biodegradation, it is essential to evaluate the relationship between the specific growth rate and the initial substrate concentration. The bacteria behave differently when exposed to different initial substrate concentration and other experimental conditions. Hence, knowledge on both the rate and the extent of degradation is essential for improvements in the process control and phenol removal efficiency. In the present study, the biodegradation of phenolic compounds in the mixed microbial consortia containing *P. aeruginosa* and *B. subtilis* has been demonstrated and the bio-kinetic parameters have been evaluated.

Materials and methods

Spray dried wattle extract of commercial grade was used. All other chemicals used were of analytical and biological grade.

Source of bacteria

The *Pseudomonas* bacteria were isolated from the soil near the wastewater treatment plant at the premises of Central Leather Research Institute, Chennai, India. The *Bacillus subtilis* strain was obtained from the Microbiology Laboratory, Central Leather Research Institute, Chennai, India.

Estimation of phenol

Modified Folin–Ciocalteu's method (Clesceri, 1998) was employed for the estimation of phenol. Phenol solution of various concentrations was prepared by adding known volumes of phenol from the stock to the media. 1 ml of phenol solution of various concentrations was added to the labeled test tubes to which, 0.5 ml of media was serially added followed by 3.5 ml of sodium carbonate and 5 ml of de-ionized water. Finally 1 ml of 1 N Folins Ciocalteu reagent was added and the absorbance was measured after a period of 25 minutes. Absorbance was measured at 760 nm in a 35 Lambda UV-visible spectrophotometer. A standard graph was plotted using the absorbance values obtained against corresponding concentrations of phenol. The blank used had the same composition of reagents except that 1 ml of phenol solution added was replaced by 1 ml of water.

Isolation of the microbe

The isolation of microbes was carried out by inoculating the microbes from the nutrient broth into the selective medium using a sterile inoculation loop in a laminar airflow chamber. The selective medium favors the growth of only a particular microbe and inhibits the growth of others. For the isolation of *Pseudomonas sp. Pseudomonas* selective medium procured from Qualigens Fine Chemicals was employed. The medium after sterilization was poured in the petri plates or test tubes and left for solidification. After solidification of the medium, the bacterial samples were inoculated in the petri plates or test tubes.

Identification of species

The bacterial species identified were subjected to biochemical analysis in order to ascertain the genus of the species. Various biochemical tests like catalase test, citrate utilization test, starch hydrolysis, methyl red test, Voges Proskauer test were carried out as per standard procedures (Cappuccino, 1996). The optimum growth conditions like pH and temperature were also determined.

Effect of pH

pH of the medium is a very important factor, which influences the growth of microorganisms. The inoculation medium was prepared by adjusting it to required pH values (4 to 11) by employing either 0.1 N NaOH or 0.1 N HCl. Optical density (OD_{400nm}) of the inoculated medium was measured at 0^{th} hour soon after the inoculation of microbe and was again measured after 24 hours to estimate the growth of microbes at respective pH's.

Synergistic degradation of phenol and wattle by P. aeruginosa and B. Subtilis

B. subtilis and *P. aeruginosa* were used as mixed culture in the degradation of phenol and wattle. The use of selective media was not recommended in this operation because the chemical present in one selective medium may inhibit the action of the other microorganism. Hence nutrient broth was used for the purpose of mixed degradation. Hence, extreme care was taken during inoculation and incubation as risk of using nutrient broth is very large. To the nutrient broth, loop full of *P. aeruginosa* and *B. subtilis* were inoculated and incubated at 37±1 ^oC in an incubator shaker. Once an initial OD_{400} of 0.8±0.2 was attained, known quantity of phenol and wattle from the stock solution was added to nutrient broth and made up to known volume, respectively and kept in an incubator shaker. The OD_{400} was monitored at regular intervals to monitor the growth of microbes. The change in substrate concentrations with time was also estimated by modified Folin's method. The growth as well as the change in the concentration of phenol was monitored using Lambda 35 UV-Vis spectrophotometer. The substrates concentration in the medium was calculated from the standard graph for the particular substrate.

Results and Discussions

Growth of microbes in the selective medium

The nutrient broth prepared and inoculated with the microbes showed immense turbidity after 24 hours of incubation. The microbial samples from the nutrient broth were grown in a *Pseudomonas* selective medium. In order to cultivate the individual species of *Pseudomonas*, the consortia were inoculated in 4 different selective media (*P. aeruginosa, P. putida, P. cepacia, P. fluroscens*). The abundant growth was observed in the *P. aeruginosa* selective medium. This proves *P. aeruginosa* is the dominant species

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present in the soil. All the other microbes were either completely absent or must be present in trace quantities.

Biochemical characterization

Biochemical characterization has been carried out in order to ensure the presence of the microbe of our interest. The results for the biochemical characterization of the bacterial species are shown in (*Table 1*). The formation of effervescence has been observed in the test tube inoculated with *P. aeruginosa and B. subtilis*, which indicates a catalase activity. Simmons citrate test has been positive in case of *Pseudomonas* and *B. subtilis* (appearance of blue color). *Pseudomonas* answered negative starch test, where as *B. subtilis* showed a visible color change. The formation of red ring has been observed with *B. subtilis*, whereas no ring was observed in *Pseudomonas*, which indicated a negative methyl red test. From the table, it is clear that the microbe employed are *P. aeruginosa* and *B. subtilis.*

Table 1. *Biochemical characterization of the microbes*

Test performed	*P. aeruginosa*	*B.subtilis*
Catalase test	+	+
Simmon citrate test	+	+
Starch test	-	+
Methyl red test	-	+
VP test	+	+

Effect of pH and temperature

To find out the favourable pH for the growth of *P. aeruginosa* and *B. subtilis*, the growth profile of the microbes have been observed over time by monitoring the change in OD_{400nm} at different pH values (*Fig. 1*). Figure clearly shows that *P. aeruginosa* and *B. subtilis* had maximum growth at pH of 7±1. Hence, for subsequent experiments the pH of the nutrient broth has been maintained at 7±0.2.

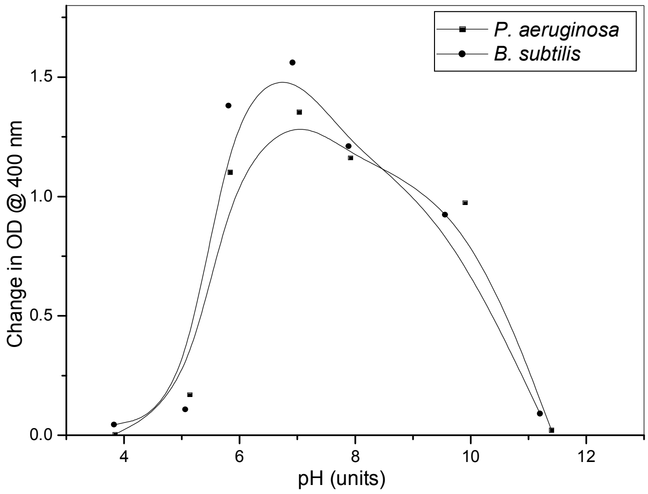

Figure 1. *Effect of pH on the growth of P. aeruginosa and B. subtilis*

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Once the pH has been optimized, the effect of temperature on the growth of microbes has been studied. The optimal temperature for the growth of these microbes is determined to be around 37±2oC. Hence, the subsequent experiments have been carried out at this temperature.

Effect of initial substrate concentration

Spray dried wattle extract, a polyphenolic material, used as a tanning and retanning agent during leather manufacture was selected as a representative substrate for phenol. In order to find out the maximum concentration with which the mixed culture of *P. aeruginosa* and *B. subtilis* can grow, the microbes were grown in the presence of different initial concentrations of phenol and wattle. The initial concentrations of phenol and wattle were varied from 25, 50, 100, 250 and 500 mgl^{-1}. The degradation profile of phenol and wattle were monitored at regular intervals using modified Folin's method. The growth of microbes was monitored over time. (*Figs. 2* and *3*) provide the phenol and wattle degradation data at an initial concentration of 250 mgl^{-1} of the substrate. The measurement of growth of microbe and the residual concentration of the substrate were followed till the substrate present got completely consumed. The results showed that the higher the concentration of the substrates higher the time taken to consume it fully. From the figures, it is very clear that the mixed microbial culture containing *P. aeruginosa* and *B. subtilis* was able to consume phenol and wattle completely. The consumption of 250 mgl^{-1} of phenol took 36 hours, while the consumption of 250 mgl^{-1} of wattle took almost 48 hours. Wattle being a polyphenolic compound took a longer time for degradation when compared to phenol alone.

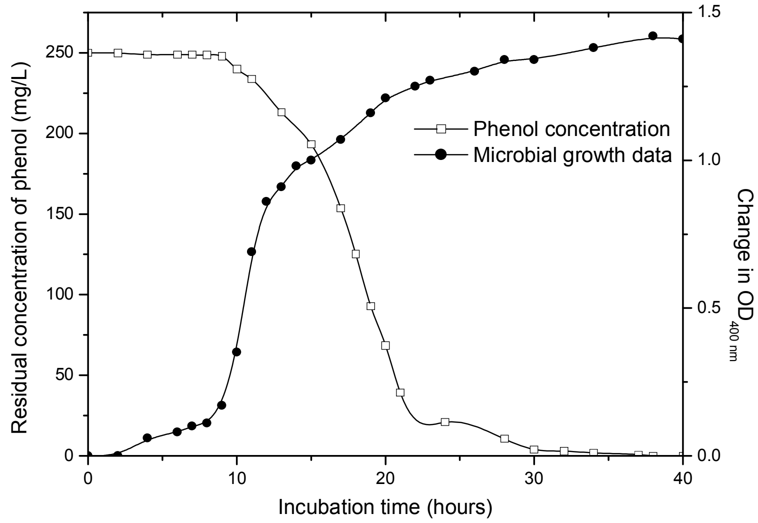

Figure 2. *Degradation of phenol by the mixed microbial culture (Initial conc= 250 mgl^{-1}; pH = 7.0±0.2; Temp = 37±2 °C)*

Growth kinetics

The growth kinetics of the mixed culture has been evaluated from the microbial growth data at different initial phenol and wattle concentration. The plot of OD against the initial phenol concentration has been used for the determination of specific growth rate (Livingston, 1989). The specific growth rate has been determined from the slope of the linear plots of OD vs Time during the exponential phase at respective initial phenol concentration.

Cell growth kinetics in a batch reactor may be modelled by the following equation (Edwards, 1970):

$$\frac{dX}{dT} = \mu_g X - k_d X = \mu_{net} X \qquad \text{(Eq. 1)}$$

For substrate:

$$\frac{dS}{dt} = -\frac{1}{Y}\left(\frac{dX}{dt}\right) \qquad \text{(Eq. 2)}$$

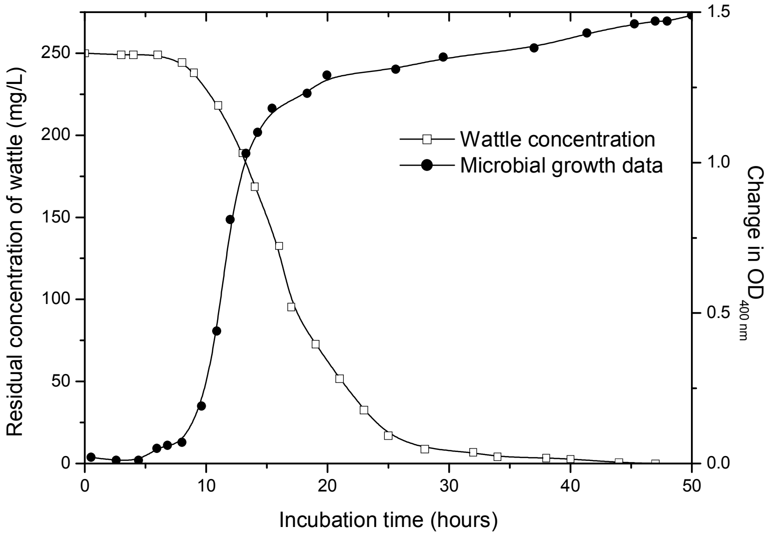

Figure 3. *Degradation of wattle by the mixed microbial culture (Initial conc = 250 mgl^{-1}; pH = 7.0±0.2; Temp = 37±2 °C)*

The specific growth rate (μg) is a function of substrate concentration (S). Monod and Haldane kinetics are the two most widely used equations to calculate the growth kinetics of any microbial systems. According to Monod's equation, the substrate is considered a non-inhibitory compound and the Monod's non inhibitory kinetics equation is given as below:

$$\mu_g = \frac{\mu_{\max} S}{K_s + S} \qquad \text{(Eq. 3)}$$

Whereas, the Haldane's kinetic equation considers the substrate to an inhibitory compound. Haldane's growth model was selected due to its mathematical simplicity and wide acceptance for representing the growth kinetics of inhibitory substrates. The Haldane's inhibitory growth kinetics equation is as follows:

$$\mu_g = \frac{\mu_{\max} S}{K_s + S + (S^{2/}K_i)} \qquad \text{(Eq. 4)}$$

At higher substrate concentrations, S>>Ks, the above equation reduces to the following:

$$\mu_g = \frac{\mu_{\max} S}{S + (S^2 / K_i)} \qquad \text{(Eq. 5)}$$

or

$$\frac{1}{\mu_g} = \frac{1}{\mu_{\max}} + \frac{S}{(K_i \mu_{\max})} \qquad \text{(Eq. 6)}$$

This is the linearized Haldane's equation.

The experimental µ data obtained have been plotted against the respective initial concentration of phenol and wattle, respectively. (*Figs. 4* and *5*) show a typical trend that the values of µ increases with increase in initial substrate concentration up to certain initial concentration of the substrates and then it starts decreasing with further increase in initial concentration. This observation infers that both phenol and wattle are inhibitory types of substrates. Thus the Haldane's growth kinetic model has been used, which represents growth kinetic data of an inhibitory compound.

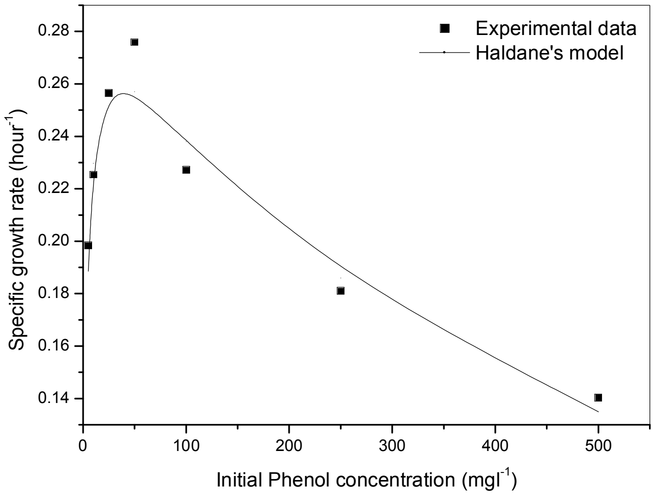

Figure 4. *Haldane's growth model fitted to the degradation of phenol by the mixed microbial consortia to determine the growth kinetic parameters.*

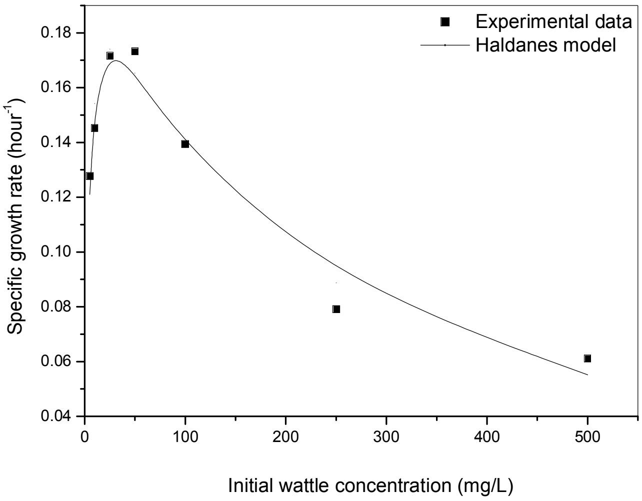

Figure 5. *Haldane's growth model fitted to the degradation of wattle by the mixed microbial consortia to determine the growth kinetic parameters.*

The estimation of the parameters using Linearized Haldane's model have been calculated and provided in (*Table 2*). Nonlinear regression technique has been adopted for the estimation of parameters of Haldane model. The experimental data have been solved for the Haldane equation using *SPSS 15 for Windows*, statistical software. The values for the linearized Haldane equation have been used as the starting guess values for the nonlinear regression fits. It could be observed from the figures that Haldane's model represents well the growth kinetics of the mixed culture. Also, the R^2 value has been observed to be above 0.930 in both the cases. The calculated parameter values are provided in (*Table 2*). The values of maximum specific growth rate μ_{max} (0.305 and 0.233 h^{-1} for phenol and wattle) and the substrate inhibition coefficient K_i (399 and 155 mgl^{-1} for phenol and wattle), were comparable to the values already published in various literatures (Pawlowsky, 1973 and Kumar, 2005). The values of substrate half saturation coefficient K_s was also well within the range (3.02 and 4.48 mgl^{-1}) (Kumaran, 1997).

Table 2. *Growth kinetics parameter values of linearized (LHM) and non-linearized (NLHM) Haldane's model for biodegradation of phenol and wattle by the mixed consortia of P. aeruginosa and B. subtilis*

Parameters		**Phenol**	**Wattle**
LHM	μ_{max} (h^{-1})	0.244	0.164
	K_i (mgl^{-1})	702	289
	R^2	0.800	0.915
NLHM	μ_{max} (h^{-1})	0.305	0.233
	K_i (mgl^{-1})	399	155
	K_s (mgl^{-1})	3.02	4.48
	R^2	0.935	0.971

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Endogenous or decay coefficient

A typical growth curve shows a decline in cell population after the complete consumption of substrate. During this declining phase some part of the cell population becomes the food for the rest of the cell population. This part of the growth curve in a batch reactor has been modelled by following equation.

$$\frac{dX}{dt} = -k_d X \qquad \text{(Eq. 7)}$$

In order to determine the value of k_d, the growth runs were not stopped, rather the measurement of cell density was continued further for another 5 to 6 days even after the complete consumption of phenol and wattle. Initial substrate concentrations were 250 mgl^{-1} for phenol and wattle, respectively. The data of the decay region were plotted as $\log_e$(OD) versus time (*Figs. 6* and *7*). The negative slope gives decay rate coefficient. The values of the decay rate coefficients obtained are 0.0069 and 0.0082 h^{-1} for phenol and wattle, respectively. These values are in agreement with the earlier studies by Kumar et al. (2005), where the decay coefficient value for phenol and catechol have been reported to be 0.0056 and 0.0067 h^{-1}. The decay coefficient is an important parameter that affects the growth kinetics. Higher the decay coefficient, the wash-out condition would occur at lower dilution rates. The observations from the experiment implies that a reactor treating wastewater containing mainly wattle should be operated at lesser dilution than when treating influent containing mainly phenol.

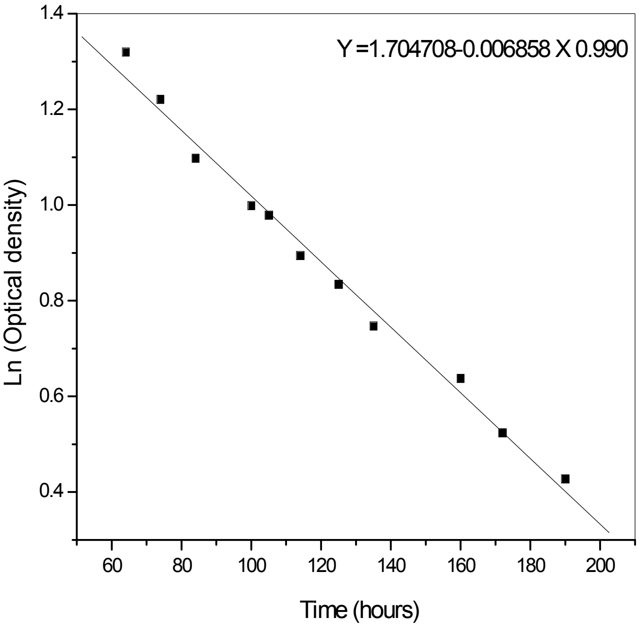

Figure 6. *Evaluation of decay coefficient K_d for the growth of the mixed consortia containing P. aeruginosa and B. subtilis on phenol using decay phase growth experimental data*

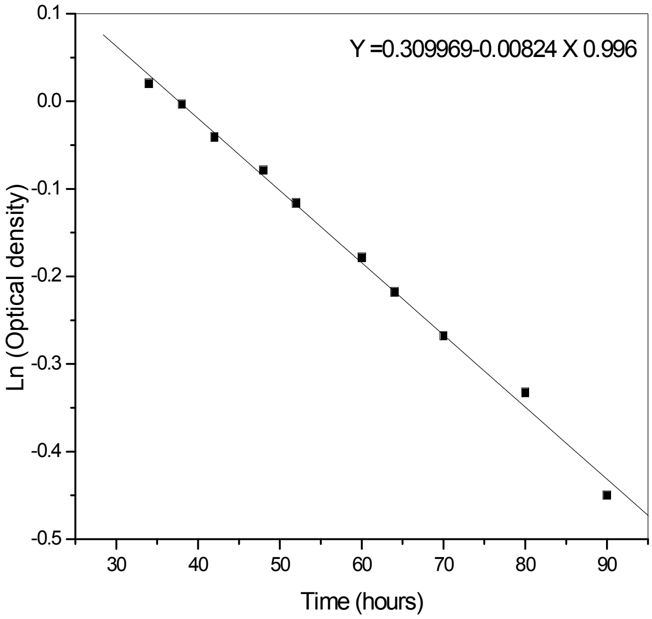

Figure 7. *Evaluation of decay coefficient K_d for the growth of the mixed consortia containing P. aeruginosa and B. subtilis on wattle using decay phase growth experimental data*

Conclusions

The degradation of phenol and a polyphenolic compound by the mixed culture containing *Pseudomonas aeruginosa* and *Bacillus subtilis* have been demonstrated. The favourable pH and temperature for the growth of bacteria has been established to be 7.0±0.5 and 37±2°C, respectively. The mixed culture was able to grow even at an initial concentration of 500 mgl^{-1} of phenol and wattle. The time taken for the complete degradation of 250 mgl^{-1} of phenol and wattle were estimated to be around 36 and 48 hours, respectively. According to growth kinetics calculation, the degradation of phenol and wattle has been well explained by the Haldane's inhibitory growth model. The growth kinetic parameters such as the maximum specific growth rate, the inhibitory and the half saturation coefficients for the degradation of phenol and wattle have been determined and found to be in accordance with the available literature values. The decay coefficient for the degradation of phenol and wattle has also been calculated from the decay phase of the growth of bacteria. The decay coefficient for the degradation of wattle was high, inferring that while treating the wastewater containing wattle, the reactor should be operated at lesser dilution.

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A STUDY ON DETECTING HEAVY METAL ACCUMULATION THROUGH BIOMONITORING: CONTENT OF TRACE ELEMENTS IN PLANTS AT MOUNT KAZDAGI IN TURKEY

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Abstract. The purpose of this study is to determine the present levels of atmospheric heavy metal pollution in the area of the Kazdagi Mountain in the Aegean region in the western part of Turkey. Twenty-five different plants were selected as potential biomonitors of trace elements including nickel, iron, zinc, lead, cadmium, and manganese (µg g^{-1}, dry weight). The samples were collected from two different heights of Mt. Kazdagi. Atomic absorption spectrometry was used to determine the concentrations of trace elements. The mean concentrations determined at 600 m altitude ranged from 0.107 to 0.442, 0.269 to 0.619, 0.873 to 9.030, 0.338 to 0.523 and 0.143 to 2.823 (µg g^{-1}, dry weight), for Ni, Zn, Fe, Pb and Mn, respectively. At 1100 m altitude, the values ranged from 0.119 to 1.806, 0.232 to 0.792, 0.618 to 5.720, 0.371 to 0.534 and 0.766 to 4.782 (µg g^{-1}, dry weight) for Ni, Zn, Fe, Pb and Mn, respectively. No Cd was found at either altitude. For the determination of the existence of any differences between the averages of the herbaceous and woody plants, comparisons were made in the independent sample *t*–test. In the statistical analysis, comparison of heavy metal pollution values of herbaceous and woody plants for Ni, Fe and Mn was significant ($P < 0.05$), while it was not for Pb and Zn.
Keywords: *Mt. Kazdagi, biomonitoring, trace element*

Introduction

Increasing anthropogenic influences on the environment have caused negative changes in natural ecosystems and altered the normal biogeochemical cycling (Ugulu et al., 2008; Dogan, 2012; Ugulu, 2012; Dogan et al., 2013). Among the anthropogenic activities, heavy metal contamination of the biosphere has increased sharply since 1900 and it poses major environmental and human health problems worldwide (Prasad & Freitas, 2003). The circulation and migration of metals in the natural environment are mainly related to such processes as rock decay, volcanic eruptions, evaporation of oceans, forest fires and soil formation. The sources of anthropogenic contamination or pollution of the environment by heavy metals include different branches of industry such as the power industry, transport, municipal waste management, waste dumping sites, fertilisers and waste used to fertilise soil (Aksoy et al., 2000; Karaca et al., 2005; Osma et al., 2012). The heavy metals from these sources are dispersed in the environment and they contaminate soil, water and air (Elekes et al., 2010). They also get into human and animal bodies, directly or indirectly, through plants (Szyczewski, 2009; Yasar et al, 2012a).

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Biological monitoring within a quality control programme involves the systematic use of living beings for obtaining quantitative information on changes in the environment, often due to anthropogenic activities (Bargagli, 1998). Biological responses can be considered more representative than data supplied by chemical or physical detectors, in that they are spatially and temporally extensive; moreover, they allow for estimating both the levels of pollutants and, even more importantly, the impact on biological receptors (Calzoni et al., 2007). For this reason, in order to evaluate, minimise and avoid detrimental effects of toxic metals, there has been an emphasis on the use of natural bioindicators to monitor atmospheric quality in both urban and rural environments (Szczepaniak & Biziuk, 2003; Ng et al., 2005).

Biomonitoring has been used to detect the deposition, accumulation and distribution of trace metals in the ecosystems. By using different types of vegetation with this method, the levels of atmospheric trace metallic concentrations have been successfully monitored (El–Hasan et al., 2002; Baslar et al., 2003; Dogan et al., 2007; Onder et al., 2007). Biomonitoring provides the cheapest and simplest method for monitoring trace metals elements in the atmosphere (Kaya & Yaman, 2008; Cayir et al., 2008).

Recently, many studies have been devoted to showing the distribution of heavy metal pollution in the urban and ruderal habitats in Turkey (Baslar et al., 2005; Yilmaz et al., 2006; Dogan et al., 2007; Cayir et al., 2008; Ozturk et al., 2008; Huseyinova et al., 2009; Akguc et al., 2010; Durkan et al., 2011; Ugulu et al., 2012; Yasar et al., 2012b). In some of these studies, mountainous areas, assumed to be unpolluted, have been used as control groups (i.e. Baslar et al., 2003, 2005, 2009; Yilmaz & Zengin, 2004; Dogan et al., 2007; Dogan et al., 2010). The present study is significant in that it aims to determine heavy metal levels in mountainous areas which are considered to be free of heavy metals, and, therefore, uses them as a reference.

The purpose of this study was to investigate and present the concentrations of lead, cadmium, nickel, zinc, iron and manganese by using plant samples from Mt. Kazdagi.

Materials and methods

Sampling area

Mt. Kazdagi (Mt. Ida) is the highest mountain on the Biga Peninsula, which is situated in the south-western part of Anatolia (*Fig.1*). It separates the Aegean and Marmara regions. It has three summits and the highest among them is 1774 m. Mt. Kazdagi possesses various climatic features due to its geographical position. On its south slopes, it is possible to see the characteristic Mediterranean climate because of its proximity to the Aegean Sea, whereas a cooler and more humid terrestrial climate is observed on its northern slopes.

Because of its climatic properties, geological structure and location, Mt. Kazdagi is ecologically and floristically diverse, containing a number of plant species endemic to Turkey. There are about 800 taxa growing naturally in the area and 79 of them are endemic to Turkey (Satil, 2008).

The location of the sampling points on steep slopes provides results that depend on altitude rather than on horizontal distance (Zechmeister, 1995). The samples were collected from altitudes of 600 m and 1100 m above sea level and 35–40 km away from the city centre.

***Figure 1**. Geographical location of the study area*

Sample collection and preparation

The samples were collected from 600 m and 1100 m during the months of July–August, 2006. Twenty-five plant species in total were collected, 11 of which were from 600 m and 14 from 1100 m. The taxonomy of the plant samples was determined according to Davis (1965–1985), Davis et al. (1988) and Guner et al. (2001).

For analysis purposes, about 100 g of aboveground parts of bushy species and well-developed leaves of other plants were collected. The samples were dried in an oven at 80°C for 24 h, milled in a micro–hammer cutter and fed through a 0.2 mm sieve. The samples were stored in clean self–sealing plastic bags under silica gel desiccant. In order to eliminate contamination from the micro–hammer cutter during the grinding, it was washed after every grinding, first with absolute alcohol then with distilled water. Therefore, the contamination was negligible.

Wet digestion procedure

The method described by the Perkin Elmer Corporation was used for plant digestion (Anonymous, 1996). The digested samples were aspirated into an air–acetylene flame and the existence of metals was determined by flame atomic absorption spectrometry (FAAS). The reproducibility was ensured by carrying out triplicate analyses. All samples were analysed immediately after digestion.

Reagents

Unless otherwise specified, all the chemicals used were of analytical reagent grade. Triple-distilled water was used throughout the experiments. By diluting the stock standard solution with water, working metal standard solutions were prepared just before use.

Instrumentation

The existence of the metals was determined with the Perkin Elmer Analyst 700 model flame atomic absorption spectrometer equipped with deuterium background

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correction, hollow cathode lamps (HCL) and acetylene burner. The absorption measurements of the metals were performed under the conditions recommended by the manufacturer. A Cole–Parmer microfiltration apparatus with membrane filter (0.45 µm pore size manufactured by Micro Filtration Systems, MFS) was used for the filtration of the aqueous phase before metal determination.

Data analysis

Statistical significance was determined by the independent sample *t*–test. In the independent sample t– test, comparisons were made in order to determine whether there were any differences between the averages of the herbaceous plants and woody plants. Differences at $P < 0.05$ were considered to be significant. A statistical package was used in the analysis of *t*–test for the data collected.

Results and discussion

The plants, used as biomonitors to investigate the levels of the trace elements, were sampled with 25 different species at two different levels of altitude on Mt. Kazdagi. The concentrations of elements were determined by atomic absorption spectrometry. The levels of the trace elements Cd, Ni, Zn, Fe, Pb and Mn (µg g^{-1}, dry weight) in plant samples from different altitudes of Mt. Kazdagi are given in *Tables 1* and *2*. As a result of experiments carried out, the following mean concentrations were determined for 600 m altitude: The contents of Ni, Zn, Fe, Pb and Mn (µg g^{-1}, dry weight) ranged from 0.107 to 0.442, 0.269 to 0.619, 0.873 to 9.030, 0.338 to 0.523 and 0.143 to 2.823, respectively (*Table 1*). As for the average for 1100 m altitude, the contents of Ni, Zn, Fe, Pb and Mn (µg g^{-1}, dry weight) ranged from 0.119 to 1.806, 0.232 to 0.792, 0.618 to 5.720, 0.371 to 0.534 and 0.766 to 4.782, respectively (*Table 2*). No Cd values were determined in the samples collected from both heights.

Table 1. *Trace element contents in plants growing in the Mt. Kazdagi (µg g–1 dry weight) (600m)*

Plant	Ni	Zn	Fe	Pb	Mn
		woody			
Celtis australis L.	0.170	0.598	2.347	0.504	0.877
Cydonia oblanga Miller.	0.138	0.521	1.432	0.523	0.226
Juglans regia L.	0.222	0.619	2.633	0.357	0.531
Malus sylvestris Miller.	0.134	0.414	1.449	0.503	0.230
Pinus brutia Ten.	0.133	0.337	0.873	0.352	1.429
Quercus cerris L. subsp. *cerris*	0.169	0.305	2.731	0.498	2.823
		herbaceous			
Dryopteris filix–mas (L.) Schott	0.107	0.269	1.332	0.398	0.925
Epilobium angustifolium L.	0.442	0.432	9.030	0.453	1.525
Hypericum lydium L.	0.196	0.538	3.179	0.454	0.519
Juncus inflexus L.	0.208	0.407	1.067	0.376	0.589
Verbascum sp.	0.150	0.366	0.897	0.338	0.143
Min.:	0.107	0.269	0.873	0.338	0.143
Max.:	0.442	0.619	9.030	0.523	2.823
Mean:	0.18±0.02	0.43±0.03	2.45±0.70	0.43±0.02	0.89±0.23

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Some plant species are useful for biomonitoring of the atmospheric deposition of pollutants (Kaya & Yaman 2008; Baycu et al., 2006; Mertens et al., 2005; Singh et al., 2005; Smodis et al., 2004). In this respect, the aggregation of investigated trace elements in plants collected from the 600 m height on Mt. Kazdagi was presented in *Table 1*. It can be seen from the table that Ni content was the highest in *Epilobium angustifolium* (0.442 µg g^{-1}), and the lowest in *Dryopteris filix–mas* (0.107 µg g^{-1}). Zn content was the highest in *Juglans regia* (0.619 µg g^{-1}), and the lowest in *Dryopteris filix–mas* (0.269 µg g^{-1}). It was determined that Fe content was the highest in *Epilobium angustifolium* (9.030 µg g^{-1}), while the lowest was in *Pinus brutia* (0.873 µg g^{-1}). In terms of Pb content, *Cydonia oblanga* (0.523 µg g^{-1}) was the highest, and *Verbascum* sp. (0.338 µg g^{-1}) was the lowest. Mn content was the highest in *Quercus cerris* subsp. *cerris* (2.823 µg g^{-1}), and the lowest in *Verbascum* sp. (0,143 µg g^{-1}). At 1000 m, the highest values were recorded as Ni and Fe in *Epilobium angustifolium,* Zn in *Juglans regia,* Pb in *Cydonia oblanga* and Mn in *Quercus cerris* subsp. *cerris.*

The results of analysis of trace element values in plants collected at 1100 m are presented in *Table 2*. The table shows that Ni content was highest in *Plantago holosteum* (1.806 µg g^{-1}), and the lowest in *Rubus idaeus* (0.119 µg g^{-1}). In terms of Zn content, it was the highest in *Fragaria vesca* (0.792 µg g^{-1}), and the lowest in *Erica manipuliflora* (0.232 µg g^{-1}). Fe content was highest in *Fragaria vesca* (5.720 µg g^{-1}), and the lowest in *Abies nordmanniana* subsp. *equi–trojani* (0.618 µg g^{-1}). It was seen that Pb content was highest in *Abies nordmanniana* subsp. *equi–trojani* (0.534 µg g^{-1}), and the lowest was in *Origanum vulgare* (0.371 µg g^{-1}). The highest Mn content was observed in *Quercus cerris* subsp. *cerris* (4.782 µg g^{-1}), while the lowest Mn content was in *Celtis australis* (0.766 µg g^{-1}). At 1600 m, Ni content was highest in *Plantago holosteum*, Zn and Fe were highest in *Fragaria vesca,* Pb was highest in *Abies nordmanniana* subsp. *equi–trojani* and Mn was highest in *Quercus cerris* subsp. *cerris.*

Sources of pollution that cause accumulation of various heavy metals have been reported by some researchers. For instance, Pb and Zn originate mainly from anthropogenic activities (Alfani et al., 2000; Blok, 2005; Oliva & Rautio, 2005). The burning of coal and oil cause the production of Cu, Ni and Pb, and mining operations, steel works and the cement industry are major anthropogenic sources of Ni (Nriagu & Pacyna, 1988). Plants have been reported to be highly affected by contamination of the soil by Fe and Mn in the Mediterranean climate zone, although airborne Mn originates mainly from soil (Loppi et al., 1999; Bargagli et al., 2003; Oliva & Rautio, 2005). Fe originates both from anthropogenic and natural sources (Oliva & Rautio, 2005).

Various researchers in different parts of the world, as well as in Turkey, investigated the accumulation of trace elements in plant parts. Some of those are given below for the purpose of comparison with our findings: Dijingova et al. (1995) (Cd: 0.10–31.20 µg g^{-1}, Ni : 0.50–4.9 µg g^{-1}, Zn: 7–302 µg g^{-1}, Fe: 100–283 µg g^{-1}, Pb: 0.80–21.30 µg g^{-1}, Mn: 44–405 µg g^{-1}), Baslar et al. (2003) (Ni: 0.88 µg g^{-1}, Fe: 57.28 µg g^{-1}, Pb: 1.4 µg g^{-1}), Dogan et al. (2007) (Ni: 3.56 µg g^{-1}, Fe: 486.35 µg g^{-1}, Pb: 4.59 µg g^{-1}), Baslar et al. (2005) (Cd: 1.7 µg g^{-1}, Zn: 63.4 µg g^{-1}, Fe: 182.6 µg g^{-1}, Pb: 2.3 µg g^{-1}) and Kapusta et al. (2005) (Cd: 6.44 µg g^{-1}, Pb: 5.64 µg g^{-1}, Zn: 304 µg g^{-1}).

Trace elements, which are an intrinsic part of nature, appear in the composition of plants. Bowen (1979) has reported the normal natural concentration intervals for land plants as Cd: 0.2–2.4 µg g^{-1}, Ni: 1–5 µg g^{-1}, Zn: 20–400 µg g^{-1}, Fe: 70–700 µg g^{-1}, Pb: 1–13 µg g^{-1}, Mn: 20–700 µg g^{-1}. Comparison of our results with these findings (*Tables 1* and *2*) clearly shows that our values are well below the accepted range. Hence, the area studied is free from the contamination of heavy metal pollution in terms of the trace elements investigated. The accumulation levels obtained are soil orientated.

Table 2. *Trace element contents in plants growing in the Mt. Kazdagi (µg g–1 dry weight) (1100m)*

Plant	Ni	Zn	Fe	Pb	Mn
		woody			
Abies nordmanniana (Steven) Spach subsp. *equi-trojani* (Asch. & Sint. ex Boiss.) Coode & Cullen	0.171	0.631	0.618	0.534	2.601
Castanea sativa Miller.	0.214	0.704	1.902	0.414	2.246
Celtis australis L.	0.223	0.615	1.816	0.404	0.766
Erica manipuliflora Salisb.	0.200	0.232	4.065	0.440	2.634
Pinus nigra Arn. subsp. *pallasiana* (Lamb) Holmboe	0.237	0.486	1.272	0.417	2.623
Quercus cerris L. subsp. *cerris*	0.187	0.321	1.833	0.420	4.782
Rubus idaeus L.	0.119	0.393	1.045	0.470	3.820
		herbaceous			
Dactylis glomerata L. subsp. *hispanica* (Roth) Nymas	0.201	0.384	0.997	0.460	1.154
Dryopteris filix–mas (L.) Schott	0.179	0.510	0.812	0.434	0.892
Euphorbia sp.	0.175	0.659	1.632	0.390	0.823
Fragaria vesca Coville	0.678	0.792	5.720	0.512	3.352
Origanum vulgare L.	0.314	0.625	5.165	0.371	1.339
Plantago holosteum L.	1.806	0.732	4.823	0.519	2.474
Verbascum sp.	0.161	0.377	1.472	0.445	1.466
Min.:	0.119	0.232	0.618	0.371	0.766
Max.:	1.806	0.792	5.720	0.534	4.782
Mean:	0.34±0.11	0.53±0.04	2.36±0.47	0.44±0.01	2.21±0.32

Baslar et al. (2009) have obtained the following results in a study they conducted on Mt. Honaz, which is another important mountain of the same region: The mean concentrations determined at 1000 m altitude ranged between 0.273 to 0.488, 0.099 to 0.488, 0.306 to 0.682, 1.017 to 3.744, and 0.148 to 0.674 (µg g^{-1}, dry weight), of Pb, Ni, Zn, Fe and Mn, respectively. At 1600 m altitude, the values ranged between 0.225 to 0.534, 0.150 to 0.842, 0.234 to 0.905, 1.082 to 3.864 and 0.023 to 0.982(µg g^{-1}, dry weight) of Pb, Ni, Zn, Fe, Pb and Mn, respectively. No Cd was detected at either altitude. Additionally, Kula et al. (2010) have studied trace element concentrations of plants on Mt. Akdag and obtained the following results: The mean concentrations determined at 1000 m altitude ranged from 0.011 to 0.882, 0.241 to 0.714, 0.532 to 9.396, 0.329 to 0.487, and 0.155 to 3.439 (µg g^{-1}, dry weight), for Ni, Zn, Fe, Pb and Mn, respectively. At 1600 m altitude, the values ranged from 0.092 to 0.600, 0.272 to 0.834, 1.130 to 8.021, 0.263 to 0.889 and 0.076 to 0.508 (µg g^{-1}, dry weight) for Ni, Zn, Fe, Pb and Mn, respectively. No Cd was detected at either altitude. The similarities of the results obtained from Mt. Honaz, Mt. Akdag and Mt. Kazdagi show the validity and credibility of all studies concerned.

In the statistical analysis, comparison of heavy metal pollution values of herbaceous and woody plants for Ni, Fe and Mn was significant ($P < 0.05$), while it was not for Pb and Zn (*Table 3*). Kula et al. (2010) found the difference between the values of Fe, Pb and Mn from herbaceous and woody plants of Mt. Akdag statistically significant, while Yildiz et al. (2010) found the values of Fe and Mn statistically significant in their work on Mt. Bozdag. When we compare our results with those of the above two studies, it can be concluded that the concentration of Fe, particularly, displays a statistically significant difference between herbaceous and woody plants of the area.

Table 3. *Statistical analysis values of herbaceous and woody plants*

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Ni	Equal variances assumed	6,856	,015	-1,564	23	,131	-,206519	,132036	-,479656	,066617
	Equal variances not assumed			-1,501	11,139	,161	-,206519	,137598	-,508911	,095872
Zn	Equal variances assumed	,023	,881	-,516	23	,611	-,032506	,063001	-,162835	,097822
	Equal variances not assumed			-,514	22,433	,612	-,032506	,063201	-,163430	,098417
Fe	Equal variances assumed	12,325	,002	-1,502	23	,147	-1,163115	,774471	2,765230	,438999
	Equal variances not assumed			-1,454	13,522	,169	-1,163115	,800065	2,884797	,558566
Pb	Equal variances assumed	,331	,570	,842	23	,408	,019756	,023450	-,028753	,068266
	Equal variances not assumed			,846	23,000	,407	,019756	,023366	-,028579	,068092
Mn	Equal variances assumed	4,524	,044	1,460	23	,158	,701558	,480539	-,292513	1,695629
	Equal variances not assumed			1,487	20,271	,152	,701558	,471746	-,281643	1,684759

In the present study, the plants used as biomonitors to investigate the levels of the trace elements Cd, Ni, Zn, Fe, Pb and Mn (μg g^{-1}, dry weight), were sampled with 25 different species at two different heights (600 m and 1100 m) on Mt. Kazdagi. The values of the trace elements we obtained were below the values obtained from control samples of other studies of clean areas, and therefore low values are concluded to be soil orientated. We are convinced that this study will contribute to future studies on pollution.

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ARBUSCULAR MYCORRHIZAL FUNGUS ENHANCES MAIZE (*ZEA MAYS L.*) GROWTH AND ORGANIC CARBON SEQUESTRATION IN WHEAT (*TRITICUM AESTIVUM L.*) STRAW-AMENDED SOILS

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Abstract. A pot experiment was conducted to investigate the effects of wheat (*Triticum aestivum* L.) straws and arbuscular mycorrhizal (AM) fungi on maize (*Zea mays* L.) growth and organic carbon (C) sequestration in a sterilized sandy loam soil. The experiment included three treatments: control, amendment with wheat straws (S), and amendment with wheat straws plus inoculation with AM fungus *Glomus caledonium* (S+M). Compared with the control, the amendment of wheat straws significantly ($P<0.05$) increased soil alkaline phosphatase (ALP) activity in maize seedling period (30 d) and plant phosphorus (P)-uptake and shoot biomass at harvest time (110 d), and also tended to increase soil organic C content as well as produced low but demiurgic crop yield at harvest time. In wheat straw-amended soils, *G. caledonium* inoculation significantly ($P<0.05$) decreased soil pH and significantly ($P<0.05$) increased plant P-uptake as well as soil ALP activity in seedling period, and tended to increase shoot biomass and grain yield in seedling and harvest periods, respectively. Furthermore, *G. caledonium* inoculation tended to increase soil organic C content at harvest time, which was significantly ($P<0.05$) higher than that of the control, suggesting potential application of AM fungi in facilitating crop production and C sequestration in straw-returned fields.

Keywords: *alkaline phosphatase, Glomus caledonium, P-uptake, rhizosphere acidification, straw return*

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Introduction

Soil organic carbon (C), an important component of terrestrial ecosystems, is a crucial regulator of C fluxes between the biosphere and the atomosphere (Zhu and Mille, 2003). Soil C storage depends on the balance between C input and output. Optimizing agricultural managements can increase soil C by increasing C input and/or decreasing C output (Lu et al., 2009). Organic amendments are used primarily to increase nutrient availability to plants, and are essential for improving soil organic C content as well (Zhong et al., 2010). For instance, straw, the dry stalks of cereal plants, is an agricultural by-product after the grain and chaff have been removed. Since recycling of nutrients is the key to nutrient management in sustainable agricultural system, the management of straw in the field as crop residue for returning the organic matter to the soils is significant in agricultural production (Tuyen and Tan, 2001). Although straw has value from both the nutrients removed and the organic matter addition it will return to the soil, the value of the organic matter that straw returns to the soil is much more difficult to calculate, and it is the activity of microbe and enzyme that regulates C accumulation via mineralization and immobilization of plant derived residues in the soil.

Phosphorus (P) is one of the major essential macronutrients limiting plant growth owing to its low bioavailability in soils (Feng et al., 2004), and improving plant acquisition of P from soil is an obvious alternative to the management of those low P soils (Zhu et al., 2003). It is commonly known that arbuscular mycorrhizae (AMs) provide a direct link between soil and roots: AM fungi help plants to capture water and nutrients (notably P) from the soil, and in return, the plant provides the fungus with relatively constant and direct access to carbohydrate (Smith and Read, 2008), which are translocated from their source to root tissue and on to fungal partners. It is also generally accepted that AM fungi receive all their carbohydrate from host plant and that the association could create a sink demand for carbohydrate, which could result in a 4–20% drain of C from the host plant and could indirectly influence C storage in soils (Graham, 2000).

Although straw return can increase C input to soil from crop residues, uncertainties still remain about the impacts of AMs on crop production and soil organic C sequestration in straw-returned soils. Then, the objectives of this study were to investigate the effects of AMs on maize (*Zea mays* L.) growth and organic C sequestration in a wheat (*Triticum aestivum* L.) straws-amended soil, and find out the main factors that influence these parameters.

Materials and methods

Soil and mycorrhizal inoculum preparation

A subsurface soil sample was collected from an arable agricultural land at Fengqiu County (35º00′N, 114º24′E), Henan Province, China. The air-dried soil sample was ground with a wooden pestle, homogenized by sieving through a 5 mm sieve, autoclaved (121°C for 1 h on three successive days), and stored for the pot experiment.

The soil is derived from alluvial sediments of the Yellow River and classified as aquic inceptisol with the following properties: soil pH (soil: water ratio of 1: 2.5) 8.6, 3.66 g kg^{-1} organic C, 0.35 g kg^{-1} total N, 0.55 g kg^{-1} total P, and 22 g kg^{-1} total K. *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerdemann 90036 was isolated from a fluvo-aquic soil in Hennan Province, China (Liao et al., 2003), and deposited at the Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China. The AM inocula were propagated on white clover (*Trifolium repens* L.) grown in an autoclaved (121°C for 1 h on 3 successive days) substrate for two successive propagation cycles (4 months each). The inocula were a mixture of rhizospheric soil containing spores, hyphae, and mycorrhizal root fragments, and were air-dried and sieved (2 mm). At the same time, the non-mycorrhizal inoculum was also prepared with the same sterilized substratum on which white clover was cultivated under the same conditions.

Pot experiment

Three treatments were established in the pot experiment: control, amendment with wheat straw (S), and amendment with wheat straw plus inoculation with *G. caledonium* (S+M). Each square polyvinyl chloride (PVC) pot (30×30×30 cm) contained 7.5 kg of soil, which was mixed with 375 g of mycorrhizal/nonmycorrhizal inoculum, and each straw amendment treatment was also mixed with 37.5 g of wheat straw (< 1 cm). Six maize seeds were sown into each box, and three seedling per box were left after emergence. Plants were grown in a sunlit glasshouse with 30/22° C day/night temperature, 40-60% relative humidity and 60-70% water-holding capacity. Pots were randomly arranged with four replicates per treatment. Two maize seedlings per pot was harvested after growing for 30 days, and the other one was harvested after growing for 110 days. At each sampling time, soil samples were also collected.

Mycorrhizal colonization, plant and soil analysis

Wheat plants were divided into roots, shoots, and grains if possible. Fresh roots were used for mycorrhizal colonization assessment by the grid-line intersect method (Giovannetti and Mosse, 1980) after rinsing with tap water, clearing with 10% KOH and staining with acid fuchsin (Phillips and Hayman, 1970). Shoots and grains were weighed after oven drying at 70 °C for 48 h, and subsamples of dried and ground shoots and grains were taken for immediate nitric-perchloric acid digestion in HNO_3 (70%): $HClO_4$ (70%) mixture (6:1 v/v) (Zhu et al., 2003), followed by molybdenum-ascorbic acid spectrophotometry (Hanson, 1950), to measure tissue P concentration. To this end, the individual P-uptake by maize plant (shoot plus grain) was calculated. Soil samples were air-dried and homogenized by sieving through a 2 mm mesh sieve. Soil pH was determined with a glass electrode using a soil-to-water ratio of 1:2.5. Soil alkaline phosphatase activity was determined by incubation at 37 °C with borate buffer (pH 9) according to the method of Tabatabai (1982), and is given in units of mg *p*-nitrophenol produced g^{-1} soil 24 h^{-1}. Then, the enzyme activity was expressed on an oven-dried soil

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weight basis by correcting for water content in the soil (105 °C, 24 h). To this end, soil organic C content was determined by dichromate oxidation (Mebius, 1960).

Statistical analysis

The means and standard deviations of four replicates were computed. An analysis of variance was carried out using the one-way procedure with SPSS software while the comparison of mean effects was based on least significant difference (LSD) multiple-comparison tests ($P < 0.05$).

Results

Mycorrhizal colonization, shoot biomass, grain yield, and P uptake of maize plant

Mycorrhization in maize roots was shown in the *G. caledonium*-inoculated treatment (*Table 1*), and the colonization rates were 31.7±3.1% and 50.3±2.9% in seedling (30 d) and harvest (110 d) periods, respectively. The amendment of wheat straw significantly ($P < 0.05$) increased maize shoot biomass and P-uptake at harvest time but not in seedling period, and produced low but demiurgic crop yield, which approached zero in the control soils. In wheat straw-amended soils, *G. caledonium* inoculation significantly ($P < 0.05$) increased maize P-uptake and tended to increase maize shoot biomass in seedling period as well, but had no significant effects on them at harvest time, except for a trend towards higher grain yield.

