University of South Bohemia
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Biological diversity: measures of ecosystem functioning and implications for restoration ecology

Ph.D. Thesis

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Annotation. The ecological significance of biodiversity mechanisms and the plant community development in a harsh environments of mined peatlands were studied. Investigations were also focused on processes affecting species coexistence in grassland and peatland communities with using various experimental approaches.

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I hereby decleare that I worked out this thesis on my own using the cited literature only.

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Introduction

Introduction

Biological diversity of the Earth and its origins have long been a source of amazement and curiosity and an area of scientific research, at least since Wallace and Darwin. Current interest in biodiversity is focused on why there are so many species and on how diversity impacts populations and ecosystem processes, questions which are both the central topic of this thesis. Many observational and review studies conclude, however, that despite all the effort, ecological consequences of biodiversity changes are still poorly understood (e.g. Kinzig et al. 2001, Levin 2001, Loreau et al. 2002, Wardle 2002, van der Maarel 2005). For example, we still do not fully understand how hundreds of plants and thousands of insects coexist on a hectare of rain forest. Because these mechanisms are poorly understood, we have only a blurry vision of the long-term impacts of habitat conversion and destruction, invasion of exotic species, nutrient enrichment, and other anthropogenic changes leading to species extinction. The accelerating effects of human activities on biodiversity and the possibility that the loss of biodiversity might impact ecosystem functioning renewed interest in the effects of diversity on ecosystem processes. Recent work on the consequences of changes in biodiversity has led to both insights and debates (e.g. Johnson et al. 1996, Bengtsson et al. 1997, Kaiser 2000, Cameron 2002), as often occurs when ideas are young and paradigms are challenged. Major debates concern the relationship between plant community diversity and productivity (or generally, ecosystem functioning) and focuses on the proper ways how to design and interpret diversity experiments (e.g. Garnier et al. 1997, Huston 1997, Allison 1999). There are no shortcuts in biodiversity research – we need more basic information about more groups, and not just species list, but the functional relationships: but who does what and with whom.

Despite the current ascent on the diversity, it is the actual species composition playing the critical role in the dynamics and functioning of ecosystems. Species composition is likely to be one of the major determinants of stability, primary productivity, nutrient dynamics, invasibility and other ecosystem traits. By definition, composition and diversity are interdependent in both natural and managed ecosystems, making it difficult to unambiguously attribute effect to one or the other based on observational studies. To attribute effects to diversity requires comparison of sites that differ in diversity, but do not differ, on average, in proportional species representation. This could be achieved experimentally by having the species composition of many plots determined by a separate, random draw of species from a species pool, and by comparing mean responses for sets of plots that differ in diversity. On average, across many such independent draws at a given level of diversity, each species would have an equal chance of being present in a plot, thus controlling for effects solely attributable to composition. Random draw can be replaced by regular selection of species assuring that each of them will be equally represented at all the diversity levels. Similarly, to attribute effect to composition, it is necessary to control for differences in diversity. Therefore, this approach is used to distinguish between effects caused by diversity versus composition in my biodiversity experiments.

Differences in plant composition may have large effects on ecosystem processes, particularly, if few species posses the traits that enable them to dominate the whole community. For example, if one species or group of species reduces soil nutrients to a lower level than do other species, then this species (or group) may dominate soil nutrients consumption in a mixture. Some studies found that composition (the identity of species or functional groups present) explained much more variance than did richness (the number of species or functional group present) (e.g. Díaz and Cabido 2001). Objective identification of functional groups is therefore of great potential interest (Simberloff and Dayan 1991, Smith et al. 1997). Functional groups are used to reduce system complexity by aggregating species by some shared role that they play in a particular ecosystem function. Functional groups are simply a convenient way how to category phenotypic or functional diversity. However, their determination is always arbitrary (to some extend), and depends on the objectives and types of the study. In particular, they are likely to differ for short-term and long-term biodiversity effects.

Testing whether increased biodiversity enhances ecosystem function would be shown via heuristic equation $F = f(\mathbf{d}, \mathbf{B}, \mathbf{f}, \mathbf{P}, \mathbf{E})$ (modified according to Naeem 2002). If we consider productivity to be a function under study, then, in a very simplified fashion, it is a function of the densities of photosynthetic species (**d**), the matrix of interactions among them (**B**), soil nutrient content (**f**), the rates of transition of these nutrients between available inorganic forms (**P**), and environmental conditions (**E**). We can say that a specific ecosystem function (F) is a function of biodiversity (**d**, **B**), associated biogeochemical processes (**f**, **P**), and the abiotic environment (**A**). Three different hypotheses that summarized much of the thinking about diversity and ecosystem function at the time were posed (Loreau et al. 2002): diversity shows (i) no relationship (null) with ecosystem function, (ii) a linear relationship, or (iii) an asymptotic relationship in which a small amount of diversity was responsible for the bulk of an ecosystem`s function. Since then, large number of hypotheses reflects the fact that a bivariate space is inadequate for the best understanding of the relationship between biodiversity and ecosystem function, as was shown by the equation.

In the first part of this thesis I present results from the biodiversity experiments conducted using plant assemblages combining various levels of species and functional group richness, investigated under various field and greenhouse conditions. In this way the experiments are able at least partly to resolve basic uncertainties in the biodiversity-ecosystem functioning approach. Experimental designs allow me to test between conflicting hypotheses and to attribute effects of biodiversity on ecosystem processes to particular components of plant biodiversity such as species and functional group richness.

According to some hypotheses, large proportion of native species richness is required to maximize ecosystem stability and to sustain function (e.g. Lepš 2005). The assessment of diversity-productivity relationships is important for conservation strategies because sustainability of ecosystem functions has been used as an argument for a precautionary approach in conservation of species and for decision-making processes (Schwartz et al. 1999). The restoration of ecological communities previously intensively managed is a major tool to counteract biodiversity losses. In this way, we need to recognize if loss of biodiversity can have negative impacts on ecosystem functioning, which is combined with uncertainty over the current and future roles of most species in many ecosystem processes (Walker and del Moral 2003). These facts led me to present here results of manipulated experiments carried out in man-disturbed habitats of mined peatlands because there is a lack of knowledge about the effects of changing biodiversity on these ecosystems (the second part of the thesis).

Certainly peatlands are vitally important to the biodiversity conservation of the planet and provide a heaven for a wealth of unusual and specially adapted organisms. In their natural state, peat bogs are a unique ecosystem in which atmospheric carbon is sequestered as peat for long-term periods (Vasander 1996). However, composed primarily of *Sphagnum* mosses and sedges, peat is extracted for horticultural or fuel purposes on large expanses by modern milling technology (Chapman 2002). When the peat deposit has been exhausted, the site is completely abandoned (e.g. Girard et al. 2002, Lavoie et al. 2003). The peatland remaining after peat extraction is not a favorable place for re-growth of many peatland species. The surface of the residual peat deposit is dry and devoid of viable seeds (e.g. Salonen 1987, Stoneman and Brooks 1997). Regardless, some plants have the ability to colonize dry peat surface, and seem to help stabilize the soil surface and facilitate the establishment of other plants (Groeneveld and Rochefort 2002). Understanding of a processes that favor the spontaneous regeneration of peatlands is thus an essential step in restoration programs (Price et al. 2003). However, in this unique environment it is possible to study not only mechanisms of early succession but also specific mechanisms of facilitation and species-by-species replacement. Studies focusing on these aspects can also help us to find some successional patterns and partly look at the mechanisms of species coexistence and exclusion, processes responsible for increasing or maintaining plant species diversity.

The main objectives of various biodiversity experiments in this thesis were: to (i) evaluate biodiversity effects, (ii) determine the effects of species number, functional group number and soil nutrients on community productivity, (iii) test if species-rich communities are more resistant to slug grazing than species-poor communities and (iv) how is the resistance to invasion affected by species and functional group richness of sown residents and how is the resistance to invasion affected by species composition of the sown mixture. Additionally, I present results of: (v) the facilitating effect of a pioneer plant *Eriophorum angustifolium* on the growth of another plants in harsh conditions of a post-mined peat bog, (vi) the effects of different levels of water table depth and other specific factors on the growth of saplings *Betula pubescens* during the course of plant succession in a post-mined peat bog, (vii) the effectiveness of dam construction on species-by-species replacement and development of mire plant assemblages in a mire forest.

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Paper I

Effect of functional group richness and species richness in manipulated productivity-diversity studies: a glasshouse pot experiment

Effect of functional group richness and species richness in manipulated productivity-diversity studies: a glasshouse pot experiment

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Abstract. Species and functional group (grasses, legumes, creeping nonlegume forbs, rosette nonlegume forbs) richness of species assemblages composed of 16 species from four functional plant groups were manipulated to evaluate the productivity-diversity relationships in a greenhouse pot experiment. Pots were filled with sand, and supplied at two levels of nutrients. The plants were grown in monocultures, 2, 4, 8 and 16 species mixtures. Individual 2, 4, and 8 species mixtures differed in the richness of functional groups. Although the two characteristics of biodiversity, i.e. species and functional group richness, were necessarily correlated, it was shown that it is possible to separate their effect statistically, and also test for their common effect without pronounced loss of test power. There was a pronounced increase of average aboveground biomass and a mild increase in belowground biomass with biodiversity. The effect of functional group richness was more pronounced than the effect of the number of species. By using the method of Loreau and Hector (2001), selection and complementarity effects were statistically separated, and the overyielding index was calculated as a ratio of the productivity of a mixture to the productivity of its most productive component (to demonstrate transgressive overyielding). Positive values of complementarity and transgressive overyielding were both found, particularly in some rich communities and under high nutrient levels. Complementarity significantly increased only with functional group richness and mainly under high nutrients in the belowground biomass. Some species, when grown in monocultures, had decreased productivity under higher nutrients, and thus were more productive in mixtures than in monocultures. It seems that those species suffered from too high nutrient levels when grown in monocultures, but not in the presence of other species, which were able to use the nutrients in high concentrations and effectively decrease the nutrient levels. As a consequence, mixtures of high diversity were always more productive under high nutrients. The difference in species proportions between high and low nutrients, characterized by chord distance, increased with species richness. The relative change in productivity decreased with the number of functional groups. This suggests that species richness might lead to stabilization of aggregate characteristics (like total productivity) under changing environmental conditions by changing the proportions of individual species.

Keywords: Complementarity, Overyielding, Selection effect, Species richness, Plant functional groups

Introduction

The critical role of species composition and richness in the dynamics and functioning of ecosystems has been discussed many times over the past two decades. The dependence of productivity on plant species diversity is a hotly debated topic (Naeem et al., 1994; Tilman et al., 1996; Tilman, 1997a; Grime, 2001). Diversity experiments, where the number of species is manipulated and ecosystem functioning (most often productivity) is measured as a response variable, became a standard part of experimental ecology (Naeem and Li, 1997; Hector et al., 1999; Tilman, 1999; van der Putten et al., 2000; Tilman et al., 2001). However, their proper design and interpretation of their results is still an open question (Johnson et al., 1996; Bengtsson et al., 1997; Grime, 1997; Huston, 1997; Tilman, 1997a, 1997b; Hodgson et al., 1998; Lawton et al., 1998; Naeem et al., 1999; Sankaran and McNaughton, 1999; Hector et al., 2000; Huston et al., 2000; Kaiser, 2000; Naeem et al., 2000; Wardle et al., 2000; Lepš et al., 2001; Cameron, 2002). The observed response of ecosystem processes to species or functional group diversity can be generated by a combination of different effects (Tilman, 1997a; Loreau, 1998a, 2000; Loreau and Hector, 2001). The complementarity effect arises from niche differentiation: When two species use resources in different ways, their mixture should be able to use the resources more effectively than corresponding monocultures, and consequently, achieve higher productivity. Unless there is intimate knowledge of the mechanisms of species interaction, we are not able to distinguish direct facilitation from niche differentiation, and consequently, both mechanisms are often subsumed under the name complementarity (Loreau and Hector, 2001). Second, the sampling effect model (Aarssen, 1997; Huston, 1997; Tilman et al., 1997) hypothesizes that species differ in their competitive abilities, and that better competitors are also more productive. Under this assumption, communities that have greater diversity should, on average, be more productive because they are more likely to contain one or more highly productive species (selection or chance effect is sometimes used in the same or very similar meaning). Whereas complementarity is generally accepted as a genuine biodiversity effect, it is often questioned whether the sampling effect is no more than an artifact of the design of biodiversity experiments (e.g. Huston, 1997).

The most convincing evidence that biodiversity is beneficial would come from a demonstration of overyielding, i.e. the productivity of a species mixture being higher than the productivity of any of its constituent species grown in isolation (trasnsgressive overyielding sensu Hector et al., 2002). Transgressive overyielding can not be achieved through the sampling effect, and is, consequently, proof that other mechanisms ("complementarity") played a role. Similarly, only overyielding demonstrates that the mixture's function (productivity) can not be replaced by any of the monocultures. Obviously, monocultures of all the species are required to demonstrate overyielding (Garnier et al., 1997). Recently, Loreau and Hector (2001) suggested a new method that enables numerical separation of the sampling and complementarity effects. Their method is based on the RYT (Relative Yield Total, De Witt, 1960) philosophy, and does not require transgressive overyielding to demonstrate complementarity. Their method is based on non-transgressive overyielding (i.e. productivity of a mixture being higher than the weighted average of the corresponding monocultures).

Many researchers consider ecosystem processes to be more consistently associated with functional composition (presence of certain plant functional types or traits) and/or functional richness (number of different plant functional types) than with species richness itself (Lepš et al., 1982; Grime et al., 1997; Hooper, 1998; Hooper and Vitousek, 1998; for a comprehensive review see Díaz and Cabido, 2001). Symstad et al. (1998) and Spehn et al. (2002) found that, whereas total plant biomass increases with diversity, most of the diversity effects are attributable to the presence of the functional group of N-fixers. According to Symstad (2000), higher functional group richness increases ecosystem stability, particularly the resistance to invasion.

The belowground environment is more heterogeneous and, consequently, provides more opportunities for complementarity than the aboveground part of the ecosystem. Also, because the importance of competition for light increases with increasing nutrients, it was hypothesized that complementarity should be expected mainly in low nutrient environments.

Lehman and Tilman (2000) predicted that species rich communities should be more stable in aggregated characteristics, like total biomass, but that this stability can be at the expense of the stability of individual populations. Using similar reasoning, the difference between total biomass of assemblages with the same species composition, but grown under a different nutrient regime, should decrease with diversity, but their compositional dissimilarity should increase.

To test these hypotheses, a glasshouse pot experiment was performed, where species richness and functional richness of plant assemblages, and fertility of their environment were manipulated. We used 16 perennial grassland species grouped in 4 functional types, planted in ten possible combinations of species and functional group richness, and at two nutrient levels. The pot experiments lack the variability of natural habitats, and consequently restrict (in comparison with field conditions) the possibility of niche differentiation. All of those restrictions have to be taken into account in interpreting the results. However, the pot experiments can be much more replicated than field experiments (which is very important, particularly as we need the monocultures of all of the constituent species), and enable better control of environmental conditions.

The aim of the study was to evaluate diversity effects on productivity in our experimental mixtures, and to separate the effects of species number, functional group number and soil nutrients on these effects. Special attention was paid to differences in the responses of above and belowground biomass.

Materials and methods

Selected experimental species

We selected 16 species which are common and often grow together in mesotrophic meadows in Central Europe. All of them are polycarpic perennials, and can be classified into four functional groups: four grass species - the narrow leaved *Festuca rubra*; the broad-leaved *Trisetum flavescens, Alopecurus pratensis* and *Holcus lanatus*; 4 rosette hemicryptophytes - *Lychnis flos-cuculi*, *Hypochaeris radicata*, *Plantago media* and *Leontodon autumnalis*; four herbs with creeping aboveground stolons - *Veronica officinalis*, *Glechoma hederacea*, *Fragaria vesca* and *Prunella vulgaris*; 4 nitrogen-fixers (legumes) - *Lotus corniculatus*, *Anthyllis vulneraria*, *Trifolium pratense* and *Lathyrus pratensis*.

Experimental design

The glasshouse experiment was set up in early May 2002. Round pots (diameter 19 cm, height 18 cm) were filled with sand. Seeds of 1 – 16 species were sown at the same density, so that each pot contained 160 seeds, and were grown under two nutrient levels. The low nutrient treatment was fertilized each week with a commercial formula (Kristalon start: N 19

Table 1. Treatments, number of species combinations and number of replications used in each, high and low nutrients pots.

Treatment	Number of species in mixture	Number of species combinations	Number of replicates
1	monoculture of each species	16	2x
2	2 species within one functional group	8	1x
3	2 species from two groups	8	1x
4	4 species (each species is from one group)	4	1x
5	4 species (each two species are from different groups)	4	2x
6	4 species that composed one functional group	4	1x
7	8 species from two groups	6	1x
8	8 species from three groups	8	1x
9	8 species from four groups	6	1x
10	16 species (i.e., four functional groups)	1	8x

%, P_2O_5 6 %, K_2O 20 % and MgO 3 %) in concentration of 10 g per 10 l of water, while the concentration in the high nutrient treatment was 20 g per 10 l of water. The experiment was set in a completely randomized design. Ten combinations of species and functional group richness were used, with different species combinations within each combination (treatments, Table 1), yielding 184 pots at the beginning of the experiment. All species were equally represented at each species richness and functional group richness. An equal number of seeds of each species was sown in each combination. As far as possible, all of the functional groups were equally represented in mixtures containing more functional groups (in mixtures with three functional groups and eight species, the ratio of species belonging to individual groups was 3:3:2). During the experiment, plants were grown under natural day-light conditions in a glasshouse and watered when needed. Six pots were lost for various reasons during the experiment. After 4 months, soil from all 178 pots was rinsed thoroughly, plants were sorted into species, dried and weighed (shoots, i.e. aboveground biomass and roots, i.e. belowground biomass separately).

Data analysis

Productivity was characterized by above and belowground biomass. For each mixture, the Overyielding index and the complementarity and selection effects were calculated and estimated. The overyielding index (*OI*) was calculated by

$$OI = Y / MAX(M_i),$$

where Y is biomass of a mixture and M_i is the biomass of i-th species grown in monoculture. *OI* is equivalent to the I_1 index of Garnier et al. (1997) and to D_{max} (Loreau, 1998a). For statistical analyses, log (*OI*) was used because it is centered around zero in the case that the productivity of the mixture is the same as the productivity of the most productive monoculture (Špaeková and Lepš, 2001). The average value of two replicates of each monoculture was used as M_i.

The method of Loreau and Hector (2001) was used for partitioning selection and complementarity effects. According to them, the net biodiversity effect, ΔY, is characterized by the difference between the observed yield of a mixture and its expected yield under the null hypothesis that there is no selection and complementarity effect:

$$\Delta Y = Y_O - Y_E = N\overline{\Delta RY}\,\overline{M} + N\,\mathrm{cov}(\Delta RY, M),$$

where ΔRY_i is the deviation from expected relative yield of species i in mixture, calculated as the difference between expected and observed relative yields. The observed relative yield of a species in mixture is the ratio of its yield in mixture and its yield in monoculture. The expected relative yield is the proportion of the species sown. The complementarity effect is proportional to the average of ΔRY over all species in the mixture, whereas selection is proportional to the covariance of ΔRY and yield of the species in monoculture. N is the number of species in a community.

The dependence of above and belowground biomass and log (*OI*), complementarity and selection effects on the number of species, number of functional groups and nutrient level was analyzed by general linear models (GLM). Species richness and functional group richness were continuous predictors, and nutrient level the categorial predictor. The number of species and number of functional groups are inevitably correlated predictors: the number of functional groups can not exceed the number of species. Also, because there was a closed species pool, the 16 species treatment means inevitably the highest number of functional groups. In GLM, the test of partial effects statistically separates the unique effects of functional group richness and species richness, and consequently, enables their comparison. However, because of correlation between the number of species and number of functional groups, there can be a large shared effect between these two. Consequently, both partial effects can be non-significant, whereas their common effect and their marginal (i.e. effects in absence of the correlated predictor) effects are highly significant. Consequently, we tested also for the common effect (called biodiversity) and for the marginal effects. As the aim of the study was whether biodiversity effects are the same under low and high nutrients, parallelisms (interactions by covariates) were also tested for. Significant result of this test means that the lines are nor parallel, and so the effects are different under low and high nutrients. To stress this, the effect will be called “Interaction” in further text. In analyzes of dependence of differences between high and low nutrients on the diversity measures, there is no categorial predictor, and, consequently, multiple regression is used (which is a special case of GLM). The common effect of the two diversity indices is then characterized by the significance of ANOVA of the entire model. Consequently, in this case, the significance of the model corresponds to the significance of “Biodiversity” in GLM.

This approach was used to comply with the methodology used in similar experiments (Hector et al. 1999). However, one should be aware that the approach is statistically correct for the biomass values only. In the derived characteristics (*OI*, complementarity, selection), the same monoculture values are used in various pots (i.e. replications), and, consequently, the values are not independent, thus the degrees of freedom are inflated (Špaeková and Lepš, 2001).

Differences between both productivity and relative species composition of pots with the same species composition but different nutrient regime were characterized in the following way: First, pairs were formed between low and high nutrient pots with the same species composition; where there were more replicate with the same species composition, the pairing

was random. (Thanks to this, all of the resulting values are independent, and can be correctly used in statistical analyses). For each pair, the following characteristics were calculated:
Plain difference between biomass in high and low nutrient level treatments

$D=W_h - W_l$

i.e., the positive value mean increase of biomass in high nutrients. Because the productivity of some combinations was higher in low nutrients, and we were interested in the stability with respect to change of nutrient level, the absolute value of the difference was calculated, i.e. $AD=|D|$, as a characteristic of resistance.

Because it could be expected that the magnitude of the difference is be proportional to the productivity of the species combination, the standardized difference (D_{st}) and standardized absolute value of difference (AD_{st}) were calculated by dividing the difference by the average productivity in high and low nutrient treatments:

$D_{st}= D/((W_h + W_l)/2)$ $AD_{st}= AD/((W_h + W_l)/2)$

Compositional similarity was characterized by the standardized Euclidean distance (chord distance, Orloci 1978)

$$CD = \sqrt{\sum_{i=1}^{s}(X_{h,i} - X_{l,i})^2}$$

where S is the number of species in the combination, $X_{h,i}$ and $X_{l,i}$ are the biomass values of i-th species in high and low nutrient pots respectively, after standardization by sample norm, so that the length of the sample vector is unity, i.e.

$$\sum_{i=1}^{S} X_i^2 = 1$$

This value was naturally not calculated for the monocultures, where no change in species composition was possible.

Table 2. GLM results for the Number of species, Functional group richness, Biodiversity, Fertilization in (A) aboveground biomass and (B) belowground biomass. The test of parallelism is included.

A) Aboveground biomass					
	SS	df	MS	F	P
Number of species	5.31	1	5.31	2.83	0.094
Functional group richness	13.53	1	13.54	7.21	**0.008**
Biodiversity	115.15	2	57.58	31.62	**0.000**
Fertilization	12.22	1	12.22	6.51	**0.012**
Interaction	17.11	2	8.56	4.81	**0.009**
Error	326.58	174	1.88		
B) Belowground biomass					
	SS	df	MS	F	P
Number of species	0.26	1	0.26	0.55	0.459
Functional group richness	0.74	1	0.74	1.58	0.209
Biodiversity	5.16	2	2.58	5.29	**0.006**
Fertilization	2.21	1	2.21	4.72	**0.031**
Interaction	1.68	2	0.83	1.99	0.139
Error	81.39	174	0.47		

Table 3. Summary of GLM analyses for log (*OI*), net effect (Net), selection (Select) and complementarity effects (Compl) in (A) aboveground and (B) belowground biomass. DF error for each GLM analysis has the same value of 172.▲ indicates significant increase in the values of biodiversity indices with the number of species and functional groups; ▼ indicates significant decrease in biodiversity values.

A) Aboveground biomass												
	Log(*OI*)			Net			Select			Compl		
	F	P		F	P		F	P		F	P	
Number of species	4.03	**0.047**	▲	1.14	0.287		12.59	**0.000**	▲	0.51	0.477	
Functional group richness	0.60	0.440		1.09	0.297		0.000	0.977		0.24	0.625	
Fertilization	13.46	**0.000**	▲	2.36	0.127		9.35	**0.003**	▼	12.02	**0.000**	▲
Biodiversity	0.42	0.659		3.94	**0.022**		0.83	0.439		1.25	0.289	
Interaction	4.66	**0.012**		0.84	0.434		2.19	0.117		0.84	0.434	

B) Belowground biomass												
	Log(*OI*)			Net			Select			Compl		
	F	P		F	P		F	P		F	P	
Number of species	1.07	0.301		5.04	**0.027**	▲	7.03	**0.009**	▼	3.19	0.077	
Functional group richness	1.06	0.304		0.12	0.725		0.12	0.724		4.92	**0.028**	▲
Fertilization	39.77	**0.000**	▲	25.24	**0.000**	▲	0.6	0.437		10.68	**0.001**	▲
Biodiversity	3.54	**0.032**		0.81	0.447		11.68	**0.000**		8.52	**0.000**	
Interaction	0.14	0.986		0.09	0.918		3.97	**0.022**		2.12	0.124	

Results

Aboveground biomass increased with both the number of species and number of functional groups (Fig. 1). The GLM analysis (Table 2) showed that the explanatory power (measured by sum of squares) of the number of functional groups was roughly three times higher than that of the number of species. Of the partial effects, only that of functional group numbers on aboveground biomass was significant. The effect was much more pronounced under high nutrients (significant interaction). Similarly, the effect of biodiversity on belowground biomass was positive, although none of the partial effects was significant (but the total effect of biodiversity was still highly significant). Although the interaction was not significant, there was still a tendency for stronger dependence under high nutrients. The effect of nutrient level was significant for both above and belowground biomass.

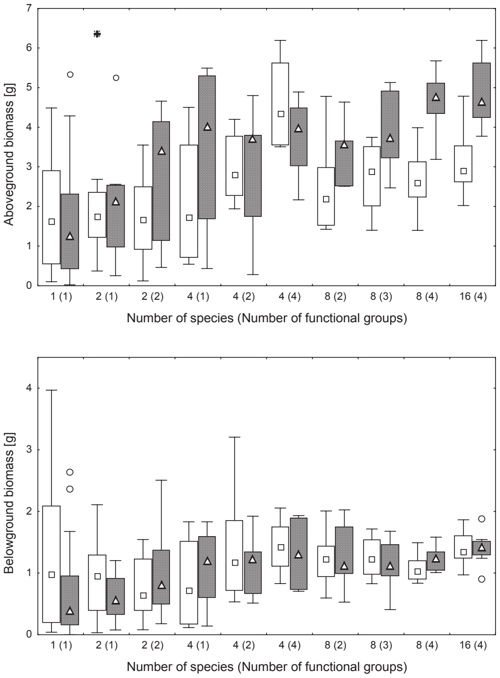

Fig. 1. Above and belowground biomass in pots. Biomass for low nutrient treatment is shown by empty boxes, for high nutrient treatment by filled boxes. The box shows the interquartile range with median, whiskers reach to the non-outlier range (i.e., range of data within {lower quartile – 1.5 x interquartile range; upper quartile + 1.5 x interquartile range}), (o) – outliers, (*) - extremes.

The relationship between biomass production in monoculture and dominance in mixtures was significantly positive under the low nutrient level for both above and belowground biomass. However, species of intermediate or low-to-intermediate monoculture biomass were the most successful ones in mixtures under high nutrient levels, both for above and belowground (Fig. 2). Biomass in monoculture was thus a poor predictor of the success of a species in mixture. Some species were even more productive in the 16 species mixture than in monoculture.

The analysis of log (*OI*) for aboveground biomass showed that its value increased slightly with the number of species; the increase was found under low nutrients only (significant interaction), but on average, the value was higher under high nutrients (Table 3). Values of log (*OI*) for belowground biomass increased similarly with biodiversity under both low and high nutrient levels.

