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Plant roots in heterogeneous soil environment

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PhD thesis

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This study focused on root response of grassland plants to geterogeneity of soil resources under field conditions. Main attention was paid to the effects of nutrient heterogeneity and to the role of mycorrhizal symbiosis.

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Prohlašuji, že jsem na této práci pracovala jako autorka nebo spoluautorka samostatně, pouze s použitím uvedené literatury.

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Marie Šmilauerová

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Contents

Introduction

Heterogeneity of soil resources is considered to play important role in coexistence of plant species in natural and semi-natural communities (Grime 1994, Casper & Jackson 1997) but only few studies documented fine-scale soil heterogeneity in non-agricultural ecosystems (Jackson & Caldwell 1993, Ryel *et al.* 1996, Šmilauer 1996, Farley & Fitter 1999).

Studies of plant response to nutrient microsites discovered that plant response can consist of morphological (Fitter 1994, Arredondo & Johnson 1999, Einsmann *et al.* 1999), physiological (Caldwell *et al.* 1992, Derner & Briske 1999, Fransen *et al.* 1999) or demographic (Gross *et al.* 1993, Pregitzer *et al.* 1993, Hodge *et al.* 1999a, b) changes on root level, but also of changes in clonal growth strategy (Humphrey & Pyke 1997, Wijesinghe & Hutchings 1997) or changes in development of mycorrhizal structures within roots (McArthur & Knowles 1992, Duke *et al.* 1993, Cui & Caldwell 1996). However, impressive morphological response observed by Drew in seventies for barley (Drew & Saker 1974, Drew 1975) was not found later in most of wild species. One possible explanation is that barley as a crop plant has specific ecological characteristics, such as rapid growth rate, high nutrient demand, and an annual habit (Fitter 1994). Other explanation is that nutrient concentration used in those studies exceeded many times the values usual in (semi-)natural ecosystems.

Even for wild plant species, behaviour noticed in glasshouse experiments has not been found in plants growing under field conditions (e.g. Fitter & Stickland 1992, West *et al.* 1993). The lack of competition in some of the experiments can be the cause of significant shift in plant behaviour (as suggested by Cahill & Casper 1999, Hodge *et al.* 1999c, Robinson *et al.* 1999, Fransen *et al.* 2001). Although presence of mycorrhizal fungi by itself influences morphological characteristics of roots (Kothari *et al.* 1990, Hetrick 1991, Fusconi *et al.* 2000) and composition of fungal community has clear effect on both above- and below-ground biomass of host species and consequently onto plant community composition and productivity (van der Heijden *et al.* 1998), most studies ignored mycorrhizal dependency of plant species or used only one fungal taxon, mostly with very special ecological behaviour (e.g. Hetrick *et al.* 1990, 1991, Fusconi *et al.* 2000). Role of mycorrhizal fungi can change during the life cycle of host plant as well as during a single growing season (Hetrick *et al.* 1994, Mullen & Schmidt 1993, Lapointe & Molard 1997).

Fitter (Fitter 1991, Fitter et al. 1991) predicted that species from nutrient-rich habitats or plants growing under higher nutrient availability will have root systems with more dichotomous topology and with shorter root links, while herringbone topology (which is considered more efficient in nutrient acquisition) would be favoured in nutrient-poor sites. Experimental results of Fitter & Stickland (1991) supported these suggestions. Taub & Goldberg (1996) found that the response of the forbs, but not of the grasses, was in agreement with Fitter's predictions. Several other studies found link length sensitive to increased nutrient availability but root topology insensitive at the same time (Fitter et al. 1988, Fitter 1994). Fitter et al. (1988), Fitter & Stickland

(1991), and Taub & Goldberg (1996) found the root topology of grasses more herringbone in comparison with the forbs. On the other hand, Gross et al. (1992) and Glimskär (2000) did not find significant difference in root topology between grasses and forbs. Zhang *et al.* (1999) referred that Arabidopsis roots exposed to a local source of NO_3^- did not increase branching frequency, but there was a localised 2-fold increase in the mean rate of lateral root elongation. They suggested that NO_3^- acts as a signal rather than a nutrient.

Emergency of a new nutrient patch can affect not only the rate of root proliferation and the root branching frequency, but also longevity of new roots in the patch (Gross *et al.* 1993, Pregitzer *et al.* 1993, Hodge *et al.* 1999a,b). Although increased birth rate inside the nutrient patches was noticed in all those studies, there was also a significant increase in root death rate. Gross *et al.* (1993) found differences between species in the magnitude and timing of root proliferation in patches due to differences in root birth and death rates.

The ability of roots exposed to higher nutrient availability within soil microsites to increase nutrient absorption is deduced from increased amount of labelled elements in shoot biomass (e.g. Hodge *et al.* 1999a,b,c, Fransen *et al.* 1999). Caldwell *et al.* (1992) assumed that the selective elevation of phosphorus uptake kinetics in fertile microsites may be of greater importance than the root proliferation, at least under some circumstances. Importance of this type of plastic response can be even greater for nutrients with relatively high diffusiveness such as NO_3^- ions (Caldwell 1994). Derner & Briske (1999), who compared morphological and physiological root plasticity in caespitose and rhizomatous grasses from mesic and semi-arid communities, found differences in root morphological plasticity among species from different habitats but not between grass growth forms, while the rhizomatous grass from the semi-arid community was the only species with significant physiological root plasticity. According to Robinson & Van Vuuren (1998) slow-growing species stimulate their rate of nutrient uptake per unit of root in nutrient-rich patches more than the fast-growing species, although their conclusions are based only on data with an uniformly nutrient-rich control. When the nutrient capture was measured per whole plant, the forbs were able to capture nutrients from a patch better than the grasses, when compared with a nutrient-deficient control.

Although many species used in the studies of root behaviour in nutrient patches are clonal plants, duration of experiments is usually too short for full expression of clonal growth characteristics. Humphrey & Pyke (1997) found root growth as important for exploitation of patchy soil nutrients as ramet placement. Huber-Sannwald *et al.* (1998) found that morphological plasticity of rhizomatous grass *Elymus lanceolatus* ssp. *lanceolatus* was influenced by root competition much more than by the local nutrient enrichment. When *Elymus* was grown in root competition with a strong competitor *Agropyron desertorum*, it changed clonal growth strategy and produced aboveground stolons. In another experiment, the authors documented that rhizome or root contact of a clonal plant with its neighbours may induce different clonal response depending on the neighbour species (Huber-Sannwald *et al.* 1997). Effects of patch scale and patch contrast on the

growth of clonal plant *Glechoma hederacea* were studied by Wijesinghe & Hutchings (1997, 1999).

Thesis aims and results

The aim of this thesis was to contribute to understanding of plant root behaviour in heterogeneous soil environment under field conditions. After obtaining essential information on morphological root characteristics of wide set of grassland species with different ecological demands and clonal growth strategies (glasshouse experiment, **Chapter 1**), I paid attention to behaviour of grassland plants under field conditions. I investigated plant response to changed nutrient availability and activity of mycorrhizal fungi on several levels. Firstly, long-term impact of suppression of mycorrhizal fungi and of phosphorus addition on grassland community composition was studied (**Chapter 2**). Two other experiments were focused on short-term effects of local nutrient enrichment and suppression of mycorrhizal development on root behaviour at the levels of whole plant community (**Chapter 3**) and of individual plants (**Chapter 4**).

To compare root morphological characteristics of larger set of grassland species and to investigate relationships between root properties of seedlings and selected ecological traits, relevant mostly to adults under field conditions, I performed a comparative glasshouse experiment (**Chapter 1**). I evaluated average length of exterior and interior links, magnitude and total length of whole root systems, two topological indices, and the leaf area - root length ratio (L:R) for seedlings of 57 species (for definition of root morphological characteristics see Fitter 1991 and Chapter 1, Table 1, p. 12). I used Ellenberg indicator values of soil moisture and available nitrogen (Ellenberg 1988) for characterisation of species ecological demands, and typology of clonal growth (Klimešová & Klimeš 1998) as a characteristic of their growth strategy. Species differing in nitrogen demand possessed significantly different root morphological characteristics: the total root length, the number of root tips (magnitude), and dichotomy of root branching increased with higher species demand for nitrogen. On the other hand, average length of links was independent of nitrogen demand and this finding disagrees with findings published earlier. Species position on the soil moisture gradient was not related to seedling root properties. It seems that type of clonal growth is manifested already in root properties of very young plants, much earlier than characteristic properties of the clonal type develop. Species with different type of clonal growth differed significantly in root topology and magnitude. When origin of clonal growth organs was considered, I found difference in average link length and in L:R ratio. This study confirmed the already known differences between root properties of grasses and forbs. Unlike the published results, the grasses produced more dichotomously branched roots than the forbs, in my experiment.

The mycorrhizal symbiosis between vascular plants and various fungi is one of the important factors that might influence structure and composition of plant community. It is traditionally expected that the main contribution of arbuscular mycorrhizal (AM) fungi to host plant is improvement of nutrients acquisition, particularly of the less mobile phosphorus (P). To assess

influence of AM symbiosis on the plant community composition and to separate effect of the symbiosis on the phosphorus nutrition of plants, we combined experimental exclusion of AM fungi by fungicide benomyl with the addition of supplementary P source. We measured aboveground biomass of important species or functional groups in four successive years (**Chapter 2**). While only suppression of forbs in favour of graminoids (due to P application) was significant after three seasons, significant effect of both P and benomyl application was found after four seasons. Mycorrhizal forb *Achillea millefolium* responded positively to fungicide application, while other mycorrhizal forbs were suppressed by both fungicide and P. The grasses and sedges profited from P application, mainly when their share in total biomass was considered. When we looked directly on the effect of P application on the AM symbiosis, the largest negative impact was found in mycorrhizal forbs, while response in grasses was much smaller.

Last two experiments studied short-term response of plant roots to soil heterogeneity on small spatial scale. In both experiments, increased availability of nutrients (N, P) in patches was combined with suppression of AM symbiosis by fungicide benomyl and with modification of substrate structure. The whole community response was measured as newly grown root biomass in the patches (**Chapter 3**). Both nutrient and fungicide application had positive effect on the root biomass grown into the patches. Interaction of effect of these two treatments was not significant. The modification of substrate structure had no effect on the root biomass in patches but it modified the effect of benomyl. The amount of root biomass proliferating into the patches was not related to species composition of vegetation near the patches, but it was significantly correlated with the number of grass tillers in close neighbourhood.

Chapter 4 presents a detailed study of morphological response of three grassland species to small-scale heterogeneity in soil resources. Non-mycorrhizal *Luzula campestris* responded little to nutrient application, but strongly to benomyl application, in all measured characteristics. Mycorrhizal *Poa angustifolia* produced the longest, most branched roots but exhibited limited sensitivity to nutrients and benomyl application. Strongly mycorrhizal *Plantago lanceolata* was the species most sensitive to nutrient application, but did not almost respond to benomyl application. It was the only one among studied species with root characteristics influenced (negatively) by increased production of total root biomass in the patches. The results indicate limited role of root topology in exploitation of soil microsites.

References

Arredondo J.T. & Johnson D.A. (1999): Root architecture and biomass allocation of three range grasses in response to nonuniform supply of nutrients and shoot defoliation. *New Phytol.* 143: 373-385.

Cahill J.F. & Casper B.B. (1999): Growth consequences of soil nutrient heterogeneity for two old-field herbs, *Ambrosia artemisiifolia* and *Phytolacca americana*, grown individually and in combination. *Ann. Bot.* 83: 471-478.

Caldwell M.M. (1994): Exploiting nutrients in fertile soil microsites. In: Caldwell M.M. & Pearcy R.W. [eds.]: *Exploatation of Environmental Heterogeneity by Plants*. San Diego, USA: Academic Press, Inc., 305-323.

Caldwell M.M., Dudley L.M. & Lilieholm B. (1992): Soil solution phosphate, root uptake kinetics and nutrient acquisition: implications for a patchy soil environment. *Oecologia* 89: 305-309.

Casper B.B. & Jackson R.B. (1997): Plant competition underground. *Annu. Rev. Ecol. Syst.* 28: 545-570.

Cui M. & Caldwell M.M. (1996): Facilitation of plant arbuscular mycorrhizas from enriched soil patches. I. Roots and hyphae exploiting the same soil volume. *New Phytol.* 133: 453-460.

Derner J.D. & Briske D.D. (1999): Does a tradeoff exist between morphological and physiological root plasticity? A comparison of grass growth forms. *Acta Oecol.* 20: 519-526.

Drew M.C. (1975): Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. *New Phytol.* 75: 479-490.

Drew M.C. & Saker L.R. (1974): Nutrient supply and the growth of the seminal root system in barley. *J. Exp. Bot. 26: 79-90.*

Duke S.E., Jackson R.B. & Caldwell M.M. (1993): Local reduction of mycorrhizal arbuscule frequency in enriched soil microsites. *Can. J. Bot.* 72: 998-1001.

Einsmann J.C. *et al.* (1999): Nutrient foraging traits in 10 cooccurring plant species of contrasting life forms. *J. Ecol.* 87: 609-619.

Ellenberg H. (1988): *Vegetation of central Europe*. - Cambridge University Press, Cambridge.

Farley A.U. & Fitter A.H. (1999): Temporal and spatial variation in soil resources in a deciduous woodland. *J. Ecol.* 87: 688-696.

Fitter A.H. (1991): Characteristics and functions of root systems. In: Waisel Y., Eshel A. & Kafkafi U. [eds.]: *Plant roots: the hidden half.* New York, USA: Marcel Dekker, Inc., 3-25.

Fitter A.H. (1994): Architecture and biomass allocation as components of the plastic response of root system to soil heterogeneity. In: Caldwell M.M. & Pearcy R.W. [eds.]: *Exploatation of Environmental Heterogeneity by Plants*. San Diego, USA: Academic Press, Inc., 305-323.

Fitter A. H., Nichols R. & Harvey M. L. (1988): Root system architecture in relation to life history and nutrient supply. *Funct. Ecol.* 2: 345-351.

Fitter A.H. *et al.* (1991): Architectural analysis of plant root systems. 1. Architectural correlates of exploitation efficiency. *New Phytol.* 118: 375-382.

Fitter A. H. & Stickland T. R. (1991): Architectural analysis of plant root systems 2. Influence of nutrient supply on architecture in contrasting plant species. - *New Phytol.* 118: 383-389.

Fitter A.H. & Stickland T.R. (1992): Architectural analysis of plant root systems 3. Studies on plants under field conditions. *New Phytol.* 121: 243-248.

Fransen B., Blijjenberg J. & de Kroon H. (1999): Root morphological and physiological plasticity of perennial grass species and the exploatation of spatial and temporal heterogeneous nutrient patches. *Plant Soil* 211:179-189.

Fransen B., de Kroon H. & Berendse F. (2001): Soil nutrient heterogeneity alters competition between two perennial grass species. *Ecology* 82: 2534-2546.

Fusconi A. *et al.* (2000): Root apical meristems of *Allium porrum* L. as affected by arbuscular mycorrhizae and phosphorus. *Protoplasma* 214: 219-226.

Glimskär A. (2000): Estimates of root system topology of five plant species grown at steady-state nutrition. *Plant Soil* 227: 249-256.

Grime J.P. (1994): The role of plasticity in exploiting environmental heterogeneity. In: Caldwell M.M. & Pearcy R.W. [eds.], *Exploatation of environmental heterogeneity by plants: ecophysiological processes above- and below-ground.* Academic Press, Inc., pp. 1-19.

Gross K.L., Maruca D. & Pregitzer K.S. (1992): Seedling growth and root morphology of plants with different life-histories. *New Phytol.* 120: 535-542.

Gross K.L., Peters A. & Pregitzer K.S. (1993): Fine-root growth and demographic responses to nutrient patches in four old-field plant species. *Oecologia* 95:61-64.

Hetrick B.A.D. (1991): Mycorrhizas and root architecture. *Experientia* 47: 355-362.

Hetrick B.A.D., Wilson G.W.T. & Leslie J.F. (1990): Root architecture of warm- and cool-season grasses: relationship to mycorrhizal dependence. *Can. J. Bot.* 69: 112-118.

Hetrick B.A.D., Wilson G.W.T. & Todd T.C. (1991): Relationships of mycorrhizal symbiosis, rooting strategy, and phenology among tallgrass prairie forbs. *Can. J. Bot.* 70: 1521-1528.

Hetrick B.A.D., Wilson G.W.T. & Schwab A.P. (1994): Mycorrhizal activity in warm- and cool-season grasses: variation in nutrient-uptake strategies. *Can. J. Bot.* 72: 1002-1008.

Hodge A. *et al.* (1999a): Nitrogen capture by plants grown in N-rich organic patches of contrasting size and strength. *J. Exp. Bot.* 50: 1243-1252.

Hodge A. *et al.* (1999b) Plant, soil fauna and microbial responses to N-rich organic patches of contrasting temporal availability. *Soil Biol. Bioch.* 31: 1517-1530.

Hodge A. *et al.* (1999c): Why plants bother: root proliferation results in increased nitrogen capture from an organic patch when two grasses compete. *Plant, Cell Environ.* 22: 811-820.

Huber-Sannwald E., Pyke D.A. & Caldwell M.M. (1997): Perception of neighbouring plants by rhizomes and roots: morphological manifestation of a clonal plant. *Can. J. Bot.* 75: 2146-2157.

Huber-Sannwald E. *et al.* (1998): Effects of nutrient patches and root systems on the clonal plasticity of a rhizomatous grass. *Ecology* 79: 2267-2280.

Humphrey L.D. & Pyke D.A. (1997): Clonal foraging in perennial wheatgrasses: a strategy for exploiting patchy soil nutrients. *J. Ecol.* 85: 601-610.

Jackson R.B. & Caldwell M.M. (1993): Geostatistical patterns of soil heterogeneity around individual perennial plants. *J. Ecol.* 81: 683-692.

Klimešová J. & Klimeš L. (1998): CLOPLA1 (CLOnal PLAnts, version 1) - a database of clonal growth in plants of central Europe. published at <http://www.butbn.cas.cz/klimes/>

Kothari S.K., Marschner H. & George E. (1990): Effect of VA mycorrhizal fungi and rhizosphere microorganisms on root and shoot morphology, growth and water relations in maize. *New Phytol.* 116: 303-311.

Lapointe L. & Molard J. (1997): Costs and benefits of mycorrhizal infection in a spring ephemeral, *Erythronium americanum. New Phytol.* 135: 491-500.

McArthur D.A.J. & Knowles N.R. (1992): Resistence responses of potato to vesicular-arbuscular mycorrhizal fungi under varying abiotic phosphorus levels. *New Phytol.* 100: 341-351.

Mullen R.B. & Schmidt S.K. (1993): Mycorrhizal infection, phosphorus uptake, and phenology in *Ranunculus adoneus*: implication for the functioning of mycorrhizae in alpine systems. *Oecologia* 94: 229-234.

Pregitzer K.S., Hendrick R.L. & Fogel R. (1993): The demography of fine roots in response to patches of water and nitrogen. *New Phytol.* 125: 575-580.

Robinson D. & Van Vuuren M.M.I. (1998): Responses of wild plants to nutrient patches in relation to growth rate and life-form. In: Lambers H., Poorter H. & Van Vuuren M.M.I. [eds.]: *Inherent variation in plant growth. Physiological mechanisms and ecological consequences.* Leiden, The Netherlands: Backhuys Publishers, 237-257.

Robinson D. *et al.* (1999): Plant root proliferation in nitrogen-rich patches confers competitive advantage. *Proc. R. Soc. London, Sect. B: Biol. Sci.* 266: 431-435.

Ryel R.J., Caldwell M.M. & Manwaring J.H. (1996): Temporal dynamics of soil spatial heterogeneity in sagebrush-wheatgrass steppe during a growing season. *Plant Soil* 184: 299-309.

Šmilauer P. (1996): *Modern approaches to analysis of ecological data.* PhD thesis, University of South Bohemia, České Budějovice, Czech Republic.

Taub D. R. & Goldberg D. (1996): Root system topology of plants from habitats differing in soil resource availability. *Funct. Ecol.* 10: 258-264.

van der Heijden M.G.A. *et al.* (1998): Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69-72.

Wijesinghe D.K. & Hutchings M.J. (1997): The effects of spatial scale of environmental heterogeneity on the growth of a clonal plant: An experimental study with *Glechoma hederacea*. *J. Ecol.* 85: 17-28.

Wijesinghe D.K. & Hutchings M.J. (1999): The effects of environmental heterogeneity on the performance of *Glechoma hederacea*: the interactions between patch contrast and patch scale. *J. Ecol.* 85: 860-872.

West H.M., Fitter A.H. & Watkinson A.R. (1993): Response of *Vulpia ciliata* ssp. *ambigua* to removal of mycorrhizal infection and to phosphate application under natural conditions. *J. Ecol.* 81: 351-358.

Zhang H.M. *et al.* (1999): Dual pathways for regulation of root branching by nitrate. *Proc. Nat. Acad. Sci. USA* 96: 6529-6534.

1 What youngsters tell about adults: Do seedling root properties reflect ecological behaviour of adult plants?

Marie Šmilauerová

Summary

This study investigated relationship between root properties of seedlings of grassland plant species grown under glasshouse conditions, and their selected ecological traits, relevant mostly to adults under field conditions. I evaluated average length of exterior and interior root links, magnitude and total length of whole root system, two topological indices, and the leaf area - root length ratio (L:R) for seedlings of 57 species. I used Ellenberg indicator values of soil moisture and available nitrogen for characterisation of species ecological demands, and typology of clonal growth as a characteristic of their growth strategy. Clonal types differed significantly in magnitude and topology of the seedling root systems. Links length and L:R ratio differed between species belonging to clonal types with different origin of clonal growth organs. Ecological preferences for soil moisture had no relation to root morphology, while species with different Ellenberg indicator values for available nitrogen differed in total length, magnitude, and topology of their root system. The grasses differed from the forbs in all measured root traits.

Introduction

Plant species growing together in a grassland community utilise common resources as light, soil water and nutrients, as well as the physical space. It is expected that a long-term coexistence of species is associated with some degree of niche differentiation (Gause 1934, May & Mac Arthur 1972). For plants as sessile organisms this differentiation can include differences in the relative demands for various resources and avoidance in space (different soil horizons) and time of activity (Fitter 1987). Physiological plasticity can be accounted for part of this differentiation, variability in anatomical and morphological structure of plant organs for another one (Fitter 1987, Robinson 1996, Casper & Jackson 1997).

