

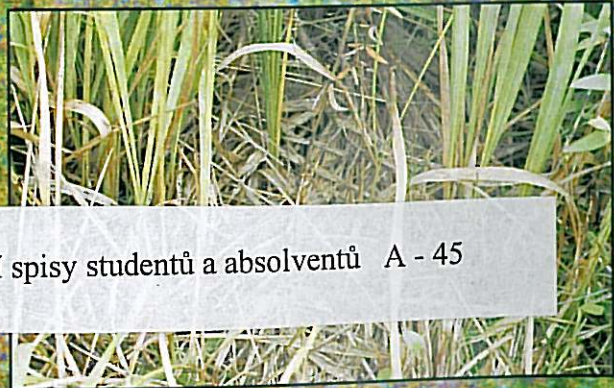
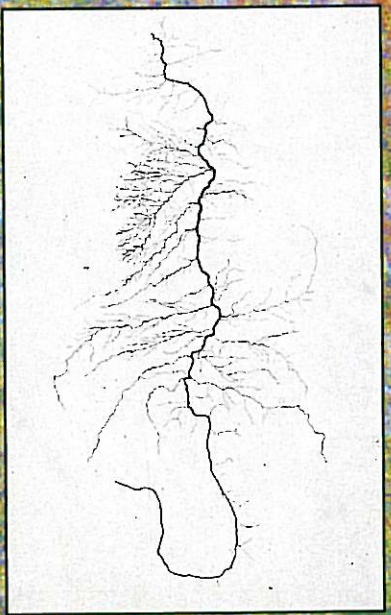
Faculty of Biological Sciences  
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# Ecological study of *Molinia caerulea*, a dominant species of wet meadows

Štěpán Janeček

PhD Thesis

2005



C - 4 Osobní spisy studentů a absolventů A - 45





FACULTY OF BIOLOGICAL SCIENCES  
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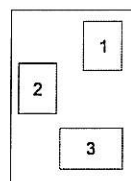
## ECOLOGICAL STUDY OF *MOLINIA CAERULEA*, A DOMINANT SPECIES OF WET MEADOWS

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ŠTĚPÁN JANEČEK

PhD. Thesis

supervisor  
JAN LEPŠ



**Cover pictures (back site)**

1/ *Carex hartmanii* in the glasshouse experiment on soil heterogeneity (Chapter IV).

2/ Root architecture of the *Molinia caerulea* (Chapter V).

3/ Accumulation of litter in abandonment part of the study site (Chapter III).

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## ANNOTATION

**Janeček, Š. (2005):** Ecological study of *Molinia caerulea*, a dominant species of wet meadows. PhD thesis [in English], University of South Bohemia, Faculty of Biological Sciences. České Budějovice, Czech Republic. 84 pp.

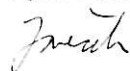
*Molinia caerulea* is a tussock grass common in wet semi-natural meadows. Due to its dominance, the species plays decisive role in plant community functioning. The aim of this thesis was to study some traits of *M. caerulea*, which can influence coexisting species and/or are important for dominant position of *M. caerulea*.

The thesis is composed of four original studies describing properties of both above-ground traits, such as tussock structure, litter or leaf cover and below-ground traits, mainly ability to exploit soil heterogeneous environment.

## DECLARATION

I hereby declare that this thesis has been fully worked out by myself and the named co-authors with the use of cited references.

Štěpán Janeček



České Budějovice, 27<sup>th</sup> February 2005

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# General Introduction

## Chapter I

### General introduction



## General introduction

Plants are often divided into species with high and low abundance. The highly abundant species has been described as matrix species (Grime 1973), dominants (Grime 1984) or core species (Wisheu & Keddy 1992), the species of low abundance are called subordinate (Grime 1984) or satellite (Wisheu & Keddy 1992). Olf & Bakker (1998) distinguished between global dominants, local dominants and subordinates. Moreover, they have shown that some species are not able to achieve dominance in any environment and so dominance or subordination can be considered to be an attribute of individual species. The dominance can be related to the allocation strategy of individual plants. Generally, an individual species allocates nutrients and energy into different plant parts or functions, so that it can grow in specific conditions and take a specific position in plant community (Tilman 1990). There are many traits supporting species survival in competitive environment of plant community (for example plants can create long rhizomes for moving in space or big reserve organs for moving in time), but it seems that only small number of traits can lead to dominance in specific environment. The traits, which seem to be generally interconnected with dominance, are size traits such as height, large biomass production or large canopy area (Keddy et al. 2002).

Tussock grasses with phalanx type growth (*sensu* Lovett Doust 1981) often dominate the grassland communities throughout the world (Walter 1984, Körner 1995). These grasses create vegetation matrix and present a great proportion of total biomass, and so they have a principal influence on processes in plant community. Tussock grasses can effect another plants by four basic competition traits, which are typical for their life form 1/ dense leaf cover of tussock grass 2/ high amount of leaf litter produced by these dominants 3/ layer of shoot bases, which creates a mechanic barrier for other species 4/ extensive root system. There is a different effect of individual competition traits of tussock grasses on individual subordinate species but also on their various ontogenetic stages (Grubb 1977). The effect of leaf layer on subordinate species is often seen as very negative thanks to asymmetry of aboveground competition for light (Weiner 1990, Schwinning & Weiner 1998, Freckleton & Watkinson 2001). Similarly the influence of litter seems to be rather negative, especially for seedlings, which have not enough energy to penetrate through (Špačková & Lepš, 2004; Jensen & Gutekunst, 2003; Xiong & Nilsson, 1999). The effects of two other competition traits are not sufficiently known. The effect of shoot bases as a specific microsite has been investigated by Kupferschmid et al. (2000); they have shown that tussock can be suitable for germination of some species. Although there are no data about tussock effect on established vegetation, we can expect that vegetative penetrating through tussock basal barrier can be very difficult. The specific impact of root competition of dominant tussock grasses on subordinate species is also unclear. Campbell et al. (1991) suggested that small species are able to coexist with dominants thanks to their ability to precisely utilise resource patches, which has been missed by the dominants. On the other hand Weiner et al. (1997) proposed that soil



heterogeneity can increase competition asymmetry between large and small plants due to the ability of large plants to reach and usurp nutrient rich patches. Likewise Rajaniemi & Reynolds (2004) did not find trade-off between foraging scale (estimated as total root mass and length of structural roots) and precision (ratio of root length in nutrient rich and nutrient poor patches).

Our understanding of competition traits and mechanisms of their functioning is very limited; fortunately, much more data on general (negative) effect of dominant tussock grasses on species richness and species evenness is available (Lepš 2004, Gurevich & Unnasch 1989, Wardle et al. 1999). Also effect of fertilisation and defoliation (mowing or grazing) on their dominance has been investigated. Whereas fertilisation increase dominance of these species and caused decrease of diversity (McCrea et al. 2004), mowing and non-selective pasture favour subordinate plant growth-forms, which lose smallish part of their biomass (Klimeš & Klimešová 2002, Zobel 1992, Liira & Zobel 2000).

Although the importance of dominants on community function is often emphasised (such as above) and we roughly know, how is this importance affected by some treatments, we have relatively limited knowledge on behavior of individual dominant species. Detailed knowledge on dominants can also be essential for understanding the behaviour of subordinates.

### *Molinia caerulea*

*Molinia caerulea* (L.) Moench (Fig 1) is a grass species, widespread throughout south-western and northern Asia, North America and Europe. *M. caerulea* grows on both calcium rich and acidic soils. We can find morphologically and physiologically very different populations (Salim et al. 1988); the species is probably not uniform, and is also taxonomically investigated (Dančák 2002). It is often dominant on wet meadows, moors, heaths, bogs, fens, mountain grasslands, cliffs and like shores (Taylor et al. 2001).

*M. caerulea* is a tufted perennial grass. Circular tufts up to 30 cm in diameter at the base are composed of dense aggregated shoots. Each stout shoot has one node towards the base. Below this node, there is an internode called "basal internode" (Jefferies 1915), which is of a special interest. Basal internodes are over-wintering organs which serve as reserve organs with starch and protein grains (Grant et al. 1996, Jefferies 1915). These reserves support growth of new shoots at the start of new vegetation season (Thornton and Millard 1993, Thornton and Bausenwein 2000, Janeček 2001).

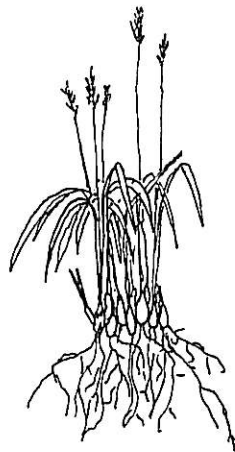


Fig. 1: *Molinia caerulea* (L.) Moench

The starch concentration decreases in old and increase in new basal internodes during the vegetation season (Fig. 2). Inflorescence stalks are from 15 up to 1.3m height (Taylor et al. 2001). The inflorescence is a variable panicle ranging from dense and spike-like to open and very loose. On the meadows of Central Europe, the flowering as well as maximal standing crop occurs late in the vegetation season (July, August). Each shoot has approximately seven functional leaves (Jefferies 1915). Leaf blade is 10-45 cm long. Leaves, in contrast to most of the other grasses, have abscission zone, which contains cells with thick walls (Salim et al. 1988).

The impact of different treatments on *M. caerulea* has been frequently studied in

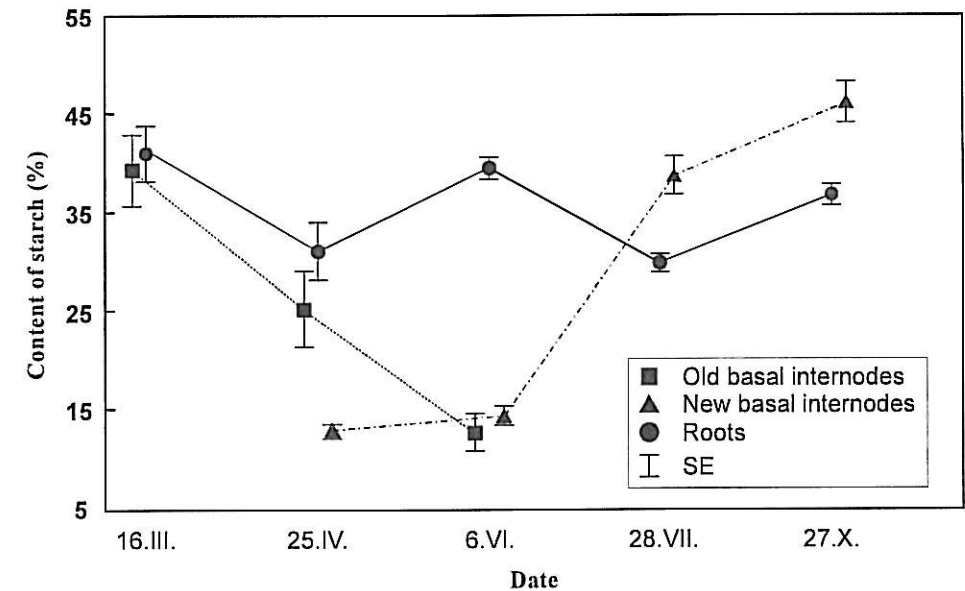


Fig.2: Changes in starch concentrations in roots, old and new basal internodes of *M. caerulea* during vegetation season (Janeček 2001).

British uplands. Grant et al. (1996) showed that tall *M. caerulea* is more utilised by cattle grazing in contrast to other smaller grass species and also basal internode size decreases in the grazed plots compared with the ungrazed exclosures. On the other hand, in extensive sheep production systems *M. caerulea* is grazed only to a small extent (Hunter 1962, King and Nicholson 1964). Todd et al. (2000) tested burning, grazing and herbicide application on *M. caerulea* dominance in six moorland plots. The cover of *M. caerulea* was most significantly reduced by herbicide application in all study sites. In contrast, the effect of burning and grazing was significant only in some of them. Milligan et al. (2004) tested effect of grazing, application of graminicide, cutting and *Calluna* brash addition. Only the cutting treatment had consistent effect on vegetation. Cutting increased proportion of bare ground, reduced vegetation height, increased species diversity and reduced *Molinia* cover.



Lepš (1999) observed the effect of fertilisation and cutting on *M. caerulea* growing in wet meadow community of Central Europe. The relative representation of *M. caerulea* decreased in mown and in fertilised plots. The strong negative effect of mowing on carbohydrate reserves of closely related species *M. arundinacea* (in contrast to weaker effect on smaller *Bromus erectus*) was demonstrated by Klimeš and Klimešová (2002).

### The aims of the thesis

The main aim of this thesis was to study some traits of *M. caerulea*, which can influence processes in oligotrophic, species-rich wet meadow community and/or help to *M. caerulea* to achieve dominance in this community. The first and second study was done with one of the very obvious traits of *M. caerulea* – its tussock structure. The goal of the first study is to describe spatial structure of genets and ramets of *M. caerulea*, which create basic matrix of wet meadow vegetation on our study site (description of the study site is presented in each individual paper in the thesis) and to detect the effect of mowing on this structure. In the second paper, I investigated the role of tussock of *M. caerulea*, litter and total leaf cover of established vegetation on seedling recruitment. The two other studies investigate the effects of competition, soil quality and soil heterogeneity on growth of *M. caerulea*. In the third study, I have compared growth of *M. caerulea* with growth of another phalanx species, *Holcus lanatus* and with guerrilla species *Carex hartmanii* in heterogeneous and homogeneous soil environment, and investigated their competition relationship in both of these soil environments. The last study describes response of root architecture of *M. caerulea* to soils of different quality and to competition pressure.

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## CHAPTER II

### ***Influence of mowing on abundance and distribution of ramets and genets of tussock grass *Molinia caerulea****

Janeček Š, Bystřická D & Lepš J  
[manuscript]



## Influence of mowing on abundance and distribution of ramets and genets of tussock grass *Molinia caerulea*

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### Abstract

Small scale spatial pattern of ramets and genets of dominant tussock grass *Molinia caerulea* was studied in two mown and two unmown squares of a larger experiment, 8 years after the start of experiment. Spatial pattern of shoots has been analysed in a 1 x 1 m plots divided into 400 0.05 x 0.05m subplots. In selected subplots, all the ramets present were subjected to genetic analysis (RAPD). Spatial pattern of ramets was analysed by the 9TLQV method, the spatial distribution of genets was characterised by the dependence of probability that two ramets belong to the same genotype and the ramet distance. Mowing causes the disappearance of tussock structure, manifested by decrease of shoot density of *M. caerulea* in tussocks and also by total decrease of shoot numbers in a plot. Relatively high number of genotypes has been detected (10 and 16 for unmown plots and 15 respectively 13 for mown plots). There was no obvious effect of mowing on genotype number. However, mowing increased genet intermingling due to tussock fragmentation and vegetative spreading into higher distances.



## Introduction

Semi-natural meadows of Central Europe evolved over centuries of human activities and represent a wide spectrum of species rich communities. Besides abiotic factors, such as moisture, temperature and nutrients, their vegetation structure and species richness are affected by the management practices, such as mowing or grazing. These affect directly but differently individual plant species. Whereas selective pasture prefers grazing tolerant plant species (Rosenthal & Kotanen 1994; Oba et al. 2001; Cingolani et al. 2005), mowing and non-selective pasture favour plant growth-forms, which lose smallish part of their biomass (Zobel 1992; Liira & Zobel 2000). The second effect leads to decrease of competition asymmetry between tall and small plants and helps maintain species diversity (Schwinning & Weiner 1998).

Functioning of meadow plant communities is largely affected by the clonal nature of most of the plant species (Klimeš et al. 1997). The clonal growth results in metapopulations of ramets (*sensu* White 1979). Some properties of clonal growth such as foraging activity (Macek & Lepš 2003) or supporting of development of daughter shoots (Alpert & Stuefer 1997) are relatively well known. On the other hand some attributes are still a mystery, such as between shoot information flow (Holzapfel & Alpert 2003) or the maintenance of genetic variability of clonal plant populations in natural communities.