The selection effect value (calculated according to Loreau and Hector 2001) for aboveground biomass increased with the number of species, but slightly decreases in belowground biomass (Table 3). For aboveground biomass, the relationship was similarly positive under both high and low nutrient levels, with higher values in the low nutrient treatment. The selection effect was negative and decreased with number of species for belowground biomass under both nutrient levels, the decrease being more pronounced in the

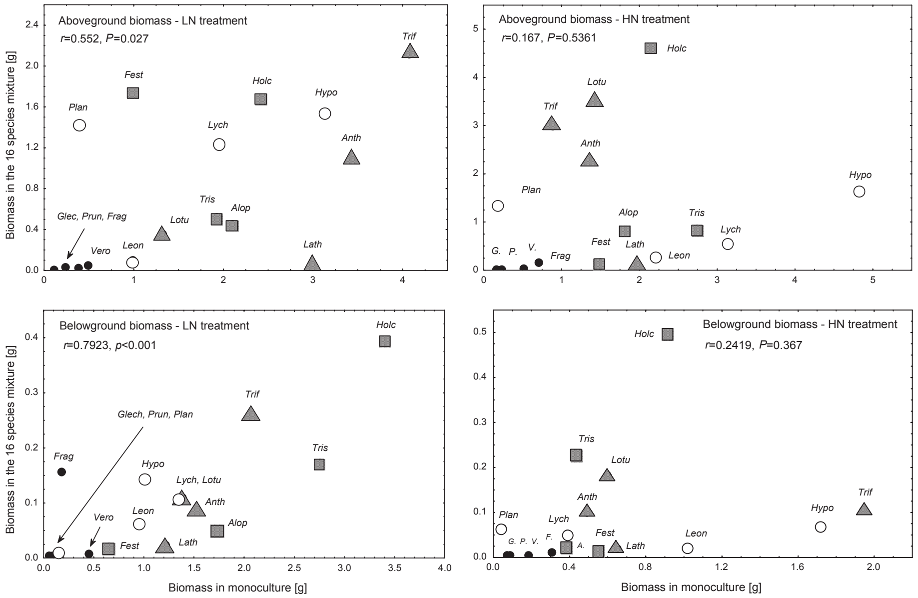

Fig. 2. The relationship between species biomass in monoculture and in mixture of all sixteen species. Above and belowground biomass for each of the low (LN) and high nutrient (HN) treatments are shown. *Alop – Alopecurus, Fest – Festuca, Holc – Holcus, Tris – Trisetum, Hypo – Hypochaeris, Leon – Leontodon, Lych – Lychnis, Plan – Plantago, Frag – Fragaria, Anth – Anthyllis, Lath – Lathyrus, Lotu – Lotus, Trif – Trifolium, G. – Glechoma, P. – Prunella, V. – Veronica*. Symbols for functional groups: triangle – legumes, square – grasses, empty circle – rosettes, full circles – creeping forbs.

low nutrient treatment. The complementarity effect did not seem to be affected by any biodiversity characteristics, but increased (mainly with functional group richness) belowground. Its value was higher under high nutrients (Fig. 3). The net effect is the combination of the selection and complementarity effects. Its value increased with diversity in aboveground biomass (only common effect significant), while for belowground biomass, it increased with species richness (Table 3).

The plain non-standardized difference (D) for aboveground biomass increased with biodiversity (the partial effects were not significant), but no dependence was found in belowground biomass (Fig. 4, Table 4). For some monocultures and low diversity mixtures, the difference was negative, i.e. their biomass was higher in low nutrients. On the contrary, in species rich mixtures, aboveground productivity was always higher under high nutrient levels. The plain standardized difference (D_{st}) and absolute non-standardized difference (AD) did not change with diversity in aboveground biomass, but the total biodiversity effect on AD was significantly negative for belowground biomass. Standardized absolute value of the difference (AD_{st}) significantly decreased with the number of functional groups in both above and belowground biomass. Chord distance increased with the number of species in the aboveground biomass (Fig. 4, Table 4).

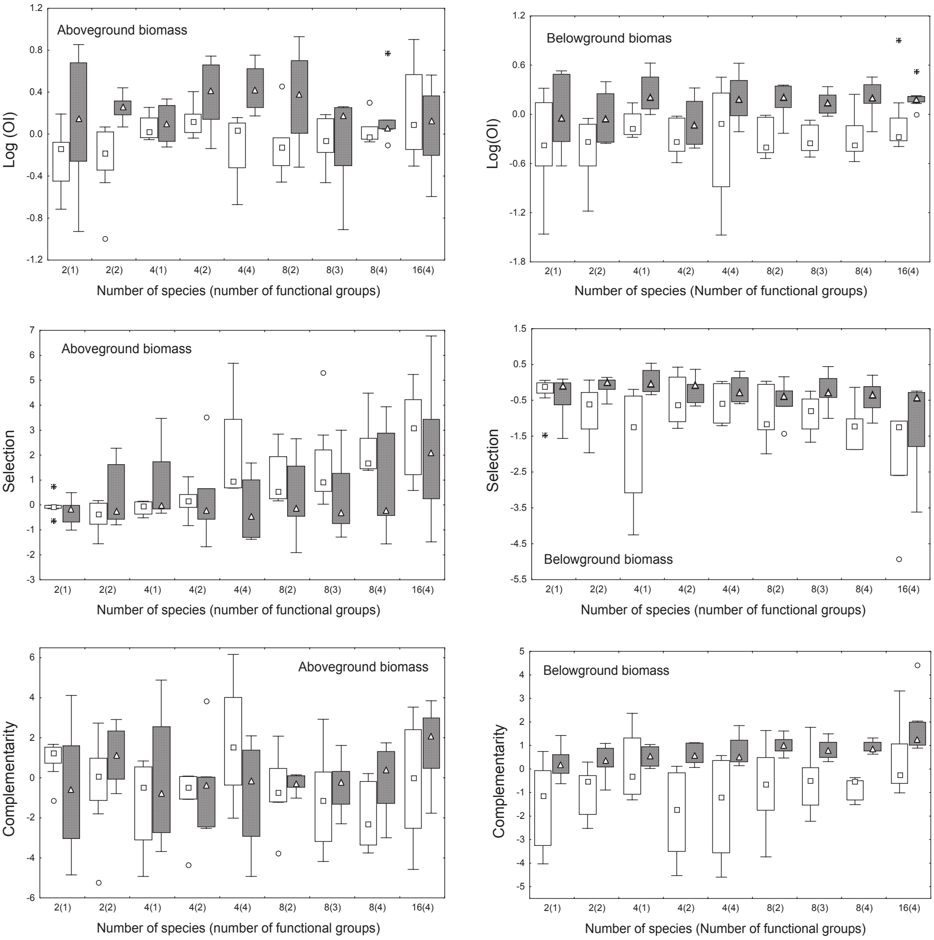

Fig. 3. Dependence of log(*OI*), selection effect and complementarity effect on species richness for above and belowground biomass. Biomass for low nutrient treatment is shown by empty boxes, for high nutrient treatment by filled boxes. The box shows the interquartile range with median, whiskers reach to the non-outlier range (i.e., range of data within {lower quartile – 1.5 x interquartile range; upper quartile + 1.5 x interquartile range}), (o) – outliers, (*) - extremes.

Discussion

Increase of productivity with diversity

This study demonstrated a pronounced increase in average aboveground biomass and a mild increase in average belowground biomass with biodiversity characteristics. In both, the effect of functional group richness was more pronounced than the effect of number of species. This supports the conclusions of Díaz and Cabido (2001) about the importance of functional differentiation. For aboveground biomass, the increase was much more pronounced under high

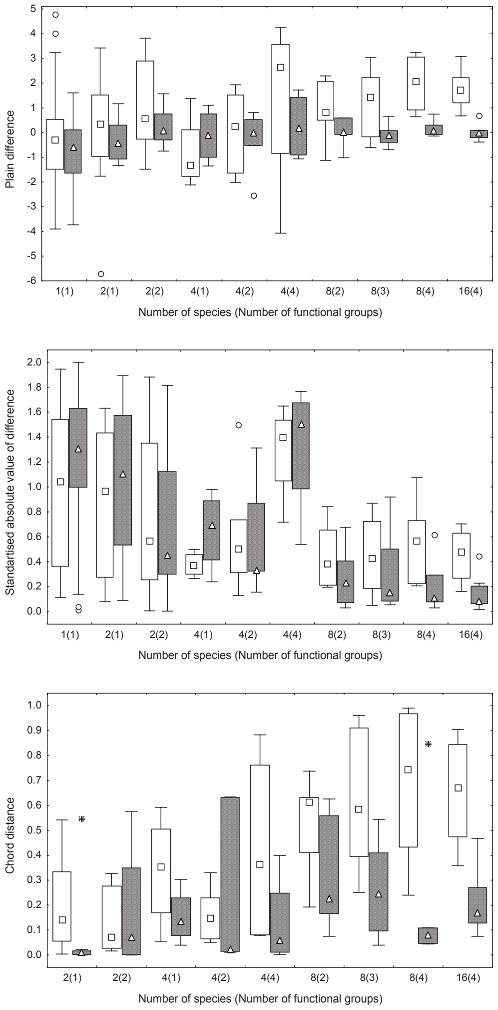

Fig. 4. Plain difference, standardized difference of absolute value and euclidean distance between biomass of high and low nutrient levels. Aboveground biomass is shown by empty boxes, belowground biomass by filled boxes. The box shows the interquartile range with median, whiskers reach to the non-outlier range (i.e., range of data within {lower quartile – 1.5 x interquartile range; upper quartile + 1.5 x interquartile range}), (o) – outliers, (*) - extremes.

nutrients. Surprisingly, some low diversity mixtures and monocultures produced more aboveground biomass under low nutrients, and some productive species were more productive in mixtures than when grown in monocultures, usually under high nutrient levels. This might be partially caused by high sowing densities of the species. Typically, *Plantago media*, *Holcus lanatus* (and some other species) suffered from high seedling density in monocultures, resulting in low final yield. This effect was more pronounced in the high nutrient treatment. It has been known for a long time in agronomy that increasing the sowing density over some threshold might lead to decreased final yield (see Silvertown and Lovett Doust, 1993, pp. 50-53 for discussion). One should be aware, however, that, if this is the case for the most productive species, decreasing its sowing density in mixture might be enough to increase the final yield of the mixture (and getting high values of all “biodiversity effect” indices, including *OI*). There is, however, an alternative explanation for this effect. This phenomenon was observed mainly under high nutrients. Some of the species might suffer from too high nutrient levels when in monoculture. The presence of other species that are not negatively affected by high nutrients might help to decrease the nutrient level to a level acceptable to other species. If this explanation is correct, then this effect would be a real case of complementarity, i.e. biomass increase caused by the differential ability of species to take up nutrients, or could be even interpreted as facilitation (nutrient uptake of one species improved the conditions for another species). The fact that the increase of aboveground biomass with diversity was much more pronounced under high nutrients, and, also, that the value of the complementarity effect was generally higher under high nutrients, supports this explanation.

Table 4. Results of multiple regression with number of species and number of functional groups as explanatory variables for D, AD, D_{st}, AD_{st} and ED in (A) aboveground and (B) belowground biomass. ANOVA is the ANOVA of the entire model. Betas are the standardized partial regression coefficients. Simple r is correlation coefficient of the variable with the response (characterizing the marginal effect of the predictor). (***) $P < 0.001$, (**) $P < 0.01$, (*) $P < 0.05$, (n.s.) $P > 0.05$.

A) Aboveground biomass						
Regression: multiple					simple	
Difference between HN and LN	Explanatory variable	ANOVA	Beta	Sign.	R	Sign.
D	Model	F(2,85) = 4.5452		*		
	Number of species		0.2934	n.s.	0.3106	**
	Number of functional group		0.0212	n.s.	0.2589	*
AD	Model	F(2,85) = 0.1473		n.s.		
	Number of species		0.0942	n.s.	0.0159	n.s.
	Number of functional group		-0.0965	n.s.	-0.0202	n.s.
D_{st}	Model	F(2,85) = 1.9386		n.s.		
	Number of species		0.1952	n.s.	0.2086	n.s.
	Number of functional group		0.0165	n.s.	0.1747	n.s.
AD_{st}	Model	F(2,85) = 8.3167		***		
	Number of species		-0.0538	n.s.	-0.3453	***
	Number of functional group		-0.3597	*	-0.4033	***
ED	Model	F(2,55) = 16.052		***		
	Number of species		0.5012	**	0.5990	***
	Number of functional group		0.1389	n.s.	0.4920	***
B) Belowground biomass						
Regression: multiple					simple	
Difference between HN and LN	Explanatory variable	ANOVA	Beta	Sign.	R	Sign.
D	Model	F(2,85) = 1.5443		n.s.		
	Number of species		0.0437	n.s.	0.1653	n.s.
	Number of functional group		0.1501	n.s.	0.1855	n.s.
AD	Model	F(2,85) = 9.2770		***		
	Number of species		-0.2384	n.s.	-0.4056	***
	Number of functional group		-0.2064	n.s.	-0.3996	***
D_{st}	Model	F(2,85) = 0.9575		n.s.		
	Number of species		0.0452	n.s.	0.1339	n.s.
	Number of functional group		0.1095	n.s.	0.1461	n.s.
AD_{st}	Model	F(2,85) = 24.551		***		
	Number of species		-0.2569	n.s.	-0.5631	***
	Number of functional group		-0.3779	*	-0.5861	***
ED	Model	F(2,55) = 0.1251		n.s.		
	Number of species		0.0655	n.s.	0.0673	n.s.
	Number of functional group		0.0025	n.s.	0.0486	n.s.

Complementarity and sampling effects

In pot biodiversity experiments, a positive effect of species richness on primary production was usually caused by the sampling effect (positive selection effect) rather than complementarity (Špaeková and Lepš, 2001; Fridley, 2002; Mikola et al., 2002). However, in those studies, the number of individuals was kept constant by thinning the germinated individuals or planting constant numbers of individuals, and was lower than in our case. In this study, the species were sown at high densities and underwent selfthinning during the experiment. This probably resulted in a much lower correlation between monoculture productivity and success in competition (compare this study with Špaeková and Lepš, 2001). The difference in the time of germination is another factor decreasing the correlation between productivity and competitive ability.

Our analyses have shown that the complementarity and selection effects, as calculated by the methods of Loreau and Hector (2001), behave differently from each other. This is not surprising, because each of them is based on different mechanisms. The matter is probably even more complicated. Although the meaning of those two parts of the biodiversity effect is intuitively clear from the formula, the biological mechanisms affecting their values probably vary from case to case. In particular, it is probably not always complementarity in resource use that leads to positive numerical values. It is based on the RYT methodology, the limitations of which have been nicely shown by Loreau (1998b). This is particularly true when the selection effect became negative.

The calculated complementarity value could also reflect the effect of facilitation (Loreau, 1998b). Legumes, the nitrogen fixers, were one of our functional groups. Of them, *Trifolium pratense* is renown as being a very efficient N_2-fixer (Spehn et al., 2002). Furthermore, agricultural intercropping experiments have shown that nitrogen-fixers in low-diversity systems commonly increase nitrogen availability for their neighbors (Vandermeer, 1989). Facilitation by legumes through nitrogen fixation leading to higher biomass production is probably common in similar experiments (Hector et al., 1999; Tilman et al., 2001). However, nitrogen facilitation by legumes should be effective mainly under low nutrient conditions. The opposite is true: when individual pots were compared, *OI* values for aboveground biomass indicate transgressive overyielding (i.e. *OI* >1) in 40.7 % and 77.2 % of the low and high nutrient pots respectively. Even bigger difference was found for belowground biomass: transgressive overyilding was found in 13.8 % and 67.2 % of low and high nutrient pots. Consequently, other causes were more likely to produce the high complementarity values than nitrogen facilitation by legumes.

Effect of individual species traits

The experimental mixtures were often dominated by *Holcus*, whereas some species (e.g. *Hypochaeris*), more productive in the monocultures, were subordinate (Fig. 2), mainly under high nutrient levels. Although *Holcus* had intermediate monoculture productivity in high nutrients, it was able to quickly elevate its canopy in mixtures. *Holcus* has the ability to overtop and shade its neighbours by allocation more resources to the stems. Therefore, to be a successful competitor for light in a mixture, a plant must sacrifice some of its potential production when growing in monoculture (Hector et al., 2002). In general, creeping forbs had very low productivity in monocultures, and were nearly eliminated in mixtures, contributing positively to the selection effect. On the contrary, rosette forbs were able to exhibit high productivity in monocultures, but were overtopped in mixtures (usually by grasses, which were not so productive in monocultures). This contributed negatively to the selection effect. It shows that, depending on the experimental setup and/or environmental conditions and species set, the high productivity in monoculture need not be a good predictor of success in competition. A combination of these effects often lead to a negative selection effect and positive complementarity. The resulting productivity depended on the relative strength of the contributing effects. Similar effects were discussed in Hooper and Vitousek (1997), Dukes (2001) and Deutschman (2001).

Effect of nutrient level

The plain difference in productivity between the high and low nutrient treatments is positively correlated with diversity. In fact, some of the low diversity mixtures and monocultures had decreased productivity with increasing nutrients. Consequently, the average change came from negative values for monocultures to highly positive values for species rich mixtures. With increased species number, the probability rises that the mixture contains a species able to take advantage of the nutrient amendment. Moreover, it seems that the presence of such species might improve conditions for other species, e.g. those that were harmed by high nutrient levels. On the contrary, the absolute value of the standardized difference decreased with diversity, particularly with functional group richness, showing that productivity of more diverse mixtures is more resistant to change in nutrient. The change in species composition characterized by the chord distance increased with diversity. This corresponds well to the predictions of Tilman (1999): the stability of the aggregate characteristic, here productivity, is achieved through a change in species composition.

Limitations of pot experiments

We are aware that pot experiments are rather limited in their realism. In particular, the spatial and temporal variability of the environment, which could be important for the manifestation of complementarity, is highly restricted in pots. However, there are two advantages to pot experiments. First, we were able to separate the roots of individual species, when rinsing the substrate carefully; this is not feasible in normal soils in field experiments. Further, field experiments impose much greater restrictions on the number of replications. Monocultures are essential for proper analysis. In calculating the indices (*OI*, selection, complementarity), monoculture values are included. This means that a possible underestimation of monoculture biomass of highly productive species causes an overestimation of biodiversity effects in many samples. Consequently, the monocultures should be replicated (we used two replicates, but more would be desirable for statistical analyses).

Also, nutrient concentrations that are high to harm some species are not common in natural conditions. This effect was also amplified by using sand as the substrate. Nevertheless, the results showing that some species are harmed by high nutrient level when grown in monoculture, but when grown in mixture, the other species are able to deplete the nutrients to acceptable levels, suggest a mechanism by which diversity (either species or functional) could improve resource use by the whole community. The fact that several of the species did grow better in mixtures (a phenomenon not observed in a similar experiment by Špaekova and Lepš, 2001) suggests that pure sand is a rather stressful environment for some species (particularly pure sand in combination with a high nutrient level was detrimental for some species). As shown several times (e.g. Pugnaire et al., 2004), facilitative interactions are more common in stressed environments, and it can be reasonably expected that the presence of roots of other species could improve soil properties for some species. As a result, the complementarity and overyielding were both observed in many cases.

Species and functional group richness

Interestingly, in biomass analyses, functional group richness was a better predictor than the number of species. Four a-priori defined functional groups were used and the statistical analysis showed that functional group richness had good explanatory power. In this study the functional groups behaved consistently. All the creeping herbs exhibited low productivity, both in low and high nutrients, and also in monocultures and mixtures. The rosette plants (with the exception of *Plantago media*) showed high productivity in monocultures, where they took the advantage of high nutrient levels, but were highly suppressed in mixtures, particularly under high nutrients. Because they did not produce any stems, they were overgrown by other species in mixtures and being shaded and outcompeted by taller plants. The legume monocultures had generally (much) higher productivity under low nutrients (with the exception of *Lotus,* where productivity was roughly the same). Under high nutrients, they mostly achieved higher biomass in mixture than in monocultures. The only exception was *Lathyrus pratensis,* a species with delayed germination, which is an important competitional disadvantage in short term experiments. The grasses did not show consistent behavior; on the contrary, the behavior of *Holcus* was somewhat similar to that of legumes. Nevertheless, in most cases, functional group membership did not only correspond to species morphology, but also reasonably predicted species behavior in the experiment.

As noted by Tilman et al. (2002), any a-priori definition of functional groups is a problem, particularly as it might be uncertain which of the species traits will be important in competitive interactions. In manipulative experiments focused directly on the effect of functional groups, however, one could select groups of species that are very similar to each other and sufficiently distinct from the others. This was the case in our experiment. Nevertheless, some of the functional traits were not reflected in the classification of functional groups, and still affected considerably the final outcome of the experiment (e.g. delayed germination in *Lathyrus pratensis*). Whereas the importance of germination speed for competition was exaggerated due to the experimental setup, similar mechanisms probably play a role in nature. For example, Hooper and Vitousek (1997) used early and late seasonal annuals and showed how annuals were able to suppress the productivity of the bunchgrasses, partially because of their phenology.

Unlike Tilman et al. (2002) we do not see much of a problem in separation the effect of functional group and species richness. Whereas we agree with Tilman et al. (2002) that “classical” GLM, with a type III sums of squares, provides a very conservative test for partial effects (i.e. high Type II error rate), in our view, it is no problem to test within GLM also for their common effect; this test has usually (much) higher power that tests for partial effects. On the contrary, we see serious problems with analyses of derived characteristics (like complementarity, OI, selection), because their values are not independent observations. They are used here only to provide results comparable with other published papers, but we are aware that the significance can be seriously inflated.

Design of biodiversity experiments

To design a biodiversity experiment is generally a difficult task. For simple logistic reasons, the possible number of replicates is limited, and consequently, one can not examine all possible species combinations. As Allison (1999) demonstrated, the power of the test is then affected by the way in which the species mixtures are assembled (i.e., how species and functional group richness are combined). Our design corresponds to the Three-tier factorial design of Allison (1999). Under this design, the number of functional groups and total number of species are necessarily correlated. However, the analysis that includes both the partial effect of species number and their common effect is able to separate the effects and concurrently provides a test which is sufficiently strong for the general “biodiversity effect”. The important fact (not reflected in Allison, 1999) is that there are two sources of “random” variation: the variability caused by species composition (various replications of the same “treatment”, i.e.

containing the same number of species and functional groups, have different species composition), and a random variability. In our experience, the first part (i.e. the species composition specific effect) of the variability is greater, particularly in strongly controlled conditions. Under those circumstances, we believe that the design varying species composition among replicates of each combination of species functional group richness is necessary whenever possible.

As has been shown by Fukami et al. (2001), the similarity between replicates increases with species richness in most designs with a limited species pool. This is also reflected by the fact that variability in biomass decreases with species richness. Fortunately, the trend of decreasing variability with species richness is not so strong that it would invalidate statistical analyses (any GLM analysis assumes homoscedascity, i.e constant variability), because there is no simple remedy. The log transformation improves homoscedascity when mean and variability are positively correlated (here they were negatively correlated). Similarly, none of the distributions commonly used in generalized linear models is based on a distribution where mean and variance are negatively correlated. Using the open species pool, however, would require a very large number of monocultures. Alternatively, some species would not be grown as monocultures, but would be part of species mixtures. In this case, however, neither *OI*, nor the other diversity indices, can be calculated.

Conclusions

In a pot greenhouse experiment, average biomass increased with both number of species and number of functional groups. The effect of functional groups was more pronounced. The increase was more pronounced under high nutrients. Contrary to many previous studies, there was low correlation between species performance in monoculture and in mixture. We demonstrated that this can be caused by a series of factors in our experimental setup. As a result, the selection effect in our experiment was low, or even sometimes negative. The relative change in productivity decreased with richness, whereas the shift in species composition increased. This suggests that richness could stabilize the community aggregate characteristics through a shift in species proportions.

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Paper II

Effects of species and functional group richness on production in two fertility environments: an experiment with communities of perennial plants

Effects of species and functional group richness on production in two fertility environments: an experiment with communities of perennial plants

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Abstract. The effects of species and functional group richness of sown species and fertilization on productivity was studied in a three year field experiment. Both species and functional group numbers positively affect the average yield of sown species, but the effect changed among the three years. The biomass of weeds (i.e. unsown species) generally decreased with the biomass of sown species, and so was negatively affected by the diversity characteristics of sown species. All three characteristics of biodiversity (selection, complementarity and overyielding effects) varied greatly during the study period, depending on both species and functional group richness and nutrient amendment. Functional differences among species led to the detection of high values of complementarity (resource use complementarity and/or facilitation), however this effect was not sufficient to cause transgressive overyielding. This result suggests that greater production with higher diversity may be restricted to particular species combinations or environmental conditions. The compositional difference between fertilized and nonfertilized control plots increased with both time and species richness, whereas the relative difference in productivity decreased. This shows that the changes in species composition toward the one best suited to the given environment can lead to optimal resource use and, in consequence, diminish variation in community functioning in a changing environment.

Keywords: Aboveground biomass, Biodiversity indices, Functional group richness, Sown species, Species richness, Weeds

Introduction

Plant species diversity has been shown to enhance ecosystem productivity (Naeem et al., 1994; Tilman et al., 1996, 1997, 2001; Hector et al., 1999; Tilman, 1999; Loreau, 2000), but the reasons for this remain controversial (Huston, 1997; Loreau et al., 2001; Huston et al., 2000; Wardle et al., 2000). In experiments where species assemblages are randomly constructed, species-rich plots have higher probability of including those species that have the greatest individual effects on production, a phenomenon called the "sampling effect" (Aarsen, 1997; Huston, 1997; Tilman et al., 1997). Determining whether sampling effects might apply in natural ecosystems has been difficult, because no experiments have examined the sensitivity of sampling effect to environmental context. Sampling effect may be particularly important in environments that promote species dominance, where one or a few species have a strong individual control over productivity (Fridley, 2001). Higher production than expected from the sampling effect ("overyielding" sensu de Witt, 1960; Trenbath, 1974; Joliffe, 1997) may be caused by complementary resource use or facilitative interactions among species (Vandermeer, 1989; Tilman et al., 1997, 2001; Hooper, 1998; Hector et al., 1999). However, this phenomenon needs further investigation (Austin et al., 1988; Garnier et al., 1997). There are two categories of overyielding. Non-transgressive overyielding occurs when the yield of a mixture is greater than expected based on a weighted average of the monoculture yields of the component species, as indicated by values greater than one for relative yield totals (RYTs; Trenbath, 1974; Vandermeer, 1989). Transgressive overyielding (Trenbath, 1974; Vandermeer, 1989) occurs when a mixture yields more than any monoculture of the component species.

Several studies have reported significant positive effects of plant species richness on ecosystem functioning (Tilman et al., 1996, 2001; Hector et al., 1999), while some studies have found functioning to be mainly influenced by species and functional group composition, i.e. the identity of the species present (Hooper and Vitousek, 1997; Wardle et al., 1997). Diversity is often equated with species richness and other components of diversity have frequently been underestimated. In several studies, rates and magnitude of ecosystem processes have been found to be more consistently associated with functional composition (presence of certain functional types or traits) and functional richness (number of functional groups) than with species richness (Lepš et al., 1982; Grime et al., 1997; Hooper and Vitousek, 1998; Reich et al., 2001; Symstad, 2000; Tilman et al., 1997). Broader issues concerning functional groups are reviewed in Mooney et al. (1996), Diaz and Cabido (2001), Diaz et al. (2003) and Hooper et al. (2002). Determining the relative contributions of functional and species richness to ecosystem functioning is important in management and conservation, because it provides information on ecological redundancy (Walker, 1992;

Lawton and Brown, 1993; Naeem, 1998), where "ecological redundancy" refers to taxonomically different species that exhibit similar or related ecological functions.

It has been demonstrated that nutrient addition brings about an increase in the average height of vegetation, which leads to a competitive advantage of tall growth forms over shorter ones (Tilman, 1988). Nutrient addition may promote light partitioning by accentuating growth form differences between species, thereby enhancing the structural complexity of vegetation. Furthermore, stratification of canopy layers promotes coexistence and increases production in some herbaceous and grassland communities (Mitchley, 1988; Liira and Zobel, 2000). On the other hand, nutrient addition usually decreases species richness, leading to competitive exclusion of some (usually shorter) species (Lepš, 1999).

To assess the relative degree to which fertilization, species and functional group diversity influence productivity in plant communities, a three year field experiment was performed in which species and functional group richness and fertilization were manipulated. Because the system was established without weeding of the plots, it could show how the diversity of resident sown species can affect weed invasion. Weeds were establishing from the soil seed bank during the course of the experiment. Specifically, our goals were to (i) evaluate diversity effects, and (ii) determine the effects of species number, functional group number and soil nutrients on these effects.

Methods

The experiment was conducted in an abandoned agricultural field, last cultivated in 2001, at Benešov in South Bohemia, CZ (49° 92` N, 15° 00` E, altitude approximately 660 m). This region experiences a temperate climate, with mean annual temperature of 6.4 °C and mean annual precipitation of 680 mm.

For this experiment, a taxonomically diverse group of polycarpic perennial species with well-known growth characteristics was chosen. These species represent major components of the surrounding grasslands. Species from four distinct functional groups were selected: four grass species - the narrow leaved *Festuca rubra*; the broad-leaved *Trisetum flavescens, Alopecurus pratensis* and *Holcus lanatus*; four rosette hemicryptophytes - *Lychnis flos-cuculi*, *Hypochaeris radicata*, *Plantago media* and *Leontodon autumnalis*; four herbs with creeping aboveground stolons - *Veronica officinalis*, *Glechoma hederacea*, *Fragaria vesca* and *Prunella vulgaris*; four nitrogen-fixers (legumes) - *Lotus corniculatus*, *Anthyllis vulneraria*, *Trifolium pratense* and *Lathyrus pratensis*.