Table 1. *Mycorrhizal colonization, shoot biomass, grain yield, and P-uptake of maize plant under different treatments.*

Sampling time	Treatment	Mycorrhizal colonization (%)	Individual shoot biomass (g)	Individual grain yield (g)	Individual P-uptake (mg)
30 d	Control	—	0.56 (0.11) b	—	0.39 (0.24) b
	S	—	0.64 (0.14) ab	—	0.59 (0.09) b
	S+M	31.7 (3.1)	0.84 (0.10) a	—	1.05 (0.20) a
110 d	Control	—	5.10 (0.40) b	—	3.09 (0.36) b
	S	—	8.28 (1.38) a	0.07 (0.12)	12.66 (1.22) a
	S+M	50.3 (2.9)	8.35 (2.55) a	2.28 (2.44)	11.93 (1.21) a

Control, without inoculation/amendment; S, amendment with wheat straw; S+M, amendment with wheat straw plus inoculation with AM fungi. Standard deviations are given in parentheses. Values within the same column and the same cultivation time not followed by the same letter differ significantly ($P < 0.05$).

Soil pH, alkaline phosphatase activity, and organic C content

The amendment of wheat straw significantly ($P < 0.05$) increased soil ALP (*Fig. 1b*) activity in maize seedling period, but had no significant effects on soil pH (*Fig. 1a*) and organic C content (*Fig. 1c*) both in maize seedling and harvest periods, except for a trend towards higher organic C content at maize harvest time. In wheat straw-amended soils, *G. caledonium* inoculation significantly ($P < 0.05$) decreased soil pH and significantly ($P < 0.05$) increased soil ALP activity in seedling period, and thereby tended to increase soil organic C content at maize harvest time, which was significantly ($P < 0.05$) higher than that of the control.

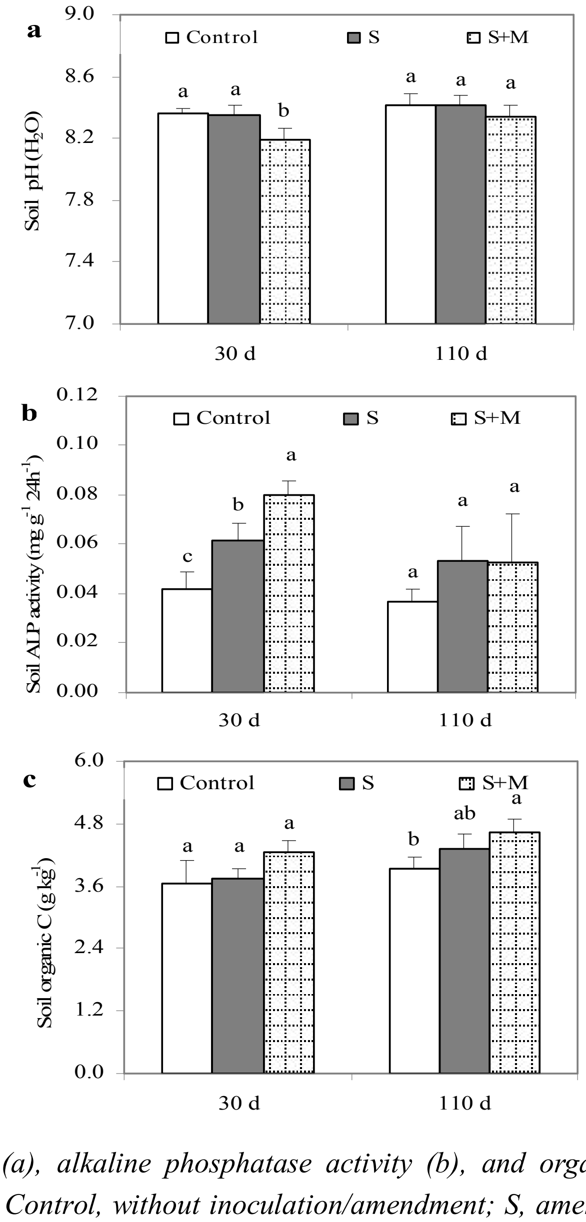

Figure 1. *Soil pH (a), alkaline phosphatase activity (b), and organic C content (c) under different treatments. Control, without inoculation/amendment; S, amendment with wheat straw; S+M, amendment with wheat straw plus inoculation with AM fungi. Vertical T bars indicate standard deviations. Bars within the same cultivation time not topped by the same letter indicate a significant difference in values (P < 0.05).*

Discussion

Organic amendments act as a source of C and other nutrients, which may improve soil organic C and favor plant growth as well (Albiach et al., 2000), owing to the enhanced plant nutrition. However, mechanisms causing increased grain yield upon AM fungal inoculation in straw-amended soils seem to be due to the rhizosphere acidification and enhanced ALP activity in the seedling period. On the one hand, plant roots alter rhizosphere soil pH by production or consumption of H^+ or by exudation of organic acids, and thereby induce changes in nutrient availability (Li et al., 1991), and such an effect is possibly enhanced by AM fungal inoculation (Bago et al., 1996). On the other hand, soil ALP activity is also involved in the mycorrhization effect on plant nutrition, since AM propagules themselves synthesize enzymes, such as ALP within the P-accumulating vacuoles of AM hyphae (Dong and Zhao, 2004), and mycorrhizal roots may release more root exudates containing enzymes than non-mycorrhizal roots because of the larger root system and/or improved nutrition (Wang et al., 2006). It is noteworthy that crops seedlings growing in straw-amended soils were still P limited in this study, and the significant increase of plant P-uptake in the seedling period upon AM fungal inoculation played a vital role in deuteric grain filling.

Straw amendment raised grain yield and plant biomass, which would also increase biomass input into soil from crop roots (Lu et al., 2009). However, in addition to facilitating plant uptake of mineral nutrients, AM fungi can also impact soil C dynamics directly by contributing to soil C inputs (Jakobsen and Rosendahl, 1990), and indirectly by modifying the decomposition of organic materials (Tu et al., 2006). Although they are thought to be unable to decompose organic materials directly due to a lack of saprotrophic capacity (Read and Perez-Moreno, 2003), AM fungi may alter rhizosphere soil directly or indirectly through changes in root exudation patterns or fungal exudates (Linderman, 1992), and still be involved in decomposition processes. For example, AM fungi have been found to proliferate in decomposing organic residues (St John et al., 1983), and therefore, may enhance wheat straw decomposition by stimulating some enzyme activities. Consequently, the combined application of wheat straw and AM fungi revealed the strongest influences on soil organic C content, which may be explained by the multitude of factors that may act in different ways, such as direct C sources and crop root exudates. Although the results indicated the potential application of AM fungi in facilitating C sequestration in straw-returned fields, the applying strategy still needs an in-depth study.

In summary, wheat straw increased maize shoot biomass and P-uptake as well as soil ALP activity, and tended to increase soil organic C content as well as produced low but demiurgic crop yield at harvest time. AM fungal inoculation greatly increased maize grain yield and soil organic C content in straw-amended soils, and seemed to be due to enhanced rhizosphere acidification and increased nutrients (notably P) uptake in the seedling period. The results indicated potential application of AM fungi in facilitating crop growth and C sequestration in straw-returned fields.

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ECOLOGICAL NICHE MODELLING WITH HERBARIUM DATA: A FRAMEWORK TO IMPROVE NATURA 2000 HABITAT MONITORING

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Abstract. The effectiveness of biodiversity conservation strategies depends on the knowledge about the distribution of habitats or single species. Despite this, efforts on biodiversity monitoring and conservation are currently hindered by a lack of information about the spatial distribution of species on large landscapes. Predictive species distribution models, can provide a powerful tool for solving this ecological problem. The vast majority of data available for modelling plants distribution are herbarium data, which lack reliable records of species absence. Although it has been found that herbarium records do not meet current standards for sampling in ecological studies, they remain often the only available source of sufficient magnitude with regard to relevant distribution data. Modifying existing statistical tools and developing new methods so that herbarium data, despite their shortcomings, can be used for modelling habitat suitability, is currently a growing field. The aim of this paper was to analyse the opportunities and bottlenecks for future application of distribution models in the mapping and monitoring of habitats of conservation interest in a complex Mediterranean area. Here we specifically concentrate on testing the Maximum entropy (Maxent) approach to estimate the distribution of a training habitat through the use of herbarium records and to explore a GIS-based integrated approach. The results obtained highlighted the important role that distribution models can have in individuating the areas where a targeted species or habitat type is most likely to be found, and in showing where to commit the limited available resources for inventories.

Keywords: *Ecological niche, GIS, Habitat distribution, Herbarium data, Maxent.*

Introduction

Identifying and mapping habitats of conservation interest around the globe represents a crucial step to individualize effective tools for biodiversity monitoring and conservation (Margules and Pressey, 2000; Groves et al., 2002). The effectiveness of conservation strategies crucially depends on the knowledge about the distribution of habitats or single species (Cianfrani et al., 2010). Alarmingly, efforts on biodiversity

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monitoring and conservation are currently hindered by a lack of information about the spatial distribution of species on large landscapes (Wilson et al., 2004). In this context, predictive species distribution models (Guisan and Zimmermann, 2000) based on complex ecological databases, can provide a powerful tool for solving this ecological problem.

Species distribution models attempt to estimate a species' niche across a geographical area by relating presence records of the species to environmental/ecological predictors. These models estimate the probability that species occur in areas where it has not directly been observed given an array of measured environmental variables (Segurado and Araujo, 2004). These environmental variables can include any sort of biotic or abiotic features that may favor the presence of the species being modeled and that can be measured spatially. Distribution models (also called 'habitat models') now have an established place within conservation biology, where they support many crucial aspects as landscape planning, reserve placement, habitat monitoring, and identification of suitable restoration sites (Funk and Richardson, 2002; Rodrìguez et al., 2007; Lobo, 2008).

Generally, distribution models use either presence-only data, or both presence and absence data (Guisan and Zimmermann, 2000; Elith et al., 2006) coming from a variety of sources as museums, herbaria, published inventories, transects and species lists (Stockwell and Peterson, 2002; Soberón and Peterson, 2004). While species data from planned surveys are ideal for modelling distributions, records for most species of the world are in a presence-only form (Cawsey et al., 2002). These are usually derived from ad-hoc observations that lack reliable records of species' absence (Dennis and Hardy, 1999; Graham et al., 2004; Lobo and Tognelli, 2011).

The vast majority of data available for modelling plants distribution are herbarium data, which are typically presence-only (Zaniewski et al., 2002; Wollan et al., 2008). However, it has been found that herbarium records provide limited accuracy in predicting distribution and do not meet current standards for sampling in ecological studies, as many populations may have been overlooked due to inadequate geographic coverage, or various collection biases (Rich and Woodruff, 1992; Heyligers, 1998; MacDougall et al., 1998; Hirzel and Guisan, 2002; Delisle et al., 2003; Ungricht et al., 2005). Nevertheless, herbarium data remain often the only available source of sufficient magnitude with regard to relevant and ample distribution data. Therefore, modifying existing statistical tools and developing new methods so that herbarium data, despite their shortcomings, can be used for modelling habitat suitability, is currently a growing field (e.g. Hirzel et al., 2002; Reutter et al., 2003; Engler et al., 2004; Hirzel et al., 2006).

A number of alternative modeling algorithms have been applied to classify the probability of species' presence as a function of a set of environmental variables and several studies have demonstrated that different modeling approaches have the potential to yield substantially different predictions (e.g. Loiselle et al., 2003; Thuiller, 2003; Brotons et al., 2004; Segurado and Araújo, 2004; Elith et al., 2006; Pearson et al., 2006). The Maximum entropy model (Maxent; Phillips et al., 2006; Elith et al., 2011) showed promising results in comparison with alternative methods (Elith et al., 2006; Pearson et al., 2006; Phillips et al. 2006; Peterson et al., 2007).

The aim of this paper is to disentangle the opportunities and drawbacks for future application of distribution models in the mapping and monitoring of habitats of conservation interest as these models could represent a straightforward but robust tool

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to improve, among others, the monitoring of Natura2000 habitats. Here we specifically concentrated on testing the Maximum entropy (Maxent) approach to estimate the distribution of habitat characterized by an intrinsic an internal complexity through the use of herbarium records.

Material and Methods

Study area

The study area covers the entire territory of the Tuscany region, situated in central Italy between 9° and 12° east longitude and 42° and 44°30′ north latitude. Tuscany is delimited by the Northern Apennines on the Northeast side and by the Tyrrhenian Sea on the West side (*Fig. 1*).

***Figure 1**. Study area (in red).*

Forests vary from the evergreen Mediterranean forests dominated by *Quercus ilex*, along the coastlines, to the *Fagus sylvatica*, *Castanea sativa* and *Abies alba* forests of mountain sites. Corine land-cover data (Bossard et al., 2000; APAT, 2005) show a surface territory of about 19,720 km^2 of which 44% is covered by forests, while the agriculture area covers about 46%. The agriculture types that take up the larger surface area include intensive non-irrigated arable land alternated with traditional agro-ecosystems, while broad-leaved forests represent the major natural class. From an environmental point of view, the region is peculiar for its extremely heterogeneous morphological and climatic features. The topography varies from the plain areas near the coast line and around the principal river valleys to the hilly and mountainous zones towards the Apennine chain. From the climatic point of view, Tuscany is influenced by

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its complex orographic structure and by the direction of the prevalent air flows: the central and southern parts of Tuscany fall within the temperate climatic zone with a dry season, the Mediterranean climate category, whereas the northern portion has some continental climate characteristics (Rapetti and Vittorini, 1995).

Target habitat and Herbarium data

The Natura2000 habitat "Arborescent matorral with Juniperus spp." (5210, corresponding to 32.13 Corine Biotope code) was selected here to test an integrated Maximum Entropy approach in habitat distribution estimation. This habitat has been selected as target habitat in Maxent distribution as: *i*) it is a habitat of conservation interest in Tuscany, *ii*) has a narrow ecological niche, *iii*) is one of the better represented habitat, in terms of characterizing species, in the Herbarium Universitatis Senensis (University of Siena, Italy).

The target habitat consists of Mediterranean and sub-Mediterranean evergreen sclerophyllous bush and scrubs, organized around arborescent junipers, and it is found in the countries bordering the Mediterranean Sea (Calaciura and Spinelli, 2008). *Juniperus* spp. are evergreen shrubs or small trees with few vital needs that, thanks to their morpho-physiological characteristics, colonize harsh environments such as rocky coasts and dry, incoherent soils (Pignatti, 1982; Jordano, 1991). Juniper, with its deep and well-developed root system, is therefore an important species for soil retention and consolidation, preventing soil erosion caused by rain and wind (Mondino and Bernetti, 1998). It is important as an ecotone, since it is often a transition area between ecosystems (Calaciura and Spinelli, 2008).

In the study area, the characteristic physiognomic combination of the arborescent matorral is: *Juniperus oxycedrus*, J. *phoenicea*, *Pistacia lentiscus*, *Rhamnus alaternus*, *Phillyrea latifolia*, *Myrtus communis*, *Lonicera implexa*, *Smilax aspera*, *Rubia peregrina*, *Clematis flammula*, *Helichrysum stoechas*, *Brachypodium ramosum* (De Dominicis et al., 1988; Chiarucci et al., 1999; Viciani et al., 2005; Foggi et al., 2006a,b).

Field data on characterizing species were gathered by the digitization and georeferencing of the essiccata preserved in the Herbarium Universitatis Senensis, for a total of 254 records (*Table 1*).

Modelling procedure

We considered 13 environmental variables as potential predictors of the target habitat distribution (*Tab.2*). An ortho-Landsat ETM+ image (path 192, row 030, acquisition date June 20, 2000; spatial resolution 30 meters) was acquired. Bands 1 (blue, 0.45 – 0.515 µm), 2 (green, 0.525 – 0.605 µm), 3 (red, 0.63 – 0.69 µm), 4 (near infrared, 0.75 – 9.90 µm), 5 (middle infrared, 1.55 – 1.75 µm) and 7 (middle infrared, 2.09 – 2.35 µm) were considered; bands 6.1, 6.2 and 8 were not taken into account due to the different pixel size from that of the other bands (60 meters of ground resolution opposed to 30 meters of the other bands). In order to obtain topographic variables we relied on a 75 meters digital elevation model of Tuscany region (DEM) resampled by a nearest neighbour algorithm at a spatial resolution of 30 m, processed with an algorithm of terrain analysis with the production of derivate images like altitude, slope, aspect and solar radiation. The climatic variables (1990-2000) were obtained by spatial interpolation (inverse distance weighting method) of 130 climatic stations operated by the Regional Agency for Innovation in Agriculture (ARSIA). All the image processing operations were performed using Grass GIS and QGIS software and resampled to 30 m pixels.

Table 1. *Number of training records used in the Maxent models and obtained average training AUC and standard deviation values.*

Maxent model	N° of records	AUC values	Standard deviation
Brachypodium ramosum	14	0.957	0.038
Clematis flammula	14	0.824	0.048
Helichrysum stoechas	17	0.980	0.026
Juniperus oxycedrus	34	0.971	0.012
Juniperus phoenicea	24	0.975	0.105
Lonicera implexa	23	0.916	0.016
Myrtus communis	18	0.911	0.015
Phillyrea latifolia	31	0.891	0.038
Pistacia lentiscus	17	0.903	0.030
Rhamnus alaternus	13	0.891	0.068
Rubia peregrina	20	0.852	0.036
Smilax aspera	29	0.913	0.024

A correlation analysis was performed, using the Spearman correlation coefficient (*cor* function within the R statistical software, R Development Core Team, 2013), in order to exclude pairs of related variables from the model. The analysis of the Spearman's rho statistic allowed the exclusion of pairs of highly correlated variables (Spearman's rho values ≥ 0.6), obtaining a set of 9 variables to be used as environmental layers in the Maxent modelling (*Table 2*).

Table 2*. Environmental variables used for Ecological niche modelling of the occurrence of Arborescent matorral with Juniperus spp. habitat.*

Spectral variables (Ortho-Landsat ETM+ image: path 192, row 030, acquisition date June 20, 2000; spatial resolution 30 meters)	band 1 (blue, 0.45 – 0.515 µm)
	band 4 (near infrared, 0.75 – 9.90 µm)
	band 7 (middle infrared, 2.09 – 2.35 µm)
Geomorphologic variables (DTM; spatial resolution 30 m)	Altitude (ALT)
	Slope (SLO)
	Solar radiation (SR)
Climatic variables (Spatial interpolation of 130 climatic stations operated by the Regional Agency for Innovation in Agriculture, ARSIA; spatial resolution 30m)	Mean winter temperature (MWR)
	Mean summer temperature (MST)
	Mean Annual Rainfall (MAR)

We applied here the Maximum Entropy modelling method (Maxent; Phillips et al., 2006; Phillips and Dudìk 2008; Elith et al., 2011) in order to estimate the potential habitat distribution for the species characterizing the selected target habitat. Maxent is a maximum entropy based machine learning program (http://www.cs.princeton.edu/~schapire/maxent/) that uses the principle of maximum entropy on presence-only data in order to approximate the species' niche and potential geographic distribution (Elith et al., 2006; Ortega-Huerta and Peterson, 2008; Hernandez et al., 2006; Pearson et al. 2007; Papes and Gaubert, 2007; Wisz et al., 2008; Kumar and Stohlgren, 2009; Warren et al., 2011). In estimating the probability distribution of a species across an area, Maxent formalizes the principle that the estimated distribution must agree with everything that is known (or inferred from the environmental conditions where the species has been observed) but should avoid making any assumptions that are not supported by the data (Shannon, 1948; Phillips et al., 2006). The approach attempts to estimate the maximum entropy (the distribution that is most spread-out, or closest to uniform) subject to constraints imposed by the information available regarding the observed distribution of the species and environmental conditions across the study area (Phillips et al., 2004; Baldwin, 2009).

The model evaluates the suitability of each pixel as a function of environmental variables and estimates the most uniform distribution given the constraint that the expected value of each environmental predictor variable matches its empirical average (average values for the set of presence-only occurrence data) (Hernandez et al., 2008; Ficetola et al., 2010). Output from Maxent models is a raster map that represents the probability of presence of a species and that is made up of pixels whose values varies from 0 to 1, where 0 is the lowest and 1 the highest probability.

In order to assess the predictive performance of the models we followed the most commonly used approach that involves the use of the Receiver Operating Characteristic curves (ROC; Hanley and McNeil, 1982; Zweig and Campbell 1993). The area under curve (AUC) value indicates the model accuracy (Phillips et al., 2006; Jimenz-Valverde, 2011). For random prediction, AUC is 0.5. The main advantage of ROC analysis is that the AUC provides a single measure of model performance, independent of any particular choice of threshold.

In this work we performed a bootstrap replicated run to do multiple runs (100) for each characterizing species (a total of 12 distribution models); through this method the training data is selected by sampling with replacement from the presence points, with the number of samples equaling the total number of presence points (Philips et al., 2006).

Then, the 12 Maxent raster outputs have been processed through overlay operations using the *raster map calculator* module of GRASS GIS software (*Fig.2*), in order to obtain a distribution map of the target Natura2000 habitat.

Accuracy assessment

In order to assess the accuracy of the distribution map of the target habitat, we generated 100 random test points, 50 in the areas characterized by an high probability to found the habitat and e 50 in the remaining areas. The threshold value of probability used to classify area with high or low suitability was equal to 0.5 (average result of mutliple runs). Once located with a high precision GPS, the test points have Been verified in the field on the basis of the presence of the characteristic physiognomic combination in a neighborhood of 30 meters.

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In order to assess the reliability of the model, the Kappa Index of Agreement (KIA) (Cohen, 1960) was computed using the following algorithm:

$$\mathbf{K = (p_o - p_e) / (1 - p_e)}$$

where **p_o** is the probability or proportion expected by chance and **p_e** is the probability or proportion observed.

The obtained KIA index allowed the assessment of the spatial agreement between the test points and the distribution map of the target habitat. The evaluation of the degree of agreement has been performed according to the interpretation scale proposed by Landis and Kock (1977),where values equal to 0 means null agreement and values equal to 1 means maximum agreement.

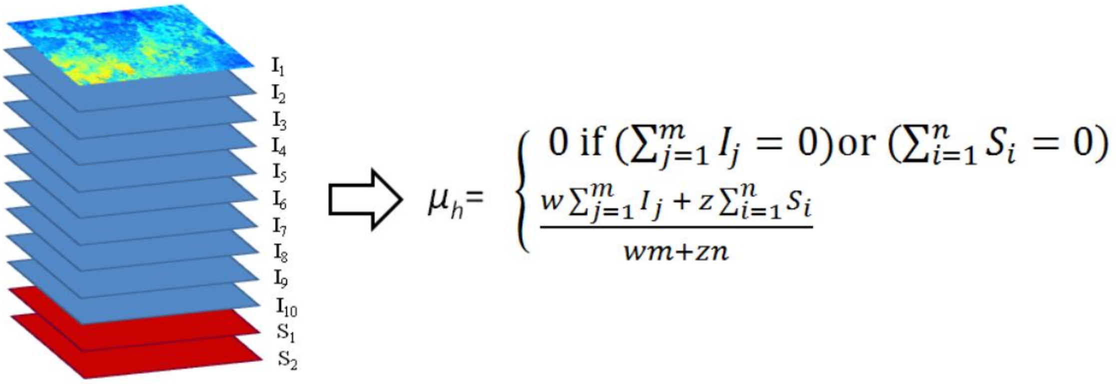

Figure 2*. Overlay algorithm used to obtain the habitat probability of occurrence (**μh**).* ***I****=key species (Juniperus);* ***m****= number of key species;* ***S****=other characterizing species;* ***n****= number of characterizing species;* ***w****= value of weight for key species;* ***z****= value of weight for characterizing species.*

Results and discussion

The approach proposed in this study yielded an habitat distribution map, highlighting how the application of a such integrated method could represent an accurate and cost-effective tool to obtain distribution maps for large and ecologically complex areas (Amici, 2011). Moreover, the modelling approach (Maxent) and the accurate geo-referencing of the herbarium specimens, allowed to use a fair amount of data that are often required to estimate species or habitat distribution for large areas. This aspect is essential as Herbarium records are often perceived as untrustworthy since they have been obtained without any planned sampling scheme, and their geographical locations are often imprecise (Elith et al., 2006; Hortal et al., 2008). Compared with contemporary data, most Herbarium data were collected using outdated methodologies, with associated uncertainties and inaccuracies and might not provide strong inference on species distribution modeling (Golding 2004; Hernandez and Navarro, 2007).

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In this context, choosing the most suitable modelling approach becomes crucial: numerous recent studies have successfully applied niche-based models, using presence-only data (e.g. data from herbarium collections; Loiselle et al., 2008) to map habitat in space (Phillips et al., 2006; Pearson et al., 2007). By isolating the niche relationships in occurrence data, presence-only habitat models supply knowledge of species' environmental and spatial distributions with less dependence on observational factors (Elith et al., 2006). In particular Maxent has been found as one of the most effective methods for species distribution modelling, as it showed high quality performance with both small and large sample sizes (Elith et al., 2006; Wisz et al., 2008) and for its capacity to predict novel presence localities for poorly known species (Pearson et al., 2007). The distribution maps provided through Maxent have a probabilistic interpretation, giving a smooth gradation from most to least suitable conditions.

In this work Maxent models output resulted in twelve maps showing the mean probability of occurrence for each characterizing species.

The Maxent internal test of model performance showed AUC values ranging from 0.98 (*Helichrysum stoechas)* to 0.82 (*Clematis flammula*), which indicate that predictive quality of the models can be considered good (*Table 1*). How close the AUC is to its potential maximum, can ultimately only be assessed if it is known how specialized is the environmental niche that the species occupies, because a wider niche corresponds generally with a lower AUC value (Phillips et al., 2006; Elith et al., 2011). In fact, lower values of AUC are related to *Clematis flammula*, that could be considered a sinantropic species, and to species as *Rubia peregrina, Phyllirea latifolia* and *Rhamnus alaternus*, that represent species with a wider ecological niche than the other characterizing species. Those species could be found in different types of Mediterranean habitats, from the thermophilous mixed oak forests to mixed forest dominated by *Quercus ilex* and Mediterranean maquis (Ahrends et al., 2011). Species with wider distributions often encompass a greater range of environmental conditions (Gaston, 2003) and thus may be harder to model, especially when the number of training points is limited (e.g. see Thuiller et al., 2004).

The highest AUC values are related to *Juniperus oxycedrus*, *J. phoenicea* and *Helicrysum stoechas* (that represent a species with a narrow ecological niche). In the study area *Helicrysum stoechas* is mostly found in scrubland and coastal maquis along the Tyrrhenian coast (Arrigoni et al., 1985; De Dominicis, 1993). The two *Juniperus* species, besides being the main species that characterize the target habitat, are found on rocky slopes, deforested areas and costal sands, mostly on calcareous substrates often with southern exposures (Angiolini et al., 2002, Landi et al., 2007).

The analysis of the contribution to the models of the selected predictors may reinforce the understanding of the ecological properties beyond the characterizing species and, consequently, of the target habitat. In this regard, analyzing the results on the contribution of each environmental variable to the models (*Table 3*), it is not surprising that the variables that have the greatest contribution to the models are, in most cases, the climatic one and in particular the rainfall and the solar radiation. In fact, the habitat type used as a test in this study presents an ecological niche characterized by xeric, warm, sunny and harsh situations and can be considered semi-arid, occupying unsheltered, steep biotopes (Blasi et al., 2005; Calaciura and Spinelli, 2008).

Concerning the spectral variables (Landsat ETM+ bands), is interesting to note that the highest contribution could be found for the models of species identified as dominant of the top layer of a shrubland (e.g. *Juniperus oxycedrus*, *Juniperus phoenicea*, *Phyllirea latifolia*) or species that are frequently associated with the Mediterranean maquis (e.g *Myrtus communis*, *Pistacia lentiscus*). Instead, the contribution of spectral reflectance with regard to herbaceous species, has generally the lowest values, since these species, in most cases, could not be identified by remote sensor due to the complexity and heterogeneity of habitats in which they are present.

***Table 3**. Relative contributions of the variables to the MAXENT models (%). For details about variables acronyms, see section Table 2. **Br** – Brachypodium ramosum, **Cf** - Clematis flammula, **Hs** - Helichrysum stoechas, **Jo** - Juniperus oxycedrus, **Jp** – Juniperus phoenicea, **Li** - Lonicera implexa, **Mc** – Myrtus communis, **Pla** - Phillyrea latifolia, **Ple** - Pistacia lentiscus, **Ra** - Rhamnus alaternus, **Rp** – Rubia peregrina, **Sa** – Smilax aspera.*

Variables	*Br*	*Cf*	*Hs*	*Jo*	*Jp*	*Li*	*Mc*	*Pla*	*Ple*	*Ra*	*Rp*	*Sa*
Band1	2.2	3.2	5.9	7.4	5.9	2.8	4.4	7.3	12.9	6.1	6.1	6
Band4	2.1	3.1	0.5	8.6	3.8	3.6	0	3.1	1.2	1.8	1.8	2.1
Band7	5.4	6.1	2.8	9.5	8.7	6.1	19.7	18.4	21.4	22	37.1	17.7
ALT	8.1	6.5	10.1	6.2	8.7	7.6	36.2	15.9	17.3	9.5	11.6	12.6
SLO	8.5	9.8	6.9	10.1	3.8	4.1	12.2	10.1	7.7	21.7	3.4	3.4
SR	8.9	14.6	16.7	5.7	8.2	18.5	12.3	10.9	19.1	6.7	5.6	12.5
MWT	7.7	8.1	12.4	12.1	24	11.7	2.4	5.9	10.6	8.8	10.8	13.2
MST	9.8	8.8	15.8	13.9	4.2	16.7	5.4	0.9	5.1	5.8	3.8	8.8
MAR	47.3	39.8	28.9	26.5	32.7	28.9	7.4	27.5	4.7	17.6	19.8	23.7

The performed overlay of the characterizing species models resulted in a raster map representing the probability of presence of the habitat “Arborescent matorral with Juniperus spp.” (*Fig.3*). The map shows the probability of occurrence values ranging from 0 to 0.58 as the result of the overlay between the individual distribution maps of the characterizing species, each representing the point-wise mean of 100 output grids. The habitat distribution map describes currently suitable areas for the target habitat, although that are delineated as suitable may in fact be unoccupied due to factors like human disturbance.

Nevertheless, as demonstrated by the accuracy assessment in a GIS environment , the results seem to be in agreement with the distribution of this habitat in Tuscany. Based on the field data, the KIA results showed a value equal to 0.82 corresponding to a very good strenght of agreement according to Landis and Koch (1977) scale. Nonetheless, unless the performed independent validation data, the expert opinion, as part of the validation process, will probably be useful for improving model accuracy (see Araùjo and Guisan, 2006 for a discussion of model evaluation).

Distribution predictions also may be improved by giving greater attention to sampling design, parameterization of the model, model selection and evaluation (Araùjo and Guisan, 2006).

In summary, despite the fact that herbaria and museums contain a vast storehouse of valuable information on biodiversity that offers great potential to advance conservation planning and decision-making (Graham et al., 2004; Soberón and Peterson, 2004), many natural history collections most species are represented by few specimens. Nevertheless, in recent years is increasing the efforts of ecologists to make available georeferenced and taxonomically verified records in electronic format for large-scale modelling (Loiselle et al., 2008). For this reason, even if in geographical areas where expert knowledge regarding species' distributions is limited because of relatively sparse geographical sampling, species distribution models might also be used by experts to improve their understanding of the geographical and environmental distributions of species.

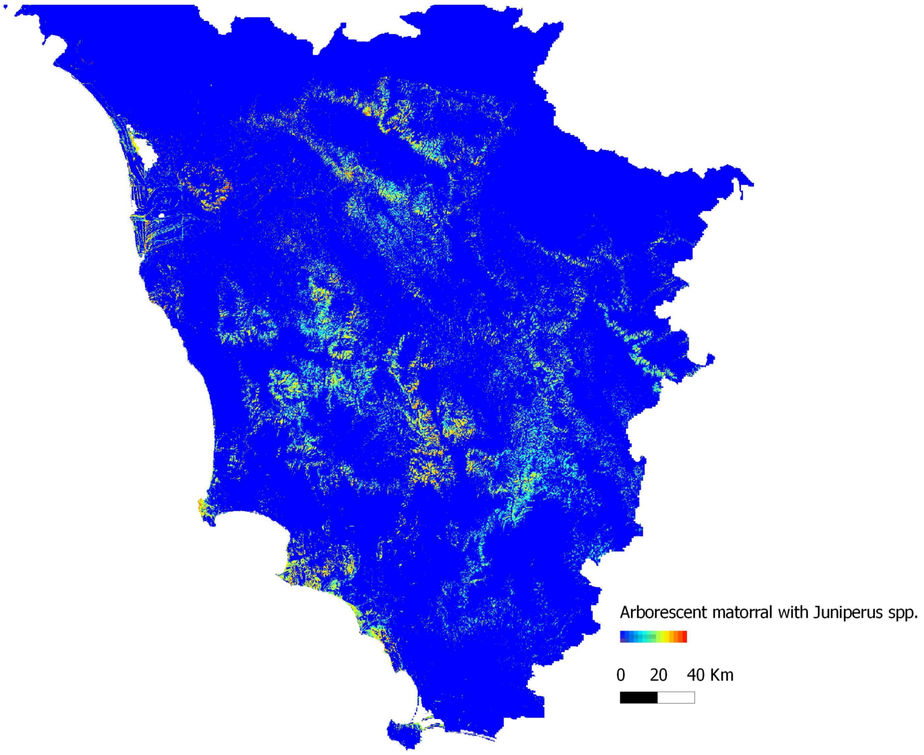

Figure 3. *Estimated distribution map of the Natura2000 habitat "Arborescent matorral with Juniperus spp." (5210) for Tuscany.*

Conclusions

The goal of this study was to test a Maximum entropy approach to predict habitat distributions in a complex Mediterranean area, given that many applications of habitat distribution modelling rely on existing natural history collections deposited at herbaria or museums.

The modelling approach followed by this study, taking into account the uncertainty proper of the ecosystems (Salski and Sperlbaum, 1991; Foody, 1999), could represent an invaluable tool for conservation management since it can aid in the process of focusing conservation action onto the right geographical locations and in ranking areas in terms of conservation values.

As also evidenced by the results of this paper, there are several advantages of using species distribution modeling to support conservation planning in complex landscapes, especially when a great amount of field data is not available. Maps based only on field occurrence data do not provide information on the likelihood of occurrence in areas that have not been surveyed, and also when surveyed, 'false absence' may occur

(MacKenzie, 2005). Moreover range maps from field and similar data are often too coarse or clustered to be useful for on-the-ground conservation action or research (Dormann et al., 2007); then accurate predictive distribution maps should be used with the aim of making field inventories more efficient and effective (Willis et al., 2003).

Our results confirm the important role that distribution models can have in highlighting the areas where a targeted species or habitat type is most likely to be found, and showing where to commit the limited available resources for inventories (Loiselle et al., 2008).

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THE IMPACT OF HUMAN LAND USE ON THE COMPOSITION AND RICHNESS OF GROUND AND DUNG BEETLE ASSEMBLAGES

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Abstract. This paper presents the results of a survey on the habitat and seasonal variation in activity density and richness of ground and dung beetle assemblages in a heterogeneous landscape of central Greece. Beetles were collected using 2,646 pitfall traps across an area of 138 ha representative of the Mediterranean mountainous landscape. Sampling was performed in cereal fields, corn fields, fallow land, grassland, *Robinia pseudoacacia* plantations, oak forest - farmland ecotone and oak forest using pitfall traps. A total of 18,275 individuals belonging to 38 species were collected, whereas only seven species constituted the 82.64% of the overall captures. *Onthophagus ovatus* was the most common species in all habitats. Beetle assemblages varied markedly regarding the number of species, while higher densities were reported during June. Significant differences in beetle richness were found considering habitat preferences with the lowest species richness in corn fields. Carabidae assemblages were both most abundant and diverse in plantations, whereas Scarabaeidae assemblages were most abundant and diverse in fallow land. Among estimated diversity indices, Margalef's index generated similar results with the observed diversity, likewise non parametric estimators. Overall, the results obtained in the present study suggest that agricultural areas in mountainous heterogeneous landscape might be the important factor for conserving rich beetle diversity, whereas supplementary biotic factors should be explored.

Keywords: *Mediterranean ecosystems; Carabidae; Scarabaeidae; habitat heterogeneity; correspondence analysis*

Introduction

Epigeic invertebrates have been successfully used as biological indicators of ecosystem health and habitat evaluation in a variety of studies (Redolfi et al., 1999; Vanbergen et al., 2005; Liu et al., 2006) focusing either on specific species or families or even on the whole order. A considerable effort has been invested in various aspects of their ecology, especially regarding effects of habitat heterogeneity and habitat loss on their activity density and distribution (Driscoll and Weir, 2005), and effects of the geographical location on their population sustainability (Thomas et al., 2001; Holland et al., 2004).

Among epigeic invertebrates, the most common ones are ground (Carabidae) and dung (Scarabaeidae) beetles. Most commonly ground beetles constitute an important group for comparative ecological studies since they are abundant, their ecology and

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systematics are well known (Lövei and Sunderland, 1996) and are sensitive to habitat changes as well (Niemelä et al., 1993). In addition, they are easily collected, allowing a standardized successful sampling, while some are highly selective and restricted to a particular habitat (Niemelä et al., 1992). Dung beetles though have proved to be key organisms in nutrient poor systems (Piñero and Avila, 2004), aerating and softening the soil (Brusaard, 1987; Herrick and Lal, 1996). However, only recently have they received increasing attention as indicators of changes in land use (Spector, 2006; Barragan et al., 2011) and the health status of pastures (Davis et al., 2004). Nonetheless, they have been scarcely studied in the Mediterranean region, except for some western European regions (France and Iberian Peninsula) and the western Rhodopes Mountains of Greece (Lobo et al., 2007). Wassmer (1995) studied the habitat selection of coprophagous beetles in southwestern Germany resulting in main differences comparing to other investigations from other geographic latitudes reflecting the variability within widely distributed species which are an ultimate reason for their expansion.

Several recent studies are dealing with beetle diversity in mountain landscapes of different geographical areas such as Iberian Peninsula (Lobo and Martin-Piera, 1999; Zamora et al., 2007) and a forested landscape of Mexico (Navarette and Halffter, 2008). It seems that habitat type and structure drive local species richness (Tews et al., 2004), whereas habitat heterogeneity is positively correlated with high diversity levels (Brose, 2003). Moreover, several studies have recorded higher diversity levels in agriculture unit (da Silva, 2008), tallgrass prairies (Larsen et al., 2003), mown meadows and riparian woodlands (Gutiérrez et al., 2004) than in other habitats. Contrasting results could reflect differences in habitat heterogeneity between study areas so there is a need for a closer examination of the effect of habitat heterogeneity on beetle diversity. Furthermore, forest edges with increased beetle species richness substantiate a significant edge effect (Báldi and Kisbenedek, 1994; Magura, 2002).

Despite the fact that Mediterranean area is one of the world’s richest regions in terms of animal and plant diversity (Myers et al., 2000; Verdú et al., 2000; Atauri and de Lucio, 2001; Burel et al., 2004; Cuttelod et al., 2008), there is only a handful of studies dealing with beetle distribution in upland landscapes of Greece. For example, Trichas (1997) studied the ecology and biogeography of ground coleoptera in the southern Aegean area, with emphasis on seasonal composition of Carabidae and Tenebrionidae assemblages, Anastasiou and Legakis (2002) studied the distribution of Carabidae in some mountains of Peloponnese, southern Greece, whereas Pitta (2009) studied the communities of soil arthropods in a mosaic of sites in different succesional stages on mountain Parnitha after fire. In northern Greece, Argyropoulou et al. (2005) described and compared the community organization patterns of Coleoptera in various sites of the Dadia forest reserve, regarding different types of vegetation and management practices. Although these studies have led to a better understanding of beetle diversity, even fewer studies have focused on seasonality and assemblage structure of ground and dung beetles (Theile, 1977; Jay-Robert et al., 1997; Errouissi et al., 2004). Finally, there has been no investigation on the relative contribution of cereal cultivation and adjacency with semi-natural habitats in mountainous landscapes on beetle richness. This lack of knowledge could lead to unsustainable land use management, as these insects play a crucial role in many ecosystem functions.

The aim of the current paper was to study the relationship between habitat heterogeneity and beetle richness in an upland Mediterranean landscape where agriculture is dominated by cereals. The specific objectives of the study were to investigate: (1) the species diversity of the landscape using beetles as an indicator taxon, (2) the seasonal distribution patterns of ground and dung beetle assemblages throughout their activity season; (3) whether habitat haterogeneity influences beetle species richness across cereal fields and neighbouring semi-natural habitats and (4) whether ground and dung beetle assemblages respond similarly to habitat heterogeneity.

Materials and methods

Study area

The study was conducted in a heterogeneous Mediterranean landscape of western Thessaly, in central Greece (Flabouresi, 33 km NE of Kalabaka town), (39°50΄44΄΄N and 21°42΄30΄΄W) (*Fig. 1A*). The study area is part of the Natura 2000 site “Antichasia - Meteora Mountains”, which is considered as an important area for bird species of european conservation concern (Meliadis and Kassioumis, 2001), the endemic plant species *Centaurea kalambakensis* (Freyn & Sint) and *Centaurea lactiflora* (Halácsy) (Asteraceae) and endangered insects such as *Rhysodes sulcatus* (Dalman) (Carabidae) (Legakis and Maragou, 2009).

The area of Flabouresi (altitude ≈ 800 m above sea level) consists of a mosaic of agricultural fields (412 ha) (*Fig.1B*) surrounded by forests dominated by *Quercus pubescens* (Willdenow) (Fagaceae). The topography of the southern slopes is moderate (average slope < 30°), whereas all other slopes are relatively low (average slope < 15°). The climate is characterized as typical sub-Mediterranean, with warm, dry summers, and mild, wet winters. The average monthly temperature ranges from 15.5 °C (January) to 26.9 °C (August), while the annual rainfall averages ≈ 700 mm (data from Kalabaka Meteorological Station, 27 km away). The dry period lasts from middle June to middle September.

Livestock (sheep, goats and cattle) grazing, along with agriculture, are the main productive activities of the area. The fieldwork was conducted in a total area of 138 ha:

- 61 ha of dry winter wheat [(Triticum aestivum Linnaeus (Poaceae)]), planted in mid September and harvested in late July;
- 6 ha of dry corn fields [(Zea mays Linnaeus (Poaceae)], planted in late May and harvested in late August;
- 9 ha of fallow land, plowed agricultural land unseeded for two or more growing seasons with short vegetation cover;
- 27 ha grazed grasslands, non fertilised, including mostly Anthemis arvensis, Avena fatua, Convolvulus arvensis, Lolium sp., Poa annua, Poa pratensis; Senecio vulgaris and Vicia cracca;
- 12 ha of managed Robinia pseudoacacia Linnaeus (Fabaceae) plantations, 10-year old trees up to 8-10 m high;
- 8 ha oak forest, 15-year old trees up to 15-20 m high dominated by Quercus pubescens, Q. frainetto and Q. ithaburensis var. cerris forming a sparse forest and
- 15 ha oak forest - farmland ecotones adjoining forests with other habitats.

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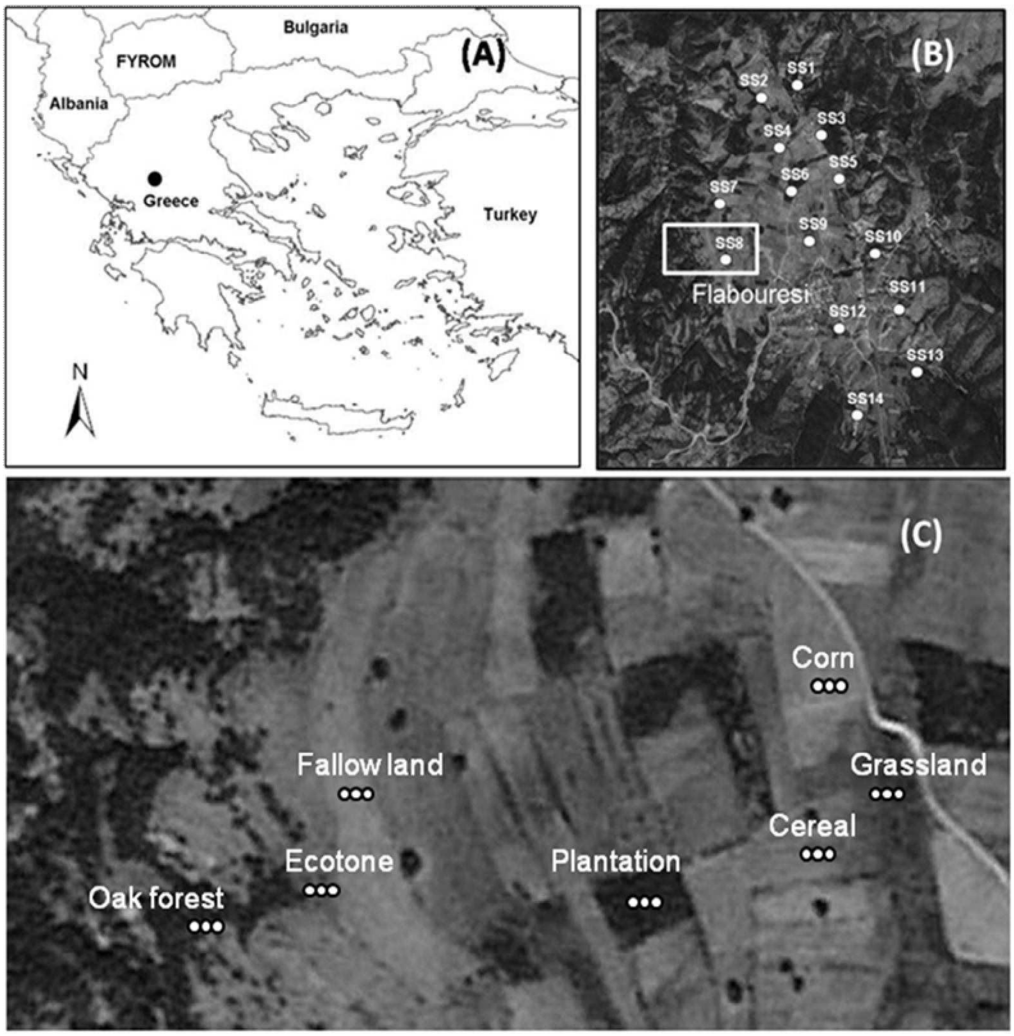

***Figure 1**. a) The study area, b) the 14 sampling sites (SS) and c) the sampling design of the pitfall traps at one of the 14 sampling sites*

Beetle sampling

Beetles were sampled at 14 sites (*Fig. 1B*) using plastic pitfall traps (diameter 9 cm, depth 13 cm) filled with 250 ml water plus 0.25% household vinegar (5% acetic acid) as a preserving solution (Thomas and Marshall, 1999). Each sampling site included all 7 habitats (see above). Pitfall traps were used to sample beetles as a very convenient and easy-to-operate method, yielding highly standardised samples (Southwood and Henderson, 2000). Collected samples (3 traps x 7 habitats x 14 sites x 9 months = total 2,646 traps) (*Fig. 1C*) were analysed in the laboratory. The distance between each trap was ≈ 10 m, for avoiding inter trap effects (Digweed et al., 1995), and within 10 m of each habitat fragment, so that trap catches represent the assemblage within that habitat. Pitfall traps were placed from 25 May 2006 until 15January 2007 for two consequent days (48 h) per month, to prevent oversampling that might have an excessive impact on the density of beetles and minimize depletion of the insect fauna. All pitfall traps were placed so that the lip of the cup to be slightly below of the ground surface in a fixed position throughout the sampling period. In few cases during the trapping period the solution was diluted by rainwater, but none of the traps overflowed. Five traps from the grasslands and 7 from the fallow land were excluded due to the severe damage by grazing animals. The geographical position of each pitfall trap was recorded using a global positioning system (GPS; e-Trex Vista, Garmin Co. Ltd.). Adult specimens were taken to the laboratory and identification to species level was accomplished using dichotomus keys (Chinery, 1993; Gueorguiev and Gueorguiev, 1995; Chinery, 2000) following the nomenclature of Fauna Europaea (Vigna-Talianti, 2007).

Statistical analysis

The numerical importance of each species in the structure of the whole community was computed by the total number of captured individuals (N), the relative activity density (%) and the species occurrence (%O) defined as the pure records of locations where a species occurred (Tsoar et al., 2007). To compare the variation of beetle assemblages among the different habitat types, the total number of activity densities and number of species per three trap complex (within habitat within site) were used. Values of variables were compared by an ANOVA, followed by the Tukey's *b* test. If assumptions of normality and homogeneity of variances (using Kolmogorov–Smirnov test and *F* test respectively, Sokal and Rohlf, 1995), were not met, data were transformed according to the method Box-Cox (Draper and Cox, 1969).

Although species richness is generally the most relevant component of the beetle diversity, alpha diversity was also calculated by three indices: i) the Shannon-Weiner index (H') (Shannon and Weaver, 1949), despite its sensitivity to the presence of rare species: $H' = -\Sigma p_i \ln p_i$, where p_i is the proportion of the ith species among the total collected; ii) the Berger-Parker's index (*BPI*), which is a measure of the numerical importance of the most abundant species: $BPI = N_{max} / N$ where N_{max} is the number of individuals of the most abundant species, and N is the total number of individuals in the sample; and iii) Margalef's diversity index (D_{Mg}), which balances the richness by the beetle numbers (Magurran, 1988): $D_{Mg} = (S-1) / \ln N$, where S is the number of species and N is the number of individuals.

To estimate species richness instead of the observed number of species (Brose, 2002; Chao, 2005), we tested the following four nonparametric estimators: i) Chao 2, ii) 1st Order Jacknife, iii) 2nd Order Jacknife and iv) Bootstrap. These nonparametric species richness estimators use the sample-based data to estimate the total number of species (Gotelli and Colwell, 2001; Chao and Bunge, 2002). All alpha diversity and nonparametric indices were generated using the software Species Diversity and Richness ver. 4.0 (PISCES Conservation Ltd 2006). The randomization test of Solow (1993) and the t-test were used to assess the significance of differences in diversity and richness along the different habitat types at the significance level 0.05.

To investigate the seasonal patterns of both beetle families mean values (± SE) of activity densities and species richness were calculated and presented graphically per month. To analyse the variation over time in activity densities and species richness of ground and dung assemblages along the various habitat types, repeated measures ANOVA was used followed by the Tukey's *b* test. The model included "habitat type" being the fixed factor (seven levels: cereal, corn, fallow, grassland, *Robinia pseudoacacia* plantation, oak forest - farmland ecotone and oak forest), "season" being the repeated factor (seven out of nine sampling periods, since there was no pitfall catches in December and January) and the interaction between the two variables. All data analyses were performed using the PASW Statistics 19 (SPSS Inc., Chicago, USA).

To explore the beetle compositional variation and find possible differences among different habitats, Correspondence Analysis (CA) (ter Braak and Smilauer, 2002) and the xlstat v. 2011.3.02 statistical analysis software (http://www.xlstat.com) were used. The multivariate data of species composition of the census points were analyzed by CA, which ordinated individual samples in a way that the differences among them would be maximized. Correspondence analysis was applied to the species x trap catches in order to reveal the relationships between a species and trap locations.