One of the most conspicuous clonal growth forms in meadows is that of tussock grasses, originating by production of new ramets in proximate distance to the maternal tiller. This growth form has been classified as phalanx type (Lovett Doust 1981) and is typical by its competition potential. In consequence, many species of this type became dominant in grassland communities throughout the world (Walter 1984; Körner 1995). Removal of such dominants can lead to pronounced responses of other species (Allen & Forman 1976; Lepš 1999, 2004). Although some authors describe tussock grasses as a classical case of clonal plant integrity (Pitelka & Ashmun 1985; De Kroon & Schieving 1990; Eriksson & Jerling 1990), others showed a high degree of physiological fragmentation of tussocks (Wilhelm 1995; Welker et al. 1991; Derner & Briske 1998). This fragmentation can be the result of a developmental programme where abscission zone is present (Wilhelm 1995), death of maternal tiller (Welker & Briske 1992) and/or by external factors such as grazing (Butler & Briske 1988).

When clonal growth is predominant and density of ramets increases, the number of genets could progressively decrease by stochastic extinction and/or by competitive exclusion (Kays & Harper 1974; Harnett & Bazzaz 1985). Such process has been documented in *Lolium perenne* (McNeilly & Rose 1984) exhibiting a drastic decrease of genotype number on 40-year old pasture as compared to 10-year old sward. Similar pattern has been observed in *Solidago canadensis* during succession on abandoned field (Hartnett & Bazzaz 1985).

These processes are more common in clonal species, where establishment is restricted to early successional stages. Such genet dynamic corresponds to the initial seedling recruitment model ISR (Eriksson 1989), in contrast to the repeated seedling recruitment (RSR), in which case there is no decrease of genotype diversity expected. Such genet dynamic has been shown by pseudo-annual clonal plant *Circaea lutetiana*

(Verburg et al. 2000). Likewise, no decrease of genetic diversity has been observed in old populations of *Cirsium arvense* (Sole et al. 2004). We can expect continuous gradient between these two extreme types of genet dynamics.

An important parameter, which affects competitive exclusion, is genet intermingling. Genets of guerrilla species tend to be more intermingled, phalanx species creating dense clusters of ramets tend to produce more separated genets (McLellan et al. 1997; for guerrilla-phalanx theory see Lovett Doust 1981). The probability of intermingling increases in time in both growth forms (Maddox et al. 1989; Steinger et al. 1996; Jonsson 1996).

The genetic structure of clonal plant populations can be also affected by disturbance regimes, in meadows specifically by mowing or pasturing. Small disturbing events can lead to maintenance of genotype diversity by creation of gaps that are important for generative regeneration (Bullock et al. 1995) and large disturbances can lead to the removal of entire genets (Sebens & Thorne 1985). The mowing affects not only dominant tussock grasses such as *Molinia caerulea* but as a consequence also vegetation vertical structure. Vertical structure characterized by tall tussocks and deep depressions between them is typical for unmown (i.e. abandoned) meadows. Regular mowing then prevents development of similar structure, or, when applied in previously abandoned meadows, causes its disappearance. The flatness of surface can permit vegetative horizontal spreading of *M. caerulea* tussocks and can also make recruitment of new seedlings of *M. caerulea* more successful. Both processes can result in higher intergenet competition. Higher degree of tussock fragmentation can also be expected in mown meadows. These changes can influence number of ramets and genets and their spatial distribution. Thanks to dominance of *M. caerulea* can these changes also affect the processes in the whole community

In our study we compare genotype and tiller distribution and abundance in two mown and two unmown experimental plots. We aimed to answer following questions:

- 1/ How does mowing affect tussock structure of *M. caerulea*?
- 2/ Does mowing influence the number of tillers of *M. caerulea*?
- 3/ Does mowing increase genotype number due to higher possibility of seedling recruitment?
- 4/ Does mowing cause higher genotype intermingling due to better ability of horizontal spreading. In this case we expect the same number of genotypes in mown and unmown plots and higher distances between shoots of the same genotype.

## Material and Methods:

### Study site

Study site is a wet, oligotrophic meadow 10 km south-east of České Budějovice, Czech Republic, 48° 57' N, 14° 38' E at 530 m a.s.l. Mean annual temperature is 7.8 °C, mean annual precipitation is 620 mm. The meadow belongs to Molinion with some species of Violion canine according to the phytosociological classification and is dominated by a tussock grass *Molinia caerulea*.



**Experimental design**

This study took place in plots of long-term experiment established by Jan Lepš (see Lepš 1999, 2004). This long-term factorial experiment started in 1994 to study the influence of mowing, fertilisation and removal of dominant species *Molinia caerulea* on vegetation. Correspondent treatments were established in a 4-m<sup>2</sup> quadrats, each combination in three replications. Mowing is applied once a year in June.

In vegetation season 2002 we selected two mown and two unmown plots (without fertilization and removal) for this genetic study. (For the purpose of this study, the mown plots are labeled 1 and 2, unmown 3 and 4). In each plot, central 1 x 1 m was divided into a grid of 400 cells (5x5 cm each). Tillers of *Molinia caerulea* were counted in each cell. Then we have randomly chosen ten 0.1 x 0.1 m squares (each of them composed of four 0.05x0.05m) in the central 0.5 x 0.5 m area, where the *M. caerulea* was present and all potentially non-interconnected tillers were sampled for genetic analysis (Fig. 1).

**RAPD analysis**

DNA was extracted from frozen leaf tissue using Invisorb Spin Plant Mini Kit (Invitex) according to manufacturer's recommendation. PCR protocol included 95 °C for 10 min., than 45 cycles of 92°C for 1 min., 35°C for 2 min., 72 °C for 3 min. followed by 72 °C for 10 min. Each reaction of 25 µl contained 1 U thermostable DNA polymerase (Biotech) and 100 pmol of primer (Operon). The obtained amplicons were separated by gel electrophoresis on 1.5 % agarose gels in 1 x TBE buffer, stained with SyberGreen and visualised under UV light. Eighty primers (series A, B, F and K, Operon Technology) were initially tested for six plants randomly selected from studied population. As a control of primer specificity we used two separately analysed leaves from the same shoot. The most useful primers were tested for twelve plants with three controls again. Finally we selected three primers efficient to discriminate genetical individuals of *Molinia caerulea*.

**Data analysis**

To analyse spatial pattern of tillers in 1x1 m plots we used 9TLQV method (Dale 1990, 1999; software Passage; Rosenberg 2001). This method is an extension of the 3TLQV (three-term local quadrat variance) of Hill (1973), for a two-dimensional grid (original 3TLQV was designed for a transect). The method is based on the contrast of the central cell of a 3 x 3 grid to the average of the 8 outer squares (and, similarly as in classical pattern analysis, the size of the cell is increased step by step). The method provides a graph of dependence of variance on the size of the cell. The peak of the graph suggests the size of the aggregations (tussocks).

Logistic regression was used to estimate the dependence of the probability that two tillers belong to the same genotype ( $p_a$ ) on tillers distance. This relationship is a good characteristic of spatial pattern of genets in the population. It is analogous to the Simpson index of dominance. This index, when used for species data, can be interpreted as the probability that two randomly selected individuals belong to the same species. The curve obtained from the logistic regression is so spatial counterpart of Simpson index (used here for genotype instead of species data). The height of the peak at distance=0

characterizes the intensity of the clumping (high value means that in the smallest quadrat, most of tillers belong to the same genet), the "tail" shows, how far the individual genets are able to penetrate. Whereas the relationship could be estimated using distance for each pair as a predictor, and value 1 for two tillers of the same genotype and 0 for two tillers of different genotypes, the statistical significance of the logistic regression is inflated, because tiller pairs are not independent observations. Consequently, we have used the Mantel test (Mantel 1967; software Passage; Rosenberg 2001), and tested the null hypothesis that the distances between tillers and binary matrix of tiller genetic identity are independent.

Table 1: Basic characteristics of analysed plots: *No. Occup. 5x5* – number of occupied cells 5x5 cm in the whole 1x1m plot; *No.Occup.10x10* - number of occupied cells 10x10 cm in the whole 1x1m plot; *Ave. Shoot 5x5* – average number of shoots in 5 x5 cm occupied plots; *Total Gen.* - total number of genets, which were determined; *Ave. Gen. 5x5* – average number of genets in occupied and genetically analysed 5x5 cm plots; *Ave.Gen.10x10* average number of genets in genetically analysed 10x10 cm plots;  $p_a(0)$ - probability that the two tillers in the square 5x5 cm (distance 0) have the same genotype;  $p_a(50)$ - probability that the two tillers 50 cm apart have the same genotype.

Square No.	treatment	No. of tillers in 1 x1 m plots	No. Occup. 5x5	No. Occup. 10x10	Ave. Shoot 5x5	Total Gen.	Ave. Gen. 5x5	Ave. Gen. 10x10	$p_a(0)$	$p_a(50)$
1	mown	344	115	68	2.99	13	1.71	2.89	0.226	0.025
2	mown	603	189	88	3.19	15	1.14	2.00	0.577	0.007
3	unmown	753	144	72	5.23	16	1.4	2.30	0.753	0.000
4	unmown	653	159	72	4.11	10	1.15	1.78	0.630	0.003

**Results**

Three selected primers produced about 10 usable polymorph bands per square. This degree of polymorphism allowed us to determine 13.5 genotypes in each plot on average. The average number of shoots in 1x1 m square was about 588 and was higher in unmown plots (Table 1).

The 9TLQV analysis of spatial pattern of tillers showed variance peaks for three squares. Unmown plot no. 3 had the highest detected peak of variance (20.5) in distance 20 cm, which indicates the best tussock definition. The second highest peak (5.0) was detected for unmown plot no. 4, with the variance peak at distance 15 cm. Mown plot no. 1 showed peak of variance (4.15) in distance 20 cm. No aggregation was observed for mown plot no. 2 (Fig. 2). Mown plots had also smaller shoot density in occupied 5x5cm plots (Table 1).

Spatial pattern analysis of genetical individuals showed aggregation of genetical tillers. Probability  $p_a$ , that two tillers are of the same genotype decreased through

Table 2: Results of the Mantel test, where the matrix of distances between genotypes and the binary matrix of genotype similarity were correlated. Number of permutations=999.

Square No.	treatment	t	p
1	mown	3.63	0.001
2	mown	7.40	0.001
3	unmown	9.73	0.001
4	unmown	21.59	0.001

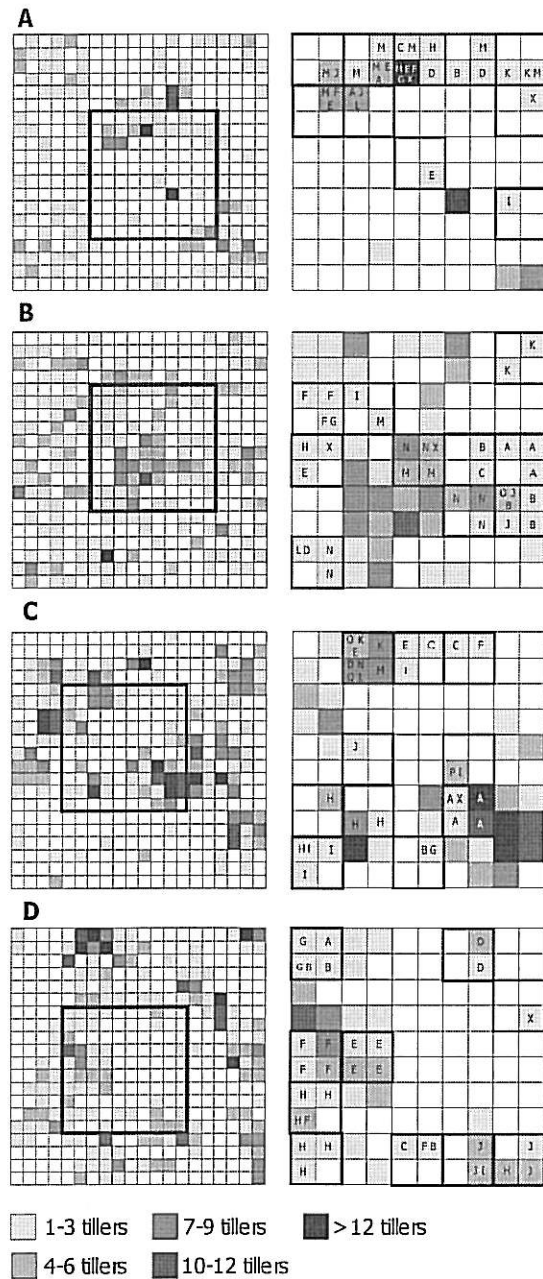


Figure 1: Spatial distribution of tillers of *Molinia caerulea* in squares 1 x 1 m plots (left) and occurrence of individual genotypes in ten 10 x 10 cm plots of sampling square 0.5 x 0.5 m squares (right), where each genotype of one 0.5 x 0.5 m square is indicated by unique letter. Letter X - non-determined genotype. The bold square in the pictures on the left indicate positions of 0.5 x 0.5 m squares, which are visualized on the right side. The bold squares in the right pictures indicates 10 x 10 cm plots where genetic analyses were performed. A-square 1 (mown), B-square 2 (mown), C-square 3 (unmown), D-square 4 (unmown).

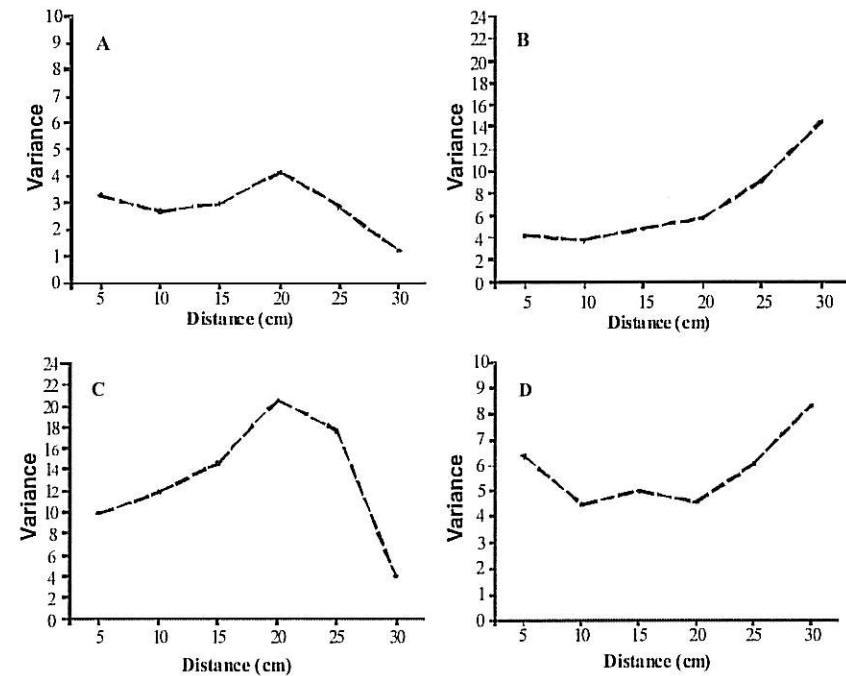


Figure 2: Results of 9TLQV analyses in each 1 x 1 m square. Peaks of variance indicate size of tussocks of *Molinia caerulea*. A-square 1 (mown), B-square 2 (mown), C-square 3 (unmown), D-square 4 (unmown).

distance in all plots significantly (Table 2, Fig. 3). The profile of  $p_a$  logistic curve for mown plots (compare to unmown plots) indicates higher vegetative intermingling, where the probability to be of the same genotype is lower for shorter distances and the probability of genotype to move into greater distances is higher (Fig. 3). Values of  $p_a$  for distance 0 cm (tillers in one 5x5 cm plot) and 50 cm are given in table 1. Nevertheless, as has been demonstrated for square 2, intermingling need not lead to increase of genet number in small microsites (5x5cm and 10x10 cm) when new microsites are occupied (compare numbers of occupied microsites with their average genet numbers Table 1 and see Fig 1).