During March 2002, treatments were established in 2 x 2 m plots in a completely randomized design. Ten combinations of species and functional group richness were used, with different species combinations within treatments (Table 1), yielding 184 plots. Mixtures of 1-16 species were sown at the same density of 3000 seeds m^{-2}, and were grown under two nutrient treatments (non-fertilized control and fertilized plots). The plots assigned to the nutrient addition treatment were amended with 40 g NPK m^{-2}. yr^{-1} (Synferta P-NPK; AGRO CS Česká Skalice, CZ), applied in April 2002 and March 2003, 2004 and 2005. All species were equally represented at each diversity combination. Equal number of seeds of each species was sown in each combination. As far as possible, all of the functional groups were equally represented in mixtures with more than one functional group. The 2x2 m^2 plots were harvested for aboveground biomass in June 2003, 2004 and 2005 by clipping at ground level in two 15 x 15 cm subplots and then completely mown. The two subsamples were then pooled, separated into individual sown species and the rest of aboveground biomass (associated with the summed biomass of colonizing species), oven-dried and weighed.

The aboveground biomass of sown and weedy species is a measure of the growth success of the sown and weedy species respectively in different biodiversity treatments. General linear models (GLM) were used to compare the influence of species number, functional group richness and fertilization on the biomass of sown and weed species. Species richness and functional group richness were continuous predictors, while nutrient level was a categorical one. In a design with a closed data set, the number of species and functional groups is inevitably correlated (in our case, the correlation was 0.813). Consequently, the strength of the test of partial effect of each of the two correlated predictors is decreased. The common test was also performed to test for the overall effect of diversity (for details, see Lanta and Lepš, 2006).

Besides biomass, each plot was also characterized by biodiversity effect indices, namely the transgressive overyielding index (*OI*), and by additive partitioning biodiversity effects technique (Loreau and Hector, 2001). Those indices are calculated on the basis of the sown species only; we are interested in the effect of sown mixture richness on productivity, and also in its ability to suppress the arable weeds. *OI* is defined as the total biomass of mixture /monoculture biomass of the component species with the largest monoculture value (Loreau,

Table 1. Treatments, number of species combinations and number of replications used in each, high and low nutrients pots.

Treatment	Number of species in mixture	Number of species combinations	Number of replicates
1	monoculture of each species	16	2x
2	2 species within one functional group	8	1x
3	2 species from two groups	8	1x
4	4 species (each species is from one group)	4	1x
5	4 species (each two species are from different groups)	4	2x
6	4 species that composed one functional group	4	1x
7	8 species from two groups	6	1x
8	8 species from three groups	8	1x
9	8 species from four groups	6	1x
10	16 species (i.e., four functional groups)	1	8x

1998). *OI* > 1 for a mixture indicates transgressive overyielding (Trenbath, 1974; Vandermeer, 1989; Hector et al., 2002). At the second step, the role of sampling effects among diversity and fertility levels were measured by using the additive partitioning biodiversity effects technique (Loreau and Hector, 2001) to separate the productivity responses of mixtures into selection effect (SE) and complementarity (CE). The net difference in yield for a mixture, ΔY, is the observed yield (Y_o) minus expected (Y_e) and is equal to the summation of both complementarity and selection effect:

$$\Delta Y = Y_o - Y_e = \mathrm{SE} + \mathrm{CE}.$$

This estimates SE for each plant mixture by calculating the covariance between species monoculture yields and their deviance from expected relative yields in mixture (based on their planted proportions), multiplied by the total number of species in mixture. CE for a mixture is calculated as the average deviance over all of the species in the mixture and over species average monocultural yields. If species of higher than average monocultural yields dominate a mixture, the associated SE is positive; dominance by less productive species leads to a negative SE. CE gives similar information as RYT (Relative Yield Total). These indices were then also subjected to the same GLM analyses as with the biomass data. This approach is generally used in biodiversity studies (e.g. Hector et al., 1999). It was used here so that the analysis is comparable with other studies. However, we are aware that each monoculture value enters into all of the mixtures where the species is present, and, consequently, the individual values are not independent. As a result, the significance of analyses could be inflated (Lanta and Lepš, 2006).

Differences between both the productivity of the sown species and the relative composition of sown species in plots with the same species composition, but different nutrient regime, were characterized in the following way: First, pairs were formed between control and fertilized plots with the same species composition; where there were more replicates with the same species composition, the pairing was random. The following characteristics were calculated for each pair:

Plain difference between biomass in control and fertilized plots

$D=W_h - W_l$

i.e., the positive value mean increase of biomass in high nutrient treatments. Because the productivity of some combinations was higher in low nutrient treatments, and we were interested in stability with respect to a change of nutrient level, the absolute value of the difference was calculated, i.e. $AD=|D|$, as a characteristic of resistance.

Because it could be expected that the magnitude of the difference is proportional to the productivity of the species combination, the standardized difference (D_{st}) and standardized absolute value of difference (AD_{st}) were calculated by dividing the respective differences by the average productivity in high and low nutrient treatments:

$D_{st}= D/((W_h + W_l)/2)$ $AD_{st}= AD/((W_h + W_l)/2)$

Compositional similarity was characterized by the standardized Euclidean distance (chord distance; Orloci, 1978):

$$CD = \sqrt{\sum_{i=1}^{s}(X_{h,i} - X_{l,i})^2}$$

where S is the number of species in the combination, $X_{h,i}$ and $X_{l,i}$ are the biomass values of the i-th species in control and fertilized plots respectively, after standardization by sample norm, so that the length of the sample vector is unity, i.e.

$$\sum_{i=1}^{S} X_i^2 = 1$$

This value was naturally not calculated for the monocultures, where no change in species composition was possible.

Results

Biomass of sown species increased with the number of species in 2003 and 2004, but not in 2005. Fertilized amendments had positive effects on the biomass of sown species in 2003 and 2004, but the differences between fertilized and control plots were not significant in 2005 (Table 2, Fig. 1). Biomass of weeds significantly decreased with the increasing diversity of residents only in 2004 (Fig. 1), with the effect of functional group richness detected in 2004 and 2005 (Table 2). Biomass production of both sown species and weeds significantly differed between years (Table 3).

Weed biomass significantly decreased with increasing biomass of sown species in control and fertilized plots, no significant relationship was found for fertilized plots in 2003 (Table 4).

The relationship between aboveground biomass production in monoculture and dominance in mixtures was significantly positive (Fig. 2) in both control ($P<0.001$) and fertilized ($P<0.001$) plots for all studied years. We regressed dominance against monoculture biomass that was normalized to proportions of the maximum value observed at each control and fertilized treatment (Fig. 2). Aboveground biomass in monoculture was a good predictor of the success of a species in a mixture. Differences among species are much more pronounced in polycultures, suggesting that the variation is strongly affected by interspecific competition.

The analysis of *OI* showed significant relationships between indices values and species richness in 2005 (Table 5, Fig. 3). However, average values of log(*OI*) were lower than zero in most biodiversity treatments. Values did not significantly differ between years of study (Table 6). However, significant fertilization x time interactions indicated that values were higher for control plots in 2003, but were higher in fertilized plots in 2005.

Table 2. Summary of GLM for aboveground biomass of sown residents and weeds.

		Sown species 2003		Weeds 2003		Sown species 2004		Weeds 2004		Sown species 2005		Weeds 2005	
	df	F	P	F	P	F	P	F	P	F	P	F	P
Species richness	1, 180	18.04	**0.000▲**	0.58	0.446	7.49	**0.007▲**	4.36	**0.038▼**	3.51	0.062	2.11	0.148
Functional group richness	1, 180	0.06	0.809	0.44	0.506	0.74	0.391	3.99	**0.047▼**	2.46	0.119	7.10	**0.008▼**
Biodiversity	2, 180	24.79	**0.000**	2.77	0.065	17.99	**0.000**	2.31	0.102	16.03	**0.000**	4.33	**0.015**
Fertilization	1, 180	12.33	**0.001▲**	1.47	0.226	5.28	**0.023▲**	0.35	0.557	1.21	0.272	1.66	0.199
Test of parallelism	2, 178	0.56	0.573	0.07	0.933	3.06	**0.049**	1.76	0.175	0.55	0.580	0.18	0.838

Table 3. Results of repeated measures ANOVA for biomass of sown species and weeds. Independent variable was fertilization, as covariates numbers of species and functional groups were used. Effect of both covariates was significant for both sown species (Rao`s *R*, $F_{(6, 360)} = 16.29$, $P < 0.001$) and weeds (Rao`s *R*, $F_{(6, 356)} = 2.77$, $P = 0.012$).

		Sown species		Weeds	
	df	F	P	F	P
Fertilization	1, 180	8.26	**0.005**	1.39	0.239
Time	2, 364	76.96	**0.000**	44.11	**0.000**
Interaction	2, 364	9.19	**0.000**	1.30	0.272

Table 4. Relationships between aboveground biomass of weeds and biomass production of sown species for three years and two nutrient treatments.

Year	Plots	Adj. R-square	R	P
2003	Control	0.033	-0.209	**0.045**
2004	Control	0.135	-0.381	**0.000**
2005	Control	0.169	-0.422	**0.000**
2003	Fertilized	0.006	-0.076	0.473
2004	Fertilized	0.059	-0.164	**0.011**
2005	Fertilized	0.236	-0.494	**0.000**

The selection effect significantly increased with the number of functional groups in 2005. This effect significantly decreased with the number of species in 2004 (Table 5, Fig. 3). The values differed under the fertilization treatment in 2003 and 2004. The second biodiversity term, complementarity effect, significantly increased with the number of species in 2004 and

Table 5. Summary of biodiversity indices calculated for sown species and 3 consecutive years (2003, 2004 and 2005).

Complementarity		2003		2004		2005	
	df	F	P	F	P	F	P
Species richness	1, 116	3.02	0.085	30.14	**0.000▲**	51.44	**0.000▲**
Group richness	1, 116	5.26	**0.024▲**	0.28	0.597	3.23	0.075
Biodiversity	2, 116	14.01	**0.000**	34.73	**0.000**	36.59	**0.000**
Fertilization	1, 116	10.13	**0.002▼**	10.89	**0.001▼**	0.65	0.421
Test of parallelism	2, 114	0.32	0.728	1.47	0.240	10.94	**0.000**
Selection		2003		2004		2005	
	df	F	P	F	P	F	P
Species richness	1, 116	0.01	0.498	7.09	**0.009▼**	0.022	0.883
Group richness	1, 116	0.46	0.908	0.03	0.854	3.986	**0.048▲**
Biodiversity	2, 116	0.59	0.555	7.87	**0.001**	3.61	**0.030**
Fertilization	1, 116	14.24	**0.000▲**	8.80	**0.004▲**	0.684	0.409
Test of parallelism	2, 114	1.42	0.246	2.98	0.055	0.61	0.543
Log(*OI*)		2003		2004		2005	
	df	F	P	F	P	F	P
Species richness	1, 116	2.27	0.134	0.53	0.468	9.96	**0.002▲**
Group richness	1, 116	3.58	0.061	0.41	0.523	9.44	**0.003▼**
Biodiversity	2, 116	1.82	0.167	0.28	0.757	5.68	**0.004**
Fertilization	1, 116	0.02	0.876	5.79	0.177	3.15	0.078
Test of parallelism	2, 114	0.16	0.849	0.06	0.944	0.01	0.992

Table 6. Repeated measures ANOVA for complementarity and selection effects and Log (*OI*). Independent variable was fertilization. As covariates number of species and functional groups were used. Effect of both covariates is significant for complementarity (Rao`s *R*, $F_{(6,\ 228)} = 20.99$, $P < 0.001$), selection (Rao`s *R*, $F_{(6,\ 228)} = 4.02$, $P < 0.001$) and Log (*OI*) (Rao`s *R*, $F_{(6,\ 228)} = 2.68$, $P = 0.016$).

		Compl		Select		Log (*OI*)	
	df	F	P	F	P	F	P
Fertilization	1, 116	19.22	**0.000**	10.94	**0.001**	0.03	0.857
Time	2, 236	19.10	**0.000**	16.32	**0.000**	2.72	0.068
Interaction	2, 236	3.95	**0.000**	6.20	**0.002**	5.04	**0.007**

Table 7. Results of repeated measures ANOVA for D, AD, D_{st}, AD_{st} and ED. Time was only one variable. As covariates number of species and functional groups were used.

		D		AD		D_{st}		AD_{st}		ED		
	df	F	P	F	P	F	P	F	P	df	F	P
Time	2, 182	9.02	0.000	41.03	0.000	4.19	0.017	3.70	0.027	2, 106	59.16	0.000
Covariates	6, 174	1.32	0.250	0.82	0.555	1.30	0.259	4.92	0.000	6, 98	2.43	0.031

2005, but significantly increased with the number of functional groups in 2003 (Table 5, Fig. 3). Fertilization influenced communities in 2003 and 2004. Complementarity values were higher in control plots, while selection values were greater in fertilized plots. Values of selection and complementarity varied significantly among years, but the variation was not parallel in fertilized and control plots (Table 6).

The plain non-standardized difference (D) for biomass did not change with covariates (neither the effect of species number nor that of functional group number was significant), but

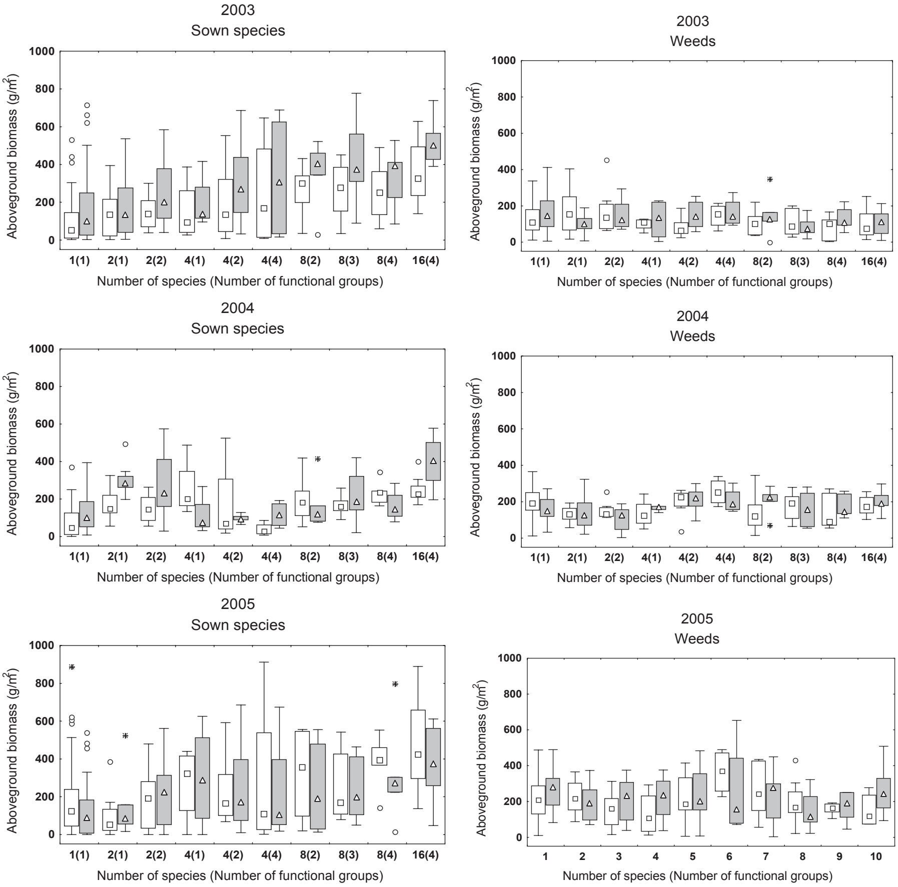

Fig. 1. Relationships between aboveground biomass of sown species for years 2003, 2004, 2005 and aboveground biomass of weeds for 2003, 2004, 2005, and species richness of the communities. Unfertilized plots: empty bars, fertilized plots: filled bars. The box shows the interquartil range with median, whiskers reach to the non-outlier range (i.e., range of data within {lower quartil – 1.5 x interquartil range; upper quartil + 1.5 x interquartil range}), (o) – outliers, (*) - extremes.

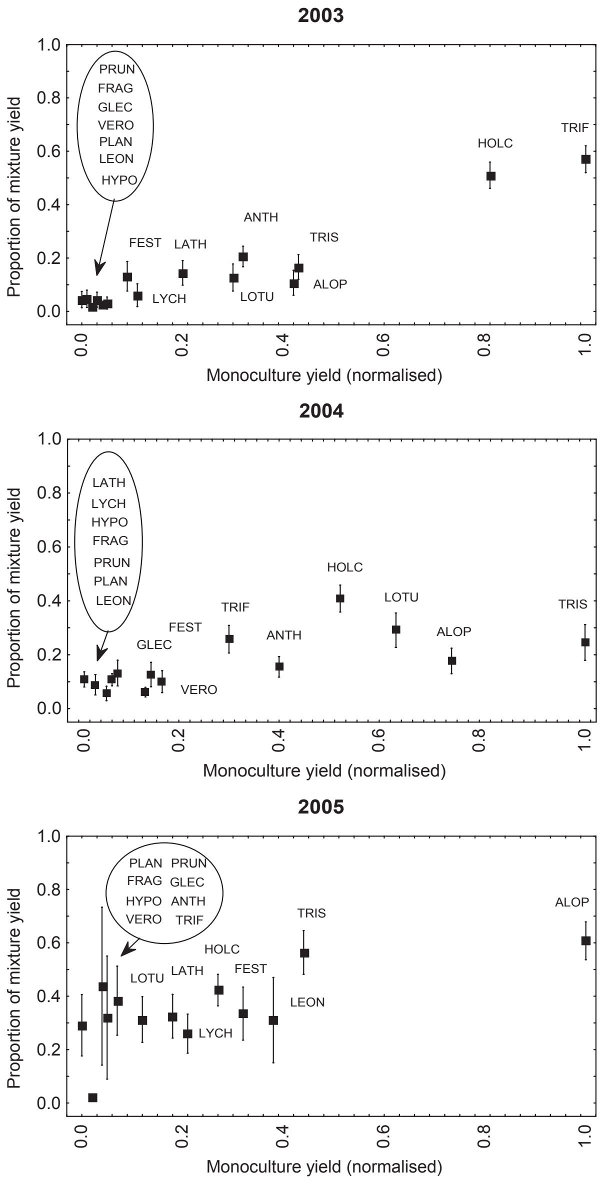

Fig. 2. Dominance of species in individual mixtures (proportion of total aboveground biomass) vs. average monoculture yields normalized to the maximum biomass. Results for fertilized plots are shown. Boxes are means, whiskers s.e. Abbreviations: HOLC – *Holcus lanatus*, FEST – *Festuca rubra*, ALOP – *Alopecurus pratensis*, TRIS – *Trisetum flavescens*, HYPO – *Hypochaeris radicata*, LYCH – *Lychnis flos-cuculi*, LEON – *Leontodon hispidus*, PLAN – *Plantago media*, FRAG – *Fragaria vesca*, VERO – *Veronica officinalis*, PRUN – *Prunella vulgaris*, GLEC – *Glechoma hederacea*, TRIF – *Trifolium pratense*, LOTU – *Lotus corniculatus*, ANTH – *Anthyllis vulneraria*, LATH – *Lathyrus pratensis*.

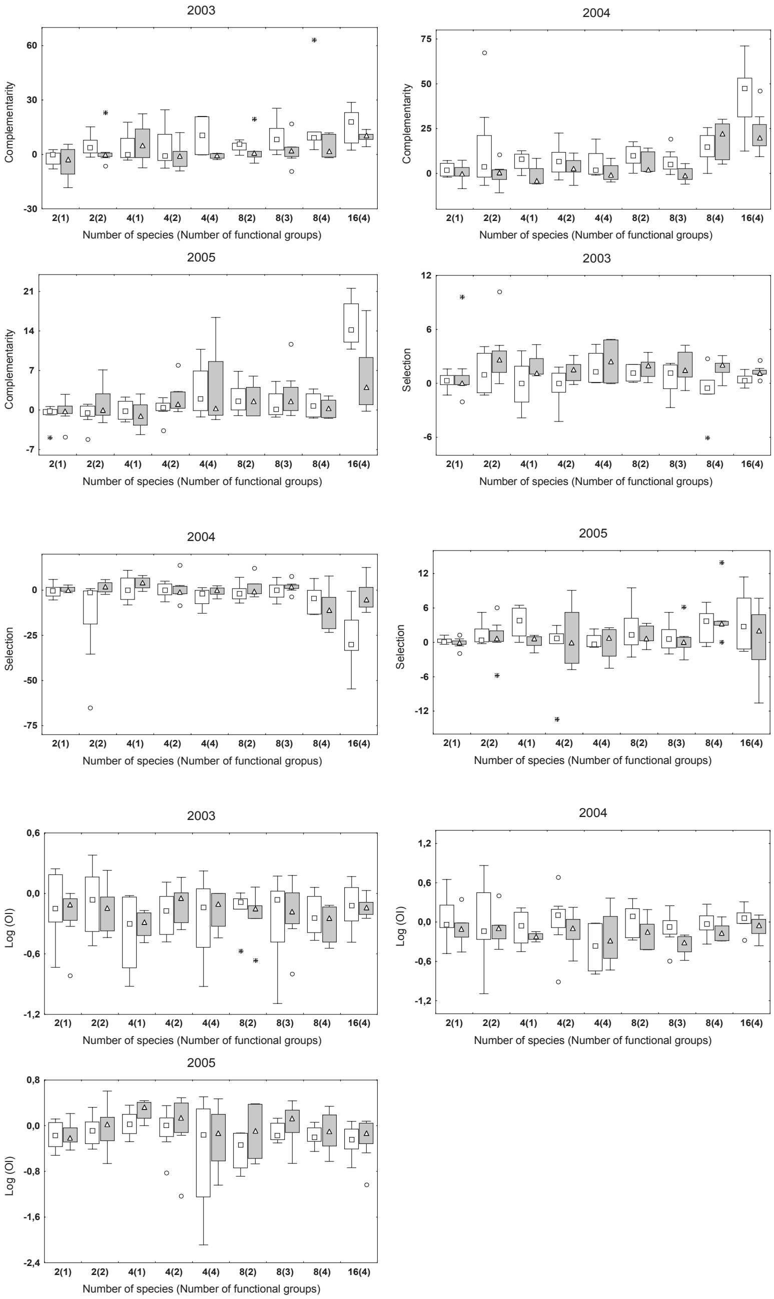

Fig. 3. Biodiversity effects selection, complementarity, and log(*OI*) regressed against biodiversity treatments used in the three year experiment. The box shows the interquartile range with median, whiskers reach to the non-outlier range (i.e., range of data within {lower quartile – 1.5 x interquartile range; upper quartile + 1.5 x interquartile range}), (o) – outliers, (*) - extremes.

the value strongly decreased with time (Fig. 4, Table 7). For some plots, the difference was negative, i.e. their biomass was higher in low nutrient treatments. The plain standardized difference (D_{st}) and absolute non-standardized difference (AD) also did not change with diversity, bud varied with time. The standardized absolute value of the difference (AD_{st}) significantly decreased with diversity. Chord distance increased with diversity (Fig. 4, Table 7) and very strongly increased with time.

Discussion

Our results demonstrate that both plant species number and functional group richness impacts aboveground biomass of sown residents and weeds. Greater plant species richness led to greater aboveground biomass of sown species, while the biomass of weeds was negatively influenced by the biomass of sown species. This agrees with previous biodiversity experiments (e.g., Hector et al., 2001; Symstad, 2000; van Ruijven et al.; 2003). These authors found a positive relationship between invasion resistance and increasing community diversity, which confirmed long-held theoretical expectations (Elton, 1958). The negative effect of diversity is often explained by increased resource use complementarity. When complementarity between species results in lower levels of available resources in high diversity, then invasion is inhibited (Knops et al., 1999).

The experiment showed that a majority of sown species grown in monocultures had higher biomass in fertilized than in control plots. This contrasted with a previous pot experiment performed for one season in a greenhouse (Lanta and Lepš, 2006), where we also manipulated diversity in two nutrient levels. In this pot experiment, productivity was higher in lower nutrient treatments particularly in monocultures, while some species, often dominants, had higher productivity in mixtures than in monocultures. In pots, some species were apparently harmed by high nutrient levels, and profited from the presence of other species, which probably decreased the nutrients to acceptable levels. No such effects were found in the field experiment. This comparison demonstrates how important it is to compare pot experiments with real field situations.

Trifolium and *Holcus* dominated the mixtures in 2003,. Both were also highly productive in our pot experiment (Lanta and Lepš, 2006). This might be a consequence of the high growth rate of *Trifolium*, as found in another experimental study (Spehn et al., 2002). However, this species gradually declined in the latter years of 2004 and 2005. This might have been partially caused by a rust fungus infection, which was observed on most plants of this species as very small gray-green spots. Again, the same effect was observed by Spehn et al. (2002). *Holcus* is a major component of wet meadows (Lepš, 2004) that can quickly form very dense tussocks due to the production of extravaginal ramets (Grime et al., 1988). This species was very productive over the whole course of the experiment. Two other grasses, *Trisetum* and

Alopecurus acquired very high biomass in 2004 and 2005 too, leading to the dominance of grasses in all plots where they were sown. Accordingly, the values of both complementarity and selection effects change pronouncedly over the course of the experiment. The dynamics of community composition and also of various indices during the first years demonstrate how caution is required in interpreting results of short term experiments with species mixtures, particularly when the desire is to generalize our results to communities not undergoing transitional dynamics.

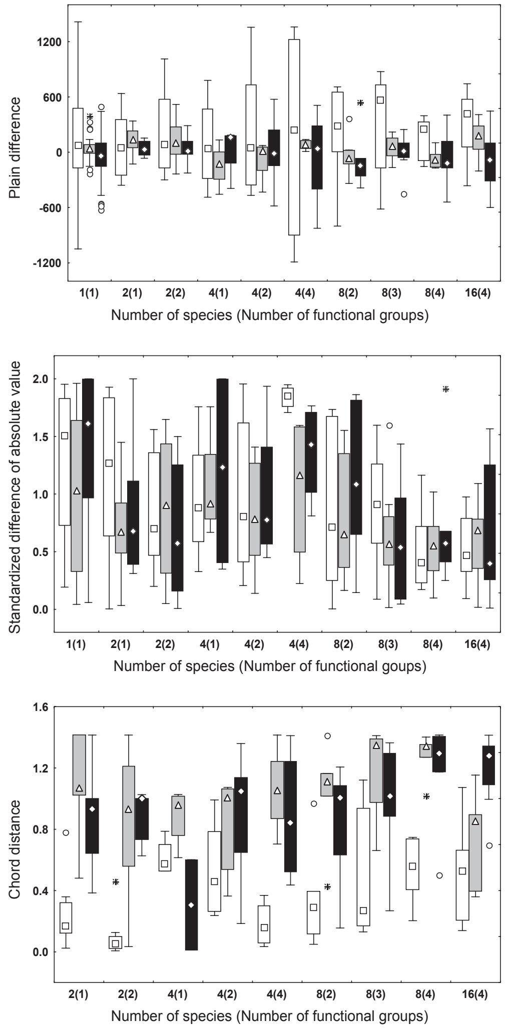

Fig 4. Plain difference, standardized difference of absolute value and chord distance between biomass of high and low nutrient levels. Differences for 2003 are shown by empty boxes, 2004 by gray boxes and 2005 by filled boxes. The box shows the interquartile range with median, whiskers reach to the non-outlier range (i.e., range of data within {lower quartile – 1.5 x interquartile range; upper quartile + 1.5 x interquartile range}), (o) – outliers, (*) - extremes.

With the exception of 2005, selection was significantly higher in the fertilized plots, and complementarity significantly higher in the unfertilized controls. Fertilization partially releases plants from competition for nutrients, but, as a consequence, the competition for light increases. Taller plants are better competitors for light and usually take over in fertile conditions (Lepš, 1999). In fertile conditions with prevailing competition for light, which is more asymmetric than underground competition, the selection for highly productive species seems to be stronger. On the contrary, in unfertilized plots with prevailing competition for underground resources, there is a better chance for complementary use of resources (e.g. due to heterogeneity of soil environment, different rooting depths of different species, etc.). Moreover, in additive partitioning, complementarity includes also facilitation, as it is impossible to distinguish it from complementarity only on the basis of compositional data (analysis of the mechanisms would be needed). Consequently, if there is an effect of legumes as nitrogen fixers (in our case mainly the effect of *Trifolium*), then this effect would be stronger in unfertilized plots. Generally, the positive effect of legumes on the productivity of the entire community is a well known phenomenon; consequently, some authors (Huston et al., 2000) consider the increase of productivity with diversity in this situation, where increasing diversity increases the probability of including a legume, as trivial.

The biomass of *Trifolium* rapidly decreased in 2004 and 2005. It is likely that the niche was then occupied by grasses. This effect was particularly seen in 2005 when values of selection effect increased with increasing diversity (the effect was significant for increasing number of functional groups in plots). The selection effect was stronger in the fertilized treatment than in the control environment in 2003. In contrast, complementarity values were lower in the fertilized plots. This fact indicates that the sampling effect estimated by the selection effect metrics promoted ecosystem productivity with fertility.