Several of the resources essential for plant existence occur below soil surface, so we can expect a strong evolutionary pressure for differentiation of physiological and structural properties of belowground organs. Over the last two decades, attention of some ecologists has been directed to traits of underground organs, which could play important role in species coexistence in plant communities. Gross et al. (1992) studied morphological properties of roots of plant species with different life histories. They found that annuals produced longer and more branched roots than biennials and perennials, and that grasses allocated more biomass to roots than the dicots. Their experiment was too short (12 days) for extensive root system development, so roots only started to

branch, and therefore all species produced root systems with herringbone branching pattern. Differences in root characteristics among the grasses and the dicots have been found in several studies. Taub & Goldberg (1996) compared root system topology of plants from habitats with different soil resource availability, and they found root topology of grasses relatively invariant, close to maximally herringbone branching, while dicots from the richer site produced more dichotomously branched roots. Campbell et al. (1991) and Grime (1994) found common British grasses, regardless of their potential status in the community, less precise in root foraging than the dicots. Robinson & Van Vuuren (1998) found that plasticity within root system in response to a nutrient patch depended on life form (grasses vs. forbs) as well as on the growth rate of studied species. Slowly growing species co-ordinated more tightly the growth within their root systems and they had in patches higher nutrient uptake rate per unit root length than the fast growing species, which produced in nutrient patches proportionally more roots. Glimskär (2000) found root topology of the grasses very similar to that of the forbs, but specific root length in the grasses much higher than in the forbs. Two slowly growing forbs in his experiment tended to produce more herringbone root systems under conditions with limited nitrogen supply.

Fitter & Stickland (1991) compared root architecture of species from habitats differing in soil nutrients supply. Dicotyledoneous species from low nutrient habitats produced, in agreement with the prediction, more herringbone branched roots with longer links than dicots from richer habitats while for grasses the root system properties agreed with prediction only in terms of geometry (link lengths). Nicotra et al. (2002) studied seedling root anatomy and morphology in relation to rainfall in the areas inhabited by those species. They found relation between rainfall quantity on one hand and proportional allocation into the main root axis, its diameter and areas of stele and xylem, as well as root topology and magnitude on the other hand.

Another trait in which plants occupying one habitat can differ is the type of clonal growth (Antos & Zobel 1984, Klimeš et al. 1997). Martínková (1999) found type of clonal growth exhibiting much larger variability among grassland species than other ecological traits she studied. Klimeš et al. (1997) described twenty-one types of clonal growth for species in central Europe, which can be used, with minor corrections, in other areas of the temperate zone and in the Arctic and Subarctic. In their classification, the origin of clonal growth organ, the initial, and the resulting position of daughter ramets with respect to the soil surface play important role. Humphrey & Pyke (1997) compared exploitation strategy of two closely related subspecies with different clonal type. They found root growth as important as ramet placement for exploitation of nutrients from soil microsites.

In this study, I analysed morphological and topological root characteristics of seedlings of a larger set of grassland species, in relation to ecological demands and clonal type of these species. Because soil moisture and nitrogen availability during growing season are important limiting soil resources in grassland communities, I used Ellenberg' indicator values (Ellenberg 1988), describing the position of studied species on the gradients of soil moisture and available nitrogen

for characterisation of species ecological demands. I used typology of clonal growth introduced by Klimeš et al. (1997).

The primary question of this study was whether differences in these ecological traits, referring to adult individuals of studied species growing under field conditions, are reflected already in differentiation of root characteristics of seedlings growing under artificial conditions. I consider this question very important, given the fact that, on one hand, most descriptors of ecological behaviour of plant species refer to adult individuals and, on the other hand, comparative experimental studies usually work with juvenile plants.

Methods

Seeds of 80 grassland species (produced by Planta naturalis, Markvartice u Sobotky, Czech republic) were sown on sterile sand in Petri dishes. Five to seven days old seedlings were replanted to containers filled with the perlite substrate. In the beginning of the experiment, each species had four replicates. Individual plants were arranged into blocks, consisting of single replicates of each species. The position of individual species within the blocks was completely randomised. Seedlings were grown in an unheated greenhouse without supplementary light, watered as needed, and supplied with commercial nutrient solution *Univerzal nové KH* (Explantex Vondruš, Czech Republic; with N:P:K ratio 7.2 : 4.2 : 9) at two times - immediately after replanting and then after two weeks.

Because of higher mortality of some species during the experiment, they had less than two replicates at the end of experiment. All remaining seedlings were harvested three weeks after transplantation and gently washed. Root system of each plant was carefully spread over the glass plate and its image, together with the aboveground parts, was scanned using a standard flatbed scanner (UMAX Astra 600S). Root architecture, recorded in the image files, was evaluated using the RootArch software (P. Šmilauer, unpublished). I evaluated several root morphological characteristics for each root system: **ELL** - the average length of exterior links, **ILL** - the average length of interior links, **μ** - the magnitude of the root system, **TotL** - the total length of the root system, and two topological indices **log(p_e):log(μ)** and **DBI** (Šmilauerová & Šmilauer, manuscript, see below for definition). All the characteristics, except DBI, are described in Fitter (1991). Primary root morphological characteristics are defined in Table 1.

Acronym	Characteristic	Definition
EL	exterior link	terminal part of root between the root tip with meristem and the nearest branching point
IL	interior link	root part joining other links, i.e. the part of root between any adjacent branches
μ	magnitude	number of exterior links (i.e. root tips) served by a root
TotL	total length of root	sum of the lengths of all exterior and interior links of the root, expressed in millimetres
p_e	total exterior path length	sum of the number of links in all paths from any exterior link to the base of the root
$max(p_e)$	maximal total exterior path length	total exterior path length of imaginary root of given magnitude if fully herringbone-style branched
$min(p_e)$	minimal total exterior path length	total exterior path length of imaginary root of given magnitude if fully dichotomously branched

Table 1: Definitions of primary root morphological characteristics used in this paper.

Topological index called **dichotomous branching index** (DBI) is calculated for a particular root system with the magnitude μ and the total exterior path length p_e as

$$DBI = [p_e - min(p_e)] : [max(p_e) - min(p_e)]$$

where $min(p_e)$ and $max(p_e)$ are, respectively, the minimum and maximum values of total exterior path length (p_e) that a root system can achieve for a particular magnitude.

DBI shows the relative position of the actual total exterior path length value of a root between the reference values $min(p_e)$ and $max(p_e)$. Its values are therefore between 0 and 1 and so it is easier to estimate the position of the root on the scale between fully dichotomously and fully herringbone-style branched roots of given magnitude.

Leaf areas were estimated from the scanned images of aboveground parts of individual seedlings. Ratio of the leaf area to the total root length (L:R ratio) was calculated separately for each seedling. All characteristics measured on seedlings (including the L:R ratio) are called **root traits** in the following text.

Dominant clonal types (sensu Klimes et al. 1997) and habitat preferences (using the Ellenberg indicator values for moisture - F and nutrients - N, Ellenberg 1988) for each species were excerpted from literature (Klimešová & Klimeš 1998, Ellenberg 1988). When a species can exhibit more than one type of clonal growth, only those types, which can be found under usual field conditions, were used for the analyses. The clonal types, which were found only in one

species, were brought together to the clonal type category *Tother*. Brief definitions of clonal types used in this study are presented in Table 2. Only species with at least two individuals were used for statistical analyses. For the final list of species, see Table 3. The differences in root properties, explainable by type of clonal growth and by the two indicator values were evaluated on subsets of the whole dataset, removing species with unknown values of the particular ecological characteristic.

In the case of Ellenberg indicator values, also the species with "indifferent behaviour" (i.e. wide tolerance to soil moisture or nitrogen availability) were omitted. To test, whether this deletion introduced bias into analyses, a multivariate hypothesis of no difference in root properties between the indifferent species and species with a narrower amplitude was also tested (using RDA, see below).

Relations between the root traits and ecological properties of studied species were analysed using a two-fold approach:

1. First, a multivariate statistical method was used to visualize and test the explanatory power of particular predictor (grasses vs. forbs, type of clonal growth, or the two Ellenberg indicator values) in respect to all response variables (representing the measured or calculated root traits). I used the redundancy analysis (RDA) which can be viewed as a constrained form of multivariate multiple regression (Ter Braak & Šmilauer 1998). The advantage of this approach lies in the fact that the inter-dependencies between various response variables are not ignored and also that the statistical test on null hypothesis (of no difference or no linear relation) is tested using a permutation test, which does not rely so much on distributional properties of the response variables. The results of a RDA are portrayed in ordination diagram, which attempts to summarise common patterns in response of root properties to particular explanatory variables.

   During the test, the hierarchical character of the data had to be taken into account: while the measurements of root system properties were taken on individual seedlings, the ecological traits refer to the particular plant species to which a seedling belongs. To test properly their relation, whole groups of seedlings belonging to the same species had to be permuted, using the split-plot design arrangement (Ter Braak & Šmilauer 1998). Therefore, the number of seedlings available for each species had to be unified. Three specimens per species were selected as a reasonable compromise: six species represented by only two seedlings were omitted and for the species with four seedlings, one specimen was selected randomly (using pseudo-random number generator) for omission. The reported estimates of Type I error from multivariate tests therefore refer to such subsets, while the ordination diagrams visualize the relations in the original dataset.

clonal type	Properties			
T0a	non-clonal annuals and biennials			
T1	main root of PRS without adventitious roots and buds; old genets disintegrate into ramets bearing parts of the main root and one or few shoots; bud bank is on the perennial bases of shoots		Root-derived OCG	
T2	main root of PRS with adventitious buds; fragmentation in the same way as in T1; bud bank is on hypocotyle and/or on tap root			
T3	lateral roots of PRS or adventitious roots with adventitious buds; the lateral and adventitious roots decay within a few years causing fragmentation of the mother plant			
T6	turf graminoids	below-ground stems formed above-ground (epigeotropic rhizomes); nodes bear green leaves, internodes are short; bad bank and roots are on rhizomes	Long-lived OCG	Stem-derived OCG
T7	rhizomes <10 cm in length			
T8	rhizomes> 10 cm in length			
T9	rhizomes <10 cm in length	below-ground stems formed below-ground (hypogeotropic rhizomes); horizontally growing part of the rhizome bears bracts and few roots at nodes and has long internodes; after some time it starts to grow vertically and forms above-ground shoots		
T10	rhizomes > 10 cm in length			
T11	above-ground stems are specialised for horizontal spreading, they may bear roots, leaves and buds		Short-lived OCG	
T13	characteristics are the same as for the types T9 and T10 except for longevity			

Table 2: Characteristics of the clonal types used in this study (full description can be found in Klimeš et al. 1997); OCG - organ of clonal growth, PRS - primary root system

Species	Family	F	N	Clonal type	Replications
Pimpinella saxifraga L.	*Apiaceae*	3	2	T7	2
Achillea millefolium L	*Asteraceae*	4	5	T7, T10	4
Achillea ptarmica L	*Asteraceae*	8	4	T10	2
Carlina acaulis L.	*Asteraceae*	4	2	T1, T2	4
Centaurea jacea L.	*Asteraceae*	NA	NA	T9	3
Crepis biennis L.	*Asteraceae*	5	5	T0a	4
Hieracium pilosella L.	*Asteraceae*	4	2	T8, T11, Tother	3
Leucanthemum vulgare L.	*Asteraceae*	4	3	T8	3
Tragopogon pratensis L.	*Asteraceae*	4	6	x	4
Myosotis nemorosa BESSER	*Boraginaceae*	8	5	T13	4
Campanula patula L.	*Campanulaceae*	5	4	T0a	3
Campanula rotundifolia L.	*Campanulaceae*	4	2	T8	3
Dianthus carthusianorum L.	*Caryophyllaceae*	3	2	T1	3
Dianthus deltoides L.	*Caryophyllaceae*	4	2	T8	3
Lychnis viscaria L.	*Caryophyllaceae*	NA	NA	T7	3
Scabiosa ochroleuca L.	*Dipsacaceae*	3	3	T1	3
Anthyllis vulneraria L.	*Fabaceae*	3	3	T1	3
Coronilla varia L.	*Fabaceae*	4	3	T3, T9	3
Lotus corniculatus L.	*Fabaceae*	4	3	T1	4
Lotus uliginosus SCHKUHR	*Fabaceae*	8	4	T1	4
Trifolium aureum POLLICH	*Fabaceae*	3	2	T0a	4
Trifolium dubium SIBTH.	*Fabaceae*	5	4	T0a	4
Trifolium pratense L.	*Fabaceae*	NA	NA	T1	4
Geranium palustre L.	*Geraniaceae*	7	8	T7	3
Geranium pratense L.	*Geraniaceae*	5	7	T8	3
Hypericum perforatum L.	*Hypericaceae*	4	NA	T3, T7, T11	3
Thymus pulegioides L.	*Lamiaceae*	4	1	Tother	4
Plantago lanceolata L.	*Plantaginaceae*	NA	NA	T7	3
Agropyron caninum (L.) P. B.	*Poaceae*	6	8	T6	4
Agrostis tenuis SIBTH.	*Poaceae*	NA	3	T10, T11	4
Alopecurus pratensis L.	*Poaceae*	6	7	T9	2
Arrhenatherum elatius (L.) J. et C. PRESL	*Poaceae*	5	7	T9	3
Avenochloa pubescens (HUDS.) HOLUB	*Poaceae*	NA	4	T7	2
Briza media L.	*Poaceae*	NA	2	T7, T9	3
Bromus erectus HUDS.	*Poaceae*	3	3	T6	3
Dactylis glomerata L.	*Poaceae*	5	6	T7, T9	3
Festuca ovina L.	*Poaceae*	3	NA	T6, Tother	3
Festuca rubra L.	*Poaceae*	NA	NA	T6, T10	4
Holcus lanatus L.	*Poaceae*	6	4	T6	4
Molinia caerulea (L.) MOENCH	*Poaceae*	7	2	T7	4
Poa angustifolia L.	*Poaceae*	3	3	T6	3
Poa palustris L.	*Poaceae*	9	7	T7, T11	4
Poa pratensis L.	*Poaceae*	5	6	T6, T10	4
Trisetum flavescens (L.) P. B.	*Poaceae*	NA	5	T6	3
Rumex acetosa L.	*Polygonaceae*	NA	NA	T7	4
Lysimachia vulgaris L.	*Primulaceae*	8	NA	Tother	4
Filipendula ulmaria (L.) MAXIM.	*Rosaceae*	8	4	T8	3
Filipendula vulgaris MOENCH	*Rosaceae*	4	2	T8	4
Potentila argentea L.	*Rosaceae*	2	1	T7	3
Potentila erecta (L.) RAUSCHER	*Rosaceae*	NA	2	T7	2
Sanguisorba minor SCOP.	*Rosaceae*	3	2	T7	4
Galium mollugo L.	*Rubiaceae*	5	NA	T10	3
Galium pumilum MURRAY	*Rubiaceae*	4	2	T8	3
Galium wirtgenii F. SCHULTZ.	*Rubiaceae*	4	3	T10	3
Veronica chamaedrys L.	*Scrophulariaceae*	4	NA	T13	2
Veronica officinalis L.	*Scrophulariaceae*	4	4	T11	3
Viola canina L.	*Violaceae*	4	2	T2, T7	4

Table 3: Final list of studied species (with at least two individuals at the end of experiment), their membership in families, Ellenberg indicator values for moisture (F) and nutrients (N) (Ellenberg 1988), prevailing types of clonal growth (Clonal type), and number of individuals evaluated at the end of experiment (Replications). NA - indifferent behaviour, i.e. wide amplitude or different behaviour in different parts of Europe; x - value not available in database. Species nomenclature after Rothmaler (1976).

The total variability of relations among the measured root traits was additionally summarised using principal component analysis (PCA, Ter Braak & Šmilauer 1998) on correlation matrix (i.e. with individual variables centered and standardised).

All the multivariate analyses, including the tests of multivariate hypotheses were performed using the Canoco for Windows 4.0 software (Ter Braak & Šmilauer 1998).

2. I supplemented the multivariate approach with the traditional parametrical tests, using general linear models (GLM) and classical significance tests. The hierarchical structure of the data was taken into account - an additional error level corresponding to separate plant species was added.

The general linear models were fitted using the S-Plus 4 software (Anonymous 1997).

The analyses performed using the methods outlined in the preceding text do not take into account the possibility that the differences in root system properties, correlated with differences in ecological traits are, in fact, both influenced by a third factor, namely the (partially) shared evolutionary history. The often-adopted approaches for adjusting the tests for the evolutionary relatedness of studied plant species (see Harvey & Pagel 1995) necessitate different selection of compared taxa. Because such a selection would conflict with the aim of this paper (to provide balanced overview of root system properties of common grassland plant species), an alternative approach to testing the effects of ecological characteristics, which are independent of the evolutionary relatedness, was adopted. I assumed that the relatedness of two species can be solely characterised by their membership in the same family. Further, I supposed that a significant relation between a particular environmental trait and root system properties, detected at the **within-family** level would provide reasonable support for the research hypothesis that root system properties are varied due to different ecological preferences even among the species sharing substantial part of their evolutionary history. These tests at within-family level were performed using the multivariate approach, by accounting for the differences in root traits among the families and then evaluating the effect of selected explanatory variable by permuting observations within the individual families. This approach cannot be used with families represented only by one (or very few) species, so these tests were performed on dataset where only the observations from families represented by more than three species were retained (species from families *Asteraceae, Fabaceae, Poaceae,* and *Rosaceae*).

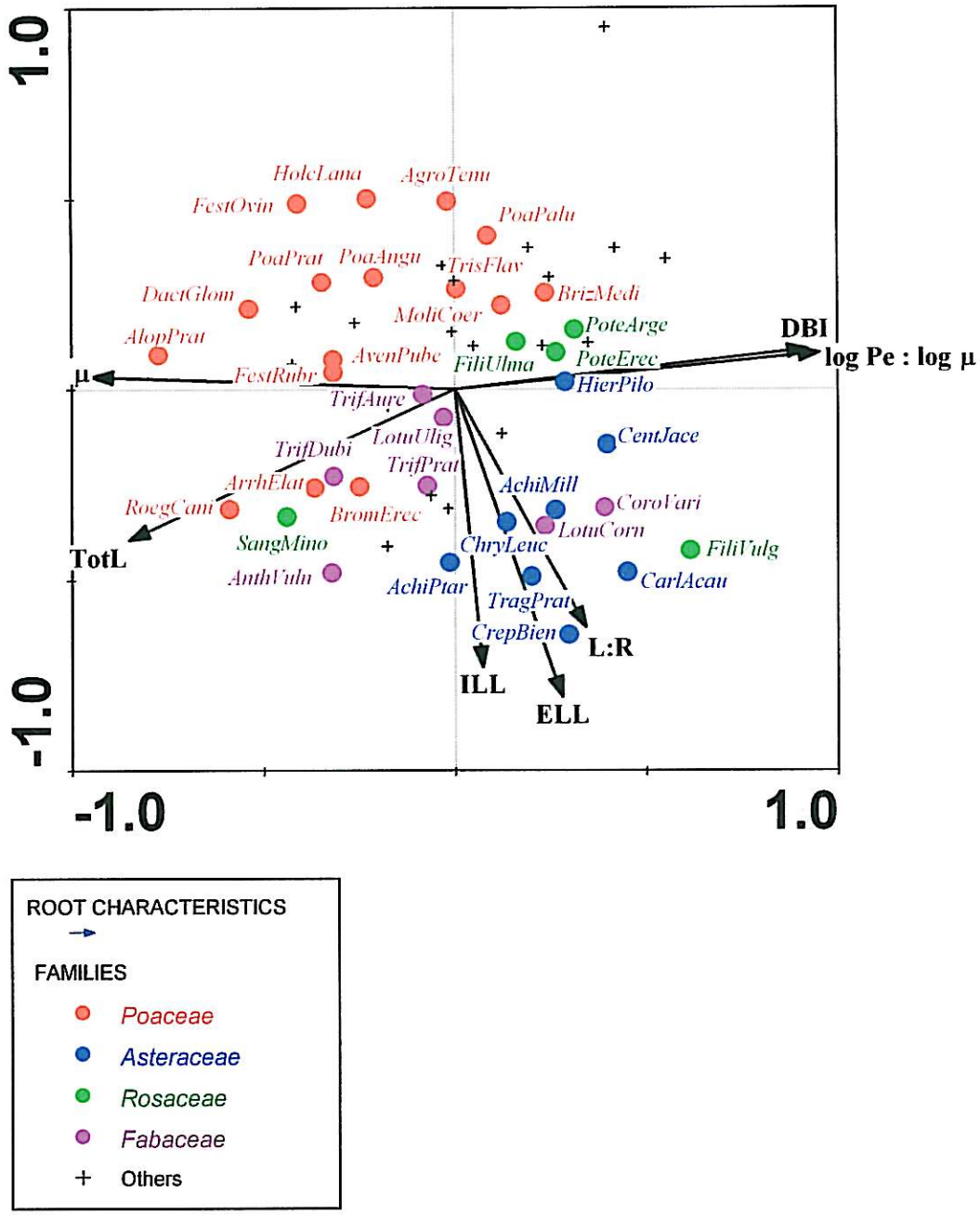

Figure 1: Differences in root traits among the grassland plant species, summarised by the first two axes of PCA. Only the species from the four families represented by at least five species are identified in the plot. Approximate ordering of species in respect to averages of particular root characteristic can be read by perpendicularly projecting species circles onto the variable' arrow.

Results

Figure 1 shows ordination diagram from PCA on complete dataset (with all plant species). The first two axes explain 76% of the total variability. The first ordination axis may be interpreted as a gradient of changes in the architectural parameters DBI and log(Pe) : log(μ), increasing from left to right, and also as a gradient of the magnitude of root systems (μ) increasing in the opposite direction. The second ordination axis is correlated with a change in average length of interior and exterior links (ILL and ELL), increasing from top to bottom. Position of studied plant species is only passively projected into this diagram and membership of species to the four most numerous families is marked. The three families of dicotyledons do not differ substantially, although some trends can be seen. The *Asteraceae* had the longest links and the largest L:R ratio, the *Fabaceae* produced the most branched roots (high μ and lower values of topological indices) and the *Rosaceae* had long, mostly herringbone branched root systems. More apparent differences can be seen between the forbs and the grasses, mainly in the average length of links and in L:R ratio. Range of values of evaluated root traits is summarised in Table 4.

	ILL [mm]	**ELL [mm]**	**μ**	**TotL [mm]**	**L:R**	**log P_e : log μ**	**DBI**
minimum	0.7	1.1	3	34.0	0.01	1.6	0.1
Q1	2.9	4.6	12	126.5	0.2	1.7	0.3
median	3.7	6.3	20	203.0	0.4	1.7	0.5
Q3	4.6	8.3	39	346.5	0.5	1.8	0.8
maximum	11.0	22.0	123	1317.0	1.5	1.9	1.0

Table 4: Range of values of root traits over all species. Q1, Q3 - lower and upper quartile, ILL (ELL) - the average length of interior (exterior) links, μ - the magnitude of the root system, TotL - the total length of the root system, L:R - ratio of the leaf area to the total length of the root system, log(p_e):log(μ) and DBI - topological indices. For definitions of primary root morphological characteristics see Table 1, definition of DBI can be found in Methods.

Several partial RDA-s were used to separate the effects of individual explanatory variables (Figures 2 to 4). Table 5 summarises the marginal tests of individual response variables, based on general linear models.