Results

Species composition and richness

A total of 18,275 adult beetles belonging to 38 species and 20 genera were collected throughout the sampling period (*Table 1*). A percentage of approximately 0.24 % of the whole captured specimens was not identified to species level because they had decayed in the trap. The number of singletons (species with only one individual) was 5 carabids, while the captured doubletons (species with only two individuals) were 2 carabids and 1 scarabid in the total 2,646 traps. The most abundant subfamilies in terms of the number of individuals recorded were: Scarabaeinae (N=15,706), Harpalinae (N=1,958) and Carabinae (N=559). The most numerous in terms of number of species within ground and dung beetle assemblages in comparison to all other genera were the genus *Pterostichus* and *Onthophagus*, respectively. Regarding ground beetle assemblages, the most frequently occurring species were: *Pterostichus nigrita* (Paykull), *P. cupreus* (Bonelli), *Carabus nemoralis* (Linnaeus) and *Myas chalybaeus* (Palliardi), whereas *Onthophagus ovatus* (Linnaeus), *O. coenobita* (Herbst) and *Gymnopleurus mopsus* (Pallas) were the most abundant dung beetles. *Zabrus tenebrioides* (Goeze), which is the most important pest for cereals of the captured species, was firstly captured in late July in the *R. pseudoacacia* plantations. A total of 67 individuals were captured throughout the entire sampling period. During the same period only 2 to 3 individuals of the species were captured in wheat and corn respectively. Twenty four out of the 38 species were classified as rare species representing less than 0.1% of all beetles collected (*Table 1*). Some of the most abundant species were captured in a small proportion of traps. For example, 2,086 individuals of *O. coenobita* were captured in 69 pitfall traps.

Habitat effects

Significantly different beetle richness was detected among habitats denoting habitat preferences (carabid species richness, $F = 7.548$, $p < 0.0001$; scarabid species richness, $F = 10.397$, $p < 0.0001$), whereas both assemblages exhibited the lowest species richness in corn fields (*Table 2*). Regarding the ground beetle assemblage, the highest number of species was recorded in grasslands, whereas similar number of dung beetle species was counted in fallow land. Oak forest was completely dominated by three species: *C. nemoralis* (total number of captured individuals N=141), *O. ovatus* (N=162) and *M. chalybaeus* (N=141). *O. ovatus* was also common in all other habitats, whereas the other two species occurred mostly in the oak forest. Similarly, significantly different beetle activity densities were found among habitats showing their habitat preferences (carabid activity density, $F = 5.986$, $p < 0.0001$; scarabid activity density, $F = 6.543$, $p < 0.0001$). Most common scarabids were captured within the plantations, whereas dung beetles were dominant in the catches from the fallow land.

***Table 1**. Overview of individuals trapped in the different habitats in pitfall traps in Flabouresi during 2006 (n=294). N: Total number of captured individuals; %: Relative activity density of each species in relation to the total number; f: Number of pitfall traps where the species was trapped, and O: species occurrence (in percentage) in total sampled traps*

Family	Species	Habitat							Total	%	f	%O
		Cereal fields	Corn fields	Fallow land	Grassland	Plantations	Ecotone	Oak forest				
Carabidae	*Acinopus sp.* L.	-	-	-	-	-	2	-	2	0,01	2	0,68
	Amara similata G.	-	1	-	-	1	-	-	2	0,01	1	0,34
	Amara aenea De Geer	-	-	-	-	1	-	-	1	0,01	8	2,72
	Amara sp. B.	-	-	-	1	-	-	-	1	0,01	2	0,68
	Amara aulica P.	14	-	1	-	-	6	1	22	0,12	1	0,34
	Anchomenus dorsalis L.	1	1	-	1	1	-	-	4	0,02	4	1,36
	Brachinus crepitans L.	3	-	1	3	26	-	-	33	0,18	15	5,1
	Carabus coriaceus L.	25	17	19	27	47	43	7	185	1,01	102	34,69
	Carabus violaceus L.	7	5	37	8	22	26	7	112	0,61	48	16,33
	Carabus nemoralis M.	2	3	7	1	39	64	141	257	1,41	89	30,27
	Carabus sp. L.	5	-	-	-	-	-	-	5	0,03	1	0,34
	Cicindela campestris L.	-	-	1	9	-	1	-	11	0,06	7	2,38
	Harpalus zabroides D.	1	-	-	-	-	-	-	1	0,01	1	0,34
	Molops piceus P.	24	7	1	20	16	1	1	70	0,38	29	9,86
	Molops striolatus F.	7	1	-	5	-	-	-	13	0,07	3	1,02
	Myas chalybaeus P.	4	1	2	2	93	44	141	287	1,57	74	25,17
	Nebria sp. L.	-	-	-	-	1	1	1	3	0,02	5	1,7
	Poecilus sericeus F.	-	-	1	1	6	8	6	22	0,12	15	5,1
	Poecilus cursorius D.	2	-	-	10	-	-	-	12	0,07	7	2,38
	Poecilus purpurascens D.	-	-	-	3	1	-	-	4	0,02	2	0,68
	Poecilus punctulatus S.	7	-	8	1	22	8	12	58	0,32	32	10,88
	Poecilus sp. B.	-	-	-	-	-	-	1	1	0,01	7	2,38
	Pterostichus cupreus L.	120	46	34	169	107	13	23	512	2,8	113	38,43
	Pterostichus nigrita P.	155	37	100	168	237	34	14	745	4,08	136	46,26
	Rhysodes sulcatus F.	-	-	-	-	-	-	1	1	0,01	1	0,34
	Sphodrus sp.	1	39	2	2	29	16	32	121	0,66	33	11,22
	Zabrus tenebrioides Gze.	2	3	6	2	44	9	1	67	0,37	7	2,38
	Zabrus curtus A.S.	13	1	2	1	-	-	-	17	0,09		
Scarabaeidae	*Copris lunaris* L.	-	-	2	-	-	-	-	2	0,01	2	0,68
	Copris hispanus L.	-	-	6	-	1	-	-	7	0,04	4	1,36
	Gymnopleurus mopsus P.	231	-	350	125	32	72	1	811	4,44	62	21,08
	Onthophagus ovatus L.	2230	276	4451	3508	664	1115	162	12406	67,86	171	58,16
	Onthophagus coenobita Hbst.	267	3	366	614	50	724	62	2086	11,41	69	23,47
	Onthophagus amyntas O.	8	-	-	-	-	-	-	8	0,04	2	0,68
	Scarabaeus laticollis L.	-	-	1	-	-	1	1	3	0,02	3	1,02
	Scarabaeus sacer L.	8	-	37	8	2	11	-	66	0,36	28	9,52
	Scarabaeus semipunctatus F.	-	-	4	1	-	-	-	5	0,03	4	1,36
	Sisyphus scaefferi L.	33	-	69	42	34	84	50	312	1,71	19	6,46

Beetle diversity

The relationships between alpha diversity indices and the beetle assemblages among the habitat types varied significantly based on the randomization Solow test (*Table 2*). According to it, carabids were much more diverse in plantations similar to their activity densities. Low Shannon-Wiener index value was recorded in grasslands although most species were caught in this habitat. *BPI* values were high, meaning dominant species were recorded in this habitat resulting in great sensitivity of *H′* values, which was different from D_{Mg} since this is influenced by the rare species of the sample. Scarabids were significantly predominant and rich in fallow land and grasslands. On the contrary, corn fields were significantly poor with a total of only 2 species recorded. However, corn hosted one of the most dominant species (*O. ovatus*) (*Table 1*). Furthermore, Margalef's diversity index generated similar results with the observed ones, meaning the lowest value at the corn fields and the highest one at the fallow land.

Table 2. *Comparison of activity densities, species richness, values of Shannon-Wiener index (H′), Berger-Parker Index (BPI), Margalef's diversity index (DMg) and estimated non parametric indices of species richness among the different habitats for the total sampling period. Significance of difference in indices values are indicated by the letters "a", "b", "c" and "d". Means followed by the same letter did not differ significantly (p = 0.05)*

Observation		**Cereal fields**	**Corn fields**	**Fallow land**	**Grassland**	**Plantations**	**Ecotone**	**Oak forest**
Carabidae	**Abundance**	380	164	215	426	692	263	383
	Species richness	19	14	17	21	18	15	15
	Individuals (mean ± SE)	0.97±0.21b	0.07±0.15b	0.55±0.08b	1.09±0.21a	1.76±0.23a	0.67±0.07b	0.97±0.17b
	Species (mean ± SE)	0.16±0.01b	0.12±0.02c	0.15±0.02b	0.16±0.01b	0.26±0.02a	0.22±0.01a	0.18±0.02b
	Alpha diversity indices							
	H′	1.93c	1.87c	2.41a	1.53d	2.13b	2.18b	1.61d
	BPI	0.35b	0.28b	0.20b	0.45a	0.32b	0.22c	0.37b
	D_{Mg}	3.03c	2.55d	3.91a	3.30b	2.60c	2.51c	2.35d
	Estimated non parametric indices of species richness (mean ± SE)							
	Chao 2	13.87±1.16a	12.56±2.20a	27.56±3.85b	14.45±1.34a	13.90±0.86b	13.15±0.55b	11.75±0.76b
	1st Order Jacknife	19.51±1.71b	15.14±1.38c	16.47±1.64a	21.08±2.15a	18.50±1.17b	15.02±0.82c	14.68±1.07c
	2nd Order Jacknife	22.46±2.43b	15.62±1.79c	19.55±2.37a	25.13±3.15b	19.58±1.95b	15.16±1.35c	16.48±1.67c
	Bootstrap	15.22±1.63a	11.24±1.34b	12.76±1.46a	16.16±1.98a	15.55±1.31b	13.56±1.14b	12.55±1.24b
Scarabaeidae	**Abundance**	2735	273	4746	4124	842	1854	279
	Species richness	6	2	9	6	6	6	5
	Individuals (mean ± SE)	19.53±0.44a	1.95±0.84c	33.9±9.29a	29.46±7.07ab	6.01±2.53c	13.24±4.44bc	1.97±3.33c
	Species (mean ± SE)	0.25±0.02a	0.08±0.01d	0.36±0.03a	0.33±0.04ab	0.20±0.03c	0.22±0.03bc	0.15±0.03cd
	Alpha diversity indices							
	H′	0.65a	0.06c	0.63c	0.59a	0.48c	0.80b	0.85b
	BPI	0.81d	0.98a	0.83a	0.81d	0.88a	0.58c	0.69c
	D_{Mg}	0.63a	0.17b	0.94b	0.60a	0.74b	0.66b	0.71b
	Estimated non parametric indices of species richness (mean ± SE)							
	Chao 2	4.82±0.28c	1.48±0.11d	7.48±0.39a	5.37±0.28b	4.64±0.28c	4.94±0.31c	3.97±0.25d
	1st Order Jacknife	6.51±0.39b	2.02±0.18d	9.06±0.61a	5.93±0.27b	5.97±0.42b	6.52±0.34b	5.02±0.42c
	2nd Order Jacknife	5.94±0.64b	2.37±0.38d	9.46±0.84a	5.66±0.44b	5.86±2.01b	6.37±0.62b	5.69±0.68c
	Bootstrap	4.96±0.45b	1.66±0.18d	7.42±0.68a	5.79±0.45a	5.18±0.49b	5.11±0.48b	4.18±0.42c

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Chao 2 and the Bootstrap generated estimates that were not higher than the observed richness, but 1st and 2nd Order Jacknife estimates, much more similar to each other, were much higher than the observed ones, irrelevant to the beetle family (*Table 2*). Regarding only Scarabaeidae assemblage, all non parametric indices generated the highest species richness at the fallow land and the lowest one at the corn fields. This is in total accordance with D_{Mg} index and the observed values. In addition, the nonparametric estimator Chao 2 showed the same performance as the index D_{Mg} referring to both Carabidae and Scarabaeidae assemblages.

Seasonal patterns of ground and dung beetle assemblages

The seasonal pattern of the 38 species and 18,275 individuals trapped through the sampling period is given in *Figure 2.* Both carabid and scarabid species richness was highest in June and September and lowest in November. Moreover, there was a high seasonal variation as far as scarabids was concerned between May (total number of individuals N = 270) and June (total number of individuals N = 11,149). In addition, during the mid July – September period, scarabids decreased and in turn carabids became predominant in the pitfall catches. Seasonal pattern of activity density was similar to that of species richness with its highest value in June and the lowest one in November. No pitfall catch occurred in December and January.

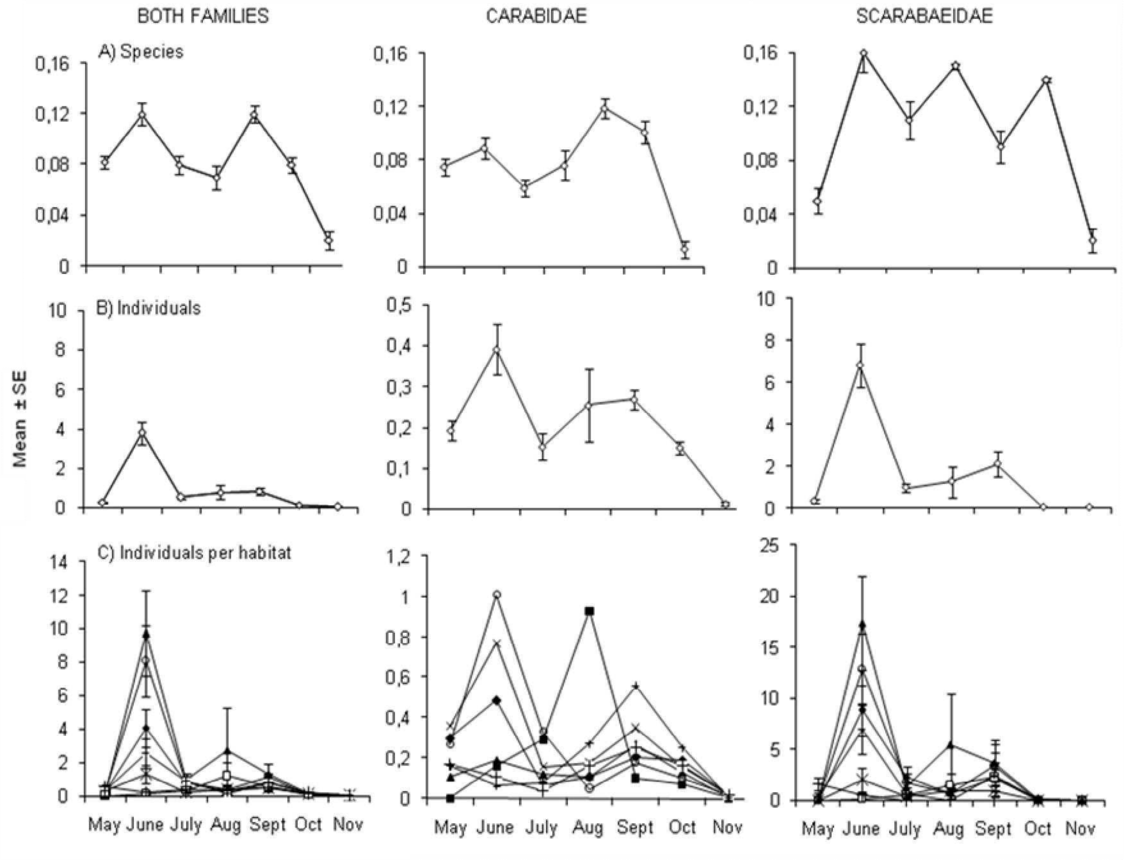

Figure 2*. Seasonal patterns of the number of beetles: (A) mean species richness (±SE), (B) mean activity densities (±SE), and (C) mean activity densities per habitat type (±SE) (—◆—, cereal fields; —□—, corn fields; —▲—, fallow; —×—, plantations; —○—, grassland; —+—, ecotone and —+— oak forest)*

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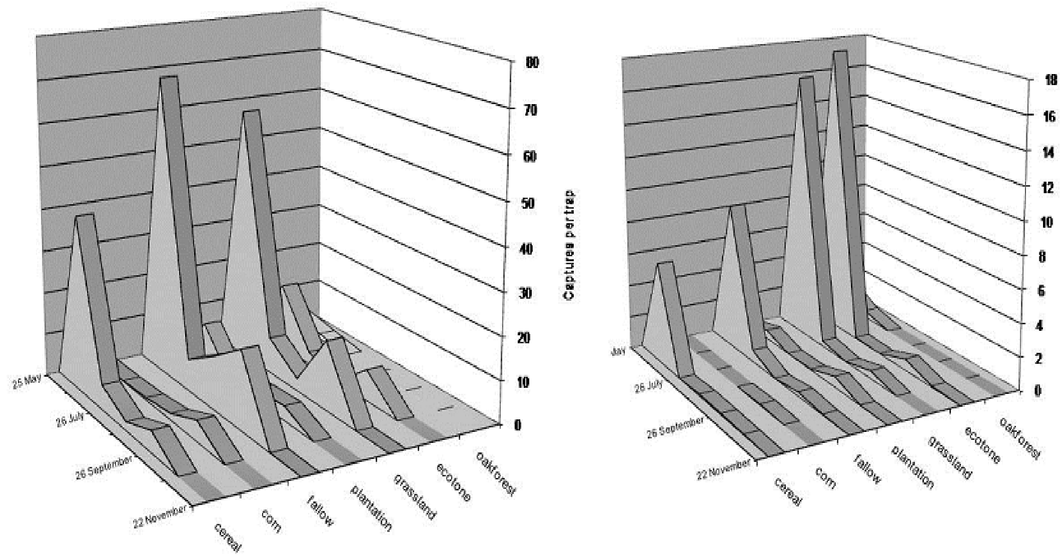
Based on seasonal activity recorded through the trapping period ground and dung beetles can be classified into two groups (*Fig. 3*).

Spring breeders appeared early in the season and their activity peaked from May until July. *O. coenobita* (*Fig. 3B*), *P. cupreus* (*Fig. 3C*) and *G. mopsus (Fig. 3F*) are characteristic species of that group. The second group, meaning *P. nigrita* (*Fig. 3D*) and *S. scaefferi* (*Fig. 3E*), was active during two periods, early in June and in the middle of September. Cereal fields hosted high beetle catches during the sampling period, while corn fields seemed to be the least preferred habitat (*Fig. 2C, Fig. 3*).

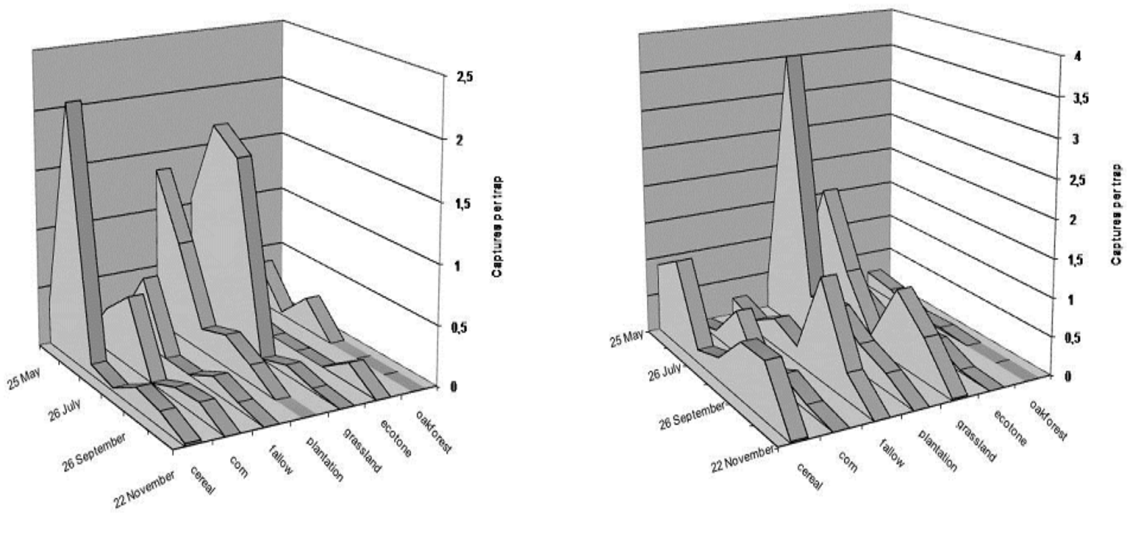
Habitat and season

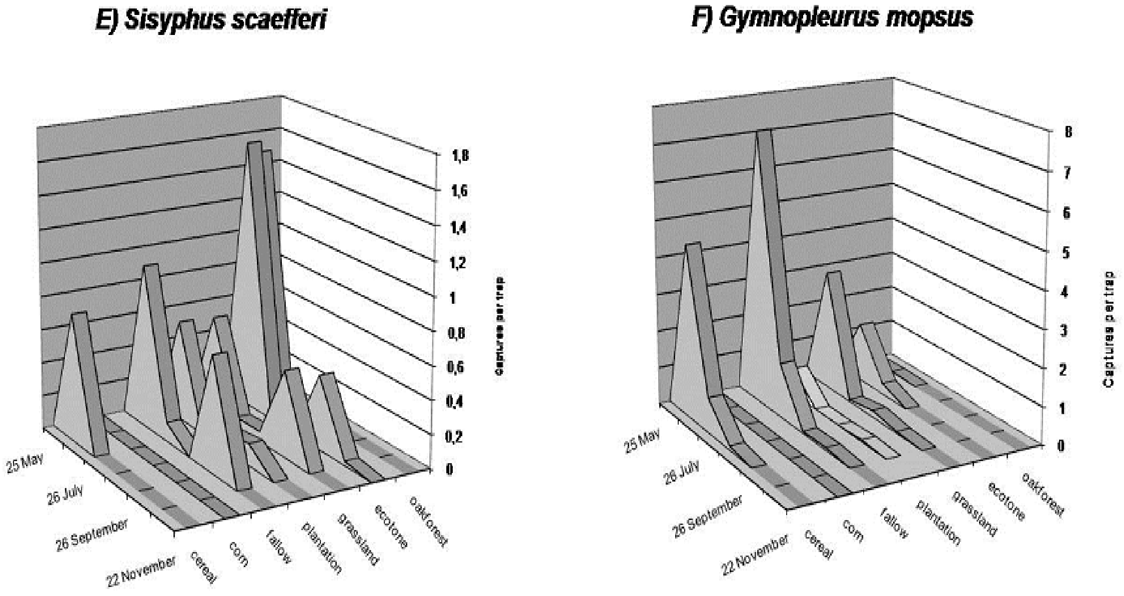
The repeated measures analysis of variance showed that there were significant differences in the activity densities and richness of ground and dung beetles caught in the seven habitats. Habitat type, season and their interaction had a significant effect on the overall number of pitfall catches (*Table 3*).

A) Onthophagus ovatus **B) Onthophagus coenobita**



C) Pterostichus cupreus **D) Pterostichus nigrita**




E) Sisyphus scaefferi

F) Gymnopleurus mopsus

***Figure 3**. Seasonal patterns of captures of the six most abundant species: A) Onthophagus ovatus B) Onthophagus coenobita C) Pterostichus cupreus D) Pterostichus nigrita E) Sisyphus scaefferi F) Gymnopleurus mopsus*

***Table 3**. Repeated measures analysis of variance (seven monthly counts made from 25 May to 25 November) of beetles in pitfall traps suspended on seven different habitats. Results of the Tukey's b test indicate which habitat type differs significantly ($p < 0.05$) from the others.*

Source of variation	df	MS	F	*p*	Tukey's *b* test
Carabidae species richness					
Habitat	6	1.703	19.179	0.001	plantation>ecotone=oak forest=grassland=cereal>fallow>corn
Error	287				
Season	6	33.638	73.848	<0.001	
Habitat x Season	36	1.716	3.768	<0.001	
Error (Season)	1722	0.456			
Carabidae activity density					
Habitat	6	12.558	7.405	<0.001	plantation> grassland=cereal=ecotone=oak forest=fallow>corn
Error	287	1.695			
Season	6	204.508	22.576	<0.001	
Habitat x Season	36	38.651	4.267	<0.001	

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Error (Season)	1.722	9.059			
Scarabaeidae species richness					
Habitat	6	1.169	11.579	<0.001	fallow=grassland=cereal=ecotone>plantation> oak forest=corn
Error	36				
Season	6	29.518	49.24	<0.001	
Habitat x Season	36	2.367	3.949	<0.001	
Error (Season)	1722	0.599			
Scarabaeidae activity density					
Habitat	6	1960.13	7.148	<0.001	fallow=grassland=cereal>ecotone>plantation= oak forest=corn
Error	287	274.21			
Season	6	54336.38	36.548	<0.001	
Habitat x Season	36	7061.82	4.750	<0.001	
Error (Season)	1722	1486.72			

The inertia percentage of the correspondence analysis for the two main axes generally explained 77.32% of the species variance. The inertia percentage of the first and the second axis was 60.83% and 16.49%, respectively. Characteristic species of the cereal fields and grasslands were *O. ovatus, P. cupreus* and *P. nigrita* (*Fig. 4*) showing relative inertia 0.032, 0.035 and 0.045, respectively. *G. mopsus, S. sacer* and *O. coenobita* were proved to be characteristic species for the fallow land. Only *C. coriaceus* (Linnaeus) with relative inertia 0.050 was proven as characteristic species of corn fields, while *P. sericeus, C. nemoralis, S. scaefferi* and *M. chalybaeus* characterized ecotone and oak forest. *B. crepitans* was strongly associated with the grassland, while *Z. tenebrioides* was most abundant in the plantations.

Discussion

Seasonality of beetle assemblages

Our results indicate that: (a) an upland landscape characterized by habitat heterogeneity shaped mainly by agriculture has rich biodiversity, (b) beetle assemblages show strong seasonality, (c) characteristic species are found depending on habitat type and (d) the intermediate disturbed agricultural land uses have higher species activity densities and richness in comparison to the wooded ones.

In the present study, in an area of 138 ha, 10 dung species were collected, whereas 40 species were sampled in Northern Alps from 250,000 ha (Lumaret and Stiernet, 1989).

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Land uses in our study area and specifically open agricultural areas and grazed grasslands, generate a rather heterogeneous landscape, even at a small scale as multi fragmentation of fields characterizes it. The number of individuals of the Scarabaeidae family (15,706 adult individuals) was high although the number of species was relatively low. Regarding singletons and doubletons, 8 locally rare species were found in our study area, such as the dung beetle *Copris lunaris* (Linnaeus) and the endangered ground beetle *R. sulcatus* (Legakis and Maragou, 2009). Although recording rare species is crucial as the collective loss of rare taxa could have significant effects on ecosystem functions as well as on the services these systems provide to humans (Lyons et al., 2005), rare insect species have been scarcely studied in Mediterranean mixed forest – agricultural landscapes (Ricarte et al., 2009; Sirami et al., 2010). In our study there were only five individuals of the pest *Z. tenebrioides* collected in crop fields (wheat, 2; corn, 3) probably due to the presence of diverse perennial habitats as Miller and Jones (1997) have reported. This species is a widespread pest in the winter and spring wheat-growing areas of our study area, western and eastern Europe, western Asia, northern Africa and south-central Asia (Bonnemaison, 1980; Burnett, 1984; Borror et al., 1989; Miller, 1991; Remaudière and Remaudière, 1997), causing severe crop losses in wheat monocultures. If it is true that habitat heterogeneity affects negatively its activity densities, then it should be explored from an agronomy point of view.

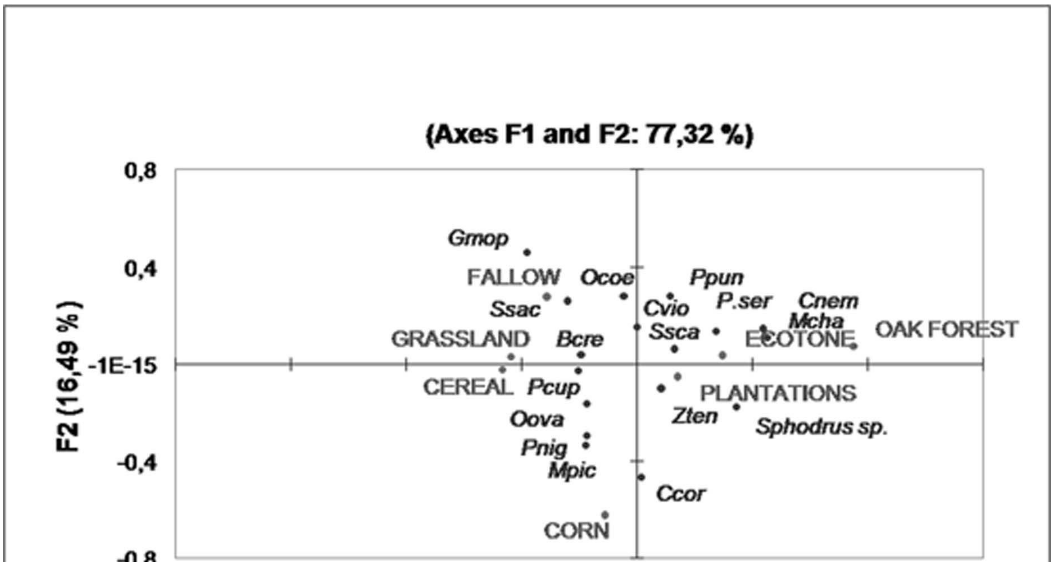

Figure 4. *Results of the correspondence analysis: spatial analysis of the species distribution among the habitats. Species names are presented with abbreviations*

Our results revealed strong seasonality, with significant differences occurring more within than between the two beetle assemblages. According to other studies, variations in community composition among different seasons are related to changes in temperature and humidity that affect activity (Moreno and Halffter, 2001; Romanuk and Kolasa, 2001), seasonal variability in availability of food resources and habitat productivity (Kaspari et al., 2000; Perner et al., 2005), prey availability and vegetation presence (Dennis et al., 1994). In our study, early summer samplings (i.e. June) yielded higher numbers of individuals for both assemblages than those carried out in late summer or winter. Maximum activity density in summer followed by a considerable decrease in late autumn has also been reported by Pinero and Avila (2004) and Sackmann and Flores (2009). Thiele's (1977) classic work suggested that the most frequent carabid captures occur during the season of mating and reproduction. Total activity density of individuals showed fluctuations within the main activity period of beetles (May – July) (*Fig. 2*). Given that seasonal crops such as wheat are present only for a few months possibly predatory ground beetles may serve as biological agents only during these months. When the cereals are harvested in the middle of July, the adjacent permanent vegetation, e.g. grasslands or fallow land, hosts abundant assemblages (*Fig. 3*). The presence of livestock and dung production across the study area during the summer is of paramount importance for the dung beetles resulting in the enhancement of their population (Romero-Alcaraz and Avila, 2000). In our study area, in late autumn and early winter there are no grazing animals, as the herds are moving to lower elevations, resulting in the significantly dung decrease and probably may affect dung beetle population.

Our results pointed out that habitat heterogeneity had a positive effect on alpha diversity of beetles, with significant variations among seasons. Our results demonstrated that habitat type affected the diversity parameters examined for beetle assemblages, while specific species characterized cereal fields, grasslands, ecotone and oak forest according to the correspondence analysis. Specifically, grasslands and fallow land had higher activity density and species richness of both assemblages in comparison to oak forest. Similarly to our study, Wassmer (1995) showed that population densities of dung beetles were higher in open areas, like pastures, than in the wooded areas, whereas da Silva et al. (2008) reached the same conclusion for ground beetles in Portugal. It has also been suggested that carabids dominate the most exposed habitats like agricultural areas, where they usually find high food availability, caused by the heterogeneity of vegetation (Niemelä, 1997), due to their high mobility and invasive ability (Larsen et al., 2003). The low carabid richness of the forest probably suggests that fewer species are adapted to relatively cool and dark forest habitats (Niemelä, 1993). In our heterogeneous landscape, crops are the most disturbed habitat, due to the complete removal of crop biomass at harvest. Forest is the least affected habitat by the removal of plant biomass in terms of timber; fallow land, plantations and grasslands can be considered intermediate disturbed habitats, falling between crops and forest, as in the latter the vegetation cover is almost permanent, and frequently reduced due to the livestock grazing. The existence of habitat diversity favors the coexistence of species in the area that have different habitat preferences, leading to effective spatial separation of them (Giller and Doube, 1994). On the contrary, some widely distributed species, such as *O. coenobita*, have shown high plasticity in habitat preference across different geographical areas, illustrating possible effects of climatic factors (Lumaret and Kirk, 1987; Wassmer, 1995).

Livestock raising and extensive agriculture should be carried out in such mountainous areas and are strongly recommended in similar landscapes as they have positive effects on the beetle fauna. Similar future studies on the effects of habitat types and elements configuration in different spatial and seasonal scales on the beetle fauna, leading to targeted agricultural applications, should be implemented. In addition, biotic factors such as the abundance of predators or prey should be explored to explain habitat heterogeneity – species diversity relationships.

In conclusion, in this upland heterogeneous Mediterranean landscape, significant differences in beetle richness and activity densities were revealed among different seasons and habitats. A striking outcome is that agriculture, which creates habitat heterogeneity in the landscape context, has rather positive effects on beetle diversity. It is also demonstrated that less disturbed habitats, such as the young oak forest in our study area, do not favor diversity. Another apparent outcome is that the estimated non parametric indices performed well comparing to the observed richness, suggesting that they could be used in biodiversity surveys avoiding cost-effective protocols.

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ADDITIONAL WEB RESOURCES

http://purl.oclc.org/estimates/
www.iucn.org/redlist/
http://www.xlstat.com

THE ECOLOGICAL INFLUENCE OF LARGE HERBIVORES – BEHAVIOR AND HABITAT UTILIZATION OF CATTLE AND HORSES

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Abstract. „Heck“ cattle (*Bos taurus primigenius*, 23 individuals) and Exmoor ponies (*Equus ferus caballus*,11 individuals) were introduced in a 171 ha tract of forest and pasture. Time budgets of behavior and habitat preferences were calculated. Horses showed significantly more grazing ($p < 0.001$), standing ($p < 0.01$) and comfort behavior ($p < 0.05$) and more lying ($p < 0.001$) and relocation ($p < 0.001$) than cattle. The overall preference indices (Chesson index) had positive values at both species in open pastures (cattle 0,42 and horses 0.33) and for the edges of the wood (0.16, 0.13). The closed wood was avoided by the cattle with -0.51 and horses with -0.57. The highest preference values based on feces dispersion were +0.84 for the cattle in open pasture and +0.64 for the horses in the alder marsh. Both species showed the highest habitat preferences for feeding in open pastures (0.81, 0.77) and for moving in edges of the wooded areas (0.75, 0.69). Horses preferred the closed wooded area for resting (0.17). The median of the daily distances walked was larger for cattle than for horses ($p<0.001$) but horses reached the larger maximal distance per day (7018 m) compared to cattle (6762 m).

Keywords: *forest, herbivores, nature protection, time budgets, feces, walking distances*

Introduction

Ungulate herbivores and spermatophytes including trees have co-evolved over long periods of time and large herbivores show distinct adaptations to semi-open habitats characterized by sparse woods. The natural vegetation is adapted in many ways to being used as pasture. The former practice of pasturing in woodlands was based on the given natural circumstances. It enabled domesticated animals to perform natural behavior in a close to natural habitat. The domesticated grazers occupied the vacant ecological niches of wild animals that had died out (aurochs, *Bos primigenius*, European bison *Bison bison*, moose, *Alces alces*, and tarpan, *Equus ferus*) or become rare (red deer, *Cervus elaphus*) and played in so far a natural role as long as they were kept in moderate density. Apart from being used as pasture, in the past, the forests have been used very intensively for fire wood, collection of foliage and, in recent times, charcoal until the rising demands of wood could not longer satisfied at the time of early industrialization. Accordingly, between 1850 and 1880 the pasturing rights in Germany were reorganized because of the conflicts between unnatural high grazing pressure of domesticated animals and forestry which led to a separation between forestry and pasturing (Küster 1998). Large areas were reforested and the grazers were restricted to open areas. According to the Forest laws in Germany, it was strictly forbidden to keep domestic animals like cattle and horses in forests. Due to this, the landscape has changed

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dramatically, and these unnatural forms of husbandry have existed until today. Recent discussions about the aim and practice of nature conservation put in question this separation and discuss the role and function of large herbivores, domestic or wild, for natural development (Riecken et al. 2001). Under certain circumstances, herbivores may favour habitat biodiversity (Zahn et al. 2007) but this depends between mainly on the spatial and nutritional behavior of the animals. Since these species have not been kept under such conditions, scientific analysis of their behavior and ecological function are mainly lacking. Consequently the aim of the study was to analyze the general behavior budgets and the habitat preferences of the two species in closed to natural population densities. It was the question if under such conditions these species would develop a behavior that cause such disturbances of wood development as assumed by most foresters.

We tested the hypothesis, if these species generally prefer the closed wooded areas or prefer the open pasture. A second question was, if these species use the different vegetation units for feeding and resting differently, such as open areas for grazing or woods for resting. In the following the question should be answered, if two herbivore species, a ruminant and a non-ruminant roughage eater, in the same area would act as competitors or as supplementary plant consumers. This would lead to the argument, if single-species or mixed-species projects for nature conservation should be preferred.

Animals, Material and Methods

Project Area

As experimental area a tract of 171 ha of forest with shares of open space has been used in the “Weserbergland” region in Lower Saxony. The area included 11ha open range without woods and 160 ha of differing types of wood. Deciduous forests were predominant (127.9 ha) and consisted of oaks (*Quercus robur* 107.4 ha), beech (*Fagus sylvatica*), hornbeams (*Carpinus betulus*) and alder (*Alnus glutinosa*). Coniferous forests (27.6 ha) consisted of spruce (*Picea abies* 23.3 ha), larch (*Larix deciduas* 4.7 ha) and some Douglas forests (*Pseudotsuga mensiesii*, 0.2 ha), alder marsh (1.7 ha), and coppice (1.4 ha).

The herbal layer included Luculo fagaetum Stellario-Carpineten, Pteridium aquilinum and other ferns. The shrubs present were *Rubus idaeus*, *Corylus avellana* and *Rubus fruticosus*. The edges of the wood were defined as an area measuring 5-m from both sides of the boundary between the wood and the open pasture.

The large open pasture of 9 ha was dominated by poaceae and white clover (*Trifolium pratensis*), and few shrubs like *Prunus spinosa*, *Crataegus laevigata/ monogyna* and *Sambucus nigra* were present beyond the wood. The clearings (1.1 ha) inside the woods were structured like the open pasture. Water (0.5 ha) was present as a brook and a pond. Forest tracks (2.4 ha) and trails of the animals (1.4 ha) pervaded all the project area. During the wintertime a hayrack was furnished.

Animals

The introduced animals were a herd of 23 adult cows with calves and one bull of the breed Heck cattle and 11 horses of the breed Exmoor pony. The Heck cattle were breed as cattle with a similar morphology of the aurochs but are smaller in size. The Exmoor pony is the oldest and purist of the British native pony breeds and comes from the open moors of south western England. The horses were forming two groups which each one stallion. The numbers were stabile during the time of the investigations. All animals could be identified by earmarks, additionally one cow and one mare were equipped with VHF radio-collars. In 2004, both species were observed for 148.25 hours, which resulted in 3025 observation points. In 2005, in total 150 hours with 3085 observations points were recorded for horses and cattle.

Behavior Recording

The observations of the cattle and horses were conducted during the growing seasons (April to October). Twice a month, observations were made of each herd, and all of them were conducted during daylight hours, from 2 hours after dusk until 2 hours before dawn. Five focus animals were chosen from each herd. The applied method for the observations of behavior was scan sampling (Altmann 1974, Martin and Bateson 1986).

At intervals of 15 minutes the occurrence of the basic behavior patterns (see *Table 1*) was recorded. The data evaluation was performed with Excel 2000 and SsS 1.0 (G-Test). Furthermore, the behavior recordings were used to calculate preference indices in relation to the habitats wood and open pastures for grazing, moving, and resting separately.

Table 1. *Definitions of the recorded parameters of behaviour*

Parameters of behaviour	Definitions
Grazing	Grazing on grasses and herbs (moving with head down)
Browsing	Browsing on twigs, leafs and buds
Drinking	Drinking from natural water sites
Standing	Standing, doing nothing else
Lying	Lying, doing nothing
Walking	Slow movement of individual animals without the herd
Running	Fast movement of individual animals with or without the herd
Relocation	Movement of all animals in the herd from one place to another
Comfort behaviour	Rubbing against surfaces, grating, licking, wallowing
Social behaviour	Physical interactions between animals (mutual rubbing, grooming, licking, suckling, pushing, knocking, biting)

Habitat use

Records were carried out by direct observation and by collection of feces. For visual location the animals were followed by means of the telemetry device (tracking), and animal positions visually identified with help of a handheld GPS-receiver, a compass, distinctive structures and positioning rods, and marked on a map. For every stay point the position of the central animal of the herd was determined. The feces depositions were located during two systematic screening tours (spring and autumn 2005). The positions were used to calculate habitat preferences independent of the behavior, as well as depending from the basic behaviors (see above).

The animal positions were analyzed with topographic raster maps (100 by 100 m) and digital maps. All these positions were transformed from the map on GIS Arc-View 3.2. Relative utilization frequencies were computed for the habitat types and tested for equal distribution by the Kolmogorov-Smirnov test (SPSS). Based on the stay frequencies habitat preferences were determined by GIS- procedures. To calculate the habitat preferences, the Chesson Index (Chesson 1978) was applied.

$$\alpha_i = \frac{\frac{r_i}{n_i}}{\sum_{i=1}^{m} \frac{r_i}{n_i}} \qquad \text{(Eq.1)}$$

r_i =relative use of the habitats i, n_i=relative share of the habitats i, m=maximum of the number of the habitats).
A selective use result in a transgression of α1=1/m.
Additionally, preferences were also estimated by mapping faeces with the same mode.

Walking Distances

The distances covered daily by the herds were computed based on the mapped positions at 15 minute intervals. The positions were linked by straight lines and their length summed up for the observation days. For testing differences of the mean distances per species the U-Test was used, for testing the difference of the absolute distances the G-Test.

Results

Behavior

The time budgets for the particular elements of cattle and horse behavior were compared (*Table 2*). Grazing occupied the greatest amount of time in both species, where the horses grazed significantly more than the cattle ($p<0.001$, G-Test). Grazing

Table 2. *The time budgets of the behavioral parameters (*$p<0.05$). n_{cattle}=6038 cattle, n_{horses}=6091*

Parameters of behavior	Cattle [%]	Horses [%]	Significances
Grazing	39.40	51.19	horses > cattle*
Browsing	3.20	3.08	not significant
Drinking	0.40	0.60	not significant
Standing	8.33	26.12	horses > cattle*
Lying	29.61	3.73	cattle > horses*
Walking	2.78	2.85	not significant
Running	0.50	1.69	horses > cattle*
Moving (relocation)	11.63	7.72	cattle > horses*
Comfort behavior	1.23	1.67	horses > cattle*
Social behavior	2.57	2.19	not significant

occupied the greatest amount of time in both species, where the horses grazed significantly more than the cattle ($p<0.001$, G-Test). The horses were sometimes observed in a remarkably running behavior, while cattle always walked slowly. However, the cattle changed their positions more frequently than the horses ($p<0.001$). During the resting periods, the cattle were observed predominantly lying ($p<0.001$) and the horses standing ($p<0.001$). The horses showed a lot more of comfort behavior than the cattle ($p<0.05$). Browsing, drinking, walking and social behavior, were not significantly different between both species.

Habtiat use, observed positions

Most of the times, both animal species were recorded in open pastures (*Table 3*). The cattle spent a little bit more time in the oak forest than the horses. On the other hand, the horses used the spruce forest more frequently than the cattle. Also, the horses were more frequently in the oak mixed forests than the cattle. The utilization frequencies of the edges of the wooded areas and of the clearings were similar for both animal groups. Comparatively, few observations were made in the larch forest, in the larch-beach forest, and in the coppice. The Douglas forest and the alder marsh habitats had the lowest utilization frequencies. The Chesson - indices confirm the preference for the open pasture and the edges of the wood by both species (*Table 3*). To a low degree, horses prefer the clearings and the Douglas forest, while cattle prefer to a low degree prefer the coppice.

The Kolmogorov-Smirnov test confirmed that the observed stays were not distributed equally or casually (all the tests with $p<0.01$). Accordingly, the animals used the different habitat structures selectively.

Table 3. Chesson-Index of habitat use by observations. n=6112

Habitats	%Area of habitats (n_i):	%Use cattle of habitats (r_i): observations	%Use horses of habitats (r_i): observations	Chesson-Index (α_1): Cattle observations	Chesson-Index (α_1): Horses observations
Open pasture	5.48	52.17	42.97	**0.42**	**0.33**
Clearing	0.63	1.33	1.64	0.09	**0.11**
Edge of the wood	2.00	7.00	6.08	**0.16**	**0.13**
Oak forest	62.04	20.58	17.17	0.01	0.01
Oak mixed forest	10.46	3.08	7.97	0.01	0.03
Coppice	0.78	1.75	1.07	**0.10**	0.06
Alder marsh	0.98	0.92	0.96	0.04	0.04
Larch forest	2.69	2.08	2.96	0.03	0.05
Larch-beech forest	1.33	0.75	1.56	0.03	0.05
Spruce forest	13.48	10.17	17.26	0.03	0.05
Douglas forest	0.12	0.17	0.41	0.06	**0.14**

Mapped feces

The feces of the cattle and horses were found in different allotments for the particular habitats. Cattle feces were distributed area-wide. The highest concentrations were found in the open pasture (*Table 4*). Frequently, cattle feces were located in the spruce forest and the oak forest. The feces of the horses were, in contrast with the cattle feces, concentrated in specific areas. The largest number of feces was found in the oak forest, followed by the spruce forest. However, there were far less horse feces than cattle feces in the open pasture. The parts of the feces of the cattle and the horses, was similar at the edges of the wood, in clearings, in the larch forest, in the alder marsh, in the coppice and in the oak mixed forest. Like the preference indices of the stays, the mapped feces were not distributed equally or casually (tests of Kolmogorov-Smirnov with p<0.01), accordingly the rate of utilization of the area was selective.

Table 4. *Chesson-Index of habitat use (α1) by faeces. n=6112*

Habitats	%Area of habitats (n_i):	%Use cattle of habitats (r_i): faeces	%Use horses of habitats (r_i): faeces	Chesson-Index (α_1): Cattle faeces	Chesson-Index (α_1): Horses faeces
Open pasture	5.48	63.00	13.01	**0.64**	**0.12**
Clearing	0.63	0.00	0.58	0.00	0.05
Edge of the wood	2.00	3.81	2.33	**0.11**	0.06
Oak forest	62.04	9.87	37.09	0.01	0.03
Oak mixed forest	10.46	1.12	5.24	0.01	0.03
Coppice	0.78	0.45	2.72	0.03	**0.18**
Alder marsh	0.98	0.45	4.47	0.03	**0.24**
Larch forest	2.69	1.57	1.17	0.03	0.02
Larch-beech forest	1.33	1.79	3.88	0.00	**0.15**
Spruce forest	13.48	17.94	29.51	0.07	**0.11**
Douglas forest	0.12	0.00	0.00	0.00	0.00

Habitat use depending on behavior

The cattle preferred the open pasture for all behaviors, especially for feeding (32,9%, α_1 = 0,81) but to a low degree for moving (*Table 5*). Wood preferred the cattle for any behavior. For moving, the edges were more preferred than the other structures. Horses instead, showed a preference for the wood for resting behavior (26,69%, α_1 =0,17), while these animals preferred the pasture for all other behaviors just like the cattle but to a lower degree for resting. Like cattle, the horses moved most frequently on the edges of the wood.

Distances

The recorded absolute distances of the cattle were between 1983 and 6539 m per day and these of the horses between 1642 and 7018 m per day (*Figure 1*). The cattle covered larger daily distances than the horses (p<0.001, G-Test). The values of means do not differ between their means (U-Test). In the beginning of the year, both species walked similar and rising distances. From the maximal values in may (horses) and june (cattle), the distances dropped until they reached a minimum in the middle of the year. After this, the values increased something and to the autumn the distances were falling gradually again in both species.

***Table 5**. Chesson-Index (α1) of feeding (grazing and browsing), resting (standing and lying) and moving (relocation): n_{cattle}=5492, n_{horses}=5526.*

Behavior (r_i)	**Open pasture**		**Closed wood**		**Edge of the wood**	
	%	α_1	%	α_1	%	α_1
Feeding cattle	32.90	**0.81**	10.38	0.02	3.37	**0.17**
Feeding horses	38.96	**0.77**	15.27	0.02	4.99	**0.21**
Resting cattle	17.77	**0.69**	19.54	0.05	3.26	**0.27**
Resting horses	3.62	**0.38**	26.69	**0.17**	2.03	**0.45**
Moving cattle	1.69	**0.20**	8.03	0.06	3.06	**0.75**
Moving horses	1.43	**0.26**	5.25	0.06	1.76	**0.69**

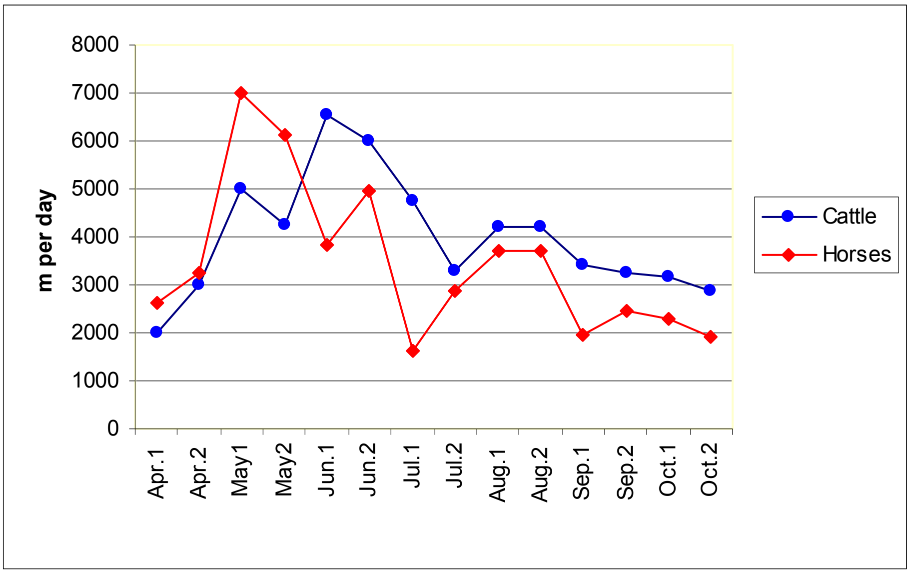

***Figure 1**. Distances of cattle and horses from April to October, 2 observation days per month.*

Discussion

In central Europe, several projects have been started to reintroduce large herbivores, wild animals such as European bison or feral animals as Konik (Polish feral horses) or Heck Cattle (re-selected primitive breeds) alone or together into close to natural areas. They follow the model of semi-open pastures and “new wilderness” (Riecken et al. 2002, Finck et al. 2004). In this project, the cattle and horses should be investigated for their possible role of wild herbivores.