Trifolium is a member of the legume functional group, which is known to strongly increase both productivity and complementarity, because members of this group do not compete with other plants for soil nitrogen, but fix atmospheric N_2. The analyses showed that species diversity had a stronger effect on community productivity than does functional group composition, but that composition has relatively stronger effects on productivity than diversity. This difference can be partly explained by the opposite effects of legumes versus grasses on biomass production. Legumes (mainly *Trifolium* presence) tend to increase concentration of soil nitrate, whereas grasses have high root biomass with high C:N ratios. Legumes are highly productive because of their ability to fix nitrogen, and grasses are highly productive because of their high efficiency of capture and nitrogen use. This means that these functional groups have similar effects on total biomass, thus causing species diversity, a measure of the chance that legumes or grasses are present, to be a more important determinant of community biomass.

Our experiment agreed with some long-term experiments showing strong transgressive overyielding effects, in which the experimenters postulated that positive complementary resource use among species may lead to increased productivity of plant communities (i.e., Tilman et al., 2001). Similarly, Hooper and Dukes (2003) found strong overyielding, which was caused by facilitation of N-fixers. Another example is Joliffe (1997), who found that mixtures with great overyielding often included N-fixers, although some mixtures with legumes significantly underyielded.

When the control and fertilized plots with the same species composition were compared, several trends were observed. First, whereas the differences in species composition increased with time, most measures of differences in productivity decreased. The increase in compositional difference with time suggests that nutrient status is an important determinant of competitive equilibria, which are being reached step by step over time. However, this increase in compositional difference signifies that the most appropriate species are selected for a given environment. This might cause the decrease in differences in productivity. In particular, the standardized absolute difference decreased both with time and also with species richness, whereas the chord distance increased. This corresponds to the theoretical predictions of Tilman (1999) that the stability of aggregated characteristics (like total productivity) might increase with diversity thanks to greater changes in individual populations (changes in individual populations are reflected by chord distance). This shows that changes in species composition toward the one best suited to a given environment can lead to optimal resource use and, in consequence, diminish the variation in community functioning in a changing environment.

Overall, this experiment presented evidence that plant species diversity does have effects in experimental plant communities. However, transgressive overyielding, the clear proof of the effect of species richness on community productivity (Garnier et al., 1997), was mostly not found. The strongest influences on primary productivity were combined effects of the number of species and functional groups planted in a mixture. The study demonstrated that ecosystem functioning depends on community composition. Functional group diversity of sown species played important role in suppressing establishment of weeds from the soil seed bank. The study showed that a system open to invasion has weaker effects of diversity on productivity weaken over time.

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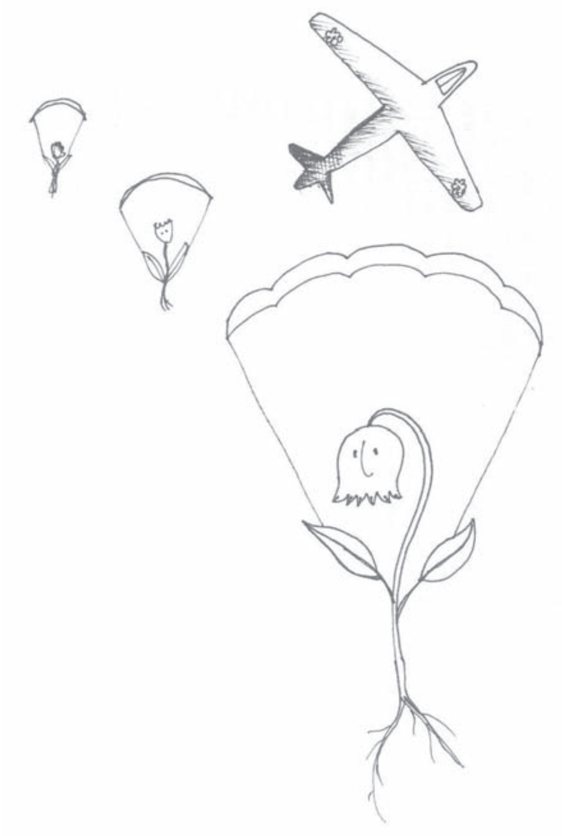

Paper III

Effect of plant species richness on weed invasion in experimental plant communities

Effect of plant species richness on weed invasion in experimental plant communities

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Abstract. Invasion of weeds (defined as all unsown species) to artificially created assemblages of grassland species was investigated in a three years field experiment. In the experiment, species richness (1, 2, 4, 8 and 16 species) and functional group richness (grasses, legumes, rosette forbs, creeping forbs) were combined in factorial design with fertilization. No hand-weeding was done. Relationships between species diversity and aboveground biomass were positive for sown species in all studied years 2003, 2004 and 2005. In the two latter years, biomass of weed invaders decreased significantly with the biomass of sown species, with their functional group richness, but not with their species diversity. The negative relationship between the number of functional groups of residents and weed biomass suggested that species assembled into groups according to similar traits could have large effect on species invasibility. Additionally, species composition of weeds significantly related to initial composition of sown residents. It showed that the resultant composition of weeds depends on the presence and proportion of individual sown species.

Keywords: Aboveground biomass, Biodiversity, Invasion, Sowing, Weeds

Introduction

Concern over impacts of biodiversity loss on the functioning of ecosystems has stimulated renewed interest in the importance of species diversity to community stability. One of components for community stability is the ability to increase their resistance to invasion. Experimental studies of the correlation between diversity and invasion measured on local scales indicate that communities decline in invasibility as species accumulate over time (are more stable), thought the role of diversity itself was often ambiguous (see review Levine and D`Antonio 1999). These results also can reflect environmental factors covarying with diversity.

The effect of species richness of a community on its invasibility has been in ecologists' focus since ninetieth, when the debates on the effect of biodiversity on its function were very contentious (i.e., Kaiser 2000, Johnson et al. 1996). The main hypothesis was that the resistance against invasion increases with resident plant diversity. That idea has been proposed already by Elton (1958) and subsequently supported by many theoretical and field studies (e.g., Rejmánek 1989, Case 1990, Law and Morton 1996, Tilman 1997, Knops et al. 1999, van Ruijven et al. 2003) investigating the relationship between biodiversity and ecosystem functioning. The positive relationship between species richness and resistance to invasion is usually explained by better utilization of resources by species-rich communities so that fewer resources are available for invading species. Similar reasoning can be used for both, invasion of alien species into communities of indigenous species, and invasion of "weeds" (any unintended species) into sown species mixtures. We use the term invasion here in wide sense, i.e. for any appearance of a new species in existing plant community. Community invasibility means lack of resistance to invasion. However, not all studies found a negative relationship between diversity and plant invasions. Positive relationship between resident species richness and the number of invasive species was also repeatedly found (i.e., Robinson et al. 1995, Palmer and Maurer 1997). These results were explained by the covariation with extrinsic factors (such as climate, disturbance, and soil fertility) with resident plant diversity and invasion rate (Naeem et al. 2000, McIntyre and Lavorel 1994, Tilman 1993, Burke and Grime 1996). Recent investigations showed that the impact of dominants rather than the impact of species richness on community productivity and invasibility could play important role in biodiversity experiments. Manipulations of plant dominance through seed addition (Foster et al. 2002) and biomass reduction of clonal dominants (Smith et al. 2004) supported this idea. Similarly, Robinson et al. (1995), in agreement with this idea, found that the invasion success is not a function of the species richness, but depends on the level of dominance exerted by a grass.

The species richness of a community is determined mainly by two extrinsic sets of factors – environmental conditions and species pool (Lepš 2004). Those determine the nature of interspecific interactions, and, through them, final composition (and consequently also the diversity) of the community. It can be expected that covariation of functional characteristic with diversity will be different, when the community diversity is determined mainly by environmental condition (most intersite comparisons), and when it is affected by species pool (e.g. comparison of mainland with islands, and most of experiments with synthetic communities). In the present study, we focus mostly on the differences caused by species pool.

Wardle (2001) argued that the relationship between the invasion success and the species richness is often negative due to the difference in species composition between species rich and poor communities. He suggested that species rich plots are less invaded because they are more likely to include aggressive plant species that fully exploit resources (i.e. the sampling effect). Thus, he argued that the invasion success is a function of species identity in the community rather than the species richness.

Only few so far reported grassland biodiversity experiments has focused on the relationships between species richness and extinction rates in invaded plant communities (e.g., Pfisterer et al. 2004). There was found that extinction rates were positively related to species richness. According to the theory of island biogeography (MacArthur and Wilson 1967), invasion should be high in species-poor communities and extinction high in species rich communities, so that combination of these factors may lead to the balanced species richness.

Competition for nutrients is a race to produce roots in areas of high nutrient availability, but low root occupancy, thus allowing a plant to preempt nutrients from competitors (Grime 2001). The race for nutrient resources can indirectly influence competition for light when stronger competitors can utilize more nutrients and quickly develop photosynthetic apparatus and production of leaves above competitors (e.g. Fahey et al. 1998). Placing leaves rapidly above those of other species requires high acquisition potential of both light and below-ground resources.

To test the invasion-biodiversity hypothesis, we designed an experiment, where between-year variation in biomass of sown species and invading weeds under different nutrient regimes was studied. Species density of plants ranged from 1 to 16 per plot. No weeding was applied there. The aim of this study is to analyze the relationship between the diversity of sown plant community, and its resistance to invasion of naturally colonizing species (mostly arable weeds). Specifically, we asked; (1) how is the resistance to invasion affected by community species richness and by richness of functional groups? (2) how is the resistance to invasion affected by species composition of the sown mixture. Because the biomass of the sown species increased with the number of sown species (as is usual in experiments with manipulated species pool), we particularly interested in.

Methods

The experiment was conducted in an abandoned agricultural field, last cultivated 2001, at Benešov village in South Bohemia, CZ (49° 92` N, 15° 00` E, altitude approximately 660 m). This region experiences a temperate climate, with the mean annual temperature of 6.4 °C and the annual precipitation of 680 mm.

For this experiment, species representing major component of the surrounding grasslands were selected. All of them are polycarpic perennials, and can be divided into four functional groups: four grass species - the narrow leaved *Festuca rubra*; the broad-leaved *Trisetum flavescens, Alopecurus pratensis* and *Holcus lanatus*; four rosette hemicryptophytes - *Lychnis flos-cuculi*, *Hypochaeris radicata*, *Plantago media* and *Leontodon autumnalis*; four herbs with creeping aboveground stolons - *Veronica officinalis*, *Glechoma hederacea*, *Fragaria vesca* and *Prunella vulgaris*; four nitrogen-fixers (legumes) - *Lotus corniculatus*, *Anthyllis vulneraria*, *Trifolium pratense* and *Lathyrus pratensis*.

In March 2002, the experimental treatments were established in 2x2 m plots, using a completely randomized design. Ten treatments differing in the number of species and functional group were used, with different species combinations within the treatments (Table 1). Monocultures and mixtures of 1-16 species were sown at the same density of 3000 seeds per 1 m^2. All species were equally represented at each combination of species and functional group richness. An equal number of seeds of each species was sown in each combination. As far as possible, all of the functional groups were equally represented in mixtures with more functional groups (only 3:3:2 ratio was used in treatment 8, i.e. three functional groups, eight species). The sown species are called residents further on. The mixtures were grown under

Table 1. Treatments, number of species combinations and number of replications used in each, high and low nutrients pots.

Treatment	Number of species in mixture	Number of species combinations	Number of replicates
1	monoculture of each species	16	2x
2	2 species within one functional group	8	1x
3	2 species from two groups	8	1x
4	4 species (each species is from one group)	4	1x
5	4 species (each two species are from different groups)	4	2x
6	4 species that composed one functional group	4	1x
7	8 species from two groups	6	1x
8	8 species from three groups	8	1x
9	8 species from four groups	6	1x
10	16 species (i.e., four functional groups)	1	8x

two nutrient levels (non-fertilized plots, further on called control plots, and fertilized plots). The plots assigned to the nutrient addition treatment were amended with 40 g NPK m^{-2}. yr^{-1} (Synferta P-NPK; AGRO CS Česká Skalice, CZ), applied in April 2002, March 2003, 2004 and 2005. The experiment yielded 184 plots.

In each 2x2 m plot, 1-m^2 subplot was fixed in the center and the cover of each naturally invading species (called weeds thereafter) was recorded in early summer 2003, 2004 and 2005. The amount of weeds is considered a measure of community invasibility. The plots were harvested for aboveground biomass by clipping all of the plants at ground level in two 15x15 cm quadrates adjacent to each 1 m^2 plot. The subsamples were then pooled, separated into biomass of sown species and weeds, dried and weighed.

To evaluate immigration potential of weeds, immigration rates for each plot were calculated as $IR=2I/(S_{03}+S_{05})$, where I is the number of species immigrations for each plot from 2003 to 2005. S_{03} is the number of species recorded in 2003 and S_{05} is the number of species recorded in 2005 (Nilsson & Nilsson 1982). I is simply the difference S_{05} - S_{03}. Rarely, more species were recorded in 2003 than in 2005; in this case, I was set to zero.

We provided statistical tests to estimate dependence of (i) total aboveground biomass of sown species (residents), (ii) weedy species (invaders), and (iii) the number of weeds per plot on both the number of residents and number of functional groups. These tests were provided using repeated measures ANOVA in general linear models (GLM) for data sampled over three years. Species richness and functional group richness were used as continuous predictors, and nutrient level as the categorical one. Linear regressions we used to find any differences in

Table 2. Effect of the number of sown species (Species), functional group richness (Group), fertilization (Fert) and vegetation season (Time) on the biomass of sown species and weeds, and on the number of weeds in 1 m^2 plots tested using repeated measures ANOVA in GLM. Symbol ** indicates significance level $P < 0.01$; *** $P < 0.001$; * $P < 0.05$; n.s.– non-significant. ▲ or ▼ indicates significant increase or decrease in the values of tested variables.

		Biomass of sown species			Biomass of weeds			Number of weeds		
	Df	F	P		F	P		F	P	
Species	2	10.44	**	▲	1.01	n.s.		0.02	n.s.	
Group	2	4.80	*	▲	4.84	*	▼	2.61	n.s.	
Fert	2	2.75	n.s.		0.96	n.s.		0.17	n.s.	
Error	180									
Time	2	4.00	*	▲	6.48	**	▲	48.70	***	▲
Time*Species	2	0.35	n.s.		1.55	n.s.		0.24	n.s.	
Time*Group	2	0.03	n.s.		0.61	n.s.		0.41	n.s.	
Time*Fert	2	5.69	**	▼	0.77	n.s.		0.32	n.s.	
Error	360									

immigration rates across biodiversity treatments. Prior to the analyses, biomass and the number of weed species were log transformed in order to achieve equal variance and normality of the input data.

To examine the relationship between composition of residents and invaders redundancy analysis (RDA) was used. RDA is a direct gradient analysis technique that is suitable for data with a low beta diversity (ter Braak & Šmilauer 2002). This requirement is accomplished in short-term experiments, such is this one. Both RDA wit and without standardization by sample norm were used. With non-standardized analyses we investigated if treatments affected species cover. With standardized analyses we studied if treatments caused changes in species proportion. RDA selects a linear combination of explanatory variables (in this case the number or seeding density of species in individual mixtures) that maximally explains variation in weed cover. A square root transformation of cover data was applied in order to dampen out the effect of abundant weeds. In RDA analyses various combinations of environmental variables, covariables and their interactions can be used with appropriate Monte Carlo permutation test to evaluate a wide range of hypothesis (ter Braak & Šmilauer 2002). Data were in the form of repeated measurements. Therefore, the permutation scheme was adjusted to the repeated measures design. A split-plot design approach, where split plots were individual observations and whole plots were records of one permanent plot repeated in time and were permuted completely at random. A significant effect of the time x treatment interaction indicates a divergent temporal development of plots under different treatments. A biplot ordination diagram was used to visualize the results of the analyses. Only the species with the highest fitting to ordination axes are shown in the ordination diagram.

Table 3. Effect of the number of sown species (Species), functional group richness (Group), biomass of residents (log transformed) and fertilization on biomass of weeds. ▲ or ▼ indicates significant increase or decrease in the values of tested variables.

		2004			2005		
	df	F	P		F	P	
Species	1	6.97	**	▲	5.19	*	▲
Group	1	0.61	n.s.		3.26	n.s.	
Biomass of residents(log)	1	32.95	***	▼	41.02	***	▼
Fert	1	0.55	n.s.		0.50	n.s.	
Error	179						

Results

Species and functional group richness of residents were both significant predictors of the total aboveground biomass of residents (Table 2). Significant interaction between the time and fertilization was found, which indicates a decrease in biomass values in time.

Only the functional group richness of residents (but neither number of resident species nor fertilization) had a significant negative effect on the biomass of weeds (Table 2). The biomass of weeds differed among years, being highest in 2004.

The number of weed species that invaded the communities was not affected by the diversity of residents (Table 2), but differed among years - the highest number of weed species was recorded in 2005 (Fig. 2).

When pooled for control and fertilized plots, only nonsignificant relationship between biomass of residents and weeds was found in 2003 (-r=0.13, P=0.087), but highly significant negative relationships in 2004 and 2005 (-r=0.32, P<0.001 and -r=0.45, P<0.001, respectively). Effect of the biomass of the residents on the weed biomass was much more pronounced than that of their richness (either characterized by species number or number of resident groups, Table 3). Surprisingly, when both richness characteristics and biomass of residents are used as predictors in the same GLM, the partial effect of the species number on the weed biomass is positive (Table 3).

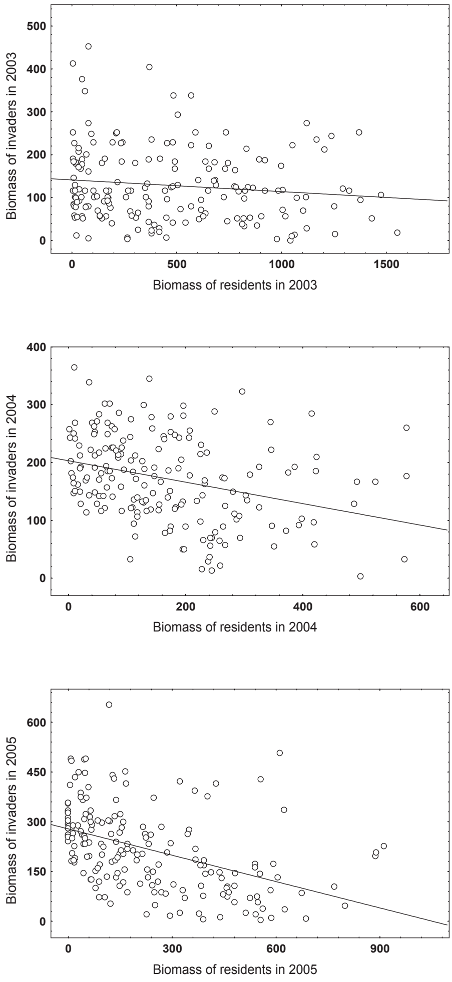


Fig. 1. Relationships between biomass of invaders and residents for subsequent years 2003, 2004 and 2005. Note that different of scales of axes are shown.

Table 4. Results of non-standardized and standardized RDA analyses of cover estimates of weeds in 1 x 1 m plots. Env. var. = Environmental variables, Covar. = Covariables, % ax 1 = % species variability explained by axis 1 – measure of the explanatory power of the environmental variables, r ax 1 = species environment correlation on axis 1, F = F – ratio statistics for the test on the trace (all axes). P = corresponding probability value obtained by the Monte Carlo permutation test (499 permutations, i. e. Type I error probability in testing the hypothesis that the effect of one explanatory variables is zero). S = number of sown species in plots; G = number of functional groups; F = fertilization treatment; I = identity of sown species, i.e., seeding density of individual sown species in particular mixtures; T = serial year number; PlotID = identifier of each plot; * = interaction between terms.

	Env. var.	Covar.	% ax 1	r ax 1	F	P
Non-standardized						
A1	S*T	G*T, F*T, I*T, T, PlotID	1.2	0.33	1.99	*
A2	G*T	S*T, F*T, I*T, T, PlotID	0.7	0.31	1.09	n.s.
A3	F*T	S*T, G*T, I*T, T, PlotID	0.5	0.25	0.87	n.s.
A4	I*T	S*T, G*T, F*T, T, PlotID	4.8	0.61	1.65	**
A1+2	S*T, G*T	F*T, I*T, T, PlotID	1.5	0.25	5.31	**
Standardized						
A5	S*T	G*T, F*T, I*T, T, PlotID	1.7	0.20	2.29	*
A6	G*T	S*T, F*T, I*T, T, PlotID	0.2	0.15	0.84	n.s.
A7	F*T	S*T, G*T, I*T, T, PlotID	0.2	0.13	0.43	n.s.
A8	I*T	S*T, G*T, F*T, T, PlotID	1.8	0.39	1.67	**
A5+6	S*T, G*T	F*T, I*T, T, PlotID	0.8	0.21	1.54	*

Table 5. Results of non-standardized and standardized RDA analyses of cover estimates of weeds in 1 x 1 m for the season 2005.

Env. var.	Covar.	% ax 1	r ax 1	F	P
Non-standardized					
S	G, F, I	4.8	0.29	8.25	**
G	S, F, I	1.5	0.19	2.57	n.s.
F	S, G, I	0.2	0.15	0.29	n.s.
I	S, G, F	24.0	0.60	4.10	**
S, G	F, I	5.9	0.32	5.37	**
Standardized					
S	G, F, I	1.8	0.23	2.94	*
G	S, F, I	0.8	0.17	1.29	n.s.
F	S, G, I	0.5	0.20	0.90	n.s.
I	S, G, F	18.3	0.57	2.77	**
S, G	F, I	1.9	0.24	1.7	n.s.

Linear regressions did not detect any relationship between the number of the species immigrations and increasing diversity of residents (r=0.03, P>0.05 for control and r=0.18, P>0.05 for fertilized plots, Fig. 3).

The RDA analyses of both non-standardized and standardized data sets showed that the species composition of invaders was significantly affected by both, the species composition

Fig. 2. Influence of initial species richness of the communities on the total number of weeds for the years 2003, 2004 and 2005. Shown are only mean values.

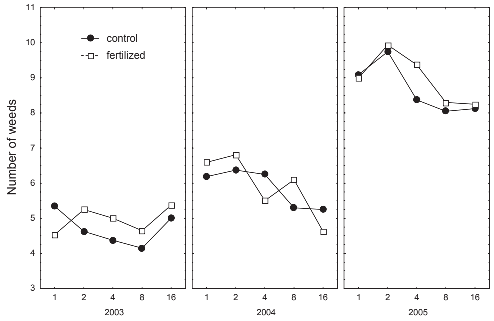
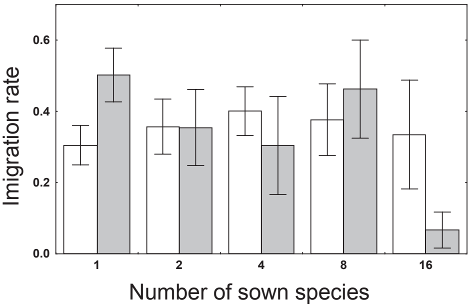
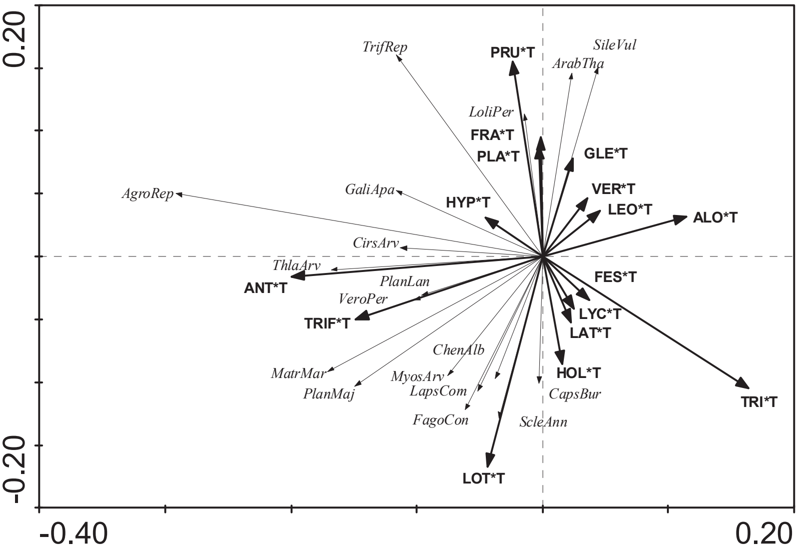


Fig. 3. Effect of initial species richness on the extinction rate. Shown are means (bars) ± s.e. (whiskers). Unfertilized plots: empty bars, fertilized plots: filled bars.



and the richness of the residents (Tables 4 and 5). While the effect of the number of sown species was significant (Table 4; analyses A1, A5), the effect of the number of functional groups and fertilization were not detected. The weed composition was significantly related to the species composition of the sown residents, indicated by RDA, in which the initial densities of each sown resident species were used as a set of environmental variables (Table 4; analyses A4 and A8). Species composition of residents and species richness had fairly greatest effect on the weed community, analyzed both as repeated measurement (Table 4), or as was shown for the final year of experiment (Table 5). The percentage explained by the first axis is not directly comparable (there are 16 variables describing the composition), nevertheless, the effect of those 16 variables together is always highly significant, similarly as the effect of the number of species, whereas the effect of the number of functional groups is not detected. The ordination diagram suggests that residents suppress most the weed species of the same functional group (Fig. 4). This is particularly true for *Agropyron repens*, a grass weed with the highest cover, which was negatively related to the sown grasses, but positively related to the densities of *Anthyllis* and *Hypochaeris*. The cover of the leguminous weed, *Trifolium repens*, was related to the densities of the sown rosettes, but not to the sown legumes. Other weeds with a high cover such as *Plantago major*, *Matricaria maritima* and *Thlaspi arvense,* were related to the presence of sown legumes.

Fig. 4. Redundancy analysis biplot (Table 3, analysis A4, cover of weeds, nonstandardized data) of the year 2003 to year 2005 difference in the individual weed response to seeding density of individual sown species in particular mixtures. Abbreviations for weeds: * - indicates interaction of environmental variables, AgroRep – *Agropyron repens*, ArabTha – *Arabidopsis thaliana*, CapsBur – *Capsella bursa-pastoris*, ChenAlb – *Chenopodium album*, CirsArv – *Cirsium arvense*, FagoCon – *Fagopyrum convolvulus*, GallApa – *Galium aparine*, LapsCom – *Lapsana communis*, LoliPer – *Lolium perenne*, MatMar – *Matricaria maritima*, MyosArv – *Myosotis arvensis*, PlanLan – *Plantago lanceolata*, PlanMaj – *Plantago major*, ScleAnn – *Scleranthus annuus*, SileVul – *Silene vulgaris*, ThlaArv – *Thlaspi arvense*, TrifRep – *Trifolium repens*, VeroPer – *Veronica persica*. Abbreviaions for residents: ALO – *Alopecurus pratensis*, ANT – *Anthyllis vulneraria*, FES – *Festuca rubra*, FRA – *Fragaria vesca*, GLE – *Glechoma hederacea*, HOL – *Holcus lanatus*, HYP – *Hypochaeris radicata*, LATH – *Lathyrus pratensis*, LEO – *Leontodon hispidus*, LOT – *Lotus corniculatus*, LYC – *Lychnis flos-cuculi*, PLA – *Plantago media*, PRU – *Prunella vulgaris*, TRIF – *Trifolium pratense*, TRI – *Trisetum flavescens*, VER – *Veronica officinalis*.



Discussion

The results of this study only partly support the Elton`s hypothesis (1958), suggesting that increasing resident diversity decreases invasibility. We found that increasing number of functional groups of sown residents suppressed invasibility of weeds (measured by their biomass). The fairly greatest negative effect on the weed invasion, however, was demonstrated by the total biomass of sown residents (similarly as, e.g. Knops et al. 1999). Relationship was significant for 2004 and 2005, and its strength increased with time. It seems that competitive effect of residents increased with time - because open space in vegetation became more closer in latter years. Differences in weed production in three studied years could be also partly a result of different recruitment from soil seed bank, survival and establishment of seedlings (Olff et al. 1994). Different reproduction life histories and dispersal ability undoubtedly played a role too (Stykstra et al. 1998).

There are two possible explanations for weak or no relationship between invasion impact and the number of sown residents. One of them is environmental heterogeneity. When different species utilize different soil resources and form a different kind of canopy structure, then increasing diversity may result in increasing environmental heterogeneity (Levine and D`Antonio 1999). This microheterogeneity could potentially increase the biomass and/or diversity of weed. The second explanation could be the phenomenon of ecological equivalency (Loreau and Mouquet 1999). The idea is that species can coexist together in a community because of competitive exclusion or plant replacement within patches. The idea suggest that communities are ecologically equivalent in such cases when the most productive species (a dominant) could vary from patch to patch independently of the species diversity (Loreau and Mouquet 1999). Plants of different traits thus could reach dominance in different patches whose dynamics is driven by colonization and extinction (Mouquet at al. 2004). This could be a likely explanation for detection of similar invaders productivity throughout all mixtures of different species richness. For instance, the annuals successfully survived in most plots likely due to a high seed production and creation of permanent soil seed bank. However, the effect of functional group richness was significant and negative. Similarly, when the residents biomass effect is added to the model, the partial effect of species richness became positive. This suggests that at the same biomass of residents, assemblages formed by lower number of species are more successful in suppression of weeds. The effect, however significant, is much weaker than the effect of biomass. Interestingly, the effect of functional group richness, even when not significant (P=0.073) is negative. This suggests that for preventing invasion, the functional diversity is more important than species diversity.