**Discussion**

We found high genet diversity in a relative small area 0.5 x 0.5 m. This finding corresponds with results of other authors. Steinger et al. (1996) determined 15 genotypes of alpine *Carex curvula* in small 2.0 x 0.4 m sampling transect. Jonsson et al. (1996) showed high genet diversity for *Carex bigelowii* populations, where among 85-88 analysed ramets for one population 41-55 genets were recognised. The samples in this study have been taken every 4m along transects. Suzuki et al. (1999) estimated the



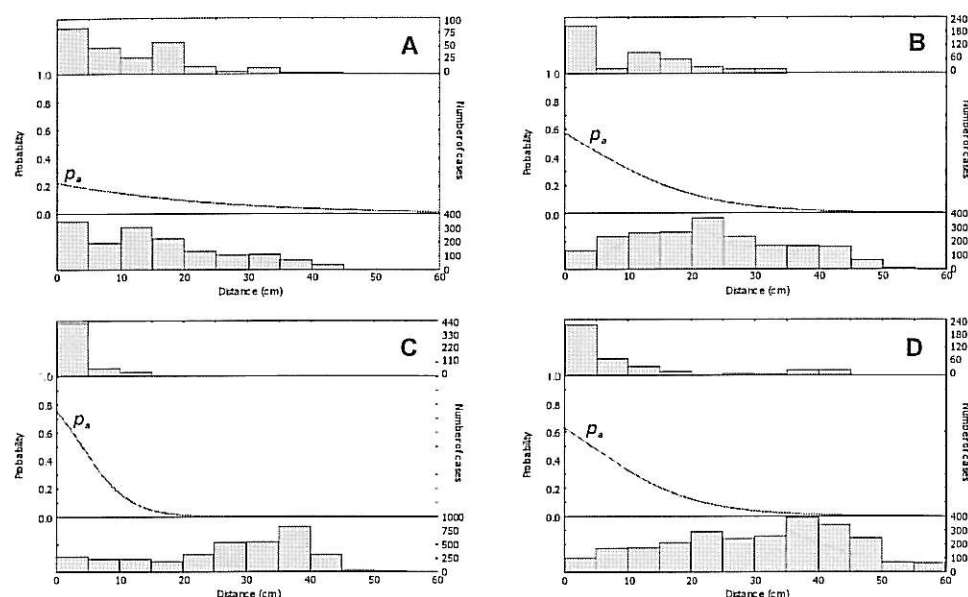


Figure 3: Dependence of probability ( $p_s$ ) that two tillers have the same genotype on their distance. Histograms show number of distances of the two tillers of the same genotype ( above) and number of distances of two tillers of different genotype ( below). A-square 1 (mown), B-square 2 (mown), C-square 3 (unmown), D-square 4 (unmown).

number of genets of *Festuca rubra* in transects 1m x 3.3cm at 18-53. Nevertheless, before acceptance of such surprising results we need to realise that there are important factors, which could affect these results. The first is that every data set includes some erroneous genotypes, which can be generated at every step through analysis (Bonin et al. 2004). Although these errors were not considered by population genetics they can considerably affect final conclusions (Bonin et al. 2004). The second factor, which influences assessment of genet number in clonal plant populations is possibility of somatic mutations (Pineda-Kirch and Lehtilä 2004). Whereas previous factors lead to over-estimation of genet number, the final number can be under-estimated due to impossibility to recognizing all genetic differences by used methods. From this point of view, it seems that comparisons based on the same molecular methods are more reliable than absolute estimates of genet number in a plot.

The total number of shoots has been lower on mown plots. This finding is in agreement with other studies that were focused on influence of mowing or grazing on *M. caerulea*. Milligan et al. (2004) tested effect of grazing, application of graminicide, cutting and *Calluna* brush addition. Only the cutting treatment had consistent effect on vegetation. Cutting increased proportion of bare ground, reduced vegetation height, increased species diversity and reduces *Molinia* cover. Likewise Grant et al. (1996) showed a negative effect of cutting on biomass and reserves of *M. caerulea*. Additionally

our study presents that negative effect is not demonstrated only by biomass or cover reduction, but also by decrease in shoot number.

For unmown plots the analysis of spatial shoot distribution indicated that tussock diameters at the base size are 15-20 cm. This result corresponds to maximal diameter 25 cm observed by Jefferies (1915) and 20 cm observed by Taylor et al. (2001). After all (according our observation) in long time unmown meadows can diameter reach up to 30 cm. However tussock structure becomes less obvious in mown plots. Several processes can cause this pattern: 1/ tussock can be disintegrated by the death of some shoots (or its clusters), what can be due to a rapid decrease of their reserves (Grant et al 1996; Klimeš & Klimešová 2002) 2/ possibility of horizontal spreading cause tussock binding and/or 3/ new plants from seedlings, fill the gaps. In addition mowing decreases microsite shoot density in 5x5 cm plots. This pattern where tussocks are less apparent and shoot microsite density decreases can permit coexistence of other species in both large and small scale (see data for studied plots in table 3, Lepš-unpublished data)

Table 3: Number of plant species in target plots.

Square No.	treatment	No. of species 10 x10 cm	No. of species 50 x50 cm
1	mown	7.4	36
2	mown	7.4	32
3	unmown	4.4	28
4	unmown	4.3	27

The analysis of genotypes did not show any clear influence of mowing on number of genotypes, indicating that

seedling recruitment was probably not more frequent in mown plots. We suggest that there is, in contrast to other species (Špačková & Lepš 2004), no effect of mowing on seedling recruitment of *M. caerulea* or that these differences can be manifested in longer time intervals only. This corresponds with finding of Grime (1981) that extremely low percentage of *M. caerulea* seeds germinate immediately. Moreover, Weiterová (2004) on the same study site found that the seeds of *M. caerulea* are practically missing in the seed bank. On the other hand Jefferies (1915) observed that *M. caerulea* produces many seeds, which colonize bare ground on moorland. This controversy indicates that more detailed studies on strategies of *M. caerulea* generative production are needed.

Due to 1/ absence of contrast between genet number on mown and unmown plots, and 2/ higher probability that two shoots have the same genotype in larger distances on the mown plots we suggest that mowing causes higher genet intermingling by simplifying of vegetative spreading of *M. caerulea*.

This study demonstrated the crucial importance of mowing for structural matrix, which is created by dominant tussock grass. This knowledge can help us understand not only the ecology of target dominant tussock grass but also the space in which subordinate species coexist.

However for generalisation of our conclusions much more or/and larger studies are needed. Although such projects are expensive and time consuming they can bring us relevant information on plant community function.

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## Chapter III

### ***Effects of litter, leaf cover and cover of basal internodes of dominant species *Molinia caerulea* on seedling recruitment and established vegetation***

Janeček Š & Lepš J  
Acta Oecologica [in revision]



## Effect of litter, leaf cover and cover of basal internodes of dominant species *Molinia caerulea* on seedling recruitment and established vegetation

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### Abstract

The effect of litter removal, leaf cover of established plants and cover of basal internodes of a dominant species *Molinia caerulea* on seedling germination and the dynamics of established plants were studied in a field experiment in an oligotrophic wet meadow.

Although the negative influence of litter on total seedling number and seedling species composition was non-significant, litter significantly affected the dynamics of the established vegetation and caused inhibition of total leaf cover development. The effects of total leaf cover of established plants on seedling establishment changed during the vegetation season. Whereas the effect of total leaf cover was positive at the start and in the middle of the vegetation season, at the end the total leaf cover negatively affected seedling establishment. Both total leaf cover and cover of basal internodes affected seedling composition. Effects of these two variables were statistically separable suggesting that they are based on different mechanisms. The response of seedling establishment to these factors was species specific and, consequently, our data support the hypothesis that that biotically generated spatial heterogeneity can promote species co-existence through the differentiation of species regeneration niches.

## Introduction

Seedling germination and establishment are often the most critical phases of plant life. The requirements for germination and establishment were defined as regeneration niche (Grubb, 1977). The main factors, which determine establishment success in meadow communities, can be divided into two groups. The first involves biotic factors such as litter production, leaf cover density and herbivory. The second group includes abiotic factors mainly soil composition, temperature, humidity and light intensity. These two groups are closely related and their effects are hardly separable without an experimental approach.

The effects of individual factors differ among plant communities and the results of studies are also affected by the methods used (Xiong and Nilsson 1999). In meadows, the influence of established vegetation on seedlings can be divided into effects of individual layers by roots, litter, moss and leaves. In meadows dominated by tussock grasses, the layer of tussock bases formed by individual tillers has often a specific effect.

Root competition has a negative effect on seedling establishment in grasslands (Hugland and Tawfig, 2001; McKendrick, 1996) and, similarly, the effect of litter on seedling recruitment seems to be negative (Špačková and Lepš, 2004; Jensen and Gutekunst, 2003; Xiong and Nilsson, 1999). Both positive (Keizer et al., 1985; Ryser, 1993) and negative (van Tooren, 1990; Špačková et al., 1998; Špačková and Lepš, 2004) effects of the moss layer on seedlings have been documented. The effects of leaf cover of established vegetation vary in similar ways. Ryser (1993) showed a positive effect of neighboring plants on conspecific seedlings thanks to protection against frost and/or drought. On the contrary, seedlings of *Reseda lutea* preferred micro-sites with low cover of established vegetation (Silvertown 1981). The established vegetation and litter can also serve as a suitable habitat for seed predators (Reader 1991). Although the effect of tussock bases can differ from the effect of leaf cover, these are usually not separated in the studies. One of the reasons is a strong spatial correlation between leaf and tussock base cover. Nevertheless, the tussock base layer has some specific characters different from the leaf layer, most notably the ability to mechanically exclude competitors (thanks to its density). On the other hand, it can provide a balanced microsite climate.

The highly specific conditions that enable seedling recruitment are often characterized as "safe site" (Harper 1967). However, safe site conditions might be species specific – conditions promoting recruitment of one species can be detrimental for another. This is one of the assumptions of Grubb's (1977) theory of maintenance of species diversity by differentiation of species regeneration niches.

The tussock base layer is well established in wet meadows dominated by *Molinia caerulea* or *Molinia arundinacea*. It is formed by the thick basal internodes of these species, which have a storage function (Taylor et al., 2001). In our study site, tussock bases of *M. caerulea* covered about 18 % of the meadow surface, being a very important determinant of vegetation structure.

Many studies pointed to a deficiency of data on year to year variation in seedling recruitment (recently Špačková and Lepš, 2004). However, data on seasonal dynamics

of seedling recruitment in meadows, and its relation to the dynamics of the established vegetation (primarily to leaf and tussock bases cover) are similarly needed (Ryser, 1993; Silvertown, 1981). In our experiment, we aimed to test the effects of litter on the seasonal dynamics of seedling recruitment and established vegetation. The second objective was to estimate and disentangle the effect of two aboveground layers, basal internodes and leaf cover, on seedling recruitment.

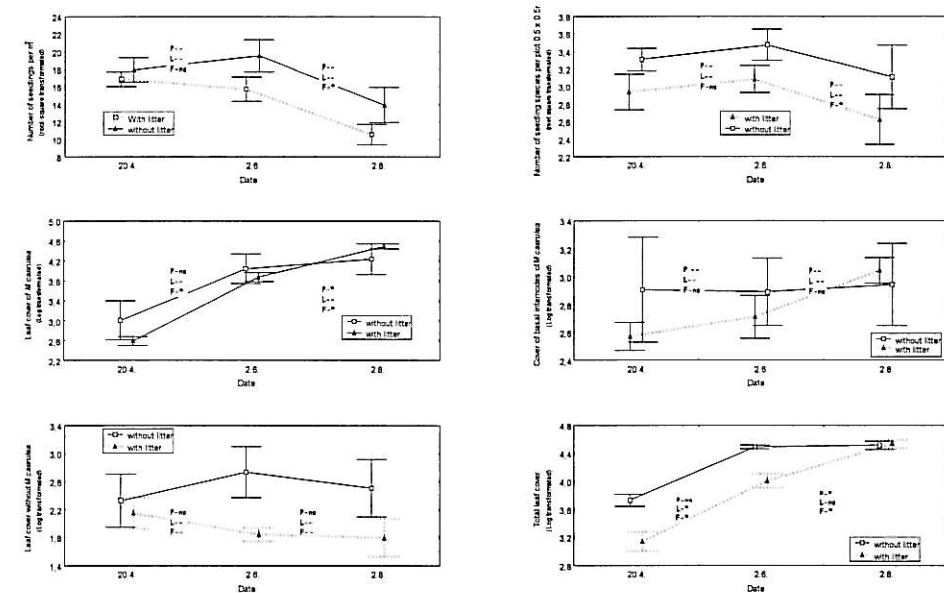


Figure 1. Effect of litter on univariate characteristics of seedlings and established vegetation. Results of the profile analysis are indicated for each time interval: P- parallelism; L-levels; F- fleetness; \* indicates  $p < 0.05$  (after Bonferroni adjustment); -- indicates that the test was not performed due to a nonsignificant result in the repeated measurement test (see Table II); ns-non significant. Means and SE (whiskers) are displayed.

## Methods

### Study site

The study plots were established on an oligotrophic, species-rich wet meadow 10 km southeast of České Budějovice, Czech Republic (48° 57' N; 14° 36' E; 510 m a.s.l.). The mean annual temperature ranges between 7 °C and 8 °C, and mean annual precipitation is 600-650 mm. The most dominant species is *Molinia caerulea*, which determines the structure of all of the community by its compact tussocks. It is late flowering (July, August) grass forming compact tussocks up to 30 cm in diameter, with flower stems up to 1.5 m tall. Plots were established in an abandoned part of the meadow, which had not been mown for several years before the start of the experiment, and where the dominance of



*Molinia* was higher than in the mown part, reaching cover over 90% at the end of vegetation season. Other common species in this part include grasses *Holcus lanatus*, *Deschampsia caespitosa*, *Festuca rubra*, graminoids *Juncus effusus*, *Carex hartmanii* and forbs *Cirsium palustre*, *Achillea ptarmica*, *Angelica sylvestris*. The amount of litter in the abandoned part of the meadow ranged from 400 to 700 g.m<sup>-2</sup> in the middle of vegetation season. There are several concurrent studies running in the locality (Špačková and Lepš 2004 and papers cited there), of them, Lepš (1999) provides detailed description of the community.

Table I. RDA analyses results performed on established vegetation data. Permutation (two lines for each test) – upper line: type of permutation on the whole plot level, lower line: type of permutation on split-plot level; % exp – percent of variability explained by all canonical axes; t1,t2,t3 – time identifiers coded as three dummy variables; Plot ID - plot identifiers coded as ten dummy variables. Nonstandardized/standardized - standardization by sample norm.

Explanatory variables	Covariables	Permutation	% exp.	F trace	P trace
<b>Nonstandardized</b>					
litter	t1,t2,t3	Freely, No	8.9	2.959	0.350
t1,t2,t3	Litter	No, Freely	12.9	2.144	0.002
t1*litter, t2*litter, t3*litter	t1,t2,t3, Plot ID	Freely, Freely	2.3	1.746	0.026
<b>Standardized</b>					
litter	t1,t2,t3	Freely, No	9.6	3.015	0.360
t1,t2,t3	Litter	No, Freely	7.3	1.137	0.002
t1*litter, t2*litter, t3*litter	t1,t2,t3, Plot ID	Freely, Freely	2.6	1.679	0.038

### Experimental design

Experimental plots were established in April 1999. Five experimental plots (1x1 m), where the litter was removed, and five control plots were arranged in a block of 2 x 5 plots. Removal plots regularly alternate with control plots. The data were recorded in 0.5 x 0.5 m plots, subdivided into 25 (10 x 10 cm) subplots, located in the centre of each 1m<sup>2</sup> plots. We visually estimated cover of individual species, cover of basal internodes of the dominant species *M. caerulea* and recorded the number of individual seedlings of all species in each 10 x 10 cm subplot three times throughout the vegetation season. Data collection took three days. For simplification, each sampling period was designated by the middle day (April 20<sup>th</sup>, June 2<sup>nd</sup>, August 2<sup>nd</sup>).

Table II. Influence of time and litter on univariate seedling and established plants characteristics, tested by repeated measures ANOVA. df - error degrees of freedom.

	litter			time			time*litter		
	df	F	p	df	F	p	df	F	p
Seedling number	8	1.978	0.197	16	43.142	0.000	16	2.566	0.108
Number of seedling species	8	3.150	0.114	16	25.029	0.000	16	1.128	0.348
Leaf cover of <i>M. caerulea</i>	8	0.119	0.739	16	244.236	0.000	16	10.665	0.001
Leaf cover of others species	8	2.019	0.193	16	0.6838	0.519	16	4.774	0.024
Total leaf cover	8	17.979	0.003	16	114.088	0.000	16	9.364	0.002
Cover of basal internodes of <i>M. caerulea</i>	8	0.1925	0.672	16	4.283	0.032	16	2.952	0.081

### Data analysis

Seedling composition and cover of adult plants were determined for the same plot three times, resulting in a repeated measures design (i.e. split-plot design with litter removal as the main plot factor and time as the within-plot factor). Changes in univariate characteristics (seedling number, number of seedling species, leaf cover of *Molinia caerulea*, leaf cover of others species, total leaf cover and basal cover of *M. caerulea*) were analysed by repeated measurements ANOVA. Profile analysis (Von Ende 2001) was used to test hypotheses about parallelism, levels and flatness between individual observation dates. Profile analysis is a type of post-hoc testing, tailored to the repeated measures design (similarly as multiple comparisons for one-way ANOVA). In the profile analysis, the levels test the differences in the average value over each tested period,

Table III. RDA analyses results performed on seedling data. Permutation (two lines for each test) – upper line: type of permutation on the whole plot level, lower line: type of permutation on split-plot level; % exp - percentage of variability explained by all canonical axes; t1,t2,t3 – time identifiers coded as three dummy variables; Plot ID - plot identifiers coded as ten dummy variables. Nonstandardized/standardized - standardization by sample norm.