Reliability of methods

Like applied method, the scan sampling was used for the stays in the behavioral

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observations and the mapping of the habitat use. It is a current method for investigations into the behavior of cattle, horses and sheep in diverse pasture systems (Hart et al. 1991, Scheibe et al. 1998b). The direct observations resulted in an accurate differentiation of the behavioral elements and should be adopted to study animals in pastures (Jensen et al. 1986). Intervals of 15 minutes were also approved by many other observers (e.g. Lofgreen et al. (1957), Krysl and Hess (1993, Menhard et al. (2002). The use of time budgets by the behavioral pattern follows other studies (Arnold 1984, Illius et al. 1987, Vulink 2001). The classification of the elements of behavior coincides with those of other authors (Gammon and Roberts 1980, Krysl and Hess 1993, Hepworth et al. 1991). All of them gave the elements grazing, walking, drinking, standing and lying. Resource selection can not alone be evaluated by utilization frequencies. The calculation of the Chesson-Index was recommended as the most convenient index for animal habitat preferences (Manly et al. 1993), and applied frequently (Garshelis 2000, Broglio et al. 2004). Most of the descriptions of cattle and horses refer to observations made during daylight, because the main activities of these animals happened during that time (Arnold and Dudzinski 1978, Zeitler-Feicht 2001). Nevertheless, the lack of nocturnal observations may be a certain disadvantage of this study, which should be overcome by a combination of GPS-telemetry and activity recordings for future investigations (Scheibe et al. 1999).

Behavior results

Horses are generalist herbivores and coexist with cattle in many ecosystems (Menhard et al. 2002). The percentage of time spent foraging, which consists of grazing, browsing and drinking, was higher in the horses than in the cattle. Our results equal to studies on cattle and ponies in the New Forest (Pratt et al. 1986), the horses spent foraging 75% of time and the cattle 57%, respectively 60% (Tubbs 1997). The physiological reason for this is the two animals differing abilities to utilize nutrients (Janis1976). Horses require a higher amount of food compared to cattle to extract the same net energy (Menard et al. 2002). Accordingly, the proportion of the time of browsing in trees and shrubs is also higher in horses than cattle (Pott and Hüppe 1994). The browsing time budgets are very low for both species and coincide with other studies (1%, Gammon and Roberts 1980, 5%, Miller and Krueger 1975) . It has been reported that cattle pasturing in wetlands were browsing more than horses, whereas horses grazed for more time (Menhard et al. 2002). The animals spent little time drinking and were drinking irregularly and infrequently. Several authors refer this to the availability of fresh food (Scheibe et al. 1998a, Zeitler-Feicht 2001). Lying during the resting time is an important behavior especially for the cattle (Metz 1985), while horses are able to rest standing with nearly no energy consumption (Budras and Röck 1997). Accordingly, the horses in our study were observed more often standing than lying. The alternation of the resting time in the horses was more irregular than in the cattle. The proportion of time the horses spent running was much higher than in the cattle and is ascribable to social discrepancies between the stallions. Also during the change of places the horses ran more than the cattle. Walking individually, independent of the group, was seldom observed in both species. For the most part it was only observed when the animals were grazing or locating water or resting sites. Cattle changed their positions significantly more frequently than of the horses. The cattle moved away across large distances and for long time, mostly in the morning. They used wooded areas only for short times for foraging. Semi-wild living cattle in the New Forest offer a value of

21% movement time (Tubbs 1997). Occasions of social and comfort behavior could only be scarcely documented for both species. Comfort behavior was observed significantly more in the horses, where wallowing was a common activity.

Habiat Use Results

The project area comprised of a wide spectrum of different vegetation and soil and was used by the animals with variable intensity. During all the observation time, the cattle and horses used mostly the open pasture. The same preferences of the open pastures by cattle and horses has been found in the New Forest (Putman et al. 1987). The animals in our investigation used open areas for 70-80% for foraging. These results are in accordance with the classification of cattle and horses as grazers (Hofmann 1989). Other free living grazing animals such as bison, wildebeest, zebra, antelope and reindeer live in very large herds and migrate across the open and semi-open areas with sufficient aliments and high visibility (Homewood et al. 2001; Lushchekina and Struchkov 2001; Howall 2002; Musiega and Kadazi 2004). In both cases, observations and feces, the cattle and the horses obvious selected the open pasture and in the edges of the wood. Selection of the coppice was identified per observation only. The deciduous woodland covered very large areas of the study area. It was used by both cattle and horses in equal measure. But the cattle crossed wide parts of the woods and stopped for foraging in clearings consisting of raspberries, grasses and herbs. Their favored resting sites were clear of trees and with little vegetation. In contrast, the horses rested in certain sites within the deciduous forests for longer periods of time. The cattle in the New Forest used woodland less, at 10-20% of their total time (Putman et al. 1987), just as the cattle of our project area avoided the woodland compared to the open pasture. The horses in New Forest showed differences in foraging behavior as did the horses in our project area. Browsing behavior predominated during the wintertime because snow covered the ground vegetation. Both of herds, the cattle and horses, frequented the coniferous woodland to shelter from hot and cold weather, insects and heavy rain. This is a common sheltering behavior in large herbivores (Hancock 1953, Sambraus 1978). The edges of the woods and the clearings were often occupied by both animal groups for foraging or resting and were preferred compared with the other habitats. The places assured shelter from the weather and offered a free view of the open land as well as a short distance to the feeding grounds.

The described behavior strategies indicate that both, cattle and horses originally are adapted to semi-open pastures (Beutler 1996, Bunzel-Drüke 1997). An obvious difference between the species was that the clearings within the forest were much less used by the cattle, as indicated by only a few stays and feces. Cattle always depose their feces in places where they are resting, whereas horses have certain sites for the deposition of their feces (Zeitler-Feicht 2001). These sites of aggregation serve as an olfactory marking of their territory. Only the stallions exhibited a distinctive marking behavior. Both the observed stays and the mapped feces did not demonstrate a stochastic distribution in the cattle or the horses. As a result, the unequal distribution follows the preferred areas, the herd structure and the social behavior (Tyler 1972, Arnold and Dudzinski 1978, Putman et al. 1991). Accumulations of feces can be regarded as an indicator for resting places, because they often were deposited after the resting time (Altmann 1988).

The results of habitat use depending on behavior show similar patterns as independent behavior. Both species preferred very clearly for feeding the open pasture and avoid the closed wood areas. Nevertheless, certain differences became obvious. Horses significantly preferred open pastures and woodland for resting. Movements occurred especially frequently at the edges of the wooded areas in both species while the preference indices for the open pasture where the lowest of all behaviors on the open pasture. Occasional use of edges of the wood like successive mosaic areas offer optimal habitats by food resources better than the wood and by shelter conditions better than the open pastures (Bokdam et al. 2003). Possibly, the edges of the wood serve like orientation. The results support clearly the evolutionary adaptation of these two species for feeding on open areas and demonstrate that they will not suppress the wood development so completely as often assumed.

Distances covered results

The daily movements of the animals were reflected in their covered distances. The distances were impacted by different factors like the availability of space, nutrients, distance from water, temperature, wind, the age of animals, pregnancy, stocking rate and pasture practices (Anderson and Kothman 1980; Phillips 1993; Karsli 1995). In contrast to the horses, the cattle covered larger distances. It is assumed that the horses, by reason of their smaller groups, require less space and therefore do not need to change their location as often as the cattle. Both animal groups moved further in the spring and the autumn than in the summer. This is possible because of exploration behavior during foraging (Krysl and Hess 1993). In the spring all the vegetation is not available and in the autumn there is a decline of the energy and crude fiber in the food, and the animals have to change their foraging sites more frequently An increase of locomotion could also result, from warm, wet or very windy weather and an over-abundance of insects (Hancock 1953, Phillips 1993, Waite et al. 1951).

Suggestions

The hypothesis is supported that the two herbivore species generally prefer the open pasture and will not destroy the forest or suppress any tree regeneration if they have a possibility to choose their positions according to their natural preferences and live in moderate population densities. A forest destruction as observed during pasturing before 1850 must not be afraid under these preconditions. Although these species share basically the same habitat structures they differ slightly in their preferences. Furthermore, they differ significantly in ecological relevant behavior elements such as locomotion, resting, or defecation behavior. Accordingly, single species grazing projects will not come close to natural herbivore-habitat interactions. These results support the theory both of additive and complementary utilization of nutrients in free nature by a complex herbivore community also in Central European biotopes. We argue for nature-protection projects in semi-open landscapes including forest with at least two different species such as horses and ruminant species.

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GROUND PENETRATING RADAR AS REMOTE SENSING TECHNIQUE TO INVESTIGATE THE ROOT SYSTEM ARCHITECTURE

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Abstract. Ground Penetrating Radar (GPR) is a remote-sensing technique widely applied in archaeology, engineering and environmental sciences. It is a non-invasive geophysical method which uses electromagnetic pulses to create an "image" of the shallow subsurface. In recent years, GPR gained popularity in forestry, thanks to its application in water content estimation, root stress evaluation, root biomass modelling, and roots location. In this paper we present the results of two radar surveys aimed at reconstructing the geometric features of root systems buried at shallow subsurface, thanks to a multi-profile data acquisition and to the use of a three-dimensional software package. Our results show the potentiality of this approach as a reconnaissance method before performing any destructive test.

Keywords: *Remote Sensing, Ground Penetrating Radar, Root, Forestry*

Introduction

Schowengerdt (2007) describes Remote Sensing as an attempt to measure something "at distance", i.e. without any "contact with the target", by means of some propagating signal. All geophysical methods can be considered, in general, Remote Sensing techniques, being able to remotely measure "the contrast" between the physical properties of the background and the target under investigation. Very few of those, however, are able to produce a full image of the subsurface, where the geometry and the position in space of the buried objects can be clearly determined. Ground Penetrating Radar (GPR) fulfils rather well these requirements, being capable to produce a detailed map of the subsurface using radio waves generated just above the ground surface (Jol, 2009; Daniels, 2004; Annan 2004). The interaction between the electromagnetic waves and the buried targets, produces an electromagnetic image similar to ultrasound images used in diagnostic medicine.

The main advantage of GPR is its capability to detect, in a non-destructive way, the vertical and horizontal dielectric anomalies associated with natural or manmade subsurface variability like: lithology, water content and bulk density changes, voids or buried objects, liquid or solid waste disposal, etc.

For this reason, GPR has been widely used in archaeology, engineering, hydrogeology, environmental sciences, and recently has also been applied in forestry, with different applications as root detection, root damages in urban context, root biomass evaluation, carbon allocation, water content variations, analyses on standing

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tree stems, control of the tree growth, as shown in Barton & Montagu, 2004; Cermák et al. 2000 ; Cox et al.,2005 ; Cui et al., 2011; Dannoura et al.,2008 ; Hirano et al., 2009 ; Hruška et al., 1999 ;Leucci, 2010 ; Stokes et al.,2002 ; and Stover et al., 2007.

Furthermore, GPR has also been tested in combination with other geophysical techniques, e.g. Amato et al., 2008 ; Perez-Gracia et al. (2010), Rahjens et al. (2003), Rossi et al. (2011), Zanetti et al. (2011), and Zenone et al. (2008), showing the validity of such a multi-technique approach in developing different strategies to preserve tree roots and trees, as also described in Danjon & Reubens (2008), and in Allred et al. (2011).

The potential use of GPR in forestry becomes even more evident if we take into account that to study the relationships between vegetation and the urban environments, or to estimate the biomass of tree roots, the traditional investigations are carried out in a very invasive way. In fact, soil and roots are usually sampled throughout cores, pits, or trenches, and the roots are collected, sieved, washed dried, and weighted, e.g. Butnor et al. 2001; Butnor et al. 2003. This method is destructive, labour-intensive, and not very useful to measure the lateral extent of a root system. Conversely, GPR is capable to map in a pseudo-3D fashion, with high vertical and horizontal resolution, the root system architecture, reducing the amount of root samples needed for quantitative analysis.

In this work, we show some examples of GPR surveys aimed at detecting and reconstructing the spatial distribution of the root system in different locations and soil conditions.

The GPR technique

GPR is a geophysical method which uses electromagnetic (em) waves, typically in the frequency range 10-3000 MHz, to image structures and features buried in the ground. The physical principle of GPR detection, is based on the dielectric contrast between the buried target and the background material. Such a contrast can be produced in several ways: by spatial changes in the physical-chemical property of the sediments or the soil, changes in water content and bulk density of the material, or even by the presence of different objects in the subsoil like voids, rocks and boulders, wood, or manmade materials like metal and plastic targets. In general, if there is a detectable contrast between different subsurface objects, this can generate strong signal reflections which can be clearly identified on a GPR image (Annan, 2004; and Jol, 2009).

The equipment used in all GPR systems consists of four main elements: a transmitting unit; a receiving unit; a control unit; and a display unit. The transmitter produces a short duration, high voltage pulse. This pulse is applied to the transmitting antenna (Tx), which radiates it into the ground. The receiving antenna (Rx) collects the signals coming from the material under investigation, which are amplified and formatted for display, by the control unit, as depicted in *Fig. 1*.

The radar measures the signal amplitude vs. time (two-way travel time), for each position of the Tx-Rx on the ground. The data are collected moving the Tx-Rx system along a profile, so that a bi-dimensional radar cross-section having the two-way travel time on the Y axis and the antennas position on the X axis is obtained for each collected profile. To estimate the depth of the target (i.e. to convert time in depth) a simple calibration technique can be used, as illustrated in Annan, 2004, even though in complicated subsurface scenarios more refine algorithms should be applied, (Annan, 2004; and Jol, 2009). The velocity and the attenuation of the radar signals depend on the electromagnetic properties of the soil, which can be frequency-dependent quantities. In particular, in common geo-materials the maximum investigation depth decreases rapidly with increasing frequency due to the signal attenuation; this explain why almost all subsurface radar systems operate at frequencies lower than 3 GHz. The antenna frequency also affects the vertical and the horizontal resolution achievable in a GPR image. The shorter is the time pulse width (i.e. the higher is the antenna frequency) , the higher is the resolution.

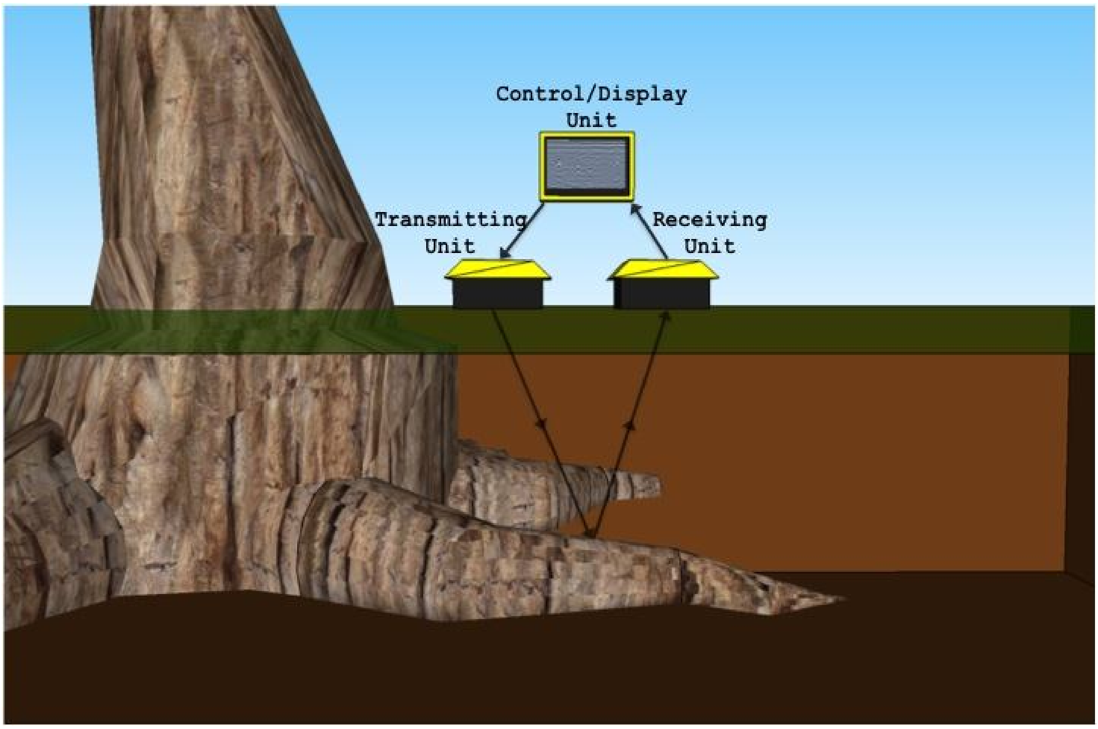

Figure 1. A schematic reconstruction of a GPR system for root investigations. The four main elements are the transmitting unit, the receiving unit, the control unit, and the display unit. A detectable contrast between different subsurface objects generates strong signal reflections which can be clearly identified on a GPR image.

In order to obtain a XY image of the subsurface, the radar data should be collected in multi-profile mode, where the profiles are acquired parallel to each other, at a fixed distance. This technique allows to create XY time (or depth) slices, in which the lateral geometry of the targets can be identified, i.e. Jol 2009. A further processing of the data collected on a XY grid, allows a pseudo-3D visualization of the subsurface, throughout the countering of the anomalies generated by the electromagnetic contrast between the target and the background material. The final results is a three-dimensional representation of the subsurface using the isosurfaces to display a surface of constant data value in three dimensions (Pettinelli et al., 2011).

Case studies for subsurface roots detection

In the following, we present two examples of radar investigations aimed at mapping the root system, performed in Rome (Italy) in different locations.

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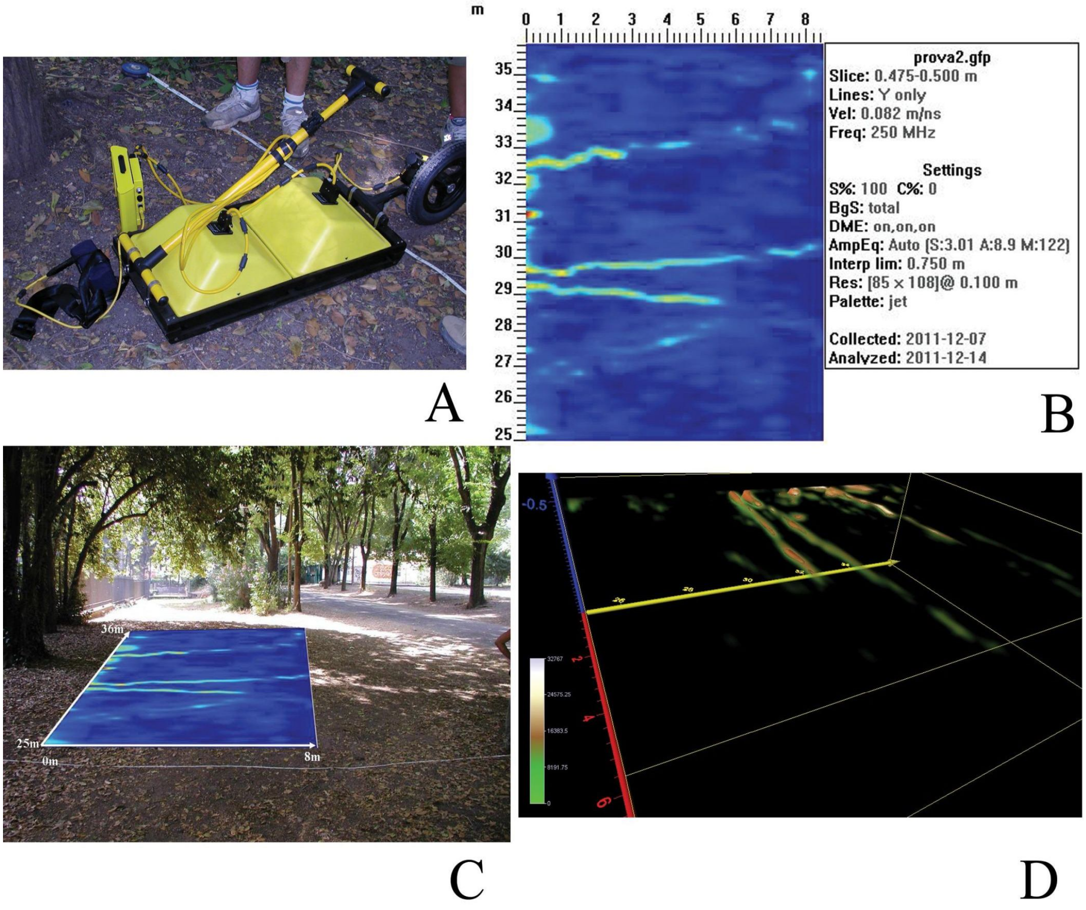
The first case study reported here, is a GPR survey collected in the San Sebastiano Park (Rome, Italy) originally planned for archaeological purposes. Indeed, the correct detection of tree roots in archaeological areas is of paramount importance to distinguish between natural and manmade targets, and to define to what extent a root system has "invaded" the ruins present at shallow depth. The area (40x10m) was investigated using a bistatic GPR system (*Fig.2A*), equipped with 250 MHz antennas (PulseEkko Pro by Sensors & Software, Inc). The survey was performed acquiring a multi-profile Y grid, (for a total of 20 parallel lines) with a line spacing of 0.50m and a step-size of 0.05m. The second case illustrates the results of a GPR survey carried out inside the cloister of the Physics Department of the University of Roma Tre. The work was aimed at detecting the buried residual roots, still present in an area where a big tree was previously removed. *Figure 3A* shows the bistatic 500 MHz Noggin Plus GPR system (Sensors & Software, Inc) used for the measurements. The area (14x12m) was investigated collecting a multi-profile Y grid (for a total of 26 parallel lines) with a line spacing of 0.50m and a step-size of 0.02m.



prova2.gfp
Slice: 0.475-0.500 m
Lines: Y only
Vel: 0.082 m/ns
Freq: 250 MHz

Settings
S%: 100 C%: 0
BgS: total
DME: on,on,on
AmpEq: Auto [S:3.01 A:8.9 M:122]
Interp lim: 0.750 m
Res: [85 x 108]@ 0.100 m
Palette: jet

Collected: 2011-12-07
Analyzed: 2011-12-14

Figure 2. *The GPR results acquired in San Sebastiano Park. Figure 2A shows the 250 MHz GPR used during the investigation; Figure 2B illustrates the 2D penetration map (about 0.50m depth) of the GPR anomalies; in Figure 2C the overlap clarifies the correlation between the GPR anomalies and the roots beneath the soil; in Figure 2D there is the pseudo-3D reconstruction of the root system.*

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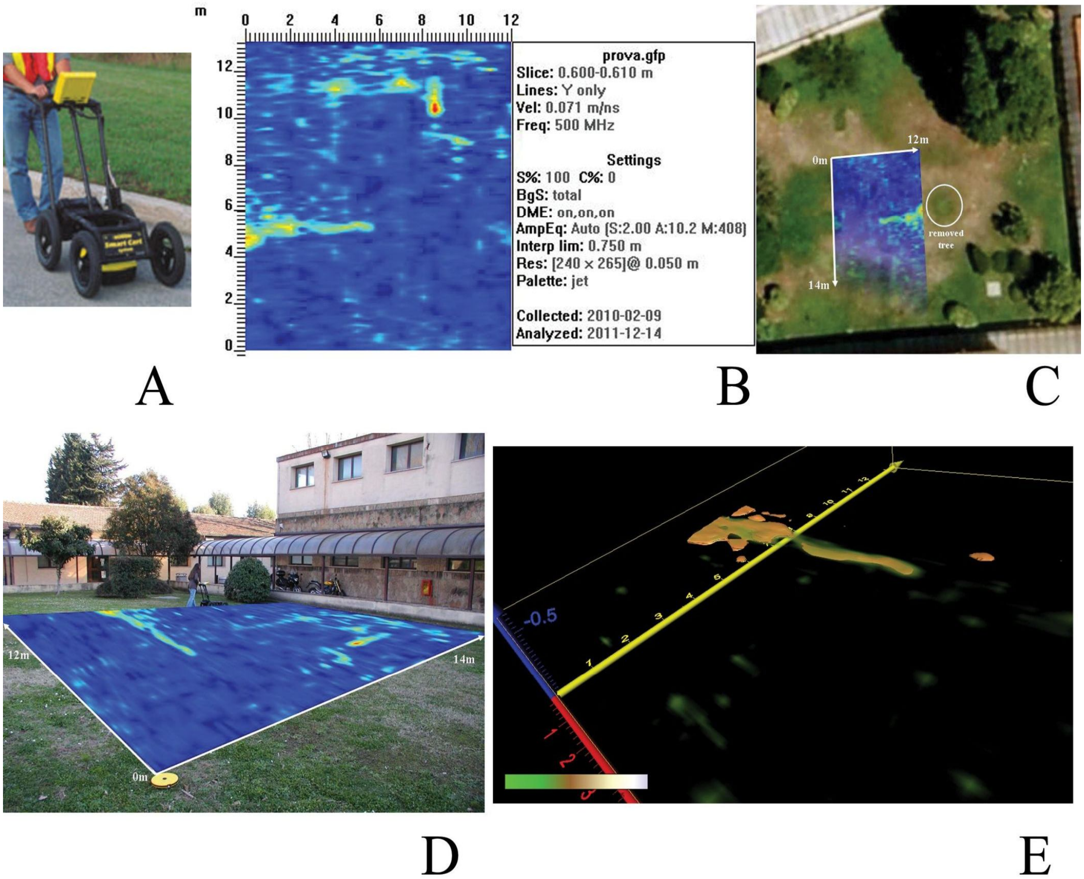
Fig.2B depicts the radar map relevant to site 1, showing a 2D depth slice (at about 0.5m) obtained interpolating the radar data collected on the single lines, using a pulse velocity in the soil of v=0.082m/ns (estimated using the hyperbola calibration technique). The superposition of the radar map to the photo of the investigated area (*Fig. 2C*), allows to correctly locate the elongated anomalies detected by the radar, and to relate those with the tree root system architecture. The geometry of the roots is better visible if a pseudo-3D representation is used, as shown in *Fig. 2D*. In fact, if the data quality is high, and the radar anomalies are well visible also at depth (as in the case study presented here), the XYZ data volume reconstruction can be useful to better identify the target orientation, dimension and shape.

The second radar map (*Fig.3B*) depicts the results of the survey collected at Roma Tre University. The depth slice, superimposed to the photo of the investigated area (*Fig. 3C*), clearly shows various anomalies present in the subsoil due to pipes or other targets buried when the yard was built. In particular, *Fig.3D* shows the presence of an elongated anomaly, located near the position of the removed tree at a depth of about



prova.gfp
Slice: 0.600-0.610 m
Lines: Y only
Vel: 0.071 m/ns
Freq: 500 MHz

Settings
S%: 100 C%: 0
BgS: total
DME: on,on,on
AmpEq: Auto (S:2.00 A:10.2 M:408)
Interp lim: 0.750 m
Res: [240 x 265]@ 0.050 m
Palette: jet

Collected: 2010-02-09
Analyzed: 2011-12-14

Figure 3. *The GPR results acquired in the cloister of the Physics Department of the University of Roma Tre. Figure 3A shows the 500 MHz GPR used during the investigation; Figure 3B illustrates the amplitude 2D map (about 0.60m depth) of the GPR anomalies; in Figure 3C it is evident the previous position of the removed tree and the GPR anomaly; in Figure 3D the overlap clarifies the correlation between the GPR anomalies and the remain roots in the subsurface; in Figure 3E there is the pseudo-3D reconstruction of the remain root.*

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0.60m (v=0.072m/ns), which indicates the presence of some root relict. Again, if the data are represented in a XYZ space (*Fig.3E*), the geometric features, i.e. orientation, shape and size, are much better defined, generating a more accurate reconstruction of the buried targets.

Final considerations

In this technical advance we have discussed the potentials of Ground Penetrating Radar as remote-sensing technique applied to forestry. The non-invasive nature of this geophysical method makes it appealing in all those applications where the common techniques used require the destruction of the samples. GPR is capable to create an electromagnetic image of the targets buried in the soil, allowing a detailed 3D reconstruction of their position and form. This is particularly important in roots system architecture study, where the alternative is the excavation or coring of the roots. Moreover, a part from very conductive soils, where the attenuation drastically reduces the maximum penetration depth of the radar pulses, this method can be successfully applied to every type of material from snow and ice to asphalt or dry sand. It is important to notice, however, that this technique is not able per se to define the nature of the object, e.g. metal, wood or rock, but can still be used as a reconnaissance method before performing any destructive test.

Another important advantage of this type of technique is its fast real-time acquisition, which allow to create a very large georeferenced (using a D-GPS) data volume. This aspect is particularly important for long term monitoring, where the evolution and development of the root system should be followed for months or years. Besides the capability to produce detailed maps of the subsurface, GPR can also be used to quantitatively estimate the water content in the soil or in the tree trunks, as well as to detect the presence of defects or voids in the wood. Moreover, the flexibility and reliability of the method permit a wide use in urban and rural environments, to address different problems like, tree protection during constructions, tree selection and landscape plan review, GIS compatible tree inventories, street tree management plans, plant health care planning and hazard evaluations.

Finally, some considerations should be made on the economic value of this type of technique: the moderate cost of the equipment with respect to those used to excavate or drill the soil, together with the fast acquisition time in large areas, make GPR quite competitive for forestry applications.

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EFFECT OF DISTURBANCE ON THE ANT COMMUNITY IN A SEMIARID REGION OF CENTRAL MEXICO

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Abstract. Ants have been used as indicators of disturbíííance, because of their fast response to environmental changes. However, it is not well defined which disturbance factors are associated with specific changes on the ant community. We evaluated the effect of disturbance on the ant community in a xeric community from central Mexico. Two sites with contrasting levels of disturbance were chosen based on a quantitative index. We compared ant abundance, ant diversity and the main disturbance factors affecting the ant community. Also, we identified the bioindicator species of ants and the feeding guilds. Soil compaction and cattle paths were the most important factors of disturbance within the study sites. These factors probably affect the community of ants by preventing them from colonizing the surrounding vegetation as well as from nesting in the soil. Ant diversity was lower in the disturbed site than in the conserved one. Four ant species were identified as bioindicators of disturbance. Although functional diversity did not differ between sites, the guild of granivore ants was the most affected by soil compaction. This is of great importance because the foraging patterns of granivore ants determining the structure and other aspects of the plant community.

Keywords: *antropogenic change; bioindicator species; feeding guilds; quantitative disturbance index; soil compaction.*

Introduction

Ants have an important role within the ecosystems because they occupy many different ecological niches. For instance, ants have numerous interactions with different plant species, including seed dispersers, leaf- and seed- predators, and in a few cases, pollinators (Vázquez 1998; Hernández 2005). Some ant species establish mutualistic relationships with a diversity of organisms. For example ants protect aphids and other homopterans from their predators, obtaining sugar-rich solutions produced by the homopterans (Delfino and Buffa 2000). Moreover, several ant species build their nests in the leaf litter, underneath the soil, within woody stems or under the rocks. In all of these cases, activities associated with gallery building by ants favor the mixing of organic matter in soil, as well as enrich and oxygenate the soils (Luque *et al.* 2002).

Among social insects, ants have the widest geographical distribution (Bolton *et al.* 2006; Fisher and Cover 2007; Vásquez-Bolaños and Mackay 2004; Vásquez-Bolaños 2007). However, ant diversity and population size are strongly affected by changes in environmental factors such as temperature, humidity, solar radiation and vegetation coverage (Rojas 2001; Grytnes and McCain 2007; Castro *et al.* 2008). In addition, anthropogenic disturbance may cause changes in the structure and diversity of the ant

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community (Davidson *et al.* 2003; Del Toro 2009; Brito *et al.* 2010). Anthropogenic disturbance may cause an increase in soil temperature, and a decrease in humidity and in the abundance of plants and leaf litter. Consequently, these changes may have a negative effect on ant-ant communication since the chemical signals left by worker ants may be washed out by the rain (Carroll and Rich 1983).

Because ants have a fast response to changes in environmental factors and occupy a great variety of ecological niches, they can be good bioindicators of disturbance (Rojas 2001). Several studies have evaluated the effect of disturbance on the abundance, diversity and composition of functional groups of ants within specific ecosystems (Majer 1983; Andersen 1995; Andersen 1997; Bustos *et al.* 1997; Lobry 1999; Kaspary and Majer 2000; Hoffmann and Andersen 2003; Redolfi *et al.* 2003; Vittar 2008).

However, those studies have classified the level of disturbance qualitatively. Therefore, it is hard to identify the factors of disturbance that are associated with changes in the ant community. More recently, Martorell and Peters (2005) have proposed a quantitative method to determine the level of disturbance of a particular community. Their method considers 15 variables, including human and cattle activities, and soil degradation. By using Martorell and Peters (2005) method, one can estimate a quantitative index of disturbance and analyze the effect of each disturbance factor on the community of study. Thus, it is possible to establish the relationship between the most important disturbance factors and changes in the community of study. This information, together with the identification of bioindicator species and feeding guilds may provide useful information on the processes and consequences of the disturbance in a particular habitat.

Therefore, through the quantitative estimation of the level of disturbance on a diverse semiarid region from Central Mexico, we selected two sites with contrasting disturbance indexes and identified the species of ants within each community. Particularly, we were interested in determining the effect of disturbance on the relative abundance and diversity of ants, identifying the species of ant that can be used as bioindicators of disturbance. We also, classified the ant community in feeding guilds, establishing the effects of disturbance factors on changes in the functional diversity of the ant community.

Material and Methods

Study site

The study was conducted in the Zapotitlan Valley, located within the Tehuacan-Cuicatlan Reserve in the Puebla state, Mexico. The valley is representative of an arid ecosystem and is located at 1400 masl. Climate is semiarid with a mean annual precipitation of 380 mm, recorded mainly during the summer. The mean annual temperature is 21°C (Montaña and Valiente-Banuet, 1998). Soils are shallow and rocky, calcic cambiosols and xerosols or litosols (Zavala-Hurtado, 1982). The vegetation in the locality is a xeric shrubland (Rzedowski, 1978), in which columnar cacti dominate the landscape (Valiente-Banuet *et al.,* 1997).

Disturbance index and site selection

We used the methods proposed by Martorell and Peters (2005) to estimate the disturbance index on 26 different sites within the locality and thus choose two sites with

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contrasting level of disturbance to conduct the study. To estimate the disturbance index, within each site we marked two 50 m transects separated by 10 m from each other. On each transect we recorded 15 factors of disturbance from three categories: i) *Livestock raising* (goat droppings frequency, cattle droppings frequency, browsing, livestock trail density and soil compaction); ii) *human activities* (fuelwood extraction, human trails density, human trails surface, settlement proximity, contiguity to activity cores, land use and evidence of wildfires) and iii) *land degradation* (erosion, presence of soil islands and totally modified surfaces). Then, we constructed a data set with the 15 disturbance factors obtained for each site, such as Martorell and Peters (2005) have suggested. A principal components analyses (PCA) was applied to this data set. Data were standardized and combined into a single index. The PCA score of a site is a linear combination of the values recorded for each factor. This allowed us to calculate an index for each disturbance factor by adding only the respective linear terms. The sum of these indices equals the PCA score, so they reflect the contribution of different forms of disturbance in a location. The analysis was conducted in MVSP v. 3.12g (Kovach, 2004).

According to the disturbance index obtained for each site, we choose two sites with contrasting indexes to conduct the present study. The sites with the lowest and highest disturbance indexes were then denominated conserved and disturbed site, respectively.

Ant sampling

Ant collection was conducted using pitfall traps. Pitfall traps are widely used for collecting terrestrial invertebrates, having the advantage of being inexpensive and facilitating the collection of a great amount of organisms (Andersen, 1991).

In each site, we established two parallel 100 m transects, separated by 10 m from each other. On each transect, we placed 10 pitfall traps every 10 m. Five traps were placed on the soil surface, whereas the other half were placed on the surrounding vegetation at 1m in height. Tuna was used as bait in each trap (Bestelmeyer and Rios-Casanova, 2010). Trapped organisms were collected from the traps every day at 7 and 19 hrs during a 10 days period. As we collected the organisms, the bait on each trap was replaced. All collected ants were preserved in 70% ethanol. Afterwards, we determined the number of ants collected per species. Ant species were determined by using the identification keys by Mackay and Mackay (1989).

Disturbance effect on the ant community

Ant abundance was compared between sites and between sampling time within each site by applying a repeated measures ANOVA (Gurevitch and Chester, 1986). A post-hoc Bonferroni test (Rice, 1989) was applied in order to determine which species showed significant differences. Data of ant abundance were transformed as $\log_e$ to fit normality. Statistic analysis was conducted on NCSS 2001 (Hintze, 2006).

A Simpson index (Magurran, 1988) of ant diversity was estimated for each site. Diversity indexes were estimated using the software MVSP v. 3.12c (Kovach, 2004).

A canonical correspondence analysis (CCA; Johnson, 1998) was applied to determine the effect of the 15 disturbance factors estimated in each site on the ant community. Then, we constructed a biplot showing the position of each ant species (from both sites) respect to each one of the 15 disturbance factors. In the biplot, disturbance factors are shown as vectors, indicating the direction in which the variable increases. The importance of disturbance factors on the distribution of each ant species is indicated by the length and the angle between each vector and the horizontal axis (Ter Braak, 1987). The analysis was conducted in MVSP v. 3.12c (Kovach, 2004).

Feeding guilds

In order to determine the effect of disturbance on the functional communities of ants in the two sampled sites, we estimated the similarity index of Sörensen modified by Silvestre (2000). The modified index includes the number of functional guilds sampled within each locality and the number of species within each guild. Functional similarity (fs) is very useful when comparing community structure from different sites, assuming that species within each functional guild are ecologically equivalent (Martínez, 1996, Silvestre *et al.,* 2003).

Results

Disturbance index

The disturbance indexes for the two sites chosen were 8.93 (disturbed site, D) and 0.27 (conserved site, C). Among the 15 disturbance factors considered for the estimation of the index, cattle paths (0.963) and soil compaction (0.266) had the greatest values for the first component. Disturbance factors associated with human activities had low values. Among these, closeness to sites with human activity (0.001), plant coverage on paths (0.022) and path surface (0.024) were the less important.

Abundance, species richness and diversity of ants

A total of 5254 ant individuals were collected. The ants collected belong to four subfamilies (Myrmicinae, Pseudomyrmecinae Formicinae Dolichorerinae), seven genera (*Crematogaster* Lund, *Pheidole* Westwood, *Pogonomyrmex* Mayr, *Camponotus* Mayr, *Brachymyrmex* Mayr, *Dorymyrmex* Mayr, and *Pseudomyrmex* Lund) and 13 species (*Table 1*).

Brachymyrmex musculus was the most abundant species within the disturbed site, whereas *Camponotus zonatus* and *Pseudomyrmex gracilis* were the less abundant in that same site. On the other hand, *Dorymyrmex pyramicus* was the most abundant species, while *Pheidole skwarrae* and *Pheidole tepicana* were the less abundant species in the conserved site (*Table 1*).

Repeated measures ANOVA, showed a significant effect of sampling time on ant abundance ($F_{3,\ 39} = 9.52$; $P = 0.0473$). However, the Bonferroni test showed that sampling time only affected the abundance of *Brachymyrmex musculus* (the most abundant species in the disturbed site) and *Dorymyrmex pyramicus* (the most abundant species in the conserved site). Both species were significantly more abundant in the morning sampling than in the evening one ($P < 0.05$). The ANOVA test also showed a significant effect of disturbance on ant abundance ($F_{12,\ 24} = 5.06$; $P = 0.0001$). The post-hoc Bonferroni test showed that *Pheidole tepicana, P. skwarrae, Pogonomyrmex barbatus* and *Dorymyrmex pyramicus* were significantly more abundant in the conserved site than in the disturbed one ($P < 0.05$). Contrastingly, *Brachymyrmex musculus* was significantly more abundant in the disturbed site.

Table 1. *Ant species, feeding guild and number of ants collected on the disturbed (D) and conserved (C) sites within the Zapotitlan Valley, Puebla.*

Subfamily	Species	Feeding guild	Individuals (D)	Individuals (C)	Total
Myrmycinae	*Crematogaster torosa* (Mayr 1870)	Generalists[2]	46	282	328
	Crematogaster distans (Mayr 1870)	Generalists[2,4]	442	179	621
	Pheidole skwarrae (Wheeler 1934)	Granivores[1]	0	3	3
	Pheidole tepicana (Pergande 1986)	Granivores[1]	0	3	3
	Pogonomyrmex barbatus (Smith 1858)	Granivores[1,5]	63	341	404
Formicinae	*Camponotus planatus* (Roger 1863)	Generalists[2]	44	59	103
	Camponotus atriceps (Smith 1858)	Generalists[4,5]	25	53	78
	Camponotus lespesii (Emery 1894)	Generalists[2]	3	25	28
	Camponotus zonatus (Emery 1894)	Generalists[2]	2	6	8
	Brachymyrmex musculus (Forel 1899)	Generalists[2,4]	1388	697	2085
Dolichoderinae	*Dorymyrmex bicolor* (Wheeler 1906)	Generalists[3]	71	13	84
	Dorymyrmex pyramicus (Roger 1863)	Generalists[4]	373	1130	1503
Pseudomyrmecinae	*Pseudomyrmex gracilis* (Fabricus 1804)	Predators[2,5]	2	4	6

[1] Rojas 2001; [2] Silvestre *et al* 2003; [3] Ríos-Casanova *et al.* 2004; [4] Fisher y Cover 2007; [5] Guzmán-Mendoza *et al.* 2010

The highest specific richness was found in the conserved site (13 species). The disturbed site had 11 species of ants. Ant diversity was higher in the conserved site (0.7) than in the disturbed one (0.54).

Effect of disturbance factors on the ant community

Results of the CCA showed that the “x” and “y” axes explained 52.3 % and 36.2%, respectively, of the total variability of ant species abundances (*Fig. 1*). *Pheidole tepicana* and *P. skwarrae* were located on the lowest values for the disturbance factors, suggesting that these species could be highly sensitive to disturbance. On the contrary, *Brachymyrmex musculus* and *Dorymyrmex bicolor* are located on the high values of disturbance factors, indicating that these species tolerate disturbed habitats.

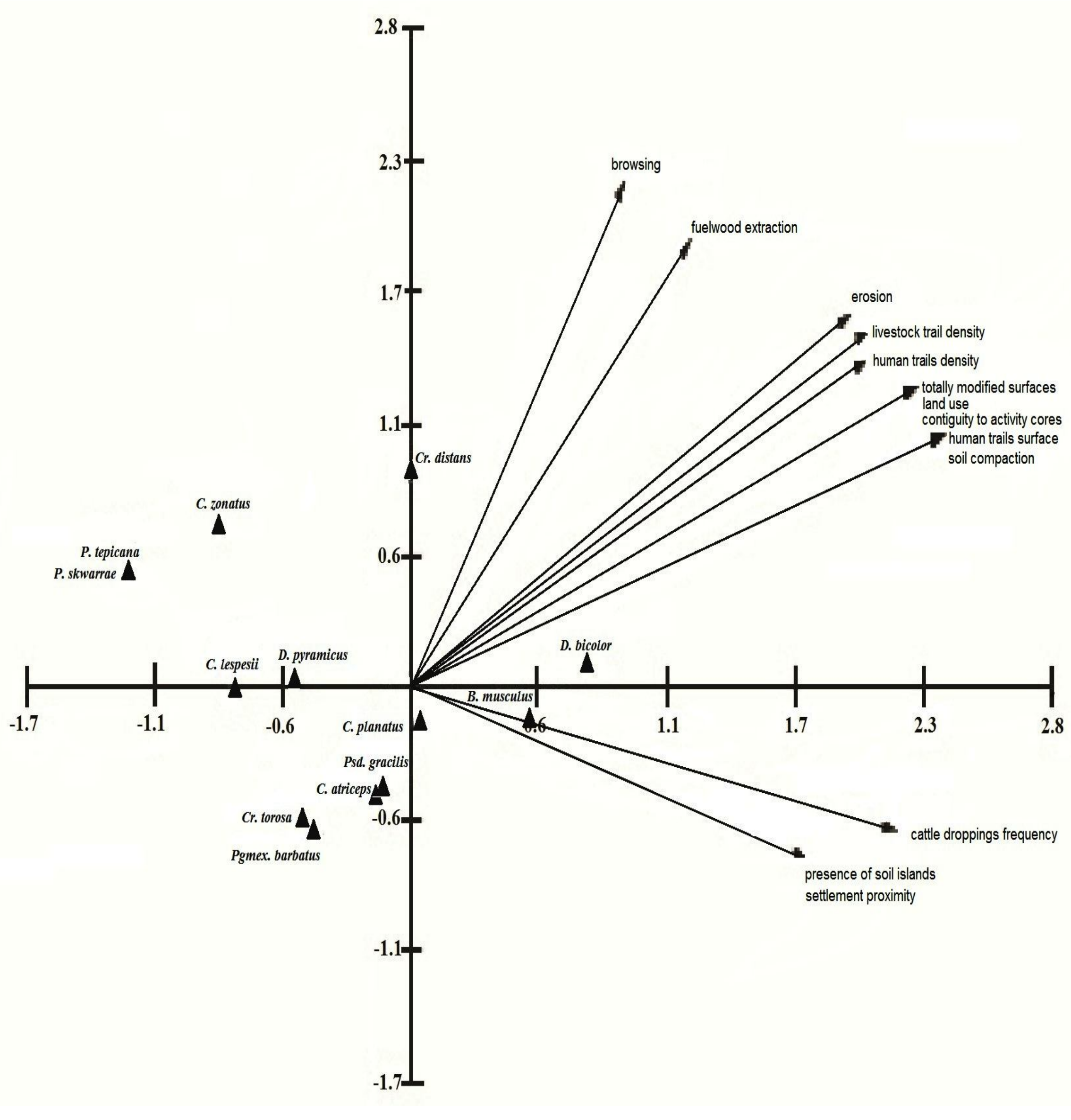

Figure1. *Ordination (CCA, box plot) of ant species (triangles) in relation to 15 disturbance factors (arrows) that were combined into a single index such as Martorell and Peters (2005) have suggested.*

Feeding guilds

From the 13 ant species collected, there were three granivore species (*Pheidole skwarrae, P. tepicana* and *Pogomomyrmex barbatus*), one predator (*Pseudomyrmex* gracilis) and nine generalist foragers (*Table 1*). The communities of ants from both sites had a functional similarity index of 72%. All the feeding guilds were found in both sites.

Discussion

Disturbance index

Soil compaction and cattle paths (the most important disturbance factors within the Zapotitlan Valley) may have a negative effect on the ant community, because soil compaction caused by cattle may prevent ants from building their nests underneath the soil surface and/or may destroy the nests already built by ants (Tizón *et al.,* 2010). Moreover, disturbance factors have an indirect effect on ant behavior. For instance, soil compaction prevents the normal development of plant roots, and favors soil impermeability to water as well as a decrease in gas exchange; thus, preventing the establishment of diverse plant species (Hallmark and Barber, 1981; Davis *et al.,* 1983; Abercombie, 1990; Carrillo, 2003; Philpott *et al.,* 2010). Consequently, there is a decrease in plant coverage together with a change in temperature and humidity. A decrease in vegetation coverage might reduce feeding resources and may expose the chemical signals of ants to rain and wind, so that communication among individuals may be affected negatively (Rojas, 2001). Consequently, ant density may decrease (Bustos *et al.*, 1997) and the ant community may change from being rich in species and feeding habits to a poor community with a few dominant species and generalist feeding habits (Carrol and Risch, 1983; Perfecto and Vandermeer, 1996).

Abundance, species richness and diversity of ants

A total of 13 ant species were collected in this study. Other studies conducted in the same locality have found a much higher species richness. For instance, Ríos–Casanova *et al.* (2004) recorded 28 ant species, whereas Guzmán-Mendoza *et al.* (2010) reported 27 ant species. The differences in species richness found in those studies and ours may be due to the number of traps and time of collection. Ríos–Casanova *et al.* (2004) and Guzmán-Mendoza *et al.* (2010) used 400 and 20 traps during three and one years, respectively; whereas we only used 20 traps (each site) on a period of 10 days. Therefore, the species richness obtained in this study is relatively high; moreover, 11 out of the 13 ant species recorded are new records for the Zapotitlan Valley. Among the species recorded in this study, *Pogonomyrmex barbatus* and *Camponotus atriceps* are the only two species out of 13 species recorded that have been previously registered in the locality (Ríos-Casanova *et al.,* 2004; Guzmán-Mendoza *et al.*, 2010).

The high number of new ant species recorded in our study may be attributed to a great variability in time and space that may be experiencing the ant community in the Zapotitlan Valley (Rico–Gray *et al.*, 1998; Guzmán-Mendoza *et al.*, 2010). Another possibility is that the number of ant species collected in our study may be associated with the methodology, since half of the pitfall traps were placed on the soil surface and the other half were placed on the surrounding vegetation at 1m in height. Contrastingly, in other studies, traps have been placed only on the soil surface (Ríos-Casanova *et al.,* 2004; Guzmán-Mendoza *et al*., 2010).

As for ant activity, it has been recorded that the highest activity of ants occurs early in the morning, when temperature is not that high (Kaspari, 2000). In our study, most ant species had a similar abundance on the morning and the evening hours. However, *Brachymyrmex musculus* and *Dorymyrmex pyramicus* were significantly more abundant during the morning. This pattern has been previously recorded for these same ant species in other studies (Tobin, 1994; Andersen, 1997; Davidson, 1997). It has been proposed that the greater abundance of these ant species during the morning is associated with their behavior as submissive opportunists (Andersen, 2010). That is, these ants constitute an unspecialized functional group, often with wide habitat distribution, but they are dominant only when the behavioral dominance of other species is low (Andersen, 2010). In our study, most ant species distribute their activity throughout the day, so that *B. musculus* and *D. pyramicus* can be more abundant and competitive during the morning. This may be the reason why *B. musculus* and *D. pyramicus* are more abundant during the morning, which is when they can compensate their submissive behavior (Cerdá and Cros, 1997).

Disturbance had a negative effect on ant diversity, such that there was a lower diversity index in the disturbed site compared with the conserved site. Besides, the ant community was dominated by only three ant species: *B. musculus, C. distans* and *D. pyramicus* (*Table 1*). These three species are generalists and either can be very successful at competing for resources with other organisms (*C. distans*) or have developed successful strategies of feeding (change their behavioural forager like *B. musculus* and *D. pyramicus*; Brown, 2000; Rojas, 2001; Ríos-Casanova *et al.,* 2004; Fisher and Cover, 2007; Andersen, 2010). In any case, the behavior of these three species may cause a decrease in the diversity of the community (Rojas *et al.,* 2004).

The conserved site showed a higher diversity of ants than the disturbed one. Moreover, *Pheidole skwarrae* and *P. tepicana* were recorded only in the conserved site. The higher diversity of ants in this site may be attributed to the availability of a greater number of microhabitats and resources that can be used by ants (Bustos *et al*., 1997). For instance, in the conserved site, ants may be able to find decomposing wood, rocks, litterfall and shrubs to build their nests as well as to obtain food (Rojas, 2001; Blüthgen and Feldhaar, 2010). Therefore, the intrinsic characteristics of the conserved site may favor niche partition by different ant species and thus, reducing ant competition and allowing the maintenance of a highly diverse community (Bustos *et al*., 1997; Cerdá and Cros, 1997).