No clear differences in community invasibility in relation with fertilization amendment were apparent. This is surprising. We expected that fertilized plots experience a lower level of invasion than the control plots because the relatively productive vegetation in fertilized plots closes gaps more quickly making conditions less favorable for invaders to establish (Morin 1999, Grime 2001). In spite of this hypothesis, our results showed that fertilized communities may be resistant to invasion in the same extent as control ones. This may be influenced by identity of both the residents and also invaders as was shown in ordination diagram. Probably their functional traits may partly determine community resistance (Díaz et al. 1999).

The grass *Agropyron repens* attained the highest cover of all the weed species , but was also the most responsive for the composition of the sown species (Fig. 4). *Agropyron* is able to spread and consecutively occupy empty space by its very fast clonal growth with underground rhizomes; this grants the species a competitive advantage in nutrient uptake (de Kroon and

Groenendael 1997). This makes the species a very aggressive weed. We observed faster growth of this grass weed immediately after the onset of the experiment mainly in plots with sown legumes *Trifolium* and *Anthyllis* and rosette forb *Hypochaeris* (Fig. 4). Both legumes might facilitate the environment through nitrogen fixation (Spehn et al. 2002). However, probably more important was the fact that both the species, similarly as *Hypochaeris,* are subcanopy herbs, with lower competitive abilities, and consequently can be easily overgrown. The second most dominant weed was legume *Trifolium repens*, species forming long aboveground stolons, which helps this species to occupy quickly patches of higher light availability (Hejcman 2005). This weed occurred mainly in plots with sown rosette and creeping forbs, and was less abundant in plots with tall residents, where these species showed lower success in competition with aggressive weeds.

A slightly higher numbers of weed species detected in species-poor communities may reflect the design of the experiment because more diverse plots had lower average population sizes of individual species than species-poor. Similar results were found by Pfisterer et al. (2004).

A major difference between this study and many other biodiversity experiments is that our experiment was carried out without topsoil removal and soil-sterilization and without weeding. The natural colonizers were mainly weeds germinating from soil seed bank, however, some additional colonization from other seed sources, such as diaspore dispersal from neighbour area cannot be completely excluded (Zobel 1997). Our experiment demonstrated that the suppression of early–successional plant species by sown later-successional species was function of both, the species diversity and the species composition in the sown mixtures. Sowing later-successional species may significantly change the initial stage of vegetation development on abandoned arable land (e.g., Hansson and Fogelfors 1998).

Presented biodiversity study showed that the composition of invaders may be suppressed by the number and identity of sown residents. Such identities can be important and, at a certain stage, may affect vegetation development. This suggests that the assembly rules (Wilson 1999) affect, although in highly stochastic manner, the composition of plant communities.

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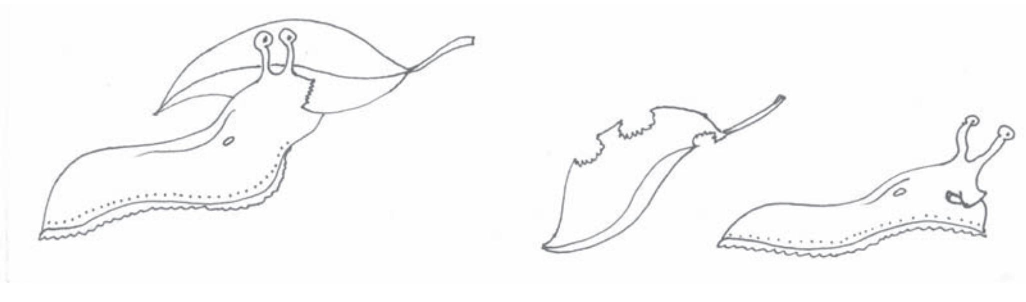
Paper IV

Effect of slug grazing on biomass production of a plant community during a short-term biodiversity experiment



Effect of slug grazing on biomass production of a plant community during a short-term biodiversity experiment

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Abstract. While recent theoretical work has demonstrated several mechanisms whereby more diverse communities can exhibit greater resistance against herbivore pressure, empirical examinations have been few and the subject of much debate. The aim of this aquarium experiment was to determine how the selectivity of herbivores affected relationships between species number and productivity within artificially created grassland communities. The influence of the slug, *Arion lusitanicus*, was assessed at three levels of plant species diversity (1, 2, 4 and 6 species per aquarium). The proportion of biomass of particular species eaten by a slug was estimated on the basis of comparison of paired plots. The biomass in control (ungrazed) plots was compared with the biomass in grazed plots. A significant interaction between the number of species and slug grazing for aboveground biomass was found, indicating a gradual decrease in effect of grazing pressure with increased species richness. Positive average values of the complementarity effect and overyielding index, and negative values of the selection effect indicated niche resource partitioning between species in both grazed and control plots. The electivity index of food selectivity suggested that food selectivity was more pronounced under higher plant species diversity.

Keywords: Diversity effects, Herbivore preference, Slugs, Species richness

Introduction

Recently, many studies have investigated biodiversity effects on ecosystem function. This is largely attributed to increased human impacts on the ecosystem functions. The results of biodiversity experiments are interpreted in different ways. Some researchers give emphasis to species richness, which could explain an ecosystem function (Hector et al., 1999; Tilman et al., 2001). Others test a functional group of organism, individual types and traits (see Diáz and Cabido, 2001; Hooper et al., 2002). The observed response of ecosystem processes to species or functional group diversity can be generated by a combination of different effects (Tilman, 2002; Loreau, 1998, 2000; Loreau and Hector, 2001). The sampling effect appears when the probability of containing species in a community that are able to perform an ecosystem function, increases with the number of species in the community. This effect is often observed during short-term experiments (Tilman et al., 2002). Contrary to the sampling effect, another effect, complementarity, is manifested mainly in long-term biodiversity experiments, and occurs through niche differentiation by individual species (Loreau, 1998).

A consequence of increasing human impact on ecosystem function includes species invasions. In Central Europe the invasive herbivore *Arion lusitanicus*, has gradually expanded in semi-natural meadows, where its grazing may influence the competitive ability of coexisting plant species (Grimm, 2001; Buschmann et al., 2005). Slugs and snails represent an important selection pressure (Harper, 1977) which may lead to the evolution of resistance to herbivory.

Studies that have examined the grazing effects of individual slug species have demonstrated that mollusc herbivory is important in grassland ecosystems (e.g., Pallant, 1972; Hanley et al., 1995; Grimm, 2001; Scheidel and Bruelheide, 2001). Others have focused on the grazing impacts of more than one slug species (e.g., Cates and Orians, 1975; Rees and Brown, 1992), showing that early successional perennials were more palatable than later successional plants. However, few studies have focused on the role of the herbivores in relation to vegetation diversity (Huntly, 1991), taking account the resistance of plant communities to mollusc herbivory (Oliveira Silva, 1992; Grime, 2001; Scheidel and Bruelheide, 2005). Herbivores may remove a sizeable proportion of plant biomass and thus alter both the resource requirements of damaged plants and the availability of resources to other plant species, either of which could influence the outcome of competitive interactions (Howe and Westley, 1988). In other words, defoliation caused by herbivores may have the potential to mediate exploitative competition. Indirect effects of biomass removal on preemptive competition, such as increasing the number of microsites and the opportunities of colonization, may also be an important mechanism (Bullock et al., 1994).

This study investigated the impact of slug grazing on plant species composition by using aquariums as microcosms. This microcosms experiment lacks the variability of natural habitats, and consequently restrict (in comparison with field conditions) the possibility of niche differentiation. All of those restrictions have to be taken into account in interpreting the results. However, the aquarium experiment can be much more replicated than field experiments (which is very important, particularly as there is need the monocultures of all of the constituent species), and enable better control of environmental conditions.

I performed an outdoor aquarium experiment to test the effect of slug herbivory on ecosystem functioning. The following questions were addressed: (i) Are species-rich communities more resistant to slug grazing than species-poor communities? (ii) Is there a biodiversity effect? If so, what is the nature of this effect? (iii) Is there any relationship between increased plant diversity and herbivore preference?

Methods

The microcosm study was conducted at the institute`s experimental garden (Academy of Sciences, České Budějovice, Czech Republic; 390 m a.s.l.). The climate is rather temperate with a summer dry-wet season extending from June to September and cool snow-wet winter.

Selected plants

I selected six perennial plant species for the slug herbivory experiment: two grasses *Holcus lanatus* and *Alopecurus pratensis*; two rosette hemicryptophytes *Lychnis flos-cuculi* and *Hypochaeris radicata* and two other forbs *Plantago lanceolata* and *Achillea millefolium*. All tested species are common at the garden. *Holcus* and *Alopecurus* dominate most of the grasslands. *Alopecurus* is weak competitive species, occurring most frequently within wet areas of the meadow. *Lychnis* (*Caryophyllaceae*) and *Hypochaeris* (*Asteraceae*) are both long-lived rosette plants, that produce a single shoot bearing reproductive organs. *Plantago* (*Plantaginaceae*) and *Achillea* (*Asteraceae*) are common herbs, occurring mainly within the drier parts of the meadow.

Experimental design

I used paired plots (grazed and ungrazed by slugs) in completely randomized design. On the 12th of July 2003, each 38 x 58 x 38 cm in diameter, were divided by glass partition into two plots. The first one was held under slug grazing activity and second one was considered the control (without slug grazing). I used *Arion lusitanicus* (Mollusca: Pulmonata) as the herbivore. This species was collected from the surroundings of greenhouses at the Academy of Sciences in České Budějovice, Czech Republic. Individual slugs were added into one plot of each aquarium 4 times during the experiment. Slugs were introduced to aquariums for two days on the 12th August, the 2nd and the 10th of September. The last introducing was conducted on the 17th of September, when slugs were left in aquariums for five days. After the last census, aboveground plant biomass was clipped (separately in grazed and control plots), sorted into species and dried for six hours at temperature of 105ˇC.

Sand and sterile peat mix (in 2:1 ratio) was used as soil substrate for successful plant growth.

The following plant assemblages were used in the experiment: monocultures (two replicates of each species), all 15 combinations of two-species mixtures (individual combinations were not replicated), all 15 combinations of four-species mixtures (combinations not replicated) and six-species mixtures (all species included, 5 replicates). Plant assemblages were sown in a replacement series design in both plots of an aquarium in the same density of 800 seeds. Aquariums were completely randomized at the experimental site.

Data analysis

The data are in the form of paired plots (two dependent variables at a time: grazed and ungrazed plots). Therefore the hypothesis is that all biodiversity treatments (of 4 levels) are affected by the difference in plots. ANOVA is performed to test this hypothesis. In all analyses, the original number of species in a plot was used as an explanatory variable.

I used the Loreau and Hector (2001) equation to separate selection and complementarity effects. The term "selection effect" is used instead of the term "sampling effect" because it is more general. It does not require complete dominance of the performing species, with extreme trait values selection (Loreau, 2000). The equation describes net biodiversity effect of plant assemblage as:

$$\Delta Y = N\overline{\Delta RY}\,\overline{M} + N\operatorname{cov}(\Delta RY, M),$$

where ΔRY is the deviation observed from expected relative yield of species i in a mixture, M is yield of species i in a monoculture and N is the number of species in a mixture. The net biodiversity effect, ΔY, is the difference between the observed yield of a mixture and its expected yield under null hypothesis that there is no selection and complementarity effect. An observed relative yield of the species in the mixture is the ratio of its yield in the mixture and its yield in the monoculture. An expected relative yield is the proportion of the species sown. The complementarity effect is proportional to the average of ΔRY over all the species in the mixture, whereas selection is proportional to the covariance of ΔRY and yield of the species in monoculture.

The overyielding index (*OI*) calculation, preferred in agricultural ecology (Vandermeer, 1989), can also be used to test for biodiversity effects. The *OI* is derived from the ratio of the biomass of the mixture and the biomass of the most productive species of the mixture. When the ratio reaches a value higher than one, the mixture overyields. When the mixture productivity is lower than the productivity of the best monoculture, the mixture underyields. The *OI* is identical to *I* (Garnier et al., 1997) and D_{MAX} (Loreau, 1998) indices. The *OI* was calculated for each mixture as follows:

$$OI = Y / MAX(M_i),$$

where Y is the biomass of the mixture and M_i is the biomass of the i-th species grown in the monoculture. Log(*OI*) was used (Špačková and Lepš, 2001): if the biomass of each mixture would reach the biomass of its most productive species in monoculture, then the expectation of log(*OI*) is zero.

There were some statistical differences in the calculation of the overyielding. I decided to calculate the transgressive overyielding because it is more conservative of complementary resource use than *RYT* equation (Huston et al., 2000; Hector et al., 2002). Evidence of complementarity in *RYT* is determined by positive deviations from expected yields (based on monoculture performance) across all mixture species (Trenbath, 1974; Vandermeer, 1989).

Positive or negative values generated from the electivity index (see Cock 1978 for a review) were used to detect the grazing preference of slugs. Electivity is defined as:

$$Electivity = \frac{N_e / S_e - N / S}{N_e / S_e + N / S},$$

where N_e is the dry mass of the species in grazed plot, S_e is the total dry mass of the grazed mixture, N is the dry mass of the species in control plot and S is the total dry mass of the control mixture. Species with lower values are more preferred by slugs. To compare the influence of the number of species on electivity index, ANOVA was used.

Table I. Results of ANOVA on the effects of number of species and herbivory on biomass production. Data were log-transformed to improve normality and homoscedasticity. Symbol ** indicates significance level P < 0.01; *** P < 0.001; n.s.– non-significant.

	df	MS	F	P
Number of species	3, 43	1.91	10.71	***
Herbivory	1, 43	1.41	85.49	***
No spec. x herbivory	3, 43	0.25	15.03	***

Table II. Results of ANOVA analyses of biodiversity indices (net effect - Net, selection effect - Select, complementarity effect - Compl) and overyielding -Log(*OI*). Symbol ** indicates significance level P < 0.01; *** P < 0.001; n.s.– non-significant.

		Net		Select		Compl		Log(OI)	
	df	F	P	F	P	F	P	F	P
Number of species	2	1.26	n.s.	11.15	***	6.19	**	10.70	***
Herbivory	1	0.62	n.s.	17.40	***	14.23	***	85.48	***
No spec. x herbivory	2	2.63	n.s.	5.49	**	10.69	***	15.03	***
Error	32								

Results

Species richness and slug grazing were both significant predictors of total aboveground biomass (Table I). Overall, aboveground biomass increased with plant species number and reached significantly higher values in control plots (Figure 1). There was a significant interaction of species richness and slug grazing, indicating a negative effect of grazing on plant biomass at low species richness.

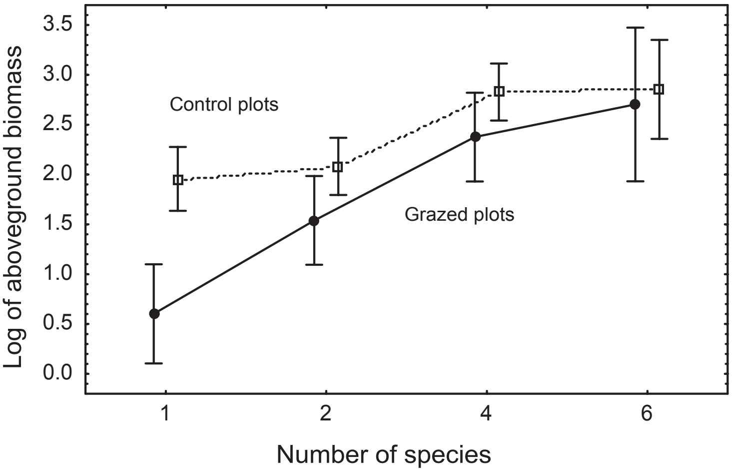

Figure 1. Mean (± SD) aboveground biomass (log scaled) in monocultures and species mixtures with variable species richness. Empty squares represent control plots, full circles represent grazed plots.

Significant differences in selection effect between diversity treatments were found, but the values of the selection effect were dependent on slug grazing (Table II, Figure 2). Average values of selection for biodiversity treatments used, were lower or equal to zero, with slightly higher averages for control plots. Significant interaction of species richness and slug grazing detected very low values of selection for 4-species mixtures which were grazed. The complementarity effect significantly increased with the number of species, but complementarity values were dependent of the slug grazing (Table II, Figure 2). Average values of complementarity for biodiversity treatments used, were higher or equal to zero, with slightly higher values of means for control plots. Significant interaction of species richness and slug grazing detected very high values for 4-species mixtures which were grazed. The values of the net biodiversity effect did not differ between mixture levels and did not depend on the presence of the slugs (Table II).

The analysis of log(*OI*) showed that its values did not increase with the number of species. The values were significantly higher under the grazed treatment (Table II, Figure 2). The interaction of species richness and herbivore grazing was not significant.

The values of electivity index significantly decreased with the number of species (ANOVA, df = 2, 32 , F = 6.69, P < 0.01). The slug *Arion lusitanicus* preferred plant species in this order: *Hypochaeris, Achillea, Alopecurus, Lychnis, Plantago* and *Holcus*. Figure 3 shows that food selectivity was more pronounced with higher diversity treatment.

Discussion

In the current study, outdoor aquariums were used to conduct a diversity experiment using plant assemblages to unfold natural processes. The purpose of the present study was to test predictions about the impact of generalist invertebrate herbivore on plant community productivity.

The number of plant species can alter biomass productivity of a plant community. The observed change in biomass of plant species may not be caused simply by the direct influence of herbivory but rather through a change in interspecific competition resulting from preferential feeding by invertebrate herbivores (Fraser and Grime, 1999). Productivity increased with increasing species richness in both grazed and control plots. Significant interaction between the number of species and slug grazing indicates that more diverse communities are more resistant against the impact of grazing. Thus, plant community composition and plant diversity simultaneously could influence resistance against grazing pressure.

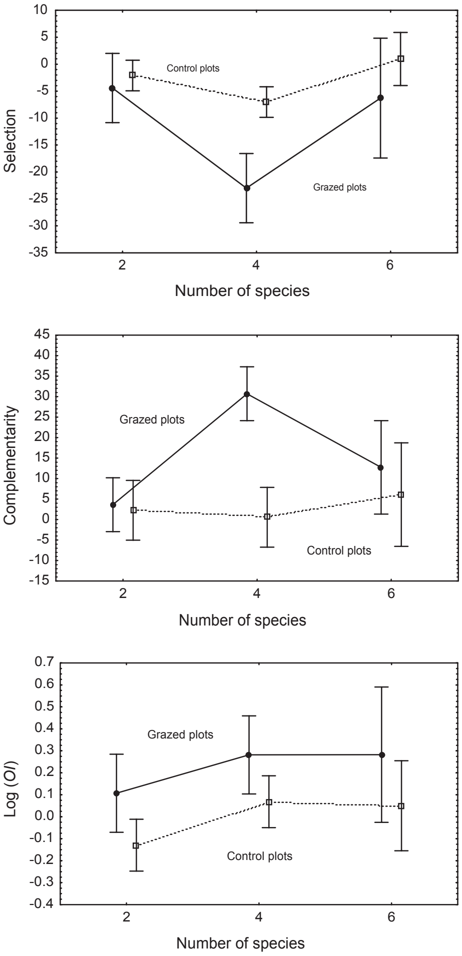

Figure 2. Mean (± SD) selection and complementarity effects, and mean (± SD) of log(*OI*) values for control (squares) and grazed (circles) plots for two, four and six-species mixtures.

I found a positive slope between the complementarity values and the number of species, suggesting that resource niche differentiation rather than the selection (or the sampling effect) for dominant plants could affect productivity of species rich mixtures. The consequence is that species rich plots have a greater productivity than the respective monoculture (Loreau, 1998). The complementarity values were found higher under grazed treatment. It was probably because of the grazing of more palatable species which might be responsible for a new gap creation in a community. Obviously, such new gaps were then colonized by weaker competitors.

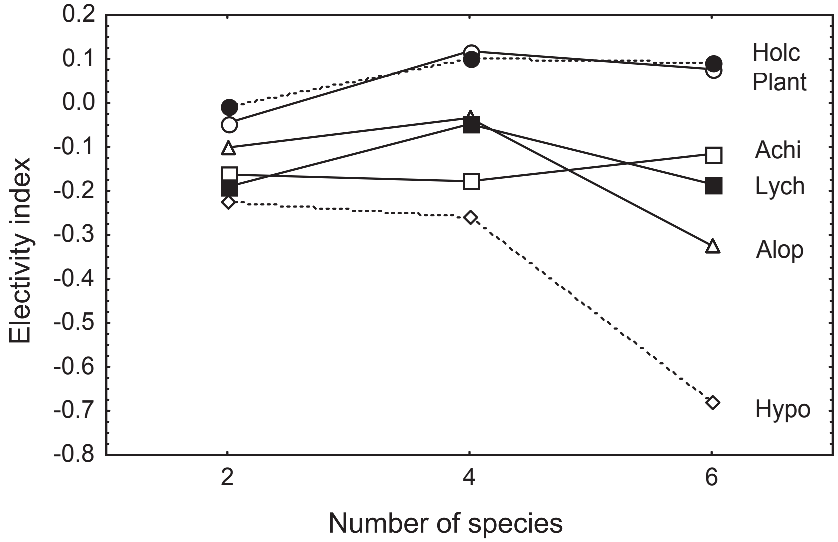

Figure 3. Mean of electivity index for all plant species used within two, four and six-species mixtures.

In this experiment, the complementary resource use is unlikely for any resource other than light, because both plots within an aquarium (grazed and no-grazed control) were relatively small and exhibited little soil heterogeneity. The evidence for light partitioning in the grazed plots is consistent with the prediction of Naeem et al. (1994). They claimed that in small-scale plots the production could be promoted by allowing a greater packing of photosynthetic tissues. It seems that the light partitioning hypothesis in the current experiment works more reliably only if the species in the lower strata of community respond well to the grazing treatment; canopy species are competing largely with themselves and should therefore differ in intraspecific densities. Therefore, herbivory probably promotes light partitioning by accentuating differences in species heights and growth forms, an effect that is extended by preferential feeding.

Results of log(*OI*) analyses, running under different grazing regimes, partly suggested that subordinate species performed better next to the interspecific neighbors. This occurred mostly in grazed plots. In this way, species interactions allowed species richness to significantly enhance production probably by light partitioning (see above). The most productive assemblages were four-species mixtures in the grazed and control plots. Thus, although six-species mixtures were on average more productive than four-species mix, any particular transition from mixtures of six species to four species may not be accompanied by lower production. This may be the reason for the non-significant relationship between the values of the transgressive overyielding and plant diversity.

The electivity index revealed that the most palatable specie is *Hypochaeris.* When *Hypochaeris* is grown in mixture, *Arion* shows marked preference for it. Once attacked it is much less likely to be completely eaten than the other species. Such plant species badly perform in mixtures. Moreover, monocotyledons (in this case *Holcus* and *Alopecurus*) probably have adapted to herbivory in mixtures and are able to achieve higher fitness when grazed. Results correspond with Hulme (1996), who argued that seedlings of the grasses were more likely to suffer severe damage as a result of mollusc attack than were the forb seedlings.

The fate of *Hypochaeris* contrasts with that of *Holcus* which has a fast growth rate. The unacceptability of *Holcus* to slugs provides enough protection to give *Holcus* a significant competitive advantage over neighboring acceptable competitors under intense slug grazing. The avoidance of grass species is well documented (Hanley et al., 1996; Wilby and Brown, 2001), and thought to be attributed to the silica contained within the shoots (Grime et al., 1968). In contrast, Pallant (1972) showed that *Holcus* leaves material with epidermal hairs was the most frequently ingested food of *Agriolimax reticulatus.* I could not to demonstrate any relation between presence of epidermal hairs and slug preference because both used hair-leaves species (*Holcus* and *Hypochaeris*) strongly differed in palatability of the slug. While *Hypochaeris* was preferred, *Holcus* was neglected.

The results of this study suggest that a generalist herbivore, can influence the resistance of differently rich plant assemblages by preferential feeding on subordinate palatable species (*Hypochaeris*, *Lychnis* and *Alopecurus*) rather than consumption of fast-growing dominant competitors (*Holcus* and *Plantago*).

Conclusion

The resistance of species-rich communities against herbivory pressure were demonstrated through the manipulation of plant diversity. With increasing species richness of plant communities, the slug searched for *Hypochaeris* and only partly fed unpalatable fast growing *Holcus* and *Plantago*. Food preference led to increasing productivity of the mixtures when regressed across all diversity treatments. The increase was caused by positive interspecific interactions between plants, and was influenced probably by resource use partitioning. I assume that the new-gap creation and colonization by weaker competitors during the herbivore impact could play role in plant assemblages.

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Paper V

Vegetation patterns in a cut-away peatland in relation to abiotic and biotic factors: a case study from the Šumava Mts., Czech Republic

Vegetation patterns in a cut-away peatland in relation to abiotic and biotic factors: a case study from the Šumava Mts., Czech Republic

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Abstract. We studied the natural regeneration of a cut-over peat bog in the Šumava Mountains, Czech Republic. The spontaneous revegetation by vascular plants has been limited by extreme abiotic conditions left after peat mining. Only 1-2 % of the total area was recolonized by *Sphagnum* mosses. This was mainly because drainage channels are still drying out the bog. Only plants tolerant to water stress such as *Juncus effusus, Molinia caerulea*, *Eriophorum angustifolium* and *E. vaginatum* were able to establish there. A key species colonizing bare surface is a clonal plant *E. angustifolium*. It forms circular polycormons of densely aggregated ramets. As in other radially spreading phalanx plants, the oldest (central) part of the system gradually dies, previously connected ramets become separated, and ring polycormon becomes open to recolonization by other plant species. We analyzed the relationships between species richness of the ring and their size, percentage litter cover, distance to seed sources, and soil fertility. The number of plant species was higher in the middle of the polycormons. The soil was more fertile in the central area than in the surroundings. We conclude that the restoration of highly disturbed habitats can be facilitated by clonal behavior of pioneer populations.

Keywords: Clonal plant, Cut-away peat bog, *Eriophorum angustifolium*, Plant colonization

Introduction

Peatlands are rare ecosystems that are being rapidly destroyed by human activities. Removal of the original vegetation, extensive drainage, and the extraction of horticultural or fuel peat are the main factors that threaten in Europe and North America these unique habitats for which few restoration measures were considered until recently (Lappalainen 1996). Attempts to restore these ecologically and economically important landscapes by natural means are often unsuccessful for the extreme abiotic conditions left after peat mining. The natural processes of recolonization and succession may take decades or more especially in those areas in which peat moss extraction was carried out by modern mechanical vacuum methods (Joosten 1995, Pfadenhauer & Klötzli 1996, Desrochers et al. 1998, Lavoie et al. 2003). The impacts of vacuum mining are more severe than those of manual block-cut mining in that it leaves a more uniform peat surface topography. Homogeneous bare peat surfaces are prone to cracking and crust formation, frost heaving and erosion by wind and water, restricting the establishment of plant propagules (Girard et al. 2002). These conditions severely inhibit the re-establishment of *Sphagnum* mosses, the primary peat forming vegetation. Hence, re-establishment of the former vegetation cover in post-mined peatlands represents an important challenge for ecologists and conservation managers.

Recent restoration measures in post-mined peatlands aim to re-establish the original vegetation cover by introducing plant diaspores and reducing moisture deficiencies in the open fields created by the vacuum mining. Blocking draining ditches is a first necessary prerequisite for improving moisture conditions and stimulating regeneration of mosses and bog plants (Price 1997, Stoneman & Brooks 1997). Other possibilities to ameliorate the surface conditions, reduce the water stress and loss of diaspores in the open fields are to create open water reservoirs that decrease lateral seepage, to alter the surface microtopography, and use shading devices and straw mulches (Price et al. 1998, Schouwenaars 1995, Robert et al. 1999, Horn & Bastl 2000). Price et al. (1998) proposed the use of mulches to assist the development of a vegetation cover and stabilize the peat surface. The mulching has been shown to ameliorate soil moisture and temperature conditions through its effect on the energy balance. Price (1997) showed that the use of mulch keeps soil water suction lower during dry summer, which increases the survival of bog plants (Campeau & Rochefort 1996). Using this and other similar restoration techniques, successful establishment of mosses and mire plants has been reported frequently, whereas case studies documenting revegetation solely through natural processes are rather rare.

In Central Europe, many peat bogs have been abandoned and left without management after the end of mining activities, usually due to various economic reasons. Some of these have gradually been overgrown by woody species, notably *Betula pubescens* and *Pinus sylvestris*, but others remained only sparsely vegetated. The plant succession appears to be particularly inhibited in abandoned mountain peatbogs of colder regions with acid and nutrient-poor bedrock (Horn & Bastl 2000). The Šumava Mts., Czech Republic, include several such cut-over and abandoned peatlands. The intent of this paper is to investigate the patterns of plant colonization/succession in post-mined peatland at a locality "Soumarský most" in Šumava Mts. The peat-bog has an extensive open area created by peat mining. Pioneer plant populations that colonize bare surface rarely progress beyond the initial invasion phase of succession. An exception is a clonal plant *Eriophorum angustifolium* which is able to form dense cover and build-up a critical standing crop. This can lead to habitat improvement and establishment of other plant species. This study aims to examine (i) the relationship among the different habitats present in disturbed and undisturbed parts of cut-away peat bog; (ii) their hydrological conditions and (iii) if the *Eriophorum* facilitates ecesis of other plants.