Explanatory variables	Covariables	Permutation	% exp	F trace	P trace
<b>Nonstandardized</b>					
litter	t1,t2,t3	Freely, No	4.7	1.649	0.790
t1,t2,t3	litter	No, Freely	22	3.899	0.002
t1*litter, t2*litter, t3*litter	t1,t2,t3, Plot ID	Freely, Freely	1.8	0.856	0.630
<b>Standardized</b>					
litter	t1,t2,t3	Freely, No	3.4	1.051	0.950
t1,t2,t3	litter	No, Freely	11.6	1.769	0.002
t1*litter, t2*litter, t3*litter	t1,t2,t3, Plot ID	Freely, Freely	1.6	0.587	0.928

*Molinia* was higher than in the mown part, reaching cover over 90% at the end of vegetation season. Other common species in this part include grasses *Holcus lanatus*, *Deschampsia caespitosa*, *Festuca rubra*, graminoids *Juncus effusus*, *Carex hartmanii* and forbs *Cirsium palustre*, *Achillea ptarmica*, *Angelica sylvestris*. The amount of litter in the abandoned part of the meadow ranged from 400 to 700 g.m<sup>-2</sup> in the middle of vegetation season. There are several concurrent studies running in the locality (Špačková and Lepš 2004 and papers cited there), of them, Lepš (1999) provides detailed description of the community.

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Table III. RDA analyses results performed on seedling data. Permutation (two lines for each test) – upper line: type of permutation on the whole plot level, lower line: type of permutation on split-plot level; % exp - percentage of variability explained by all canonical axes; t1,t2,t3 – time identifiers coded as three dummy variables; Plot ID - plot identifiers coded as ten dummy variables. Nonstandardized/standardized - standardization by sample norm.

Explanatory variables	Covariables	Permutation	% exp	F trace	P trace
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t1,t2,t3	litter	No, Freely	22	3.899	<b>0.002</b>
t1*litter, t2*litter, t3*litter	t1,t2,t3, Plot ID	Freely, Freely	1.8	0.856	0.630
<b>Standardized</b>					
litter	t1,t2,t3	Freely, No	3.4	1.051	0.950
t1,t2,t3	litter	No, Freely	11.6	1.769	<b>0.002</b>
t1*litter, t2*litter, t3*litter	t1,t2,t3, Plot ID	Freely, Freely	1.6	0.587	0.928



flatness tests whether there is any change during the tested interval, and parallelism tests whether the changes over the interval are the same in all the groups (interaction between time and the main plot factor, tested separately for each interval). Within - subject repeated measures data were transformed for parallelism and flatness analyses to a set of contrasts (i.e. differences between two subsequent measurements) and were tested by t-tests. To test the hypotheses about levels (test of main effect), values of two adjacent dates were averaged and then the means were compared between groups (for details see Von Ende, 2001). Because two t-tests were established between three observation dates in profile analysis the Bonferroni adjustment of  $\alpha=0.025$  was used in each individual test to maintain an overall  $\alpha=0.05$ . Influences of total leaf cover of adult plants and cover of basal internodes of *M. caerulea* on seedling number in small 10 x 10 cm plots were tested by general linear models. For both, repeated measures and general linear models, data on count and percentage were square-root and log-transformed respectively to improve normality and homoscedasticity.

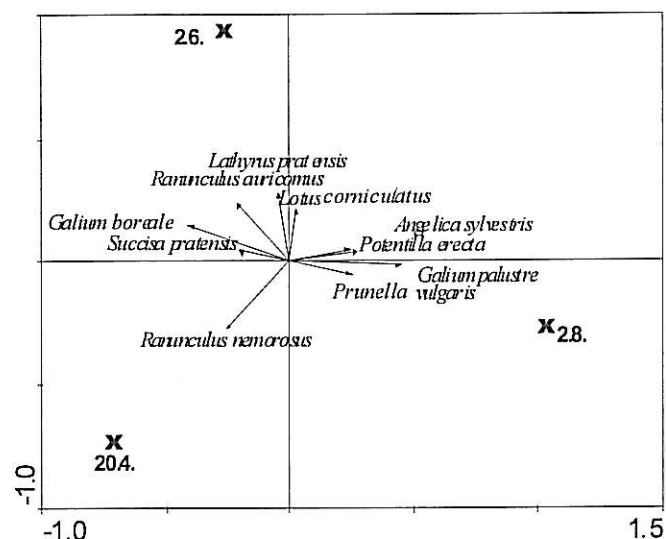


Figure 2. Changes in species composition of seedlings on individual sampling dates. Results of standardized RDA, with time (coded as three dummy variables) as environmental variables, and litter presence as a covariable. Differences were highly significant ( $F=1.769$ ,  $P=0.002$ )

RDA (redundancy analysis, constrained ordination method based on linear species response, Lepš and Šmilauer, 2003) was used to analyze the multivariate data because the species composition of both established plants and seedlings in the plots was rather homogenous, litter as the main factor was categorical and the response of seedlings to the continuous predictors (cover of *M. caerulea*, total leaf cover) was expected to be

linear. Plot identifiers (coded as dummy variables) were used as covariates, when the influence of litter on time changes in plant and seedling compositions was tested. Time was considered categorical variable (we created three dummy variables for three observation dates). Data in the whole 50x50 cm plots were used for those analyses. Split-plot permutation tests were used where split plots were the individual observations and the whole plot was the 50x50cm plot. RDA was conducted with and without standardization by sample norm. RDA without standardisation includes both the effects of total seedling number and seedling community composition, RDA with standardization takes into account the species proportions only.

Further testing of the influence of total leaf cover and cover of basal internodes of *M. caerulea* was performed by RDA analysis. Microsites data (10x10 plots) were used to separate the effect of *M. caerulea* internode cover and total leaf cover. Species data in all RDA analyses were  $\log(x+1)$  transformed. Analyses were performed using the CANOCO software (ter Braak and Šmilauer, 1998).

## Results

### Effect of litter on seasonal development of established vegetation

Both standardized and nonstandardized RDA analyses of cover values of all the species showed a significant effect of litter on established vegetation development (significant time by litter interactions, table I). Both the development of total leaf cover (table II) and the development of species composition were significantly affected by litter. Development of total leaf cover on plots without litter was faster and reached on average 88 % in June (in contrast to plots with litter where the total cover was only 55%). In August, leaf cover was approximately equal in both groups (slightly above 90%, figure 1.). Interestingly, the response of leaf cover development for the dominant species *M. caerulea* differed from that of other plants. Litter affected negatively leaf cover of other species, but leaf cover of *M. caerulea* increased during the vegetation season and on August 2<sup>nd</sup> it was slightly higher in plots with litter present (figure 1; table II). Cover of basal internodes increased throughout the vegetation season. This increase was not caused by development of new basal internodes but by basal internode thickening (Janeček-unpublished data).

Table IV. Results of the GLM analysis, testing the effects of time, total leaf cover (TLC), cover of basal internodes of *M. caerulea* (CBI) and interactions of TLC and CBI with time on the number of seedlings in 10 x 10 cm plots. Plot identifiers were used as a random factor, all the tested effects were fixed. Error df=741.

	d.f. (effect)	F	p
time	2	4.374	0.013
TLC	1	4.670	0.031
CBI	1	3.754	0.053
Time* TLC	2	5.321	0.005
time*CBI	2	1.426	0.241

Table V. RDA analyses results performed on seedling data, testing the effect of total leaf cover (TLC), cover of basal internodes of *M. caerulea* (CBI) and time. Permutation (two lines for each test) – upper line: type of permutation on the whole plot level, lower line: type of permutation on split-plot level; Def. by cov. – permutation in blocks defined by covariables, % exp – percent of explained variability by all canonical axes. t1,t2,t3 – time identifiers coded as three dummy variables; Plot ID – plot identifiers coded as ten dummy variables. Nonstandardized/standardized – standardization by sample norm.

Explanatory variables	Covariables	Permutation	% exp	F trace	P trace
<b>Nonstandardized</b>					
TLC, CBI	t1,t2,t3 Plot ID	Freely, No	4.8	23.757	0.002
TLC*t1, TLC*t2, TLC*t3,CBI*t1, CBI*t2, CBI*t3	Plot ID, TLC, CBI, t1, t2, t3	Def. by cov.	0.9	2.343	0.012
<b>Standardized</b>					
TLC, CBI	t1,t2,t3 Plot ID	Freely, No	3.7	16.802	0.002
TLC*t1, TLC*t2, TLC*t3,CBI*t1, CBI*t2, CBI*t3	Plot ID, TLC, CBI, t1, t2, t3	Def. by cov.	0.9	2.148	0.004

#### Effect of litter on the development of the seedling community through the vegetation season

Seedling number, number of seedling species and seedling composition changed during the vegetation season (figures 1,2; tables II, III). The numbers of seedlings and seedling species were lower in plots with litter, but this effect was not significant. Maximum seedling number per m<sup>2</sup> (395) was recorded in June in plots without litter. On the contrary minimum seedling number per m<sup>2</sup> (117) was recorded at the end of the vegetation season in August in plots with litter. A similar development was observed in number of seedlings species, with the maximum species number per plot (12) recorded in June in plots without litter and the minimum (7.2) in August in plots with litter (figure 1).

#### Effect of total leaf cover and cover of basal internodes of *M. caerulea* on seedlings

The effect of total leaf cover of established vegetation (TLC) on seedling number changed during the season (significant time x TLC interaction, table IV). Whereas in April and June, the effect was positive, the effect was negative in August. Seedling species composition was affected significantly by both leaf cover and cover of *M. caerulea* basal internodes (CBI) in the microsites (10 x 10 cm) and this effect changed through the vegetation season (table V). TLC and CBI explained different parts of the variability at the

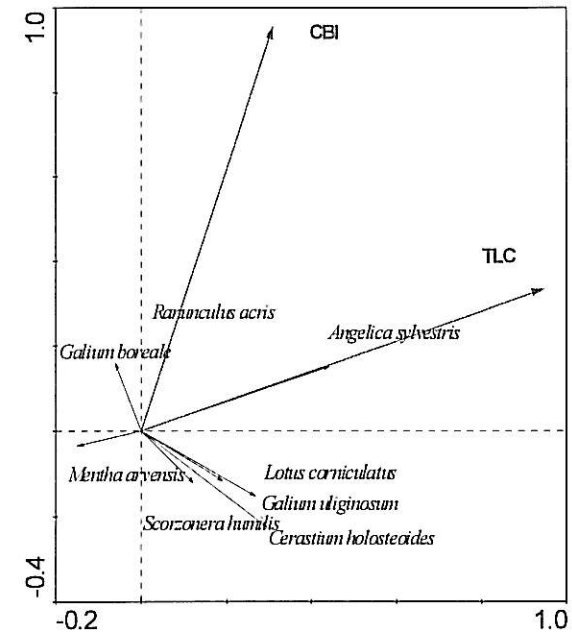


Figure 3. Results of non-standardized RDA analysis. Relation of seedling species to total leaf cover (TLC) and cover of basal internodes of *M. caerulea*(CBI) for the April 20<sup>th</sup> sample.

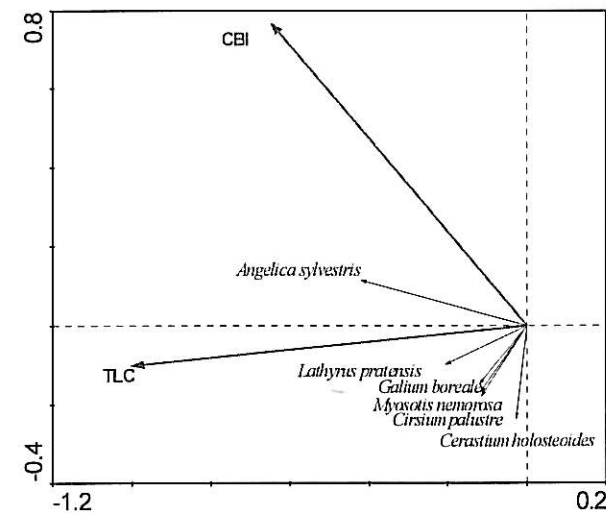


Figure 4. Results of non-standardized RDA analysis. Relation of seedling species to total leaf cover (TLC) and cover of basal internodes of *M. caerulea*(CBI) for the June 2<sup>nd</sup> sample.



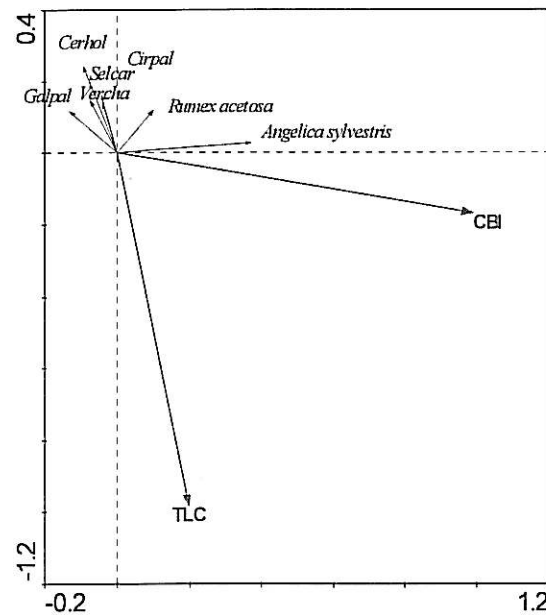


Figure 5. Results of non-standardized RDA analysis. Relation of seedling species to total leaf cover (TLC) and cover of basal internodes of *M. caerulea* (CBI) for the August 2<sup>nd</sup> sample. Abbreviations of seedling species names: Cirpal- *Cirsium palustre*; Selcar- *Selinum carvifolia*; Vercha- *Veronica chamaedris*; Galpal- *Galium palustre*; Cerhol- *Cerastium holosteoides*.

start and end of the vegetation season (table VI). Part of the explained variability was shared between CBI and TLC only in the middle of the vegetation season (June) (figures 3-5, table VI). The explanatory power of TLC decreased while that of CBI increased during the season (figures 3-5, table VI). Relationships between CBI, TLC and individual seedling species also changed during the vegetation season (figures 3-5). Increasingly seedlings of most species were not found in plots with high TLC. This effect was probably caused by increasing total leaf cover. Only seedlings of *Angelica sylvestris* were positively correlated with TLC and even with CBI at the end of vegetation season in August. The highly significant results of the standardized RDA (table V) confirmed that different species preferred different microsites for their species recruitment.

### Discussion

Špačková and Lepš (2004) on the same study site did not find any effect of litter removal on established vegetation over a four year experiment. In contrast, we observed the effect of litter on changes in plant composition in a single vegetation season. Litter

Table VI. Results of non-standardized RDA analyses. Separation of total leaf cover (TLC) and cover of basal internodes of *M. caerulea* (CBI) effects on seedling data for individual dates. Note that only in June the effect of both (i.e. CBI, TLC) is considerably higher than both the partial effects, suggesting shared effect of the two explanatory variables; at the beginning and end of the season, the two effects seem to be independent.