Disturbance factors and changes in the community of ants

Results from the CCA showed that the horizontal and vertical axes explained 52.3% and 36.2% of the total variability in species abundance. Actually, it has been shown that factors explaining more than 30% of variability are very important (Ter Braak, 1987). The CCA showed that most ant species were grouped by the lowest values of the disturbance factors (left side in Fig. 1). The ant species *Pheidole tepicana* and *P. skwarrae* were negatively affected by disturbance, such that they were found only in the conserved site. Therefore, these species seem to be sensitive to environmental changes and thus, may be considered as indicators of disturbance. According to Kremen *et al.* (1993), an indicator species of disturbance is defined on the basis of its presence/absence in sites with different levels of disturbance and/or on differences in its abundance when comparing sites experiencing different levels of disturbance. The ant species *Pogonomyrmex barbatus* was significantly less abundant in the disturbed site so, we can consider *P. barbatus* as an indicator species. However, the CCA grouped *P. barbatus* with species that were not significantly affected by disturbance. This result suggests that *P. barbatus* is not directly affected by the disturbance factors estimated, but that may be indirectly affected by them through changes in microclimate and availability of resources associated with disturbance. Similar results have been previously recorded for this same ant species, Johnson (2000) found that the pattern of microdistribution of *P. barbatus* may be caused indirectly by soil texture, which has a direct effect on plant species distribution and hence the seeds available to ants. Moreover, Gordon *et al.* (2005) showed that the requirements imposed by external conditions have a strong influence on the distribution of this ant species and their colonies sequence.

Other ant species with elevated abundance on disturbance site were *B. musculus* and *D. bicolor.* These species were more abundant in sites with high values of disturbance factors, especially of those related with cattle and land degradation. Because *B. musculus* was significantly more abundant in disturbed sites, it can be considered as an indicator species, tolerant and/or favored by disturbance. MacGown *et al.* (2007) reported that populations of *B. musculus* grow and spread out explosively in anthropogenically-disturbed habitats.

Although, *Dorymyrmex bicolor* also showed to be tolerant to disturbance factors; its abundance was not significantly affected by disturbance, which prevents us from considering it as an indicator species.

Feeding guilds

Six feeding guilds of organisms living in soil and litterfall have been recognized for Mexico (Rojas, 2001; Ríos-Casanova *et al.,* 2004): predators, granivores, omnivores, mycophagous, parasitic and generalists. Most of the ant species collected in our study are generalists (*Table 1*); except for ants within the *Pseudomyrmex, Pheidole* and *Pogonomyrmex* genera, the former being predator and the other two, granivores (Ríos-Casanova *et al.,* 2004). In our study, *Pheidole* ants were recorded only in the conserved site and their abundance was not high, therefore, species from this genus seem to be sensitive to disturbance factors (Torres, 1984). In the Zapotitlan Valley, soil compaction and the abundance of cattle paths prevent the establishment of plants from which *Pheidole* species may feed (Philpott *et al.,* 2010). Besides, those disturbance factors may cause microclimatic changes that affect negatively the distribution of *Pheidole* ants in the disturbed site. However, in order to determine the microclimatic factors that are affected by disturbance as well as the intensity and directionality of their change, more studies are needed.

The other granivore ant, *Pogonomyrmex barbatus,* was also sensitive to disturbance factors. Although it was recorded on both sites, its abundance was significantly lower in the disturbed site. This result shows that, like *Pheidole* species, disturbance has a negative effect on the abundance of *P. barbatus*, probably because in the disturbed site less plants can establish and, therefore, there is a lower availability of seeds. Therefore, is reasonable considering that generalist species use a great variety of resources throughout the year (*i.e.* insects or parts of them, animal and plant exudates), so that they do not depend upon the seasonal availability of specific resources (Whitford, 1978). Contrastingly, granivore species depend upon the availability of seeds and fruits, which are more abundant after the rainy season; and the rest of the year these species depend from stored food. Therefore, if there is a lost of vegetal species by disturbance, granivore ants will be seriously affected and it will be hard for them to find food (Brown *et al.*, 1986).

Functional similarity (fs) of the ant community between the disturbed and conserved sites was elevated (72%). This suggests that even when the granivore guild was negatively affected by disturbance, the functional structure of both ant communities is maintained. Moreover, our results suggest that ecological equivalents must exist in both sites. Silvestre *et al* (2003) used the same similarity index to compare ant communities in Brazil. They found that between a conserved and a disturbed site, the species composition was different but ecological equivalents existed on both sites (fs = 78.1%). Results from Silvestre *et al.* (2003) showed that the only difference between sites was the number of species of the generalized Camponotini guild. Therefore, they considered the guild as a disturbance indicator. Accordingly, in our study, the granivore guild of ants can be considered an indicator group of soil compaction and cattle paths whthin the Zapotitlan Valley.

Our sampling method was restrictive to a single type of bait and two strata where ants could be found. Therefore, it is possible that not all of the feeding guilds of ants within the community were collected and thus, the similarity between the two ant communities may change if the sampling method is modified and a greater diversity of baits are used, as has been recorded by Bestelmeyer and Ríos-Casanova (2010).

Summarizing, the use of a quantitative method to determine the index of anthropogenic disturbance allows an objective selection of sites with contrasting disturbance. Moreover, this method facilitated the determination of the disturbance factors affecting the ant community. Our results showed that actually soil compaction and cattle paths are affecting the ant community in the Zapotitlan Valley. Differences in the abundance of each ant species between sites and results from the CCA suggest that approximately half of the species are affected by these disturbance factors. This pattern is also shown by the differences found in ant diversity between sites. The CCA and ANOVA analyses showed that *Pheidole tepicana*, *P. skwarrae* and *P. Barbatus* were highly sensitive to disturbance, whereas *Brachymyrmex musculus* was favored by disturbed habitats; therefore, they can be considered as indicator species. Even when there were not differences in the functional diversity between sites, the granivore species were negatively affected by disturbance, whereas all other species were not affected. Granivore organisms have a strong influence on the structure of the plant community, depending upon the pairwise granivore-plant interactions (Golley and Gentry, 1964; Wight and Nichols, 1966). Therefore, local extinction of granivore species may have an effect on other interactions within the community (*i.e.* seed dispersal; Rissing, 1986). Moreover, the local loss of an ant species can cause a decrease in species diversity within the community, and thus, a greater sensitivity to environmental changes (Begon *et al.*, 1996). Then, even when our results showed a negative effect of disturbance only in the granivore ant guild, we cannot discard other effects through the trophic web.

The results obtained in this study can be useful for future studies in which disturbance is evaluated. Thus, by identifying the presence/absence of the five indicator species that we recorded, disturbance on a specific site can be evaluated rapidly and inexpensively.

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GRAZING OF SELECTED GENERA OF GREEN, RED AND BROWN MACROALGAE

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Abstract. Macrobenthic algae and grazers like gastropods, amphipods etc. eventually form a key component of intensified grazing ecosystem in rocky intertidal area. Grazing gastropods suppresses the abundance of canopy forming algal species, thus lead to a reduced algal diversity in the long term. We conducted feeding trials with single and mixed diet during a 28–Days indoor experiment to examine the grazing intensity among green, red and brown algae namely Ulva lactuta, Enteromorpha linza, Gelidium and Padina tetrastromatica following a gut content analysis of experimental gastropods such as Euchelus asper and Nerita oryzarum. Feeding experiments revealed that Euchelus asper consumed 70% Enteromorpha, 10% Ulva and about 10% Gelidium whereas, Nerita oryzarum consumed 45% Ulva and 10% Enteromorpha during single diet experiment. Euchelus asper showed its preference towards all experimental diet where as Nerita preferred Ulva to a greater extends than Enteromorpha and rest other combined diet. Our results showed a higher grazing pressure exerted by Euchelus asper might pose a threat for algal culture and form competition in animals of same trophic level intern affecting their population. Similar experiment may be useful to control mass canopy forming algal growth and habitat studies for grazing animals will also be attributed.

Keywords: *grazer, herbivory, seaweed*

Introduction

Macrograzers strongly affect the abundance and distribution of seaweed in both temperate and tropical communities (Hay, 1985). Lubchenco and Cubit (1980) have suggested that the upright and boring stages of ephemeral macroalgae are adapted for high growth rates and reproduction due to lower grazing pressure, and on the other hand they are adapted for surviving during high grazing pressure. *Littorina littorea* is the most prevalent herbivore in the intertidal zone which shows a specific preference towards *Enteromorpha intestinalis* than *Chondrus crispus* among the algal foods (Lubchenco, 1978). A comparative grazing experiment conducted by Underwood and Jernakoff (1981) brought out the differential grazing features of *Siphonaria denticulate* on established algal growth and *Cellana tramoserica* on early sporeling stages of algae. Grazers maintain algal diversity by depressing the abundance of dominant canopy forming species, and that

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despite an initial increase in number of algal species exclusion of grazers reduced algal diversity in the long term. Herbivores can be a determining factor in seaweed distribution directly through grazing which has been proved by Forslund et al. (2012). Result showed that the range of the herbivore *Idotea balthica* is known to decrease the abundance of fucoids *Fucus radicans* through grazing along the Swedish coast. Hence the present investigation has been conducted to examine the grazing preference of *Euchelus asper* and *Nerita oryzarum* during a 28 - days feeding experiment.

Materials and Methods

Indoor grazing experiment set up

Two gastropods namely *Nerita oryzarum* (Recluz) and *Euchelus asper* (Gml) were chosen to conduct laboratory experiment for measuring rates of grazing and seaweed consumption in single feeding trials. Seaweeds used for feeding experiments were *Ulva lactuta* Linnaeus (1753*), Enteromorpha linza* Linnaeus (1753) *and Gelidium* Lamouroux (1813), *Padina tetrastromatica* Hauck (1887) which were collected from intertidal zone along Bandra (19^o 02’41. 58”N, 72^{o}49’08. 80”E) and Colaba (18^{o}54’08. 40”N, 72^o 48’12. 22”E) region of Maharashtra coast of India.

Collected animals were starved and acclimatized for 24 hours in filtered seawater at temperature 25ºC and at salinity 30-32 ppt. These conditions were similar for both the experiments conducted with a period of 28-days. A group of ten gastropods (consisting 5 nos. *Nerita oryzarum* and 5 nos. *Euchelus asper*) were placed in the plastic tub filled with 10 liter filtered seawater. In experiment -1, four sets of treatments in triplicate (comprising first with *Ulva lactuta* as feed, second with *Enteromorpha linza* and the third with *Gelidium*; and the fourth one with *Padina tetrastromatica*) were set. The second experiment was the repetition of the previous. During 28-days treatment phase, seaweeds were exposed to gastropod. Rate of grazing in 7-days interval were calculated by loss of algal fresh weight. Gastropods were dissected after 12 hours interval of grazing to examine the gut content. Single factor ANOVA was performed separately for treatments and replicates of both the experiments to find out the significant difference in feeding preference and grazing intensity in the experimental gastropods.

Gut Content Analysis

The experimental gastropods (*Euchelus asper* and *Nerita oryzarum)* were dissected in laboratory and their gut content had been examined for the availability of seaweed fragments using stereoscope and compound microscope (Hund, Germany) and images were taken using Nikon digital camera (Nikon 310) attached to the microscope. Grazing preference of the gastropod was estimated using Chi-Square test.

Results

Single factor analysis of variance was performed in within treatment to compute the significant difference in feeding preferences of the gastropods, while similar analysis was carried out within replicates to find out the significance difference in grazing intensity of gastropods (*Table 1*). Experiment-I revealed a significant difference in feeding preferences of *Nerita oryzarum* as this organism has shown preference towards algal diets, while there is no significant difference in grazing interval since the feeding intensity was almost similar during the study period. On the other side, *Euchelus asper* showed significant difference within the replicates as it shows similar attraction towards all kind of seaweed except *Padina*. During experiment-II, *Euchelus asper* had shown significant difference in feeding preferences within the treatment and on the other hand within replicates there was no significance as the organisms preferred all types of alga except *Padina.* Whereas, *Nerita* did not show any significant difference within the treatment and within the replicates since *Nerita* had fed upon only in *Ulva* and most of the time they were found with empty gut.

Table 1*. Analysis of Variance showing significance difference in grazing intensity of the experimental organisms (* indicates significance)*

Source of Variation	SS	df	MS	F
Experiment - 1				
Nerita oryzarum				
Within Treatments	2.379456	4	0.594864	8.204568*
Within Replicates	0.394296	4	0.098574	0.573899
Euchelus asper				
Within Treatments	0.577464	4	0.144366	0.982469
Within Replicates	1.532824	4	0.383206	3.863976*
Experiment - 2				
Nerita oryzarum				
Within Treatments	0.474944	4	0.118736	2.580769
Within Replicates	0.396784	4	0.099196	1.987259
Euchelus asper				
Within Treatments	1.92692	4	0.48173	7.301816*
Within Replicates	0.35052	4	0.08763	0.605205

Feeding experiment set up (as shown in *Figure 1*) was installed to estimate the feeding preference of the experimental grazers (*Figure 2*) for 28-days. Loss of individual algal weight was noted down with an interval of 7 days and was represented graphically to find out rate of grazing. The graph interpreted that *Enteromorpha* was being grazed much faster than the other three algae as it losed much weight within similar period of time (*Figure 3*). The overall rate of grazing was observed higher in *Enteromorpha* than *Ulva*, *Gelidium* and *Padina*. In both the experiments *Padina* was not grazed by the gastropods which may be due to presence of phenolic compounds which shows antiherbivory defense mechanism among all other seaweed given as feed to the grazer.

***Figure 1**. Grazing Experimental Set up*

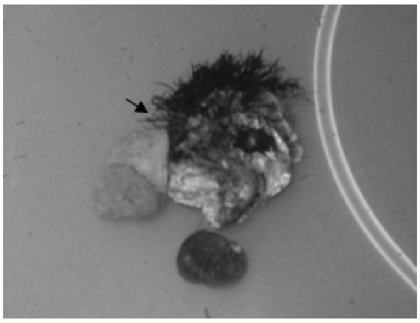

***Figure 2**. Gelidium being Grazed by Euchelus asper*

Gut Content Analysis

A total of 20 gastropods (*Euchelus asper* and *Nerita oryzarum*) from each of four single diet treatments (Treatment I – *Ulva lactuta*, Treatment II – *Enteromorpha linza*, Treatment III - *Gelidium* and Treatment IV – *Padina tetrastromatica*) were dissected. The experimental organisms were dissected after 12 hours of grazing and gut content were observed under microscope for presence of algal fronds. The analysis had shown semidigested *Enteromorpha linza* frequently in the gut of gastropods during feeding experiments. The bar diagram (*Figure 4*) revealed that *Euchelus asper* preferred *Enteromorpha linza* since their stomach contained algal fronds very often. They consumed 70% of *Enteromorpha linza*, 10% *Ulva lactuta* and about 10% *Gelidium. Nerita oryzarum* were observed with only 45% of *Ulva lactuta* and 10% *of Enteromorpha linza*. Gut contents of *Nerita* were examined empty on very frequent observations. In fact *Euchelus* *asper* stretched out its preference towards all experimental diet where as *Nerita oryzarum* confined towards *Ulva* in a greater extends than *Enteromorpha linza*. Fronds of *Padina tetrastromatica* were absent totally during gut content analysis of both *Euchelus asper* and *Nerita oryzarum* which may be due to presence of extractive phenolic compound and also may be due to the hardy nature of the thalli.

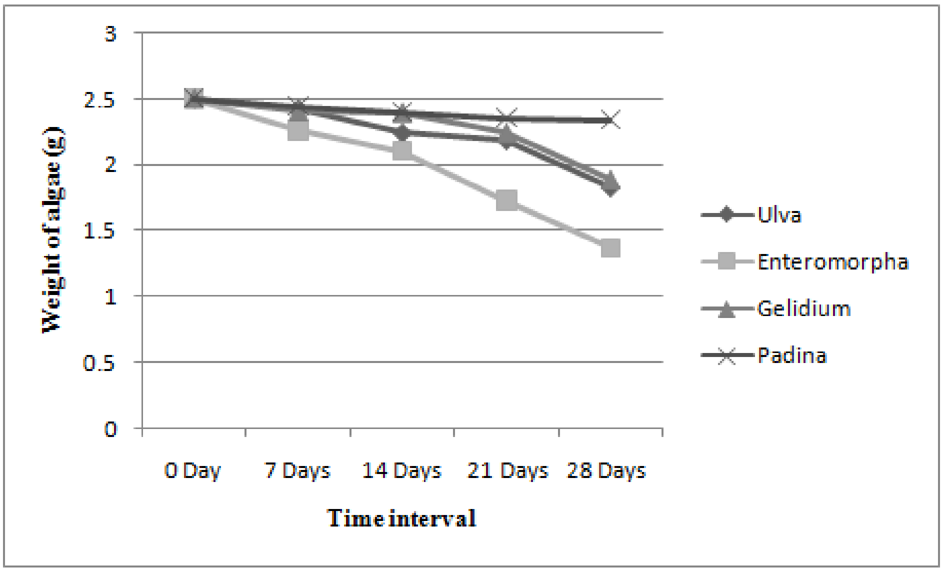

Figure 3. *Loss of algal weight in single diet experiment in a week interval*

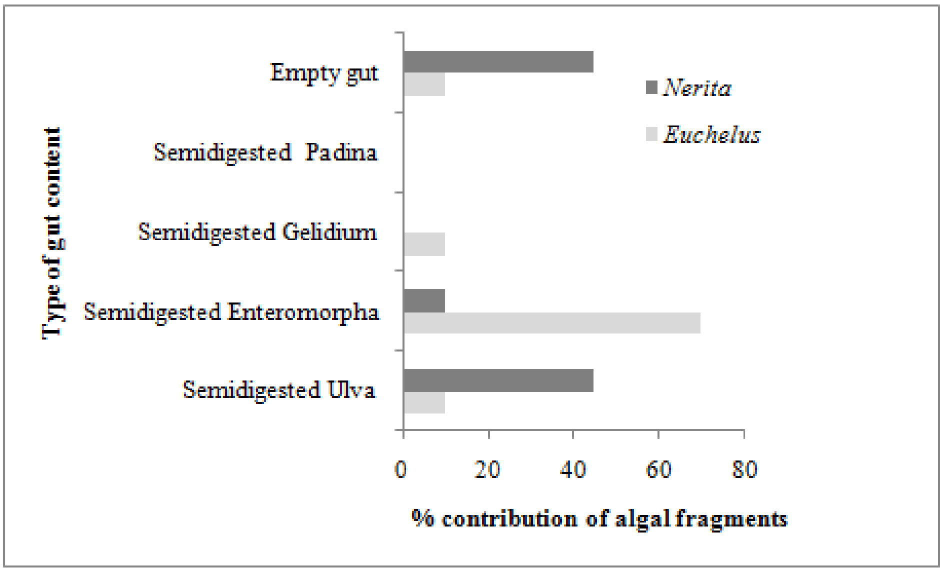

Figure 4. *Percentage contribution of algal fragments in gut of Nerita and Euchelus*

Semidigested algal fragments were observed in the gut of *Euchelus asper* on frequent basis (*Figures 5 and 6*) as compared to *Nerita oryzarum*. A Chi-square test was carried out for each treatment to check the type of gut content in organisms in relation to their food preference (*Table 2*). In experiment-I, among all of the single diet treatment *Enteromorpha linza* had been ingested at a significantly greater rate (P<0.05) than the other algae, whereas *Ulva lactuta, Gelidium* and *Padina tetrastromatica* did not show any significance within the treatments. On the other hand, during experiment-II, except *Padina* all three other algae show significance within the treatment.

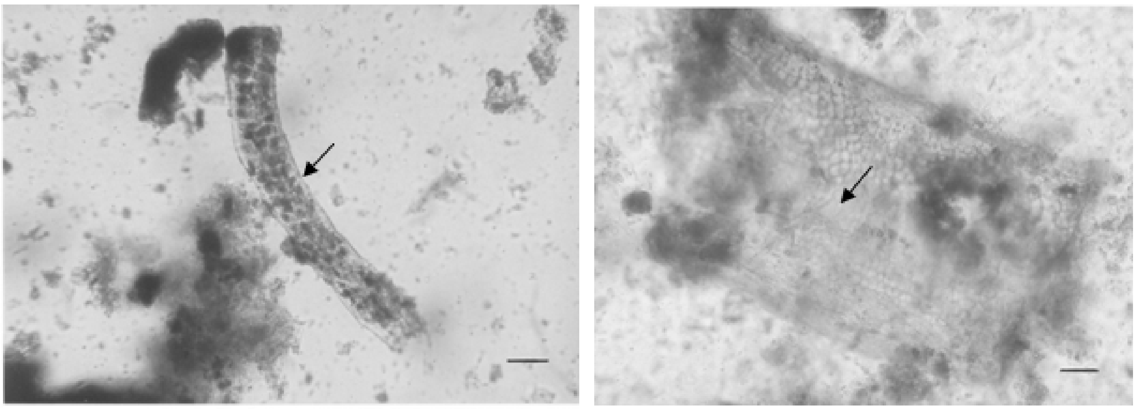

Figure 5. *Semi digested Enteromorpha and Ulva fragments in the Gut of Euchelus in Single Diet Experiment (Scale bar = 50 µm)*

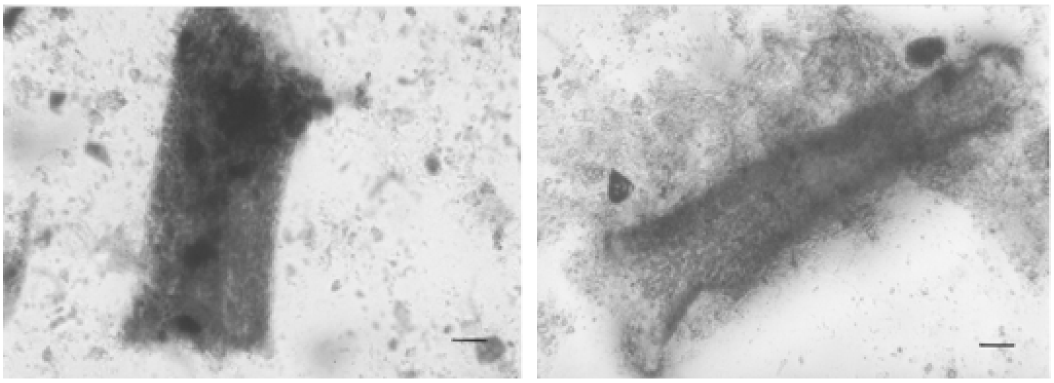

Figure 6. *Semi Digested Gelidium Thalli in the Gut of Euchelus During Single Diet Experiment (Scale bar = 0.2 mm)*

Discussion

The algal turf is reduced to dominance of species like *Gelidiopsis*, small turfs of *Ulva* and *Enteromorpha*, *Centroceras* – a filamentous algae. Lubchenco and Cubit (1980) showed direct relationship between grazers algal abundance. The Lubchenco - Cubit model has been developed to justify the heteromorphic life histories in marine algae. Their

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experiment on cleaning the area off grazers proved that ephemeral fronds do grow in absence of grazer supports present findings.

***Table 2**. Chi-Square test showing feeding preferences of the grazing gastropods among the algal diets (* indicate significance)*

Experiment 1	
Treatment	Chi^2
Ulva lactuta	2.1429
Enteromorpha linza	10.8*
Gelidium	0.83333
Padina tetrastromatica	0.2608
Experiment 2	
Treatments	Chi^2
Ulva lactuta	10.995*
Enteromorpha linza	16.425*
Gelidium	20*
Padina tetrastromatica	0.2857

In a grazing experiment, Vadas (1985) had suggested direct assessment of grazing by removal or addition of grazers, physical manipulation and algal manipulation. In the present study, *Euchelus asper* showed wide significance within algal treatment (diet) and grazing intensity as they might have fed on the respective diet with similar interval of time while *Nerita oryzarum* feds on algal diet but not in similar time interval as differ significantly within the treatments.

The result of the single diet experiment has been unveiled in terms of loss of algal fresh weight varied among experiments related to the studies of Hambrook and Sheath (1987). Grazing rate calculated from single diet feeding experiment in present study has concluded in greater rate of grazing. Similar observation was noticed by Nicotri (1980) where he studied the comparative attractiveness of algal species against artificial plants towards isopods and amphipods and by Lawrence (1975) for sea urchin grazing. Various scientific studies have also justified the present outcomes where algae offered singly have been consumed at faster rate than in combined form (Leighton, 1971; Vadas, 1977). Abundant colonization of green algae *Enteromorpha intestinalis*, *Ulva* have been observed within period of six month and finally appearance of perennials as Sargassaceans, *Padina,* Corallinaceans have become dominant in the tidal pools without the grazer snails, *Nerita albicilla* in the lower intertidal rocky shore (Arai and Arai, 1984).

Comparative analysis of gut content of both *Euchelus asper* and *Nerita oryzarum* have shown the occurrence of semidigested algal diet in their gut. Maximum number of *Euchelus* has been recorded with semidigested algal fronds in gut as compared to *Nerita oryzarum*. Results of Chi-square test between four algal treatments have given a detail account of occurrence of algal fragments in *Euchelus asper* and *Nerita oryzarum*. The analysis has demonstrated that treatment I (*Ulva*) differs in greater significance than treatment II (*Enteromorpha*), treatment III (*Gelidium*) and treatment IV (*Padina*) in single diet experiment. In bar diagram representation *Euchelus asper* pulls out maximum preference towards *Enteromorpha linza* which may be considered to be a highly preferred seaweed followed by *Ulva lactuta* and *Gelidium* as they show lower level of significance in Chi-square test.

The feeding experiment has also emphasized that *Euchelus asper* and *Nerita oryzarum* together prefer higher percentage of green algae (*Enteromorpha linza* and *Ulva lactuta*) than red algae i.e. *Gelidium*. Present study has also brought into light the fact that *Euchelus asper* and *Nerita oryzarum* have fully avoided *Padina tetrastromatica* during the study period which may be due to the presence of phlorotannins (Ragan and Glombitza, 1986). These polymers of phloroglucinol units hold an important position when plant-herbivore interactions and anti-fouling studies are being conducted (Hay et al., 1987; Cronin, 2001; Targett and Arnold, 2001; Amsler and Fairhead, 2006). And it is also proved that phaeophytes are resistant to meso-hervivores owing to concentrations of phlorotannins (Targett and Arnold, 1998; Wikstro ¨m et al., 2006; Jormalainen and Ramsay, 2009) which also supports the present study. High rate of grazing pressure by *Euchelus asper* may pose a threat to algal culture and may build up a competition for particular food item to other grazer intern affecting the trophic food chain. The hardy cell wall structure and polyexctractives compounds of *Padina* may prevent the grazer from not being consumed during the feeding experiment which was also supported by Arai and Arai (1984). Feeding experiment in this investigation has concluded that grazing gastropods prefer more of green and red macroalga in comparison with brown algae such as *Padina* due to presence of polyextractive compounds. Hence, present study addresses threats of cultivating edible seaweed like *Ulva* in mass scale production system.

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CHARACTERIZATION OF SPATIAL VARIABILITY OF SPECTRAL IRRADIANCE IN TOBACCO CANOPY

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Abstract. Characterization of light micro-environment in plant canopy is vital in ecological and agricultural research. In this study, spatial heterogeneity of light micro-environment in tobacco canopies was characterized in biologically active radiation of blue, red, far-red, as well as photosynthetically active radiation (PAR) based on field measurements. The results revealed that spectral composition of light penetrating into these canopies varied significantly with both depth and horizontal locations, featured with abrupt drop in PAR and relatively mild decrease in far-red. The variations had good accordance of linear correlations between PAR, blue and red, whereas quadratic relations between far-red and other biologically active radiation in PAR. These spectral variations were manifested pronouncedly by R/FR (red to far-red ratio), a light signal perceived by plant organs for modulating their growth and development. The main contribution of this study is introducing a generic measurement method for charactering the spatial and spectral variation of light micro-environment within canopy. It is a basal and vital step to the profound study of the interaction between plants and their natural light micro-environment.

Keywords: *plant; light environment; spatial heterogeneity; red to far-red ratio; biologically active radiation*

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Introduction

The light micro-environment in plant canopy can vary dramatically with time and location not only in quantity but also in quality (spectral characteristics) (Grant, 1997; de Castro, 2000; Hertel et al., 2011). Plants had evolved themselves to possess a variety of photo-sensory systems with photoreceptors for perceiving tiny fluctuations in their light environment and responding with delicate modulation on their development and growth for capturing more energy and resources (Gilbert et al., 1995; Santner and Estelle, 2009; Stamm and Kumar, 2010). Phytochrome is the most well-characterized family of photoreceptors perceiving the ratio of red (R: 655-665 nm) to far-red (FR: 725-735 nm) and triggering shade avoidance response in the early phase of canopy development of higher plant (Ballaré et al., 1990; Aphalo et al., 1999; Smith, 2000). The variations in the blue (B: 400-500 nm) domain can be sensed by cryptochrome which is another family of photoreceptors (Ahmad and Cashmore, 1993; Ballaré, 1999; Pierik et al., 2004; Sellaro et al., 2010).

Kasperbaue (1971) and Kasperbaue and Peaslee (1973) reported that in the environment-controlled laboratory, far-red irradiated tobacco plants developed longer internodes, longer, narrower and thinner leaves with fewer stomata and less chlorophyll, owing to the modulation of phytochrome. Two decades later, three phytochrome genes, designated *Nt-PHYA1, Nt-PHYA2* and *Nt-PHYB1,* were isolated in tobacco plant. Their structures, expression patterns and levels were characterized in detail (Adam et al., 1997). The phytohormone signal network in photo -sensory system was being revealed (Achard et al., 2006; Santner and Estelle, 2009; Stamm and Kumar, 2010). Further studies on the interaction between tobacco plant and its natural light environment were expected.

Light environment in plant canopy has traditionally been quantified in the limited range of the photosynthetically active radiation (PAR, 400–700 nm) as photosynthetic photon flux density (PPFD). The spectral composition of light and its regulating function on plant's morphogenesis were ignored. This study aims to characterize the light in photoreceptor sensitive wavebands of B, R and FR as well as PAR. By measuring the above- and within-canopy spectral irradiance in the range of 350-1100 nm, the spatial heterogeneity of irradiance in these biologically active radiations (Lee and Downum, 1991; Grant, 1997) and the spectral ratio of R/FR are examined.

Materials and methods

The field experiment was conducted at Yanhe Experiment Station (24.23˚N, 102.49˚E, Altitude: 1634 m), Yuxi, Yunnan province in the southwest of China. Two tobacco (*Nicotiana tabacum*) cultivars, Y87 and K326, were employed in this study. Seedlings were transplanted to field plots (paddy soil) on May 11, 2011, in deep rooting way on northeast-southwest rows with 100 cm row distance and 50 cm plant-plant distance. The ridge was earthed up from 30 cm to 35 cm in height after planting. Fertilization, irrigation and pest control were performed according to the local standard cultivation of tobacco (Lei and Shi, 1999).

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Radiation measurements were performed in these two tobacco canopies (named as Y87P and K326P) from 11:00 to 12:00 on August 3, 2011. The plant height was about 100-120 cm and there was 20-25 leaves per plant. Leaf length ranged from 25-70 cm and maximum width from 10-30 cm. Leaf area indexes (LAI) were 2.61(Y87P) and 3.26 (K326P), respectively. The sky was clear with casual broken cloud.

FieldSpec3 field spectroradiometer (ASD Inc., USA) was employed in this study and it was equipped with a Remote Cosine Receptor (RCR) (model *A124505,* ASD Inc.) with 180° field of view. It had a detection range of 350-2500 nm, with spectral resolution of 1.4 nm for the region 350 -1000 nm and 2 nm for the region 1000-2500 nm. Radiometric calibration of RCR has been performed by manufacturer for irradiance (W m^{-2} nm^{-1}) calculation. The RCR is primarily designed for measuring hemispheric sky irradiance. In this study it was also used for measuring the irradiance within canopy in different depth and locations. To position the measurement precisely, we fixed the RCR facing upwards and horizontally on a 1-meter-length ruler which was bonded on a self-designed holder (*Fig. 1*). The holder was constructed with a vertical stick fixed on a stable base and a horizontal stick jointing on it. A horizontal plate was mounted on the horizontal stick for bonding the ruler. The horizontal stick can move freely in vertical direction along the vertical stick, and the horizontal plate can move in horizontal direction along the horizontal stick. The sticks were labeled evenly with 5 cm intervals in horizontal range of 60 cm, and vertical range of 150 cm.

***Figure 1**. The picture of the self-designed holder for mounting the Remote Cosine Receptor and AccuPAR external sensor*

Radiation measurement for each canopy was conducted in a sample plot including 2x2 (rows x plants in each row) plants at the center of the whole canopy. *Fig.2* presented the position of measuring points in one sample plot. We set the vertical measuring range 0-135 cm and horizontal (in the direction of cross rows) range 0-60 cm

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respectively. Since the maximum height of two canopies was 120 cm and their row distance was 100 cm, the measurement can cover from above-canopy down to canopy bottom in depth, and cross a half of one sampling row in horizon.

For monitoring solar spectral irradiation above the canopy instantaneously with within-canopy measurements, it was preferable to make above and within canopy spectral irradiance measurements simultaneously. Considering we had only one spectroradiometer, we employed AccuPAR (model LP-80, Decagon Devices inc. USA) with an external sensor to monitor the photosynthetic photon flux density (μmol m^{-2} s^{-1}) of solar irradiance instead of spectral irradiance when we used spectroradiometer for within-canopy measurement, based on the fact that the spectral composition of solar irradiation does not change significantly around midday in clear days (Jacovides et al., 2000). The external sensor was horizontally mounted on the top of the vertical stick of the holder.

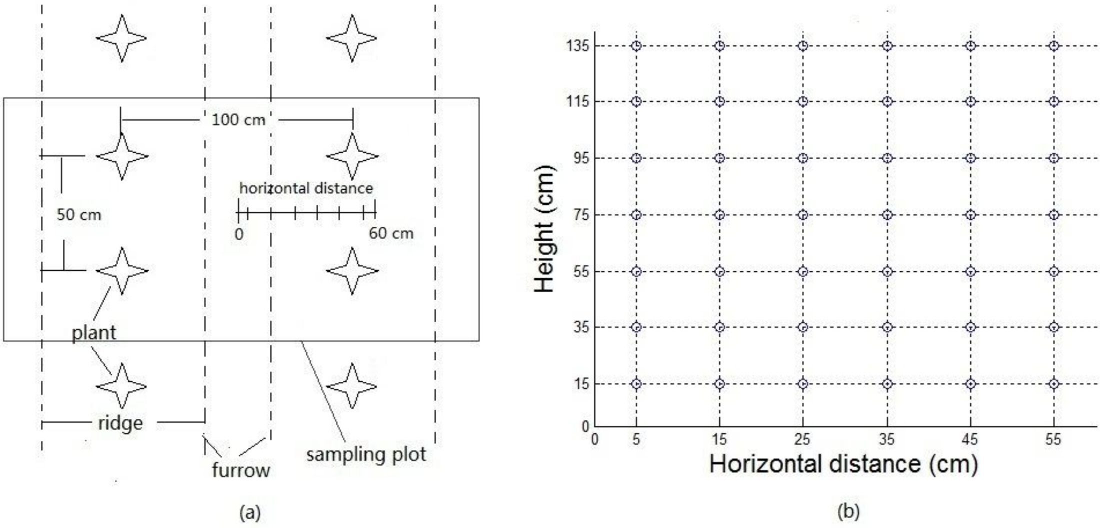


Figure 2. *Scheme of measuring points in a sample plot: (a) The sampling scheme in horizontal direction, (b) The sampling scheme in both horizontal and vertical directions. The reference point (0,0) is in the plane of ridge crest lines.*

Before and after each within-canopy measurements, we took measurements of both spectral irradiance and corresponding PPFD for incident solar radiation instantaneously with interval of 1 minute. These observations were used to build the relationship between the above-canopy solar spectral irradiance with PPFD values.

For each measurement position, spectral irradiance was recorded by taking an average of 25 continuous scans from 350 to 1100 nm in 1 nm step. In the mean time, PAR readings above canopy were recorded. During the measurement, optical optimizing of spectrometer was performed when strong variation occurred, especially when the solar radiation increased dramatically, for getting response with high quality signal and avoiding signal saturation.

Unary linear regression was used to build the relationship between the above-canopy solar spectral irradiance and PPFD values (E_{PPFD}), with spectral irradiance (E_{λ_ac}) of each wavelength as a dependent variable, and E_{PPFD} as the independent variable:

$$E_{\lambda\_ac} = C_\lambda E_{PPFD} \quad \text{(Eq.1)}$$

The coefficient C_λ is wavelength dependent, it was figured out with a least squares fit on observations, by minimizes the sum of the squares of the deviations between observed and modeled spectral irradiance as:

$$\min = \sum_n (E_{\lambda\_ac} - C_\lambda E_{PPFD})^2 \quad \text{(Eq.2)}$$

Where n is the number of observations.

For quantifying the percentage radiation penetrating into canopy, spectral transmittance (τ_λ) was defined as the ratio of within-canopy spectral irradiance (E_{λ_wc}) to above-canopy spectral irradiance which is estimated from the measurement of PPFD by Eq. 1:

$$\tau_\lambda = E_{\lambda\_wc} / (C_\lambda E_{PPFD}) \quad \text{(Eq.3)}$$

Transmittance in biologically active radiation of PAR, B, R and FR were calculated using following equations:

$$\tau_{PAR} = \sum_{\lambda=400}^{700} E_{\lambda\_wc} \Big/ \left(\sum_{\lambda=400}^{700} C_\lambda E_{PPFD} \right) \quad \text{(Eq.4)}$$

$$\tau_B = \sum_{\lambda=400}^{500} E_{\lambda\_wc} \Big/ \left(\sum_{\lambda=400}^{500} C_\lambda E_{PPFD} \right) \quad \text{(Eq.5)}$$

$$\tau_R = \sum_{\lambda=655}^{665} E_{\lambda\_wc} \Big/ \left(\sum_{\lambda=655}^{665} C_\lambda E_{PPFD} \right) \quad \text{(Eq.6)}$$

$$\tau_{FR} = \sum_{\lambda=725}^{735} E_{\lambda\_wc} \Big/ \left(\sum_{\lambda=725}^{735} C_\lambda E_{PPFD} \right) \quad \text{(Eq.7)}$$

R/FR ratio was calculated with:

$$R/FR = \sum_{\lambda=655}^{665} E_{\lambda} / \sum_{\lambda=725}^{735} E_{\lambda} \quad \text{(Eq.8)}$$

Results

Above-canopy observations of solar spectral irradiance and corresponding PPFD were recorded before and after each of the two within-canopy measurements with 10 and 6 samples, respectively. The above-canopy observations for Y87P were grouped in group1 and those for K326P in group2. The measuring time for group1 was from 11:05 to 11:30 and from 11:35 to 12:00 for group2. It showed that the solar radiance spectrum had no significant variation in its shape within one-hour measurement, although its PPFD and magnitude changed pronouncedly with time (*Fig.3*).

The correlation analysis illustrated good linear correlation between solar irradiance at each wavelength with PPFD for each of the two groups, with correlation coefficients all around 0.9 (*Fig.4a*). In contrast, very poor correlation was found when pooling data from both groups (*Fig.4a*). So it is necessary to build local linear regressions for each group of observations. *Fig.4b* displayed the wavelength dependent linear regression coefficients C_{λ} (Eq. 1) of these two groups of observations calculated with Eq. 2.

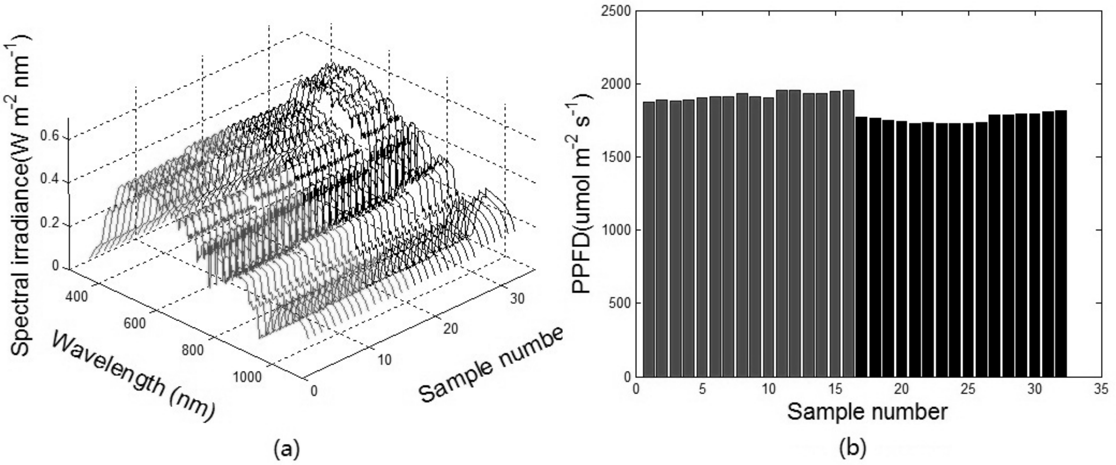
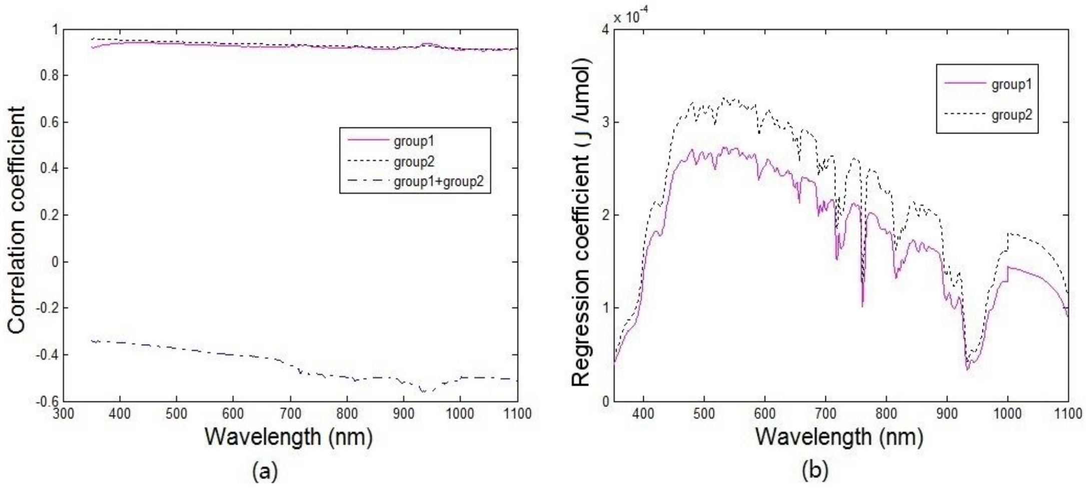


Figure 3. *Observations (group1-gray, group2-black) of solar spectral irradiance (a) and corresponding PPFD (b) above Y87 and K326 canopies collected from 11:00 to 12:00 on August 3, 2011*

Spectral irradiances were collected at all the measuring points including above and within canopies. The spectral transmittances at these measuring points were calculated with Eq.3, in which the wavelength dependent coefficients illustrated in *Fig.5b* were used to estimate solar spectral irradiances above canopy from PPFD measurements.

Fig.5 displayed the measured irradiances and calculated transmittances at four different depths which referring to above-canopy, upper, middle, and lower levels of K326P, respectively.



(a) (b)

Figure 4. *The relationship between above-canopy solar spectral irradiance and PPFD (a) Correlation coefficients (b) Regression coefficients*

The measured spectral irradiances above canopy (*Fig.5a* left) manifested the same feature of the solar irradiation with relatively higher irradiance in PAR region and lower irradiance in far-red region. For upper part of canopy, the spectra collected near the center of sample row showed sharp drop in PAR while only small decrease in far-red region (*Fig.5b* left). With the increase in canopy depth, more measured points distant from center showed similar spectral variation (*Fig.5c*-d left).

The transmittance spectra above canopy were constant with the same value of 1.0 (*Fig.5a* right). As they were calculated from PPFD measurements and spectral irradiance measurements, it illustrated the good agreement of solar spectral irradiance between the estimations from PPFD and measurements with spectroradiometer.

Spectral composition in the transmittance spectra within canopy (*Fig.5b-d* right) varied in large decrease of PAR and small decrease of FR with changes of depth and horizontal position. In the upper level of canopy, only a few of transmittances were lowered at the points near the center of row. With the increase in canopy depth (decrease of height), more points distant from row center had lower transmittances. This trend of variation kept good accordance with that of irradiance (*Fig.5b-d* left), and transmittance spectra manifested the spectral variation in irradiance distinctly. For instance, it is not easy to observe the variation of spectral composition in irradiance spectrum at horizontal distance of 5 and 15 cm with height of 95 cm (*Fig.5b*), but it was clear in corresponding transmittance spectra that they were lower in PAR than FR.

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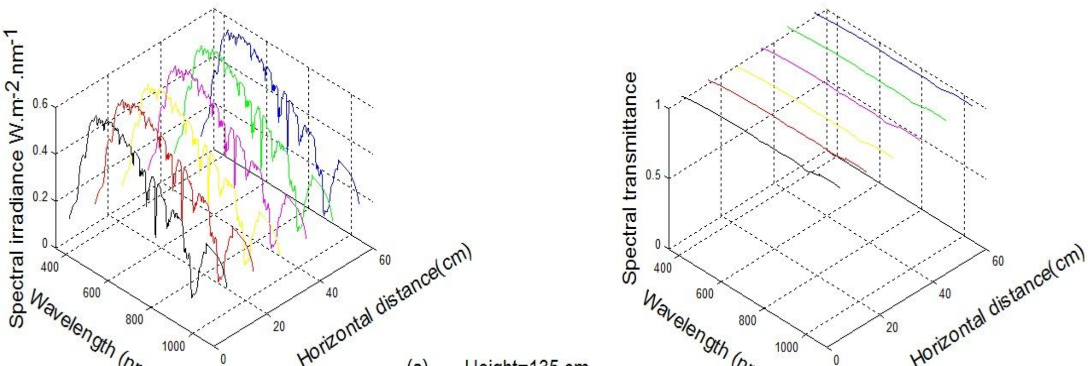
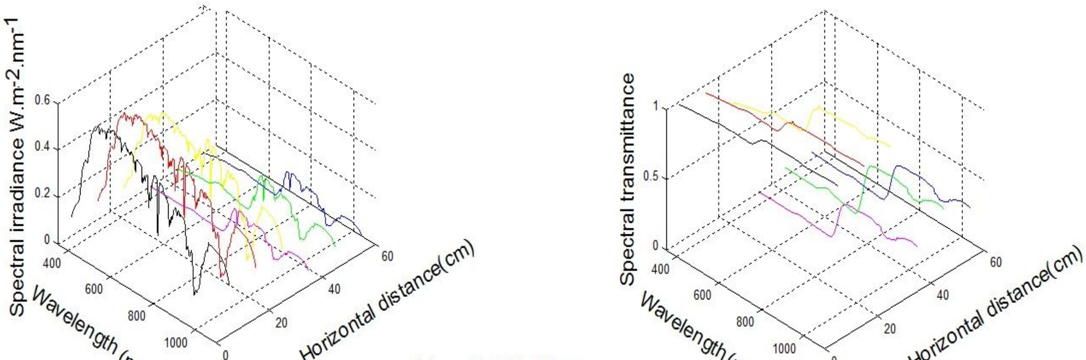
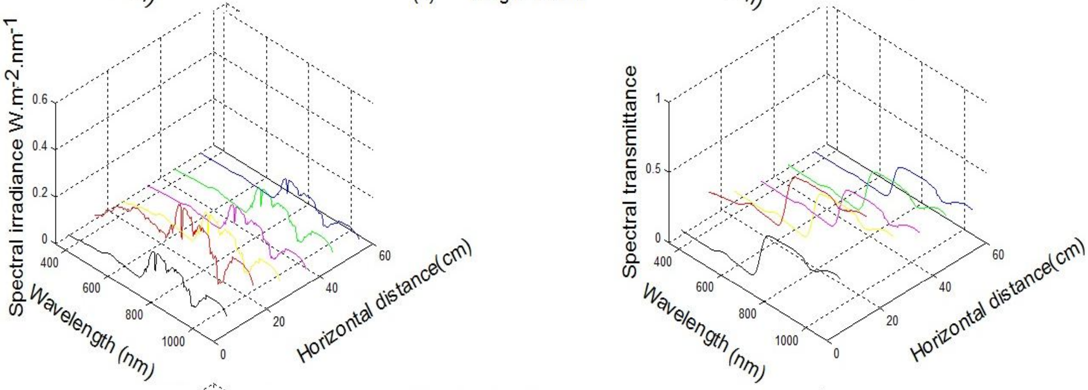


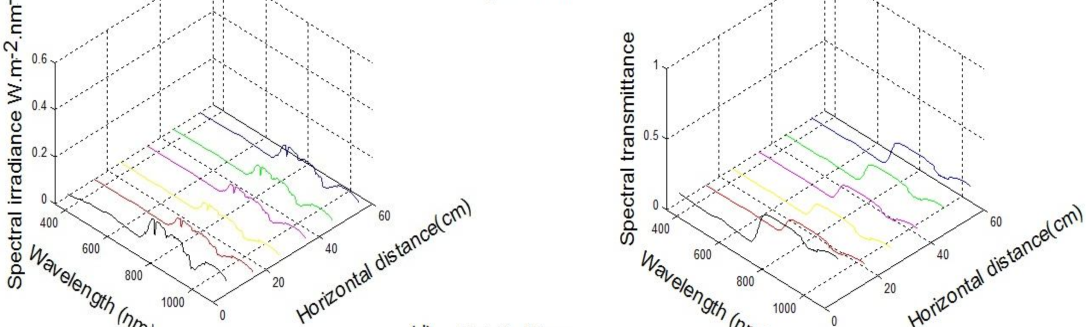
(a) Height=135 cm



(b) Height=95 cm



(c) Height=55 cm

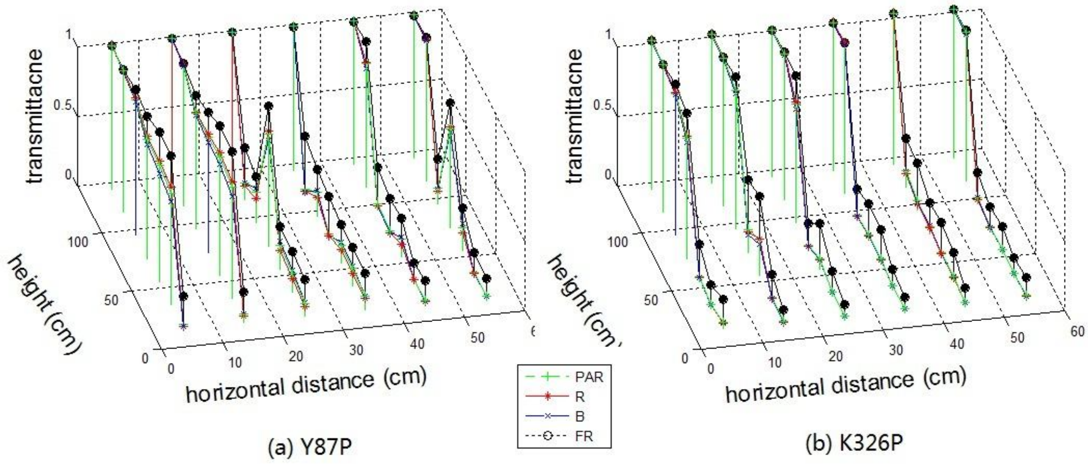


(d) Height=15 cm

Figure 5. *Spectral irradiance (left) and transmittance (right) at different height of K326 canopy*

The transmittances in PAR, B, R and FR were calculated for all measured points (*Fig.6*). Transmittances in all these wavebands had a trend of decrease with the increase of depth on each vertical profile at the same horizontal position, with few exceptions which might be caused by the offset of measuring points for avoiding canopy disturbance. Abrupt decreases occurred in the profiles near the center of measuring row in a contrast to the relatively mild decrease in profiles near row's edge. This kind of contrast was more prominent in Y87P than in K326P. *Fig.7* illustrated the average and deviation of transmittances in these four wavebands at different canopy heights. It is evident that Y87P had higher variation across horizontal planes than K326P.