Materials and methods

Study area

The data were collected at “Soumarský most” peat bog in the Šumava Mts., South Bohemia, Czech Republic (Fig. 1). The “Soumarský most” peat-bog is a continental raised bog (sensu Neuhäusl 1972) dominated by *Pino rotundatae-Sphagnetum* community, *Oxycocco-Sphagnetea* class. The bog covers an area of 90 ha at an elevation of 650 m in Vltava river floodplain. The bedrock consists of acid, nutrient-poor granit and clay (Kodym 1961). The region is characterized by a humid climate with ca. 1000 mm mean annual precipitation (the Lenora meteorological station, 4 km north-west of “Soumarský most”).

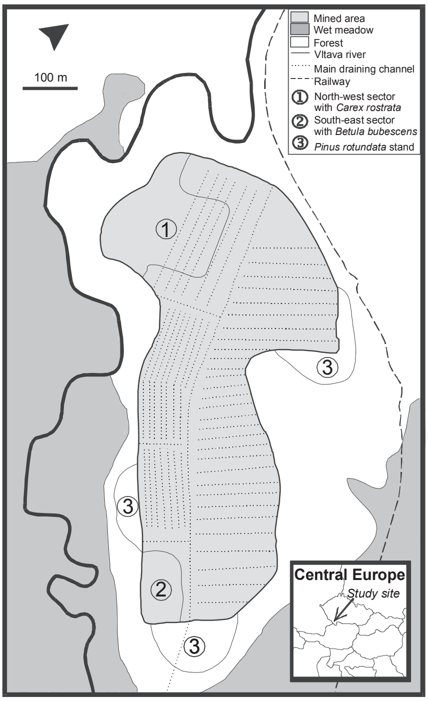

Fig. 1. The study area and its location within Central Europe.

The “Soumarský most” peat-bog was disturbed several times during the last 200 years. Between 1958 and 1999, the site was intensively mined for horticultural peat using the milling method, which needs effective drainage because heavy machinery is used and milled peat is dried on the surface of the strips to a moisture content of 40 % (Frilander et al. 1996). A grid of drainage ditches was first dug out creating rectangular units. The surface vegetation was than removed and put aside. The mining of moss and peat layers followed, altering the relief and hydrological properties of the site. The bog surface is now flat, with maximum of residual peat reaching the depth of approximately 1 m. One main drainage channel demarcates the peatland into N and S part. The channel was excavated to the present depth of 2.5 - 3 m in order to drain away water from secondary ditches, which are running alongside and across the main channel in S and N parts, respectively. In 2000, the secondary ditches in S part were already manually blocked by the breakdown of palisade dams cut through the peat (see Stoneman & Brooks 1997). Water accumulates successfully only in some ditches, with no distinguishable flow.

Data collection

Plant distribution

Plant species distribution was recorded in a total of 50 randomly located quadrates, distributed across seven habitats delineated within the peatland area and the nearest surroundings of: (i) bare post-mined surface; (ii) drainage channels; (iii) old primary *Pinus rotundata* forest; (iv) bog spruce forest; (v) periodically flooded area, i.e., wet meadows; (vi) forest composed of dominant *Pinus sylvestris* trees and (vii) secondary young forest. Cover of all vascular plants were estimated by using the Braun-Blanquet scale. The area of sample quadrates ranged from 25 to 200 m^2, because different scale in forest and open area had to be taken into account (see Moravec et al. 1994). For statistical analysis, species cover values were transformed using 1-9 scale (van der Maarel 1979). Hydrological data were recorded one to three times per month at six sites during vegetation season 2000 for a totaling of 11 measurements in each site. Water table depth was measured relative to the soil surface in perforated 1.5 m long, polyvinyl-chloride pipes (6 cm in diameter) inserted permanently in the peat.

Eriophorum angustifolium

Fig. 2. A key species colonizing bare surface is a clonal plant *E. angustifolium* which forms circular polycormons of densely aggregated ramets.

E. angustifolium (Cyperaceae) is a clonal plant which forms distinct rings of densely aggregated ramets on bare surfaces left after peat mining. *E. angustifolium* develops new ramets intravaginally within each shoot. The older shoot increments are re-oriented downwards to form a creeping stem covered with dead leaves. The ramet population expands radial and after some time a wide ring of densely aggregated ramets is formed. As in other radially spreading phalanx plants such as *Carex humilis* (Wikberg & Mucina 2002), the oldest (central) part of the system dies and previously connected parts become separated, i.e. *E. angustifolium* forms rings rather than filled circles. Consequently, the ring habitat can be divided into three concentric zones: Centre - the zone with low ramet density in the middle of ring and high accumulation of litter; Ring - the band with high ramet density around the Center, Surroundings - the bare surfaces among the rings (see Fig. 2).

In August-September 2002, we described fifty randomly selected *Eriophorum* rings localized within the whole harvested area in terms of size, shape, litter area, spatial location and the abundance of bryophytes and other vascular plants. One 80 x 80 cm relevé was recorded in the center of each ring and then four relevés were recorded in the Ring zone of *Eriophorum* (in southern, northern, western and eastern part). Due to a small diameter of several poycormons, only one relevé was recorded from the Ring zone. In total, we sampled 184 relevés. We further counted the number of seedlings (< 10 cm in height), saplings (10-130 cm in height) and trees (>1.30 m) of *Betula pubescens* and *Pinus sylvestris* in each ring. In addition, distance (in m) from the nearest drainage channel, i.e. from a diaspores source, was measured. The area of Ring and Centre zone was estimated as an area of ellipse based on two perpendicular measurements approximating major and minor axes. From each ring and the nearest surrounding, 120 ml soil samples were collected from 5-15 cm depth. Each sample was weighted and then dried to constant weight at 105°C to determine water content. We further collected 40 soil samples, always 20 from Centre zone and 20 from Surroundings, to determine extractable phosphorus concentration by a modified ammonium molybdate-ascorbic acid method (Olsen & Sommers 1982). Nomenclature follows Rothmaler (1976) for vascular plants and Kučera & Váňa (1997) for mosses.

Data analysis

The phytocoenological relevés were classified by UPGMA (average clustering) hierarchical procedure with Ward's method using squared euclidean distance coefficient (Sokal & Rohlf 1995). The variability in plant distribution was also analyzed by method of unconstrained ordination, detrended correspondence analysis (DCA), using the program CANOCO (ter Braak & Šmilauer 1998). DCA ordination diagrams showing plant species scores on the first two axes were produced using CANODRAW software (ter Braak & Šmilauer 1998). For each of phytocoenological relevés, obtained in Centre and Ring zones of *Eriophorum* circles, the Shannon-Wiener and Evenness diversity indices (Magurran 1988) were calculated. Both indices describe general relationship between species and their percent covers. Obtained values compared using a GLM (STATISTICA software,

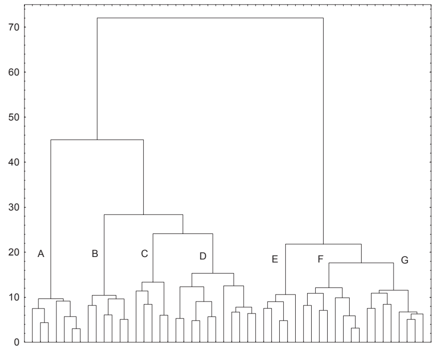

Fig. 3. Dendrogram of clustering of 50 reléves representing seven habitate types in a peatland. The main types of habitat are marked with letters: A – secondary young forest, B – wet meadow, C – bare peat surface, D – bog spruce forest, E – forests by channels, F – forest with planted *Pinus sylvestris*, G – old forest with *Pinus rotundata*.

Anon. 1996). Differences in underground water level between contrasting peat bog habitats were evaluated by one-way ANOVA. Vegetation of *Eriophorum* rings was analyzed by constrained ordination, redundancy analysis (RDA), with the ring zone type (Center versus Ring) as the only explanatory variable. The species score on the first (i.e. constrained) axis corresponded to the relative position of species' abundances with respect to Centre and Ring zone. A Monte Carlo permutation was used to test for the significance of the RDA model (499 permutations).The number of species, number of tree individuals and ring diameters were log(*x+1*) transformed prior to the analysis, in order to improve compliance with equal variance and normality assumption.

Results

Plant communities and water table fluctuations in relation to disturbance

Plant species composition differed between the seven habitate types of the peatland area. A total of 50 relevés were divided into 7 main clusters in that corresponded to 7 different vegetation types (Fig. 3). Similarly, DCA ordination determined 7 different groups connected with similarity in species composition, although the boundaries among them were not pronounced (Fig. 4). According to the dendrogram based on species composition, first vegetation type is a late successional *Pinus rotundata* community. Second type is a species rich wet meadow, occurring on flooded zone along the Vltava river. Third is secondary young forest composed mainly of juvenile stage of *Betula pubescens* and *Pinus sylvestris*. Fourth type is early succession habitat of bare peat surface, with low vegetation cover and dominance of *Juncus effusus*, *Carex canescens* and *Carex rostrata*. Fifth type is spruce forest, which is considered as a natural habitat in the neighboring area of the

Table 1. Differences between Center and Ring zones and differences between *Eriophorum* circles in Shannon-Wiener index and evenness.

		Shannon-Wiener index		
	df	MS	F	P
Circle	1	0.02	1.15	0.28
Zone	1	0.42	20.35	<0.001
Error	202	0.02		
		Evenness		
Circle	1	4.50	0.03	0.84
Zone	1	54.99	0.44	0.50
Error	202	122.77		

Table 2. Results of RDA analyses of cover estimation. % expl. variability = species variability explained by all ordination axes (measure of explanatory power of the explanatory variables). P-value is corresponding probability value obtained by the Monte Carlo permutation test.

Tested hypothesis	Explanatory variable	% expl. variability	F	P
A1: There are differences among *Eriophorum* rings. NO	Circles	4.0	1.04	0.3
A2: There are differences between Centre and Ring zones. YES	Zone	38.0	142.29	0.002
A3: There are differences between zones in relation to *Eriophorum* rings. YES	Circles*Zone	38.6	72.46	0.002

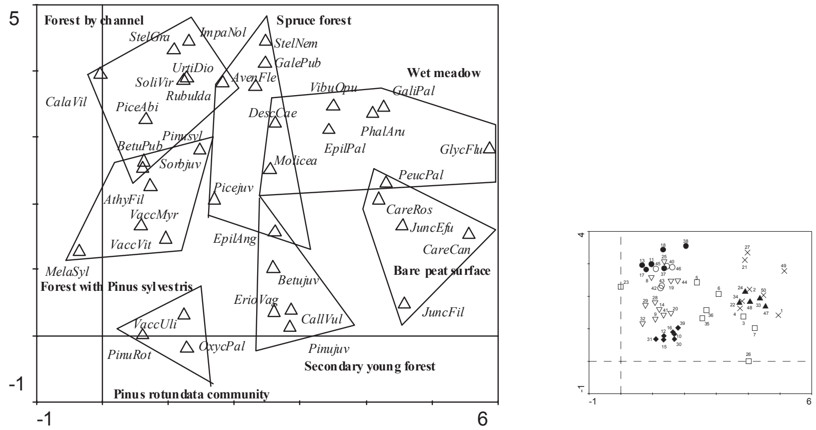

Fig. 4. DCA ordination diagram for vegetation reléves (lower diagram) and plant species sampled at "Soumarský most" peat-bog. Different groups of samples arise from the UPGMA classification. First two ordination axes explain 17.5 % variability of species data. Only species with fit range from 5 to 100 % are shown. Abbreviations: *OxycPal* = *Oxycoccus palustris*, *PinuRot* = *Pinus rotundata*, *VaccUli* = *Vaccinium uliginosum*, *Pinujuv* = *Pinus sylvestris* (in juvenile stage), *CallVul* = *Calluna vulgaris*, *ErioVag* = *Eriophorum vaginatum*, *Betujuv* = *Betula pubescens* (in juvenile stage), *EpilAng* = *Epilobium angustifolium*, *Picejuv* = *Picea abies* (in juvenile stage), *AvenFle* = *Avenella flexuosa*, *GalePub* = *Galeopsis pubescens*, *StelNem* = *Stellaria nemorum*, *DescCae* = *Deschampsia caespitosa*, *Molicea* = *Molinia caerulea*, *VibuOpu* = *Viburnum opulus*, *EpilPal* = *Epilobium palustre*, *PhalAru* = *Phalaris arundinacea*, *GaliPal* = *Galium palustre*, *GlycFlu* = *Glyceria fluitans*, *PeucPal* = *Peucedanum palustre*, *CareRos* = *Carex rostrata*, *Carecan* = *Carex canescens*, *JuncEfu* = *Juncus effusus*, *JincFil* = *Juncus filiformis*, *ImpaNol* = *Impatiens noli-tangere*, *RubuIda* = *Rubus idaeus*, *UrtiDio* = *Urtica dioica*, *StelGra* = *Stellaria graminea*, *SoliVir* = *Solidago virgaurea*, *CalaVil* = *Calamagrostis villosa*, *PiceAbi* = *Picea abies*, *BetuPub* = *Betula pubescens*, *PinuSyl* = *Pinus sylvestris*, *Sorbjuv* = *Sorbus aucuparia* (in juvenile stage), *AthyFil* = *Athyrium filix-femina*, *MelaSyl* = *Melampyrum sylvaticum*, *VaccMyr* = *Vaccinium myrtilus*, *VaccVit* = *Vaccinium vitis-idaea*. Symbols in lower diagram: squares - Secondary young forest, open circles – Bog spruce forest, filled circles – Forest by channels, crosses – Wet meadow, down-triangles – Forest with *Pinus sylvestris*, up-triangles – Bare peat surface, diamonds – *Pinus rotundata* community.

"Soumarský most" bog. Sixth type is artificial forest dominated by *Pinus sylvestris*. The last type is a young natural stands along draining channels with dominating *Betula pubescens* trees.

Fig. 1 shows a high cover of *Carex rostrata* and *Juncus effussus* (vegetation type of the bare peat surfaces in DCA diagram) in the northwest sector. The northwest sector was characterized by higher water levels that seems crucial for the establishment and survival of mire vegetation. In contrast, the southeast part of the peatland was abandoned earlier and was rapidly recolonized by a dense cover of shrubs and trees. The southeast part is located at a lower plateau. Here, protecting effect of surrounding trees of *Pinus sylvestris* may had evolved. This stand has the higher abundance of *Betula pubescens* saplings, and *Molinia caerulea* and *Juncus effusus* tussocks. *M. caerulea* is considered as a typical grass of peat bogs affected by severe summer droughts. Its presence indicates more favorable conditions for mineralization and mineral release. Along the draining channels *M. caerulea* is replaced by *J.*

effusus and *Eriophorum vaginatum,* plants behaving typically as early colonists of bare peat with sufficient water supply.

The depth and seasonal variations in water table differed mainly between undisturbed natural *Pinus rotundata* stand and disturbed peatland (Fig. 5). Water table was the highest in natural forest of *Pinus rotundata* and the lowest in a young stand of *Betula pubescens* (Fig. 5). Water table fluctuation during the vegetation season 2000 was highest at the bare peat surface and lowest in *Pinus rotundata* forest.

Eriophorum angustifolium ring polycormons

Eriophorum had a mean ring diameter of 4.43 ± 0.26 (mean ± standard error) m, with some polycormons 12 m wide. Both the number of species and of tree individuals increased significantly

with diameter of *Eriophorum* rings, especially with increasing the Centre zone. Both characteristics tend to decrease with distance from the nearest draining channel (Fig. 6). The average number of species per ring was 3.86 ± 0.29; average number of *Pinus* seedlings, saplings and tree individuals per *Eriophorum* ring were 0.58 ± 0.17, 2.58 ± 1.39, 0.06 ± 0.04 respectively, and the number of *Betula* seedlings, saplings and tree individuals per ring were 5.82 ± 1.55, 13.46 ± 3.27, 0.5 ± 0.20 respectively. Results of GLM analyses are summarized in Table 1. Shannon-Wiener diversity index (SW) differed significantly between Centre and Ring zones within the ring, but evenness indices did not. This indicates that more species is concentrated in the Centre than in the Ring zone, but in terms of evenness there is relatively uniform proportional percent cover between species. Values of SW and evenness indices did not differ among *Eriophorum* rings.

RDA analyses show that species composition significantly differed between the Centre and Ring zones (Table 2). Obviously, *E. angustifolium* is abundant in the Ring zone, while other species with rather low percent cover are concentrated in the Centre zone (Fig. 7). Species composition among *Eriophorum* rings was rather similar, i.e. it showed little changes with the ring size (Table 2). The circle x zone interaction was significant. Thus, it depended

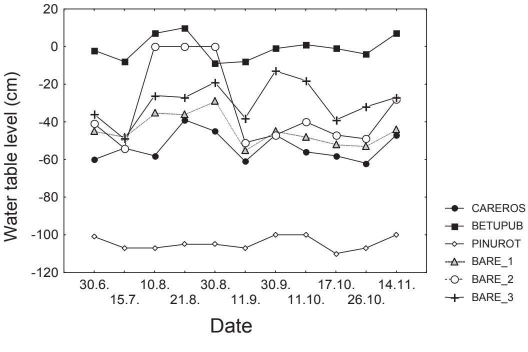

Fig. 5. Water table fluctuation in six sites. Sites: CAREROS-northwest sector with high *Carex rostrata* cover; BETUPUB-southeast sector with *Betula pubescens*; PINUROT-*Pinus rotundata* stand; BARE_1, BARE_2, BARE_3-sites on naked peat bog.

where the plant communities were positioned.

Comparison of water content in soil samples taken from *Eriophorum* rings did not reveal any significant difference between the Centre litter-rich zone and the outer Ring zone (t = -1.62, df = 98, *P* = 0.1084). The mean soil moisture was 59.28 ± 1.79 g/120 ml soil (max 92.07) and 63.23 ± 1.66 (max 92.50) ml inside and outside *Eriophorum* rings respectively. However, the soil in Centre zone had a higher phosphorus content (1.62 ± 0.06 µg/1 g of dry soil; max 2.13) than in Surroundings (1.36 ± 0.08; max 2.12). Analysis of exchangeable phosphorus content showed that Centre zone differed significantly from Surroundings (*t* = 2.4, df = 38, *P* = 0.0214).

Discussion

Large scale pattern in vegetation of the cut-away bog

The "Soumarský most" peat-bog was highly disturbed during the last 100 years, mainly from 1960's on. The mining of 70 % of the peatland was by far the main disturbance. During this period, the vegetation was removed and an extensive network of drainage ditches created. This led to high water removal from the rest of the peat deposit. After the cessation of mining, the spontaneous revegetation by vascular plants and mosses was successful only along the draining channels, presumably because of higher water supply and a protective shading by established *Betula pubescens*. The high moisture habitats were recolonized almost exclusively by typical fen and peatland species such as *Juncus effusus, Carex rostrata, Carex canescens, Juncus filiformis, Potentilla palustris* and *Eriophorum vaginatum*. Although the spontaneous revegetation by vascular plants was successful in some parts, *Sphagnum* mosses (*S. russowii, S. magellanicum*) colonized only 1-2 % of the peat surface. In fact, the drainage ditches are still drawing off a large quantity of water during the vegetation season, resulting in high water table fluctuation across the cut-away peatland (Wind-Mulder et al. 1996, Price 1997). All these factors contribute to drying out the bog and restrict recolonization by *Sphagnum* mosses. Overall, most of the cut-away peatland is scarcely vegetated, especially in those places where mining activities ceased 3 years ago. It is known that the course of revegetation in cut-away peatlands frequently relies on moisture conditions (Girard et al. 2002), but also the initial species pool after disturbance (Campbell et al. 2003). We recorded a high cover of *Carex rostrata* and *Juncus effusus* in the northwest sector of the peatland (Fig. 1), which is characterized by relatively high and stable water table level. This factor seems crucial for the establishment and survival of mire vegetation (Schouwenaars 1995, Price 1997, Stoneman & Brooks 1997), and could lead to dominancy of several plants as was observed in our case. *C. rostrata* and *J. effusus* are both clonal plants with fast rhizome (*C. rostrata*) and tiller (*J. effusus*) growth, and are thus able to occupy nearly whole part of the northwest sector. Only some subordinate species such as carrot *Selinum carvifolia* and sedge *Carex canescens* survived there.

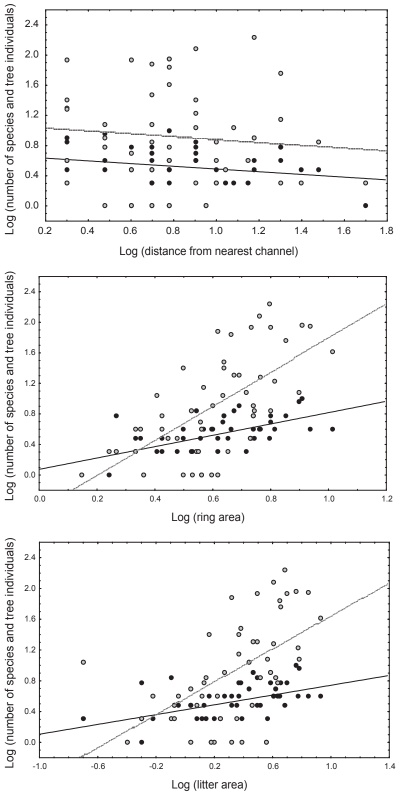

Fig. 6. Relationships between number of species (full line, black circles) and number of tree individuals (dotted line, gray circles) and independent variables, which are distance from the nearest channel, litter amount and ring area. The total number of species depended on the ring area (R^2=0.32, P<0.001) and litter amount (R^2=0.19, P<0.001), but did not correlate with the distance from the nearest channel (R^2=0.04, P=0.08). The number of tree individuals depended on the ring area (R^2=0.41, P<0.001) and litter amount (R^2=0.31, P<0.001), but not on the distance from the nearest channel (R^2=0.00, P=0.50).

A different situation occurred at the southeast part, where the peatland was abandoned earlier and was rapidly recolonized by a dense cover of shrubs and trees. The southeast part is located at a lower plateau. Here, protecting effect of surrounding trees of *Pinus sylvestris* may had evolved. Therefore, saplings of the downy birch (*Betula pubescens*) could establish here in high density. Further, *Molinia caerulea* and *Juncus effusus* tussocks also occurred here.

Molinia caerulea is considered as a typical grass species of mined peat bogs that are affected by severe summer droughts. Its presence indicates more favorable conditions for mineralization and mineral release (Schouwenaars 1995). *Molinia* belongs together with *Eriophorum vaginatum* to colonizers which are able quickly spread over the bare peat surface in the first phase of the vegetation succession. We found that this phenomenon is valid for conditions of our study locality. The fact that *Molinia* is regarded as species of relatively drier sites was supported by our observation, when it was replaced by *Juncus effusus* and *Eriophorum vaginatum* on wetter sites along the draining channels.

Compared to disturbed areas, natural undisturbed pine stands had lower water table fluctuation, possibly by retaining water in active peat layer (acrotelm) during extreme weather conditions (Stoneman & Brooks 1997). Kučerová et al. (2000) showed that water table fluctuation is closely connected with a plant species dominating in a particular environment. In our case, the water table level highly fluctuated in *Betula pubescens* stands. *Betula* with deeper roots is more effective in sucking water than *Sphagnum* carpets which are dependent on water diffusion and capillarity.

Schowenaars (1995) documented more intense evapotranspiration in the tree stands than those dominated by *Sphagnum* mosses.

Small scale pattern in vegetation of cut-away bog

This study shows that spontaneous revegetation of the bare peat surface is facilitated by clonal nature of *Eriophorum* growth. *E. angustifolium* was able to establish locally and covered the bare peat surface at high densities. This led to habitat improvement and establishment of other plant species. We see the facilitation effect (sensu Callaway 1995) through the high abundance of several plant species and mosses in the Center zone of *Eriophorum* polycormons (Fig. 7). Their establishment was probably caused by accumulating litter that substantially changed microclimatic conditions and soil fertility. Newly created favorable environment inside the rings increased the possibility not only for other plant species to become established but also for faster growth rates, especially in tree saplings. This positive association can be referred to as the "nurse plant effect" (Callaway & Walker 1997, Groeneveld & Rochefort 2002). The number of plant species was positively correlated with the area of Ring and Centre (litter) zone. Significant size effect of *Eriophorum* ring on establishment of a number of plant species supports the hypothesis that intensity of facilitation increases with benefactor size in adverse environment (Callaway et al. 1996, Chambers 2001, Haase 2001). The mechanism of *Eriophorum* facilitation probably involves the direct positive effect on moisture and evapotranspiration. The occurrence of successfully established vascular plants and mosses native to fens and peat bogs in the Centre zone of the *Eriophorum* circle suggest this mechanism. For example, Salonen (1987) compared the species number and composition in the seed rain with those of the actual vegetation, and found a sparse germination of species abundant in the seed rain. He explained this by unfavorable moisture conditions on the post-mined peat surface. It is the same situation which was occurred at the "Soumarský most", however, in our case, the facilitation mechanism

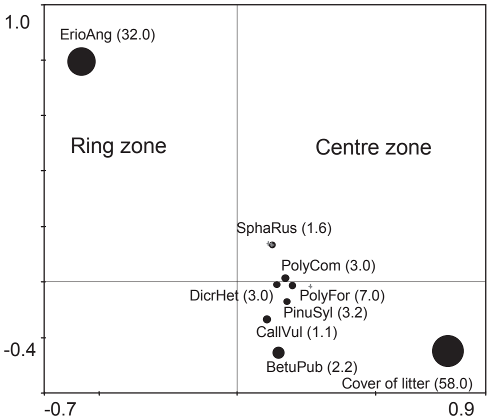

Fig. 7. Result of RDA analysis (attribute plot), where zones are used as nominal explanatory variables (only species with fit range 2-100 % are shown). In brackets: cumulative fit per species (=in % explained variability by a species). Abbreviations: ErioAng = *Eriophorum angustifolium*, SphaRus = *Sphagnum russowii*, PolyCom = *Polytrichum commune*, Polyfor = *Polytrichum formosum*, DicrHet = *Dicranella heteromala*, PinuSyl = *Pinus sylvestris*, CallVul = *Calluna vulgaris*, BetuPub = *Betula pubescens*.

improved the bare peat surface to faster recolonization by vascular plants. Similar study was conducted in Finland, where Tuittila et al. (2000) showed that many peatland species benefited from the sheltering effect of *Eriophorum vaginatum* tussocks in cut-away peat fields.

We found non-significant relationship between litter accumulation and soil moisture. However, phosphorus content was significantly higher in Centre zone of litter accumulation. Consequently, the plant species establishment inside the rings can be at least in part explained by increased phosphorus content. Sundberg & Rydin (2002) likewise found that the establishment of *Sphagnum* spores was promoted by *Betula pubescens* litter and the amount of phosphate released. Generally, phosphorus together with nitrogen are limiting ions in fens and peat-bogs (Wheeler & Proctor 2000). A floristic composition in peatlands is often strongly correlated with phosphorus and nitrogen availability. Recently, peatland vegetation is becoming less limited by nitrogen because of increased nitrogen input from air-borne pollutants (Aerts et al. 1992, Økland et al. 2001).

Conclusively, this study shows that natural revegetation process at the “Soumarský most” has been initiated through the facilitation effect of *Eriophorum* vegetation. The mechanism of litter accumulation is important to the better establishment of tree seedlings and saplings. We are aware that such process can lead to the development of dominant birch and pine forest, but not to peat formation. Regardless of this, the facilitation effect seems to be the main process that provides suitable conditions for establishment and growth of mire plant species at the highly disturbed “Soumarský most” peat-bog.