Explanatory variables	Covariable	Explained variability (%)	F	P
<i>April 20th</i>				
TLC	CBI	6.6	17.799	0.002
CBI	TLC	2.6	7.149	0.002
CBI, TLC		9.1	12.365	0.002
<i>June 2nd</i>				
TLC	CBI	4.6	12.475	0.002
CBI	TLC	2.7	7.284	0.002
CBI, TLC		8.7	11.716	0.002
<i>August 2nd</i>				
TLC	CBI	0.8	2.056	0.044
CBI	TLC	4.7	12.388	0.002
CBI, TLC		5.6	7.284	0.002

also affected development of total leaf cover, leaf cover of *M. caerulea* and leaf cover of other species. Moreover we found that, in contrast to other species, *M. caerulea* was able to increase its leaf cover faster in the second half of the vegetation season on plots with litter and so compensate for thinner leaf cover at the start of the vegetation season on these plots. Unlike Špačková and Lepš (2004), we used an unmown part of the meadow, which has both a higher amount of litter and also a higher representation of *M. caerulea*. This comparison suggests that, in unmown meadows with high dominance of *M. caerulea*, litter can inhibit the development of a mature plant community throughout a vegetation season.

In contrast to mature vegetation, there was no significant effect of litter on seedlings. This result is in contrast to other studies (Bosy and Reader, 1995; Facelli and Facelli, 1993; Fowler, 1988) which have shown a negative effect of litter on seedling establishment. Špačková and Lepš (2004) found in the same study site (but on the mown part) only a shared negative effect of moss and litter on seedling recruitment. The absence of a significant effect on seedling recruitment can be also caused by the small number of replications; litter removal was the main plot factor, consequently with a low error degrees of freedom. Accordingly, in all of the cases, removal increased (albeit non-significantly) seedling recruitment. In contrast, the effects of foliage and basal internode cover were based on their variation in split plots, which provided a stronger test.

The effect by leaf cover of established vegetation on seedlings varied throughout the vegetation season. During spring and early summer, leaf cover was positively correlated with seedling numbers (suggesting a protection against desiccation), but in late summer, the correlation was negative, probably due to an increasing effect of competition for light. Other studies revealed both negative and positive effects. The negative relation of *Reseda lutea* seedlings to micro sites of dense leaf cover was demonstrated by Silvertown (1981). On the other hand the positive effect of vegetation shelter on *Primula veris* and *Arabis hirsuta* seedlings was described by Ryser (1993). Likewise Flower (1988) showed that safe sites for seedling survival can be near neighboring plants.

Variation partitioning in seedling data sets showed that it is possible to separate the effect of leaf cover from that of the basal internode layer of *M. caerulea*. The ability of leaf cover to explain the variability in the seedling data decreased as the cover on the meadow became more closed. Concurrently, the explanatory power of *M. caerulea* basal internode cover increased. Whereas dense leaf cover had a negative effect on the seedlings at the end of the vegetation season, tufts of *M. caerulea*, represented by cover of basal internodes provided suitable conditions for seedlings of some species (especially *Angelica sylvestris*). Therefore basal internode layer created a specific microhabitat for seedling recruitment. The central part of a tuft is formed by a dense layer of basal internodes and is raised above the surface. However, the densest cover of leaves is on the circumference of the tuft. This part of the tuft is probably also influenced the most by *M. caerulea* litter. The fact that each of *Molinia* litter, leaf cover and cover of basal internodes affects the seedling germination differently, enables many possible combinations of their joint effects. This suggests potential for differentiation of microsites. These factors in combination with large interannual variation of weather and corresponding variation of seedling recruitment (Špačková and Lepš 2004) can support the species coexistence through the regeneration niches differentiation (Grubb 1977).

These results correspond to the findings of Kupferschmid et al. (2000), who showed that tussocks of *M. arundinacea* created unfavourable microsites for germination of *Arabis hirsuta*, but they supported seedling survival. Analogous to our results for *Angelica sylvestris*, Kupferschmid et al. (2000) described better germination of another umbellifer *Daucus carota* on tussocks of *Molinia arundinacea* in abandoned plots. Likewise Clarke & Davison (2001) did not find any obvious effect of grass tussocks removal on seedling emergence and survival.

In agreement with many previous studies (Jensen and Gutekunst, 2003; Bullock et al., 1995; Pons & Toorn, 1988; Gross, 1984; Grime et al., 1981) our study showed that "safe site" is highly species specific. It supports the idea that biotically generated spatial heterogeneity can promote species coexistence through the differentiation of species regeneration niches.

#### Acknowledgements

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## Chapter IV

### ***Influence of soil heterogeneity and competition on growth feature of three meadow species***

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## Influence of soil heterogeneity and competition on growth features of three meadow species

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### Summary

The effect of competition and artificially created soil heterogeneity was studied in a pot experiment. A tussock grass, *Holcus lanatus* and rhizomatous sedge *Carex hartmanii* were grown alone and in combination with another tussock grass, *Molinia caerulea*, in homogeneous and heterogeneous soil treatments. The heterogeneous treatment consisted of four compartments, two nutrient rich and two nutrient poor, in the homogenous treatment total nutrient content was the same as in the heterogeneous treatment. Soil heterogeneity increased *M. caerulea* total production, and increased *C. hartmanii* root:shoot ratio; no effect on *H. lanatus* was observed. Both *M. caerulea* and *C. hartmanii* were able to place their underground organs preferentially into nutrient rich patches. *M. caerulea* and *H. lanatus* total biomass was lower in the presence of the competitor; *C. hartmanii* responded to competition only by increased allocation to rhizoms. *M. caerulea* was more affected by competition in the heterogeneous environment.



## Introduction

Soil heterogeneity affects both growth and competition processes in plant communities. The existence of nutrient-rich patches in space and time has been described in several studies (e.g., Jackson & Caldwell 1993; Ryel et al. 1996; Cain et al. 1999), as well as spatiotemporal variability for non-nutrient soil parameters (Jackson & Caldwell 1993). Individual plant species differently take advantage of soil heterogeneity by placing their organs into preferable places (Birch & Hutchings 1994; de Kroon & Hutchings 1995; Humphrey & Pyke 1997; Fransen et al. 1998; Cahill & Casper 1999; Einsmann et al. 1999; Kleijn & Groenendael 1999; Fitter et al. 2000; Wijesinghe & Whigham 2001). Differences in plant response to soil patches depend on different plant requirements (i.e., nutrient, water, O<sub>2</sub> and pH requirements) and on the ability of plant species to find and exploit soil resources (Veresoglou & Fitter 1984; Gross et al. 1993) or on the ability for retranslocation of nutrients in stoloniferous plants (Dong et al. 2002). Furthermore, foraging speed is an important factor because patches are dynamic. (El-Shatnawi & Makhadmeh 2001; Pickett et al. 2000). Plants may effectively exploit nutrients by both physiological and morphological root plasticity. Derner & Briske (1999) suggested that there is no tradeoff between these two mechanisms and that they may represent complementary, rather than alternative, foraging strategies.

Experiments including cespitose grasses and other "phalanx" species (for phalanx-guerrilla theory see Lovett Doust 1981) demonstrated a foraging ability of this growth form (e.g. Fransen et al. 1999, 2001; Wijesinghe et al. 2001) and showed that root systems of phalanx plants are able to search for favorable soil-patches. After nutrient depletion below a tussock, the phalanx underground strategy, in the sense of overproducing biomass to obtain competition superiority, does not have any advantage: the roots need to explore new nutrient resources, outside the depleted zone. This is especially important for K and P ions, which diffuse slowly in soil compared with rates at which roots and microbes can absorb them (Robinson 1994). De Kroon & Hutchings (1995) reformulated foraging concepts and recommended incorporation of the foraging behavior of shoots and roots into studies of foraging strategies of clonal plants.

Although many experiments have investigated foraging of numerous plant species in heterogeneous environments, the overwhelming majority of these studies did not include the competition effect. The few studies dealing with the effect of soil heterogeneity on competition suggest that different foraging behaviors in heterogeneous soil environments can separate plant niches and change the relative competitive ability of individual plant species (Shwinning & Weiner 1998; Cahill & Casper 1999; Fransen et al. 2001). Weiner et al. (1997) suggested that soil heterogeneity can increase competition asymmetry between large and small plants due to the ability of large plants to reach and usurp nutrient rich patches. This hypothesis was experimentally supported by de Fransen et al. (2001). Alternatively, Campbell et al. (1991) proposed that dominant species with large root systems tend to be less selective in placing their roots into nutrient rich patches

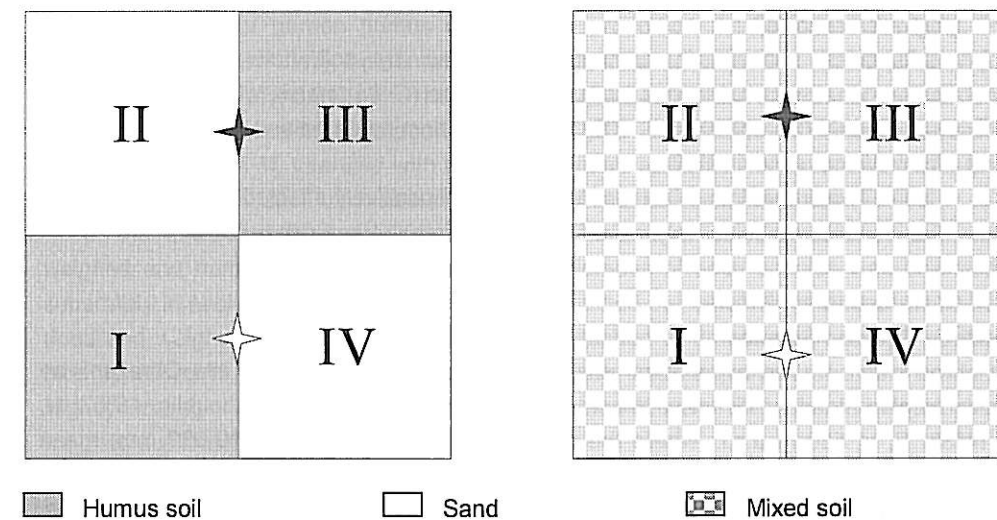


Fig. 1. The experimental design. Both heterogeneous (left) and homogeneous (right) treatments were performed in square 19 x 19 cm pots. I, II, III, IV-sector numbers. +The place, where one growth unit of *M. caerulea* in plant combinations 1,4 and 5 was planted. +The place, where one growth unit of *H. lanatus* (combination 3 and 5) or one growth unit of *C. hartmanii* (combination 2 and 4) was planted (for plant combination see Table 1). Note, that for the monospecific combinations 1, 2 and 3, one of the positions remained empty.

than species with smaller root systems and described the dominant species as "high scale" foragers and the subordinate species as "high precision" foragers. According to this hypothesis there is a tradeoff between scale (size of root system) and precision of root allocation into nutrient rich patches. This hypothesis was recently supported by Wijesinghe et al. (2001). However, all of these authors point to the insufficient number of experimental studies that deal with heterogeneity effects on competitive ability of individual plant species.

In our experiment, two species with different growth strategies (*Carex hartmanii* Cajand. and *Holcus lanatus* L.) were grown alone and with *Molinia caerulea* (L.) Moench as a competitor, both in homogeneous and heterogeneous soil, where the overall nutrient content was retained. All three species coexist in natural conditions in a wet oligotrophic meadow near České Budějovice (Czech republic). There, *M. caerulea* is dominant, forming a matrix of dense tussocks. Consequently it is the main competitor of the other species. *C. hartmanii* is a typical guerrilla species, forming long underground rhizomes, and *H. lanatus* is a tussock grass. This study complements the field experiments carried at the locality (Lepš 1999 and references therein). Lepš (1999) suggested that species similar to *M. caerulea* take more advantage of *M. caerulea* removal than do dissimilar ones. This would confirm the idea that niche differentiation (i.e., differences in growth form) leads to reduced competition.

We aimed to answer the following questions:

1. Are plants able to place their roots preferentially into nutrient rich patches and does soil heterogeneity lead to increased plant biomass? Does the ability to do this differ between species?
2. Is species success in competition affected by soil heterogeneity? Is soil heterogeneity advantageous for guerrilla species (*C. hartmanii*), where high precision is expected (Campbell et al. 1991), or for the dominant phalanx species (as follows from Weiner et al. 1997)?
3. Is the investment into rhizomes in *C. hartmanii* positively affected by competition? Is the plant able to selectively place its rhizomes into nutrient-rich patches?

## Materials and methods

### Study Site and Species

The plants for the experiment were collected at our experimental site Ohrazení, an oligotrophic meadow 10 km southeast of České Budějovice, Czech Republic, 48°57' N, 14°38' E, at 530 m a.s.l., where the mean annual precipitation is 600-650 mm, mean annual temperature is 7.8 °C. *M. caerulea* is the dominant species, and *H. lanatus* and *C. hartmanii* are the major species at the locality.

Individual growth units of *M. caerulea*, *C. hartmanii* and *H. lanatus* were randomly selected from different plant individuals on a study field, where the species coexist in nature. *M. caerulea* and *H. lanatus* (*Poaceae*) are perennial non-rhizomatous tufted grasses. *M. caerulea* is dominant in several vegetation types (Taylor et al. 2001), including the vegetation of our study site, where it reaches up to 50% cover, and in samples taken in June 2000 constituted up to 30 % of biomass. *Carex hartmanii* is a perennial rhizomatous sedge, penetrating vegetation by long rhizomes (Dostál 1989); in our locality, the plant is able to form rhizomes over 0.5 m long. *H. lanatus* cover increased in our study site after *M. caerulea* had been experimentally removed, but the reaction of *C. hartmanii* was negligible (Lepš 1999 and unpublished data).

### Experimental design

Randomly selected growth units of *M. caerulea*, *C. hartmanii* and *H. lanatus* from different individual plants were transplanted into plastic pots (19 cm x19 cm, 15 cm deep) at the end of April 2001 (*H. lanatus* at early May), and placed in a greenhouse (Fig. 1). The non-competitive pots contained one unit, the competitive two units, each belonging to different species. This corresponds to the simplified target-neighbor design (Gibson et al. 1999; Goldberg & Landa 1991). The transplanted units consisted of two interconnected basal internodes in *M. caerulea*, of two interconnected shoots of *C. hartmanii*, or one shoot of *H. lanatus*. Weight of each individual unit including its roots was estimated before planting using calibration (to avoid cleaning of roots of tillers to be planted). Calibration was based on the regression of weight on easily nondestructively measurable characters (shoot height in *C. hartmanii* and *H. lanatus*, height of basal internodes in *M. caerulea*) in an extra "training sets" of growth units of individual species.

Pots were divided into two soil treatments, heterogeneous and homogeneous. The overall nutrient content in both treatments was retained. In the heterogeneous treatment, each pot was divided into four patches (sectors) of two types: the nutrient-rich patches contained a mix of garden humus and peat in proportion 2:1 (N total=12.5 g/kg, P total=120 mg/kg, pH=5.47), nutrient-poor patches contained only sand (N total<0.2 g/kg, P total<6 mg/kg, pH=6.04). The homogeneous treatment was composed of garden humus, peat and sand in the proportion 2:1:3 (Fig.1). Five species combinations (each species alone, and *M. caerulea* with each of the other species) were combined with the two soil-treatments. Each of combinations was replicated at least five times (Table 1).

In the middle of August 2001 aboveground biomass was harvested, dried and weighed and number of shoots recorded; root biomass of each species was collected separately in each sector, remnants of soil was washed out carefully, and the roots were dried and weighed. The roots were mostly still attached to the aboveground parts. The unattached roots were identified according to their color and structure, which differs slightly among the species. Rhizomes of *C. hartmanii* were also counted, dried and weighed separately in each sector.

### Data analysis

In all the analyses, we used the weight of tillers before planting as a covariate (to account for the possible variability caused by unequal size of tillers at the beginning of the experiment). Although the effect of the covariate was not significant in all the analyses, in some cases, dropping of the covariate lead to decrease of the significance in the main effect (clearly because the covariate was able to account for part of the variability in the response, and in this way to decrease the unexplained variation). Because of this, and because the effect of covariates could be expected a priori, we decided to keep the covariates in all the models, regardless whether they were significant or not.

Table 1. Number of pots analyzed in individual treatments and plant combinations. M - *M. caerulea*, C - *C. hartmanii*, H - *H. lanatus*.

Combination	Plant species	Heterogeneous treatment	Homogeneous treatment
1	M	8	8
2	C	7	8
3	H	5	5
4	M*C	7	8
5	M*H	8	8

We first analyzed the characteristics of each species. We used Analysis of Covariance (using STATISTICA 5.5, Anon 1996) to test the effect of heterogeneity and presence of competitor on total biomass, R:S ratio, and number of shoots.