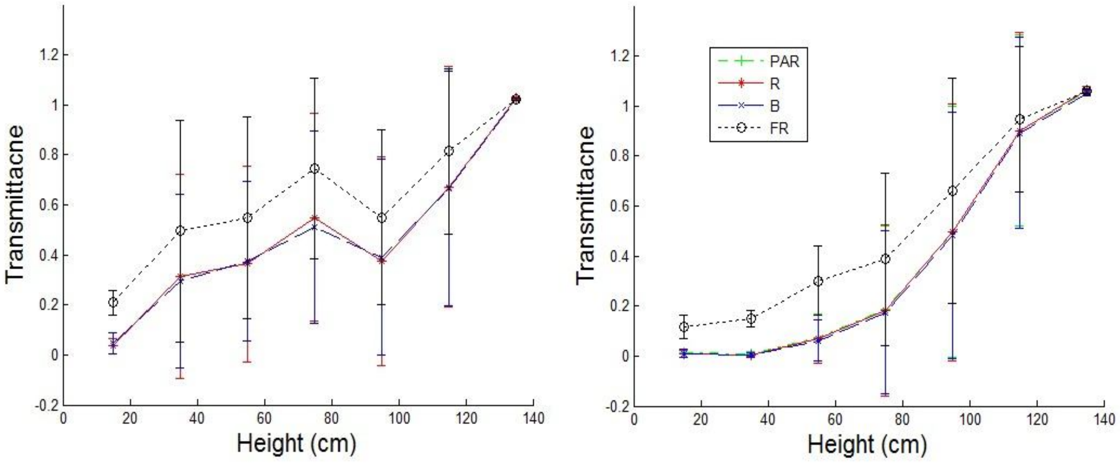
Transmittances in PAR, B and R were very close at the same points except for FR which had much higher value when those in other wavebands were low. The scatter plots of transmittances for all the measurements between these wavebands demonstrated that transmittances in PAR, B and R had strong linear relations with coefficient of determination (r^2) all greater than 0.99. Transmittance in FR also had positive correlation with PAR, B and R, but their relationship are more close to quadric rather than linear ones (*Fig.8*) and the coefficients of determination was higher for K326P (r^2>0.99) than Y87P (0.95>r^2>0.97).



(a) Y87P (b) K326P

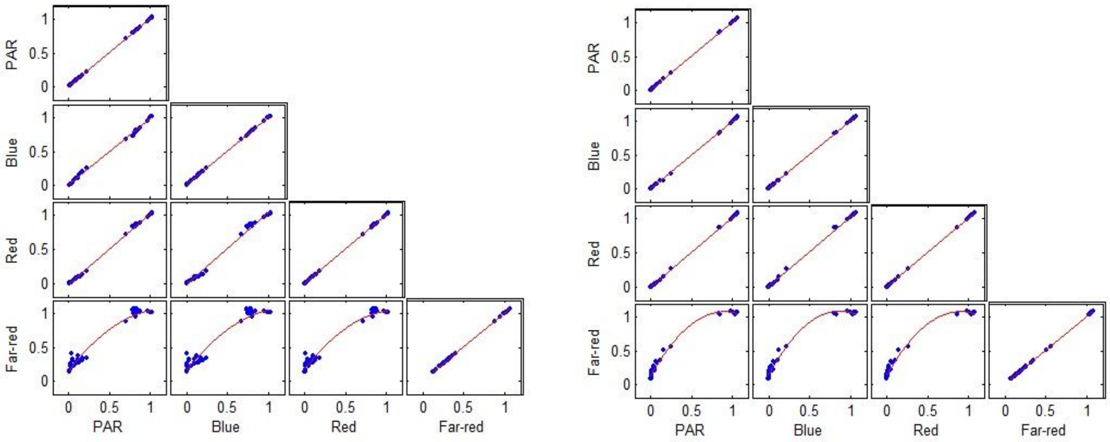
***Figure 6**. Transmittances at different measured points in Y87 and K326 canopies*

R/FR ratio at each measuring point was calculated for investigating its spatial variation within canopy (*Fig.9*). They had significant spatial variations within and between canopies. R/FR ratios were rather low at most points downwards from middle layers in K326P. In contrast, only a few points close to the bottom of Y87P canopy had low R/FR. The decrease of R/FR with increase of depth was milder in Y87P than that of K326P.



(a) Y87P (b) K326P

Figure 7. Average transmittances in vertical profiles of Y87 and K326 canopies



(a) Y87P (b) K326P

***Figure 8**. Scatter plot of transmittance between PAR, Blue, Red and Far-red in Y87 (a) and K326 (b) canopies*

Discussion

Although the spectral irradiance above canopy might vary with time according to the change of solar elevation and atmosphere conditions, its spectral composition did not have significant variation around midday under clear condition. As the spectral irradiance at each wavelength had good linear correlation with PPFD, it is acceptable to use measured PPFD for monitoring the solar irradiance and estimating solar spectral irradiance above canopy in clear midday. But we noticed that magnitude in spectral irradiances (*Fig.3a*) in group1 were lower than those in group2, which were in the

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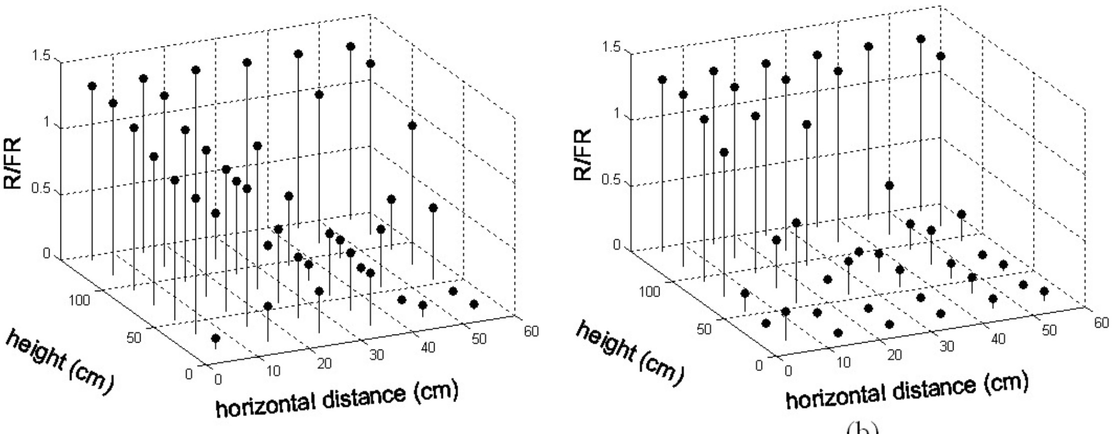
reverse of PPFD values in these two groups (*Fig.3b*). The reason resulting in this system deviation was not clear yet. So it is recommended that their relationship should be examined frequently (at least with half an hour interval).



(a)

(b)

***Figure 9**. Spatial distribution of R/FR in Y87 (a) and K326 (b) canopies*

The measurements of within-canopy spectral irradiance demonstrated significant spatial and spectral variations in canopy light micro-environment, and their variations keep good accordance with spectral transmittance. As spectral transmittance in a medium is mostly determined by the property of medium, we could conclude that the variation of irradiance in spectrum composition within canopy was dominated by the property of canopy rather than solar spectral irradiation in clear midday. The spectral characteristic of lowest transmittance in blue and red waveband attributed to the selective absorption of blue and red light by chlorophyll in the leaves within canopy, and the spatial variation of spectral irradiance within canopy was affected by canopy architecture which was determined by spatial distribution of leaves (Panferov et al., 2001)

The decrease of transmittance in B, R and PAR were all caused by selective absorption of chlorophyll in leaves (Walter-Shea and Norman, 1991). It made the variation of transmittance in PAR, B and R having good linear relations. High scattering and no significant absorbance of FR in leaves made the variation of FR quite different from those of PAR, R and B and kept more information of the spatial distribution of leaves. So it was reasonable to take the spatial variation of R/FR as the indicator of spatial heterogeneity of canopy structure. The spatial distribution of R/FR in two canopies inferred that most places downward from middle in K326P were shaded by upper canopy apparatus. On the contrary, Y87P was much sparser in most part of the canopy except for the central part. It agreed to the fact that K326P had higher LAI than Y87P.

Quadric relationship was found between transmittances of FR with PAR, B and R in both Y87P and K326P, and it fit better for K32P which had high LAI. It might reveal some kind of relationship between quality and quantity of light within canopies. More theoretical and experimental investigations are needed for characterizing these relations.

In spite of the great achievements in the understanding of plant's photo-sensory system, most of the knowledge about the role played by various photoreceptor systems in plant–plant interactions was derived from physiological studies in which plant responses to specific light signals rather than actual plant neighbors in the field (Ballaré, 1999; Aphalo et al., 1999). Long-term field observation of canopy architecture and its light micro-environment in morphogenetic stage is necessary to fill this gap. Furthermore, orientation of the photoreceptorshould be taken into account since it has been revealed that in natural environment plants use the increase in horizontal FR to sense and respond to neighbors with increased elongation growth (Ballaré et al., 1990; Grant, 1997).

However, due to the constraint of experimental conditions, field measurement of spectral irradiance within canopy was limited to the hemispherical irradiance, and most of them were carried out in tree canopies (Turnball and Yates, 1993; de Castro, 2000; Leuchner, 2007; Hertel et al., 2011). It is always difficult to measure directional spectral irradiance on the surface of plant organisms within a field-grown crop canopy which has a complicated structure. Computer simulation of radiation transfer within three-dimensional canopy can be a good supplement tool (Panferov et al., 2001; Chelle and Andrieu 2007; Lao et al. 2010). But it should be based on the field measurement for providing primary experimental data and validating the results of simulation. Based on this study, we could build the relationship between field measurements and the simulations by three-dimensional radiative transfer model.

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POTENTIAL OF AGRICULTURAL LAND MANAGEMENT ACTIVITIES FOR INCREASED SOIL CARBON SEQUESTRATION IN AFRICA- A REVIEW

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Abstract.This paper reviewed the potential for agricultural land management activities for increased soil carbon sequestration with particular reference to Africa. The sections of this review covered different land management activities (including agroforestry, conservation tillage, fallow management, mulching/cover crops, water management, rotation, manure, fertilizer use, grazing management) which lead to carbon sequestration in different farming systems. This review has revealed that there is high potential to sequester additional carbon through selected land management practices. The performance of these practices depends on soil properties and climatic conditions, and the degree of soil degradation at the time of time of intervention. There is need to integrate these land management practices for carbon sequestration into larger sustainable development and livelihoods strategies and practices in order to enhance an holistic approach and reduce some of the constraints that may inhibit these positive effect of land management practices for carbon sequestration.

Keywords : *climate change, land management, soil carbon, conservation*

Introduction

Africa, the second largest continent, has a wide diversity of climates, ecosystems and soil conditions. Since the 1960s, the continent has been experiencing serious economic and environmental problems. The growth of its population, the highest in the world, has placed pressure on many ecosystems. In addition, there has been political and social turmoil in many countries. Farming systems in Africa have been evolving towards land-use intensification in response to population growth and the scarcity of land suitable for long-fallow shifting cultivation. In many agro(eco)systems the soil has been degraded, due mainly to deforestation, overgrazing, agricultural mismanagement, and over exploitation. These degraded systems can be managed to reduce carbon emissions and increase carbon sinks in vegetation and soil (*Table 1*), thus contributing to global climate change mitigation (WBBGU, 1998).

The Green House Gas (GHG) mitigation potential of Sustainable Land Management (SLM) in agricultural lands is very large (Liniger, et al 2011). SLM strategies and practices can prevent land degradation, restore degraded lands, and reduce the need for further conversion of natural forests and grasslands. Farmers can, reduce GHG emissions, increase carbon sequestration, and maintain above- and below-ground carbon stocks at relatively low cost, while also improving food production and livelihoods.

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Improved agricultural practices can reduce carbon emissions from soil erosion and disturbance, and capture carbon from the atmosphere to store long-term in soils. Practices like cover cropping, applying crop residues, mulch, manuring, reduced tillage, and rotational cropping with legumes increase organic matter in soil, while also increasing crop yields. With better agronomic practices, nutrient and water management, reduced tillage and crop residue management, African croplands could potentially reduce GHG emissions by 2.0–3.5 million tons of CO_2eq per hectare per year or a total of 52.3–91.5 million tons of CO_2eq equal to 5-9% of annual African fossil fuel emissions in 2005 (Liniger, et al 2011). As part of SLM, farmers grow trees in and around their farm fields, to harvest useful products such as fruit, livestock fodder and medicines. This benefits the climate as well as ecosystems. In humid zones of Africa, retaining shade and understory trees in cacao can provide vast carbon stores. For example, mature cacao agroforestry systems in Cameroon store 565 tons of CO_2eq per hectare. Even in semi-arid lands, agroforestry systems like intercropping or silvopasture, with 50 trees per hectare, can store 110 to 147 tons of CO_2eq per hectare in the soil alone (Liniger, et al 2011).

Table 1. *Average stock and density of soil organic carbon for the world, Africa and West Africa (in first 1m of soil)*

Soil organic carbon	World	Africa	West Africa
Soc stocks (Pg C)	1462 – 1548	170-180	25-27
SOC density (kg C m^{-2})	10.9-11.6	6.4-6.7	4.2-4.5

Source: Batjes, 1996

Sanchez (2000) reported the carbon uptake rates, time average carbon stocks and differences in carbon stocks from land transformation at margins of the humid tropics. Similarly specific agricultural management and land-use options are available for C sequestration in tropical and subtropical soils. These include conservation tillage (no-till/minimum-till) in combination with planting of cover crops, green manure and hedgerows; organic residue management mulch farming, particularly in dry areas; water management, including *in-situ* water conservation in the root-zone, irrigation, and drainage to avoid potential risk of salinization and water-logging and soil fertility management, including use of chemical fertilizers and organic wastes, rhizobium inoculation, liming and acidity management in order to take advantage of the CO_2-fertilization effect. Others are introduction of agroecologically and physiologically adapted crop/plant species, including agroforestry; adapting crop rotations and cropping/farming systems, with avoidance of bare fallow; controlling of grazing to sustainable levels and stabilizing slopes and terraces.

The following sections consider these land use practices in details, documenting the GHG mitigation potentials as reported in scientific journals and reports.

Nutrient management

Nutrient management aims at managing soil by combining different methods of soil fertility amendment together with soil and water conservation. It takes into account all farm resources and is based on 3 principles: (1) maximizing the use of organic sources of fertilizer; (2) minimizing the loss of nutrients; (3) judiciously using inorganic

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fertilizer according to needs and economic availability. In Sub-Saharan Africa, soil fertility depletion is reaching a critical level, especially under small-scale land use. These techniques can rehabilitate degraded soils and then maintain soil fertility by using available nutrient resources in an efficient and sustainable way. Given the high cost of inorganic fertilizer, however, micro-fertilization has been a cost-saving entry point. The nutrient management practices considered in this review were the use of chemical fertilizer and animal manure.

Chemical fertilizer

There were several studies on the use of fertilizer in Africa, and the impact on soil nutrient particularly soil carbon. Snapp et al, (1999) stated that recommendations for fertilizer use on cereals in Malawi and Zimbabwe are in the range of 40-90 kg N per ha -- approximately ten-fold the amount of current fertilizer use by smallholders. Fertilizer use rates in Africa are only 8 kg ha-1 with most of fertilizer applied to cash crops for export (Buerkert et al., 2000; Morris et al., 2007), while FAO, (2005) indicated that only fourteen percent of the total land area of South Africa receives sufficient application rates of 46 to 92 kg P2O5/ha for decreasing soil P- levels. Similarly, Minot (2009) stated that the overall, fertilizer application rates in Africa is 10-12 kg of nutrients per hectare.

The impact of fertilizer application cannot be widespread due to the fact that fertilizer is often not available for farmers in SSA and when available, it is not timely for farm operations and usually very expensive. For all the different treatments covered in this review, the mean impact of the fertilizer application was 626 kg C ha^{-1} yr^{-1}. Many of the studies focused on different levels of Nitrogen and the combination of fertilizer with locally available manure sources.

According to ICRISAT, (2009), micro-fertilization and microdosing involve the application of small, affordable quantities of fertilizer onto the seed at planting time, or a few weeks after emergence. The microdosing technique increases the efficiency of fertilizer use, and helps improve productivity. The method uses about one-tenth of the amount typically used on wheat, and one-twentieth of the amount used on corn in the US. The small dosage needed illustrates just how depleted of nutrients African crops are. The microdosing method has been introduced to Zimbabwe, Mozambique and South Africa, making fertilizer use a productive and economically viable option for the farmers. Abdoulaye and Sanders (2006) reported that many Nigerian farmers have been using micro-fertilization such that small quantities of inorganic fertilizer (compound fertilizer of NPK: nitrogen 15%, phosphorous 15%, potassium 15%) up to 25 kg/ha are put in the planting hole along with manure and the seed. Farmers repeatedly point out that the micro-fertilization increases the vigor of the seedlings and therefore there is less replanting required. Moreover, there is now substantial empirical evidence for the significance of the yield gains from micro-fertilization and their profitability from widespread trials over two years (2002 and 2003, an adverse and a good rainfall year) in Niger, Mali, and Burkina Faso (Tabo et al., 2005,).

Animal manure

Closely related to the issues of fertilizer experiment and trials was the use of animal manures. Most of the experiment conducted across the ecological zones shows that animal manure increased soil carbon tremendously. This might have been popular due to the prevailing farming system in many countries in Africa where livestock production is integrated with field crops.

Manure application to agricultural soils can reduce net GHG emissions in two ways. First, by the displacement of more typical anaerobic storage options with aerobic decomposition of organic material, CH_4 emissions can be reduced. Second, soil application can sequester soil C and displace N fertilizer use, also potentially reducing field N_2O emissions. Some of these benefits have already been recognized in efforts to divert organic waste from landfills. The impact of composting on emissions post-land-application is of further interest. Fronning et al. (2008) examined GHG fluxes following land application of solid beef manure and composted dairy manure over a three-year period. Net CH_4 flux was minimal (< 0.01 t CO_2e ha^{-1} yr^{-1}), whereas untreated manure application generated higher N_2O emissions than did compost (0.9 versus 0.7 t CO_2e ha^{-1} yr^{-1}). However, these land emission impacts were small when compared to soil C sequestration rates, which were 1.8 times greater for compost than for manure, suggesting that the organic matter stabilization during the compost process reduces post-application respiration losses. The net sequestration of C in the manure that is used for compost feedstock versus direct land application difference may also be affected by respiration losses during the composting process.

No/Minimum Tillage

There are varying degrees of carbon sequestered due to the levels of tillage reduction, it is a general trend that no tillage and reduced tillage increased carbon stocks in African soils; this particularly true for experiments conducted in the humid and forest regions. The main principle of conservation agriculture (CA) is minimal soil disturbance through reduced or no tillage. This favors soil life, and builds up of soil organic matter (less exposure to oxygen and thus less soil organic matter mineralization). Compared to conventional tillage, CA increases the organic matter content of soils, increasing their porosity and hence improving their ability to absorb and retain water. This has two positive effects: first, there is more water to support crop growth and the biological activity that is so important for productivity, and second, less water accumulates and thus doesn't flow across the surface, causing floods and erosion. Liniger et al (2011) noted that the potential for C Sequestration (tonnes/ha/year) in East Africa is 0.57 ± 0.14. Under 'minimum tillage and direct planting', land is prepared by slashing the existing vegetation and allowing regrowth up to 30 cm height. Herbicide is sprayed with a knapsack fitted with a low-volume nozzle. The residue is left on the soil surface without burning. After 7–10 days, direct planting is carried out in rows through the mulch. The mulch layer helps to increase and maintain water stored in the soil, reduces soil erosion, helps to improve soil fertility (after crop residues have decomposed in subsequent seasons) and it efficiently controls weeds by hindering their growth and preventing weeds from producing seeds.

Residues

These residues ranged from slaughter wastes to household wastes as well as crop residues. Composting is an important organic practice and a very important tool for adaptation to climate change. Composting involves the accumulation and proper mixing of animal manure, crop residues, weeds and organic household waste so that they can be broken down into compost, which is used as a soil conditioner. During the composting processes fungi and bacteria decompose the raw material so that nutrients are stabilized within the organic matter. It also kills or reduces weed seeds, pests and diseases. Composting increases soil fertility by holding and gradually releasing nutrients and building up organic matter levels in the soil. Composting also improves the water holding capacity of the soil and makes crops better able to survive droughts and floods.

Mulches

The practice of mulching is naturally associated with the cultivation of some crops as it helps reduce loss of soil moisture; however the deliberate use of mulches and dry materials is usually considered a labor-intensive exercise. Most of the food crops that share the characteristics of cover crops were used for dual-purpose in Africa. Traditionally, these crops feature in the farming system and cropping pattern of most farmers. Some of the species used for cover crops as reported in the studies reviewed were Calliandra, Tithonia, Tephrosia, *Chamacrista rotundifolia, Pueraria phaseoloides, Aeschynomene histrix, Centrosema pascuorum, Crotolaria ochroleuca, Mucuna deeringiana, M. pruriens, M. eracruz, Stylosanthes capitata* and *C.brasilianum*.

Agroforestry

In Africa, of all agricultural land management activities suggested for GHG mitigation, agroforestry practices has been the most widely applied and studied, with the majority of research. Agroforestry has the potential to sequester significant amounts of carbon for 2 reasons. First, the area currently in crops and pastoral systems is large. Second, although the density of carbon storage is low in comparison with forests, the woody biomass of agroforestry systems could provide a source of local fuel. This fuel would reduce pressure on the remaining forests in the area and, at the same time, provide a substitute for fossil fuel. These effects are important because the most effective way to use land for stabilization of atmospheric carbon is not through reforestation but through the substitution of wood fuel for fossil fuel. Takimoto et al 2008 reported that *Faidherbia albida* parklands stored more C than improved agroforestry systems (live fence and fodder bank) or abandoned land. Similarly carbon sequestration potential of agroforestry systems varies greatly from under 100Mt to over 2000Mt of carbon dioxide equivalent per year particularly the use of *Faidherbia albida*, in Malawi and Niger.

Forests would continue to hold the carbon accumulated, but they would accumulate no additional carbon after that time. In contrast to the temporary effect of reforestation, the sustainable use of wood fuels provides a solution that could last indefinite. The biophysical and spatial potential for carbon sequestration in Africa is high; the socio-political conditions related to land usage, ownership and permited land management practices are not, constituting a serious dilemma for carbon storage on the continent—and a similar dilemma for biofuel projects. The primary problem within this dilemma is land tenure, and no clear way ahead exists, despite the well-intentioned recommendations in the afforestation and reforestation carbon sequestration literature for the development and implementation of Western notions of property rights, along with improved governance, local participation, and sustainable development

(KauppiandSedjo, 2001; Brown, 2002; Smith, 2002). Land tenure will be the most fundamental issue regarding how trees are to interact with African landscapes.

Intercropping and Alley farming

The practice of growing crops or pasture between rows of trees (alley farming) sequestered soil carbon. Beedy et al (2010) indicates that the use of the gliricidia intercrop had proportionately greater positive effects on rebuilding of soil fertility capital than on carbon sequestration, though both results are modest compared to impacts on maize yields over the course of 14 years in a semi-arid tropical maize cropping system in southern Africa. Particulate organic matter pools, which act as a reservoir of crop nutrients, were enhanced by the gliricidia/maize intercrop.

Tree-crop farming

Tree crops such as cocoa plantation in Ghana and Cameroon, Coffee plantation in Burkina Faso, indigenous fruit trees in South Africa, oil palm plantation in Cote d'Ivoire, exotic tree species in Ethiopia, rubber plantation in Nigeria and Ghana, Cashew and Teak plantation in Nigeria were covered. Ofori-frimpong et al (2010) noted that Cocoa planted at low plant density and under shade stores more carbon per unit area of soil than an equivalent area of cocoa planted at high density without shade. In addition to C sequestration in biomass and soil, tropical plantations are needed for timber, and more importantly, as fuel wood for cooking. Thus, the area under tropical plantations has increased drastically since the 1960s, from 7 Mha in 1965 to 21 Mha in 1980, 43 Mha in 1990 (Evans, 1986) and 187 Mha in 2000 (FAO, 2003). In Western Nigeria, Ekanade et al. (1991) reported that the SOC pool under forest was 29 g/kg and that under cocoa was 19 g/kg. Similar observations were made by Adejuwon and Ekanade (1988) in Oyo state, Nigeria. Also in southern Nigeria, Ogunkunle and Eghaghara (1992) observed that the SOC concentration under 10-year old cocoa plantation was 25 g/kg compared with 35 g/kg under forest. In Nigeria, Aweto (1987) reported that the SOC concentration was 14 g/kg under primary forest and 12 g/kg under a 18-year old rubber plantation. The SOC concentration under rubber increased over time. In Kade, Ghana, Duah- Yentumi et al. (1998) reported that the SOC concentration of a soil under 40-year old rubber plantation was lower than that under virgin forest or 20-year old cocoa. Both rubber and cocoa received no fertilizer or manure.

Land-use changes

The impact of land-use changes on carbon sequestration has also been documented in the literature. Liniger et al (2011) reported that afforestation has high potential for C sequestration at a rate comparable to the use of conservation agriculture of no and minimum tillage. In this review, land-use changes focused on afforestation practices, grazing and cropping intensity. Lal (2004) noted that afforestation, the establishment of tree plantations has a large potential for SOC sequestration in the tropics. SOC accumulation under 18-year plantation of *Acacia senegal* in northern Senegal at the rate of 0.03%/yr under the tree canopy and 0.02%/yr in the open ground, corresponding to SOC sequestration rates of 420 and 280 kg C/ha/yr for a soil bulk density of 1.4 Mg/m3.

They also estimated, at a very general level, a potential sequestration capacity of 45.7 Tg C per year through cessation of overgrazing, although research also has found that some grasslands sequester more carbon in response to heavier grazing intensities. Reeder and Schuman (2002) reported higher soil C levels in grazed – compared to un-grazed – pastures, and noted that when animals were excluded, carbon tended to be immobilized in above-ground litter and annuals that lacked deep roots. After reviewing 34 studies of grazed and ungrazed sites (livestock exclusion) around the world, Quattara et al (2006) reported that the average carbon stock in continuous cultivation system was 10.05 Mg $ha-^1$ in Burkina Faso.

Soil Management

The arrangement of crops in time and space is a common practice among farmers in Africa that allows for crop rotation and mixed cropping practices as dictated by their taste preferences and culture.

Crop rotation is the practice of growing a series of dissimilar types of crops in the same area in sequential seasons for various benefits such as to avoid the build- up of pathogens and pests that often occurs when one species is continuously cropped (Pearson et al, 1995). Crop rotation also seeks to balance the fertility demands of various crops to avoid excessive depletion of soil nutrients. A traditional element of crop rotation is the replenishment of nitrogen through the use of green manure in sequence with cereals and other crops. It is one component of polyculture. Crop rotation can also improve soil structure and fertility by alternating deep-rooted and shallow-rooted plants.The sequence is usually cereal, cover crop/leguminous crop, tuber crops and cereals such maize, cowpea, cassava and maize. Pearson et al (1995) indicated that crop rotation pattern in West- African Sahel usually include Millet/sorghum, maize, groundnuts, cowpea, sesame, cassava, yams, tree legumes; while in Ethiopia the sequence follows Maize/barley, sorghum, millet, and tef.

Natural and Improved fallow

Fallow periods during which there is no crop on the land, can be reduced or managed to increase soil C stocks, especially if fallow coincides with conditions that could permit some vegetative growth (primary productivity). Depending on the region and cropping system, both the elimination of summer fallow and the use of winter cover crops have significant GHG mitigation potential. Ganry et al (2001) have shown that to ensure soil fertility management, a range of practices can be envisaged, such as spontaneous long fallow and cultivated parkland, improved herbaceous and bush fallows, cover-crop legume system, alley cropping system, compost and manure. Kotto-same (1997) reported that where bush fallow was well-established two years after land abandonment, dominated by *Chromolaena odorata.* The tree fallows consisted of dense understorey and nearly closed canopy of pioneer tree species. The most common tree species were *Albizia zygia, Ficus exasperata* and *Myrianthus arboreus* with understoreys of *Aframomum longiscarpum* and *Haumania danckelmaniana. C. odorata* in the tree fallows.

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Water management

Water management practices covered in this review were rain water harvesting, slope/barriers and terracing.

Rainwater harvesting

This practice is particularly important to framers in the semi-arid and arid region where there were few days of rains but rain –fed agriculture is practiced. Rainwater Harvesting (RWH) refers to all technologies where rainwater is collected to make it available for agricultural production or domestic purposes. RWH aims to minimize effects of seasonal variations in water availability due to droughts and dry periods and to enhance the reliability of agricultural production. A RWH system usually consists of three components: (1) a catchment / collection area which produces runoff because the surface is impermeable or infiltration is low; (2) a conveyance system through which the runoff is directed e.g. by bunds, ditches, channels (though not always necessary); (3) a storage system (target area) where water is accumulated or held for use - in the soil, in pits, ponds, tanks or dams. When water is stored in the soil - and used for plant production there - RWH often needs additional measures to increase infiltration in this zone, and to reduce evaporation loss, for example by mulching. Furthermore soil fertility needs to be improved by composting / manuring, or micro-dosing with inorganic fertilizers. Commonly used RWH techniques can be divided into micro-catchments collecting water within the field and macro-catchments collecting water from a larger catchment further away with potential for C sequestration (tonnes/ha/year) 0.26-0.46.

A Smallholder Irrigation Management (SIM) unit is typically a plot covering an area less than 0.5 ha. SIM schemes may be managed either by an individual land user or by groups / communities. The guiding principle of sustainable SIM is ‘more crop per drop’, in other words efficiency of water use. This can be achieved through more efficient (1) water collection and abstraction; (2) water storage; (3) distribution and; (4) water application in the field. Two main categories of SIM can be distinguished, traditional surface irrigation systems and recent micro-irrigation systems including drip irrigation. Micro-irrigation systems are commonly used for, and are very important in, the production of vegetables, fruits and flowers. Potential for C sequestration (tonnes/ha/year) is 0.15 (Liniger et al 2011).

Cross slope barriers and terracing

The practices of cross slope barriers and terracing sequester additional soil carbon . This is very critical in regions of high undulating surfaces and highlands. Cross-slope barriers are measures on sloping lands in the form of earth or soil bunds, stone lines, and / or vegetative strips for reducing runoff velocity and soil loss, thereby contributing to soil, water and nutrient conservation. This is achieved by reducing steepness and / or length of slope. Terraces are not usually constructed per se, but rather develop gradually behind earth bunds, vegetative strips (usually grass) or stone barriers, due to soil movement from the upper to the lower part of the terrace. Erosion between the barriers helps to achieve the leveling of the terrace bed. Potential for C Sequestration (tonnes/ha/year) 0.5-1.0 (Liniger et al 2011)

Biochar

These covered the use of Biochar from different plant sources in Kenya, Ghana and Cameroon to improve soil. Biochar is charcoal made from organic material burned during conditions with low oxygen level (pyrolysis). Previous trials have showed that biochar can or will in small scale farming: Add nutrients and improve uptake of applied fertilizers, Increase water holding capacity of the soil, Increase carbon concentration in the soil, Remain resident in the soil over a long period of time, Affect decomposition rates of organic compounds. Alvum-Toll et all 2011 noted that Due to the high stability the constituent carbon, biochar added to soil may act as a carbon sink and might be part of a solution to the global warming problem by reducing the concentrations of carbon dioxide (CO2) in the atmosphere. Besides acting as a carbon sequester, biochar has several beneficial effects on soil properties, such as increased water holding capacity, enhanced CEC, BS, as well as add nutrients and improve the plant uptake of nutrients (Lehmann & Joseph, 2009). However, research on biochar's effect on soil CEC is not coherent. Novak et al. (2009) did not find a significant increase in CEC after biochar addition. Biochar affects soil fertility in many ways; it can add nutrients by itself or make them more available for plant uptake by enhance the decomposition of organic material- or, possibly, reduce decomposition rates of other organic material thereby increasing soil C concentration in the long run. Moreover, the large surface area results in increased CEC, which may prevent nutrient leaching and thus eutrophication (Lehmann & Joseph, 2009). Lehmann et al. (2003) found a significant decrease in leaching of applied fertilizers after charcoal addition.

Apart from the beneficial effects of drawing CO2 from the atmosphere, bio-char applications to soil are also able to reduce the emissions of other greenhouse gases. Nitrous oxide emissions were reduced by up to 50% when bio-char was applied to soybean and by 80% in grass stands. These low emissions may be explained by better aeration (less frequent occurrence of anaerobic conditions) and possibly by greater stabilization of C. The lower nitrous oxide evolution may also be an effect of slower N cycling (possibly due to a higher C/N ratio).

Soil Amendment

In this review the use of soil amendments such as of Cocoa husk, rice bran/mill spent grain, ash and saw dust were covered. The literature suggest the great potential for the addition of a suite of amendments containing organic and inorganic energy related byproducts to improve degraded land increase sequestration of C, and utilize energy byproducts. However, the optimal strategy for rapid enhancement of C sequestration is not currently known. In areas limited by specific elements, these products can supply micronutrients Micronutrient amendment from coal fly ash with sewage sludge was applied to an acid boron deficient loamy soil in China with resulting higher yield for cucumber and corn than either the control treatment or a boron-containing fertilizer. Ash additions to an easily clodded clay loam soil increased bulk density and desirable aggregates and decreased the modulus of rupture. Fly ash has been shown to increase porosity, water holding capacity, pH, conductivity, sulfate, carbonate, bicarbonate, chloride and metals, although the effect is reduced in high clay soils. While surface soil P and Mn were also important, soil depth appears to be a positive factor in establishing white pine on mine spoil

Conclusion

This review has revealed that there is high potential to sequester additional carbon through selected land management practices. Prominent practices include use of animal manure, residues, improved fallow, tree inclusion on farm field and use of slope and cross barriers and natural fallow. The performance of these practices depends on soil properties and climatic conditions, and the degree of soil degradation at the time of time of intervention. There is need to integrate these land management practices for carbon sequestration into larger sustainable development and livelihoods strategies and practices in order to enhance an holistic approach and reduce some of the constraints that may inhibit these positive effect of land management practices for carbon sequestration.

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MODELLING THE EFFECTS OF GLOBAL TEMPERATURE INCREASE ON THE GROWTH OF SALT MARSH PLANTS

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Abstract. Gradual increases in temperature and atmospheric CO_2 concentrations have resulted from the increased human use of fossil fuels since the beginning of industrial activity. In coastal wetland ecosystems, salt marshes constitute important habitats because they play important ecological roles, acting as carbon sinks by capturing atmospheric CO_2 and storing it in living plant tissue. Ecological models are important tools for understanding the results of anthropogenic impacts on a global scale. Global warming poses threats to salt marshes through different effects, e.g., increases in sea level. The objectives of this study were i) to assess how temperature increases will influence the growth of salt marsh plants, ii) to infer the carbon budget of salt marshes under temperature increase scenarios and iii) to predict how salt marsh plants will keep pace with increases in sea level. These goals were achieved by developing growth models of three different plants (*Spartina maritima*, *Scirpus maritimus* and *Zostera noltei*) found in the Mondego estuary. Models were developed for C_3 and C_4 plant species. The results suggest that a temperature increase enhances the aboveground biomass of salt marsh plants. According to the predictions of the models, the sedimentation rate of *S. maritima* and *Z. noltei* can keep pace with increases in sea level, but this is apparently not the case for *S. maritimus*. If *S. maritimus* disappears from the Mondego estuary, the carbon sequestration ability of the system should decrease due to the loss of active plant tissue. This conclusion is based on the fact that *S. maritimus* accumulated more than 80% of the total carbon sequestered in the tissues by the three studied species.

Keywords: *plant growth model, sea level increase, sedimentation, salt marsh, estuaries*

Introduction

Because of increasing human fossil fuel use since the beginning of intensive industrial activity (Houghton, 1999), the world has experienced an increase in atmospheric CO_2. It is widely believed that anthropogenic additions of CO_2 to the atmosphere are contributing to increase surface temperatures worldwide, a phenomenon known as the ‘‘greenhouse effect’’ (Bluemle *et al.,* 1999). IPCC (2007) reports show that the global temperature increased approximately 0.3 °C per decade from 1979 to 2005. Coastal wetlands such as salt marshes are excellent carbon sinks, as they withdraw CO_2 from the atmosphere and store it in living plant tissue (Williams, 1999). Salt marshes reduce the rates of greenhouse gas emissions (Magenheimer *et al.*, 1996) and have a carbon sequestration capacity per unit area approximately one order of magnitude higher than other wetland systems (Bridgham *et al.*, 2006), with the potential to sequester carbon continuously over thousands of years (Brevik et al, 2004). In

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addition to their carbon storage capacity, salt marshes are key areas for estuarine systems because of their role in primary production and nutrient regeneration (Caçador *et al.*, 2009). They represent one of the most productive ecosystems on the planet (Lefeuvre *et al.*, 2003). However, global warming can threaten salt marsh areas through, e.g., increases in sea level. Salt marsh ecosystems play a vital role in the dissipation of wave energy, accretion of sediment and filtration of nutrients and as habitats supporting commercially important fisheries (Mudd et al. 2009). These ecosystems are vulnerable to changes in sea level (Cundy & Croudace, 1996, Rybczyk & Cahoon, 2002), and sea level increases have become a severe threat to coastal zones and estuaries at low elevations (Vandenbruwaene et al. 2011). It is recognised that many marshes are able to keep pace with increases in sea level (Friedrichs and Perry, 2001); the response of coastal marshes to relative increases in sea level depends upon their ability to maintain their relative elevation through sedimentation (Reed, 1990). IPCC (2007) reports show that the most pessimistic prediction for sea level change is an increase of approximately 0.97 cm per year, which will endanger certain areas around the world that are influenced by the tides, including, in particular, the Mondego salt marsh. Ecological models have been used to clarify the effects of anthropogenic impacts on a global scale by integrating processes related to the biota of the ecosystem in its conceptual structure (Fragoso Jr., *et al.* 2009). Accordingly, an ecological model focuses on the objects of interest for a considered and well-defined problem. There can be many different ecological models of the same ecosystem, as the model version is selected according to the goals of modelling (Jørgensen & Fath, 2011). Because *Spartina maritima, Scirpus maritimus* and *Zostera noltei* together occupy approximately 50% of the salt marsh vegetation of the Mondego estuary (Neto, unpublished data), these species were selected as the focus of the present study. Our goal was to examine the influence of temperature increase on the carbon accumulation of the salt marsh area of the Mondego estuary. Specifically, our objectives were to investigate how temperature increase influences the growth of salt marsh plants (*Spartina maritima*, *Scirpus maritimus* and *Zostera noltei*) and their carbon storage capacity. Furthermore, we attempted to estimate whether the sedimentation rate of the three salt marsh plants can keep pace with the increase in sea level.

Materials and methods

Study site

The Mondego estuary (Fig. 1) is located on the Portuguese Atlantic coast (40°08 N, 8°50 W) (Marques and Nogueira, 1991). The estuary is approximately 8.6 km^2 in area, and its upstream limit extends up to 21 km from the mouth of the estuary (Teixeira *et al.*, 2008). The final portion of the estuary (approximately 7 km) is divided into two arms (north and south) by Murraceira Island (Marques *et al.*, 2003). The sample sites for this study (Fig. 1) were located in the south arm of the estuary. The south arm is shallower than the north arm (2–4 m during high tide) and is characterised by large areas of intertidal mudflats (almost 75% of the area) (Neto *et al.*, 2008). The sediments of the south arm contain a high percentage of silt and clay, and the area is considered to be a rich estuarine habitat in terms of productivity and biodiversity (Marques *et al.*, 1993).

Field data sampling and laboratory procedures

Field data sampling was conducted seven times, once in each season, between 2010 (spring, summer and autumn) and 2011 (winter, spring, summer and autumn). For the aboveground biomass, three replicates (size= 0.3 x 0.3 m squares) of each salt marsh plant species were randomly selected in each area and clipped (Caçador *et al.,* 2004). In the laboratory, all samples were washed with ultrapure water (18.2 MΩ cm). The aboveground tissues were then dried at 60 °C until a constant weight was achieved, and the dried tissues were pulverised with a grinding ball mill (Glen Creston MM2000) (Gross *et al*., 1991). Total carbon content was determined using a CHNS/O analyser (Fisons Instruments Model EA 1108). The carbon pool (grams) for each species and for each season analysed was calculated by multiplying the results from the CHNS/O analyser, expressed as a percentage (%), by the biomass according to equation 1.

(Eq.1)

Sedimentation rates were measured using lengths of wood with millimetre marks, which were buried up to the zero mark level in the area occupied by each species. This procedure was performed in February 2011 in the sampling areas corresponding to the three salt marsh plants. One year later (February 2012), the wooden markers were checked to measure the level corresponding to sediment accumulation in each of the three sampling areas.

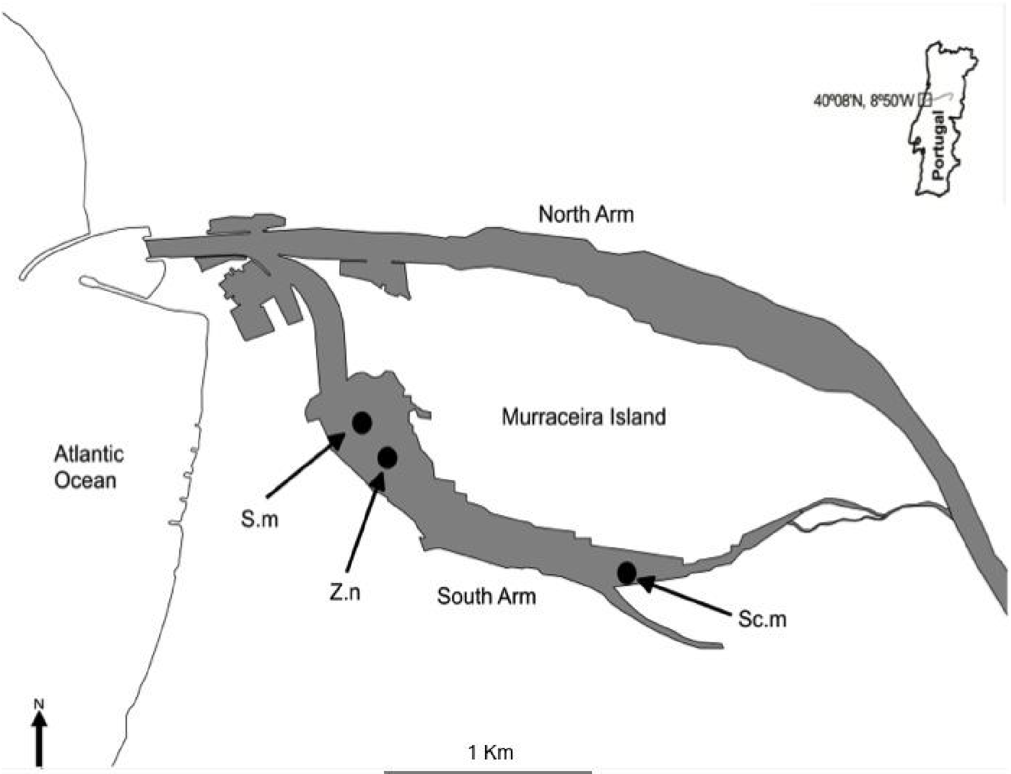

Figure 1. *Sampling locations in the south arm of the Mondego estuary. S.m (Spartina maritima), Z.n (Zostera noltei) and Sc.m (Scirpus maritimus).*

Model

To simulate the growth of a primary producer, a model must include the principal physiological processes, such as photosynthesis, respiration and exudation (Duarte, 2011). The present study follows the work of Simas *et al.* (2001), where fluxes are expressed as gC dry weight m^{-2}; the key equation is

$$\frac{dB}{dt} = \mu - R - L_m - E \qquad \text{(Eq.2)}$$

where B is plant biomass, μ is gross productivity, R is respiration, L_m is leaf mortality and E is exudation. Gross production (μ) is represented by equation 3:

$$\mu = P_{max} \times f(T) \times f(I) \times f(N) \qquad \text{(Eq.3)}$$

where P_{max} is the plant's maximum production, $f(T)$ is the air temperature function, $f(I)$ is the light function and $f(N)$ is the nutrient function.

P_{max} values for each studied plant species are shown in table I. The air temperature function $f(T)$ is described in equation 4 (Bach, 1993):

$$f(T) = \gamma_1^{(T-T_{opt})} \qquad \text{(Eq.4)}$$

where γ_1 is the temperature coefficient for growth, T is the daily air temperature and T_{opt} is the optimum temperature for growth.

The daily temperature variation (T) throughout the year was obtained from a modified sine equation (Anastácio *et al.*, 1995) (Equation 5):

$$T = \frac{(T_{max}+T_{min})}{2} + \frac{(T_{max}-T_{min})}{2} \times SIN\left(\left(\frac{time}{365} - 0.23\right) \times 2 \times \pi\right) \qquad \text{(Eq.5)}$$

where T_{max} is the maximum air temperature (39.2°C) and T_{min} the minimum air temperature (-2.4°C) during the study period. The maximum and minimum temperature values were obtained from the Portuguese Meteorology Institute (IPMA).

The light function was defined as a Michaelis-Menten equation:

$$f(I) = \frac{I}{I_k + I} \qquad \text{(Eq.6)}$$

where I is the light intensity and I_k is the half-saturation constant. Daily values of light intensity at 40° latitude were obtained from the Portuguese Environment Agency (APA), and half-saturation constants for the three plant species were obtained from the literature (Morris, 1982).

The regulation of growth by nitrogen ($f(N)$) is expressed in Equation 7:

$$f(N) = \frac{N}{(N+\eta)} \qquad \text{(Eq.7)}$$

where N is the concentration (%) of nitrogen in the leaves and η is the half-saturation constant (%) for nitrogen.

Leaf mortality (L_m) is dependent on temperature (equation 8):

$$L_m = L_{max}\left(\gamma_2^{T-T_{opt}}\right) H \qquad \text{(Eq.8)}$$

$L_m = L_{max}\left(\gamma_2^{T-T_{opt}}\right)$ where L_{max} is the maximum leaf mortality rate, γ_2 is the temperature coefficient for leaf mortality and H is the loss of aboveground biomass due to wave motion, calculated using equation 9:

$$H = 1 + W_1 e^{-W_2\, h} \qquad \text{(Eq.9)}$$

where W_1 and W_2 are constants and h is the tidal height.

Exudation (E) (equation 10) is the release of dissolved organic carbon and can be calculated as a fraction of gross production (Baretta-Bekker *et al.* 1997):

$$E = \mu[\alpha + (1-\alpha)(1 - f(N))] \qquad \text{(Eq.10)}$$

where α is the fraction of gross production that is excreted under nutrient stress conditions (Simas *et al.* 2001).

The respiration rate (R) is dependent on the temperature and on the assimilated biomass (Baretta-Bekker *et al.*, 1997) and can be calculated as in equation 11:

$$R = r^{bas} Q_{10}^{T/(T_{opt}-1)} + q^{res}\,(\mu - E) \qquad \text{(Eq.11)}$$

where r^{bas} is the basal respiration, Q_{10} is the Q_{10} value and q^{res} is the fraction of production lost by respiration.

Model calibration and scenario simulations

The model was built using STELLA software (version 9.0) (Isee systems). As in Simas *et al.* 2001, the model was calibrated independently for C_3 (*Scirpus maritimus*) and C_4 (*Spartina maritima*, *Zostera noltei*) species. The model was run for a two-year period corresponding to the sampling period, and the predictions were compared with the observations. The parameter values used in the model are shown in table I.

To test the influence of temperature on the biomass production of the three plant species in the model, the temperature was increased by 1.5 °C.

Sensitivity analyses

A sensitivity analysis was performed on selected key parameters to assess the variation in the biomass of the three salt marsh plants when the parameters were changed. The parameters were changed in ±10% steps (Jørgensen and Fath, 2011).

Sea level increase

To study the sedimentation rate associated with the area occupied by each studied salt marsh species relative to sea level increase, the bathymetry of the area occupied by each plant was considered along with the average height of each plant species, namely, 1.20 m for *S. maritimus*, 0.7 m for *S. maritima* and 0.3 m for *Z. noltei*. A sea level increase of 0.97 cm per year was simulated and compared with the sedimentation rate of each species. Bathymetry data for the Mondego estuary were extracted from Kenov *et al*. (2012), and the average maximum and minimum tidal heights were obtained from the Portuguese Hydrographic Institute (IH). A constant sedimentation rate (2.7 cm y^{-1} for *Z. noltei*, 1 cm y^{-1} for *S. maritima* and 0.2 cm y^{-1} for *S. maritimus*) and constant rate of sea level increase (0.97 cm y^{-1}) were assumed.

Statistical analysis

To compare the biomass results between the model simulation using the actual temperature and the simulation with the 1.5 °C increase in temperature, a one-way ANOSIM test using Primer software, version 6 (Clarke, 1993) was performed. The model predictions and observations were compared using a regression analysis.

Table 1. *Parameters values used in the model. A major part of the values were gathered from the works of Simas et al. 2001 and 2007.*

Code	Description	Units	*Scirpus maritimus*	*Spartina maritima*	*Zostera noltei*	References
P_{max}	Maximum production rate	gC dw m^{-2} d^{-1}	1.23	2.4	0.27	Curtis *et al.*, 1989a; Curtis *et al.*, 1989b; Sousa *et al.*, 2010; Bach, 1993
γ_1	Temperature coefficient for growth	-	1.1	1.08	1.08	Bach 1993
T_{opt}	Optimum temperature for growth	°C	21	25	25	Giurgevich and Dunn 1981
I_k	Light half saturation constant	W m^{-2}	177	250	250	Morris 1982
η	Nitrogen half saturation constant	%	0.36	0.36	0.36	Morris 1982
L_{max}	Maximum leaf mortality rate	gC dw d^{-1}	0.812	0.5774	0.0641	*
γ_2	Temperature coefficient for leaf mortality	-	1.07	1.07	1.07	Bach 1993
W_1	Coefficient for H (Eq. 8)	-	30	30	30	Bach 1993
W_2	Coefficient for H (Eq. 8)	m^{-1}	1.5	1.5	1.5	Bach 1993
α	Exudation under nutrient stress	-	0.2	0.2	0.2	Bach 1993
r^{bas}	Basal respiration	d^{-1}	8.4×10^{-4}	5.52×10^{-4}	5.52×10^{-4}	Simas et al. 2001
Q_{10}	Q_{10} value	-	2	2	2	Baretta-Bekker et al. 1997
q^{res}	Production respired fraction	-	0.1	0.1	0.1	Baretta-Bekker et al. 1997

- Values estimated at calibration.