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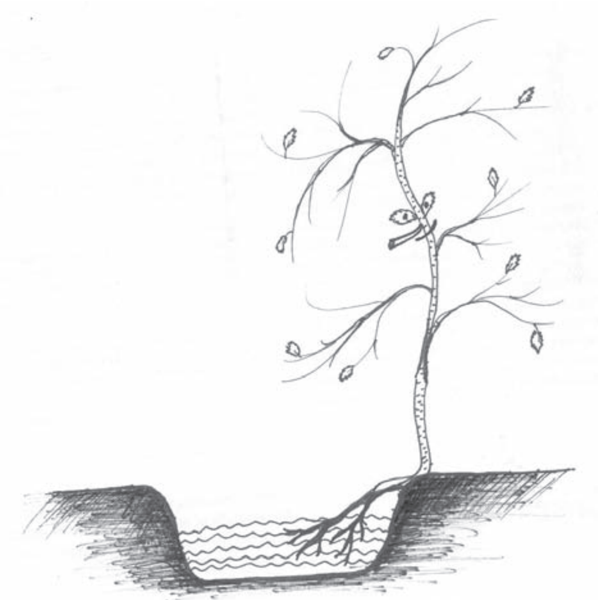
Paper VI

Growth response of downy birch (*Betula pubescens*) to moisture treatment at an cut-over peat bog in the Šumava Mts., Czech Republic



Growth response of downy birch (*Betula pubescens*) to moisture treatment at an cut-over peat bog in the Šumava Mts., Czech Republic

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Abstract. The factors affecting the adaptation of downy birch (*Betula pubescens*) to the different environmental conditions were studied in 2002-2003 by measurement of production characteristics of saplings, experimentally planted in contrasting habitats at the cut-away peatland in the Šumava Mts, Czech Republic. These habitats varied in groundwater table depth. Together with this factor, we tested for the effect of shading by shade cloth on sapling growth. We conducted greenhouse manipulative experiment to investigate the growth response of the downy birch saplings to varying groundwater table depth and soil type. We searched whether spatial distribution of naturally established birch stems changes along pronounced moisture gradient represented by transects placed at three distances from draining ditches. In the field, birch plants exhibited higher growth increments as well as higher leaf production under high water table depth. This result is fully opposite to manipulated greenhouse experiment that showed higher biomass increments for saplings under low water table. This was probably because downy birch, in principle, indicate terestrification of peat bogs and high water table depth in greenhouse inhibited its growth. In field, birch saplings were stressed by drought and overheating. Positive shading effect on sapling growth was proven by leaves production measurement. The two term local variance method (TTLQV) revealed two contrasting dispersion patterns, for the birch population at drier and wetter sites of the post-mined peatbog. The aggregation was obvious mainly at the drier site with lower abundance of birch plants within each sampled square. Higher abundance of individuals were found in squares of the wetter site, whereas the pattern was random there.

Keywords: *Betula pubescens*, Cut-over peatland, Increment, Facilitation, Spatial pattern, Water table depth

Introduction

During mechanical peat harvesting the original bog vegetation is completely destroyed. Thereafter, plants colonizing harvested peat surface must survive stress because the ecological conditions are extreme. Peat is dry, black and powder-like. During a summer sunny day, surface temperatures may be very high (Pfadenhauer & Klötzli 1996) and the water level fluctuates greatly (Lanta et al. 2004) and often abruptly. These conditions result in killing-off of the colonized plants.

Exploitation of bogs for horticultural peat cause in the complete removal of the original vegetation, and lead to the replacement by a new plant cover consisting of species which have often reduced survival probability in undisturbed bogs (Stewart & Lance 1991). The downy birch (*Betula pubescens*) is such "pioneer" tree species in disturbed bogs, where often forms stands nearby draining channels with favorable moisture conditions (Schouwenaars 1995). The birch and some other herbs such as *Eriophorum vaginatum, E. angustifolium* and *Carex rostrata* are suitable plants for a rapid establishment and spread over unvegetated peat areas (Poschold 1990). The birch together with several grass species (for example *Molinia caerulea*) have been repeatedly observed as the rapidly invading species of cut-away bogs, however, this often results in an expansion of these species and decline of mire and fen species. Recent studies in disturbed peat bogs provided evidence that water table fluctuation is also an important factor for the invasion of these species (Schouwenaars 1995, Price 1997, Robert et al. 1999). By contrast, the true mire species (e.g. *Sphagnum* sp.) grew on those sites where the water level fluctuations are small and the peat layer is thick and has low nutritional value (Wind-Mulder et al. 1996).

The distribution of individuals of the downy birch is unlikely restricted by water accessibility (Stewart & Lance 1991) with seedling and sapling growth being the most critical stages. Generally, the downy birch is intolerant of shade as a juveniles and is replaced by slow-growing later-successional species (Silwertown & Doust 1993). Although shade-intolerant, at the early stages of post-mined succession, the shade effect of adult trees may help saplings to growth and survive adverse conditions. Furthermore, as saplings are more likely to survive at wetter than drier sites (Dierssen 1992), their growth rate is expected to be positively correlated with spatial distribution of adults, as their canopies may improve moisture conditions.

The aim of this study is to evaluate the effects of different levels of water table depth and shading on the growth of downy birch saplings at the cut-away peatland in Šumava Mts.

(Czech Republic). This was achieved through measurement of production characteristics of downy birch saplings, experimentally planted in contrasting habitats varied in water table depth. The factor shading was used to asses microclimatic conditions on birch saplings growth. We were particularly interested in changes in spatial distribution of naturally established birch stems along pronounced moisture gradient represented by transects placed at three distances from draining ditches.

The second aim of this study is to evaluate the effect of different water table depth and two levels of soil type on growth of downy birch saplings in a pot experiment under greenhouse conditions. Peat and sand were used as two different soil types because of assessing the growth of birch under two contrasting soil fertility.

Methods

Study species

Downy birch is a polycormic, pioneer tree species, growing on moisture and peat rich soils. It is dominant tree at our study site and occupies mainly four stands: (i) is scattered as saplings in the middle of *Eriophorum angustifolium* rings that probably made environmental characteristics more favorable for establishing other plant species (Lanta et al. 2004), (ii) at the draining channels, (iii) at shaded dry and (iv) moist lower parts of the cut-off peatland. Canopy expansion takes place via the production of long shoots, which also produce axillary buds (Maillette 1982). Despite *B. pubescens, B. pendula* is scattered there. *B. pubescens* was distinguished according to the presence of sparsely or densely hair leaves and densely hair branches (Rothmaler 1986).

Study site

The field study was carried out in 2002-2003 on a cut-away peatland, Soumarský most peat bog (Šumava Mountains), Czech Republic, 48’54’’, 13’ 49’’, 650 m a.s.l. Mean annual precipitation is 810 mm and mean annual temperature 5.5 °C. The average effective growing season is 160 days (the Lenora meteorological station, 4 km north-west of Soumarský most). Peat harvesting at this location ended in 2001, however, the locality was step by step abandoned during 1990s. The residual peat layer thickness is 1 m on average. The area of the field was 8 ha and it was divided by ditches into 10-70 m wide strips. Nowadays, the drainage system is still partly functioning. The area was originally covered by *Pino rotundatae-Sphagnetum* community (*Oxyccoco-Sphagnetea* class). At present, the spontaneous revegetation occurs mainly along draining channels, with dominant trees and saplings of *Betula pubescens* and *Pinus sylvestris,* and *Carex canescens* with *C. rostrata*, cottongrasses *Eriphorum vaginatum* and *E. angustifolium* and grass *Molinia caerulea* dominating the understorey layer.

Greenhouse experiment

The experiment was run in an unheated greenhouse at the Faculty of Biological Sciences, České Budějovice. For the experiment, 11 – 36 cm saplings of *Betula pubescens* with peat soil were collected on 29 March 2003 at the Soumarský most peat bog. On 1 April, 84 individuals were weighted fresh and immediately planted into 84 pots (19 cm in diameter). The experiment was arranged into seven blocks. The following experimental treatments were imposed: (i) nutrient level - pots were filled by a sand or by peat soil collected at the Soumarský most bog, and (ii) two water level (for this experiment abbreviated WL). The WL was practically realized in the way when the one-half of pots were watered as plants required into a 2 cm deep, dish and the second-half into a 7 cm deep dish, where the stabile WL was held. A 1 x 2 x 2 (species x nutrient level x WL) randomized block design was adopted with three replicates in each of seven blocks. The height of each birch plant as “high increment” was nondestructively measured six times during the experiment until 15 June at which time plants were harvested. Then the soil was washed from roots, leaves were clipped, dried for 6 hours at 105 °C and weighted. To calculate an “sapling increment”, whole individuals including stem, branches and roots (but without leaves) were weighted fresh. “Sapling increment” was estimated as biomass of each plant at the end of the experiment minus biomass at the onset of the experiment.

Field experiment

Within the whole mined area of Soumarský most bog we selected four drier sites with –34.3 cm of average water table depth (for field experiments abbreviated WTD) of 5 measurements during vegetation season, and wetter parts (with average WTD of –11.7 cm). The wet part was always located under the dry part in small 0.5 – 1 m deep depressions, roughly 10 x 10 m in diameter. One week before the start of the experiment on October 2002, 160 saplings (15 – 30 cm tall) were carefully sampled in the vicinity of the study site; individuals were washed, weighted fresh (leaves were shed at this time) and stored for 3 days in a greenhouse. After this, twenty birch saplings were planted in regular distances of 15 cm in each dry and wet part within a block. Always 10 saplings were arranged into shaded and unshaded *Betula* groups.

The number of birch plants surviving the winter season was recorded and all dead individuals were replaced on March 2003. On 22 April, the experimental treatment shading was imposed to simulate two microclimatic conditions on sapling growth. A woody skeleton was installed approximately 30 cm above each *Betula* group at the drier and wetter sites. Then a well-ventilating shade cloth (with 1.5 x 1.5 cm mesh) was fixed to the skeleton. A 2 x 2 (WTD x shading) incompletely randomized blocks were set up with ten replicates in each of four blocks (Fig. 1). Plants were harvested on 12 September 2003, about four weeks before the leaf shedding. After clipping leaves, plants were carefully rinsed to get rid of soil in root system and then weighted fresh immediately in the field. Leaves were dried in the laboratory at 105°C for 6 hours and weighted.

Spatial pattern of Betula pubescens

Within the area of the Soumarský most peat-bog two sites – wetter one (average WTD of 3 measurements during vegetation season was -0.63 cm) and drier one (with average WTD of –29 cm) were selected. Six longitudinal transects, each 50 m long, were established (D0, D5, D15 and W0, W5, W15 at drier and wetter site, respectively) at 0 m (D0, W0), 5 m (D5, W5) and 15 m (D15, W15) distances from the draining channels on July 2003. Number of all birch seedlings and saplings were counted in each of 167 30 x 30 cm quadrates along each transect. Seedlings and saplings were regarded as plants attained stem height of 1 m in maximum. At both field experiments, WTD was measured relative to the soil surface in perforated 1.5 m long, polyvinyl-chloride pipes (6 cm in diameter) inserted permanently in the peat.

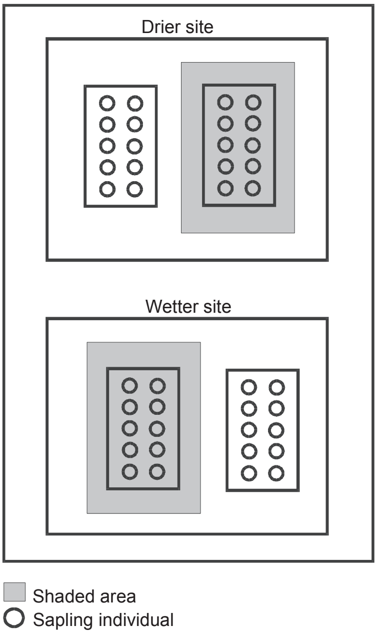

Fig. 1. A block of the field experiment with two levels of treatments: soil moisture and shading.

Data analysis

Linear scale data measured in the field and greenhouse (leaf biomass, stem-root increment, sapling height) were analyzed using ANOVA (for a block design), including repeated measures when needed, and ANCOVA (with stem weight at the start of the experiments used as a covariate).

Plant growth during the greenhouse and field experiments were analyzed for saplings using the methods of classical growth analysis (Hunt 1982) based on the increase of total plant biomass (*B*) between time *t*1 at the start of experiment and time *t*2 at the end of experiment:

$$RGR = \frac{\ln(B2) - \ln(B1)}{t2 - t1} \quad \text{(1) Eq.}$$

where *RGR* is mean relative growth rate in % d^{-1}. Differences in *RGR* were subsequently evaluated by testing the treatment interaction effect in ANOVA (for a block design).

Analysis of spatial data

Hill`s (Hill 1973) two-term local quadrat variance method (TTLQV) was applied for analyzing the transect data. The method has been proposed for studying the spatial pattern of a single species in one dimension along which there is no environmental gradient (Dale 1999). The kind of data under consideration is density collected in a string of contiguous quadrats. The TTLQV analyzes data by examining how mean square (MS) depends on the size of blocks of quadrats which are lumped together in the analysis. For the analysis is supposed that the data form a single transect consisting of $n + m$ basic units, where n is integer power of 2. The transect is represented by a sequence of birch quantities in particular sampling units x_i:

$$x_1, x_2, \ldots, x_n, x_{n+1}, \ldots, x_{n+m}. \quad \text{(2) Eq.}$$

The MS is defined as an average of overlapping terms, for example, for block size 1:

$$MS(1) = \frac{1}{m+n+1} * \left(\frac{(x_1 - x_2)^2}{2} + \frac{(x_2 - x_3)^2}{2} + \ldots + \frac{(x_{n+m-1} - x_{n+m})^2}{2} \right) \quad \text{(3) Eq.}$$

The mean square is defined analogously for any block; for example, at block size 3, the MS is defined as the average of overlapping terms:

$$\frac{(x_1 + x_2 + x_3 - x_4 - x_5 - x_6)^2}{6}, \frac{(x_2 + x_3 + x_4 - x_5 - x_6 - x_7)^2}{6}, \ldots etc. \quad \text{(4) Eq.}$$

This pattern analysis is conveniently presented as graphs of mean square against block size, in which peaks indicate the scales of pattern present. An pattern analysis was always stopped at a block size 56 to avoid decreasing reliability with the decreasing number of averaged terms (Dale 1999). This method is considered as purely descriptive one. *Betula pubescens* was most abundant towards the left end of the transects and was absent in < 43% and < 41%, on average, at the drier and wetter site, respectively.

Results

Greenhouse experiment

The analysis of repeated measures ANOVA is given in Table 1. Statistically significant effects were identified for soil treatment and interactions between time and soil, as well as for

Table 1. ANOVA for effects of depth of water (i.e., WL, low and high water table levels) and substrate (peat and sand) on growth dynamics, leaf biomass and sapling increment of downy birch grown under greenhouse standard conditions. Significance levels are: n.s.: Not significant; *: *p*<0.05; **: *p*<0.01; ***: *p*<0.001.

		Growth dynamics		Leaf biomass		Increment	
Source	df	F		F		F	
Substrate	1	29.40	**	9.28	*	0.01	n.s.
WL	1	4.63	n.s.	13.55	*	6.89	*
RepFactor	5	183.55	***	–		–	
Block	6	0.77	n.s.	0.39	n.s.	0.63	n.s.
Substrate*RepFactor	5	19.17	***	–		–	
WL*RepFactor	5	5.87	***	–		–	
Substrate*WL	1	0.00	n.s.	6.52	*	1.21	n.s.

Table 2. ANOVA for effects of shading (shaded and un-shaded subplots) and depth of water (i.e., WTD, high and low water table levels) on leaf biomass and sapling increment of downy birch planted in field conditions. Significance levels are: n.s.: Not significant; *: *p*<0.05; **: *p*<0.01; ***: *p*<0.001.

		Leaf biomass		Increment	
Source	df	F		F	
Shading	1	10.64	*	6.19	n.s.
WTD	1	20.32	*	25.28	*
Block	6	1.88	n.s.	0.39	n.s.
Shading*WTD	1	3.43	n.s.	1.45	n.s.

WL. The results for WL and soil type are shown in Table 1 and Fig. 2. On average, birch plants growing at peat soil were 1.3 x taller at the harvest than when growing in sand. High increment values differed under WL treatment, when values of low WL were 1.2 x higher than values for high WL. The WL x time interaction indicates that plants held under low WL attained higher growth rates than plants under high WL during the experiment. Peat soil under the low WL resulted in a significant increase in increment values (Table 1 and Fig. 3). Sapling increment characteristics significantly differed between WL conditions, but did not differed in soil type. Leaves biomass significantly differed of soil x WL condition interaction (Table 1). Higher productivity of leaves was measured at plants growing on the peat soil depending on WL treatment level (Fig. 3).

The mean *RGR* was lower, but not significant (Fig. 3; F = 4.38, *P* = 0.08), under the high WL treatment when compared to the low WL conditions. Under the soil treatment, there were no differences in *RGR* values (F = 0.34, *P* = 0.57). The soil x WL interaction was not significant (F = 1.18, *P* = 0.31). Thus, plants evolved similarly and independently of the treatments during the growth period lasted for 61 days.

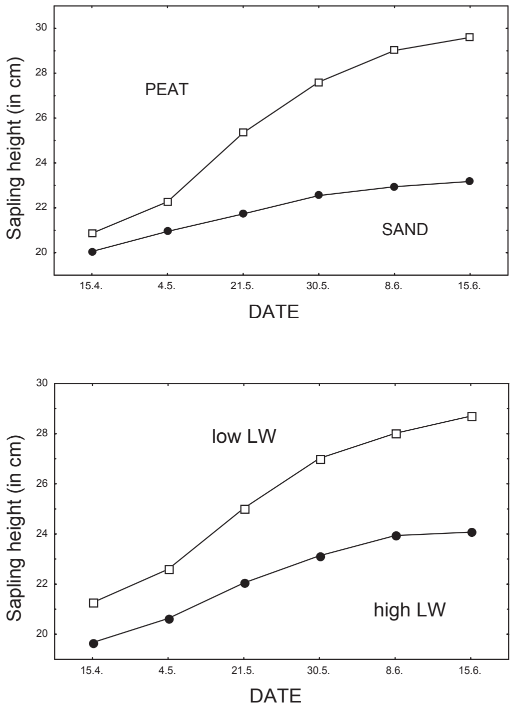

Fig. 2. Mean heights of saplings repeatedly measured during greenhouse experiment.

Field experiment

When focusing on plant survival on March, the number of survived plants varied between 3 and 10 (within a treatment combination). However, an contingency tables analysis did show no significant difference in plant survival between drier and wetter site (χ^2 = 0.48, df =3, P = 0.92). Plant survival was not analyzed at the harvest time because of very high survival of plant individuals (7-9 plants within a treatment combination), resulting in 124 survived plants (from 160 planted) in total.

Table 2 shows the effects of moisture and shading on birch increment. Significant effect of moisture indicates that birch had higher increment at the wetter than drier site (mean averages over the treatments were 2.44 vs. 1.18 g, respectively, Fig. 4). Shading treatment had not the expected effect, with birch increment being greater in shaded plants (2.28 vs. 1.33 g, respectively). There was no evidence of an interaction between shading and water table depth.

An analysis of the leaf biomass is given in Table 2. The biomass was significantly higher for the wetter than the drier site (mean averages over the treatments were 0.47 and 0.18 g, respectively, see Fig. 4). There were significant differences in the leaves biomass between the shaded and unshaded plots (0.44 vs. 0.21g, respectively). However, significant effect of the moisture x shading interaction was not detected.

The mean *RGR* was higher for the high WTD plots than for the low WTD plots, although this result was not significant (Fig. 4; F = 8.00, *P* = 0.06). Significant differences were detected in the *RGR* for the shading treatment, with higher values for shaded plots (Fig. 4; F = 13.64, *P* = 0.03). The moisture x shading interaction for *RGR* values was not significant (F = 0.65, *P* = 0.48), indicating similar growth under both treatments during the study period of 289 days.

Pattern analysis

The TTLQV revealed differences in spatial pattern between populations of the birch growing at wetter and drier sites. However, because of the large number of quadrates, the TTLQV curves were rather smooth.

The Fig. 5 shows distinct peaks at distances of 0.9 - 1.2 m in the D0, D5, D15 of drier population and at the 2.1 m distance in the W0 of wetter population. The peaks correspond with the patches composed of stems originating from seedlings of established young birch trees. At the drier site, less distinct peaks at higher block size of 40 (12 m; D5) and 50 (15 m; D0) correspond to gaps between *B. pubescens* aggregations. Curve of D15 shows that birch formed relatively homogenous growth there without creation of clear aggregations (random pattern). At the wetter site, a pronounced peak was detected at the block size of about 20 units (6 m; W5). Spatial aggregation was not apparent in higher block size units at both transects of W0 and W15. The increase in MS values at the W15 detected environmental heterogeneity at higher scale (Dale 1999).

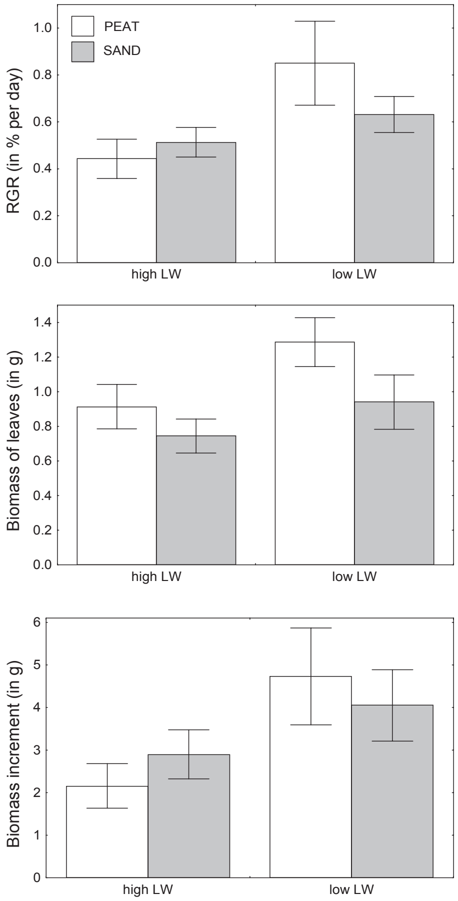

Fig. 3. Mean (columns) and standard errors (whiskers) for relative growth rate, leaf biomass and sapling increment of birch saplings grown under greenhouse conditions.

Discussion

The effects of large water table level fluctuations on natural revegetation of peat bogs after abandonment of mining activities have been repeatedly reported (Stoneman & Brooks 1998, Price et al. 1998, Robert et al. 1999, Goodyear & Sliva 2000, Girard et al. 2002, Vasander et al. 2003). These findings shows that the changes in water table level have profound effects on functioning of disturbed peatland ecosystems including further vegetation development. In this study we quantified effects of different WTD on the growth characteristics and leaf production

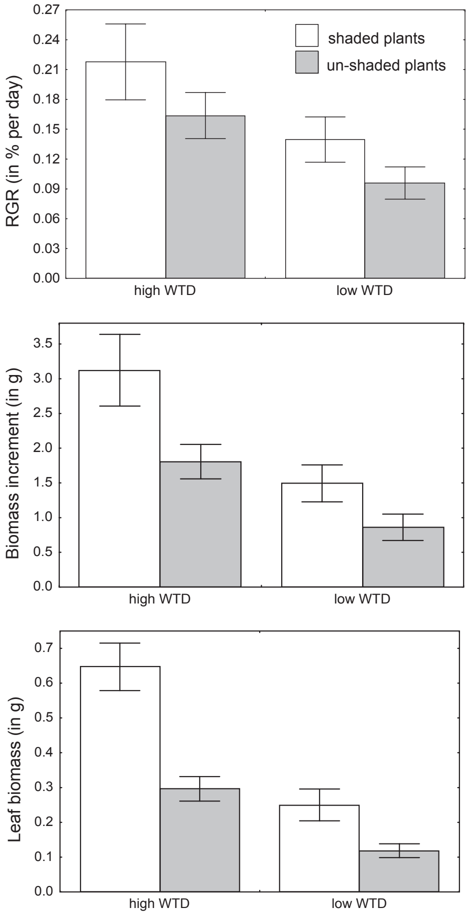

Fig. 4. Means (columns) and standard errors (whiskers) for relative growth rate, plant increment and leaf biomass of saplings planted in field conditions.

of individual tree saplings under field conditions. Additionally, we investigated similar effects under standardized greenhouse conditions, and than we interpreted both experiments. Thus, our study could partly fill a gap between investigations of the effects of water table fluctuation on ecosystem level (see references above) and several experimental studies interested in plant growth of selected species under natural environmental conditions (Weih & Karlsson 1999, 2002, Hagen et al. 2003). We are aware of limitation of our field experiment, mainly its short duration, covering only one winter and the subsequent growing season. However, we would to evaluate the growth responses of the downy birch to two levels of water table levels in the greenhouse and field experiments and also show how realistic is the pot experiment in comparison with the field experiments (Diamond 1986).

Greenhouse and field experiments

The greenhouse experiment showed different growth responses in tested birch saplings to two soil treatments. At the first sight, results seem to be a little complicated which offer to resolve questions: (i) why the growth dynamics of birch saplings differed under soil treatment but not under the WL, and (ii) why the leaf biomass differed under the WL, not under the soil treatment and why the interaction term was significant? A possible explanation could be that the plants are able to allocate assimilates more rapidly under the low WL. Furthermore, water did not fluctuated randomly (in the high WL conditions) during the experiment as is characteristic for the conditions of cut-away peatlands where the water table highly fluctuate (Joosten 1992, Schowenaars 1993, Price 1997). In our experiment, we held stabile WL which suggests that the root system in the high WL conditions could be affected by some microbial and anoxic processes (anaerobic stress). However, the description of root systems was not investigated in this study. There is possibility that plant growth could be abrupt by lower soil respiration at high WL (Kim & Verma 1992).

The peat soil containing more nutrients and water is more advantageous for birch growth. This probably resulted in increasing growth dynamics and leaf biomass of the downy birch growing on peat substrate (Table 1), although the response on soil substrate was not obvious in RGR. Similar values of RGR for plants planted on sand and peat could be a result of adaptations to past environments and could predispose plants to delay their growth responses to current environmental conditions (Kramer & Kozlowski 1979).

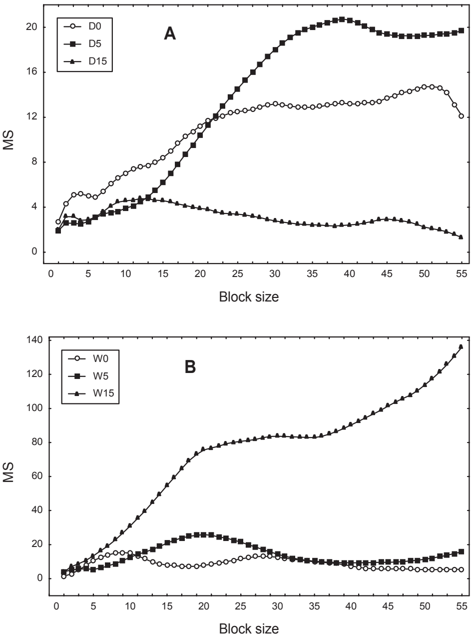

Fig. 5. Pattern analysis of two populations of *B. pubescens*. A – drier and B – wetter site of mined peat-bog. Results of analysis of transects within respective populations are shown.

The growth of the downy birch was influenced by different WTD in the field. As we expected, the downy birch grew better under wetter conditions (high WTD), particularly under shaded treatment. Under wetter conditions, the downy birch exhibited higher growth increments as well as higher leaf production what is the opposite result of manipulated greenhouse experiment (higher increment values were under low water table level). As we proposed earlier, it might be explained by fluctuated water table level in natural field conditions. The downy birch does not tolerate flood (because of anaerobic conditions in soil) and indicates terestrification of peat bogs (e.g. Salonen 1994, Lanta et al. 1994). Thus, the high WL in greenhouse inhibited the growth. In contrast, the water level decrease during vegetation season in the field, peat dried out and birch saplings were stressed by drought and overheating. Therefore, in wetter sites the effect of drought was milder, and the growth characteristics could be higher than in drier sites.

We are aware that results of our study could be partly influenced by some environmental factors, such were differences in start (field experiment – October; greenhouse experiment – March) and duration of both experiments. At the field, environmental conditions could be partly influenced in the winter season, when could play a role differences in soil temperatures or an effect of winter desiccation (Price et al. 1998). It is a reason why cut-away peat surface is dark and overheated for a long time (Girard et al. 2002). This highly variable environment could not be provided in greenhouse experiment.

Higher values of the leaf biomass and RGR for shaded saplings might be a cause of the effect of higher allocation to assimilation apparatus as was suggested by Henriksson (2001). Differences in leaf biomass could be explained by existence of the facilitative mechanism, if we consider the shaded treatment as a simulated protection effect by adult plants on the sapling growth. However, we are aware that the "real" effect of facilitation (defined in Callaway & Walker 1997) could be proven only by longer experiment and studying of survival of saplings. The facilitation of adult trees is important effect for survival and successful establishment of saplings in adverse environmental coditions (Egerton et al. 2000, Haase 2001). Our observations from the studied locality showed that the downy birch occupies wetter sites especially at the draining channels. There was already induced relatively successful spontaneous revegetation presumably because of higher water supply and partly by protective shading of birch trees.

Environment and pattern

Although the downy birch occurs in wide range of habitats at the study locality, the moisture is the most decisive environmental factor influencing its spatial pattern. The aggregation was obvious at the drier site (transects D5 and D0) probably because of the competition from neighbourhood species. The patches of aggregated saplings bordered sites of lower canopy of other plant species. These patches were characterized by lower competition effect or full absence of *Molinia caerulea* grass and *Eriophorum angustifolium* sedge, both dominant plants at drier sites. Both these dominants are capable of fast vegetative spread, which help them to modify its immediate environment and to form a major component of the community. Consecutively, they could produce marked spatial heterogeneity through their light competition on other species (Greigh-Smith 1979) and to impose the aggregate pattern of the downy birch. Conversely, at the wetter site the pattern was random probably because of the absence of strong competitors. On this sites we occurred only sparsely distributed vegetation of mosses (*Polytrichum* sp. and *Dicranella heteromalla*) and sedges (e.g. *Carex canescens* and *C. rostrata*).