The root morphology of grasses differed significantly from the root morphology of forbs (Figure 2, P=0.001). The canonical ordination axis explains 9.2 % of the total variability. The individual tests based on GLMs indicate significant differences in all root traits (Table 5). The forbs had longer interior and exterior links, but because the magnitude of their root systems was substantially lower than for grasses, they had lower total root length. The forbs also had a higher L:R ratio and less dichotomously branched roots.

	log μ		log TotL		log ILL		log ELL		log DBI		log P_e : log μ		L:R	
Forbs	$<10^{-3}$ (14.74)	⇓	<0.05 (3.99)	⇓	<0.05 (4.16)	⇑	$<10^{-3}$ (15.75)	⇑	0.005 (8.42)	⇑	0.004 (9.04)	⇑	0.001 (11.98)	⇑
Clonal Type	0.023 (2.33)		n.s.		n.s.		n.s.		0.05 (2.01)		0.037 (2.13)		n.s.	
N	0.01 (7.15)	⇑	0.007 (7.86)	⇑	n.s. (0.03)		n.s. (0.10)		$<10^{-3}$ (15.65)	⇓	$<10^{-3}$ (13.61)	⇓	n.s. (0.98)	
F	n.s. (0.02)		n.s. (0.48)		n.s. (0.25)		n.s. (1.94)		n.s. (1.26)		n.s. (0.98)		n.s. (0.09)	

Table 5: The results of partial tests of relations between the individual root system characteristics and different ecological traits of studied species. The tests are based on a fitted general linear model. The entries show test significance, together with the calculated F statistics (in parenthesis). Model DFs and residual DFs are (1,55), (10,45), (1,44), and (1,44) for, respectively, Forbs, Clonal Type, N, and F predictors. Arrows indicate direction of significant relations (upwards pointing arrow in Forbs row implies higher value for forbs, compared with grasses). ILL (ELL) - the average length of interior (exterior) links, μ - the magnitude of the root system, TotL - the total length of the root system, L:R - ratio of the leaf area to the total length of the root system, log(p_e):log(μ) and DBI - topological indices. For definitions of primary root morphological characteristics see Table 1, definition of DBI can be found in Methods.

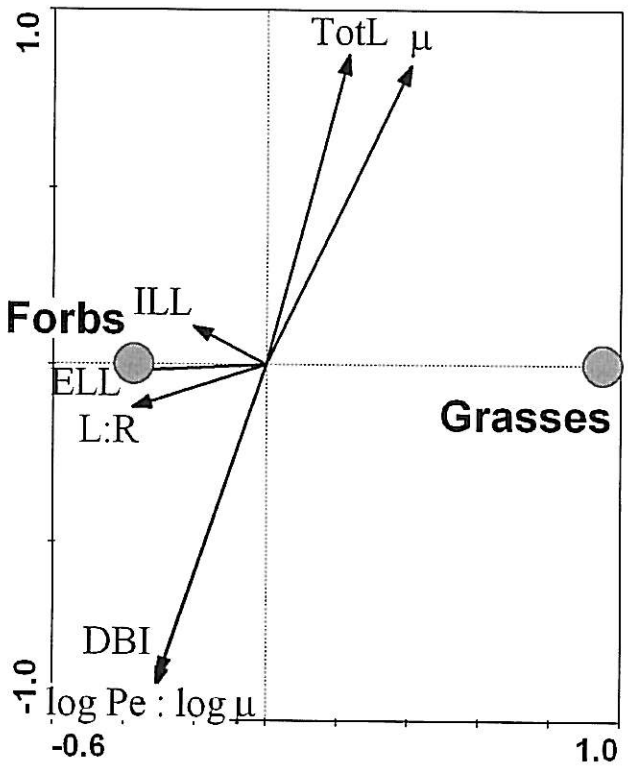

Figure 2: Difference in root traits between forb and grass species, summarised by RDA method. As only the first axis is constrained, the root trait arrow tips should be projected on horizontal axis.

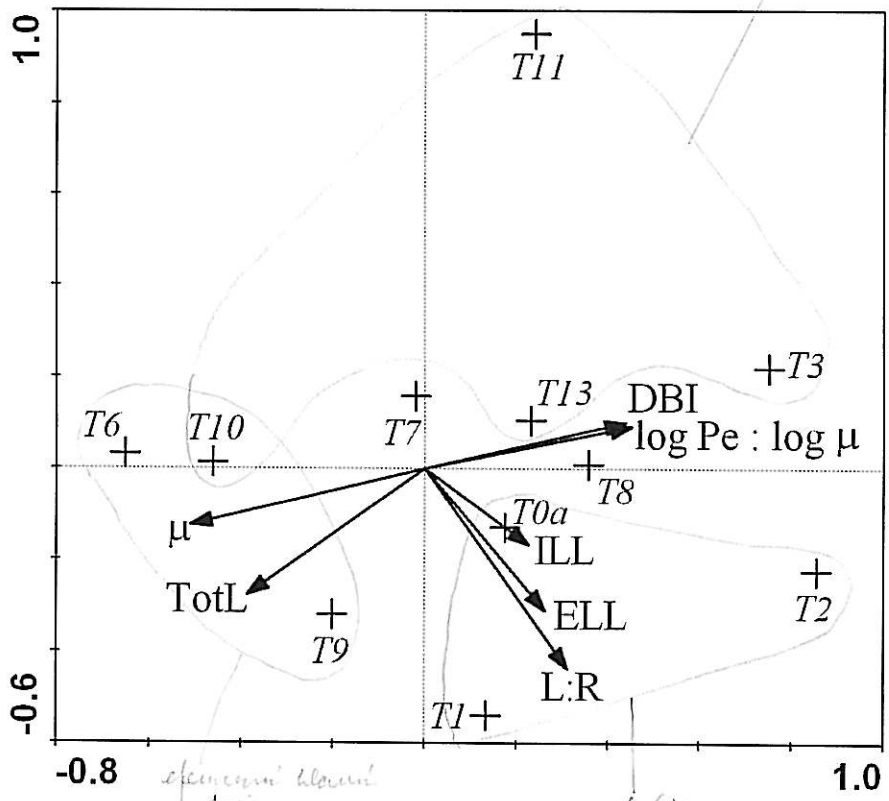

Figure 3: Differences in root traits between individual clonal growth types, summarised using the RDA method. Approximate ordering of clonal type categories in respect to particular root trait can be read by perpendicular projection of the crosses onto the particular arrow.

Figure 3 shows an ordination diagram from analysis focusing on differences in root properties among the clonal types. The differences are significant at the level P = 0.032. The first two canonical ordination axes explain 20.5%. of the total variability in root system properties. The species with different type of clonal growth differed significantly in magnitude and topology of root systems (Table 5). Some trends can be deduced from the Figure 3. Species with clonal growth types T2 and T3 (both with root origin of organs of clonal growth) had root branching restricted mainly to the main root (the herringbone branching architecture), and the least branched roots (the smallest magnitude). They had, together with species of clonal type T1, the longest links and the highest L:R ratio. Root systems of clonal type T6 (turf grasses) had opposite properties - short links, long, largely dichotomously branched roots with numerous root tips, and small L:R ratio. Types T9 and T10 (with organs of clonal growth originating belowground from belowground stems) had also long, intensively and predominantly dichotomously branched roots. Clonal type T11 (with aboveground creeping stems) had the shortest links, relatively short, poorly branched roots, and the smallest L:R ratio.

When the differences of root properties among the clonal types were evaluated at the within-families level, they were not found significant.

I did not find significant relation of any root trait to the Ellenberg indicator value for moisture (F) (Table 5). Relation of species to the nitrogen availability during the vegetative period (N) had, on the other hand, a significant effect upon several root traits (Table 5). Topology of root systems, the total length, and the magnitude of roots were the root traits most closely related to species occurrence on the gradient of nitrogen availability (Figure 4). Species from nutrient-rich habitats had more dichotomously branched and longer roots, with more abundant root tips. Length of interior and exterior links and the L:R ratio were not related to this indicator value.

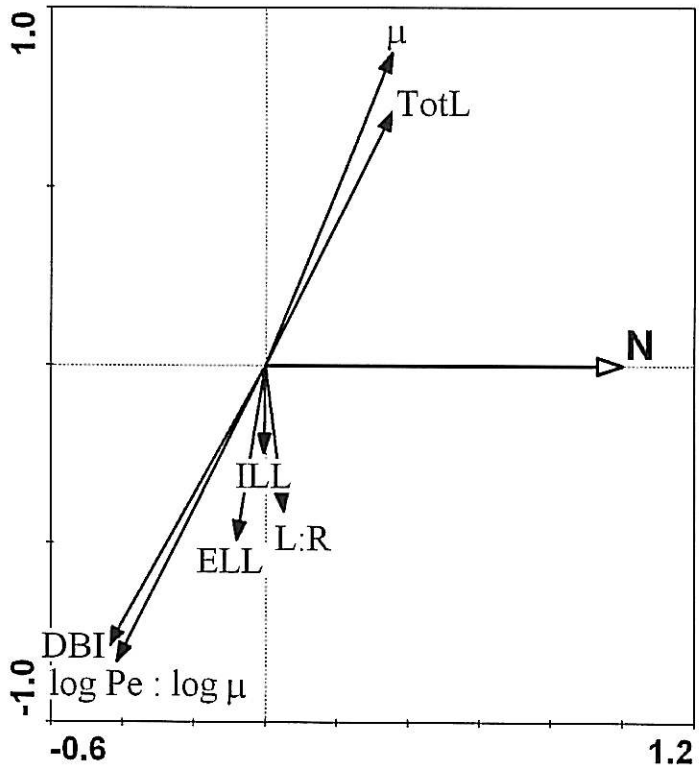

Figure 4: Effect of position of species on the gradient of soil nitrogen availability (measured by Ellenberg N value) on the measured root traits. Correlation between the nitrogen availability indicator value and particular root trait is approximated by the cosine of angle between the two corresponding arrows.

When the effect of N indicator values upon root properties was evaluated at the within-families level, it was found nearly significant, at $P = 0.06$.

When I tested for differences in root traits between the species with a particular position on the gradient of nitrogen availability and the "indifferent species" (with no particular indicator value allocated), no significant difference was found.

Discussion

The ordination diagram of PCA (Figure 1) summarises relations between root traits of more than fifty grassland species, measured on young individuals. While root topology was strongly

correlated with the root system size (the larger root systems were more dichotomously branched), the average length of both interior and exterior links was relatively independent of those characteristics. The above-mentioned relation between root system size and topology can be possibly explained by differences in growth rate among species. At the end of a short-term experiment (as the presented one is), species with lower growth rate of roots would not be able to reach such root branching order as species growing faster (Gross et al. 1992).

I found the most extensive differences between studied species, when I divided them to the grasses and the forbs. This finding agrees with the previous studies (e. g. Fitter et al. 1988, Taub & Goldberg 1996), but one specific conclusion from my experiment differs. The grasses had more dichotomously branched roots than the forbs in my experiment, while Fitter et al. (1988), Fitter & Stickland (1991), and Taub & Goldberg (1996) found root topology of the grasses more herringbone in comparison with the forbs. Gross et al. (1992) and Glimskär (2000) did not find significant difference in root topology between grasses and forbs, but the former study found that grasses allocated greater proportion of biomass to roots and tended to produce more root length per day than dicots, what is in agreement with my results.

Fitter (Fitter 1991, Fitter et al. 1991) predicted that species from nutrient-rich habitats will have root systems with more dichotomous topology, while herringbone topology (which is more expensive and more efficient in the nutrient acquisition) would be favoured in nutrient-poor sites. These predictions are supported by the presented study. Seedlings of species from habitats with higher nitrogen availability had, at the same time, longer roots and more root tips then species from habitats with less available nitrogen. Such type of root growth can be viewed as an attribute of species from nutrient-rich sites. On the other hand, this can be only a consequence of higher growth rate of such species (Grime 1994, Fransen 1999), which create more root length per day and their roots can exhibit branching earlier than the roots of more slowly growing species.

Fitter et al. (1991) predicted that plants growing under higher nutrient availability will have shorter root links and supported their hypotheses by experimental results (Fitter & Stickland 1991). Several other studies found link length sensitive to increased nutrient availability while root topology was insensitive at the same time (Fitter et al. 1988, Fitter 1994). In my experiment, the length of either interior or exterior links did not change with the position of species optimum on the gradient of nitrogen availability. This finding did not give evidence about insensitivity of link length to actual nitrogen availability because all species had comparable nutrition regime in my experiment. However, this result suggests that a long-termed existence of species in habitats with different nitrogen availability does not act selectively onto link length of seedling roots.

Soil moisture preferences of studied species did not influence significantly the seedling root properties. It is possible that root morphological adaptations to high or low soil moisture manifest themselves either in older plants or only when plants are exposed to such different conditions. It is also possible that under water stress more important role is played by the root physiological plasticity than by their morphological adaptations.

Klimeš et al. (1997) compared distribution of clonal types among plant communities and explained some differences in their occurrence in ecosystems by their ecological traits and by specific environmental conditions. Although several studies dealt with plasticity of clonal organs of species in heterogeneous soil environment (e.g. Humphrey & Pyke 1997, Wijesinghe & Hutchings 1997, Huber-Sannwald et al. 1998, Kleijn & van Groenendael 1999), relation between properties of clonal organs and root morphology (and its plasticity) has never been studied. Results of my experiment show that species differing in type of clonal growth differed also in root traits of seedlings. Species with root origin of clonal growth organs (T1, T2 and T3) had the longest root links and the highest L:R ratio. Extreme topology of root systems (fully herringbone) of clonal types T2 and T3 can be a consequence of slower root growth, because species of these two types had at the same time the shortest root systems, with the smallest magnitude.

Clonal type T6 (turf grasses) differed from the others mainly in root topology, magnitude, and total root length. Vegetative spreading of those turf grasses, which use only this type of clonal growth, is slow because their rhizomes with short internodes serve as storage organs and bud bank rather than for spreading. Root traits found in these species can enable them to colonise quickly soil volume in a close neighbourhood of the seedling. The fact that some turf grasses, e.g. *Festuca rubra* or *Poa pratensis*, can also spread by fast-growing rhizomes (clonal type T10), was not much reflected in their root traits. Although clonal types T6 and T10 differ in clonal growth characteristics (see Table 2) root traits of species belonging to them were very similar. The species of clonal type T11 (the only ones with aboveground creeping stems) differed apparently from the species of other clonal types with stem-derived organs of clonal growth. Found combination of their root traits (short, poorly branched roots with short links) can be explained by the characteristics of their clonal growth (above-ground stems, specialised for fast spreading) or by a very slow growth of these species in my experiment (small total length of roots together with small L:R ratio).

Grassland species used in my experiment belonged to 18 families, but only four families were represented by more than three species. When I tested whether the relation of ecological characteristics with the root traits was independent of the evolutionary relatedness at the within-family level, I found the differences between clonal types non-significant, and the effect of nitrogen availability indicator nearly significant. These findings can be explained in several ways:

1. the partial, within-family tests had lower power than the tests ignoring the phylogenetic relatedness
2. although species differed in their ecological demands and in clonal types also within families, the range of their ecological traits (predictor variables) was on this level narrower than for the whole set (e.g. species in *Poaceae* belong only to clonal types T6, T7, T9, T10, and T11) and some indicator values or some clonal types were restricted only to one or few families (e.g. clonal type T6 to *Poaceae* family)

3. root traits (the response variables) were less variable on the within-family level (e.g. grasses differed significantly in all measured root traits from the other families)
4. finally, based on the data, I cannot exclude the possibility that the relations are purely consequence of common evolutionary history without an adaptive significance, although this seems very improbable.

Conclusions

In this study, I found significant relationship between ecological demands or growth strategies of grassland plant species and root properties of their seedlings. Species differing in Ellenberg indicator value for nitrogen possessed significantly different root traits. Larger nitrogen demand of species at their adult stage was connected with longer, more intensively and more dichotomously branched roots of seedlings. This finding could be explained by higher root growth rate of species from more nutrient rich habitats. Average length of interior and exterior root links was independent of nitrogen demand of species, which contradicts other published findings. I did not find relation of root traits to species preferences in respect to soil moisture. The clonal architecture of species was significantly correlated with the seedling root properties. The plants with different type of clonal growth differed significantly in topology and magnitude of their root systems. The origin of clonal growth organs was related mainly to the average length of root links and to L:R ratio, and plants spreading by aboveground stems had poorly developed root systems of their seedlings. This study also confirmed the already known differences between root properties of grasses and forbs.→viz klonalita

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References

Anonymus (1997): S-PLUS 4 Guide to Statistics. Data Analysis Products Division, MathSoft, Seattle

Antos J.A. & Zobel D.B. (1984): Ecological implications of belowground morphology of nine coniferous forest herbs. *Bot. Gaz.* 145: 508-517.

Campbell B.D., Grime J.P. & Mackey J.M.L. (1991): A trade-off between scale and precision in resource foraging. *Oecologia* 87: 532-538.

Casper B.B. & Jackson R.B. (1997): Plant competition underground. *Annu. Rev. Ecol. Syst.* 28: 545-570.

Ellenberg H. (1988): *Vegetation of central Europe.* - Cambridge University Press, Cambridge.

Fitter A.H. (1987): Spatial and temporal separation of activity in plant communities: prerequisite or consequence of coexistence? In: Gee J.H.R. & Giller P.S. [eds.]: *Organization of communities. Past and present.* Blackwell Sci. Publ., Oxford, pp. 119-137.

Fitter A.H. (1991): Characteristics and functions of root systems. In: Waisel Y., Eshel A. & Kafkafi U. [eds.]: *Plant roots: the hidden half.* New York, USA: Marcel Dekker, Inc., 3-25.

Fitter A.H. (1994): Architecture and biomass allocation as components of the plastic response of root system to soil heterogeneity. In: Caldwell M.M. & Pearcy R.W. [eds.]: *Exploatation of Environmental Heterogeneity by Plants.* San Diego, USA: Academic Press, Inc., 305-323.

Fitter A.H., Nichols R. & Harvey M.L. (1988): Root system architecture in relation to life history and nutrient supply. *Funct. Ecol.* 2: 345-351.

Fitter A.H. & Stickland T.R. (1991): Architectural analysis of plant root systems 2. Influence of nutrient supply on architecture in contrasting plant species. - *New Phytol.* 118: 383-389.

Fitter A.H., Stickland T.R., Harvey M.L. & Wilson G.W. (1991): Architectural analysis of plant root systems. 1. Architectural correlates of exploitation efficiency. *New Phytol.* 118: 375-382.

Fransen B.L.L. (1999): *Root foraging. The consequences for nutrient acquisition and competition in heterogeneous environments.* PhD thesis, Wageningen University, Wageningen, The Netherlands.

Gause G.F. (1934): *The struggle of existence.* Reprinted 1964. Hafner, New York.

Glimskär A. (2000): Estimates of root system topology of five plant species grown at steady-state nutrition. *Plant Soil* 227: 249-256.

Grime J.P. (1994): The role of plasticity in exploiting environmental heterogeneity. In: Caldwell M.M. & Pearcy R.W. [eds.]: *Exploatation of environmental heterogeneity by plants: ecophysiological processes above- and below-ground.* San Diego, USA: Academic Press, Inc., pp. 1-19.

Gross K.L., Maruca D. & Pregitzer K.S. (1992): Seedling growth and root morphology of plants with different life-histories. *New Phytol.* 120: 535-542.

Harvey P.H. & Pagel M.D. (1995): *The comparative method in evolutionary biology.* Oxford University Press, Oxford.

Huber-Sannwald E., Pyke D.A., Caldwell M.M. & Durham S. (1998): Effects of nutrient patches and root systems on the clonal plasticity of a rhizomatous grass. *Ecology* 79: 2267-2280.

Humphrey L.D. & Pyke D.A. (1997): Clonal foraging in perennial wheatgrass - a strategy for exploiting patchy soil nutrients. *J. Ecol.* 85: 601-610.

Kleijn D. & van Groenendael J.M. (1999): The exploatation of heterogeneity by a clonal plant in habitats with contrasting productivity levels. *J.Ecol.* 87: 873-884.

Klimeš L. et al. (1997): Clonal plant architecture: a comparative analysis of form and function. - In: de Kroon H. & van Groenendael J. [eds.]: *The ecology and evolution of clonal plants,* Backhuys Publishers, Leiden, pp. 1-26.

Klimešová J. & Klimeš L. (1998): CLOPLA1 (CLOnal PLAnts, version 1) - a database of clonal growth in plants of central Europe. published at <http://www.butbn.cas.cz/klimes/>

Martínková J. (1999): *Niche differentiation of coexisting grassland species. A multivariate analysis of ecological and morphological traits.* Bc thesis, University of South Bohemia, Ceské Budejovice, Czech Republic.

May R.M. & MacArthur R.H. (1972): Niche overlap as a function of environmental variability. - *Proc. Nat. Acad. Sci. USA* 69: 1109 - 1113

Nicotra A. B., Babicka N. & Westoby M. (2002): Seedling root anatomy and morphology: an examination of ecological differentiation with rainfall using phylogenetically independent contrasts. *Oecologia* 130: 136-145.

Robinson D. (1996): Variation, coordination and compensation in root systems in relation to soil variability. *Plant Soil* 187: 57-66.

Robinson D. & Van Vuuren M.M.I. (1998): Responses of wild plants to nutrient patches in relation to growth rate and life-form. In: Lambers H., Poorter H. & Van Vuuren M.M.I. [eds.]: *Inherent variation in plant growth. Physiological mechanisms and ecological consequences.* Backhuys Publishers, Leiden, The Netherlands, pp. 237-257.

Rothmaler W. (1976): *Exkursionsflora für die Gebiete der DDR und der BRD. Kritischer Band.* Volk und Wissen Volkseigener Verlag, Berlin.

Šmilauerová M. & Šmilauer P. (2001): Field study of morphological response of plant roots to heterogeneity of soil resources. manuscript

Taub D.R. & Goldberg D. (1996): Root system topology of plants from habitats differing in soil resource availability. *Funct. Ecol.* 10: 258-264.

Ter Braak C.J.F. & Šmilauer P. (1998): *CANOCO reference manual and user's guide to Canoco for Windows: software for canonical community ordination (version 4).* Microcomputer Power, Ithaca.

Wijesinghe D.K. & Hutchings M.J. (1997): The effects of spatial scale of environmental heterogeneity on the growth of a clonal plant. - An experimental study with *Glechoma hederacea. J. Ecol.* 85: 17-28.

2 Effect of AM symbiosis exclusion on grassland community composition

Petr Šmilauer & Marie Šmilauerová

Abstract

Experimental data on grassland community response to exclusion of arbuscular mycorrhizal (AM) symbiosis using fungicide benomyl and to phosphate addition were evaluated after four years of continued manipulation using multivariate direct gradient analysis. While the community responded only to phosphate application in the third year (phosphate application suppressing forbs in favour of graminoids), the data from the fourth season indicate relative suppression of some forbs (including *Plantago lanceolata, Cerastium holosteoides*) with both phosphate and fungicide applications. Positive response of graminoids to phosphate addition seems to interact with fungicide application, while an important community constituent - *Achillea millefolium* - seems to profit primarily from fungicide applications. The effect of the fungicide on the AM symbiosis was confirmed on roots of *Plantago lanceolata*. The direct evaluation of frequency of AM fungal structures in plant roots also revealed a diverse response of different plant species to phosphate application. Nevertheless, in all species (except non-mycorrhizal *Luzula campestris*), phosphate decreased frequency of arbuscules and increased frequency of noninfected root segments.