Biomass allocation into individual organs (roots, shoots and rhizomes) of *C. hartmanii* was analyzed by MANCOVA (STATISTICA, Anon 1996). Heterogeneity, presence of competitor and interaction were used as predictors and percentage allocation into individual organs as dependent variables. ANCOVA was used to test differences in allocation to different plant organs.



We then analyzed the selective placement of underground organs into differing sectors of the heterogeneous treatment. This was analyzed by ANCOVA with split-plot design, where humus-rich soil versus sand placement was used as a within plot factor.

Data on biomass placement and number of shoots or rhizomes were log-transformed and square-root transformed respectively to improve normality and homoscedasticity.

## Results

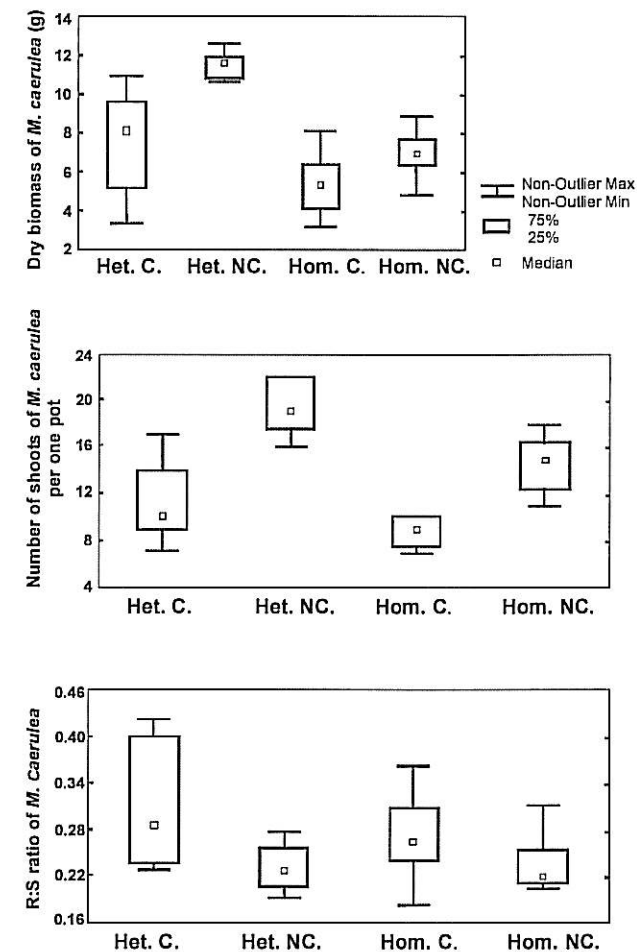
### Response of individual plant species to the heterogeneity and competition

At the end of our experiment plant canopies in the competition environment exhibited little overlap. Hence, we can reasonably assume that competition was predominantly underground.

Total biomass production and number of shoots of *M. caerulea* was higher in the heterogeneous soil environment (Table 2). Both total biomass and number of shoots of *M. caerulea* were negatively affected by competition with *C. hartmanii*. Competition effect of *C. hartmanii* on total biomass of *M. caerulea* was higher in heterogeneous environment

**Table 2.** Effect of heterogeneity, presence of competitor and their interaction on biomass, root:shoot ratio (R:S) and number of shoots of the species *M. caerulea*, *C. hartmanii* and *H. lanatus* tested by ANCOVA. Symbol \* indicates significance level  $0.05 < p < 0.1$ . The data on number of shoots was square-root transformed prior to analysis. Initial growth unit weight was used as a covariate. Superscript + at the significance value means positive effect of heterogeneity or competitor on the variable under consideration, superscript - means negative effect. For replication number see Tab. 1.

Species (competitor)	Dependent variables	Predictors					
		Heterogeneity		Competitor		Interaction	
		p	F	p	F	p	F
<i>M. caerulea</i> ( <i>C. hartmanii</i> )	Total biomass	0.0001 <sup>+</sup>	19.8759	0.0001 <sup>-</sup>	20.8338	0.0237	5.7777
	R : S ratio	n.s.	0.6545	0.0109 <sup>+</sup>	7.5116	n.s.	1.0347
	Number of shoots	0.0332 <sup>+</sup>	5.0607	0.0000 <sup>-</sup>	29.3354	n.s.	2.2371
<i>(H. lanatus)</i>	Total biomass	0.0016 <sup>+</sup>	12.3352	n.s.	0.1261	n.s.	0.7405
	R : S ratio	n.s.	0.0662	n.s.	0.6442	n.s.	0.0007
	Number of shoots	0.0007 <sup>+</sup>	14.6838	n.s.	0.3662	n.s.	0.0343
<i>C. hartmanii</i> ( <i>M. caerulea</i> )	Total biomass	n.s.	1.6835	n.s.	0.3515	n.s.	0.5412
	R : S ratio	0.0093 <sup>+</sup>	7.2852	n.s.	1.8519	n.s.	0.5159
	Number of shoots	n.s.	0.0931	n.s.	1.7419	n.s.	1.5871
<i>H. lanatus</i> ( <i>M. caerulea</i> )	Total biomass	n.s.	0.7985	0.0413 <sup>-</sup>	4.7252	n.s.	2.3468
	R : S ratio	n.s.	0.9134	n.s.*	3.3540	n.s.	0.0381
	Number of shoots	n.s.	0.0025	0.0029 <sup>-</sup>	11.3357	n.s.	1.2777



**Fig. 2.** Effect of soil treatment and presence of *C. hartmanii* as a competitor on total biomass, number of shoot and R:S ratio of *M. caerulea*. Het. - heterogeneous soil treatment, Hom.- homogeneous soil treatment, C -competition environment NC - non-competition environment. Statistical analyses are given in Table 2.

and R:S ratio of *M. caerulea* was positively affected by the presence of *C. hartmanii* as a competitor (Table 2, Fig. 2). There was no significant effect of *H. lanatus* on *M. caerulea* (Table 2, the non-significant results are not portrayed in Figures).

There was no effect of either soil heterogeneity or competition (of *M. caerulea*) on the total biomass production and number of shoots of *C. hartmanii* (Table 2). However, both heterogeneity (MANCOVA, Wilk's  $F=0.74$ ,  $p=0.029$ ) and competition (MANCOVA, Wilk's  $F=0.68$ ,  $p=0.009$ ) affected *C. hartmanii* biomass allocation into shoots, rhizomes and roots; their interaction was not significant (MANCOVA, Wilk's  $F=0.95$ ,  $p=0.563$ ). Biomass allocation to the rhizomes was positively affected by the presence of the competitor (ANCOVA,  $F=5.94$ ,  $p=0.022$ ). Allocation of biomass to the roots was higher in

heterogeneous treatment (ANCOVA,  $F=5.49$ ,  $p=0.027$ ), whereas allocation to the shoots was higher in homogeneous treatment (ANCOVA,  $F=9.46$ ,  $p=0.005$ ) and in the non-competitive environment (ANCOVA,  $F=5.23$ ,  $p=0.031$ ). This results correspond to significant effect of heterogeneity on R:S ratio of *C. hartmanii* (Table 2, Fig. 3).

*M. caerulea* competition negatively affected the number of shoots and total biomass of *H. lanatus* (Fig. 4) and the increase in the R:S ratio of *H. lanatus* in the presence of *M. caerulea* was only marginally significant (Table 2). There was no significant effect of heterogeneity on either total biomass or biomass allocation of *H. lanatus* (Table 2).

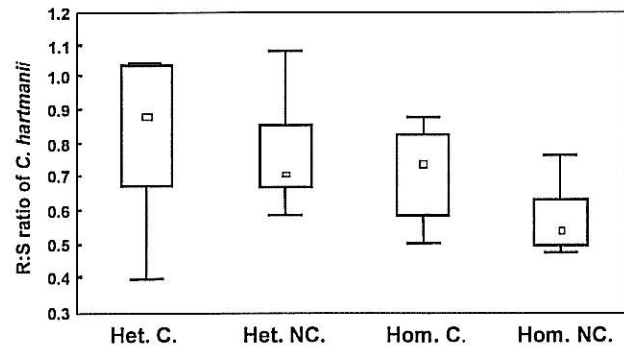


Fig. 3. Effect of soil treatment and presence of *M. caerulea* as a competitor on R:S ratio of *C. hartmanii*. Het.-heterogeneous soil treatment, Hom. – homogeneous soil treatment, C-competition environment, NC-non-competition environment. Statistical analyses are given in Table 2.

**Root placement**

In heterogeneous treatments, *M. caerulea* and *C. hartmanii* significantly translocated root biomass into nutrient rich patches, whereas the root biomass of *H. lanatus* was similar in nutrient rich and nutrient poor patches (Table 3, Fig. 5). There was no significant effect of presence of competitor on root placement of either species (Table 3). *C. hartmanii* translocated a significantly higher proportion of root biomass into nutrient-rich patches (66.9%) than did *M. caerulea* (62.8%) in the absence of competitor (t-test,  $t=2.44$ ,  $p=0.03$ ), but there was no significant difference in translocation between *M. caerulea* and *C. hartmanii* in the presence of competitor (t-test,  $t=1.02$ ,  $p=0.32$ ).

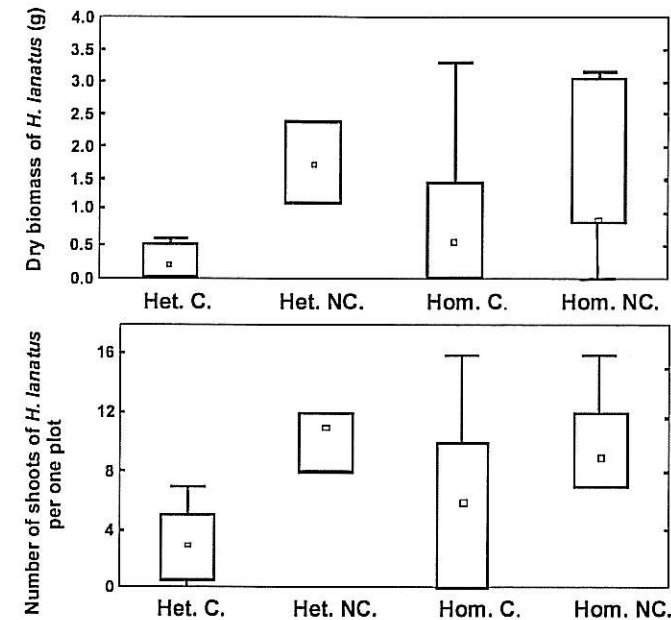


Fig. 4. Effect of soil treatment and presence of *M. caerulea* as a competitor on total biomass and number of shoots of *H. lanatus*. Het. – heterogeneous soil treatment, Hom.- homogeneous soil treatment, C –competition environment NC – non-competition environment. Statistical analysis are given in Table 2.

**Rhizome translocation of *C. hartmanii***

Both rhizome number and biomass were greater in the nutrient-rich patches (Table 4, Fig.6). Moreover, the presence of a competitor affected the mode of rhizome biomass translocation with the preference for nutrient rich patches stronger in the absence of a competitor (Interaction effect, Table 4, Fig. 6).

Table 3. Effect of presence of a competitor on root production and placement (interaction) of studied species and placement-effect in the heterogeneous treatment (see text). Analyzed by split-plot analysis of covariance. Initial growth unit weight was used as a covariate. Humus-rich soil versus sand placement was used as a within plot factor. Symbol \* indicates significance level  $0.05 < p < 0.1$ . The data were log-transformed.

Species	Competitor		Placement		Interaction	
	p	F	p	F	p	F
<i>M. caerulea</i>	n.s.	1.2531	0.0000	68.35695	n.s.	0.50007
<i>C. hartmanii</i>	n.s.	0.0479	0.0000	217.9538	n.s.	0.4710
<i>H. lanatus</i>	n.s.*	5.0266	n.s.	1.3491	n.s.	1.7886



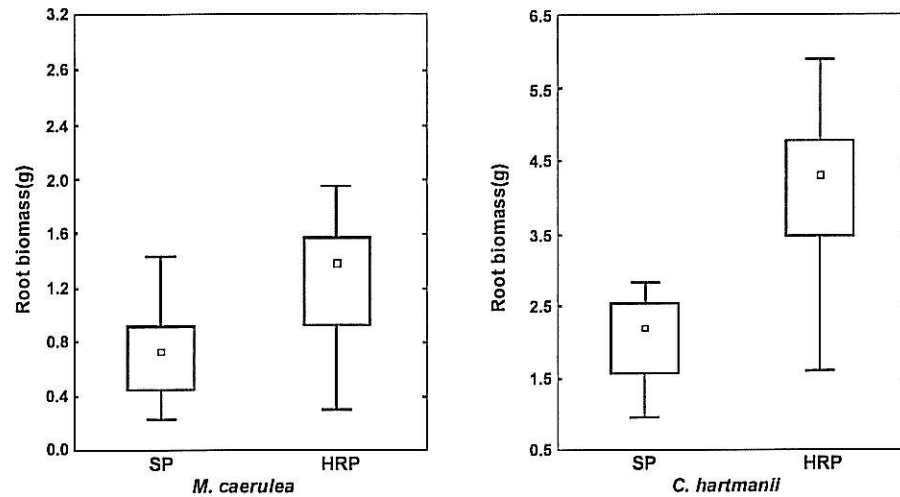


Fig. 5. Translocation of root biomass of *M. caerulea* and *C. hartmanii* into sand (SP) and humus-rich (HRP) patches. Statistical analysis is given in Table 3. As the competition had no effect on the root placement, the results are pooled for the competition/without competition treatments.

**Discussion**

Both *C. hartmanii* and *M. caerulea* roots exhibited foraging behavior, i.e., they were able to translocate their biomass into nutrient-rich patches, whereas *H. lanatus* did not so. Under competition-free conditions, precision foraging by *C. hartmanii* was higher than by *M. caerulea*. However, in the presence of a competitor, this difference disappeared. *H. lanatus* behavior contradicted the results of Fransen et al. (1999), who found its ability to utilize both spatial and temporal nutrient patches. We assume that the differences were

Table 4. Effect of presence of a competitor, placement-effect and interaction on rhizome biomass production and rhizome orientation of *Carex hartmanii* in the heterogeneous treatment. Analyzed by split-plot analysis of covariance. Humus-rich soil versus sand placement was used as a within plot factor. Initial growth unit weight was used as a covariate. Rhizome biomass data were log-transformed, and number of rhizomes was square-root transformed prior to analysis. Symbol\* indicates significance level  $0.05 < p < 0.1$ . Superscript +/- at the significance value means positive/negative effect of competition or increased amount in the nutrient rich sectors. For replication number see Tab. 1.

Source Dependent variable	Competition		Placement		Interaction	
	p	F	p	F	p	F
Rhizome biomass <sup>a</sup>	n.s.*	5.036	0.0001*	31.2504	0.0394	5.339
Number of rhizomes <sup>b</sup>	0.0404*	6.143	0.0365*	5.5403	n.s.	2.5199

<sup>a</sup> translocation of rhizomes biomass to the sand or humus-rich soil patches.  
<sup>b</sup> number of rhizomes penetrating to the sector II (sand) or sector III (humus-rich soil), see Fig. 1.

caused by different experimental design and/or by the very small root biomass of *H. lanatus* in our experiment. Moreover the study of Fransen & de Kroon (2001) has shown that *H. lanatus* is able to distinguish nutrient-rich patches at a high overall level of nutrient availability only.

Total biomass production was positively affected by environmental heterogeneity in *M. caerulea* only. Heterogeneity also affected biomass allocation in *C. hartmanii*, which, in the heterogeneous environment invested more into roots and less into shoots, and showed very high precision foraging by placing its roots into nutrient-rich patches. It seems that *C. hartmanii* was able to take advantage of the soil heterogeneous environment by increasing its R:S ratio.

In concordance with other studies (e.g. Einsmann et al. 1999) we can answer the first question: Plant species differ in their ability to find and exploit nutrient rich patches. Two of the three studied species exhibited the ability to forage, but only one of them (the dominant plant, with lower foraging precision) increased its total biomass. However, we should be aware that plant success over the studied time interval need not be manifested by an increase in total biomass production.