Except of the competition, disturbances such as frost heaving and crust formation could influence the size of birch aggregation due to variable intensity of erosion at the bare peat surface. Drier sites are more prone to environmental extremes, where the highly fluctuating WTD and the drought are both harmful to birch survival, and might lead to the increase of heterogeneity in the birch population. Hence, it was the reason why we recorded fewer individuals in transects sampled at the drier site.

Expansion of the B. pubescens

Conclusion of the study is that the tested variables (water table level, shading, soil substrate) influenced growth characteristics of the downy birch in different ways.

Furthermore, spatial pattern of the downy birch can be influenced by different water table level and competition of neighbour species. The fact, that draining ditches are now rapidly colonized by a dense population of birch, supports the idea that the downy birch probably will expand in near future over the mined peat surface at the locality. For that reason, we can expect that it may lead to the development of a mix-forest composed from dominant trees of *B. pubescens* and *Pinus sylvestris* than to a peat formation and accumulation.

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Paper VII

The effect of dam construction on the restoration succession of spruce mires in the Giant Mountains (Czech Republic)

The effect of dam construction on the restoration succession of spruce mires in the Giant Mountains (Czech Republic)

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Abstract. Spruce mires are rare and endangered plant communities of Central and Western Europe. In the Czech Republic, they were intensively destroyed and drained during 1970`s. To start the regeneration of spruce mires, palisade dams sealed with peat were used to block draining ditches in the Giant Mountains. Four years after dams construction, there were significant differences in vegetation above and below the dams. Vegetation above dams successfully developed towards plant communities characteristic to spruce mire forests. Below dams, the colonization by forest floor species continued. These differences indicate that palisade dams effectively retain water and help the regeneration of spruce mire forests. Our results support construction of palisade dams in such habitats.

Keywords: Community structure, Dam, Drainage ditch, RDA, Restoration, Spruce mire

Introduction

Spruce mires are rare plant communities of Central and Western Europe. In recent decades, they were intensively destroyed by human activity and their area decreased dramatically throughout Europe. Drainage of spruce mires has a long tradition in the Giant Mountains (Krkonoše in Czech). The first attempt to drain them comes from the 19th century. Paradoxically, the catastrophic flood of 1897 stopped this effort. Drainage of mire forests and deforestation of mountain belts were considered to be the main reasons for the catastrophe (Lokvenc 1994). In 1970 – 80's, spruce mires were destroyed by severe drainage and air pollution in the Giant Mountains. Drainage was performed despite the conservation status of the Krkonoše National Park. In the Czech Republic, all types of wetlands were drained as a part of existing government policy, which tried to increase agricultural and horticultural land production. Wetland drainage causes mineralisation, peat decomposition and leaching of minerals (Kuntze 1988, Wind-Mulder et al. 1996, Sundström et al. 2000). These processes result in a decrease or disappearance of stress-sensitive species with weak competitive ability and narrow ecological amplitudes, and an invasion or increase of ubiquitous and nitrophilous strong competitors, often with wide ecological amplitudes (Lavoie et al. 2003).

Excavation of draining ditches leads to an increase in lateral seepage from the wetlands (Schouwenaars 1995, Horn & Bastl 2000). To reduce water loss caused by ditching, a key activity is usually blocking or preferably filling of any ditches. To regenerate wetlands, blocking requires insertion of a series of impermeable (or nearly so) barriers (dams). The damming raise water table levels in the ditch (Mawby 1995, Price 1996) and stimulates establishment and spreading of mosses and mire plants (Pfadenhauer & Klötzli 1996, Robert et al. 1999, Price et al. 2003). Many successful attempts have been recorded from the insertion of plastic, plywood or palisade dams at regular distances to complete refilling of drains with peat (Rowell 1988, Wheeler & Shaw 1995, Stoneman & Brooks 1997).

To start regeneration of spruce mires, palisade dams sealed up with peat were used to block draining ditches in the Giant Mountains. Palisade dams were used because of low cost and because they do not disturb landscape patterns. The aim of our study was to evaluate the effectiveness of dam construction on vegetation development in draining ditches.

Methods

Site description

The study site (Mrtvý vrch) is located 3 km north of the Harrachov town in Czech Republic (50°48’15’’, 15°26’45’’). The altitude of the site is 1058 m a. s. l. and the mean annual temperature is 4°C and mean annual precipitation is 1400 mm (Harrachov and Szrenica meteorological stations). The Mrtvý vrch is a topogenic raised peatland (Dohnal 1965). According to the phytosociological nomenclature (Moravec et al. 1995) the vegetation of the study site was classified as *Sphagno-Piceetum* (spruce mire) with some refuges of *Oxycocco-Empetrion hermaphroditi* (mire vegetation). *Avenella flexuosa* and *Calamagrostis villosa* were the most dominant species, co-occurring with *Molinia caerulea*, *Vaccinium myrtillus, Galium saxatile, Agrostis tenuis, Carex canescens* and *Eriophorum vaginatum*. In early 1980’, spruce trees (*Picea abies*) dominants died out because of air pollution and were removed. At the same time, draining ditches were excavated to lower the water table level and to increase of the stand productivity. The spruce mire is situated in a slightly shallow depression of a granite pluton form. The peatland surface is moderately south-face sloping. The maximum depth of peat accumulation is 2 m. A mire macrotope of the peatland is composed from two raised mire mesotopes (here called locality 1 and locality 2).

First mesotope (locality 1) has a central drainage ditch (75 cm deep) that was dug out around the central mire. Two ditches were excavated to collect water from the central ditch. To control water run-off and to stimulate regeneration of the mire, palisade dams were installed in ditches and sealed up by peat from the neighborhood in the summer of 1997. A total of 14 dams were installed, approximately in 10 – 15 m distances apart from each other.

In the second mesotope (locality 2) only one drainage ditch (75 cm deep) was excavated. Three dams in 15 m distances were installed there in the summer 2000. Damming resulted in effective retaining the water (above dams), shown as large differences in water level between above and below parts of dams.

Data collection

To monitor the effectiveness of dam construction, 28 relevés were laid out at the locality 1 in July 2001. We estimated visually the vegetation cover using pairs of 1 m^2 plots established in draining ditches. Each pair consists of plots on either side of a particular dam, and 1 m away inside of the ditch. This experimental design is based on the presumption of the same vegetation in each pair of plots before the dams were established in 1997.

To compare alternative ways of restoration succession we used permanent plots to monitore temporal changes in vegetation at the locality 2. Vegetation cover was estimated visually in permanent 1 m^2 plots using a continuous grid of nine 0.33 x 0.33 subplots in three paired plots established in draining ditch. Each pair consists of plots on either side of a particular dam, and ca 0.75 m away (for spatial arrangement of the experiment see Fig. 1). An initial vegetation recording was conducted immediately in August 2000, after the dams building in order to obtain baseline data for each plot. First vegetation changes after dams building we monitored in three consecutive years (August 2002, July 2003 and July 2004). To evaluate the effect of damming to species diversity of both vascular species and mosses, the number of species per a plot was counted.

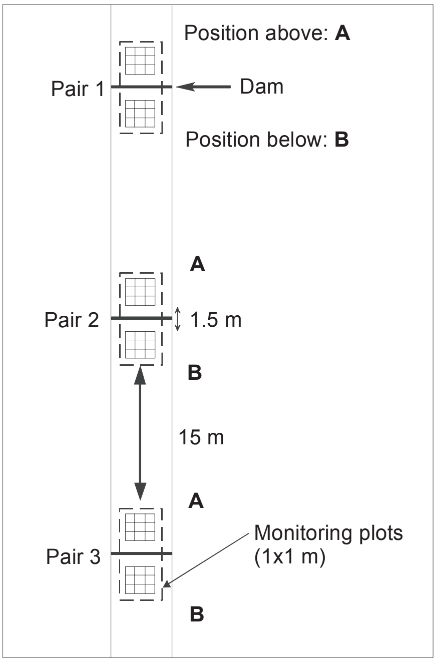

Fig. 1. Spatial arrangement of the experiment at the locality 2. Vegetation cover was estimated in permanent 1x1 m plots using a continuous grid of nine 0.33x0.33 m subplots in three paired plots established in draining ditch.

We measured (immediately after damming of the ditch) the actual water table as a height of water-column above and below the dam six times during the vegetation season of 2001 at the locality 2. In further years, we do not measure the water table because there were remarkable differences between above and below parts of a dam.

Nomenclature: for vascular plant species (Neuhäuslová & Kolbek 1982), for *Senecio hercynicus* (Hodálová 1994) and for bryophytes (Váňa 1997).

Data analysis

We used constrained ordination method redundancy analysis (RDA) to analyze differences in vegetation of locality 1 caused by damming. Redundancy analysis is a direct gradient analysis method based on the assumption of a linear response, and was used because data sets were relatively homogeneous (the length of the first DCA axis was 3.114). The species score on the first (i.e. constrained) axis corresponded to the relative position of species' abundances with respect to position above and below dams. A Monte Carlo permutation was used to test for the significance of the RDA model (299 permutations). We analyzed data using both non-standardized data and standardized by sample norm. With non-standardized analyses we investigated if damming affected species cover. With standardized analyses we studied if damming caused changes in species proportion.

We used the RDA to study successional change in vegetation of the locality 2. We used RDA models because the data set was relatively homogenous (the length of the first DCA axis was 2.819) and the explanatory variables were categorical. For hypotheses testing with Monte Carlo permutation tests (499 permutations) we used split-plot design appropriate for our experimental design (ter Braak & Šmilauer 2002). Permutations were performed within each pair of plots. For RDA analyses of the species composition of the established plant community, data were taken from 0.33 x 0.33 subplots with permutation test reflecting this hierarchical procedure (i.e. permuting the whole 1 m^2 plots together). As the data form repeated observations that include the baseline (before treatment) measurements, the interaction of time and treatment (plot position above or below dams) is of greatest interest and corresponds to the effect of the experimental manipulation. The significant effect of time and position interaction indicates divergent temporal development of plots located above and below dams. Plot identifiers (coded as dummy variables) were used as covariates, when the influence of plot position (above or below a dam) on time changes in plant composition was tested. Time was considered as a categorical variable (we created four dummy variables for four observation years). The biplot ordination diagram was used to visualize the results of the analyses. Only the species with highest fit to ordination axes are shown in the ordination diagrams.

Species scores on the constrained axis of the non-standardized analysis (that was processed on coverage data sampled above dams), where time was the only linear variable and codes of individual plots treated as covariables, were considered characteristic of the species response to time: cover of species with negative values of the RDA score decreased and cover of species with positive values increased. Then posterior classification of species into simple functional types, using data from the local flora of Šourek (1969) for vascular plants, was tested as possible predictor of this response. We recognized these three categories: mosses, forest vascular species and mire vascular species. Then we processed the data by using a simple ANOVA analysis. We used the ANOVA for repeated measurements to analyze univariate data of species diversity. To improve normality and homoscedasticity, we log-transformed species diversity data.

Table 1. Results of the RDA analyses of cover estimates in 1 m x 1 m plots at the locality 2. Data are centered by species and either standardization by sample norm (Y = yes) was applied or data were not standardized (N = no). Env. var. = Environmental variables, Covar. = Covariables, % ax 1 = % species variability explained by axis 1 – measure of the explanatory power of the environmental variables, r ax 1 = species environment correlation on axis 1, F = F – ratio statistics for the test on the trace (all axes). P = corresponding probability value obtained by the Monte Carlo permutation test (499 permutations, i. e. Type I error probability in testing the hypothesis that the effect of one explanatory variables is zero). Position = location of the plots above or below a dam; Yr = time coded as four dummy variables; PlotID = plot identifiers of each plot coded as many dummy variables; * = interaction between terms.

	Env. var.	Covar.	St	% ax 1	r ax 1	F	P
A1	Yr*Position	Yr, PlotID	N	6.8	0.667	8.890	0.002
A1st	Yr*Position	Yr, PlotID	Y	1.6	0.548	2.394	0.002
A2	Yr	Yr*Position	N	6.9	0.668	8.077	0.002
A2st	Yr	Yr*Position	Y	4.0	0.585	5.415	0.002

Results

Water table measurements

Building of dams resulted in an increase of the belowground water table level. The values varied from -8 to -18 cm (mean value -15) below and from -4 to -14.5 cm (mean value -7) above dams.

Species composition at the locality 1

Four years after the dams were built, there was a significant difference in plant species composition between above and below dams. Damming affected species cover (RDA; Non-standardized test: the first canonical axis - RDA I - explained 7.9% variation, F = 2.604, P = 0.038) as well as the relative proportion of the species (RDA; Standardized test: RDA I - 8.1%, F = 2.905, P = 0.025). Above dams, *Sphagnum capillifolium* and *S. fallax* were only bryophytes present, but below dams, *Dicranella heteromalla* and *Dicranum scoparium* had highest cover from bryophytes. From vascular plants, *Carex canescens, Eriophorum* *vaginatum* and *Vaccinium vitis-idaea* were more abundant above dams, while *Avenella flexuosa, Agrostis tenuis* and *Galium saxatile* were more abundant below dams (Fig. 2).

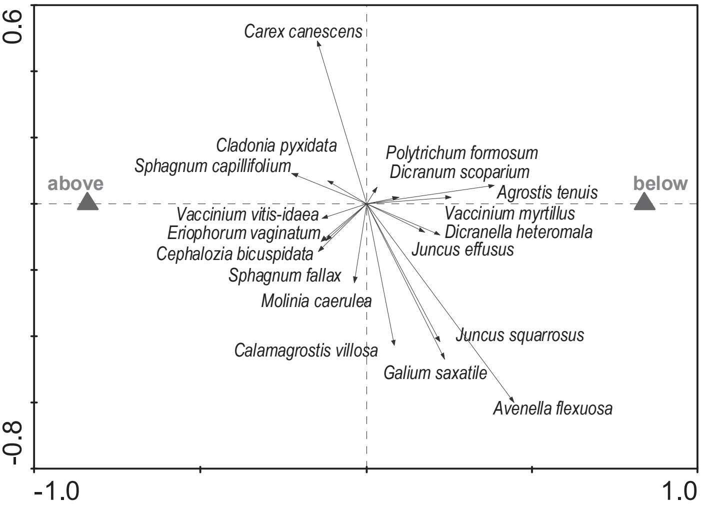

Fig. 2. Ordination diagram showing the results of RDA analysis for the locality 1 where the position of reléves above and below dams was used as only one environmental variable (nonstandardized test).

Species composition at the locality 2

Regardless of the type of analysis used (standardized and non-standardized RDA), the species composition of plant and mosses community varied significantly between years (both tests P < 0.01, see Table 1). However, no clear directional trend was observed during the four years study that would be common to all sample plots (Fig. 3).

Significant differences between positions above and below a dam were detected (Table 1, analyses A1 and A1st). The mire mosses as *Dicranella cerviculata*, *Sphagnum* sp. and *Brachythecium rutabulum*, and vascular plants as seedlings of *Salix caprea*, grasses *Avenella flexuosa*, *Calamagrostis villosa*, and forb of *Cirsium palustre* were the species most thrived above dams (Fig. 4). Typical forest species as *Galium saxatile, Trientalis europaea* and *Gnaphalium sylvaticum* better survived below the dams. The moss *Polytrichum commune* performed better above dams, whereas *P. formosum* increased below dams. *Viola palustris* was the mire species most promoted by damming, however performed better below dams (Fig. 4). The total cover values did not differ between plots (cover close to 100 %), therefore the results of standardized and non-standardized analyses were very similar (Table 1).

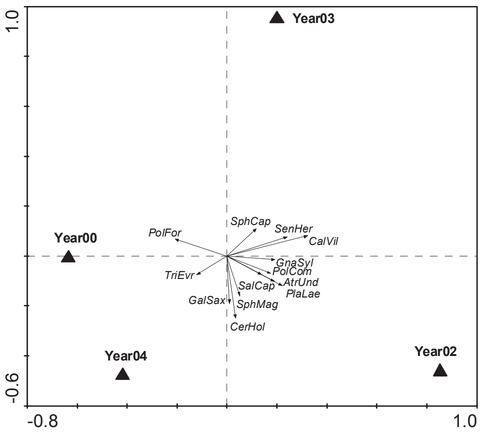

Fig. 3. The distribution of plant species and mosses during different years of the experiment (locality 2). Results of non-standardized RDA test (Table 1, analysis A2, species cover). Time was coded as four dummy variables. AtrUnd – *Atrichum undulatum,* CalVil – *Calamagrostis villosa*, CerHol – *Cerastium holosteoides*, GalSax – *Galium saxatile*, GnaSyl – *Gnaphalium sylvaticum*, PlaLae – *Plagiothecium laetum*, PolFor – *Polytrichum formosum*, PolCom – *Polytrichum commune*, SalCap – *Salix caprea*, SenHer – *Senecio hercynicus*, SphCap – *Sphagnum capillifolim*, SphMag – *Sphagnum magelanicum*, TriEur – *Trientalis europaea*.

The difference between the results of standardized and non-standardized RDA analyses shows that variation in species composition in relation to position above or below a dam in both studied localities is a consequence of the general response of plants to positions (non-standardized RDA) rather than of changes in species proportions itself (standardized RDA).

There was a significant difference in the response to the time between three functional types of grouped species in the data set sampled above dams (Fig. 5; ANOVA, df = 2, F = 6.71, P = 0.003). Tukey post hoc comparison test showed that the response of forest vascular species significantly differed from the response of mosses (P < 0.05). Responses of the mosses and mire plants to the time were rather similar (Tukey test, P > 0.05). The scores of the canonical axis indicated that cover of species with positive values increased and with negative values decreased with time in relation of time as an environmental factor. Therefore, the Fig. 5 showed that species with increased cover were mostly mosses such as *Sphagnum capillifolium* and *Polytrichum commune*. The cover of forest species declined except of three species (*Calamagrostis villosa*, *Avenella flexuosa* and *Galium saxatile*) those had positive values of canonical scores.

Species diversity

The time-position interaction term in univariate repeated measures ANOVA was not significant (df = 3, F = 0.30, P = 0.82) during the study period. Thus, the total species diversity was not influenced by damming of the draining ditch and the succession was similar above and below dams in term of species diversity. However, the values of the number of species were higher for position above the dams (see Fig. 6).

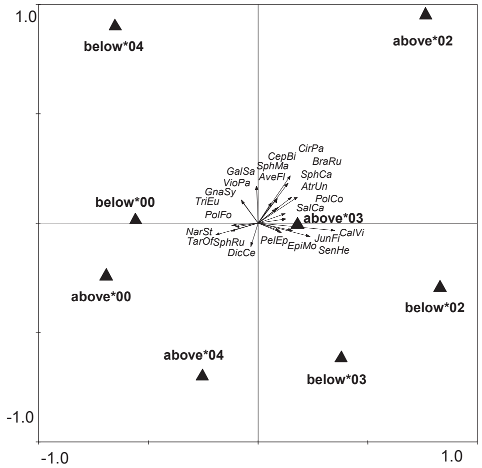

Fig. 4. Redundancy analysis biplot (Table 1, analysis A1, species cover) of the year to year difference in the individual species response to the positions above and below dams (locality 2). Abbreviations: * - indicates interaction of environmental variables, AtrUn – *Atrichum undulatum*, AveFl – *Avenella flexuosa*, BraRu – *Brachythecium rutabulum*, CalVi – *Calamagrostis vilosa*, CepBi - *Cephalozia bicuspidata*, CirPa – *Cirsium palustre*, DicCe – *Dicranum cervicullata*, EpiMo – *Epilobium montanum*, GalSa – *Galium saxatile*, GnaSy – *Gnaphalium sylvaticum*, JunFi – *Juncus filiformis*, NarSt – *Nardus stricta*, PelEp – *Pellia epiphylla*, PlaLa – *Plagiothecium laetum*, PolCo – *Polytrichum commune*, PolFo – *Polytrichum formosum*, SalCa – *Salix caprea*, SenHe – *Senecion hercynicus*, SphCa – *Sphagnum capillifolium*, SphMa – *Sphagnum magelanicum*, SphRu – *Sphagnum rusowii*, TarOf - *Taraxacum* sect. *Ruderalia*, TriEu – Tientalis europaea, VioPa – *Viola palustris*. Each centroid (triangles) represents position of plot in a year. Only species with highest fit are shown.

Discussion

Vegetation monitoring in the draining ditches revealed changes in plant vegetation above and below dams during the study period 2000-2004. Construction of the woody dams lead to clear differentiation of the vegetation at both studied localities within the spruce mire “Mrtvý vrch”.

The sites above dams were occupied mainly by mosses which were infrequent below dams. However, at the locality 1, we recorded several mosses below dams; e.g. *Polytrichum formosum, Dicranum scoparium* and *Dicranella heteromala*. These mosses are considered as forest floor mosses (Pilous & Duda 1960) and were overgrown by the mire mosses above dams in our study. At the locality 2, such early mosses colonists were *Polytrichum formosum, Dicranella cerviculata* and *Funaria hygrometrica.* Similarly to our results Robert et al. (1999) observed rapid replacement of early successional mosses by *Sphagnum* species after blocking a draining ditch.

More favorable hydrological conditions above dams lead to a colonization of all *Sphagnum* mosses recorded in plots. Lower suitability of drained sites for *Sphagnum* canopy regeneration rather then in blocked sites was observed because of the lowest water tension there (Price 1997). *Sphagnum* colonization also depends on a source of diaspores (Salonen 1987) and suitable environmental conditions for their establishment (Campbell et al. 2002, Price et al. 2003). *Sphagnum* is often regarded as a characteristic component of mire vegetation, but also because it helps to re-establish acrotelm and to stabilize the hydrochemical conditions, and also to moderate microclimatic fluctuations (Rochefort 2000).

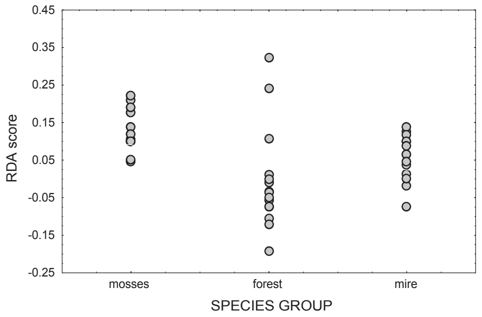

Fig. 5. Relationships between functional types and species responses to the time as an environmental factor.

Above dams, the coverage of dominant grasses was gradually decreased, although the coverage of some clonal plants as *Agrostis tenuis, Avenella flexuosa* and *Calamagrostis villosa* was higher at the locality 2. It could be explained as a reason of lower moisture content above dams for some parts of the year. Furthermore, wet profiles were partially retarded because of temperature increasing in spring and reducing the release of nutrients, oxygen supply, length of growing season, and therefore the speed of peat mineralization (Wheeler & Proctor 2000). Our study demonstrated that the presence of clonal grasses may play an important role in the vegetation dynamics of developing communities in drained mires as found earlier by Laine et al. (1995).

Eriophorum vaginatum and *Carex canescens* were two vascular plants most abundant above dams (Fig. 2). Both indicate differentiation of water conditions in relation to position. Similarly, Komulainen et al. (1999) found *E. vaginatum* and *C. canescens* to benefit on rewetting. According to Tuittila et al. (1999) *Eriophorum vaginatum* behaves as an early colonist of bare peat surfaces after disturbance.

Rather xerophilous lichen *Cladonia pyxidata* occupied bare peat among the dwarf shrubs of both *Vaccinium* species below dams, corresponding with findings that lichen grows only on hummocks (Laine & Vasander 1996).

Excavation of draining ditches lead to the colonization by forest species that continued, in particular, below dams after damming of drainage ditches. Prostrate herb *Galium saxatile* is moderately shade tolerant and can coexist with more robust species of higher competitive ability, also in mires and aquatic habitats. *Vaccinium vitis-idaea* and *V. myrtillus* are long-lived and slower-growing shrubs of acid soils. Leaves of *Vaccinium* species have larger leaf area when compared to other mire species – this characteristic enables them to maximize production per unit of limiting minerals in nutrient poor conditions (Kučerová et al. 2000). Both species that are generally more abundant in mires in Czech Republic (Dohnal 1965, Neuhäusl 1972) benefited in weter conditions.

The succession studies over longer period of time usually confirm year by year variation (Tuittila et al. 2000). The reason for these variations are most likely weather conditions (Price et al. 2003). Therefore, a slow decrease in species diversity during four years of the study period after construction of dams can be a cause of several summer droughts in 2002 and 2003. In our case, as indicated by Fig. 5, this effect of droughts was more pronounced with delay in 2003 and 2004. In our plots, where the diversity was similar in all years, grouping the

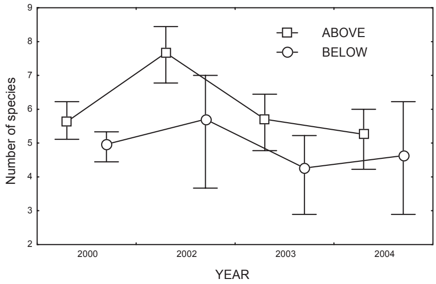

Fig. 6. Mean numbers of plant species per 1 m^2 over four years of investigation at the locality 2. Min and Max values are indicated by the vertical lines.

plant species into three ecological types showed that species grouping is a good predictor of species response to the time. In other words, each group responded to the time as a unit. It could be caused by subjective classification into three distinct groups that may have, however, fully different physiological and environmental requirements.

Our results showed that location of dams in 15 m distance supported restoration succession of forest mires in giant mountains, which are considered very specific and endangered habitats of the Czech Republic. They are characterized by specific site conditions and they are suitable for the survival of very specialized flora and fauna. Drainage and removal of the original vegetation destroyed the functional relationships founded within an intact mire. The restoration by damming can help the area to revert to its original habitat type, although the longer the area has been drained, the more difficult is to fully recreate the original state (Laine et al. 1995). The construction of dams for hydrological management of disturbed mires is the simplest mean for creating a large water-storage capacity. It aim to lead to a “new state”, often different from the original, but nevertheless recognizable as some other peatland habitat type. Blocking of drainage ditches seems to be fairly good for achieving spruce mires regeneration in the Giant Mountains. To start forest mire regeneration, palisade dams sealed with peat seem to be a sufficient and inexpensive means for water retention and is a necessary step for restoration of spruce mires in the Giant Mountains. The study showed that raising the water level to, or above, soil surface promotes conditions wet enough for rapid succession towards closed mire vegetation.

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Conclusions

Conclusions

In this thesis I have investigated ecosystem functioning (i) by experimental manipulations of species richness and (ii) by studying specific processes in man-disturbed peatlands. I have explored productivity-diversity relationships (papers 1, 2 and 3), provided specific examples of factors affecting development of plant communities (papers 4) and determined factors affecting the establishment of plants in disturbed peatlands (papers 5, 6 and 7).

In the research of biodiversity-productivity relationships, I have suggested a new way of evaluating biodiversity data (papers 1 and 2) that is expected to increase the precision of our data analyses The experiments were designed to enable separation of the effect of species richness and functional group richness as far as possible within the experiments with species assemblages constructed from a closed species set. Statistical methods used in biodiversity experiments help solve difficulties in separation the effects of species and functional group. The comparison of greenhouse and field experiments reveals that a system open to invasion has weaker effects of diversity on productivity weaken over time. It has a great potential to increase our understanding of mechanisms of coexistence in plant communities.

To show how important invasion of weeds to artificially created assemblages of grassland species. I studied if diversity and invasion resistance are positively related through diversity effects (paper 3). Because I combined species and functional group richness in factorial design with fertilization. It enables me to identify that species assembled into groups according to similar traits could have large effect on species invasibility and that the resultant composition of weeds depends on the presence and proportion of individual sown species.

The resistance of differently species-rich communities against herbivore impact was assessed through grazing of an invasive slugs (paper 4). This paper used similar methodology proposed in other biodiversity papers. The paper explore the important topic of selective grazing on plant community structure. The strength of the study presented here compared to all the previous studies is the connection of the herbivory study with testing biodiversity effects. By doing this I demonstrate a gradual decrease in effect of grazing pressure with increased species richness, which I consider as a main output of the study.

In the last three papers (papers 5, 6 and 7) I present three case studies focusing on the effects influencing species survival in a harsh conditions of mined peatlands. The methods applied here extends traditional approaches to assess the effects of biotic and abiotic factors on revegetation patterns during early phases of plant succession. I follow the approach of biodiversity papers when I demonstrate that the extrinsic factor water supply importantly affects not only the survival of a woody species (paper 6) but also the community structure (paper 5 and 7), and is responsible for species distribution patterns. The major strength of the approach used here is to understand some processes, which can be also applied to nature conservation issues.

The results of this thesis suggest that both species richness and community composition are crucial for ecosystem functioning measured through plant productivity. Presented case studies show that our knowledge of factors determining species coexistence in real and artificial communities, in agreement with other studies focusing on this topic, are very incomplete. Many more efforts should be devoted to effects of biodiversity on ecosystem processes because there are many uncertainties and questioning remaining. The results also demonstrate that integration across ecological subdisciplines can provide a new valuable insight into the biodiversity-ecosystem functioning research.

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