Keywords: benomyl, phosphorus availability, Achillea millefolium, RDA, arbuscular mycorrhiza

Introduction

The mechanisms responsible for structuring plant communities and for maintaining particular level of their diversity represent an important issue in the current ecological research. One of the important factors that might influence structure and composition of plant communities is the mycorrhizal symbiosis between vascular plants and various types of fungi. Among the various kinds of mycorrhizal symbiosis, the most important in non-forest communities is the arbuscular mycorrhizal (AM) symbiosis. Studies of the effects of AM symbiosis on functioning of whole plant communities are still rare and their results are partly controversial (Fitter 1986, Koide et al. 1988, Newsham et al. 1994). Other studies have focused on the effect of AM symbiosis on competitive relations between different plant species and have found a large impact of symbiosis on the competitive balance within plant community (Hartnett et al. 1993, Hetrick et al. 1994, van der Heijden et al. 1998a, 1998b).

The only effective approach to studying influence of AM symbiosis on community level processes is the experimental modification of the studied system using a systemic fungicide suppressing AM symbiosis development (for example Fitter & Nichols 1988, Paul et al. 1989). Because the effect

of the AM symbiosis is traditionally attributed to the acquisition of nutrients with low mobility - namely phosphorus (P) (Bolan 1991, Koide 1991), the experimental exclusion of AM symbiosis might be profitably combined with enhancement of P availability. In this way, the AM symbiosis effects can be contrasted between standard conditions and those with the supposed beneficial effects of the symbiosis being offset by P application.

The effects of AM symbiosis on plant fitness are probably not limited to enhanced P supply, however. One of the possible benefits for a plant symbiont is the protection against fungal root pathogens (Newsham et al. 1995, St-Arnaud et al. 1997). Here also lies the difficulty with the use of a fungicide for excluding AM symbiosis: all the available fungicides suppress, beside the AM fungal symbionts, at least part of the spectrum of pathogenic fungi (Paul et al. 1989). Additionally, other types of soil organisms might be affected by the fungicide applications (Paul et al. 1989, van Faassen 1974).

The objectives of this study were to assess influence of AM symbiosis on the plant community composition of a nutrient-poor grassland and to isolate the effects of the symbiosis on the phosphorus nutrition status of the plant populations by combining, in factorial way, the experimental exclusion of AM symbionts with the addition of supplementary P source.

Materials and Methods

Research site

Our research site is located near Zvíkov village (10 km east of České Budějovice, 48°59'N, 14°36'E, 500 m a. s. l.). The vegetation is an oligotrophic, traditionally managed meadow on a shallow valley slope. The meadow is cut once a year, usually in the mid of June.

The site has been used as a permanent grassland for more than 120 years. Because of its low accessibility for heavy machinery, it seems to have never been ploughed or to have had industrial fertiliser applied. The site is influenced by fertilisers leaching from the adjacent field, but both chemical analyses of the available nutrients in soil (phosphorus and nitrogen, unpublished data) and the plant community composition show a limited extent of that impact. The concentrations of water-soluble P (Watanabe & Olsen 1965) in soil is approx. 30 mg of P per 1 kg of dry soil.

In the context of Zürych-Montpellier classification of plant communities, the research site represents a real mix of community types including moist mesotrophic meadow from alliance *Alopecurion pratensis PASSARGE*, through mesotrophic hay meadows from alliance *Arrhenatherion KOCH*, up to oligotrophic grassland from alliance *Violion caninae SCHWICKERATH* (Moravec et al. 1995).

The plant species nomenclature follows Rothmaler (1976).

Experimental design

The experiment was started in the spring of 1994 by the establishment of two blocks of experimental plots, positioned near the two ends of a clearly visible gradient of soil depth. Two experimental factors (phosphate and benomyl application), each of them with two levels (applied and non-applied), were combined in a factorial way, yielding four experimental treatments. Each block has four replicate plots for each experimental treatment, arranged into a 4 x 4 Latin square. The description of experimental factors is as follows:

a) AM symbiosis was suppressed by (partially) selective fungicide benomyl. This fungicide was applied using the commercial product Benlate (DuPont Company) until 1996; during 1997 the newly approved Fundazol was used, both in the form of 50% WP preparation. From 1998, an alternative fungicide Bavistin (BASF company) was used, which is also based on the benomyl compound. The fungicide was applied every 5 weeks throughout the growing season, in amounts of approx. 8 g of benomyl per square meter (4.5 g . m^{-2} in 1994 and 1995) on each application.

b) enhancement of phosphorus availability to the plant roots was achieved by the application of a sodium phosphate (Na_3PO_4 * 12 H_2O) solution at the beginning (April) and end (September or October) of each season, using approx. 37 g of phosphate (3 g of P . m^{-2}) per application. Controls received equivalent amounts of water at each application time for both benomyl and P.

The individual experimental plots are 1 x 1 m, separated by 0.5 m wide separation zones.

In 1995, three further experimental blocks were placed in different parts of the research site with different community composition and soil type. Two blocks (**B3** and **B4**) are situated on a "plateau" with the same altitude as the adjacent arable field, with deeper soil and a community dominated by tall grasses like *Avenochloa pubescens, Arrhenatherum elatius, Alopecurus pratensis*. These blocks have the same 4 x 4 Latin square design as the original two blocks (**B1** and **B2**). The third block (**B5**) is placed in the part of the research site where community composition corresponds to the most oligotrophic grasslands in the area (with species like *Nardus stricta, Galium boreale, Potentilla erecta, Scorzonera humilis*). To maintain compositional homogeneity of that block' vegetation, we had to adjust its layout, so that the 16 plots were arranged in two parallel linear transects and the plot size was reduced to 0.5 x 0.5 m.

Data collection

The effects of the experimental treatment on community composition were measured using the aboveground biomass dry weight in the peak of the season (end of May to June). Because this is a long-term experiment where sampling should not affect the community dynamics, we used the standard hay-cut event to estimate biomass composition. The aboveground biomass was therefore clipped at a height of approx. 2 cm; clipping the plants at the ground level would have induced irreversible changes in the community composition. The biomass was collected from 0.5 x 0.5 m squares, centered over the 1 x 1 m plots (or representing the whole plot for plots from the **B5** block). Plant biomass was sorted (without differentiating live biomass and standing dead parts) into six functional groups and species of particular interest in our other research projects: **grasses,**

sedges, *Plantago lanceolata*, *Cerastium holosteoides*, *Achillea millefolium*, and **other forbs**. Additionally, the plant litter collected from the plot was used as the seventh category. The biomass was dried to a constant weight at 85°C. Biomass measurements were done on block **B1** in years 1994, 1995, and 1998, and on the other blocks (**B2** - **B5**) only in the years 1997 and 1998. Block **B4** was set aside for study of the hay-cut effect, so that only half of it was sampled. Only the more complete data from years 1997 and 1998 are presented in this paper.

To check directly the effects of both the benomyl and phosphate applications on mycorrhizal infection, AM infection levels were checked in June 1997 on the individuals of selected species. The effect of benomyl on the suppression of AM symbiosis was studied only for *Plantago lanceolata* which is a species known to have high intensity of AM symbiosis under normal conditions (e.g. Sanders & Fitter 1992). The effectiveness of benomyl in suppressing AM infection was an *a priori* expectation for the success of the experiment, while there were only limited assumptions about the effect of phosphate addition on the AM symbiosis. Therefore, the effect of phosphate was further studied (without its interaction with benomyl application) for several species, also including one with generally low to nil AM symbiosis (*Luzula campestris*).

In one of the experimental blocks (block **B1**), one individual of *Plantago lanceolata* was carefully removed with a substantial part of its root system from each of the 16 plots. Similarly, other species (*Poa angustifolia*, *Achillea millefolium*, *Holcus lanatus*, *Luzula campestris*) were sampled from the same block, but using only half of the plots this time, excluding those with benomyl application. The plant roots were washed with tap water and stained using the modified Phillip & Hayman (1970) procedure, with Chlorazol Black E stain. The percentage of arbuscular, vesicular and hyphal infection was estimated using three subsamples from each root system and a combination of Nicon binocular microscope (at magnification 80x) with preceding inspection of the slide on the Olympus research microscope at magnification 100x or 200x. The classification of root segments refers to the prevailing AM fungal structure, not directly to a particular structure under the intersection line (a root intersection point is classified as "arbuscular" if it is surrounded by a root segment where the arbuscular phase of AM symbiosis dominates).

Statistical analysis

Because our attention was focused on the community response, we summarised and tested the effects of the experimental treatments by constrained ordination models - also known as direct gradient analysis (Ter Braak 1994). A linear type of the constrained ordination - redundancy analysis (RDA) was used on the log-transformed data. The constrained models used the experimental factors as explanatory variables. The "nuisance effects" (like the differences between blocks), were removed from the analysis using the corresponding indicator variables as covariates (see Ter Braak & Šmilauer 1998 for details). The data from the year 1997 and 1998 were analysed separately and using two types of analyses:

- In the first type, the log-transformed biomass weight was analysed, with the data matrix being centered by variables' (plant taxonomic categories and plant litter weight) means. This way, the

change in absolute biomass of individual species (categories) can be judged by inspecting the corresponding ordination diagram.

- In the second type of analyses, the biomass weights were transformed to percentages of the total aboveground biomass. In this case, the change in relative importance of particular species can be inferred from the ordination diagram. When analysing percentage data, the log-transformation was applied, followed by centering of data matrix both by sample' and by variable' means. Such analysis corresponds to Aitchison's log-ratio analysis, which allows us to portray correctly the changes in fractional composition of samples (Aitchison 1986).

When analysing the AM infection percentage data, the RDA was done on log-transformed percentages (exact transformation was $X' = \log_{10}(10*X + 1)$), with the response data matrix being centered (by subtracting means) by both rows and columns, as for the percentage biomass data above.

For all the constrained ordination models, any detected effect of particular explanatory variables was first tested using a Monte-Carlo permutation test (Ter Braak & Šmilauer 1998). The permutations were (where appropriate) done only within blocks and 999 randomizations were done on each test run. The reported **P** values are estimates of the Type I Error probability. If the effect of the explanatory variables (factors) provided was found significant, the results were summarized using a biplot diagram (Gower & Hand 1996, Ter Braak 1994, Ter Braak & Šmilauer 1998), summarizing in few dimensions the main relationship between the response variables and the explanatory variables. Most of the constrained analyses resulted in only one or two constrained ordination axes so that the solution fitted well into two dimensions.

The biplot diagrams resulting from the constrained ordination method provide a powerful summary of the relations within and between multivariate datasets. Yet their interpretation must proceed carefully because of the danger of over-interpretation. This can be illustrated by the example of biplot diagram in Fig. 1a: The fact that all the available categories are displayed in the graph does not imply that all the categories significantly responded to the phosphorus application. The significance level from a Monte Carlo permutation test refers only to a change at the community level. The relative length of the arrows allows us to compare the extent of response of individual categories to the explanatory variable. Note also that because just the first (horizontal) ordination axis is constrained by the **Phosphate** factor, only the perpendicular projections of arrowtips on that horizontal axis approximate the extent and direction of response of particular category to the phosphate application.

All the multivariate analyses, including significance tests and graphical display of results, were done with the Canoco for Windows, 4.0 package (Ter Braak & Šmilauer 1998).

The multivariate analysis provided the framework for generating more specific, less-dimensional research hypotheses. Some of those hypotheses were then addressed by regression models, using family of generalized linear models (GLM) as the modelling methodology. Differences in total biomass or in the ratio of graminoids (grasses plus sedges) biomass to biomass of forbs were

tested using a generalized linear model (GLM, McCullogh & Nelder 1983) with a gamma distribution for the stochastic component and the logarithmic link function. In such analyses, block membership was used as a covariate. The 1998 biomass data of individual taxa were analysed in the same way. Significance tests on GLMs were done using the approximate F-test in the analysis of deviance. GLMs were fitted using S-Plus for Windows 4.5 package (MathSoft 1997).

Results

Change in community composition after three seasons

Raw biomass data

The analysis of biomass data from June 1997 demonstrated a significant effect of phosphorus application on the absolute dry weight of the aboveground biomass of individual categories (P=0.002), but no significant effect of benomyl application. The results are summarised in Fig. 1a.

Many forbs (with the possible exception of *Achillea millefolium*) responded negatively to the phosphate addition, in contrast to grasses.

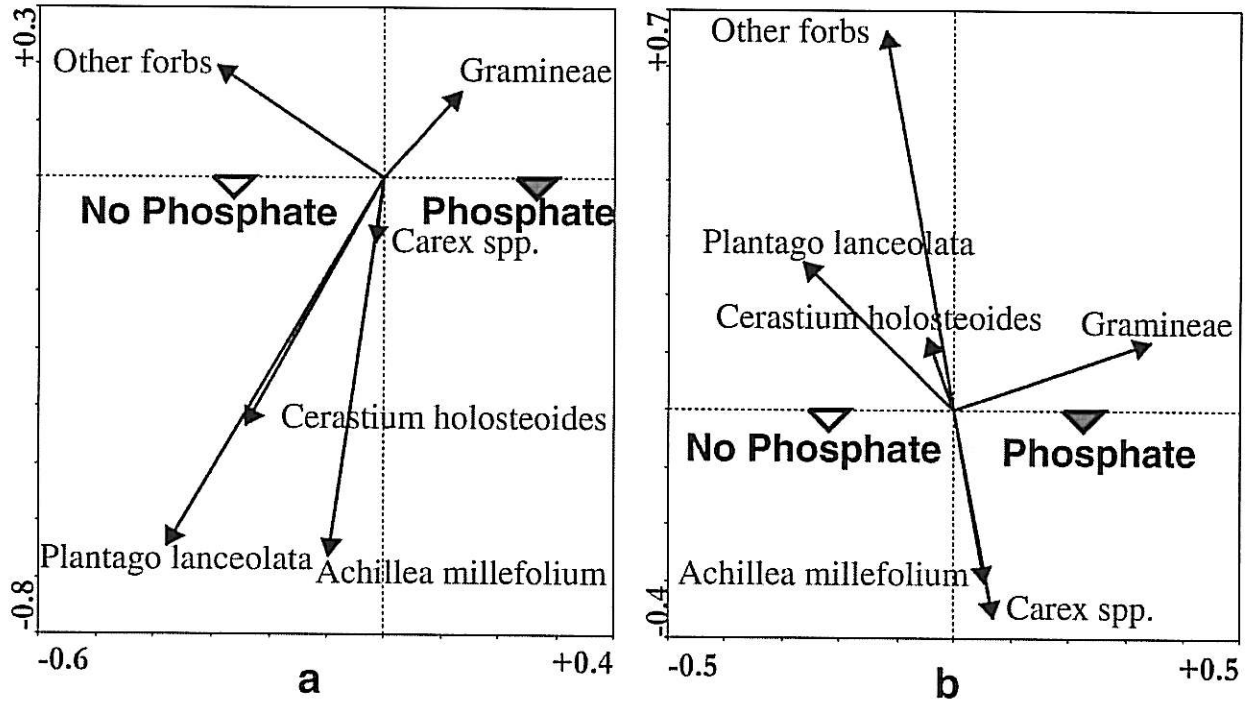

Fig. 1: Ordination biplot diagrams from two redundancy analyses (RDA) displaying response of biomass categories to phosphate application. The analyses are based on the biomass sampling in the year 1997. **Plot a** is based on the analysis of the original biomass weights, so that the changes in absolute biomass implied by phosphate addition can be deduced. **Plot b** displays results from the analysis of percentages of total aboveground biomass so that changes in relative proportion can be deduced. Block identity was used as a covariate (to exclude block-related differences).

Percentage data

When analysing the same data set after transformation to percentage values, the results are quite similar: again only the effect of phosphate application is supported by the data (P=0.036). By inspecting the corresponding ordination diagram in Fig. 1b, we can see a more pronounced increase of the share of grasses in the total biomass amount and a decrease (with comparable extent) of the *Plantago lanceolata* percentage share. The changes in the other forbs percentages are much smaller.

Change in community composition after four seasons

Raw biomass data

Analysis of biomass data from the year 1998 revealed significant effects of both the phosphate addition (P=0.002) and the benomyl application (P=0.017). After accounting for these two main effects, the interaction between them was not significant. Therefore, the ordination diagram in Fig. 2a, with the first two (constrained) ordination axes of RDA, summarizes the effects of the two studied factors on the plant community. Arrows pointing in the direction (or in the opposite direction) from the "No Benomyl" to "Benomyl" symbol (e.g. category *Gramineae* in Fig. 2a) correspond to categories that responded primarily to application of fungicide, and similarly for phosphate application. The largest change was for *Plantago lanceolata*, *Cerastium holosteoides* and "Other forbs" categories, which were suppressed by the application of phosphate or of the fungicide (or of both). The actual influence of the two treatments must be judged by more detailed models for individual categories (see section Response of individual taxa to experimental treatments, below). A negligible response is seen for sedges and plant litter biomass.

Percentage data

Analysis of percentage biomass data from 1998 also demonstrated significant effects of both phosphate addition (P=0.004) and fungicide treatment (P=0.016). As for the raw biomass data, the extent of the effect was larger for phosphate. From the ordination diagram in Fig. 2b, we can again see that *Achillea millefolium* responded positively to fungicide application and *Plantago lanceolata* and the "Other forbs" category responded negatively to the addition of the phosphate and / or of the fungicide. In percentage data, in contrast to raw biomass data, the grasses seem to respond more and in relatively larger extent to the phosphate application.

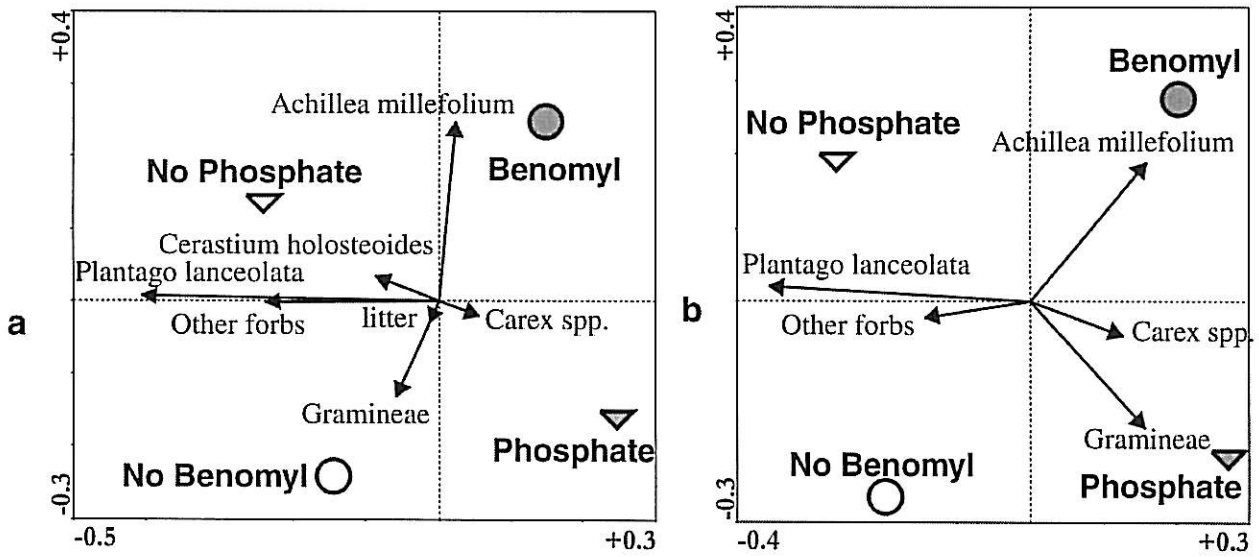

Fig. 2: Ordination biplot diagrams from two redundancy analyses (RDA) displaying response of biomass categories to both phosphate and benomyl applications. The analyses are based on the biomass sampling in the year 1998. **Plot a** is based on the analysis of the original biomass weights so the changes in absolute biomass can be implied by perpendicular projections of treatment symbols on the individual species arrows. **Plot b** can be used to interpret changes in relative proportion of individual categories. Block identity was used as a covariate (so that block-related differences are excluded).

Response of individual taxa to experimental treatments

Biomass data from the year 1998 were used to check for response of individual taxa to experimental treatments using generalized linear models (GLM). Results based on both the absolute biomass weight and the percentage of total biomass are summarised in Table 1 and Table 2.

Change in:	Absolute DW of biomass				Percentage share of biomass			
	phosphate		benomyl		phosphate		benomyl	
Achillea millefolium	▼	*	▲	*	▼	+	▲	*
Plantago lanceolata	▼	**	—	NS	—	NS	—	NS
Cerastium holosteoides	—	NS	—	NS	—	NS	—	NS
Gramineae	—	NS	▼	+	▲	+	—	NS
Other forbs	▼	*	—	NS	▼	+	—	NS
Carex spp.	▲	*	—	NS	▲	**	—	NS

Table 1: Response of biomass of individual taxa (or functional groups) (collected in year 1998) to experimental manipulation, tested using the GLM framework. Results of testing the significant relation of a response variable (dry weight of aboveground biomass or percentage of the total aboveground biomass) to particular predictors combination are summarised using following codes: NS for non-significant relation, + for $0.05 < p < 0.10$, * for $0.01 < p <= 0.05$, and ** for $p <= 0.01$. Tests of term between the benomyl application and the phosphate addition are not displayed, as they never were significant. On left side of each column is an indication of the direction of response to phosphate or benomyl application (▲ for increase, ▼ for decrease, and — for no change).

These results are, more or less, in agreement with the constrained multivariate analysis and provide further insight into the experimental results. For absolute biomass dry weight changes, we can see a significant change of *Achillea millefolium* biomass with the application of both the benomyl and the phosphate, but with no interaction among those two factors. Grasses also responded weakly to benomyl, while *Plantago lanceolata* or "Other forbs" responded negatively to phosphate addition. Sedges responded weakly to the P addition.

For percentage share of total biomass, we can again see the effect of benomyl and phosphate (less pronounced this time) on *Achillea millefolium* biomass and the positive response of grasses and sedges and negative response of "Other forbs" to phosphate additions.

The extent of the changes of biomass for individual categories is further summarized in Table 2, both in terms of absolute biomass weight and of percentage shares of the total biomass.

	control	Benomyl	Phosphorus	Benomyl + Phosphorus
Achillea millefolium	61	106	51	86
Plantago lanceolata	64	48	46	19
Cerastium holosteoides	5	6	5	6
Grasses	346	325	352	306
Other forbs	120	106	104	102
Sedges (Carex spp.)	24	22	38	59
Achillea millefolium [%]	9.3	16.9	8.7	15.7
Plantago lanceolata [%]	11.1	8.4	7.9	3.8
Cerastium holosteoides [%]	1.0	1.5	1.2	1.5
Grasses [%]	54.4	50.9	59.4	52.3
Other forbs [%]	19.9	18.1	16.4	16.9
Sedges (Carex spp.) [%]	4.2	4.2	6.5	9.8

Table 2: Average values of absolute biomass (g . m-2) and percentages of total biomass for differentiated categories from plots with different treatments, year 1998. The columns corresponding to factors, which were found to have significant effects in univariate (GLM-based) tests, are shaded.