Results and discussion

Model predictions and observed biomass

The model results followed the same pattern as the observed values (Fig. 2), with plant aboveground biomass values usually higher in the warm seasons than in the cold seasons, as also shown by several other studies (Caçador *et al.*, 2004; Couto *et al.,* 2013; Duarte *et al.* 2012; Lillebø *et al.,* 2003). Because the observed values in the spring of 2010 for *S. maritimus* and *Z. noltei* were unusually high, they were excluded from the linear regression calculations. The regression (Fig. 2) demonstrated that the observed and model results showed similar variation, although the data for *S. maritima* showed the best fit, followed by *S. maritimus* and *Z. noltei*. The sensitivity analysis (table II) showed that the parameters with the greatest influence on the biomass values of the three plant species were the maximum temperature (T_{max}) and the maximum leaf mortality rate (L_{max}). A change of ±10% in these parameters could increase or decrease the average biomass by more than 50%.

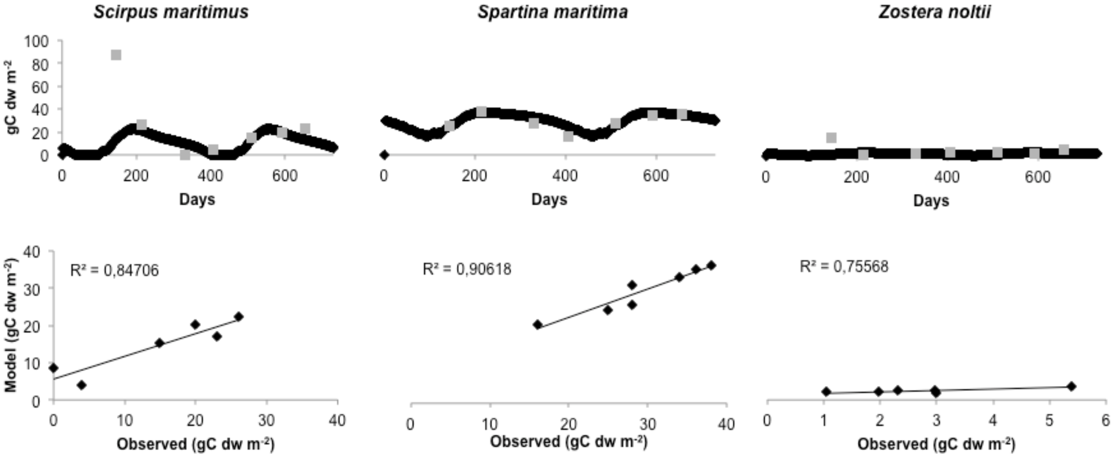

***Figure 2.** Observed (grey squares) and model (black line) values during 730 days and linear regression comparing both results for each species.*

Temperature increase

IPCC reports (IPCC, 2007) state that the global temperature has increased approximately 0.3 °C every 10 years since 1979. Fig. 3 shows the results of a comparison between the model results with the actual temperature and the model results assuming an increase of 1.5 °C. The results indicate that in such a scenario (a temperature increase of 1.5 °C), *S. maritimus* and *Z. noltei* would show an increase of approximately 30% in maximum biomass compared with the simulation using the actual temperature, whereas *S. maritima* would show an increase of 10% in maximum biomass.

The increase in temperature apparently has a positive effect in the model, as the increased plant biomass promoted by the increased temperature will absorb more carbon from the atmosphere. However, temperature increase will certainly affect the system in other ways. For example, it will cause sea level to increase due to oceanic thermal expansion, and also due to the melting of Arctic and Antarctic ice (Titus *et al.*, 1991). This process could be detrimental to salt marshes because the sea level increase would place increased stress on certain marshes; these marshes might ultimately be submerged (Silliman *et al.*, 2009).

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***Table 2.**. Sensitivity analyses of the average biomass of the total study period in relation to key parameters. The results are in percentage of the standard model. Sc.m: S. maritimus; S.m: S. maritima; Z.n: Z. noltei.*

	Sc.m		**S.m**		**Z.n**	
Parameters	10%	-10%	10%	-10%	10%	-10%
I_k	-40	52	-37	40	-49	76
T_{max}	322	-83	43	-30	79	-44
T_{min}	0,2	-0,1	-4	1	-1	0
N	41	-41	23	-30	44	-40
η	-38	46	-27	27	-37	49
r^{bas}	-0,3	-0,3	-4	-4	-3	2
Q_{10}	-0,4	-0,3	-4	-4	-3	2
q^{res}	-14	14	-10	6	-17	16
L_{max}	-72	187	-70	80	-76	145

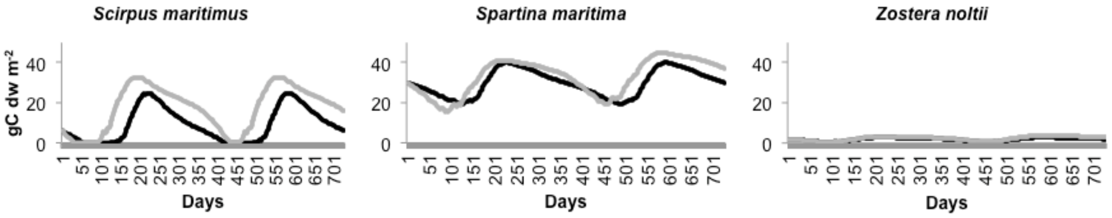

***Figure 3.** Model results for the actual temperature (black line) and for the 1.5 °C increase in the temperature (grey line) during 730 days.*

Sea level increase and sedimentation

Kenov *et al.* (2012) have shown that the bathymetry data of the sand banks for the three studied species in the Mondego estuary correspond to an elevation of approximately 2 m above sea level. The average heights of the studied species are 1.20 m for *S. maritimus*, 0.7 m for *S. maritima* and 0.3 m for *Z. noltei*. The sum of the heights of the banks and the average height of each species is approximately 3.2 m for *S. maritimus*, 2.7 m for *S. maritima* and 2.3 m for *Z. noltei*. In 2011, the average low tide was 0.9 m, and the average high tide was 3 m. *Z. noltei* had the highest sedimentation rate for a one-year period (2.7 cm y^{-1} ± 0.2), followed by *S. maritima* with 1 cm y^{-1} ± 0.1 and *S. maritimus* with the lowest sedimentation rate (0.2 cm y^{-1} ± 0.04). Along western European coasts, the sea level has been increasing at least since the Roman Period, and this increase has accelerated during the Modern Period (Pye, 1995). Salt marshes can keep pace with the sea level increase, but this ability depends on the sedimentation rate (Reed *et al.,* 1990; Pont *et al.*, 2002). In this case, *S. maritimus* could be at risk in the Mondego estuary. Fig. 4 shows that in approximately 37 years, if the sedimentation rates and rates of sea level increase remain the same (which is unlikely), the *Z. noltei* area would not be immersed during a high tide. As a result, this species would, most likely, disappear from this area. The *S. maritima*

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sedimentation rate (1 cm y^{-1}) and sea level (0.9 cm y^{-1}) are increasing to a similar extent, and the *S. maritima* area should not be greatly influenced by the increasing sea level during the next 50 years. Unlike *Z. noltei*, *S. maritimus* could be completely covered during every high tide in approximately 40 years. Of the three study species, *S. maritimus* should show the greatest effects of the sea level increase because its sedimentation rate (0.2 cm y^{-1}) is lower than the expected rate of increase of sea level. Before the *S. maritimus* area becomes completely submerged, this species will, most likely, be influenced by the salinity increase that accompanies an increase in sea level. Lillebø *et al.* (2003), working with *S. maritimus* in the same area, showed that salinity affects the dynamics of *S. maritimus*. In fact, the findings of previous studies have indicated that lower biomass values are associated with high salinity, showing that the survival of this species could be threatened by high salinity. Couto *et al.* (2013) showed that *S. maritimus* accumulated approximately 21 tons of carbon in the tissues in 21 months, compared with 1.9 tons of carbon accumulated in the tissues by *S. maritima* and 0.9 ton by *Z. noltei*. If the species that accumulates the highest amount of carbon of the three species disappears, the carbon sequestration ability of the Mondego estuary may decrease. Silliman *et al.*, 2009 suggest that as much as 90% of tidal marshland worldwide could be in jeopardy by 2100 because of the increase in sea level.

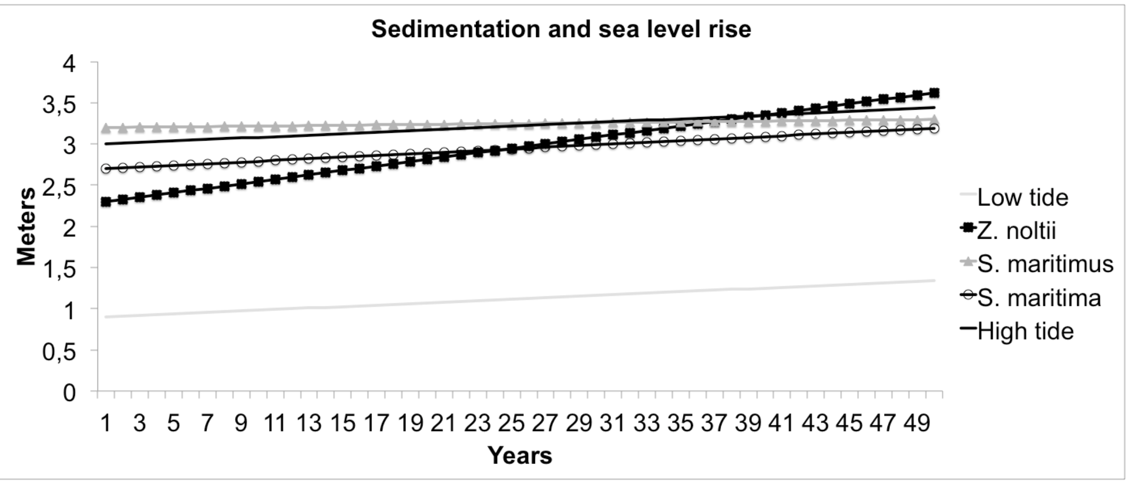

Figure 4. *Predicted sea-level rise and sedimentation of the three studied species through 50 years*

Conclusions

The fit of the models for the C_3 and C_4 plants to the observed biomass values was highly satisfactory. The assumption of a temperature higher than the actual temperature yielded simulated plant biomass values higher than the actual values. This result indicates that the biomass of both C_4 and C_3 plants would increase and that as a result, carbon sequestration would also increase. The increase in sea level should be prejudicial to salt marsh species. The *S. maritima* sedimentation rate can apparently keep pace with the sea level increase, and *Z. noltei* shows a high sedimentation rate. In contrast, because the *S. maritimus* sedimentation rate is very low, this species could disappear from the Mondego estuary. This scenario would have important implications for the system's carbon sequestration ability because, of the three species examined, *S. maritimus* accumulates the highest quantity of carbon.

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SEASONAL WEED STRUCTURE OF MAIZE IN THE LIGHT OF FARMING SYSTEMS

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Abstract. The differences between the cultivation of conventional and organic fields can result in changes in weed flora. Although herbicides do not affect all weed species equally, their use can have long-term effects on weeds and may prevent their emergence for weeks after treatment. On the other hand, the common mechanical weed control of organic farms is not selective, but weeds can reappear immediately after the treatment. Nonchemical techniques of weed control are not only used under organic conditions, but also in conventional fields, because of the increasing demands for the restricted use of chemicals in food production. The main aim of our research is to see how different weed control practices result in different weed populations, and how these populations change during the growing season of maize. This work was based on data from 93 conventional and organic maize fields in south-eastern Hungary surveyed between 2007 and 2011. The studied fields were divided into four groups, according to the farming system employed and the season of the survey (conventional in spring, conventional in summer, organic in spring, organic in summer). The aim was to explore the seasonal change of weed flora and the differences between farming systems. Our analysis, which included a comparison of the main weed species, shows that fields managed conventionally and organically are different in terms of both their early and late vegetation. Conventional fields in spring were the most distinct from the other three field groups, as they were not well characterized by any weed species. All the other field groups had dominant species present.

Keywords: *weed structure, weed flora, organic farming, maize*

Introduction

The organic approach differs from conventional farming in many of its attributes. Systems may be quite diverse in their cultivation structure: for example, the use of pesticides and fertilizers is not permitted in every system. Synthetic chemicals can be used in integrated and conventional systems, yet their use is forbidden in organic farming (EC 834/2007).

In organic farming, cultural, mechanical, physical, and biological methods are all important in weed control, and crop rotation is a generally used technique. The rotation of crops produces a yearly change of ecological conditions, and decreases the seed bank and weed biomass in fields (Brainard et al., 2008). The management of soil outside of the growing period, and the use of cultivars that compete better with weeds, can also serve as elements of prevention (Bond and Grundy, 2011).

The organic approach differs from conventional farming in many ways. An idealistic industrial approach has appeared in intensive agricultural production, whose aim is to become independent from the environment, obtain absolute human control, and to replace natural resources by artificial ones (Ángyán and Lőrinczi, 2003).

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Controlled organic farming has been present in Hungary since 1986, and has become a dynamically developing part of Hungarian agriculture over the last twenty-five years. The total area employing the system increased to over 120,000 hectares by 2004, and the value has been constant since then (Roszik et al., 2012; Kovács, 2012). Organic farming can be regarded as a system of improving vegetable quality (Kolbe et al., 1995, Weibel et al., 2004), and it has become an increasingly popular way of adding extra value to farm products (Dobbs and Smolik, 1996)

In this way, organic farming has become a factor that supports the maintenance of the environment, produces healthy food, carries out agricultural structure reform, uses environment friendly methods of production, and gains social acceptance for agriculture (Bedő, 2006).

The structure of Hungarian farms differs somewhat from the Western European average. While most area is utilized by medium-sized farms in Western Europe, Hungarian acreage is dominated by large farms (Eurostat, 2009). This difference is also visible in organic farming. Although most organic farms are small, organic farming can also be practices on a large scale, though for this to succeed, particular attention needs to be paid to the application of high-level technologies with respect for the environment and knowledge of local conditions. Apart from the many small organic farms, numerous large (many-hundred hectare), long-standing organic farms exist in Hungary, for example the Körös-Maros Biofarm, the Kishantos Ecological Model Farm, and the Galgafarm Co-operative.

Numerous well-known types of organic farming have been developed based on the environmental systems approach. (Bedő, 2006). The use of organic farming has been shown to produce benefit for the environment (Chamberlain et al., 1999; Reganold et al., 2001). When conventional and biodynamic cereals are compared, earthworm casts and weed seedlings are found more frequently in the biodynamic plot. The disaggregation of soil particles in the conventional plots leads to a smoother soil surface (Mäder et al., 2002).

Additionally, one crucial part of the crop protection of organic fields is weed control. Weeds are major factors in yield losses, as they use the water and nutrient reserves of the soil, suppress crop plants, may be reservoirs of crop diseases, and can serve as hiding places for pests. They can increase production costs, cause yield loss, and decrease the value of the product. On the other hand, it is necessary to employ some useful properties of weeds if the unity of the environment is to be maintained. For example, they can decrease wind and water deflation, may be employed as ingredients of pesticides and conditioning agents, and can be used as green manure. Weeds can also help to preserve biodiversity (Radics et al., 2004). Avoiding the use of pesticides and fertilizers has also been shown to lead to changes in weed flora (Pimentel et al., 1997; Gabriel et al., 2006, Szentes et al. 2012).

Additionally, weed flora is affected by the intensity of cultivation. Some segetal species need, to a certain extent, yearly cultivation. The disturbance can create the possibility of beginning a succession, and in this way, annual species can become dominant in the first few years, to be followed by biennial species, and then by perennial. Finally, in some cases, woody species take over the main role of the plant association (Bornkamm, 1985; Tatoni and Roche, 1994). In contrast, Bonet and Pausas (2004) found that annuals were dominant in the first ten years. The structure of pioneer succession is chiefly affected by the crop and by the time of the last cultivation (Hüppe and Hofmeister, 1990). These pioneer successions are present in the fields as weed flora.

During the intensive use of landscape, natural ecosystems turn to agroecosystems, and the living spaces became sectioned significantly. This process results in a change in the botanical composition and diversity of the natural habitats, and in the number of species. Inevitably, diversity decreases in the habitats (Edwards et al., 2007).

Additionally, the weed composition does not remain constant during the growing season, with the greatest difference being on account of the annuals. While winter annuals germinate in autumn, or sometimes in early spring, and disappear by summer, summer annuals prefer high temperatures and germinate in spring or summer, finishing seed production by autumn. The frequency of perennials is also higher in summer (Hakansson, 2003).

Material and methods

Research circumstances

During our research, the weed species present were identified and classified, and the weed cover and weed structure of both organic and conventional fields were surveyed at different stages.

The aim of our research was to characterize the weed flora of organic and conventional maize fields in the Fehér-Körös River area, and to find differences in weed flora between the different approaches (organic and conventional) used on farms. Surveys were made on an organic farm of more than 2500 hectares (Körös-Maros Biofarm), and at neighbouring conventional farms in the Fehér-Körös River region, near the town of Gyula in southeast Hungary, between 2007 and 2011.

The organic fields were controlled for weeds by harrowing twice (within a week after seeding, and between the 1 and 3 leaf stages of maize) and by use of an inter-row cultivator once or twice. The fields with most weeds were also hoed for weed control. Conventional fields were treated by pre-emergent or early post-emergent herbicides and by inter-row cultivator, and were treated between the 6 and 9 maize leaf stages.

All of the organic fields studied were managed conventionally with intensive use of herbicide prior to the early 1990s.

A total of 93 fields were surveyed in the study. These fields were divided into four groups, according to the farming system used and the season of the survey (conventional in spring, conventional in summer, organic in spring, and organic in summer). The spring surveys were carried out at the end of April and in May, while the summer surveys were performed between the middle of June and August.

We attempted to answer the following questions during our work on the fields of both farming systems: (a) Which weed species are most typical of conventional and organic maize in the region, and what is the extent of their cover? (b) Does the weed flora of the previously conventionally managed organic fields differ from that of the neighbouring continuously conventional fields? (c) Can weed control without herbicides keep weeds to an acceptable level in maize? (d) Which weed species are the most dangerous and problematic in organic farming? (e) Are there any differences between the organic and conventional systems in terms of weed structure? (f) How do the weed-species structures of spring (April–May) and summer (June–July) vegetation on organic and conventional fields differ?

The rate of cover of individual weed species was recorded by direct covering percentage (Németh and Sárfalvi, 1998). The weed cover of each field was estimated based on the mean values of four replicates. Species found only outside the sampling areas were registered as contributing 0.1 percent cover value (Zalai et al., 2012). Weed species were classified as winter annuals, summer annuals, biennials, creeping perennials, or stationary perennials according to Hakansson (2003).

Statistical analysis based on the structure of weed species

Prior to the analysis, the cover of each weed species was normalized by dividing it by the total weed cover of each field, to show the relative incidence of weed species per field. For preanalysis, one-way MANOVA (Wilks' lambda test) was used to test the difference between groups (Krzanowski, 1988). The main analysis was carried out using linear discriminant analysis (LDA), with the relative incidences of weed species as structure coefficients for the four field groups (conventional in spring, conventional in summer, organic in spring, and organic in summer) (Ripley, 1996; Venables and Ripley 2002). Weeds with lower total cover than 0.5% of the 93 studied fields were excluded from the analysis. In the figures, for clarity, only the 15 weed species with the highest effects on the model are represented. Linear discriminant number 1 (LD1) with LD2, as well as LD1 with LD3, was also used, on account of the relatively high trace of LD3. All statistical analyses were carried using R (R Development Core Team, 2011).

Results

Weed flora of conventional maize in spring

The weed flora of conventional maize fields mainly included creeping perennial and summer annual species in the spring surveys (*Table 1*).

Convolvulus arvensis and *Cirsium arvense* were the most frequent perennial species. This is unsurprising, as these species are problematic in most spring-seeded crops in Hungary. They are also important in the Fifth National Weed Survey (Novák et al., 2009), although they were not the most common weed in the Survey.

Table 1. *Most frequent weeds of conventional maize in spring*

OD*	Weed species*	Cover[%]
1.	*Convolvulus arvensis*	0.38
2.	*Cirsium arvense*	0.31
3.	*Stellaria media*	0.14
4.	*Echinochloa crus-galli*	0.10
5.	*Capsella bursa-pastoris*	0.09
6-7.	*Fallopia convolvulus*	0.04
6-7.	*Persicaria lapathifolia*	0.04
8-10.	*Helianthus annuus*	0.03
8-10.	*Setaria pumila*	0.03
8-10.	*Setaria viridis*	0.03
	other	0.30
	TOTAL	1.49

*OD order of dominance

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Summer annuals were also significant, and these species accounted for approximately half of the total coverage. Both monocotyledonous (such as *Echinochloa crus-galli* and *Setaria spp.*) and dicotyledonous species were frequent.

The coverage of winter annuals was low, but *Stellaria media* and *Capsella bursa-pastoris* took the third and fifth places, respectively, in the order of dominance.

The total weed cover was low.

Weed flora of conventional maize in summer

By summer, the creeping perennial species (*Convolvulus arvensis*, *Cirsium arvense*) managed to only slightly increase their coverage in conventional maize (*Table 2*).

The increase in the coverage of summer annuals was more intense. The order of dominance of these species changed, but *Echinochloa crus-galli* remained most frequent. Apart from this, other grassy weeds (such as Setaria pumila) and broad-leaved weeds (like *Hibiscus trionum*) were significant.

A stationary perennial plant (*Cichorium intybus*) was also present among the top weeds, but the frequency of this group was low.

The total weed coverage increased until late summer, but remained on a tolerable level.

Table 2. *Most frequent weeds of conventional maize in summer*

OD*	Weed species	Cover[%]
1.	*Echinochloa crus-galli*	1.13
2.	*Hibiscus trionum*	0.68
3.	*Convolvulus arvensis*	0.44
4.	*Cirsium arvense*	0.34
5.	*Persicaria lapathifolia*	0.22
6.	*Chenopodium hybridum*	0.18
7.	*Setaria pumila*	0.11
8.	*Abutilon theophrasti*	0.08
9.	*Cichorium intybus*	0.06
10.	*Digitaria sanguinalis*	0.04
	other	0.20
	TOTAL	3.48

*OD order of dominance

Weed flora of organic maize in spring

In the spring survey, *Cirsium arvense* was the most frequent weed in organic fields. This species was also important for conventional areas, but its coverage was many times greater in organic farming. Conversely, the coverage of *Convolvulus arvensis* (also a creeping perennial) differed only slightly from its value on conventional fields (*Table 3*).

The summer annual weeds were also significant. *Echinochloa crus-galli* was the first of these, with more than 1%. Beside this, only one monocotyledon was frequent (*Setaria pumila*). The number of dicotyledonous annuals was high, and all of the other top species came from this group (including *Xanthium strumarium* and *Abutilon theophrasti*).

The total weed coverage was less than 10%. This is still a tolerable value, but is much higher than for conventional maize.

Table 3. *Most frequent weeds of organic maize in spring*

OD*	Weed species	Cover[%]
1.	*Cirsium arvense*	1.30
2.	*Echinochloa crus-galli*	1.09
3.	*Xanthium strumarium*	0.47
4.	*Abutilon theophrasti*	0.42
5.	*Persicaria lapathifolia*	0.41
6.	*Convolvulus arvensis*	0.40
7.	*Chenopodium album*	0.29
8.	*Amaranthus retroflexus*	0.28
9.	*Setaria pumila*	0.23
10.	*Ambrosia artemisiifolia*	0.18
	other	1.79
	TOTAL	6.86

*OD order of dominance

Weed flora of organic maize in summer

By summer, monocotyledonous annuals had taken the lead in terms of their coverage of organic fields. *Echinochloa crus-galli* had the highest coverage, as with conventional fields, while *Setaria pumila* was also important (*Table 4*).

From the dicotyledonous annual group, *Hibiscus trionum* showed the greatest coverage. This is the same as on conventional farm, which shows that, just like grassy weeds, *Hibiscus trionum* is an important species in the studied region. Its high incidence is significant only in late summer.

However, the coverage of perennials (*Cirsium arvense* and *Convolvulus arvensis*) multiplied by early summer, and although their incidence was significantly higher than on conventional farms, it was not so high as to allow them to play the main role for organic maize in summer.

Table 4. *Most frequent weeds of organic fields in summer*

OD*	Weed species	Cover[%]
1	*Echinochloa crus-galli*	6.92
2	*Setaria pumila*	3.52
3	*Cirsium arvense*	3.08
4	*Hibiscus trionum*	2.72
5	*Convolvulus arvensis*	1.78
6	*Amaranthus retroflexus*	1.29
7	*Chenopodium album*	1.28
8	*Abutilon theophrasti*	0.68
9	*Ambrosia artemisiifolia*	0.51
10	*Datura stramonium*	0.33
	other	2.52
	TOTAL	24.63

*OD order of dominance

The total weed coverage was close to one quarter of the total area. This is higher than the damage limit value, in spite of the employment of continuous weed management.

Weed structure of organic and conventional maize fields

According to linear discriminant analysis with LD1 with LD2 (79.08% of the total trace), conventional fields in spring differ significantly from the other field groups (*Fig. 1*). Conventional and organic fields differ more in spring, and this difference was no longer significant by summer. The change in the structure of the weed flora was greater in conventional fields than in organic fields from spring until summer.

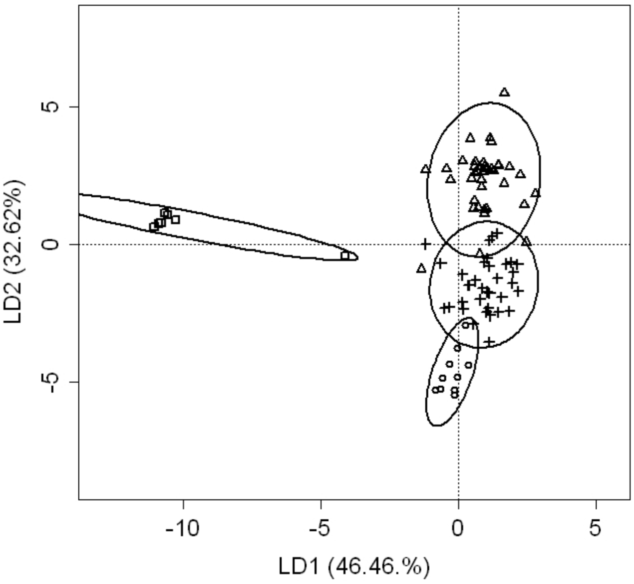

Figure 1. *Discrimination of organic and conventional fields in spring and in summer by weed structure according to LD1 and LD2 (□ conventional in spring, ○ conventional in summer, △ organic in spring, + organic in summer)*

Using LD1 with LD3 shows the same difference between conventional fields in spring and the other field groups (*Fig. 2*). The position of the organic fields interchanged from spring to summer, meaning that conventional and organic fields also differed significantly in summer. The weed structure of the organic fields in spring did not differ significantly from that of conventional fields in summer. Organic fields in different seasons did not vary, regardless of whether LD1 with LD2 or LD1 with LD3 was used.

On the basis of LD1 with LD2, most impact species can be classified into four groups. Weed group 1 includes the winter annual species (such as *Capsella bursa-pastoris* and *Lamium spp.*) that were present mainly on organic fields in spring. These are wintering or early spring germinating annual plants, and are less typical in maize than they are in cereals. Their cover was also low on organic fields in spring, but almost no winter annuals were present in the other field groups (*Fig. 3*).

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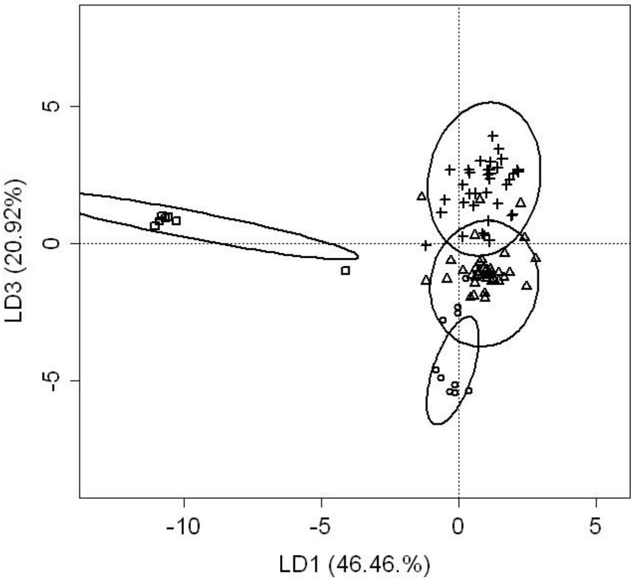

Figure 2. *Discrimination of organic and conventional fields in spring and in summer by weed structure according to LD1 and LD3 (□ conventional in spring, ○ conventional in summer, △ organic in spring, + organic in summer)*

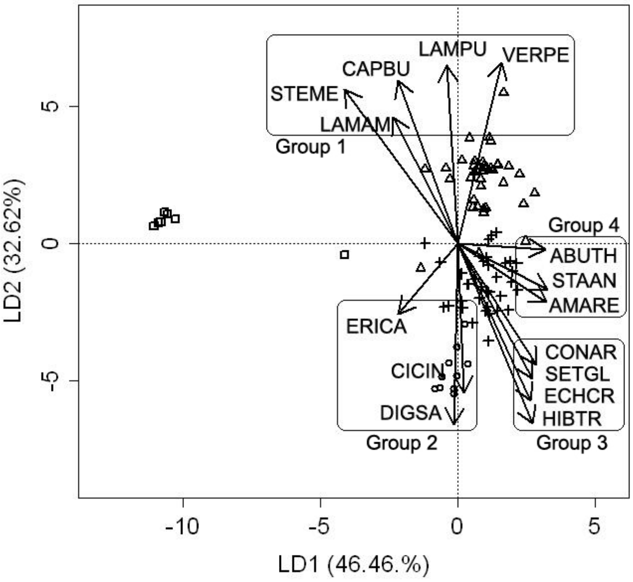

Figure 3. *Discriminating species and they effect on the model according to LD1 and LD2 (□ conventional in spring, ○ conventional in summer, △ organic in spring, + organic in summer,* ABUTH *Abutilon theophrasti*, AMARE *Amaranthus retroflexus*, CAPBU *Capsella bursa-pastoris*, CICIN *Cichorium intybus*, CONAR *Convolvulus arvensis*, DIGSA *Digitaria sanguinalis*, ECHCR *Echinochloa crus-galli*, ERICA *Conyza canadensis*, HIBTR *Hibiscus trionum*, LAMAM *Lamium amplexicaule*, LAMPU *Lamium purpureum*, SETGL *Setaria pumila*, STAAN *Stachys annua*, STEME *Stellaria media*, VERPE *Veronica persica.)*

The weeds in group 2 (*Cichorium intybus*, *Conyza canadensis*, and *Digitaria sanguinalis*) described conventional fields in summer, while those of group 4 (*Abutilon theophrasti*, *Amaranthus retroflexus*, and *Stachys annua*) described organic fields in summer. The members of groups 2 and 3, except for *Cichorium intybus*, are summer annuals that appear in early summer and flower continuously until late summer. These are common in maize fields (Novák et al., 2009; Tóth et al., 2011a, 2011b; Penksza et al., 2010).

The species of weed group 3 are typical of both farming systems in the summer. One of these (*Convolvulus arvensis*) is a perennial, while the other species (*Echinochloa crus-galli*, *Hibiscus trionum*, and *Setaria pumila*) are summer annuals whose incidence is nonetheless common in the same period. These species are also characteristic of Hungarian arable lands during summer (Novák et al., 2009).

No important species characterized conventional fields in spring, as the weed cover and the number of weed species were low in these fields, and frequently weeds also varied between fields. On the other hand, *Convolvulus arvensis* (the most frequent weed in these fields) continued to increase in frequency until summer. *Cirsium arvense* (the second most frequent weed) was common in both farming systems and in both seasons, and so was not able to characterize the ‘conventional in spring’ group.

The structure coefficients show slight differences, according to LD1 with LD3 and LD1 with LD2 (*Fig. 4*).

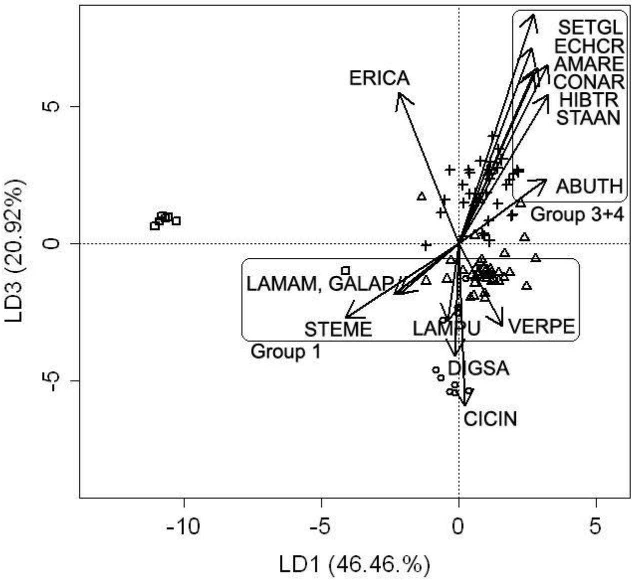

Figure 4. *Discriminating species and they effect on the model according to LD1 and LD3 (□ conventional in spring, ○ conventional in summer, △ organic in spring, + organic in summer,* ABUTH *Abutilon theophrasti*, AMARE *Amaranthus retroflexus*, CAPBU *Capsella bursa-pastoris*, CICIN *Cichorium intybus*, CONAR *Convolvulus arvensis*, DIGSA *Digitaria sanguinalis*, ECHCR *Echinochloa crus-galli*, ERICA *Conyza canadensis*, HIBTR *Hibiscus trionum*, LAMAM *Lamium amplexicaule*, LAMPU *Lamium purpureum*, SETGL *Setaria pumila*, STAAN *Stachys annua*, STEME *Stellaria media*, VERPE *Veronica persica.)*

The species of groups 3 and 4 are not distinguished, and describe only the organic fields in summer, not the conventional fields in summer.

From group 2, *Cichorium intybus* and *Digitaria sanguinalis* describe conventional fields well in summer, while *Conyza canadensis* is among the worst descriptors.

The species of group 1 are mainly characteristic of organic fields in spring. The analysis using LD1 with LD2 was more effective than the analysis using LD1 and LD3 in characterizing both group 1 and the two species *Digitaria sanguinalis* and *Cichorium intybus* taken together. It was also superior in segregating organic fields in spring from conventional fields in summer.

Conclusions and suggestions

The evidence of the surveys conducted between 2007 and 2011 is that the organic and conventional areas differ according to many parameters.

The weed species of the summer annual and creeping perennial types are present to a greatest extend in both organic and conventional maize fields, during both survey periods. The cover of species of both types decrease during the growing period of maize. The size of the increase is greater in the case of the summer annuals than with the perennials.

Among summer annual monocotyledons, *Echinochloa crus-galli* and the *Setaria* species were common. Other monocot annuals were present only rarely. Organic and conventional fields differed completely in terms of dicotyledonous weeds. *Amaranthus retroflexus* and *Hibiscus trionum* had high cover on organic fields in all years. The presence of volunteer *Helianthus annuus* was common only in conventional fields because of the differences between crop rotations. In addition, the *Chenopodium* and *Persicaria* species, as well as *Abutilon theophrasti*, were frequent in both farming systems. The most common perennials were *Cirsium arvense* and *Convolvulus arvensis*, which were found in both farming systems.

Interestingly, the allergenic *Ambrosia artemisiifolia* was not prominent in either organic or conventional maize fields, although it is common generally in maize fields in Hungary (Novák et al., 2009).

The total weed coverage was higher on organic fields than on conventionals in both periods, and this total coverage could increase during the growing season in both farming systems.

Apart from weed coverage, the structure of the weeds also differed in the two farming systems. Organic fields are characterized by *Abutilon theophrasti*, *Amaranthus retroflexus*, and *Stachys annua*; conventional fields by *Digitaria sanguinalis* and *Cichorium intybus* in summer. On organic fields, winter annual species (such as *Galium aparine*, *Lamium spp*, *Stellaria media*, and *Veronica persica*) were frequent in spring. Conventional spring fields are not characterized by any species, because the weeds that were frequent in such fields were also important in the other field groups.

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THE PROMISING ROLE OF EXOGENOUS S-METHYLMETHIONINE IN AGRICULTURE, IN THE CASE OF MAIZE CULTIVATION

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Abstract. Abiotic and biotic stress conditions have negative effects on growth and yield of crop plants worldwide. The need to improve the nutritional value, stress and disease tolerance of cultivated plants is of great importance. The problem is usually solved by breeding or by the production of transgenic plants; however, the application of biologically active substances with favourable properties can be an alternative, cost- and time-effective possibility. S-methylmethionine (SMM) is an intermediate compound in the plant sulphur metabolism, being ubiquitous in the plant kingdom. SMM has important role in stress and disease tolerance of plants by taking part in plant sulphur metabolism. Previous investigations have shown that SMM has beneficial effects on the physiological status of plants and provides protection against various stress factors. The present review aims to sum up the main results concerning the beneficial effects of SMM treatment against Maize dwarf mosaic virus infection and cold stress in maize, and to reveal the possible applications of SMM in agricultural practice.

Keywords: *abiotic stress, biotic stress, maize, MDMV, S-methylmethionine*

Introduction

S-methylmethionine (SMM; $(CH_3)_2$-S-$(CH_2)_2$-CH(NH_2)-COOH) was first detected and isolated from cabbage by McRorie et al. (1954). Later its presence has been confirmed in an increasing number of flowering plants (Sato et al., 1958; Giovanelli et al., 1980; Bourgis et al., 1999; Rácz et al., 2008). SMM is most abundant in the members of the Brassicaceae family, which plants are known for their high frost resistance (Sato et al., 1958; Greene and Davis, 1960; Giovanelli et al., 1980). SMM is a naturally occurring, biologically active non-proteinogenic free amino acid (Rácz et al., 2008). Based on previous publications, SMM was revealed as one of the most important sulphur-containing compounds in the plant metabolism, taking part in methylation processes and the regulation of methionine biosynthesis (Pimenta et al., 1998; Bourgis et al., 1999; Ranocha et al., 2000; Kocsis et al., 2003). SMM plays a vital role in the biosynthesis of osmoprotectant sulphopropionates, polyamines and prevents chlorophyll loss during stress conditions (Kocsis et al., 1998; Ko et al., 2004). SMM also promotes the incorporation of selenium into selenocysteine in selenium-tolerant plants (Neuhierl et al., 1999). Plants are characterised with sulphur autotrophy, a process in which sulphur is taken up from the nearby soil in (various) oxidised forms, than reduced and incorporated into cysteine and methionine. The latter compound can be transformed to S-adenosylmethionine (SAM), a molecule that is involved as a methyl donor in the synthesis of SMM from methionine (Kim and Leustek, 2000; Ranocha et al., 2000; Hacham et al., 2002). SMM can be reconverted to methionine, resulting in a circular

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pathway, known as the SMM cycle. Based on previous results, SMM metabolism could be connected with polyamine biosynthesis (Lásztity et al., 1992; Kissimon et al., 1994; Gyetvai et al., 2002). This alternative pathway could involve the decarboxylation of SMM, resulting in an intermediate compound which can be decomposed into volatile dimethyl-sulphide and propylamine. Propylamine is the initial compound in the polyamine biosynthesis (Bagga et al., 1997). When stress factors are present first polyamine, than phenylpropanoid pathways are upregulated, resulting in production of certain phenolics, flavonoids, anthocyanins and polyamines. The utilisation of exogenous SMM increases the amount of these protective compounds, therefore it enhances the plant's self-protection (Rácz et al., 2008; Szegő et al., 2009; Kósa et al., 2011; Ludmerszki et al., 2011; Páldi et al., 2013). Ogawa and Mitsuya (2011) determined that the intensity of the SMM cycle increased in *Arabidopsis thaliana* after salt treatment. Nevertheless there are no publications discussing a similiar result of SMM treatment on plants, especially on maize. Researches about SMM focused on crop plants such as grape, pea and tomato. Loscos et al. (2008) measured the amino acids content present in Petit Manseng grapes and found that SMM occurs naturally and in great amounts in the plants. However, they didn't investigate the effects of SMM on plant physiology. Tan et al. (2010) determined that SMM has positive effects on seed development in pea plants, but they didn't use exogenous SMM treatment to further investigate the beneficial effects. Our investigations concerning maize plants therefore are novel; since we use exogenous SMM and focus on the beneficial effects against different stress factors. In this review we would like to present the major works of our team, focusing on the positive effects of SMM on maize plants against cold stress and viral infection.

Maize (*Zea mays* L.) is one of the most widely grown crops in Hungary, and worldwide as well. Modern maize (*Zea mays* ssp. *mays*) was domesticated in Mexico about 9,000 years ago. Nowadays, selection has focused on better yield, grain quality and agro-ecosystem adaptations (Buckler et al., 2006). Maize is mainly used as a food and feed plant, but it is getting an increasing attention as raw material for industrial and pharmaceutical products.

The protective nature of SMM against abiotic and biotic stressors in maize

Studies concerning the effects of SMM against chilling temperatures

Maize is sensitive to chilling temperatures, since it has subtropical origins, and therefore it is incapable of cold acclimation (Messing et al., 2004; Buckler et al., 2006; Kósa et al., 2009). Under 10°C injuries can be observed, among them wilting, chlorosis, necrosis are the most common, but also the reduction in the fluidity of the membranes, the loss of efficiency in the electron transport chain and a decrease in the number of membrane bound or cold-sensitive enzymes can be observed (Kocsy et al., 2004; Saibo et al., 2009). Chilling stress is often accompanied by UV-B stress causing an increased difficulty for the plant defence system. Freezing regularly occurs in late spring and can deeply influence the vegetation period of plants, altering CO_2 supplies through stomatal closure and transpiration, and affecting water uptake. Chilling tolerance is mainly related to the ability of the photosynthetic apparatus to withstand the stress caused by low temperature. Exposure to low temperature induces the synthesis of polyamines, salicylic acid and abscisic acid. UV-B radiation causes DNA damage, formation of reactive oxygen species and lipid and protein oxidation (Hannah et al., 2006; Saibo et al., 2009).) Plants evade these reactive oxygen forms by producing antioxidant enzymes, and by up-regulating the phenylpropanoid biosynthetic pathway and making secondary metabolites with sunscreen effects (such as flavonoids and hydroxycinnamic acids) (Kramer et al., 1991; Bieza and Lois, 2001; Jenkins et al., 2001).

In temperate zones, because of chilling temperature, crop yields suffer great losses in the beginning of the vegetation period (Pál and Nagy, 2002; Janda et al., 2005). Szegő et al. (2009) carried out an investigation on the protective effects of SMM treatment of maize seedlings exposed to cold stress. *Zea mays* cv. 10-day old Norma seedlings were grown for 24 h on Hoagland solution containing 0.01% SMM. After SMM treatment, seedlings were cold-stressed (5 °C for 1- 24 hours) at reduced light intensity to avoid light stress. To monitor the physiological effects, the changes in the variable fluorescence (Fv/Fm) values were recorded. Fv/Fm indicates the maximum quantum efficiency of photosystem II therefore it's a useful parameter to determine photosynthetic activity. After the cold exposure the initial 0.7 values dropped to 0.4, however, when seedlings were SMM-pretreated before cold stress, Fv/Fm values dropped only to 0.5. Results suggest that SMM pretreatment moderated the reduction in photosynthetic activity caused by cold stress. Alongside physiological measurements, Szegő et al. (2009) carried out molecular studies, where the expression of the genes involved in the polyamine biosynthesis were investigated. The greatest change was observed in the expression of arginine decarboxylase (ADC), which increased within a few hours of all the treatments, and than dropped to the level of the untreated control by the end of the 24-hour period. The increase of the gene expression was the greatest (6-7 times), when SMM treatment was followed by cold stress. In the case of ornithine decarboxylase no similar changes were observed. These data confirm previous results, showing that putrescine level rises in response to SMM (Rácz et al., 2008) and is also in good agreement with earlier observations indicating that the pathway of putrescine biosynthesis starts from arginine and is activated in response to cold stress (Rácz et al., 1996). Szegő et al. (2009) observed a similar tendency in the expression of two enzymes catalyzing the synthesis of spermidine from putrescine: SAM-decarboxylase (SAMDC) and spermidine synthase (SPDS). Based on their results, both SMM and cold treatment caused a slight increase in the gene expression; however, when the combination of the treatments was applied, higher increase was observed. The increase in the level of spermidine did not correlate with the changes in the expression level of SAMDC and SPDS, since the increase in spermidine level was greater (approximately 6 times). The data suggest that the synthesis of spermidine proceeds via a SAMDC-independent pathway; however SMM influences its expression through changing SAMDC levels and also in a direct manner (see also Lásztity et al., 1992). They also investigated the gene expressional changes of CBF1, which is a regulatory protein factor of the ABA-independent pathway of stress-induced responses. The synthesis of this protein is induced by cold, drought or salt stress and is regulated by several factors. According to their results, following SMM treatment the expression of CBF1 increased to a slight extent. This rise became more pronounced after cold stress, and when the combination of the treatments was applied, the increase became even intensified. These results suggest that the rapid increase of CBF1 level aims to help the plant's defence against cold stress, moreover this protection can be promoted with the application of exogenous SMM. Overall, Szegő et al. (2009) proved the protective nature of exogenous SMM in the course of cold stress on maize, by stimulating metabolic pathways related to the plants sulphur metabolism and polyamine biosynthesis.

Similar investigation was carried out by Kósa et al. (2011), who studied the effects of SMM and chilling temperatures on maize plants, by measuring chlorophyll *a* fluorescence induction and imaging, gas exchange, anthocyanin content by spectrophotometric determination and chlorophyll content. In the investigation the same cultivar of maize (*Zea mays* L., cv. Norma) was used. In this study, maize seedlings were grown for a longer period and the SMM treatment was applied on the 22nd day with the same concentration of SMM (0.01%). During the investigation a temperature gradient was set (6-8-10-12-14 °C) and measurements were carried out 1, 2, 3 and 4 days after chilling treatments. The chlorophyll *a* fluorescence parameter Fv/Fm changed similarly compared to the results of Szegő et al. (2009). When maize plants were exposed to cold stress, a decrease was observed in the Fv/Fm values, however, when SMM treatment was applied prior to the cold stress, the decrease was much lesser. The protective effect was manifested to the greatest extent during longer periods of chilling and at the lowest chilling temperatures applied. Changes in the chlorophyll content exhibited a similar tendency. The protective effect of SMM can also be attributed to the reduction of membrane damage. This statement is further validated by the photochemical quenching data derived from the chlorophyll *a* fluorescence, which were higher than those of non-photochemical quenching parameters when SMM treatment was applied before the chilling treatments. This indicates that the photosynthetic apparatus was able to utilise the excitation energy in a more efficient way in the electron transport chain, resulting in a better CO_2 fixation. With the application of fluorescence imaging technique, an increase in the quantity of certain plant phenolic compounds was observed during combined cold and SMM treatment, as compared to the individual treatments. The results of Kósa et al. (2011) clearly demonstrates the protective effects of SMM against chilling temperatures, supplementing the results of Szegő et al. (2009).

Páldi et al. (2013) further investigated the beneficial effects of SMM treatment against cold stress by measuring the content of phenolic compounds and anthocyanins in leaves and stems, respectively, and by determining the relative gene expression of cinnamate-4-hydroxylase (C4H) and chalcone synthase (CHS). C4H and CHS are key enzymes of the phenylpropanoid pathway, leading to the synthesis of flavonoids or anthocyanins (Fraser and Chapple, 2010). Similarly, as in the case of Szegő et al. (2009) and Kósa et al. (2011), *Zea mays* L. cv. Norma was used as plant material. SMM treatment was carried out on 9-day-old plants, applying 0.001% SMM, and 10-day-old plants were cold stressed. During chilling temperature stress, 6 °C constant temperature was applied. Besides molecular measurements, Páldi et al. (2013) investigated the variable fluorescence parameters as well, and they acquired similar results as Szegő et al. (2009) and Kósa et al. (2011). In regard of phenolic content, chilling alone and the combined treatment of both chilling and SMM increased their amount. However, SMM did not significantly affect phenolic levels at control temperature. Based on their results it can be concluded, that SMM pretreatment enhances the response to chilling, resulting in elevated biosynthesis of some secondary metabolites. The results validate previous observations: Christie et al. (1994) demonstrated that the phenylpropanoid pathway is induced by cold stress; moreover Pourcel et al. (2007) proved that flavonoids have a direct protective role under stress conditions. Hernández and Van Breusegem (2010) stated, that flavonoids, apart from their well known protective function, may act as energy valves during abiotic stresses, which statement is further validated by the work of Páldi et al. (2013). Observing the transcript levels of C4H, they found that the effect of SMM alone was slight; on the other hand, cold exposure raised the transcript level significantly. This increase was more pronounced when SMM treatment was applied prior to cold stress. Changes in the expression of CHS were similar to C4H, with smaller amplitude but with the same tendency. According to their results, C4H expression was increased to a greater extent by cold stress than CHS. Since C4H is an earlier step in the phenylpropanoid pathway, this indicates a general activation of the pathway either by SMM treatment or cold stress. As concluded, SMM pretreatment stimulates the phenylpropanoid pathway and helps the plant to avoid cold injuries. The results are in great correlation with Szegő et al. (2009) and Kósa et al. (2011).

The protective nature of SMM treatment against Maize dwarf mosaic virus infection

Apart from abiotic stressors, investigations were carried out to study the beneficial effects of SMM against biotic stressors as well, namely in the case of Maize dwarf mosaic virus (MDMV) (Ludmerszki et al., 2011). The virus spreads in natural or in agro-environments, via aphids, seed or pollen transmission (Astier et al., 2007; Tóbiás et al., 2008). The symptoms of the infection are reduced growth and chlorotic bands on the leaves, giving a mosaic pattern. The infection usually causes 10-45% crop losses; however, the damage can reach 100% (Tóbiás et al., 2008). Ludmerszki et al. (2011) used a different subspecies of maize (*Zea mays* cv. *saccharata* Koern, sweet corn), which was susceptible to the infection. MDMV infection was carried out one day after the SMM treatment on 11-day old plants, and the infection was repeated two days after the first one to ensure successful infection. MDMV Dallas-A strain was used for the infection. In infected plants the chlorophyll content decreased. At the same time, an increase in the 520 nm of fluorescence was observed, indicating that MDMV triggers molecular pathways involved in the synthesis of protective compounds with a fluorescence peak of 520 nm (see also Pineda et al., 2008). SMM pretreatment resulted in the protection of chlorophylls and an enhancement of fluorescence at 440 nm, indicating an increase in the concentration of compounds with a fluorescence peak at 440 nm. Based on the ELISA results, SMM pretreatment decreased the amount of viral coat proteins present in infected leaves. A close look at the data indicates that SMM may inhibit the replication and/or the spread of MDMV.