Our study showed that *M. caerulea* is more negatively affected by *C. hartmanii* in a heterogeneous than in a homogenous environment. This might be caused not only by increased competition pressure when the resources are concentrated into smaller soil volume, but also by increased investment of *C. hartmanii* into root production in a heterogeneous environment. Moreover, the presence of *M. caerulea* increased the relative investment of *C. hartmanii* into rhizomes, but did not affect its total biomass. These results correspond to the hypothesis of Campbell et al. (1991) about the relative advantage of high precision foraging of subordinate species in heterogeneous

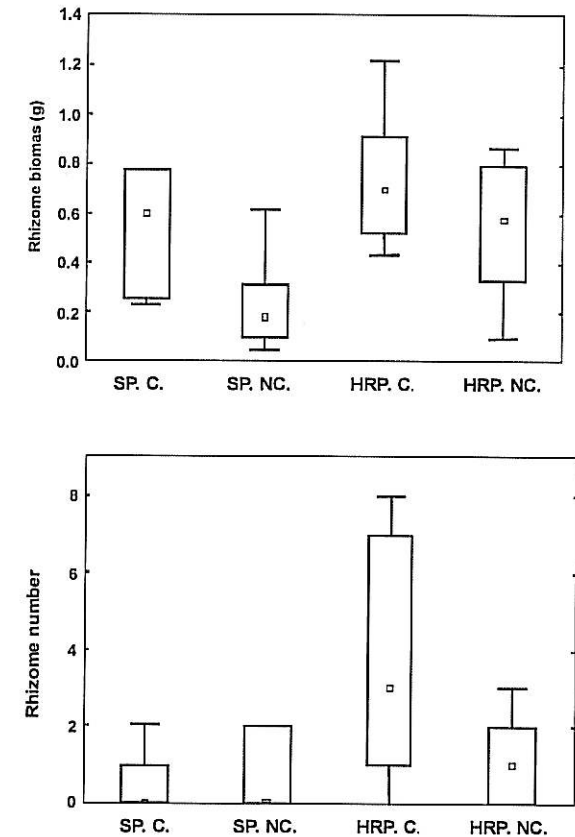


Fig. 6. Translocation of rhizome biomass of *C. hartmanii* into sand (SP) or humus-rich (HRP) soil patches in competition (C) versus non-competition (NC) treatments and number of rhizomes penetrating to the sand patch (SP) and humus-rich patch (HRP), see Fig. 1. Statistical analyses are given in Table 4.

environments. On the other hand, the increased biomass of the dominant species *M. caerulea* in a heterogeneous environment shows that this advantage of *C. hartmanii* may only be temporary. As demonstrated by Humphrey & Pyke (1998), the competition outcome could change during time. Their study demonstrated a decrease in biomass of guerrilla *Elymus lanceolatus* ssp. *lanceolatus* compared with phalanx *E.l. ssp. wawawaiensis* in the second year of the experiment. Recently Fransen et al. (2001) demonstrated a competition shift between *Festuca rubra* and *Anthoxanthum odoratum* in a heterogeneous environment in the second year of their experiment. One should, however, be aware of the limitations of pot experiments. Under natural conditions, *C. hartmanii* is able to escape the competition of *Molinia caerulea*, because in two years its daughter ramets can reach more than 0.5 m apart from the mother ramet (and consequently, also from the competitive *Molinia* tussock). Owing to its long spacers, *C. hartmanii* is able to forage on much larger spatial scale. *C. hartmanii* is able to acquire a competitive advantage by rapid investment of roots into nutrient-rich patches, whereas the dominant *M. caerulea*, although able to put roots preferentially into nutrient rich patches, does not change its R:S ratio. The behaviour of *C. hartmanii* is probably more favorable in dynamically changing heterogeneous environments, whereas that of *M. caerulea* in more stable heterogeneous environments. Whereas the first part of the second question could be unequivocally answered: Competition is clearly affected by soil heterogeneity, the answer to its second part is less clear. We suppose that both subordinate guerrilla plants (as suggested by Campbell et al. 1991) and dominants (as suggested by Weiner et al. 1997) can under certain circumstances benefit from soil heterogeneity – probably the spatial scale and temporal dynamics of the spatial mosaic determines which of them will be more successful.

The third question yielded also unequivocal answer: Rhizome formation in *C. hartmanii* is positively affected by the presence of a strong competitor, and more rhizomes were placed into favorable locations in the heterogeneous treatment. Rhizome foraging behavior (without the competition effect) has been demonstrated in several other rhizomatous and stoloniferous species (e.g., Birch & Hutchings 1994; Kleijn & Groenendaal 1999; de Kroon & Knops 1990; Salemaa & Sievänen 2002). Our study demonstrated not only a strong effect of heterogeneity, but also an interaction between heterogeneity and competition (see Fig. 6). Preference for nutrient rich patches was weaker when the nutrient-rich patches had high root density of the competitor. Generally, the rhizome biomass was higher in the presence of competitor in both treatments. Without competitor, rhizome biomass in the sand patches was much lower than in humus patches, whereas in the presence of the competitor, the difference was considerably smaller. *C. hartmanii* rhizomes are not only able to differentiate between rich and poor spots in the soil, but this differentiation is also affected by the presence of a competitor.

Lepš (1999) suggested that *H. lanatus*, because of its similarity to *M. caerulea*, increased in cover after *M. caerulea* removal. This suggestion is consistent with our results that in contrast to *C. hartmanii*, *H. lanatus* is more affected by *M. caerulea* (Table 2). Thus, the result is consistent with the idea of increased competition with increasing species similarity.

For a generalization of this hypothesis more competition studies with plants of various growth forms are required. Pot experiments are necessarily limited in their ability to mimic natural conditions. This is particularly true for experiments studying effects of spatial heterogeneity. Further studies should include effects of both temporal and spatial heterogeneity, effects of different patch size and nutrient content and longer time span. Not only biomass allocation, but also physiological and morphological responses of species to the environments should be investigated. In addition, the creation of experimental environments should reflect the field situation as far as possible.

Nevertheless, even with the limitations typical for the pot experiments, the present study was able to demonstrate the foraging behavior of underground structures of *Molinia caerulea* and *Carex hartmanii* (in contrast to *Holcus lanatus*), and how it is affected by the competition and soil heterogeneity.

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## Chapter V

### ***Effect of competition and soil quality on root topological plasticity of perennial grass Molinia caerulea***

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[manuscript]



## Effect of competition and soil quality on root topological plasticity of perennial grass *Molinia caerulea*

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### Summary

Changes in root topology of the tussock perennial grass *Molinia caerulea* were studied in a pot experiment. The target species *M. caerulea* was grown alone and with *Holcus lanatus* or *Carex hartmanii* as a competitor. The root topology in three different soils (sand, humus rich soil and a mix of both) was measured. Influence of competitive pressure on root topology was determined as root biomass surrounding the target root. Whereas no simple significant changes in root topology due to soil quality were observed, an increase of competition pressure caused a shift of root topology into a more herringbone structure. This shift was largest in the nutrient poor sand and smallest in the humus-rich soil. Moreover, we observed influence of individual competitors on topological changes in humus-rich soil after excluding the effect of total root biomass. This indicates the possibility of interspecific recognition.

## Introduction

Due to the complexity of the soil environment, new plant below-ground traits, which affect resource exploitation and both biotic and abiotic interactions, are still being discovered. It has been shown that one of the most important features of this complexity is resource heterogeneity (Jackson and Caldwell 1993, Ryel et al 1996). This heterogeneity is a very important factor affecting the outcome of plant competition (Janeček et al. 2004, Rajaniemi and Reynolds 2004, Fransen et al. 2001, Cambell et al. 1991), due to differences in plant root precision (in the sense of root biomass allocation), foraging scale and rate. Furthermore, the ability of plants to utilise nutrient rich soil patches can be modified by changes in root morphology (Šmilauerová and Šmilauer 2002, Arredondo and Johnson 1999), root physiology (Derner and Briske 1999) and co-operation with other organisms, especially with mycorrhizal fungi and N-fixing bacteria (Cruz et al 2004, Šmilauerová 2001, Šmilauerová and Šmilauer 2002).

Roots can accommodate to this soil heterogeneity by changing their topology. The centripetal topological model based on root links was suggested by Werner and Smart (1973) for topological classification of channel networks. This concept was adopted by Fitter (1985, 1986, 1987) to describe plant root systems. The fundamental element in this model is the link; in the case of roots it is a segment between two branching points or between the apical meristem and the first branch. The topology of a root ranges from a herringbone to a dichotomous type. Whereas a fully herringbone system is composed only of the main axis and its laterals, in a fully dichotomous system the new branches arise equally likely on each exterior link.

Simulation models of space exploration, root expenses and transport efficiencies showed differences between both extreme types of topology (Fitter 1987, Fitter et al 1991). The herringbone system seems to be more efficient in space exploration, but it is costlier and less transport-efficient. Although these models predict that a particular topology is well suited to a specific environment type, experimental results are not so clear. Fitter and Stickland (1991) and Taub and Goldberg (1996) supported the prediction that nutrient rich soils favour the dichotomous topology of dicots. On the contrary, Arredondo and Jonson (1999) showed an opposite effect of nutrients on root topology for grasses. A similar shift of root topology into a more herringbone structure under more fertile conditions was detected for *Luzula campestris*, whereas no effect was observed for *Poa angustifolia* and *Plantago lanceolata* (Šmilauerová and Šmilauer 2002).

It has been shown that root competition can change root behaviour (Robinson et al. 1999, Robinson 2001). Although some parameters, such as R/S ratio and root placement, are relatively often studied (Janeček et al 2004, Bliss et al 2001, Fransen et al 2001), we have only sparse knowledge about the reaction of root topology to competition (Šmilauerová and Šmilauer 2002). Moreover, the findings of Šmilauerová and Šmilauer (2002) contradicted predictions based on the centripetal topological model (Fitter et al. 1991).

To help solve the discrepancies between model predictions and experimental field studies, a study was conducted testing the effect of soil quality and competition on root topological plasticity of the tussock grass *Molinia caerulea*. In a glasshouse experiment,

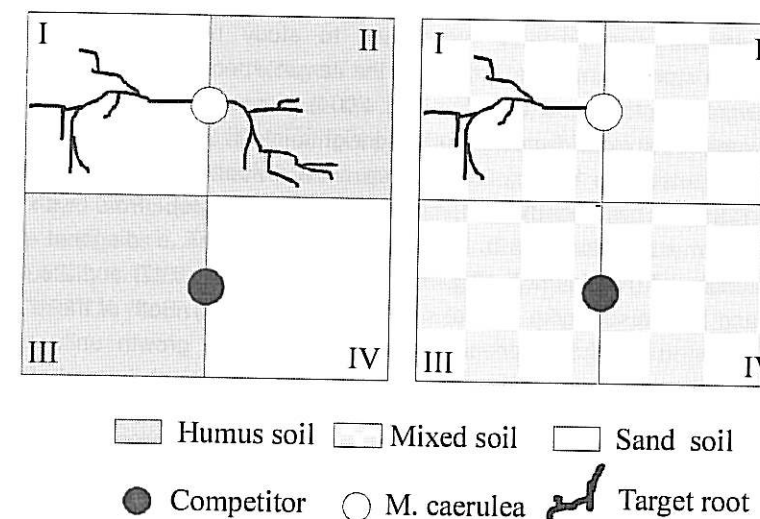


Fig 1: The experimental design. Both heterogeneous (left) and homogenous (right) treatments were performed in square 19 x 19 pots. I, II, III, IV – sector numbers.

root parameters (topological and dimensional) of this species were studied in three different soils and under three competition treatments: no-competition (respectively self-competition) and competition with one of two other meadow plant species (*Carex hartmanii* and *Holcus lanatus*). The aim was to answer the following questions:

1. Are there any differences in root parameters between individual soil types?
2. Are the changes in root parameters dependent on intensity of competition?
3. Are the changes in root parameters dependent on the species of competitor?

## Materials and methods

### Species

Individuals of studied species were collected from oligotrophic meadow at Ohrazení, which is 10 km south-east of České Budějovice, Czech Republic, 48°57'N, 14°38'E, at 530 m a.s.l. Mean annual precipitation and mean annual temperature are 600-650 mm and 7.8°C respectively.

Root penetration of *Molinia caerulea*, a dominant species in several vegetation types (Taylor et al. 2001) can exceed more than 80 cm depth (Jefferies 1915, 1916). It creates the vegetation matrix by its dense tussocks in the study site. *Holcus lanatus* is a tufted grass, which is able to increase in cover when *M. caerulea* is removed (Lepš 1999 and unpublished data). *Carex hartmanii* is a rhizomatous sedge penetrating throughout the vegetation by rhizomes longer than 0.5 m (personal observation).



### Experimental design and procedure

The pot experiment was primarily established to study the influence of soil heterogeneity and competition on the growth of the target species, characterised by above- and belowground production (Janeček et al. 2004). However, it was realised that changes in root morphology could provide further insights into the mechanisms of root competition. Plastic pots 19 x 19 cm, 15 cm deep were divided into two treatments, heterogeneous (19 pots analysed) and homogenous (20 pots). Pots with the heterogeneous treatment were divided into four equal parts. Two diagonal parts contained humus rich soil (N total 12.5 g/kg, P total 120 mg/kg, pH 5.47) and the other two contained sand (N total <0.2 g/kg, P total < 6 mg/kg, pH = 6.04). Both of these soils were mixed together in the homogeneous treatment. Individual growth units of *M. caerulea*, *C. hartmanii* and *H. lanatus* were transplanted into the pots at the end of April 2001. The transplanted growth units consisted of two interconnected basal internodes of *M. caerulea*, two interconnected shoots of *C. hartmanii*, or one shoot of *H. lanatus*. Two plants were grown in each pot in the competition treatment. *M. caerulea* was grown with either *C. hartmanii* or *H. lanatus* as the competitor. There was only one plant of *M. caerulea* per pot in the no-competition treatment (Fig. 1).

Each pot was divided into four sectors, at the end of the experiment in the middle of August 2001 (see Fig. 1). One root of *M. caerulea* was randomly selected from sector I in the homogenous treatment and sectors I (sand soil) and II (humus soil) from the heterogeneous treatment (Fig. 1.). The remaining root biomass was harvested separately for each sector and species.

Separated roots were spread on a glass plate and their images were scanned using a flatbed scanner with a resolution of 300 DPI. Root parameters, recorded in the image files, were evaluated using the ROOTARCH software (Šmilauer, unpublished). To describe the root, three dimensional parameters were used: *ILL* - the average length of interior links, *ELL* - the average length of exterior links,  $\mu$  (magnitude) - the number of root tips as well as two topological indexes:  $\log(Pe)/\log(\mu)$  (Fitter 1996), where *Pe* is the sum of the number of links in all paths from any exterior link to the root base and  $\mu$  is magnitude and *DBI* - dichotomous branching index (Šmilauerová and Šmilauer 2002). Both of the topological indices increase with a shift of the root architecture from dichotomous to a herringbone structure and seem to be independent of root size. The advantage of the dichotomous branching index (DBI) is that its values are between 0 (fully dichotomous system) and 1 (fully herringbone system) (see Šmilauerová and Šmilauer 2002)

### Data analysis

Because of the possible dependence of two roots from one plant in the heterogeneous treatment and independence between roots from the mixed soil and both sand and humus rich soil treatments were three t-tests performed (one for dependent and two for independent samples) to analyse differences between soil types. A Bonferroni correction:  $\alpha_{\text{used}} = \alpha_{\text{nominal}}/3$ ; (three t-tests) to keep the probability of Type I error at the nominal

significance level, was used. In effect, all *P* values from individual t-tests were multiplied by 3.

The influence of root biomass on the studied parameters was analysed using linear regression. Root biomass from the sector where a given root was taken was used. Analysis of competitor effect on root parameters in individual soil types was performed by ANCOVA analysis, where individual competitors were established as an independent factor and root biomass in the corresponding sector was used as a covariate. The aim of these analyses was to detect competitor effects, which are independent of root productivity in individual competition treatments. Data were analysed using the program STATISTICA (Anon 1996)

Table 1: Differences between individual soils in the measured root parameters. Three t-tests were performed for each parameter. Whereas the sand x humus soil difference was tested by t-test for dependent samples, the other two contrasts were tested by t-test for independent samples. Correction for Type I error was performed by Bonferroni adjustment (*p* in table = *p* corresponding with *t* statistics in brackets\* 3)

	ILL	ELL	$\mu$	Log(Pe)/Log( $\mu$ )	DBI
Sand x humus soil	ns (1.20)	ns (1.87)	ns (2.20)	ns (0.72)	ns (0.41)
Sand x mixed soil	ns (1.87)	0.002 (3.69)	ns (1.76)	ns (1.18)	ns (1.44)
Humus x mixed soil	ns (1.32)	ns (1.71)	ns (0.36)	ns (0.67)	ns (1.03)

Table 2: Linear regressions of the measured parameters on total root biomass in individual soils. The *p*-value is followed (in brackets) by the *F*-ratio statistic. Superscript + at the significance value means a positive effect of total root biomass on studied parameter, superscript - means a negative effect.