Response of other univariate measures to experimental treatments

The two different kinds of constrained ordination models applied above (RDA on percentage data versus RDA on raw biomass data) enable us to contrast responses attributable only to change in relative importance of constituent plant species (or categories) and the responses resulting both from the change in relative frequencies and the change in the absolute biomass weight. These analyses can be usefully complemented by testing the difference in total biomass between the studied treatments.

Differences in total aboveground biomass

Total biomass differed significantly between individual blocks and between years ($p < 0.001$), with the highest biomass amount in the **B3, B4** and **B5**. There was no significant effect of benomyl or phosphate application on the total biomass. The biomass averages from control plots of individual blocks and years are summarised in Table 3.

Block	1997	1998
B1	n. a.	453 (185.9)
B2	1251 (424.8)	597 (148.3)
B3	986 (67.7)	700 (72.8)
B5	906 (177.0)	640 (149.9)

Table 3: Average aboveground biomass [g . m^{-2}] in experimental plots in 1997 and 1998. Note that these values do not represent the **total** aboveground biomass, because the stand was cut approx. 2 cm above the ground. Only control plots were used to calculate the average. The values in the parentheses are standard deviations calculated from the four replicates in the particular block. Just two control plots were sampled in block **B4**, so it was excluded from the table.

Differences in ratio of graminoids to forbs

This ratio also differed significantly between individual blocks ($p < 0.001$), with the largest ratio in block **B2** (14.3), followed by **B4**, **B5**, and **B3** (with values 2.5, 1.9, and 1.8, respectively), and the lowest ratio in block **B1** (value 0.8). The ratio also differed significantly ($p < 0.05$) between plots with and without phosphate addition (with average value 6.5 for phosphate-treated plots and value 2.5 for plots without phosphate addition). There was no significant effect of benomyl application.

Treatments' effects on AM symbiosis of plant populations

Effect of benomyl and phosphate on AM symbiosis of Plantago lanceolata

The effect of phosphate addition on AM colonisation in *Plantago lanceolata* was not significant, while the effect of benomyl was both significant (P=0.004) and substantial (Fig. 3). The benomyl application increased the percentage of the root segments without any fungal structures, decreased the frequency of both arbuscules and vesicles and only small increase in hyphal frequency is indicated. The differences in the average relative frequency of the arbuscular, vesicular, hyphal and empty segments are also summarised in Table 4.

treatment	empty segments [%]	segments with hyphae [%]	segments with vesicles [%]	segments with arbuscules [%]
Benomyl	79	17	1	3
control	27	44	13	16

Table 4: Average percentage fractions of root segments with arbuscules, vesicles, hyphae or without fungal structures for *Plantago lanceolata* plants from plots with and without benomyl application. Each average is based on quantification of 8 plants (both from P treated and non-treated plots), each from a different experimental plot in the block **B1**.

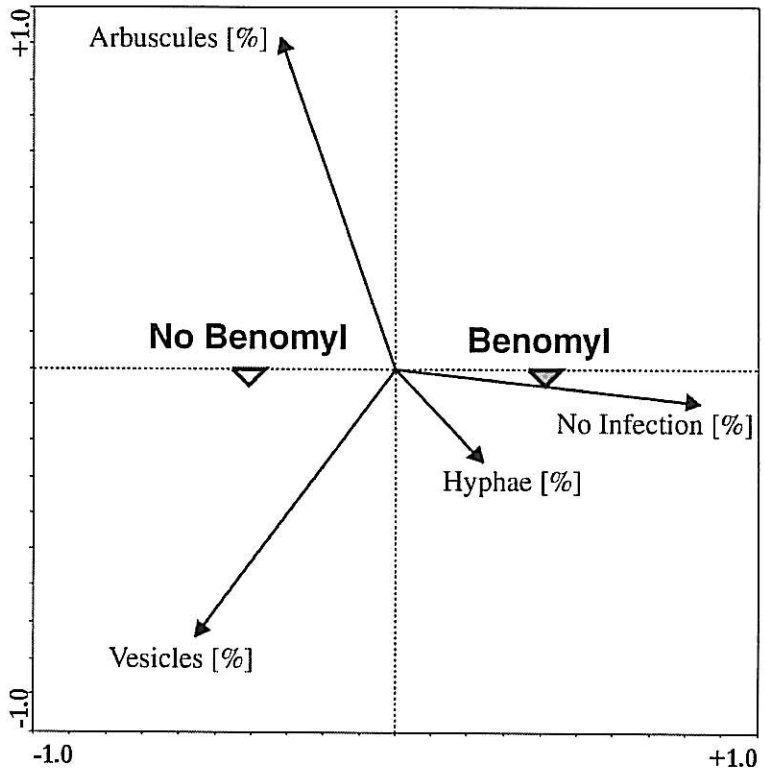

Fig. 3: Ordination biplot diagram from redundancy analysis (RDA) displaying the change in relative proportion of segments with various types of AM infection of *Plantago lanceolata* roots after application of the benomyl fungicide. Arrows point in direction of the steepest increase in the percentage of the particular infection type. We can see the strongest increase of percentage of non-infected segments after benomyl application, together with a substantial decrease in frequency of segments with vesicles and arbuscules and comparable increase in percentage of hyphal segments. No covariates were used in this analysis.

Effect of phosphate on AM symbiosis of selected species

The effect of phosphate application on relative frequency of root segments with various stages of AM symbiosis was studied for five species (*Achillea millefolium*, *Holcus lanatus*, *Luzula campestris*, *Poa angustifolia*, and *Plantago lanceolata*). First, the five species were taken as blocks and so the common (or prevailing) effect of phosphate application on AM symbiosis was studied (Fig. 4a). The effect of phosphate application was significant (P=0.014). Phosphate addition decreased the frequency of arbuscules , and correspondingly increased the number of segments with no infection. The frequency of segments with vesicles and hyphae was little influenced by phosphate application.

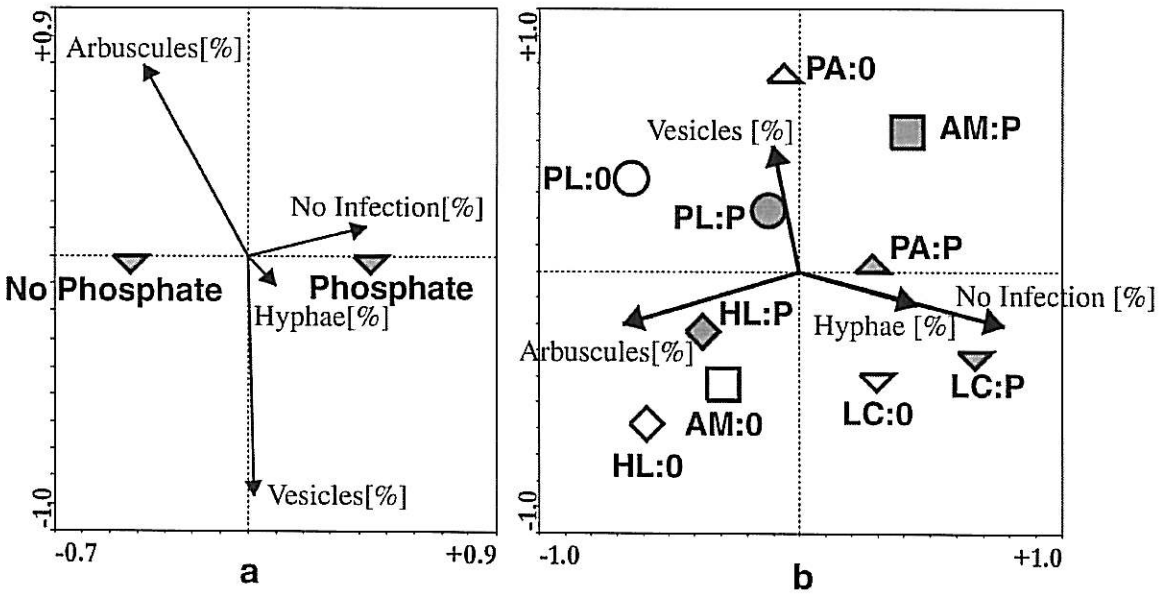

Fig. 4: Ordination biplot diagrams from redundancy analysis (RDA) displaying response of relative proportion of segments with various types of AM infection to phosphate addition. **Plot a** is based on an analysis focusing on average change in plant infection structure between plots with and without phosphate addition, irrespectively of the studied plant species (species identity indicators were used as covariates here). **Plot b** is presenting the response of individual studied species to phosphate addition. Based on the direction of arrows in the plot, we can interpret a shift in symbol position from left to right as an increase of percentage of segments with no infection and with hyphal infection and a decrease of segments with arbuscular infection. On the other hand, change in symbol position from top to bottom implies decrease in the frequency of vesicles in root segments. The codes for species names in plot **b** are as follows: PL = *Plantago lanceolata*, HL = *Holcus lanatus*, AM = *Achillea millefolium*, PA = *Poa angustifolia*, LC = *Luzula campestris*. In this analysis, the variables representing interaction between species identity and P application indicators were used as the explanatory variables.

Fig. 4b displays a biplot from an analysis where the explanatory variables were the interaction terms between plant species the particular root sample belonged to and phosphate application. This provides a fuller picture of the response but reduces reliability because there are fewer replicates (three or four) for each class. We can see again the trend of decreasing frequency of arbuscular root segments (and increasing occurrence of segments with no infection) with phosphate application. This decrease is largest for species like *Achillea millefolium* or *Plantago lanceolata*, but much smaller for *Holcus lanatus* or *Poa angustifolia*. *Poa angustifolia* responded to phosphate addition by decreased frequency of vesicles, while opposite trends can be seen for *Achillea millefolium*.

Discussion

The results of this study show the negative response of forbs to phosphate application. This probably does not imply any direct negative effect of phosphate on those species, but rather the shift in competitive balance in favour of grasses and sedges. Those species might be better able to utilise the additional phosphate pool and, consequently, are better able to suppress the forbs. If we

check values in Table 2, then particularly striking is the increase of both absolute and relative biomass of sedges, especially when benomyl was applied as well.

The decrease in performance of *Achillea millefolium* in phosphate-supplemented plots is more than outweighed by the benomyl application. This might indicate a limitation of that species' population dynamics by pathogenic fungi (similarly to the results of Newsham et al. 1994), but further study is needed. Alternatively, we might hypothesize that the relation of AM fungi with this species is not mutually profitable and application of benomyl might provide a release from a parasitic relation (mechanism suggested already by Gange et al. 1990). This explanation seems, in fact, more plausible than the previous one, as our regular observations (two seasons, every three weeks) of the AM and non-AM fungi on roots of this species show much lower incidence of non-AM fungi (including also the pathogenic fungi) compared with the other two intensively studied species (*Plantago lanceolata* and *Poa angustifolia*, unpublished data).

Plantago lanceolata is known to exhibit standardly very high levels of AM infection and respond negatively to its reduction (Gange & West 1994). The direct effect of AM symbionts on the performance of this species might be compounded with the more subtle ones, like the increased palatability of the non-mycorrhizal plants for its herbivores related to the change in the concentration of chemical deterrents in leaves (Gange & West 1994). In fact, a similar mechanism is also possible for the increased phosphate availability.

Another outcome of the study is the indication of adverse effect of AM symbiosis exclusion on grasses. From the values in Table 2, we can see that the decrease in absolute biomass of grasses due to benomyl application was approx. 10%. We can again interpret this either as a "passive consequence" of increased fitness of *Achillea millefolium*, which might be able to partly suppress the grasses or as an evidence of their dependency on the AM fungi. The scope of the "grasses" group is, nevertheless, too broad to support any specific interpretation. We can expect substantial differences in the group of approximately 17 species as different as *Poa angustifolia*, *Alopecurus pratensis* or *Agrostis tenuis*. Further study, which would include sorting the plant community components completely down to species level, must be performed in the future.

Van der Heijden et al. (1998a) suggested the essential role of mycorrhizae in maintaining the diversity of temperate plant communities (with one example being a calcareous grassland) and illustrated in their microcosm experiment the increased fluctuation in species performance in treatments without AM fungi. They obviously used young plants (starting from seedlings), so their conclusions might be limited by that fact. Our results on stabilized long-term grassland community do not indicate any change in species richness, but we can see a shift in the performance of individual plant populations. Nevertheless, a change in species richness might take a longer time, as one of the mechanisms involved in diversity reduction in non-mycorrhizal stands is the reduction in the frequency of new species arrivals and seedlings establishment (Gange et al. 1993, Zobel et al. 1999) and these do not obviously proceed quickly in the stabilized grassland community.

Our experimental results do not fit the model that Grime et al. (1987) present, where the effect of AM symbiosis exclusion on the increase of dominant species performance relative to the subordinate species was explained by the disturbance of a "common mycelial network" exporting part of the assimilates from dominant to subordinate species. In our case, the disturbance of the mycelial network leads to the suppression of both the dominant graminoids and the subordinate forbs, with the *Achillea millefolium* population possibly taking advantage of that process. In that context, we should point out that AM symbiosis exclusion can also increase the diversity by influencing the dominant species more than the subordinate ones (Hartnett & Wilson 1999).

Direct evaluation of effects of the phosphate application on the AM infection level and structure (see for example Fig. 4b) shows the variability in response of AM symbiosis for individual hosts, but it should be taken just as a first, very rough indication. A more detailed study is needed, possibly combined with differentiation of various fungal symbiont taxa. This can be important also in respect to effects of benomyl, which might exercise different damage on different AM fungal species.

It is surprising how widely the methods of benomyl application vary across different studies. Some authors applied a high dosage, but only once in a season (e.g. Zobel et al. 1999 with 30 $g.m^{-2}$) while others applied very low dosage (like Fitter 1986 with only 0.3 $g.m^{-2}$). This alone might explain some of the inconsistencies in the achieved results.

Another reason for observed differences in the response of species categories in our study might lie in the high absorbance of the benomyl on soil particles. Shallow-rooting species might be more influenced than the deep rooting ones.

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References

Aitchison J. (1986): *The statistical analysis of compositional data.* Chapman and Hall, London

Bolan N.S. (1991): A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant & Soil* 134: 189-207

Fitter A.H. (1986): Effect of benomyl on leaf phosphorus concentration in alpine grasslands: a test of mycorrhizal benefit. *New Phytol.* 103: 767-776

Fitter A.H. & Nichols R. (1988): The use of benomyl to control infection by vesicular-arbuscular mycorrhizal fungi. *New Phytol.* 110: 201-206

Gange A.C., Brown V.K., Farmer L.M. (1990): A test of mycorrhizal benefit in an early successional plant community. *New Phytol.* 115: 85-91

Gange A.C., Brown V.K., Sinclair G.S. (1994): Vesicular - arbuscular mycorrhizal fungi: a determinant of plant community structure in early succession. *Funct. Ecol.* 7: 616-622

Gange A.C. & West H.M. (1994): Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata L. New Phytol.* 128: 79-87

Gower J.C. & Hand D.J. (1996): *Biplots*. Chapman and Hall, London.

Grime J.P. et al. (1987): Floristic diversity in a model system using experimental microcosm. *Nature* 328: 420-422

Hartnett D.C., Hetrick B.A.D., Wilson G.W.T., Gibson D.J. (1993): Mycorrhizal influence on intra- and interspecific neighbour interactions among co-occurring prairie grasses. *J. Ecol.* 81: 787-795

Hartnett D.C. & Wilson G.W.T. (1999): Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology* 80: 1187-1195

Hetrick B.A.D., Hartnett D.C., Wilson G.W.T., Gibson D.J. (1994): Effects of mycorrhizae, phosphorus availability, and plant density on yield relationships among competing tallgrass prairie grasses. - *Canad. J. Bot.* 72: 168-176

Koide R.T. (1991): Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytol.* 117: 365-386

Koide R.T., Huenneke L.F., Hamburg S.P., Mooney H.A. (1988): Effects of applications of fungicide, phosphorus and nitrogen on the structure and productivity of an annual serpentine plant community. *Funct. Ecol.* 2: 335-344

MathSoft (1997): *S-Plus User's Guide*. Data Analysis Products Division, MathSoft, Seattle

McCullagh P. & Nelder J.A. (1983): *Generalized linear models.* Chapman and Hall, London

Moravec J. et al. (1995): *Red list of plant communities of the Czech Republic and their endangerment.* Okresní vlastivědné muzeum, Litoměřice.

Newsham K.K., Fitter A.H., Watkinson A.R. (1994): Root pathogenic and arbuscular mycorrhizal fungi determine fitness in asymptomatic plants in the field. *J. Ecol.* 82: 805-814

Newsham K.K., Fitter A.H., Watkinson A.R. (1995): Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends Ecol. Evol.* 10: 407-411

Paul N.D., Ayres P.G., Wyness L.E. (1989): On the use of fungicides for experimentation in natural vegetation. *Funct. Ecol.* 3: 759-769

Phillip J.M. & Hayman D.S. (1970): Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. - *Trans. Brit. Mycol. Soc.* 55: 159-161

Rothmaler W. (1976): *Exkursionsflora für die Gebiete der DDR und der BRD. Kritischer Band.* Volk und Wissen Volkseigener Verlag, Berlin.

Sanders I.R. & Fitter A.H. (1992): The ecology and functioning of vesicular-arbuscular mycorrhizas in co-existing grassland species. I. Seasonal patterns of mycorrhizal occurrence and morphology. *New Phytol.* 120: 517-524

St-Arnaud M. et al. (1997): Inhibition of Fusarium oxysporum f.sp. dianthi in the non-VAM species Dianthus caryophyllus by co-culture with Tagetes patula companion plants colonized by Glomus intraradices. *Canad. J. Bot.* 75: 998-1005

Ter Braak C.J.F. (1994): Canonical community ordination. Part I: Basic theory and linear methods. *Ecoscience* 1: 127-140

Ter Braak C.J.F. & Šmilauer P. (1998): *CANOCO Reference Manual and User's Guide to Canoco for Windows. Software for Canonical Community Ordination (version 4).* Microcomputer Power, Ithaca.

Van Faassen H.G. (1974): Effect of the fungicide benomyl on some metabolic processes, and on numbers of bacteria and actinomycetes in the soil. *Soil Biol. Biochem.* 6: 131-133

Van der Heijden M.G.A. et al. (1998a): Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69-72

Van der Heijden M.G.A. et al. (1998b): Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. - *Ecology* 79: 2082-2091

Watanabe F.S. & Olsen S.R. (1965): Test of ascorbic acid method for determining phosphates in water and sodium bicarbonate extracts from soils. *Soil Sci. Soc. Amer. Proc.* 29: 677-680

Zobel M. et al. (1999): Small-scale dynamics of plant communities in an experimentally polluted and fungicide-treated subarctic birch-pine forest. *Acta Oecol.* 20: 29-37

3 Plant roots response to heterogeneity of soil resources: effects of nutrient patches, AM symbiosis, and species composition

Marie Šmilauerová

Abstract

Response of the underground parts of whole grassland community to heterogeneity in soil resources was studied under field conditions. Increased availability of nutrients (N, P) in patches was combined with suppression of arbuscular mycorrhizal symbiosis (by fungicide benomyl) and with modification of substrate structure (sieved soil, sieved soil mixed with coarse or fine sand). Three experimental runs were established in 1999, each with seven replicate blocks. Root and rhizome biomass was determined before the experimental treatment and after 53 days of experiment. Plant community composition in neighbourhood of patches was recorded. Nutrient application had the most pronounced effect on the root biomass grown into the patches (the root biomass increased 1.64 times). The root biomass in patches with fungicide application was 1.17 times higher than in the untreated patches. The modification of substrate structure had no effect on the root biomass in patches but it modified the effect of benomyl. The amount of root biomass proliferating into the patches was not related to species composition of vegetation near the patches. However, higher frequency of grass tillers in patch neighbourhood and mycorrhizal status of neighbouring species correlated significantly with the amount of root biomass in experimental patches. In pre-treatment community, amount of root biomass was significantly related to abundance of grasses and mycorrhizal forbs surrounding the sampling points.

Keywords: grassland community, grasses, forbs, root biomass, benomyl, nitrogen, phosphorus

Introduction

Temporal and spatial heterogeneity of resources is one of the characteristic features of natural ecosystems (Caldwell & Pearcy 1994, Vogt et al. 1995) and it can play a principal role in coexistence of numerous species in a single community (e.g. Fitter 1982). Detailed measurements of soil properties on a fine scale with respect to plant position were reported by Jackson & Caldwell (1993) and Ryel et al. (1996) from a sagebrush-steppe. They found that nitrogen concentration varied considerably in both space and time. Šmilauer (1996) measured soil nutrients in a semi-natural grassland on a small scale (in a grid with cells 4x4 cm) and recorded plant species rooting in the individual samples. He found a significant relation between the availability of water-soluble P and plant cover composition.

In the seventies, Drew demonstrated in a series of experiments with barley that roots can respond to the nutrient heterogeneity by proliferation within the nutrient-rich patches (e.g. Drew et al. 1973, Drew & Saker 1975, Drew 1975). It was shown later that many wild plant species do not respond in this way or so intensively (Caldwell et al. 1992, Robinson & Van Vuuren 1998, Einsmann et al. 1999, Fransen & de Kroon 1999, Farley & Fitter 1999). Response of individual plants can consist of changes in biomass allocation, in root architecture, and in kinetics of nutrient uptake (Fitter 1994, Caldwell 1994). Some competitive, dominant species do not respond to nutrient patches by any of the changes mentioned above, but their roots represent a large part of root biomass in patches simply due to their high growth rate (Campbell et al. 1991, Grime 1994). Plant response depends also on the patch characteristics such as its size, spatial and temporal frequency, concentration of nutrients, and their chemical nature (Fransen et al. 1999a, 1999b, Hodge et al. 1999a, 1999b, Wijesinghe & Hutchings 1999, Duke & Caldwell 2000). Even the timing of the patch establishment can change substantially the plant response (compare results of Hodge et al. 1998 and 1999c). Mycorrhizal symbiosis may also play a role in nutrient acquisition from a patch (Cui & Caldwell 1995), mainly for species with small root systems (Farley & Fitter 1999).

Most experiments have been performed under greenhouse conditions and mostly on plants not entering competition. However, the response of a single plant to nutrient patches can widely differ from the response of a plant growing under competition (Huber-Sannwald et al. 1998, Cahill & Casper 1999, 2000, Fransen et al. 1999b, Robinson et al. 1999, Hodge et al. 1999c). Caldwell et al. (1991a, 1991b, 1996) found that root exploitation of nutrient patches by a plant of one species depends on the species identity of neighbouring roots and on the size of competing plants. McConnaughay & Bazzaz (1992) found considerable differences among species in their sensitivity to space fragmentation by artificial root systems. The reduction in growth of plants constrained in the deployment of their roots was not detected at higher nutrient levels.