Uzarowska et al. (2009) previously showed that in infected maize plants the expression levels of S-adenosylmethionine synthase (SAMS) and a 14-3-3-like protein GF14-6 significantly increased, probably due to the complex biochemical pathways of plant defence. Our results support these data, showing that SAMS and GF14-6 both play a crucial role in the plant defence mechanism against MDMV infection. SMM and MDMV individually trigger pathways that are related to high levels of GF14-6 expression, however, when the combination of the treatments was applied, an inhibition of GF14-6 expression was observed. SMM pretreatment delayed the increase of SAMS expression, but also prolonged it, providing a slow, but constant rise. Results of Ludmerszki et al. (2011) demonstrated that the natural compound SMM has beneficial effects on biotic stress response reactions, resulting in an increase in the defence potential of maize plants during MDMV infection.

Possible applications of SMM in agricultural practice

Based on the investigations listed above it is clearly demonstrated that in maize SMM treatment provides protection against chilling temperatures and MDMV infection. SMM was applied to at least 9-days old plants in the investigations, but previous studies indicated, that germination of the seeds in SMM-containing solutions may also provide protection against environmental factors (unpublished data). Our team is still carrying out investigations concerning SMM treatment during germination. In the 1980's when Kékfrankos grape was sprayed with SMM wine quality, anthocyanin content, free acid content and crop mass increased (unpublished data). On tomato SMM induced the synthesis of certain polyamines, being important in plant defence. Our ongoing studies indicate that SMM pretreatment could also be successfully applied in the case of drought and salt stress. All of these results lead to the conclusion, that the application of SMM can achieve a success in the field of agriculture, by moderating harmful effects of certain stress factors.

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THE MOVING SPLIT WINDOW (MSW) ANALYSIS IN VEGETATION SCIENCE – AN OVERVIEW

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Abstract. Boundaries are key entities in understanding ecological patterns and mechanisms. If challenges of fragmentation and global changes are to be met, objective techniques are needed to describe boundary characteristics and predict future dynamics. In vegetation science, the moving split window (MSW) technique has been efficiently used for boundary analysis. However, a wider but more rigorous application of the method could considerably promote our understanding of vegetation pattern and organization. First, we briefly describe the MSW method and its history. Next, we review publications that used MSW for field data to analyse vegetation boundaries. We also evaluate the studies that investigated MSW properties. Since such studies are scarce, our own additional tests are also provided. The MSW has been used in a multitude of different environments to answer highly diverse study questions. Study layouts (e.g. transect length, plot size, window width) have been plentiful. As for future directions, the MSW could broaden our knowledge on vegetation continuity vs. discontinuity and abiotic-biotic relations. It should more widely be used to study boundary dynamics. Real cross-scale studies could be achieved by using considerably different window widths without averaging the resulting dissimilarities. A significance test is needed for the correct interpretation of the results.

Keywords: *boundary, ecotone, transect, dissimilarity profile, dissimilarity indices*

Introduction

In vegetation science, knowledge on boundaries (i.e. locations that separate adjacent biotic zones) is essential in understanding basic ecological patterns and processes on several scales (Holland, 1988; Cadenasso et al., 2003; Ries et al., 2004). Boundaries may be defined as positions where the rate of change in the measured properties is the highest (Fortin and Drapeau, 1995; Fortin et al., 2000). Boundaries play an important role in landscape ecology, community ecology and nature conservation (Yarrow and Marín, 2007). Given the intensive habitat fragmentation and boundary alterations due to global changes, a profound ecological knowledge on boundaries is crucial, if the natural values of landscapes are to be maintained and correctly managed (Hansen et al., 1988; di Castri and Hansen, 1992; Risser, 1995; Laurance et al., 2001).

The most important boundary characteristics include spatial position, boundary width, sharpness, geometric shape and dynamics (cf. Strayer et al., 2003). To study these features, objective methods are needed. For this purpose, several techniques have been used during the past few decades (Kent et al., 1997; Jacquez et al., 2000; Fortin and Dale, 2005; Hufkens et al., 2009). For boundary analysis, the most commonly used methods include ordination (e.g. Werger et al., 1983; Carter et al., 1994; Erdős et al., 2008), wavelet analysis (e.g. Bradshaw and Spies, 1992; Dale and Mah, 1998; Camarero et al., 2006) and wombling (e.g. Fortin, 1994; Fortin and Drapeau, 1995;

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Fortin et al., 2000). Beside them, the moving split window technique is probably the most popular.

The moving split window technique (MSW) detects boundaries along one-dimensional (transect) data (cf. Kent et al., 1997). Its popularity is due to the fact that it is relatively simple, robust and powerful (Ludwig and Cornelius, 1987; Johnston et al., 1992). Consequently, the number of studies using the MSW is relatively high. However, we think there is a considerable potential in this method that has not been exploited yet. In this article we provide a brief description of the MSW method and a summary of how MSW emerged as a valuable tool in ecology. Next, we review 40 published studies that used the MSW for field data. In addition, we evaluate the studies that used simulated data for the analysis of the behaviour of the MSW under known circumstances. Since studies focusing on MSW-properties are scarce, a few of our own tests are also summarized in the present article. We provide some suggestions on the appropriate MSW layouts, on the significance tests and randomization procedures, and identify the main directions where MSW holds great promise.

The MSW technique: a brief description

For MSW studies, a field transect has to be established, consisting of a series of contiguous or non-contiguous plots. The transect is usually gradient-oriented, that is, it is placed perpendicular to the presumed boundary/boundaries (this study design is also called gradsect; Gillison and Brewer, 1985) (*Fig. 1a*). Variables such as species presence/absence data or cover values are registered within each plot. Then, at one end of the transect, a window is assigned, which is split into two half-windows. In the simplest case, a half-window consists of one plot. The two half-windows are compared, using a dissimilarity function. The window is then shifted along the transect by one plot in each step, and the function is computed in all positions. The procedure is repeated until the end of the transect. If the dissimilarity function is plotted against spatial position, boundaries appear as peaks (*Fig. 1b*). Half-window size can be increased, thus the analysis can be done on several spatial scales (Webster, 1978; Brunt and Conley, 1990; Fortin and Dale, 2005; Körmöczi, 2005). High and narrow peaks indicate sharp boundaries, whereas low and wide peaks form where boundaries are blurred (Ludwig and Cornelius, 1987). Double peaks that merge at larger window widths indicate transitional zones positioned between the two peaks. In contrast, double peaks that do not merge at increasing window widths indicate distinct patches that are situated in a homogeneous matrix (Körmöczi, 2005 Erdős et al., 2014).

A short history of the MSW technique

The first analysis similar to what is known today as moving split window analysis, was carried out by Whittaker (1960). However, there were two differences compared to the MSW. Firstly, similarities were calculated instead of dissimilarities; secondly, and more importantly, similarity profiles were drawn in a topological space (that is, the order of plots in the profile did not reflect their order in the real space). In subsequent studies, dissimilarity profiles were prepared in a topographical space. Beals (1969) used the approach to reveal potential discontinuities along altitudinal vegetation gradients in Ethiopia. Van der Maarel (1974, 1976) also suggested that vegetation boundaries may be delineated effectively by drawing differential profiles. This method is essentially identical to the moving split window analysis, although the possibility of increasing the window width was not considered yet.

The MSW method in the strict sense was used first in soil science (Webster and Wong, 1969; Webster, 1973, 1978; Nwadialo and Hole, 1988). Since the late 1980s, MSW has been applied increasingly in vegetation science (some early examples include Wierenga et al., 1987; Cornelius et al., 1991; Ludwig and Tongway, 1995) and later in animal ecology as well (Ross et al., 2001; Magura, 2002; Gallé et al., 2007; Torma and Körmöczi, 2009). Moreover, MSW was used to analyse satellite images (e.g. Chang et al., 2003), in which case the plots are the pixels of the image, and they are compared based on their reflectance. Also, there have been important contributions testing the properties of the MSW, using both real and simulated data (Brunt and Conley, 1990; Choesin and Boerner, 2002; Körmöczi, 2005; Erdős et al., 2013, 2014).

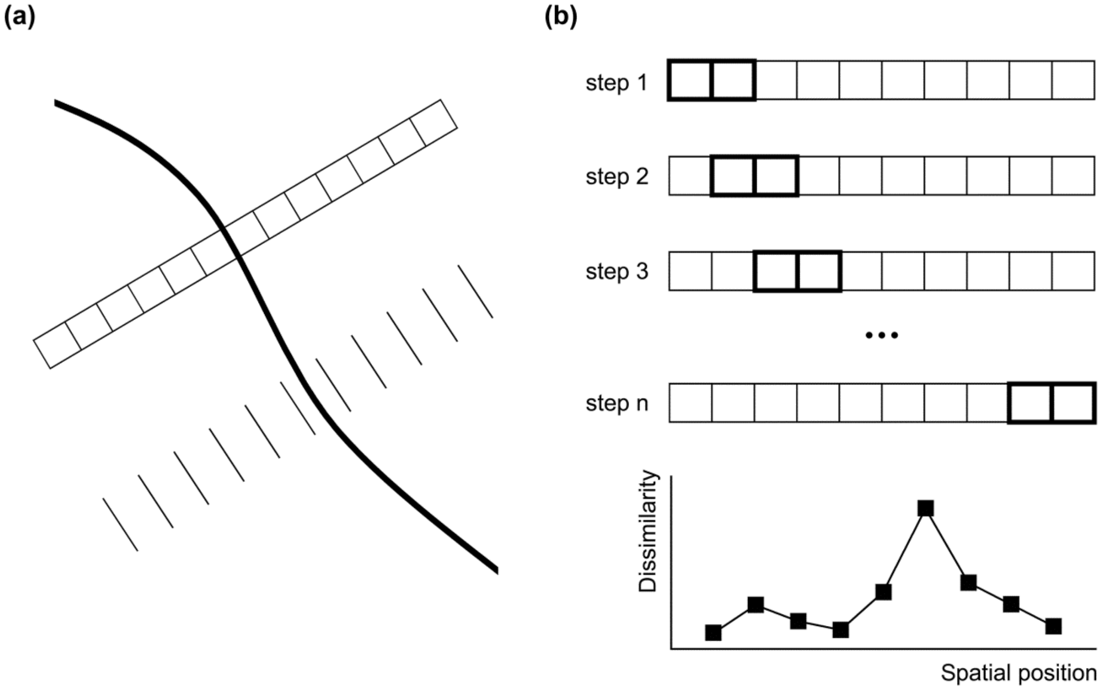

Figure 1. *Transect analysis in MSW. (a) Gradient-oriented transects (gradsects) of contiguous quadrates (up) and lines (down) across a presumed boundary. (b) Schematic representation of the MSW analysis and the resulting dissimilarity profile (based on Fortin and Dale, 2005)*

The use of the MSW in vegetation science

In vegetation science, moving split window analysis has been used in several environments (*Table 1*) of every continent except Antarctica. The majority of these studies were carried out in arid or semi-arid regions (xeric grasslands, shrublands and deserts). The method can successfully be used to analyse boundaries between woody and herbaceous vegetation (e.g. forest-grassland), as well as to study boundaries between different types of forests (e.g. coniferous-deciduous) and those between grasslands (e.g. open-closed).

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Table 1. *Habitats under scrutiny and the main study focus of 40 vegetation studies using MSW. 'b' means boundary*

Author(s)	Habitat	Main study focus
1 Azevedo and Caçador 1999	holm-oak forest	Boundary location
2 Bátori et al. 2014	mesic forests	Distinctness of doline bottoms
3 Beals 1969	semiarid shrublands	Continuity or discontinuity?
4 Boughton et al. 2006	shrubland	Boundary location Boundary dynamics (sharpness - fires) Elevation - communities
5 Camarero et al. 2006	alpine treeline (*Pinus uncinata*)	Boundary location Canopy boundary - plant richness boundary
6 Chang et al. 2003	mountain birch forest/ coniferous forest	Boundary location and width
7 Choesin 2001	alkaline wetland	Visual boundaries - MSW-boundaries
8 Choesin and Boerner 2002	alkaline wetland	Visual boundaries - MSW-boundaries
9 Dupuch and Fortin 2013	boreal forest edge	Depth of edge influence
10 Erdős et al. 2011	shrubforest/rock sward	Boundary location and width Edge diversity
11 Erdős et al. 2012	mesic and xeric forests and grasslands	Boundary sharpness Boundary densities on different slopes
12 Erdős et al. 2013	mesic and xeric forests and grasslands	Boundary location at multiple scales Landscape hierarchy
13 Harper and Macdonald 2001	boreal forest edge (selected plant species)	Boundary location
14 Hennenberg et al. 2005	tropical forest/savanna	Boundaries of different vegetation layers Depth of edge influence
15 Ibanez et al. 2013	rainforest/savanna	Boundary dynamics
16 Kirkman et al. 1998	wetland/upland	Boundary location Vegetation boundaries - soil boundaries
17 Körmöczi and Jusztin 2003	sandy grassland	Boundary location and sharpness Boundary dynamics
18 Kröger et al. 2009	wetland/upland	Boundary location Vegetation b. - topographic b. - soil b. Boundaries in 2 dimensions
19 Kröger and Rogers 2005	wetland/upland	Boundary location Grazing animals' habitat preference
20 Laliberte et al. 2007	wetland/upland	Boundary location
21 Li et al. 2005	coniferous forest/cultivated land, broadleaved forest/grassland	Depth of edge influence
22 Li et al. 2007	coniferous forest/cultivated land, broadleaved forest/cultivated land	Depth of edge influence Soil properties edge influence - vegetation edge influence
23 Ludwig and Tongway 1995	semiarid forest/grassland	Boundary location at multiple scales Landscape hierarchy
24 Muños-Reinoso 2001	Mediterranean shrubland	Boundary location Geomorphology - vegetation boundaries
25 Muños-Reinoso and García Novo 2000	Mediterranean shrubland	Boundary location Geomorphology - soil - vegetation b.
26 Muños-Reinoso and García Novo 2005	Mediterranean shrubland	Boundary location Geomorphology - soil - vegetation b.
27 Muños-Reinoso 2009	Mediterranean shrubland	Boundary location Landscape hierarchy
28 Nishimura and Kohyama 2002	subalpine coniferous forests	Boundary location Topography - soil depth - vegetation b.
29 Pärn et al. 2010	wetland/upland	Boundary location Soil b. - topographic b. - vegetation b.
30 Ross et al. 2001	wetland/upland	Boundary location Vegetation b. - algae b. - invertebrate b. - physical b.
31 Stanisci et al. 2000	mountain beech timberline	Timberline structure Boundary dynamics
32 Tolman 2006	savanna/fen	Vegetation boundaries - soil boundaries
33 Torma and Körmöczi 2009	sandy grassland	Boundary location Vegetation b. - true bug community b.
34 Watkins et al. 2003	hardwood forest	Depth of edge influence
35 Wierenga et al. 1987	desert	Boundary location Vegetation boundaries - soil boundaries
36 Cornelius et al. 1991	desert	Boundary location Vegetation boundaries - abiotic boundaries
37 Xu et al. 2012	arid-semiarid sandy land/ steppe	Boundary location Vegetation boundaries - soil boundaries
38 Zalatnai and Körmöczi 2004	alkaline grassland	Visual boundaries - MSW-boundaries Boundary sharpness Vegetation boundaries - microtopography
39 Zalatnai et al. 2007	alkaline grassland	Visual boundaries - MSW-boundaries Vegetation boundaries - soil boundaries Boundary sharpness - abiotic stress
40 Zalatnai et al. 2008	alkaline grassland	Visual boundaries - MSW-boundaries Vegetation boundaries - soil boundaries Boundary sharpness

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Study questions that can be answered by the MSW method are highly diverse (*Table 1*). In most cases, the exact and objective identification of boundary location is the primary and most important purpose. After the delineation of the boundaries, transect segments are usually further analysed. This may provide useful information for nature conservation (e.g. Kröger and Rogers, 2005; Erdős et al., 2011; Bátori et al., 2014).

The correspondence between visually delineated boundaries and boundaries identified by the MSW can also be studied (e.g. Choesin and Boerner, 2002; Zalatnai and Körmöczi, 2004). According to Zalatnai et al. (2007), the greater the environmental stress (in that case, salinity), the stricter the coincidence.

Another exciting question is whether or not different types of ecological boundaries coincide. Numerous studies have analysed how boundaries in abiotic parameters (such as soil factors and topography) influence vegetation boundaries. In some cases, the two boundary types are in the same position (e.g. Wierenga et al., 1987; Kröger et al., 2009), while in other cases, they are not (e.g. Zalatnai et al., 2007; Pärn et al., 2010; Xu et al., 2012). Similarly, the boundaries of different vegetation components or vegetation layers may be compared (e.g. Hennenberg et al., 2005; Camarero et al., 2006). The study of the correspondence between vegetation boundaries and animal community boundaries is also possible (Ross et al., 2001; Torma and Körmöczi, 2009).

The MSW method can also be used to determine boundary width or depth of edge influence, as was done by Watkins et al. (2003) in a temperate hardwood forest, by Hennenberg et al. (2005) in tropical forest patches and by Dupuch and Fortin (2013) in old-growth boreal forests.

Based on the MSW technique, detailed studies on vegetation dynamics are also possible, using either direct (long-term researches) or indirect (space for time substitution) methods. The results of Stanisci et al. (2000) indicate that Italian beech timberlines with shrubby mantle may move upwards, while those without a mantle are stable. Körmöczi and Jusztin (2003) identified stable, shifting and directionally moving boundaries in a sandy grassland. The study of Boughton et al. (2006), conducted in a Florida shrubland, suggests that fire makes boundaries sharper, but as time passes after the fire event, boundaries become more diffuse. From sharp and blurred boundaries, Ibanez et al. (2013) inferred that stable and unstable phases alternate in rainforest-savanna boundary dynamics. Interestingly, Dupuch and Fortin (2013) found that in uncut forests adjacent to logged areas, the depth of edge influence increased as the logged areas regenerated.

MSW may help to put boundaries in a broader context (i.e. the role of community boundaries in the landscape can be studied). The analysis of Muñoz-Reinoso (2001) showed that boundaries appear in a regular pattern, appr. 1500 m away from one another in a Spanish shrubland. In a comparison of differently exposed slopes, Erdős et al. (2012) found that the density of boundaries is higher on south-facing mountain slopes than on north-facing ones, probably due to different soil properties and microclimate.

Since MSW operates on several spatial scales, it allows the study of hierarchical organization of the vegetation. Boundaries of at least two different organizational levels have been detected in an Australian vegetation mosaic by Ludwig and Tongway (1995). Using large window widths, the MSW identified boundaries between alternating tree groves and treeless intergroves. Smaller window widths revealed a pattern of a lower level, where the undergrowths of the individual trees, shrubs and log mounds were separated by open areas. Boundaries of three nested hierarchical levels were demonstrated in the Mediterranean shrublands of Spain (Muñoz-Reinoso, 2009). On a regional level, a boundary separates the coastal shrubland zone from the inland grasslands and marshes. On an intermediate level, boundaries separate vegetation types of different aged dune series. The lowest hierarchical level deals with neighbouring shrub communities on the dune slopes. In a Hungarian mountain region, Erdős et al. (2013) used strongly different window widths and identified a boundary type between two community-complexes on a coarse scale, as well as the community boundaries within each complex on a finer scale.

Height and width of the peaks in an MSW profile indicate the abruptness of the spatial changes along the transect, thus the method provides an excellent opportunity to characterize boundary sharpness. Regular sampling (transect) along a gradient and the objective boundary delineation of the MSW may significantly contribute to the theories associated with a basic ecological controversy, namely the community-unit vs. continuum debate. Comparing the plant communities of steep and gentle slopes, Beals (1969) concluded that in some cases, vegetation discontinuity develops even when the environmental gradient is continuous, which is similar to the Clementsian view of plant communities. However, under different conditions, the vegetational change is more or less continuous, which is close to the Gleasonian concept. Other studies have confirmed that in nature, both sharp and blurred boundaries occur, and in some cases changes are so gradual, that no boundaries can be identified (Kirkman et al., 1998; Erdős et al., 2012). It is a related topic where and how gradual changes in abiotic parameters result in sharp boundaries in vegetation. For example, Zalatnai et al. (2008) found that in alkaline grasslands, vegetation boundaries are usually sharp and narrow, while soil boundaries are wider and more gradual.

A major drawback of the MSW is that it is confined to one dimension. However, from a study design of several parallel transects, two-dimensional information can be gained, as shown by Kröger et al. (2009). This may be less reliable than the methods specifically designed for two-dimensional spatial analyses (e.g. wombling). Nevertheless, it can provide reasonably useful results, yet it is less labour-intensive and less destructive.

MSW layouts: transect length, sampling units, window width and dissimilarity indices

In literature, transect lengths vary between 9 m and 300 km, but about half of the studies apply transects of 50-500 m (*Table 2*), which are usually appropriate for identifying the boundaries between vegetation units on the landscape scale. As for the sampling units, quadrates are by far the most popular, but the line intercept approach is also efficient. Circular plots (Ross et al., 2001) and point quadrates (Camarero et al., 2006) have also been used in some studies. The necessary resolution clearly depends on the purpose of the study and the structure of the vegetation under scrutiny: in the case of quadrates, sampling unit sizes range from 100 cm^2 to 250 m^2. For most studies, quadrate size of 1-25 m^2 has proved to be appropriate. Quadrates are most often contiguous, but in the case of very long transects, this is not possible or not needed. However, it should be kept in mind that too large distances between non-contiguous plots risk that some spatial phenomena remain undetected, especially if the vegetation is patchy (*Fig. 2*). The distance between adjacent sampling plots is almost always constant along the whole transect. The two exceptions we found were the studies of Beals (1969) and that of Watkins et al. (2003).

Table 2. *MSW layouts in 40 vegetation studies. Sampling unit type: C: circular plot, LI: line intercept, P: point quadrat, Q: quadrat. Basic sampling unit size: A: canopy, B: shrub layer, C: herb layer; S: seedlings and saplings. MSW window width: 'a' means that the results were averaged over several window widths, numbers in parenthesis indicate window widths tested in pilot studies but not further evaluated. MSW index: BC: Bray-Curtis index, CB: city block, CD: coefficient of dissimilarity, CH: chord distance, D: difference of species cover, DREN: complement of the Renkonen-similarity index, ED: Euclidean distance, SCB: squared city block, SED: squared Euclidean distance, SMD: squared Mahalanobis distance*

Author(s)	Transect lenght	Sampling unit type	Basic sampling unit size	Distance of sampling units	MSW window width	MSW index
1 Azevedo and Caçador 1999	25 m	LI	10 m	5 m	2	SED
2 Bátori et al. 2014	10-243 m	Q	1 m×1 m	–	2-30	DREN
3 Beals 1969	20-300 km	Q	10 m×20 m	inconstant	10	CD
4 Boughton et al. 2006	30-147 m	LI	1 m	–	12, 16, 20 a (2-20)	SCB
5 Camarero et al. 2006	100-140 m	P	d=2 mm	1 m	10 (2-20)	D
6 Chang et al. 2003	510 m	Q	1 m×2 m	–	2-30	SED
7 Choesin 2001	72-134 m	Q	1 m×0.5 m	–	8	SED
8 Choesin and Boerner 2002	49-134 m	Q	1 m×0.5 m	–	8 (2, 4, 8, 16)	SED
9 Dupuch and Fortin 2013	300 m	Q	2 m×2 m AS 1 m×1 m C	10 m	7-15	SMD
10 Erdős et al. 2011	20-40 m	Q	1 m×1 m	–	2-20 a	SED
11 Erdős et al. 2012	200 m	Q	1 m×1 m	–	2-20 a	SED
12 Erdős et al. 2013	382 m	Q	1 m×1 m	–	10-20 a, 30-40 a, 50-60 a, 70-80 a	SED
13 Harper and Macdonald 2001	200 m	Q	10 cm×10 cm	–	40, 200 (20, 40, 80, 120, 160, 200)	CB
14 Hennenberg et al. 2005	260-305 m	Q	5 m×10 m ABC 5 m×30 m AB 5 m×50 m A	–	2-20 a	ED
15 Ibanez et al. 2013	120 m	Q	1 m×20 m	–	10, 20, 30, 40 a	ED
16 Kirkman et al. 1998	87 m	Q	1 m×3 m	3 m	4	SED
17 Körmöczi and Jusztin 2003	55 m	Q	25 cm×25 cm	–	2-40 a	SED, DREN
18 Kröger et al. 2009	ca. 150 m	Q	0.5 m×0.5 m, 1 m×1 m	–	2-10	SED
19 Kröger and Rogers 2005	ca. 150 m	Q	0.5 m×0.5 m	–	?	SED
20 Laliberte et al. 2007	30 m	Q	1 m×1 m	1m	2-4	SED
21 Li et al. 2005	50 m	Q	2 m×5 m	–	6-10 (2-14)	SED
22 Li et al. 2007	50 m	Q	2 m×5 m	–	6, 8	SED
23 Ludwig and Tongway 1995	300 m, 500 m	Q	1 m×1 m	–	10, 100	ED
24 Muños-Reinoso 2001	10.5 km	LI	30 m	100 m	4	CH
25 Muños-Reinoso and García Novo 2000	10.5 km	LI	30 m	100 m	4-12 a	CH
26 Muños-Reinoso and García Novo 2005	10.5 km	LI	30 m	100 m	4-12 a	CH
27 Muños-Reinoso 2009	110 m	Q	5 m×5 m	–	4	ED
28 Nishimura and Kohyama 2002	50 m	LI	?	–	10 m	SED
29 Pärn et al. 2010	125-195 m	Q	1 m×20 m	–	4	ED
30 Ross et al. 2001	3.9 km	C	d=10 m	100 m	4	BC
31 Stanisci et al. 2000	14-38 m	Q	1 m×1 m	–	6 (4-8)	SED
32 Tolman 2006	9-24 m	Q	1 m×1 m, 5 m×5 m	–	4	SED
33 Torma and Körmöczi 2009	55 m	Q	25 cm×25 cm	–	2-40 a	SED, DREN
34 Watkins et al. 2003	150 m	Q	1 m×1 m	5 m-30 m	2	SED
35 Wierenga et al. 1987	2.7 km	LI	30 m	30 m	8 (4-12)	SED
36 Cornelius et al. 1991	2.7 km	LI	30 m	30 m	6	SED
37 Xu et al. 2012	110 km	Q	1 m×1 m C 2 m×2 m C 10 m×10 m B 5 m×20 m B	2 km	8 (2-12, 16)	SED
38 Zalatnai and Körmöczi 2004	17 m, 19 m	Q	10 cm×10 cm	–	4-60 partly a	DREN
39 Zalatnai et al. 2007	15 m	Q	10 cm×10 cm	–	2-40 a	SED, DREN
40 Zalatnai et al. 2008	30 m	Q	10 cm×20 cm	–	2-40 a	SED, DREN

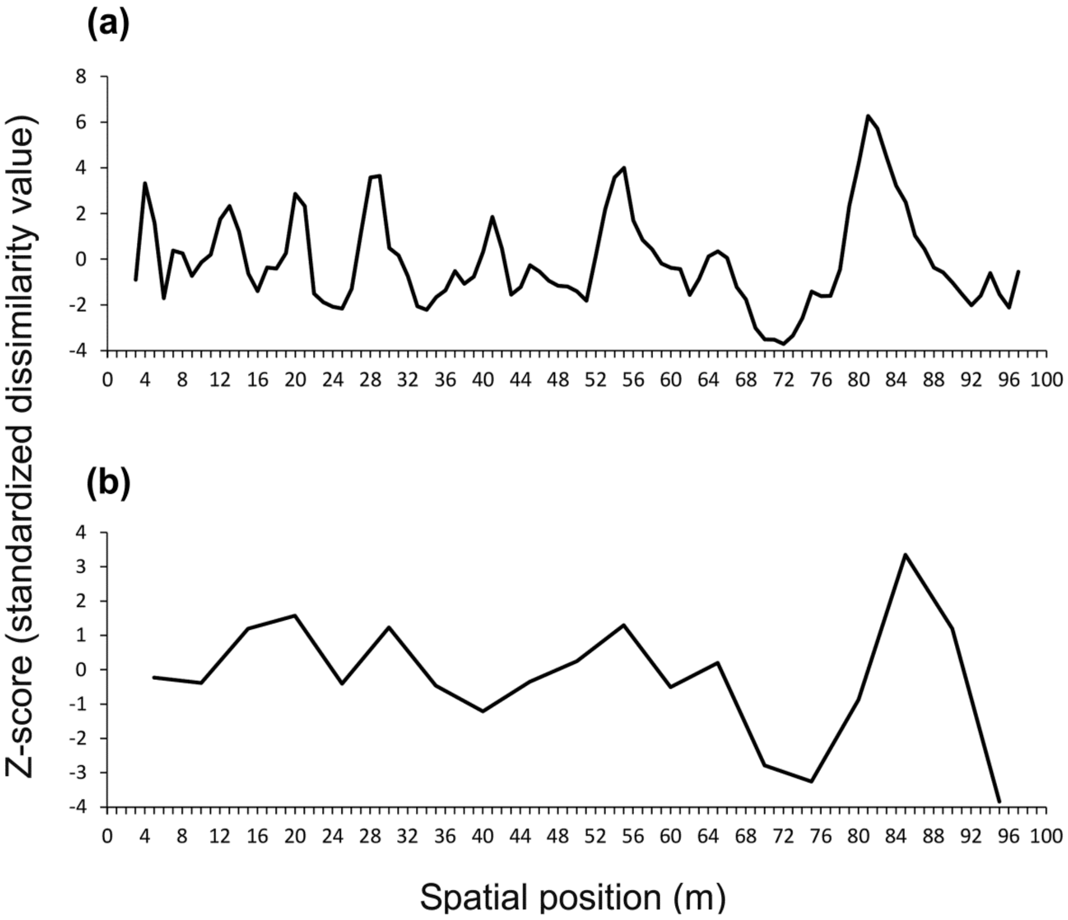

Figure 2. *If vegetation is patchy, some spatial information may be lost when applying non-contiguous quadrates. More peaks appear in the case of (a) contiguous quadrates than in the case of (b) non-contiguous ones, where a distance of 4 m was set between neighbouring plots. In both cases, z-scores were averaged over window widths that were equal to 6 m – 16 m. Note the different scale of the vertical axes. Data originate from a 100 m long transect in a xeric grassland-forest mosaic*

In the 40 studies we reviewed, a total of ten different comparative indices were used (*Table 2*), with the Squared Euclidean Distance (SED) as the most common among them. The SED index is favoured because it is quite sensitive and usually results in high peaks, although it does have some undesirable characteristics as well. Firstly, it is scale-dependent (this property can be reduced by averaging standardized values over several window sizes). Secondly, it is very sensitive to differences in species abundaces, overemphasizing even minor differences, but cannot distinguish between abundance differences and compositional differences (Brunt and Conley, 1990; Körmöczi, 2005).

The window widths used in different studies vary considerably (*Table 2*), since the choice depends on the focus of the study and on the data resolution (Choesin and Boerner, 2002). Generally, too small window widths often make results hard to interpret due to high sample-to-sample noise (Brunt and Conley, 1990; Choesin and Boerner 2002). This phenomenon is a result of internal inhomogeneity of the vegetation patches (Whittaker, 1960; Zalatnai and Körmöczi, 2004). On the other hand, it is assumed that valuable information may be lost when using too wide windows, because this can

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homogenize small patches (Choesin and Boerner, 2002; Kröger et al., 2009). Based on his experience with soil transects, Webster (1978) suggested that, in a patchy environment, window widths equal to two third of the assumed patch sizes should be used. In practice, usually various window widths are tested before the seemingly best width is chosen for final data analysis (e.g. Nishimura and Kohyama, 2002; Camarero et al., 2006; Xu et al., 2012). To reduce scale-dependence of the analysis, it is advisable to average the results from several window widths (e.g. Muñoz-Reinoso and García Novo, 2000, 2005; Boughton et al., 2006) because peaks in the resulting MSW profile are less scale-dependent than peaks from individual window widths.

Concerning window widths, the overwhelming majority of the studies using MSW fall into one of the following three categories (*Table 2*). 17 of the 40 reviewed articles (42.5%) opted for a single window width that was considered “not too small” and “not too large”. Four studies (10%) used a few very similar window widths. Finally, 12 researches (30%) were carried out using strongly different window widths, but the results were averaged (i.e. dissimilarity values from all window widths were averaged). Beside their advantages, all of the above procedures have a serious drawback: they cannot differentiate between coarse-scale and fine-scale boundaries. As Ludwig and Tongway (1995) pointed out, the detection of boundaries at different spatial scales requires the use of different window widths without averaging them. Wide windows identify coarse-scale boundaries, while small windows detect fine-scale boundaries. Erdős et al. (2013) drew the same conclusion from simulated and field data. In sum, if the complex pattern of a landscape is to be revealed, including both coarse-scale and fine-scale boundaries, small and large windows should also be used in the MSW analyses.

Significance tests

The most challenging issue concerning the MSW method is the significance test of the peaks (Körmöczi, 2005). Of the studies under review, 45% did not use any test to determine which peaks in the profile can be considered significant (*Table 3*), whereas another 7.5% used an arbitrary confidence limit.

The simplest way of selecting significant peaks is to assume normal distribution of dissimilarity. In this case, peaks over the value of P probability should be considered significant. Randomizations provide an opportunity to test whether a detected peak differs significantly from those appearing in a random pattern (Fagan et al., 2003). Some authors apply random reference to compute the expected mean and standard deviation (SD), and consider peaks significant if they exceed mean plus one (Muñoz-Reinoso and García Novo, 2000, 2005; Muñoz-Reinoso, 2001; Laliberte et al., 2007) or two standard deviations (Cornelius and Reynolds, 1991; Körmöczi and Jusztin, 2003; Zalatnai and Körmöczi, 2004; Körmöczi, 2005). Expected mean value, however, depends on the window width and the type of randomization (Cornelius and Reynolds, 1991; Körmöczi, 2005). Therefore, *z*-score transformation is often used to make the dissimilarity profile less scale dependent (Cornelius and Reynolds, 1991). *Z*-score transformation is a normalizing transformation, and calculated as follows:

$$z_k = \frac{d_{i,k} - d_{\exp,k}}{SD_{\exp,k}} \qquad \text{(Eq.1)}$$

where z_k is the standardized dissimilarity value, $d_{i,k}$ is the dissimilarity value at position i if the half-window size is k, $d_{exp,k}$ is the overall mean of the dissimilarity value from randomized data for half-window size k (expected mean), and $SD_{exp,k}$ is the standard deviation of the dissimilarity values from the randomized data for half-window size k. The probabilities of standard normal distribution may be applicable as confidence limits (Hennenberg et al., 2005; Boughton et al., 2006; Zalatnai et al., 2007, 2008; Erdős et al., 2011).

Overall mean dissimilarity and standard deviation vary with transect length and boundary numbers. This has profound influences on the statistical significance of the boundaries. It has been demonstrated that the shorter the transect is and the more boundaries are along it, the higher the chance that the MSW peaks will not be significant (Körmöczi, 2005).

In a recent study, we compared the distribution of the expected dissimilarities with normal distribution (Erdős et al., 2012). Unlike Cornelius and Reynolds (1991), we found significant deviation from the normal distribution: the observed distribution was skewed to the right, and the confidence limits proved to be higher than for the normal distribution. Therefore, z=1.85 was applied as critical value for P=0.05, instead of z=1.65. Pärn et al. (2010) also mention the deviation of the distribution of Euclidean distances from normal distribution, without any further consideration or analysis.

Randomization procedure

As mentioned above, the type of the randomization is also a crucial point of the analysis since it provides the null model. Expected mean dissimilarity and standard deviation can be calculated from random reference. Randomization of data can be carried out in several different ways (*Table 3*). Usually, Monte Carlo procedure is applied in the form of either completely mixing the abundance values of the species (*Fig. 3a*), or with random relocation of plots (*Fig. 3b*) (Cornelius and Reynolds, 1991; Hennenberg et al., 2005). However, Palmer and van der Maarel (1995) and Fortin et al. (1996) suggested the random shift method, during which the species distributions are randomly shifted relative to one another (*Fig. 3c*). According to Fagan et al. (2003), complete randomization should be avoided. In fact, both random relocation of plots and random shift have a sound ecological meaning: plot relocation preserves within-plot co-occurrences of species, while random shift preserves spatial pattern of species distributions (cf. Horváth, 1998).

In a comparison of the above three randomizations, we found that z-scores remain more stable with increasing window widths in the case of random shift than in the case of the other two randomizations (*Fig. 4*). The phenomenon appeared in several different habitat types as well as in analyses of simulated patterns. This should be considered a potential advantage of random shift, since it may ease interpretation of the z-score profile when strongly different window widths have to be used.

As for the number of randomizations, usually 1000 iterations are applied (*Table 3*). This number is high enough, since the expected mean and standard deviation are stabilized over 100 randomizations, as reported by Körmöczi (2005).

***Table 3.** Significance tests and randomization procedures applied in 40 vegetation studies using the MSW. Question marks appear where details were not given in the cited article*

	Author(s)	Significance test	Distribution considered	Randomization type	Number of randomizations
1	Azevedo and Caçador 1999	–	–	–	–
2	Bátori et al. 2014	z>1.85	random reference	plot r.	1000
3	Beals 1969	–	–	–	–
4	Boughton et al. 2006	z>1.65	normal distribution	plot r.	1000
5	Camarero et al. 2006	D>90%	–	–	–
6	Chang et al. 2003	–	–	–	–
7	Choesin 2001	SED>3500	–	–	–
8	Choesin and Boerner 2002	SED>3500	–	–	–
9	Dupuch and Fortin 2013	–	–	–	–
10	Erdős et al. 2011	z>1.65	normal distribution	r. shift	99
11	Erdős et al. 2012	z>1.85	random reference	r. shift	99
12	Erdős et al. 2013	z>1.85	random reference	r. shift	99
13	Harper and Macdonald 2001	–	–	–	–
14	Hennenberg et al. 2005	z>1.65	normal distribution	plot r.	1000
15	Ibanez et al. 2013	z>1.65	normal distribution	?	?
16	Kirkman et al. 1998	–	–	–	–
17	Körmöczi and Jusztin 2003	SED>mean+2SD, DREN>mean+2SD	normal distribution	r. shift	1000
18	Kröger et al. 2009	–	–	–	–
19	Kröger and Rogers 2005	–	–	–	–
20	Laliberte et al. 2007	SED>mean+SD	no consideration	–	–
21	Li et al. 2005	–	–	–	–
22	Li et al. 2007	–	–	–	–
23	Ludwig and Tongway 1995	–	–	–	–
24	Muños-Reinoso 2001	z>mean+SD	no consideration	?	?
25	Muños-Reinoso and García Novo 2000	z>mean+SD + MRPP	no consideration	plot r.	?
26	Muños-Reinoso and García Novo 2005	z>mean+SD + MRPP	no consideration	plot r.	?
27	Muños-Reinoso 2009	MRPP	distribution free	?	?
28	Nishimura and Kohyama 2002	–	–	–	–
29	Pärn et al. 2010	one tailed, 95% limit	random reference	plot r.	1000
30	Ross et al. 2001	–	–	–	–
31	Stanisci et al. 2000	–	–	–	–
32	Tolman 2006	–	–	–	–
33	Torma and Körmöczi 2009	z>1.65	normal distribution	r. shift	1000
34	Watkins et al. 2003	SED>mean+2SD	normal distribution	–	–
35	Wierenga et al. 1987	–	–	–	–
36	Cornelius et al. 1991	–	–	–	–
37	Xu et al. 2012	–	–	–	–
38	Zalatnai and Körmöczi 2004	DREN>mean+2SD	normal distribution	complete	1000
39	Zalatnai et al. 2007	z>1.65	normal distribution	r. shift	1000
40	Zalatnai et al. 2008	z>1.65	normal distribution	r. shift	1000

Softwares for MSW-computations

The computation of dissimilarity metrics in the MSW analysis is not a big challenge, it can be performed even with a spreadsheet software (e.g. Choesin, 2001). This works well with a single window width, but dealing with multiple window widths and handling randomized data are rather difficult in such a way. The PASSaGE software also contains the computation of a dissimilarity profile (Rosenberg and Anderson, 2011). This software package is declared as “a free, integrated, easy-to-use software package for performing spatial analysis and statistics on biological and other data” (http://www.passagesoftware.net/). Unfortunately, it is also limited to a single window size, and is without random reference. Computations can be done in the R environment.

For example, the software of Rossiter (http://www.itc.nl/personal/rossiter/teach/R/R_OptPart.pdf) enables to carry out MSW-analyses using multiple window widths and it offers two dissimilarity indices. However, it does not offer any randomizations.

We used in this paper and in earlier works our own software that was developed in the statistical language R (version 2.10.1, www.R-project.org). The source code was published in Erdős et al. (2014).

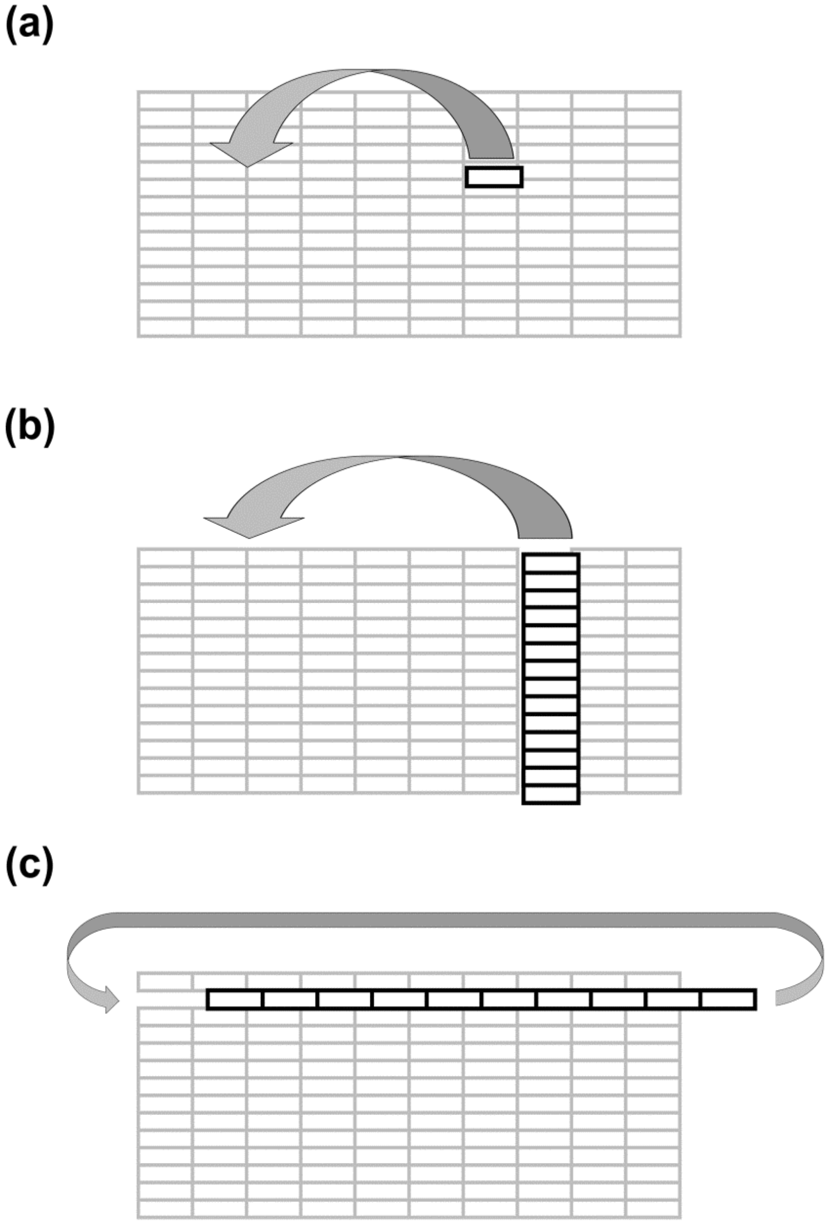

Figure 3. *Three possible randomization types, applied in earlier MSW studies. Each row corresponds to a species and each column to a relevé. (a) In the case of complete randomization, data are randomly relocated within their own row. (b) Plot randomization relocates each relevé into a new position along the transect. (c) During random shift, data belonging to the same species are shifted along the transect. Values shifted beyond the end of the transect are wrapped back on to the opposite end*

Conclusions and future directions

An ever increasing literature on the moving split window clearly indicates the method's usefulness in vegetation science. We conclude that it is a reliable technique for the analysis of boundaries, and may prove effective in any habitat type.

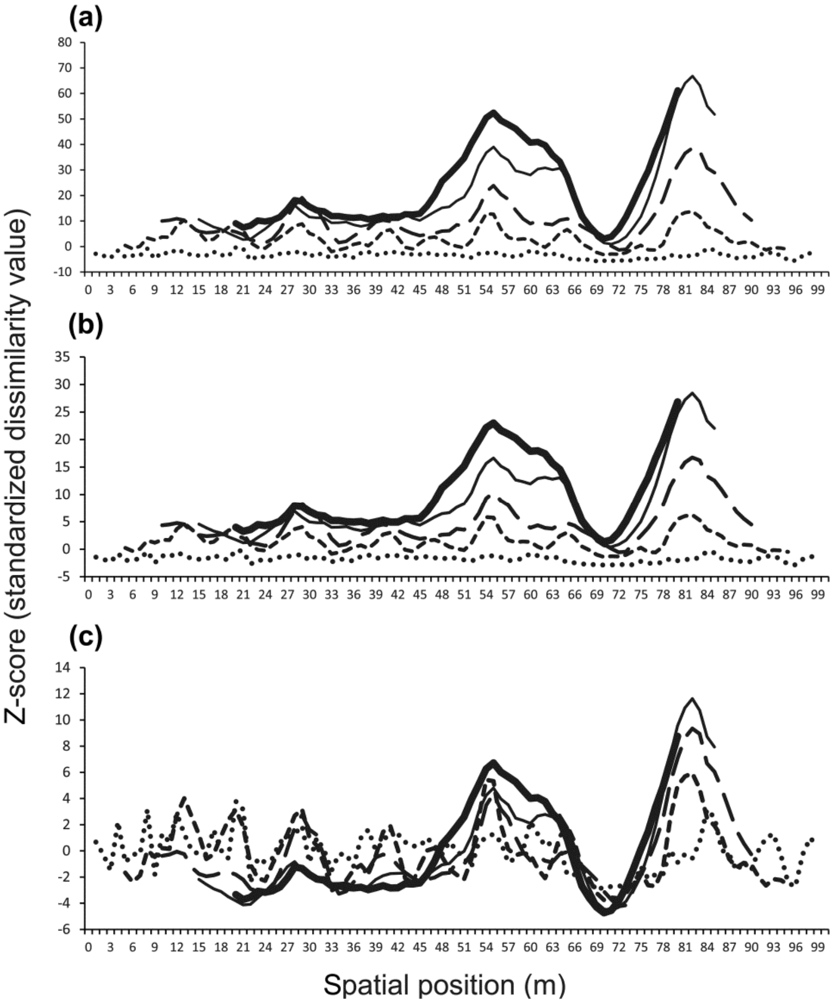

Figure 4. *Dependence of z-scores on window width. If window width is changed, z-scores vary considerably in the case of (a) complete randomization and (b) plot randomization, but there is small variability if (c) random shift is used. Note the different scale of the vertical axes. Data originate from a 100 m long transect in a xeric grassland-forest mosaic. Different lines indicate different window widths*

As we see it, a wider application of the MSW, accompanied by appropriate setting of the parameters (mainly window width) and correct statistical tests would result in a deeper understanding of the vegetation pattern and organization. We identified four areas where the MSW method holds promise for future research.

Firstly, MSW has the potential to provide objective and accurate information for some of the basic controversies in vegetation science: it can broaden our knowledge on vegetation continuity vs. discontinuity as well as on abiotic-biotic relations, issues connected to the Clements-Gleason debate. Although there have been important contributions in these topics, we think that a more wide-spread use of the MSW, followed by correct interpretation would support further development. It is generally accepted that in nature, both sharp and blurred boundaries occur, and continuous transitions (without boundaries) are also possible (e.g. Hobbs, 1986; Cadenasso et al., 2003; Fagan et al., 2003), and this observation has been verified by MSW analyses as well (e.g. Beals, 1969; Kirkman et al., 1998; Ibanez et al., 2013). However, it should be emphasized that this does not say much about the Clements-Gleason controversy, since Clements' and Gleason's perspectives are often misinterpreted. For example, Gleason (1926) did not deny the existence of sharp boundaries between plant communities. To have a more correct understanding of the original concepts, abiotic-biotic relations should be taken into consideration. According to Clements (1936), relatively sharp vegetation boundaries form even if the gradient of the background factors is continuous. In contrast, Gleason (1926) predicts that sharp vegetation boundaries appear only where there are abrupt changes in background factors (for a more complete view on this topic, see Daubenmire, 1966; Whittaker, 1967; Ricklefs, 1973; Begon et al., 1990; Collins et al., 1993; Callaway, 1997; Hoagland and Collins, 1997; Kent et al., 1997; Wilson, 2004, 2009; Austin, 2005). Through rigorous analyses of biotic and abiotic gradients, MSW may contribute to the knowledge on this topic.

Secondly, it is presumed that boundaries react sensitively to local, regional and global changes (Gosz and Sharpe, 1989; Fortin et al., 2000). Therefore, long-term monitoring projects are necessary to track alterations in boundary position and structure. Using the MSW method, it can be studied whether boundaries can change position or remain stable, whether their sharpness changes or not, whether they can disappear or new boundaries appear where they were not present earlier. Unfortunately, to the best of our knowledge, only five studies used MSW to answer similar questions. Four of them (Stanisci et al., 2000; Boughton et al., 2006; Dupuch and Fortin, 2013; Ibanez et al., 2013) used indirect methods (i.e. they inferred temporal dynamics from spatial information). The fifth one (Körmöczi and Jusztin, 2003) provided a real temporal analysis, but only for four years.

Thirdly, cross-scale studies are among the most challenging and promising directions in the ecology of boundaries (Gosz, 1993; Laurance et al., 2001). With the help of MSW, it is possible to analyse boundaries on different spatial scales and on different hierarchical levels at the same time. This can be used for a better understanding of the hierarchical organization of landscapes and vegetation. However, this capacity has rarely been used so far. If boundaries of different scales are to be distinguished, strongly different window widths have to be used, without averaging the results. This would enable a multi-level vegetation study, as was carried out by Ludwig and Tongway (1995) and Erdős et al. (2013, 2014).

Finally, Kröger et al. (2009) have shown that MSW may help reveal two dimensional vegetation pattern. In our opinion, if the huge data requirements of the wombling method cannot be supplied, the approach of Kröger et al. (2009) should be used, provided the somewhat uncertain results are handled carefully.

As for the MSW layouts, we suggest that contiguous quadrates be used whenever possible. Although several dissimilarity indices have been used, we think that SED is probably the best among them. Also, since the majority of the former studies used SED, using SED in any future studies will make their results easier to compare to the former ones. Since distance functions are insensitive to the common information that the neighbouring plots share, functions other than distance functions (e.g. complement of the Renkonen index) may also be used in additional analyses.

Carrying out a significance test is strongly advisable for the correct interpretation of the results. On the basis of a great number of analyses we found that z-transformed peaks over 1.85 should be accepted as significant at $P<0.05$. Exploration of the distribution and the scale-dependence of significance values is in progress, and will be reported later.

When comparing the three different randomization procedures, results were found similar, though random shift can be considered the best choice for two reasons, namely because z-scores are less scale-dependent with random shift, than with the other two randomizations (*Fig. 4*), and because the random shift method preserves the population patterns, which should be regarded as a desirable property.

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