	ILL	ELL	$\mu$	Log(Pe)/Log( $\mu$ )	DBI
Sand soil	ns (0.23)	ns (0.26)	0.010 <sup>-</sup> (8.30)	0.000 <sup>+</sup> (27.68)	0.000 <sup>+</sup> (49.78)
Mixed soil	0.001 <sup>+</sup> (15.77)	ns (3.30)	0.001 <sup>-</sup> (18.11)	0.002 <sup>+</sup> (13.06)	0.000 <sup>+</sup> (21.90)
Humus soil	0.129 (2.55)	ns 2.67	0.013 <sup>-</sup> (7.79)	0.005 <sup>+</sup> (10.43)	0.002 <sup>+</sup> (12.72)

### Results

Roots of *M. caerulea* showed high plasticity in their topology. The dichotomous branching index (DBI) ranged from 0.05 to 1 (mean 0.36). Means of ELL and ILL in all root systems were 5.72 mm and 3.63 mm, respectively. The mean number of exterior links (magnitude) was 311.5.

There appeared to be only one significant effect of soil quality on the measured root characteristics (Table 1): The average exterior link length was significantly higher in sand

(6.8 mm) than in mixed soil (4.8 mm). Total root biomass in the sector of the target root was the most important factor determining root architecture and number of exterior links (magnitude) (Table 2). In all soil types, root structure became more herringbone branched as root biomass increased. Moreover, the regression slope decreased in the order sand – mixed – humus rich soil. Likewise, the explained variability ( $R^2$ ) decreased in this sequence (Fig. 2). The regressions were non-significant when root biomass of *M. caerulea* and root biomass of competitor were used separately.

Surrounding root biomass negatively affected root magnitude. The most negative regression slope occurred in the mixed soil. A significant positive effect of root biomass on average length of interior links was detected in the mixed soil.

There were found significant effects of the competitor on the averaged exterior length and DBI index in humus rich soil when root biomass was used as a covariate to search for specific competitor effects on root parameters (Table 3).

Table 3: ANCOVA analyses of the effect of different competitors (*C. hartmanii*, *H. lanatus* and no-competitor) on the measured parameters; total root biomass was used as a covariate to exclude the different root productivities in the competition treatments. The p-value is followed by the F-ratio statistic (in brackets) and corresponds to the competitor identity effect.

	ILL	ELL	$\mu$	Log(Pe)/Log( $\mu$ )	DBI
Sand soil	ns (0.40)	ns (0.10)	ns (0.59)	ns (2.28)	ns (2.11)
Mixed soil	ns (0.37)	ns (1.12)	ns (1.66)	ns (3.10)	ns (1.71)
Humus soil	ns (0.34)	<b>0.038</b> (4.11)	ns (1.41)	ns (3.35)	<b>0.039</b> (4.05)

### Discussion

Of the several parameters tested, the difference in average length of exterior links between roots growing in sand and roots in the mixed soil was the only significant effect of soil quality found. Nevertheless the reaction to competition was largest in nutrient poor sand and smallest in humus rich soil, indicating that the root reaction depends on soil quality. This is in agreement with some studies, which did not detect any influence of soil fertility on root parameters for grasses (Taub and Goldberg 1996, Šmilauerová and Šmilauer 2002, Fitter and Stickland 1991). However, other authors have observed significant changes. Arrendo and Johnson (1999) have shown that

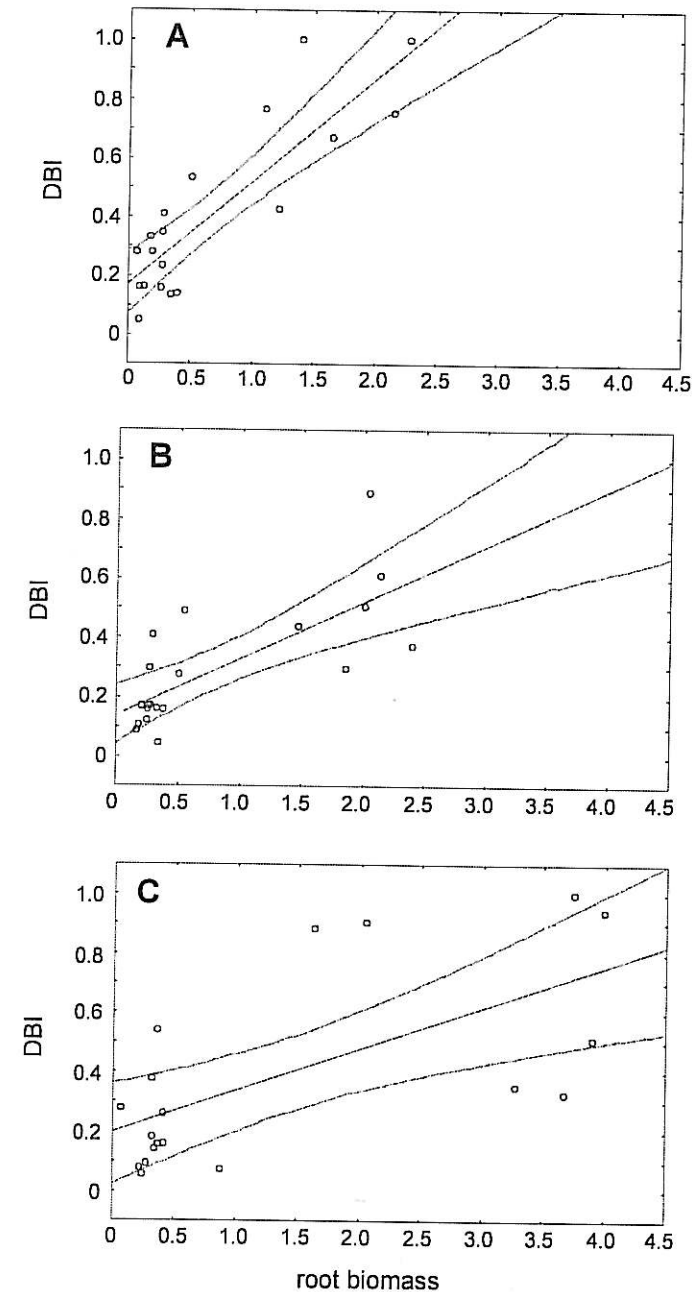


Fig. 2. Dependence of the dichotomous branching index (DBI) on root biomass in individual soils. Statistical analyses are given in Table 2. (A) sand soil,  $y=0.175+0.351x$ ,  $R^2=0.75$ . (B) mixed soil,  $y=0.140+0.190x$ ,  $R^2=0.54$ . (C) humus soil,  $y=0.190+0.139x$ ,  $R^2=0.43$ . DBI ranges from 0 for completely dichotomously branched to 1 for herringbone-style branched roots.



root topology of three grasses shifted from a herringbone to a dichotomous structure in a low nutrient environment. Dawson et al. (2003) obtained similar results for *Festuca ovina*. However, the response for *Lolium perenne* in the same study was in the opposite direction, which agrees with the model of Fitter et al. (1991). Generally, our results, together with the previously mentioned studies, suggest that changes in grasses' root topology along a fertility gradient need not (in contrast to dicots) follow theoretical predictions (Fitter et al. 1991) and increase dichotomous branching in nutrient rich soils (Taub and Goldberg 1996, Fitter and Stickland 1991).

Whereas there was no effect of competition on root placement into nutrient rich patches (Janeček et al. 2004), a clear effect of competition on root topology was shown. This finding is in agreement with the theoretical background for plasticity in root topology, which shows that a herringbone structure is more advantageous in a competitive environment by minimizing intraplant and probably also interplant competition (Fitter et al. 1991). On the other hand, Šmilauerová and Šmilauer (2002) found no response to root competition for the grass *Poa angustifolia*; *Plantago lanceolata* even exhibited the opposite response. If high root topological plasticity in *M. caerulea* is considered to be an adaptation to competition pressure, then there are two possible advantages of this adaptation for tussock grasses: 1/ The roots can respond to high root density below the tussock. 2/ Due to the extension of the main axes, the herringbone structure seems to be an adaptation to root foraging; an increase in competition pressure results in an increased effort to search for new rich patches with lower competition pressure. Root foraging can be extremely useful for tussock "phalanx" grasses (compared to guerrilla plants), which are not able to move when near soil resources are depleted (for phalanx-guerrilla theory see Lovett Doust 1981).

The fact that root topology was not affected by soil quality (differing considerably in nutrient content), whereas it changed with root biomass in soil, suggests that *M. caerulea* does not perceive competition only through a decrease in nutrient content (indirect competition). Other signals can play an important role in root development.

New results investigating the means of root communication were published recently. Gruntman and Novoplansky (2004) and Falik et al. (2003) have shown that plants have the ability to distinguish between self- and non-self roots. Even roots of twins (two individuals originating from one plant by mechanical division) are able to be distinguished. The results from this study, that in the humus rich soil root topology and the average length of exterior links are dependent on identity of the competitor species, can be to a certain extent an indication of between-species recognition. Common signal vectors, such as phenolics or plant hormones (Kaska et al. 1999, Hirsch et al. 2003, Borch et al. 1999), are not specific enough to make this distinction. The most promising vectors seem to be hormonal or/and electrical oscillations (Ortuno et al. 1990, Shabala 2003). Although there is a great effort to discover these processes, the extent and means of inter- and intraplant communication are still a mystery.

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### Summary of results

## Chapter VI

### Summary of results



## Summary of results

I have studied selected traits of *M. caerulea*, which can be important for its dominant function in wet meadow plant community: 1/ tussock structure of *M. caerulea* and its effect on other species; 2/ traits interconnected with high biomass production (leaf cover and litter); 3/ ability to exploit heterogeneous soil environment and 4/ root architectural plasticity under competition in soils of different quality.

### **Tussock structure**

The tussock is a specific, highly structured microsite affecting the seedling recruitment (Chapter III). Generally, tussock seems to be a hostile environment for seedling recruitment. Nevertheless, the seedlings of individual species differ in their ability to germinate and grow in this microsite, and this ability changes during the vegetation season. In addition, for one of the species (*Angelica sylvestris*), tussocks of *M. caerulea* are the preferred microhabitats. It seems that for some species the advantages such as protection against herbivores or extremes of weather can sufficiently compensate high competition pressure inside the tussocks.

Development of tussocks of *M. caerulea* is inhibited by traditional mowing management. Mowing causes not only disappearing of the vertical structure, but also effects horizontal shoot distribution (Chapter II). The contrast in shoot density between tussocks and inter-tussock space decreases under mowing (tussocks become less apparent). Decrease of microsite shoot density (microsite 5x5 and 10x10cm) of *M. caerulea* can permit coexistence of more plant species on these scales. In addition, the genetic study of mown versus unmown plots has shown that more flat inter-tussock space on the mown plots is probably more suitable for vegetative spreading. In contrast to most other meadow species it seems that mowing did not facilitate generative establishment of *M. caerulea*. The effect of mowing on shoot distribution of dominant tussock grasses such as *M. caerulea* can be together with that of high proportion of biomass loss responsible for coexistence of other subordinate species.

### **Leaf cover and litter**

There are various ways how plants affect other plants, both other established species, and particularly seedling recruitment. In the studied locality, effects of leaf cover and litter on seedling recruitment were studied (Chapter III). *M. caerulea* represents essential compound of both, total leaf cover and is also the most important producer of litter (see Fig. 1, Chapter III).

The negative effect of litter on seedlings was non-significant, but litter changed development of established vegetation. Both, leaf cover of *M. caerulea* and cover of other plants was small at the start of the vegetation season. However *M. caerulea*, in contrast to other plants, was able compensate negative effect of litter on its cover during

vegetation season. *M. caerulea*, which create large part of litter amount can so inhibit growth of other species.

The effect of leaf cover on seedling recruitment changes during vegetation season. At the start of the vegetation season, the effect was mostly positive, but became negative at the end of the vegetation season.

### **Exploitation of heterogeneous soil environment**

The species of phalanx growth form are limited in vegetative spread by rhizomes or stolons; this can be very important for their behavior in heterogeneous environment. The ability of *M. caerulea* to exploit heterogeneous soil environment was investigated in the pot experiment (Chapter IV). This ability has been compared with another tussock grass *Holcus lanatus* and rhizomatous sedge *Carex hartmanii* which coexist with *M. caerulea* in the study site. *M. caerulea* profits from soil heterogeneity and is able to achieve higher biomass in heterogeneous soil. Nevertheless, this advantage is affected by the presence of *C. hartmanii* as a competitor. The negative effect of *C. hartmanii* on biomass of *M. caerulea* was much more pronounced in heterogeneous than in homogenous soil environment. However, this effect of *C. hartmanii* in the heterogeneous soil was not accompanied by an increase of its total biomass during the experiment. This corresponds with observed increase of investment into root production by *C. hartmanii* in heterogeneous treatment, although *C. hartmanii* has been very successful (more than *M. caerulea*) in root placement into nutrient rich patches. It was suggested that this "usurpation strategy" of *C. hartmanii* can be more advantageous in dynamically changing heterogeneous environment, whereas "equal-investment" strategy of *M. caerulea* can be more useful in stable heterogeneous environment.

The usurpation ability of *C. hartmanii* can be also facilitated by its ability to penetrate into favorable conditions by long rhizomes, which are more frequently created under competition and penetrate into nutrient rich patches. *H. lanatus*, which has a similar growth form to that of *M. caerulea*, was heavily negatively affected in competition with *M. caerulea*. In contrast to *M. caerulea* and *C. hartmanii*, *H. lanatus* was not able to place roots into nutrient rich patches. This experiment has shown that different species use different strategies to exploit soil heterogeneous environment. There are many scales of both temporal and spatial heterogeneity, which can separate plant strategies and permit coexistence of subordinate species with dominant plants, represented in this study by *M. caerulea*.

### **Root architectural plasticity under competition in soils of different quality**

*M. caerulea* profits from soil heterogeneity and its biomass and shoot number can be negatively affected by competition (Chapter IV). Which are the morphological changes connected with the species response? In Chapter V, we describe the changes of root architecture under various competition and soil quality levels. Soil quality did not change simple root architecture; however, the roots became more herringbone branched when competition increased. This effect of competition was manifested differently in each soil type. In the nutrient rich soil, changes in root architecture were mild and in nutrient poor

sand soil were very pronounced. One of the advantages of herringbone root architecture is ability to grow into longer distances. From this point of view, herringbone branching can be correlated with ability of underground foraging. We have seen that *M. caerulea* is more negatively affected by competitor than *Carex hartmanii* (Chapter IV). When nutrients are concentrated in patches (Chapter IV), the ability to change the root topology can be seen as a virtue of necessity, which enable *M. caerulea* to escape from sites of high competition pressure, rather than a trait which can cause its underground superiority.

### **Conclusions**

The *M. caerulea* as a tall tussock grass is well adapted for its role in the plant community of wet meadows. It creates high biomass resulting in high leaf cover and production of high amount of litter, dense tussocks, which inhibit seedling recruitment of most of the coexisting species. But to be dominant on semi-natural meadow brings specific disadvantages which handicap *M. caerulea* in comparison with other subordinates. It loses higher proportion of biomass by mowing and/or eventually by non-selective grazing. Mowing causes also damage of its tussock structure and probably causes increase of inter-genet competition. Other disadvantages are caused by trade-off between individual traits. For example, the investment into dense tussocks does not enable exploitation of distant resources (as in some other species by long spacers); similarly, the investment into biomass production can disable the "usurpation strategy", which has been observed for *C. hartmanii*. These limitations can lead to the development of "secondary" traits such as to the ability of roots to leave patches with high competition pressure. However, many more studies are needed for generalization of these PhD theses hypotheses.

**Take home message:** The detail knowledge of dominant species behavior is essential for understanding plant community functioning.