Despite of our increasing understanding of root response to soil heterogeneity on individual level, we have poor knowledge of belowground response to nutrient microsites at the level of whole community. The root response to nutrient patches differed in these two communities: significant increase of The total root length in nutrient patches was increasing significantly in tropical forests (St. John et al. 1983), while only small response of root density to increased nutrient availability was found in *Artemisia* plantation where species identity of neighbouring plants was more important for the root behaviour in patches (Caldwell et al. 1991b, 1996).

In this study, I focus on the response of underground parts of grassland community as a whole to newly created nutrient patches. This field experiment was performed in species-rich semi-natural grassland with relatively low availability of nutrients. Most grassland plant species have endomycorrhizal symbiosis (Harley & Harley 1987, Stanton 1988) and the infection level was found higher if the plants were under nutrient stress (Read et al. 1976). Therefore, I combined nutrient application with suppression of mycorrhizal fungi by a selective fungicide benomyl, to estimate the role of mycorrhiza for the nutrient acquisition in a patchy environment. To compare

community response to nutrient patchiness and suppression of mycorrhizal symbiosis in soil microsites differing in substrate structure, I used three different substrate modifications. Sand grains of different size may influence penetration rate of roots, being a physical obstruction fragmenting the belowground space, but they can also change the water regime and nutrient concentration in patches. As species composition of neighbouring vegetation seems to play important role in root behaviour in soil microsites, species identity of neighbouring rooted morphological units (modules) was recorded. This study was part of a project in which attention was also paid to response of selected plant species to manipulation of resource patchiness. The results of the species - centred approach will be published elsewhere (Šmilauerová & Šmilauer 2001, manuscript). This study addresses following questions:

1. Does the grassland community respond to the emergence of a nutrient patch by increased allocation of root biomass into this volume?
2. Do plant roots avoid growth into soil volume with suppressed development of mycorrhizal fungi and does their response depend on the nutrient availability in this volume?
3. Does the response of roots to nutrients and/or to availability of mycorrhizal fungi differ between patches with different substrate structure?
4. Does the amount of roots entering a newly established soil volume depend on the composition of plant community immediately surrounding the patch?
5. Is the total root biomass in the top soil layer correlated with the composition of plant community on a small spatial scale?

Materials and methods

Research site

The research site is located near Zvíkov village (10 km east of České Budějovice, 48°59'N, 14°36'E, 500 m a.s.l.). The vegetation is an oligotrophic, traditionally managed meadow on a shallow valley slope. The meadow is cut once a year, usually in the middle of June. More information can be found in Šmilauer & Šmilauerová (2000). The plant species nomenclature follows Rothmaler (1976).

Experimental design and procedure

Three independent replications of the experiment described below were performed during the year 1999. I refer to them as to **experimental runs,** in this paper. Table 1 provides details of timing, soil properties, and species composition of vegetation of individual experimental runs. The first and the last experimental runs were located in close proximity, while the plot for middle run was established approximately 15 meters apart.

experimental run	timing	most frequent neighbour species	soil properties
1	18.3. - 10.5.	LC, PA, AM, PL, CH	well drained, very nutrient poor soil with poorly developed H horizon
2	1.4. - 25.5.	PA, CP, AM, FR, PL	soil with more developed H horizon, wetter and more nutrient rich than two others experimental runs
3	1.6. - 24.7.	PL, LC, PA, FR, HL	same as in the experimental run 1

Table 1: The characteristics of the three experimental runs. The codes for frequent neighbour species are: AM - *Achillea millefolium*, CH - *Cerastium holosteoides*, CP - *Campanula patula*, FR - *Festuca rubra*, HL - *Holcus lanatus*, LC - *Luzula campestris*, PA - *Poa angustifolia*, PL - *Plantago lanceolata*.

There were seven replicate blocks in each experimental run. Each replicate block contained all 12 combinations of the experimental treatments (2 levels of nutrient factor * 2 levels of fungicide factor * 3 types of substrate modification) arranged as 3 x 4 sampling points in a rectangular grid with the distance of 25 cm between adjacent points. The treatment combinations were completely randomised within each of the replicate blocks. To establish a patch, soil from the soil profile was removed closely to the particular sampling point, with the aid of soil corer (diameter 4.5 cm, depth of 10 cm). The soil was then sieved to remove roots and rhizomes (sieve mesh size 3 mm) and then placed back into the holes either (i) unmixed or mixed with (ii) fine (Ø < 1 mm) or (iii) coarse (Ø 3 - 5 mm) sand in a 1:1 volume ratio. Nutrients (supplied as 0.2 g $Na_3PO_4 \cdot 12\ H_20$, 0.24 g $NaN0_3$, and 0.15 g NH_4Cl per patch) and the fungicide benomyl (using Bavistin, BASF company; 0.15 g of Bavistin per one patch) were then applied in 0.1 l of water solution to each patch. The control patches received 0.1 l water instead. The application of nutrients, fungicide, and water was repeated after three weeks. Patches together with the surrounding soil and plants (soil columns with 11cm diameter) were excavated after 53 days of the experimental run. The samples were taken only from five replicate blocks in each experimental run; the remaining patches were used for other purposes, including chemical analyses. Number of rooted morphological units (**modules**) of each species growing within a circle (having diameter 11 cm) centred on the patch was recorded. For analyses of effects of functional plant groups upon root biomass grown into soil microsites, present species were divided into three groups: **grasses** (all grass species possessed mycorrhizal symbiosis), **mycorrhizal forbs**, and **non-mycorrhizal species** (both graminoids and forbs).

All roots and rhizomes were separated from each patch volume. Roots and rhizomes collected at the beginning and at the end of the experiment were washed, dried (80°C) and weighed. Several roots from each patch were used for separate analyses and their weight is therefore not included in the total root biomass analysed in the present study. Nevertheless, their contribution to total biomass was negligible. In this study, I use the following terms for the biomass categories determined at the start of the experiment: **initial root biomass** (**Mroot1**) and **initial rhizome biomass** (**Mrhiz1**) for the dry weight of roots and rhizomes respectively, **total initial**

belowground biomass (**Mall1**) for the summary weight of both these categories and the **ratio of rhizome biomass to root biomass** (**Rh:Ro**). To evaluate effects of experimental treatments, **in-grown root biomass** (**Mroot2**) representing dry weight of the roots is used. The biomass of rhizomes, which grew into the patches, was negligible (zero or < 0.01g per patch) and therefore not considered.

To estimate soil moisture dynamics during the experiments, sensors for measuring the water saturation deficit of soil (by a portable digital metering device, based on soil resistance measurement, Watermark 30KTCD, Irrometer Co., Riverside, U.S.A.) were placed in patches with the three applied substrate modifications near the experimental plots during the experimental runs. Additionally, two sensors were placed into soil profile among the plots of the three experimental runs, for permanent measurements in non-manipulated soil profile during the whole season.

Statistical analysis

All the response variables expressing absolute amounts of biomass (initial root biomass, total initial belowground biomass, and in-grown root biomass) were log-transformed in the analyses, based on the expectation of the relative (multiplicative) change in the biomass values, rather than of an absolute (additive) change (Cleveland 1993, p. 48). The ratio of initial rhizome biomass to initial root biomass was not transformed.

The differences in the studied response variables between the experimental runs were tested using an ANOVA model. Multiple comparisons used to identify the differing experimental runs are based on the method using a family-wise error rate protection. ANOVA model was also used to test for differences between the replicate blocks within the experimental runs and to tests the effects of experimental treatments upon the in-grown root biomass. The interaction of experimental treatments with experimental runs was tested by comparing ANOVA model with assumed additivity of the experimental run effect and the effect of the experimental factors with another ANOVA model, where the experimental effects (N, B, Substrate) were nested within the experimental run factor levels. The effect of abundance of plant modules within the patch neighbourhood was tested using a general linear model where the factors of experimental treatments and identity of experimental run were used as covariates. When testing effect of abundance of individual plant species in the patch neighbourhood, the Bonferroni correction was applied to the threshold significance level (α= 0.0025 =0.050/20). The factor of 20 in the Bonferroni correction is based on the 20 pre-selected plant species entering the tests (all the species occurring in neighbourhood of at least 10% of the patches). All the above given models were estimated using the S-Plus 2000 software (Anonymous 1999).

The effect of community composition upon the initial biomass of roots and rhizomes (as well as upon the ratio between the rhizomes and roots) and upon the amount of in-grown root biomass was tested using a multivariate statistical approach - redundancy analysis (RDA, Ter Braak & Šmilauer, 1998). In this analysis, the roles of the community composition (as the cause of the observed pattern) and of the observed biomass amount (initial root biomass, initial total

belowground biomass, ratio of rhizome biomass to root biomass, in-grown root biomass) were exchanged. Community composition was used as the primary data matrix (set of response variables) and one of the biomass measurements as the explanatory variable (predictor). The RDA represents a multivariate extension of the linear regression model, which is often used in context where causal relations between variables are swapped in respect to their roles as the response variables and the predictors. The counts of plant species units were square-root transformed prior to the analysis, the primary data matrix was centred by species and standardised by their error-variance (Ter Braak & Šmilauer, 1998).

Additionally, the membership of patches within experimental runs was used as a covariate in the redundancy analysis. When analysing the relation between the composition of vegetation surrounding the patches and the proliferated root biomass, I also used the experimental treatments as additional covariates. While these treatments are expected to influence the value of the predictor in this analysis (Mroot2) - not the composition of the vegetation, this approach is still valid because, technically, the analysis with covariates is identical to an analysis, where the predictors (explanatory variables) are first regressed upon the covariates and then the regression residuals are used as new predictors (Ter Braak & Šmilauer 1998, p. 187). The multivariate analyses were performed with the Canoco for Windows, version 4.0 software.

Results

Averages and variability of pre-treatment belowground biomass are summarised in Fig. 1A. Three experimental runs did not differ in total initial belowground biomass ($F_{2,215}$=0.08, n.s.), but differed in initial root biomass ($F_{2,225}$=15.38, $P<10^{-6}$), and in the ratio of rhizome biomass to root biomass ($F_{2,215}$=37.65, $P<10^{-14}$). These significant differences were due to the middle experimental run which had higher percentage of rhizomes than the other two experimental runs. I found significant differences among the replicate blocks, within experimental runs, only for the initial rhizome biomass ($F_{16,199}$=2.17, P=0.007).

At the end of experiment, there was a significant difference in the in-grown root biomass between the three experimental runs ($F_{2,184}$=12.04, $P = 1.2 * 10^{-5}$), explainable by lower values of root biomass in patches of the first (earliest) experimental run (Fig. 1A). The root biomass in-grown into the patches did not differ among the replicate blocks within each experimental run ($F_{14,169}$=1.6, n.s.).

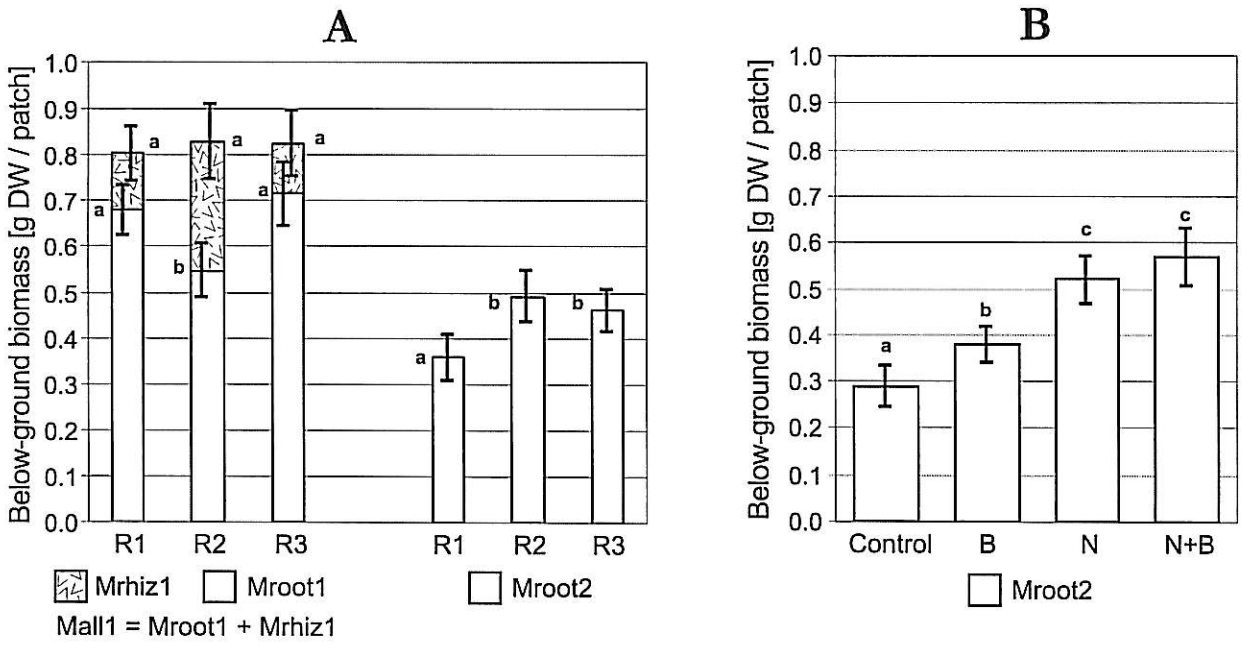

Fig. 1: The patterns of belowground biomass distribution. (A) The average initial root biomass (Mroot1), the average total initial belowground biomass (Mall1), and the average in-grown root biomass (Mroot2) in individual experimental runs; (B) The average in-grown root biomass (Mroot2) in patches with different treatment combinations (nutrients and benomyl application). The biomass quantity is expressed using the dry weight (DW) values. The white bars represent the root biomass, while the patterned bars represent the initial rhizome biomass (Mrhiz1). The averages are plotted together with their 95% confidence intervals. The confidence intervals at the top of stacked bars refer to the total belowground biomass values. The triples of lower case letters (in part A) show results of multiple comparisons among experimental runs (R1, R2, R3). Averages of runs with identical letter (for each biomass category) are not significantly different. Similarly, the lower case letters in part B indicate differences between experimental treatments. The presented averages for experimental runs R1 to R3 are based on following numbers of observations: 63, 63, 60 for Mroot1 and Mroot2; 63, 53, 60 for Mall1. The presented averages for experimental treatments Control, benomyl application without nutrients (B), nutrients application without benomyl (N), and application of both nutrients and benomyl (N+B) are based on following numbers of observations: 48, 46, 46, 46.

The effects of experimental treatments upon the root proliferation into the patches are summarised in Table 2 and in Fig. 1B. The nutrient application had the most pronounced effect: the root biomass in patches with added nutrients was 1.64 times higher than the biomass in the non-fertilised patches. Benomyl application had a significant, but not so extensive effect upon root proliferation. The root biomass in patches with applied benomyl was 1.17 times higher than in the untreated patches. The root biomass in patches with application of both nutrients and benomyl was significantly (but only by 0.04%) lower than what one would expect in the case of independent effects of these two factors. The modification of substrate structure had no significant effect upon the root biomass in patches, but it modified the effect of benomyl. Positive effect of benomyl application was more pronounced in patches with substrate with added sand, particularly the coarse sand. The measured soil moisture did not differ between the patches filled with the

substrate with different structure. The response to experimental treatments did not differ between the experimental runs: the ANOVA model with experimental effects nested within the effect of experimental runs was not significantly better than the model presented in Tab. 2 ($F_{22,173}$=1.52, n.s.).

I found no significant effect of the initial root biomass upon the root biomass grown into the patches after accounting for the effects of experimental treatments and difference between experimental runs ($F_{1,181}$=0.01, n.s.).

Term	DF	Sum of Squares	Mean Square	F statistics	P
Experim. runs	2	4.81	2.40	21.51	**$<10^{-5}$**
Nutrients (N)	1	13.36	13.36	119.52	**$<10^{-5}$**
Benomyl (B)	1	1.76	1.76	15.78	**0.0001**
Substrate (S)	2	0.32	0.16	1.45	n.s.
N x B	1	0.64	0.64	5.68	**0.018**
N x S	2	0.12	0.06	0.53	n.s.
B x S	2	0.75	0.38	3.37	**0.036**
N x B x S	2	0.43	0.22	1.93	n.s.
Residual	173	19.34	0.11		

Table 2: Response of the in-grown root biomass to experimental factors. Results of analysis of variance are presented with degrees of freedom (DF), Sum of Squares explained by individual model terms, corresponding Mean Square, F test statistics, and corresponding Type I Error estimate (P). P values larger than 0.05 are replaced by "n.s." (not significant). The response variable was log-transformed.

I found total of 48 plant species in the close neighbourhood of patches, including 13 grass species and 5 non-mycorrhizal taxa (*Luzula campestris, Carex* spp., *Cerastium holosteoides, Stellaria graminea*, and *Dianthus deltoides*). Nineteen species occurred in all three experimental runs. Five most frequent species of each experimental run are listed in Tab. 1. Experimental runs differed significantly in absolute number as well as in relative proportion of grass modules and non-mycorrhizal plant modules (Tab. 3). Number of modules of neighbouring mycorrhizal forbs was more uniform among experimental runs. Species composition of vegetation near the experimental patches differed among the experimental runs (P = 0.0005, number of permutations N = 1999) and within them (P = 0.0005, N = 1999).

	Tot	Grass	Grass: Tot [%]	AMForb	AMForb: Tot [%]	NonAM	NonAM: Tot [%]
P ($F_{2,185}$)	$<10^{-3}$ (9.4)	$<10^{-5}$ (17.5)	$<10^{-5}$ (26.1)	0.045 (3.16)	n.s. (0.57)	$<10^{-5}$ (24.1)	$<10^{-5}$ (43.6)
Exp. run 1	20.2 ± 1.30	5.2 ± 0.64	25.7 ± 2.78	7.8 ± 0.86	38.8 ± 3.38	7.0 ± 0.83	34.9 ± 3.44
Exp. run 2	22.5 ± 2.16	10.8 ± 1.10	51.2 ± 5.03	8.9 ± 1.48	37.7 ± 4.24	2.6 ± 0.75	10.2 ± 2.41
Exp. run 3	17.2 ± 1.40	6.6 ± 1.04	36.7 ± 4.06	6.0 ± 0.58	36.7 ± 3.59	4.0 ± 0.72	23.1 ± 3.76

Table 3: Results of fitting ANOVA models, describing the differences between the experimental runs in absolute and relative frequencies of rooted morphological units (modules) of species from different categories: grasses (Grass), mycorrhizal forbs (AMForb), non-mycorrhizal species (NonAM), and total count of modules (Tot). The Type I Error estimates and corresponding F statistics are given in the second row. Means and their 95% confidence intervals for the individual experimental runs are given in the third to fifth row. The plant modules were recorded in a band 3.5 cm wide around the experimental patch.

In multivariate analyses, I found no relation between the root biomass in-grown into the patches (with the root biomass differences due to the experimental treatments taken into account, in this case) and the neighbouring community composition aboveground. When the influence of individual species was tested, I found no relation of any individual species to the root biomass that grew into the patches.

Results of analyses of functional group effects upon the in-grown root biomass are summarised in Tab. 4. Amount of in-grown root biomass was significantly influenced by the absolute number of grass modules (positive correlation); relative proportion of non-mycorrhizal species among neighbouring modules had a nearly significant effect (negative correlation). If Bonferroni correction is applied, only the grass effect (Fig. 2) remains significant. Nutrient application had the strongest effect on the root behaviour in patches (see Tab. 2) and it could overshadow finer effects of functional groups in patch neighbourhood. Therefore, impact of functional groups upon root regrowth was further studied only in patches with no nutrients applied. In these more detailed analyses, only negative impact of relative proportion of non-mycorrhizal species in patch neighbourhood remained significant after Bonferroni correction ($F_{1,90}$=13.48, $P<10^{-3}$). When Bonferroni correction was ignored, another three neighbour plant categories had significant effect: the absolute amount of non-mycorrhizal modules had a negative impact ($F_{1,90}$=6.626, P=0.012), and both the absolute amount of grass modules ($F_{1,90}$=5.95, P=0.017) and the relative proportion of grasses ($F_{1,90}$=4.91, P=0.029) had positive impact onto root biomass in patches.

	Mroot2	Mroot1	Mall1
Residual DF	180	182	172
Grass	0.004 (F=8.33) *	0.004 (F=8.72) *	0.002 (F=9.80) *
Grass : Tot	n.s. (F=3.09)	$<10^{-3}$ (F=13.03) *	0.002 (F=10.41) *
AMForb	n.s. (F=2.83)	0.009 (F=7.00)	n.s. (F=2.35)
AMForb : Tot	n.s. (F=0.02)	$<10^{-3}$ (F=11.25) *	0.004 (F=8.30) *
NonAM	n.s. (F=0.02)	n.s. (F=0.08)	n.s. (F=0.01)
NonAM : Tot	0.059 (F=3.61)	n.s. (F=0.03)	n.s. (F=0.11)

Table 4: Results of fitting general linear models, describing dependence of in-grown root biomass (Mroot2), initial root biomass (Mroot1), and initial total belowground biomass (Mall1) on the absolute or relative frequency of rooted morphological units (modules) of species from different categories: grasses (Grass), mycorrhizal forbs (AMForb), and non-mycorrhizal species (NonAM). In all the fitted models, experimental run identity was used as a covariate. For the regression models with Mroot2, the experimental treatments were also used as covariates. The response variables were log-transformed. Significance of each fitted model was tested and the Type I Error estimates (significance levels), as well as the corresponding F statistics are given in the table. The significance level values larger than 0.05 are replaced by "n.s." (not significant). The two parameters of the shown F statistics are 1 and the value given in the second row for each respective response variable (column). Combinations of response variables and predictors where relation was considered significant even after Bonferroni correction are marked with an asterisk.

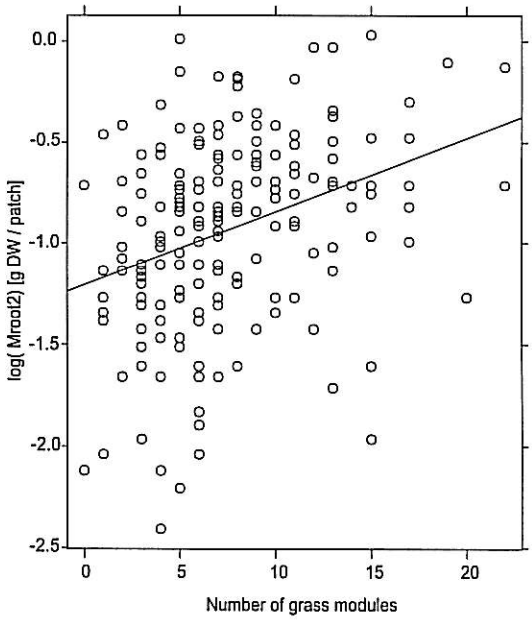

Fig. 2: Relation of in-grown root biomass (dry weight, DW) to number of grass modules in patch neighbourhood. This diagram displays simpler model than the one presented in results, with the response to number of grass modules averaged over the experimental treatments and runs. The fitted model equation is log(Mroot2) = -1.2079 + 0.0365 * (number of grass modules), R^2=0.12, $F_{1,184}$=24.22, $p < 10^{-5}$.

When similar multivariate and regression analyses as described above for in-grown root biomass were done for pre-treatment community, following results were obtained. I found no relation between the initial root biomass, the initial total belowground biomass or the ratio of the rhizome biomass to the root biomass on one side and the neighbouring community composition aboveground on the other side. Both the biomass of roots before the experiment and the total initial belowground biomass were positively correlated with the absolute number as well as with relative proportion of grass modules, and negatively correlated with the relative proportion of mycorrhizal forbs in the patch neighbourhood (Tab. 4). I found no relation of any initial belowground biomass characteristics to the total number of modules of neighbouring plants or to the abundance of non-mycorrhizal plants. The ratio of rhizome biomass to root biomass had no significant relationship with any predictor used in these analyses.

When testing contribution of individual species to the effect of neighbouring modules categories upon belowground biomass in pre-treatment community, I found only the abundance of *Festuca rubra* to be a significant predictor for the amount of initial root biomass ($p=0.0011$, compared with the Bonferroni-corrected threshold level 0.0023). The patches with a higher abundance of *F. rubra* in their neighbourhood had a higher root biomass.

Discussion

Nutrient application had a strong effect on root proliferation into the patches in all experimental runs (Tab. 2, Fig. 1B). This finding is in agreement with results of numerous authors (e. g. Hodge et al. 1998, Farley & Fitter 1999, Fransen et al. 1998, 1999a) concerning response of individual plant species. Nevertheless, it is a novel information for the community-level response in grasslands. Some species are known to respond to local nutrient enrichment by change of their uptake kinetics (Caldwell et al. 1992, Jackson & Caldwell 1996) or by morphological (architectural) changes (see e.g. Fitter 1994, Arredondo & Johnson 1999, Farley & Fitter 1999) rather than by increased biomass allocation. Results presented here do not allow me to differentiate among these components of response but provide evidence of significant biomass allocation into nutrient-enriched microsites. Amplitude of growth response could be enlarged by use of inorganic form of nutrients and it is probable that application of an organic resource would result in less impressive root proliferation (Hodge et al. 1998, 2000). However, investigation of plant response to soil microsites enriched with inorganic form of nutrients is not unrealistic, as the application of inorganic fertilisers is quite frequent in semi-natural grassland communities.

The fungicide application increased root biomass in patches (Tab. 2, Fig. 1B). One explanation may be the increased availability of assimilates for root growth in plants with suppressed mycorrhizal symbiosis. The estimates of carbon amount allocated to fungal associates range from 4 to 20 % of plant's total budget (Jacobsen & Rosendahl 1990, Johnson et al. 1997). A plant can hypothetically continue to transport assimilates into belowground parts independently of whether the carbon is used for construction and maintenance of new roots or consumed by fungal symbionts. Possibly, the plants with suppressed mycorrhizal colonisation produced more of fine

roots to occupy the newly available soil space and to extract the available nutrients (Hetrick et al. 1988, Cui & Caldwell 1996). Hetrick et al. (1988) and Hetrick (1991) suggested that plants highly dependent on mycorrhizal symbiosis reduce the metabolic cost of their roots by developing coarse root systems and that these changes in root architecture may be induced directly by mycorrhizal fungi. Fusconi et al. (2000) showed in *Allium porrum* that mycorrhizal fungi blocked root apical meristem activity. As most of the grassland plant species are mycorrhizal, root growth without fungal symbionts could induce significant changes in root morphology and subsequently in root biomass. A direct effect of benomyl is unlikely, since Merryweather & Fitter (1996) found no substantial increase in soil phosphate concentration because of benomyl application. Several authors have demonstrated a negative effect of benomyl on non-mycorrhizal fungi (Carey et al. 1992, West et al. 1993, Newsham et al. 1994), soil bacteria, and actinomycetes (Van Faassen 1974). The suppression of pathogenic fungi or of microorganisms can be another explanation of more intensive root growth in the patches treated with benomyl.

The root proliferation into patches with application of both nutrients and benomyl was almost as intensive as in the case of independent effects of both factors. The small negative interaction could be caused by physiological limits of root growth.

Substrate structure had no effect on root proliferation into the patches what contradicts the results of McConnaughay & Bazzaz (1992). They found that root biomass decreased as density of artificial neighbour root systems increased, and this negative response was reduced by nutrient addition. Although sand used in the present study differs from the artificial root systems used by McConnaughay and Bazzaz (1992), the sand grains, mainly those of coarse fraction, may still provide physical obstruction for root proliferation and fragment the belowground space. Differences in root morphological characteristics of two graminoid species (*Luzula campestris* and *Poa angustifolia*) penetrating into patches with different substrate structure were recorded in a study centred on particular species (Šmilauerová & Šmilauer 2001, manuscript). Nevertheless, these fine changes of root morphology (e.g. number of root tips) which are not accompanied with changes of root length or root diameter, cannot cause root biomass changes. In individually potted plants, the response to density of artificial neighbouring root system differed among the species (McConnaughay & Bazzaz 1992). Species responses in real grassland community are complementary and such a summary variable as the total root biomass can be much less sensitive then the corresponding measurements on individuals of a single species. Different root response to benomyl application in patches with different substrate type can be perhaps explained by larger adsorption of benomyl to soil particles (Van Faassen 1974, Liu & Hsiang 1994).

Grass modules abundance in close neighbourhood of patches had strong effect on the amount of in-grown root biomass (Tab. 4, Fig.2). Grass species growing in heterogeneous environment can allocate proportionally more biomass to roots than the dicotyledoneous species (e.g. Gross et al. 1992, Robinson & Van Vuuren 1998), but seedlings or single tillers were used in those studies. Data from presented experiment do not allow us to estimate how large is the fraction of root biomass produced by grasses. However, following findings support the hypothesis that the

increase of root biomass in patches surrounded by larger number of grass modules corresponds to increase of biomass of grass roots.

a) positive correlation between the root biomass in patches and number of grass modules was found not only at the end of experiment, but also in the pre-treatment community

b) number of forb modules in patch neighbourhood did not have significant relation to root biomass in patches at the end of experiment; negative correlation between these two variables was found in pre-treatment community

c) in the part of the project, centred on particular species, the average length of *Poa angustifolia* roots grown into the patches was 2.66 times longer than those of *Plantago lanceolata* and 5.18 times longer than those of *Luzula campestris* (Šmilauerová & Šmilauer 2001, manuscript). This was mainly due to much denser branching of *Poa* roots. *Poa* was also the most frequent grass species in the patch neighbourhood in all experimental runs in present study

From previous facts follows that grasses have a determining effect onto soil space occupation pattern both on the long-term scale as well as in the short-term response to availability of nutrient-rich patches.

Negative relation of relative proportion of non-mycorrhizal species modules with root biomass grown into the patches was significant only in non-fertilised patches. The fact that this effect was not found in pre-treatment community suggests that the non-mycorrhizal species responded to patch establishment more slowly than the other species. This is also supported by the above-mentioned finding, that the non-mycorrhizal species *Luzula campestris* produced in experimental patches the shortest roots among the three studied species (Šmilauerová & Šmilauer 2001, manuscript). Less crowded soil volume in patches surrounded by non-mycorrhizal species can be more slowly occupied by their roots or the roots of other species can invade that space.

The situation seems to be different with mycorrhizal forbs. They does not seem to play any determining role in occupation of a new patch but they seem to be able to maintain soil volume in their close neighbourhood less crowded in the long term. Their apparently small effect at the beginning of root proliferation into new patch can be due to large variability in species of forb group in the size of their modules and their root characteristics.

Pecháčková et al. (1999) studied relationship between spatial distribution of aboveground and belowground parts of seven plant species in species-poor montane grassland. They found that conspecific correlation between frequencies of roots and plant presence aboveground differed among species as well as between soil layers. Most of the significant correlations occurred at shorter spatial lag (up to 2 cm) and their number decreased dramatically with soil depth. In my experiment, the band of vegetation surrounding a patch, where number of species modules was recorded, was 3.5 cm wide. Although this distance is larger than the spatial lag of 2 cm, it is quite probable that not all the plant modules, which produced roots found in the cores, were recorded. Pecháčková et al. (1999) focused their study on relation between plant parts in undisturbed community so their results correspond in my study mainly to the results from the pre-treatment

community. Moreover, the montane grassland studied in their project was very poor in species. The higher species richness of grassland on my study site and the consequent high variability in floristic composition of vegetation surrounding the patches can be the main reason for not finding any relation of root biomass in patches to species composition aboveground or to presence of any particular species aboveground, in contrast to Pecháčková et al. (1999). Interesting is the fact that both studies found root biomass positively correlated with *Festuca rubra* presence aboveground.

Design of this experiment does not allow me to evaluate differences in community response during growing season as the spatial variability is confounded with seasonal differences, so the experimental runs were treated as random-effect blocks. For example, the experimental runs 1 and 3 were situated in close proximity, but species composition recorded in patches neighbourhood significantly differed between them. Because the average number of neighbour modules is significantly smaller in the experimental run 3, I suppose that this difference is at least partially caused by a drought during the third experimental run and disappearance of aboveground parts of some early-season species. Smaller amount of roots grown into the patches in experimental run 1 could be caused by higher relative abundance of non-mycorrhizal species in the patch neighbourhood as well as by the early start of this experimental run. Although absolute amount of in-grown roots was smaller, the relative response to treatments in comparison with control patches was as large as in the other two experimental runs. The most interesting finding from this point of view is that in spite of all the differences between experimental runs in timing, soil properties, species composition, and relative and absolute abundance of rooting modules of recognised plant groups, the whole community response to experimental treatment did not differ among the experimental runs.

The results presented here originate from an experiment established under field conditions. Although patch introduction disturbed existing root systems of the studied community, this disturbance is similar to the natural occurrence of corridors, built by small rodents or moles, gradually refilled with soil or dragged-in material. In comparison with greenhouse experiments, the state of plant community as well as of the communities of soil organisms was much more close to a natural situation. On the other hand, the complexity of experimental conditions can obscure responses of the studied part of whole community. This should be taken into account when results of such an experiment are compared with results of other studies.

In summary, I demonstrated selective allocation of root biomass into nutrient-rich microsites at the whole community level. Suppression of mycorrhizal fungi did not substantially change that pattern, but the root proliferation increased with fungicide application. Root biomass in experimental patches, as well as root biomass of the undisturbed community, were significantly correlated with the number of grass tillers in close neighbourhood. This finding suggests that grasses may form a principal matrix in the grassland structure not only aboveground, but also belowground.

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References

Anonymous (1999): *S-PLUS 2000 Guide to Statistics*, Volume 1 & 2. Data Analysis Products Division, MathSoft, Seattle.

Arredondo J.T. & Johnson D.A. (1999): Root architecture and biomass allocation of three range grasses in response to nonuniform supply of nutrients and shoot defoliation. *New Phytol.* 143: 373-385.

Cahill J.F. & Casper B.B. (1999): Growth consequences of soil nutrient heterogeneity for two old-field herbs, *Ambrosia artemisiifolia* and *Phytolacca americana*, grown individually and in combination. *Annals Bot.* 83: 471-478.

Cahill J.F. & Casper B.B. (2000): Investigating the relationship between neighbor root biomass and belowground competition: field evidence for symmetric competition belowground. *Oikos* 90: 311-320.

Caldwell M.M. (1994): Exploiting nutrients in Fertile soil microsites. In: Caldwell M.M. & Pearcy R.W. (eds.), *Exploatation of environmental heterogeneity by plants: ecophysiological processes above- and below-ground.* Academic Press, Inc., pp. 325-347.

Caldwell M.M., Manwaring J.H. & Jackson R.B. (1991a): Exploatation of phosphate from fertile soil microsites by three Great Basin perennials when in competition. *Funct. Ecol.* 5: 757-764.

Caldwell M.M., Manwaring J.H. & Durham S.L. (1991b): The microscale distribution of neighbouring plant roots in fertile soil microsites. *Funct. Ecol.* 5: 765-772.

Caldwell M.M., Dudley L.M. & Lilieholm B. (1992): Soil solution phosphate, root uptake kinetics and nutrient acquisition: implications for a patchy soil environment. *Oecologia* 89: 305-309.

Caldwell M.M. & Pearcy R.W. (eds.) (1994): *Exploatation of environmental heterogeneity by plants: ecophysiological processes above- and below-ground.* Academic Press, Inc.

Caldwell M.M., Manwaring J.H. & Durham S.L. (1996): Species interaction at the level of fine roots in the field: Influence of soil nutrient heterogeneity and plant size. *Oecologia* 106: 440-447.

Campbell B.D., Grime J.P. & Mackey J.M.L. (1991): A trade-off between scale and precision in resource foraging. *Oecologia* 87, 532-538.

Carey P. D., Fitter A. H. & Watkinson A. R. (1992): A field study using the fungicide benomyl to investigate the effect of mycorrhizal fungi on plant fitness. *Oecologia* 90: 550-555.

Cleveland W. (1993): *Visualizing Data.* Hobart Press, Summit.

Cui M. & Caldwell M.M. (1995): VA mycorrhizae and plant acquisition of nitrate and phosphorus when in uniform and patchy distributions. In: *80th annual ESA meeting. Program and abstracts*. Part 2: Abstracts. - Suppl. of Bull. Ecol. Soc. Am., Vol. 76: 57.

Cui M. & Caldwell M. M. (1996): Facilitation of plant arbuscular mycorrhizas from enriched soil patches I. Roots and hyphae exploiting the same soil volume. *New Phytol.* 133: 453-460.

Drew M.C. (1975): Compensation of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. *New Phytol.* 75: 479-490.

Drew M.C., Saker L.R. & Ashley T.W. (1973): Nutrient supply and the growth of the seminal root system in barley I. The effect of nitrate concentration on the growth of axes and laterals. *J. Exp. Bot.* 24: 1189-1202.

Drew M.C. & Saker L.R. (1975): Nutrient supply and the growth of the seminal root system in barley II. Localized, compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of the root system. *J. Exp. Bot.* 26: 79-90.

Duke S.E. & Caldwell M.M. (2000): Phosphate uptake kinetics of *Artemisia tridentata* roots exposed to multiple soil enriched-nutrient patches. *Flora* 195: 154-164.

Einsmann J.C., Jones R.H., Pu M. & Mitchell R.J. (1999): Nutrient foraging traits in 10 co-occurring plant species of contrasting life forms. *J. Ecol.* 87: 609-619.

Farley R.A. & Fitter A.H. (1999): The responses of 7 co-occurring herbaceous perennials to localized nutrient-rich patches. *J. Ecol.* 87: 849-859.

Fitter A.H. (1982): Influence of soil heterogeneity on the coexistence of grassland species. *J. Ecol.* 70: 139-148.

Fitter A.H. (1994): Architecture and biomass allocation as components of the plastic response of root system to soil heterogeneity. In: Caldwell M.M. & Pearcy R.W. (eds.), *Exploatation of Environmental Heterogeneity by Plants*, Academic Press, Inc., pp. 305-323.

Fransen B., de Kroon H. & Berendse F. (1998): Root morphological plasticity and nutrient acquisition of perennial grass species from habitats of different nutrient availability. *Oecologia* 115: 351-358.

Fransen B. & de Kroon H. (1999): Root proliferation, root turnover rates and biomass production of two perennial grass species from habitats of contrasting nutrient availability: the long-term disadvantages of selective root placement. In: Fransen B., *Root foraging. The consequences for nutrient acquisition and competition in heterogeneous environments.* PhD thesis. Wageningen University, Wageningen, The Netherlands.

Fransen B., Blijjenberg B. & de Kroon H. (1999a): Root morphological and physiological plasticity of perennial grass species and the exploatation of spatial and temporal heterogeneous patches. *Plant Soil* 211: 179-189.

Fransen B., de Kroon H. & Berendse F. (1999b): Nutrient heterogeneity changes competition between populations of *Festuca rubra* L. and *Anthoxanthum odoratum* L. In: Fransen B., *Root foraging. The consequences for nutrient acquisition and competition in heterogeneous environments*. PhD thesis. Wageningen University, Wageningen, The Netherlands.

Fusconi A. et al. (2000): Root apical meristems of *Allium porrum* L. as affected by arbuscular mycorrhizae and phosphorus. *Protoplasma* 214: 219-226.

Grime J.P. (1994): The role of plasticity in exploiting environmental heterogeneity. In: Caldwell M.M. & Pearcy R.W. (eds.), *Exploatation of environmental heterogeneity by plants: ecophysiological processes above- and below-ground.* Academic Press, Inc., pp. 1-19.

Gross K.L., Maruca D. & Pregitzer K.S. (1992): Seedling growth and root morphology of plants with different life-histories. *New Phytol.* 120: 535-542.

Grubb P.J. (1976): A theoretical background to the conservation of ecologically distinct groups of annuals and biennials in the chalk grassland ecosystem. *Biol. Conserv.* 10: 53-76.

Harley J.L. & Harley E.L. (1987): A check-list of mycorrhiza in the British flora. *New Phytol.* 105: 1-102.

Hetrick B.A.D. (1991): Mycorrhizas and root architecture. *Experientia* 47: 355-362. 47: 355-362.

Hetrick B.A.D., Leslie J.F., Wilson G.T. & Kitt D.G. (1988): Physical and topological assessment of effects of a vesicular-arbuscular mycorrhizal fungus on root architecture of big bluestem. *New Phytol.* 110: 85-96.

Hodge A., Stewart J., Robinson D., Griffiths B.S. & Fitter A.H. (1998): Root proliferation, soil fauna and plant nitrogen capture from nutrient-rich patches in soil. *New Phytol.* 139: 479-494.

Hodge A., Robinson D., Griffiths B.S. & Fitter A.H. (1999a): Nitrogen capture by plants grown in N-rich organic patches of contrasting size and strength. *J. Exper. Botany* 50: 1243-1252.

Hodge A., Stewart J., Robinson D., Griffiths B.S. & Fitter A.H. (1999b): Plant, soil fauna and microbial responses to N-rich organic patches of contrasting temporal availability. *Soil Biol. Biochem.* 31: 1517-1530.

Hodge A., Robinson D., Griffiths B.S. & Fitter A.H. (1999c): Why plants bother: Root proliferation results in increased nitrogen capture from an organic patch when two grasses compete. *Plant Cell Environ.* 22: 811-820.

Hodge A., Stewart J., Robinson D., Griffiths B.S. & Fitter A.H. (2000): Competition between roots and soil microorganisms for nutrients from nitrogen-rich patches of varying complexity. *J. Ecol.* 88: 150-164.

Huber-Sannwald E., Pyke D.A., Caldwell M.M. & Durham S. (1998): Effects of nutrient patches and root systems on the clonal plasticity of a rhizomatous grass. *Ecology* 79: 2267-2280.

Jackson R.B. & Caldwell M.M. (1993): Geostatistical patterns of soil heterogeneity around individual perennial plants. *J. Ecol.* 81: 683-692.

Jackson R.B. & Caldwell M.M. (1996): Integrating resource heterogeneity and plant plasticity - modelling nitrate and phosphate uptake in a patchy soil environment. *J. Ecol.* 84: 819-903.

Jakobsen I. & Rosendahl L. (1990): Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytol.* 115: 77-83.

Johnson N.C., Graham J.H. & Smith F.A. (1997): Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytol.* 135: 575-585.

Liu L.X. & Hsiang T. (1994): Bioassays for benomyl adsorption and persistence in soil. *Soil. Biol. Bioch.* 26: 317-324.

McConnaughay K.D.M. & Bazzaz F.A. (1992): The occupation and fragmentation of space: consequences of neighbouring roots. *Funct. Ecol.* 6: 704-710.

Merryweather J. & Fitter A. H. (1996): Phosphorus nutrition of an obligately mycorrhizal plant treated with the fungicide benomyl in the field. *New Phytol.*, 132: 307-311.

Newsham K. K., Fitter A. H. & Watkinson A. R. (1994): Root pathogenic and arbuscular mycorrhizal fungi determine fecundity of asymptomatic plants in the field. *J. Ecol.* 82: 805-814.

Pecháčková S., During H.J., Rydlová V. & Herben T. (1999): Species-specific spatial pattern of belowground plant parts in a montane grassland community. *J. Ecol.* 87: 569-582.

Read D.J., Koucheki H.K. & Hodgson J. (1976): Vesicular-arbuscular mycorrhiza in natural vegetation systems I. The occurrence of infection. *New Phytol.* 77: 641-653.

Robinson D. & Van Vuuren M.M.I. (1998): Responses of wild plants to nutrient patches in relation to growth rate and life-form. In: Lambers H., Poorter H. & Van Vuuren M.M.I. (eds.), *Inherent variation in plant growth. Physiological mechanisms and ecological consequences.* Backhuys Publishers, Leiden, The Netherlands, pp. 237-257.

Robinson D., Hodge A., Griffiths B.S & Fitter A.H. (1999): Plant root proliferation in nitrogen-rich patches confers competitive advantage. In: *Proc. R. Soc. London, series B-Biol. Sci.* 266: 431-435.

Rothmaler W. (1976): *Exkursionsflora für die Gebiete der DDR und der BRD. Kritischer Band.* Volk und Wissen Volkseigener Verlag, Berlin.

Ryel R.J., Caldwell M.M. & Manwaring J.H. (1996): Temporal dynamics of soil spatial heterogeneity in sagebrush-wheatgrass steppe during a growing season. *Plant Soil* 184: 299-309.

Stanton N.L. (1988): The underground in grasslands. *Ann. Rev. Ecol. Syst.* 19: 573-589.

St. John T.V., Coleman D.C. & Reid C.P.P. (1983): Growth and spatial distribution of nutrient-absorbing organs: Selective exploatation of soil heterogeneity. *Plant Soil* 71: 487-493.

Šmilauer P. (1996): *Modern approaches to analysis of ecological data.* PhD thesis, University of South Bohemia, České Budějovice, Czech Republic.

Šmilauer P. & Šmilauerová M. (2000): Effects of AM symbiosis exclusion on grassland community composition. *Folia Geobot.* 35: 13-25.

Šmilauerová M. & Šmilauer P. (2001): Morphological response of plant roots to heterogeneity of soil resources. *manuscript*

Ter Braak C.J. F. & Šmilauer P. (1998): *CANOCO reference manual and user's guide to Canoco for Windows: software for canonical community ordination (version 4).* Microcomputer Power, Ithaca, 352 pp.

Van Faassen H.G. (1974): Effect of the fungicide benomyl on some metabolic processes, and on numbers of bacteria and actinomycetes in the soil. *Soil Biol. Biochem.* 6: 131-133.

Vogt K.A., Vogt D.J., Asbjornsen H. & Dahlgren R.A. (1995): Roots, nutrients and their relationship to spatial patterns. *Plant Soil* 168-169: 113-123.

West H. M., Fitter A. H. & Watkinson A. R. (1993): Response of *Vulpia ciliata* ssp. *ambigua* to removal of mycorrhizal infection and to phosphate application under natural conditions. *J. Ecol.* 81: 351-358.

Wijesinghe D.K. & Hutchings M.J. (1999): The effects of environmental heterogeneity on the performance of *Glechoma hederacea*: the interactions between patch contrast and patch scale. *J. Ecol.* 87: 860-872.

4 Field study of morphological response of plant roots to heterogeneity of soil resources

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Summary

- Root morphological response to experimentally induced soil heterogeneity was studied on three grassland species under field conditions.
- Nutrient application was combined with suppression of mycorrhizal infection and with substrate structure modification in experimental patches. For each isolated root, we determined five dimensional characteristics and two topological parameters, including newly introduced topological index (dichotomous branching index).
- Non-mycorrhizal Luzula campestris responded little to nutrient application, but strongly to benomyl application, in all measured characteristics. Mycorrhizal Poa angustifolia produced the longest, most branched roots but exhibited limited sensitivity to nutrients and benomyl application. Strongly mycorrhizal Plantago lanceolata was the species most sensitive to nutrient application, but almost did not respond to benomyl application. It was the only one among studied species with root characteristics influenced (negatively) by increased production of total root biomass in the patches. Substrate structure influenced dimensional characteristics of Poa and Luzula roots, but not the topological indices.
- Results indicate different strategies of soil microsites exploitation for the three studied species. Root topology seems to play limited role in this process.

Keywords: root morphology, root topology, nutrients, benomyl, arbuscular mycorrhiza, grassland community

Introduction

The soil environment of natural ecosystems is heterogeneous both in time and space, even on a small scale (Jackson & Caldwell, 1993, Ryel *et al.*, 1996, Šmilauer, 1996). Plant response to nutrient patchiness can include changes in biomass allocation, in uptake kinetics or in root morphology (e.g. Caldwell *et al.*, 1992, Fitter, 1994, Fransen *et al.*, 1998, Derner & Briske, 1999, Einsmann *et al.*, 1999, Farley & Fitter, 1999, Ryser & Eek, 2000). Some of the competitive, dominant species do not respond to nutrient patches by changes listed above, but their roots represent a large part of root biomass in patches thanks to their high growth rate (Campbell *et al.*, 1991, Grime, 1994). Grime (1994) suggested that subordinate species forage by their roots more precisely than dominant species and that the dicotyledoneous species forage more precisely than

the grasses. According to Grime (1994), a trade-off exists between the scale (high for dominant plants) and the precision (high for subdominant plants) in resource foraging. Study of Einsmann *et al.* (1999) did not confirm Grime prediction: they found scale and precision positively correlated in herbaceous species. Robinson & Van Vuuren (1998) analysed published data of root response to nutrient patches for 27 wild plant species, which differed in growth rate (RGR_{max}) and in life form (grasses and forbs). They found that roots of fast-growing species proliferate into nutrient patches more precisely but only relatively to uniformly nutrient-deficient control, and that forbs proliferate more precisely than grasses.

The plasticity of root architecture was predicted to play an important role in the root response to soil heterogeneity (e.g. Fitter, 1994), but it has rarely been measured, especially as a response to nutrient patchiness (Fitter, 1994, Arredondo & Johnson, 1999). In an experiment where root response to increased nutrient availability was studied irrespective of spatial distribution of nutrients (Fitter *et al.*, 1988) root topology was generally insensitive to changes in nutrient supply while the length of exterior and interior links (sensu Fitter, 1991) and the specific root length changed with nutrient availability. Fitter & Stickland (1991) and Taub & Goldberg (1996) found root topology of grasses less sensitive to changes of nutrient availability than in the dicotyledoneous species.

Although role of mycorrhizal fungi in nutrient acquisition by host plants, mainly in nutrient-poor soils (Read *et al.*, 1976), is generally accepted (e.g. Koide, 1991, Wilcox, 1991), their contribution to nutrient acquisition from soil microsites has been rarely studied (St John *et al.*, 1983a,b, Cui & Caldwell, 1996, Farley & Fitter, 1999). Tibbett (2000) ascribed a higher importance in nutrient patch exploitation to mycorrhizal mycelia than to plant root response. Moreover, the root morphology seems to be directly influenced by mycorrhizal symbionts. Hetrick *et al.* (1988) and Hetrick (1991) suggested that plants highly dependent on mycorrhizal symbiosis reduce the metabolic cost of their roots by developing coarse root systems and that these changes in root architecture may be induced directly by mycorrhizal fungi. Fusconi *et al.* (2000) showed in *Allium porrum* that mycorrhizal fungi blocked root apical meristem activity. Nutrient requirements of plants change during the season depending on their phenological stage. These changes are reflected, for example, in the seasonal dynamics of mycorrhizal symbiosis (Hetrick *et al.*, 1988, 1994a, 1994b, Mullen & Schmidt, 1993, Hartnett *et al.*, 1993, Lapointe & Molard, 1997).

Most experiments on root response to soil nutrient microsites have been performed under greenhouse conditions, usually with young plants and with no competition involved. The response of plant roots is often different when plants are grown individually and when under competition (Huber-Sannwald *et al.*, 1998, Cahill & Casper, 1999, Fransen *et al.*, 1999b, Robinson *et al.*, 1999). Caldwell *et al.* (1991a, 1991b, 1996) found that root exploitation of nutrient patches by a plant of one species depends on the species identity of neighbouring roots and on the size of competing plants. McConnaughay & Bazzaz (1992) found considerable differences among species in their sensitivity to space fragmentation by artificial root systems. This reduction in

growth of plants constrained in the deployment of their roots was not detected at higher nutrient levels.

The aim of present study was to compare response of several distinct plant species from a grassland community to nutrient patchiness established under field conditions and to estimate the role of mycorrhizal symbiosis in this process. For this study, we chose three species occurring together in a nutrient-poor semi-natural grassland, one forb - *Plantago lanceolata* L. and two graminoids - *Luzula campestris* (L.) DC. and *Poa angustifolia* L.. They are dominant species on this site and differ in ecologically important characteristics (e.g. type of clonal growth, phenological separation in season, extent of mycorrhizal dependence). Attention was paid only to root morphological response; physiological plasticity was not studied.

The following questions were addressed in this study:

1. Do roots of the coexisting species respond similarly to a spatially localised increase in the nutrient availability?
2. Is their response modified by suppression of symbiotic mycorrhizal fungi?
3. Does substrate structure modify the morphological and architectural characteristics of roots growing into a newly created soil space?

As the total root production in the experimental patches can differ not only due to the type of treatment, but it can also depend on the surrounding community composition (Šmilauerová 2001), we asked an additional question:

4. Is the response of target species correlated with the growth response of neighbouring community (quantified by the total belowground biomass in-grown into the patches)?

Materials and methods

Research site

The research site is located near Zvíkov village (10 km east of České Budějovice, 48°59'N, 14°36'E, 500 m a. s. l.). The vegetation is an oligotrophic, traditionally managed meadow on a shallow valley slope. The meadow is cut once a year, usually in the middle of June. More information can be found in Šmilauer & Šmilauerová (2000). The plant species nomenclature follows Rothmaler (1976).

Target species

Three target species with a different role in the structure of the grassland community and with a different mycorrhizal dependence were chosen for this study: *Luzula campestris*, a rhizomatous non-mycorrhizal species with early spring activity, *Poa angustifolia*, a rhizomatous mycorrhizal species flowering in June, and *Plantago lanceolata*, a rosette forb with strong mycorrhizal

colonisation and with a long reproductive period from June to the end of summer. While *Poa* and *Plantago* are dominant species in most of the study site area, *Luzula* dominates its poorest parts.

Experimental design and procedure

Three separate experimental runs with an identical design were established during the 1999, each one represented by a single experimental plot. Each of the runs started at that part of season when one of the target species flowered so these species were in a comparable phenological stage (for characterisation of these three experimental runs see Table 1).

Target species	Timing	Most frequent neighbour species	Soil properties
Luzula campestris	18 March - 10 May 1999	LC, PA, AM, PL, CH	well drained, very nutrient poor soil with poorly developed H horizon
Poa angustifolia	1 April - 25 May 1999	PA, CP, AM, FR, PL	soil with more developed H horizon, wetter and more nutrient rich than in other blocks
Plantago lanceolata	1 June - 24 July 1999	PL, LC, PA, FR, HL	as for the run with *Luzula campestris*

Table 1: The characterisation of the three experimental runs. The codes for frequent neighbour species are: AM - *Achillea millefolium*, CH - *Cerastium holosteoides*, CP - *Campanula patula*, FR - *Festuca rubra*, HL - *Holcus lanatus*, LC - *Luzula campestris*, PA - *Poa angustifolia*, PL - *Plantago lanceolata*

In each of these three experimental runs, there were seven replicate blocks. Each replicate block contained all 12 combinations of the experimental treatments (2 levels of Nutrients factor * 2 levels of Fungicide factor * 3 types of Substrate modification) randomly allocated to 3 x 4 sampling points arranged in a rectangular grid with the span of 0.25 m. To establish a patch, soil from the soil profile was taken near an individual of the target species (situated close to one of the sampling points) with the aid of soil corer (diameter 4.5 cm, depth 10 cm). The soil was then sieved to remove roots and rhizomes (sieve mesh size 3 mm) and either put back into the hole or mixed with fine (Ø < 1 mm) or coarse (Ø 3 - 5 mm) sand in 1:1 ratio and then placed back into the hole. Nutrients in the form of phosphate, nitrate, and ammonia ions (using 0.2 g Na_3PO_4*12 H_20, 0.24 g $NaN0_3$, and 0.15 g NH_4Cl per patch and application) and the fungicide benomyl (Bavistin, BASF; 0.15 g Bavistin per patch) were applied in 0.1 l of water per patch. The control patches were treated with the same amount of water. The application of nutrients, fungicide, and water was repeated 3 weeks after the first application.

Patches with surrounding soil and plants (soil columns with diameter of 11cm and depth approx. 12 cm) were collected after 53 days of the experimental run. The samples were taken only from five or six replicate blocks in each experimental plot; the remaining patches were used for other purposes, including chemical analyses. From each patch, in-grown roots of one or more individuals of the target species were isolated in dry way by hand by means of needles, tracking their connections to aboveground parts. Only the root parts growing within the patches were used

for analyses of root characteristics. After isolation of the target plant roots all the remaining roots were separated from the patch substrate. Roots and rhizomes collected in the beginning and at the end of experiment were washed, dried (80°C, for 24 hours) and weighed.

Separated roots of the target species were spread on a glass plate and their images scanned using a flatbed scanner with resolution of 600 DPI (UMAX Astra 1220, with transparency adapter). Root architecture, recorded in the image files, was evaluated using the RootArch software (P. Šmilauer, unpublished). We used several morphological root characteristics for the statistical analyses: ELL - the average length of exterior links, ILL - the average length of interior links, μ - the magnitude of the root, TotL - the total length of the root, TotL:μ - the average root length per root tip, and two topological indices $\log(p_e)$:$\log(\mu)$ and DBI (see below for its definition). All the characteristics, except the last one, are described in Fitter (1991). Primary morphological characteristics are defined in Table 2. In statistical analyses, non-branched roots were considered to consist of a single exterior link.

Characteristic	Definition
EL - exterior link	terminal part of root between the root tip with meristem and the nearest branching point
IL - interior link	root part joining other links, i.e. the part of root between any adjacent branches
μ - magnitude	number of exterior links (i.e. root tips) served by a root
TotL - total length of root	sum of the lengths of all exterior and interior links of the root, expressed in millimetres
p_e - total exterior path length	sum of the number of links in all paths from any exterior link to the base of the root
max(p_e) - maximal total exterior path length	total exterior path length of imaginary root of given magnitude if fully herringbone-style branched
min(p_e) - minimal total exterior path length	total exterior path length of imaginary root of given magnitude if fully dichotomously branched

Table 2: Definitions of primary root morphological characteristics used in this paper

A newly introduced topological index called **dichotomous branching index** (DBI) is calculated for a particular root system with the magnitude μ and the total exterior path length p_e as

$$DBI = [p_e - \min(p_e)] / [\max(p_e) - \min(p_e)]$$

This index shows the relative position of the actual total exterior path length value of a root between the reference values $\min(p_e)$ and $\max(p_e)$. Its values are therefore between zero and one and so it is easier to estimate the position of the root on the scale between fully dichotomously and fully herringbone-style branched roots of the given magnitude. Moreover, this index seems to be scale-independent, which is not true for other commonly used topological indices (e.g. p_e:$\max(p_e)$ or p_e:$E(p_e)$, where $E(p_e)$ is the expected value of the parameter p_e in the case of random branching).

The analysed parameters can be divided conceptually into two groups. One group (ILL, ELL, μ, TotL, and TotL:μ) are referred to as **dimensional parameters** in the results and discussion, while the other group contains the **topological indices** $\log(p_e):\log(\mu)$ and DBI.

Supplementary measurements

For estimation of soil moisture dynamics during the experiments, sensors for measuring the water saturation deficit of soil (by a portable digital metering device, based on soil resistance measurements, Watermark 30KTCD, Irrometer Co., Riverside, U.S.A.) were placed in patches with one of the three applied substrate modifications near the experimental plots during the experimental runs. Additionally, two sensors were placed into soil profile among the places of the three experimental runs, for permanent measurements in non-manipulated soil during the whole season.

The effectiveness of benomyl application in suppression of mycorrhizal colonisation was evaluated on root samples from 145 soil patches with all treatment combinations, from all the experimental runs. Roots were stained using the modified Phillip & Hayman (1975) procedure, with Chlorazol Black E stain. The arbuscular mycorrhizal colonisation was examined using a microscope Olympus BX50, at magnification of 400x and 200x. The percentage of root length colonised was then estimated for the whole sample at magnification of 100x and 45x.

Statistical analyses

For statistical analyses where the response variable was measured on the individual roots, an analysis of variance including additional error level (corresponding to plant identity, below the error level of individual cores, at which the experimental treatments were applied) was used (nested ANOVA). All the response variables, except log(Pe):log(μ), were log-transformed to suppress their heteroscedasticity. Additionally, effect of the experimental blocks within each of the three runs was modelled using a factor with a random effect. When analysing the effect of in-grown root biomass upon the root morphological properties, the same nested model approach was used, this time using a quantitative predictor. When using the two topological indices as response variables, only the roots with more than three root tips were included, as the two extreme topologies (the herringbone and dichotomous branching) cannot be distinguished for less branched systems.

When the amount of in-grown root biomass was used as a predictor, it was also log-transformed, because we expected its effect upon the root characteristics to be multiplicative (invoking a unit change in the response variable with biomass increasing by a constant percentage amount). Additionally, if the effect of root biomass was significant, we tested its conditional effect, exhibited in addition to the effects of experimental treatment. A significant conditional effect can be then interpreted as an effect of root biomass amount, not explainable by the experimental manipulation.

When analysing the response of root characteristics to experimental factors or to amount of in-grown root biomass, an attempt was always made to build a common model for all three species, and we included the target species effect into the ANOVA model. Further, we compared this model, where the effects of target species identity and of experimental factors were additive, with an alternative model, where the treatment effects were nested within the host species effect. If the latter model was significantly better (as judged by an F-ratio test on the drop of the residual sum of squares), the ANOVA model was then fitted separately for each of the three target species, because the test outcome implied that the three species responded in a different way to the same combinations of experimental factors.

Analysis of the experimental treatment effects on the mycorrhizal colonisation used a generalized linear model (GLM, McCullagh & Nelder, 1989) with an assumed Poisson distribution and logarithmic link function, because the subjective estimates of percentage colonisation cannot be modelled with the assumption of binomial distribution. No additional error level was assumed here, because we had just one root sample from each core and the replication occurred at the cores level. The dependence of percentage of non-branched roots upon the experimental treatment was modelled using a GLM with an assumed binomial distribution and logit link function. To partly suppress the effect of over-dispersion, the F-ratio based test in analysis of deviance was used (McCullagh & Nelder, 1989).

All the statistical models were fitted and tested using the S-Plus for Windows 4.5 software (MathSoft, 1999)

Results

The values of morphological characteristics of roots that grew into the patches, averaged over all the experimental treatments, are summarised for each of the target species in Table 3. *Poa* produced the longest roots, which had the largest proportion of dichotomous branching, and had numerous, very short exterior links. The in-grown roots of *Luzula* were the shortest ones among the three species, with branching restricted almost exclusively to the main axis (herringbone type of branching) and with their interior and exterior links slightly longer then in *Poa*. *Plantago* produced the smallest number of root tips per in-grown root, but both its exterior and interior links were the longest ones among the three species. The branching pattern of its roots was more dichotomous than for *Luzula* roots and its average root was twice as long as the average root of *Luzula*.

	Luzula campestris		*Poa angustifolia*		*Plantago lanceolata*	
	median	mean ± SE	median	mean ± SE	median	mean ± SE
ILL [mm]	1.20	2.14 ± 0.50	0.96	1.21 ± 0.13	3.80	4.77 ± 0.43
ELL [mm]	1.78	5.15 ± 1.08	1.59	1.77 ± 0.05	5.90	8.04 ± 0.86
μ [number of root tips]	27	74 ± 8.2	161	266 ± 21.0	16	25 ± 1.8
TotL [mm]	79	223 ± 24	409	709 ± 58	154	239 ± 15
log(p_e):log(μ)	1.81	1.80 ± 0.003	1.74	1.73 ± 0.01	1.79	1.77 ± 0.004
DBI	0.96	0.83 ± 0.02	0.48	0.52 ± 0.02	0.84	0.74 ± 0.01

Table 3: Medians, means, and standard errors of the morphological characteristics of roots that grew into newly created patches, summarised over all treatments. As many of the variables do not have symmetrical distribution, they are also characterised by the median estimates. ILL - the average length of interior links, ELL - the average length of exterior links, μ - the magnitude of the root, TotL - the total length of the root, log(p_e):log(μ) and DBI - two topological indices. For definitions of primary root morphological characteristics see Table 2, definition of DBI can be found in Methods.

The morphological characteristics of the in-grown roots responded to experimental manipulation in a qualitatively different way for the three species [$P<0.001$ for ILL ($F_{22,251}=2.481$), $P<10^{-4}$ for ELL ($F_{22,251}=2.744$), $P<0.001$ for DBI ($F_{22,251}=2.506$), $P<10^{-5}$ for TotL ($F_{22,251}=3.390$), $P<10^{-4}$ for TotL:μ ($F_{22,251}=2.726$), $P<10^{-7}$ for μ ($F_{22,251}=4.100$), $P<10^{-7}$ for log(p_e):log(μ) ($F_{22,251}=4.147$)], therefore separate analyses were done for individual target species (experimental runs).

The effect of experimental treatments upon the morphological characteristics of the in-grown roots of all three species are summarised in Table 4 (for *Luzula*), Table 5 (for *Poa*), and Table 6 (for *Plantago*), and in the Figures 1 and 2.

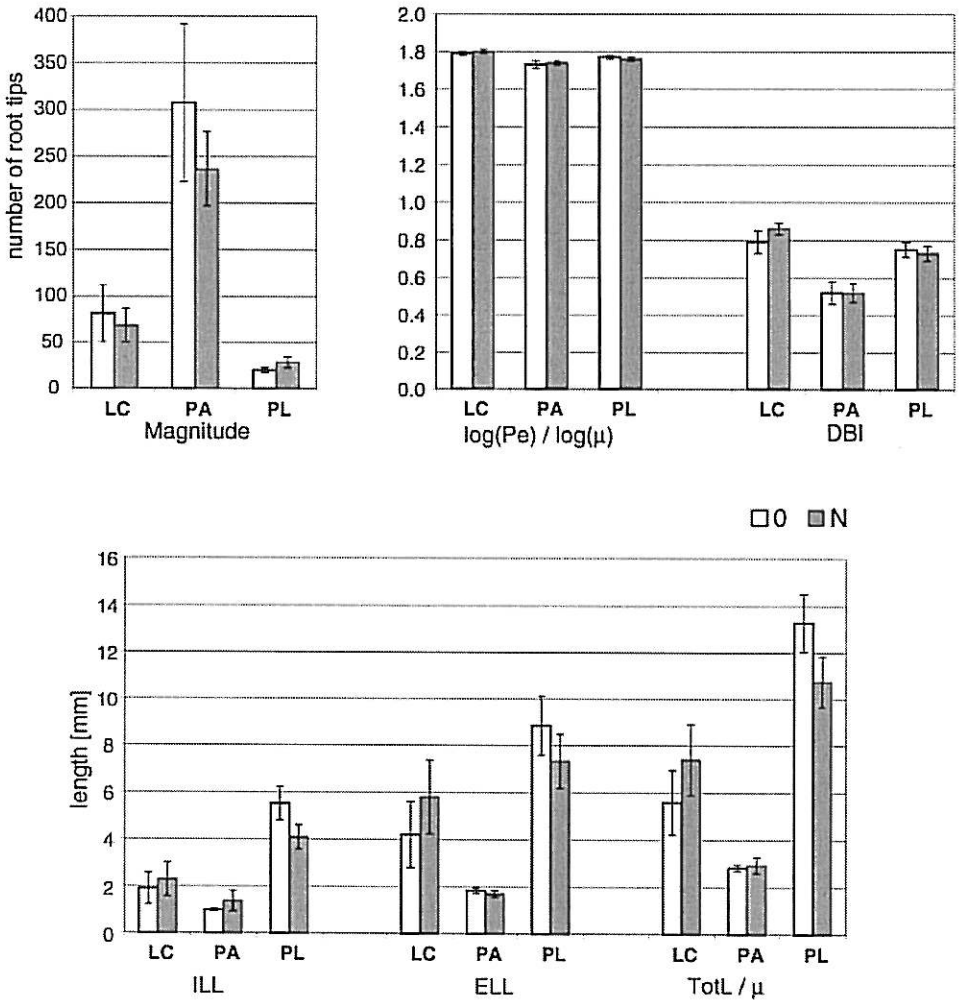

Figure 1: Effect of nutrients application on the measured morphological characteristics of roots, that grew into experimental patches. Averages are shown by vertical bars separately for the three studied species (empty bars for patches without supplementary nutrients, filled bars for patches with addition of nutrients). Vertical lines indicate 95% confidence intervals. The significance of differences in respect to nutrients application can be found in tables 4, 5, 6 for - respectively - *Luzula campestris* (LC), *Poa angustifolia* (PA), and *Plantago lanceolata* (PL). The averages for the two levels of the **Nutrients** treatment are taken over all levels of the other two experimental factors (**Fungicide** and **Substrate structure**). ILL - the average length of interior links, ELL - the average length of exterior links, μ - the magnitude of the root, TotL - the total length of the root; $\log(P_e):\log(\mu)$ and DBI (dichotomous branching index) are two topological indices (see Methods for DBI definition). For definitions of primary root morphological characteristics see Table 2.

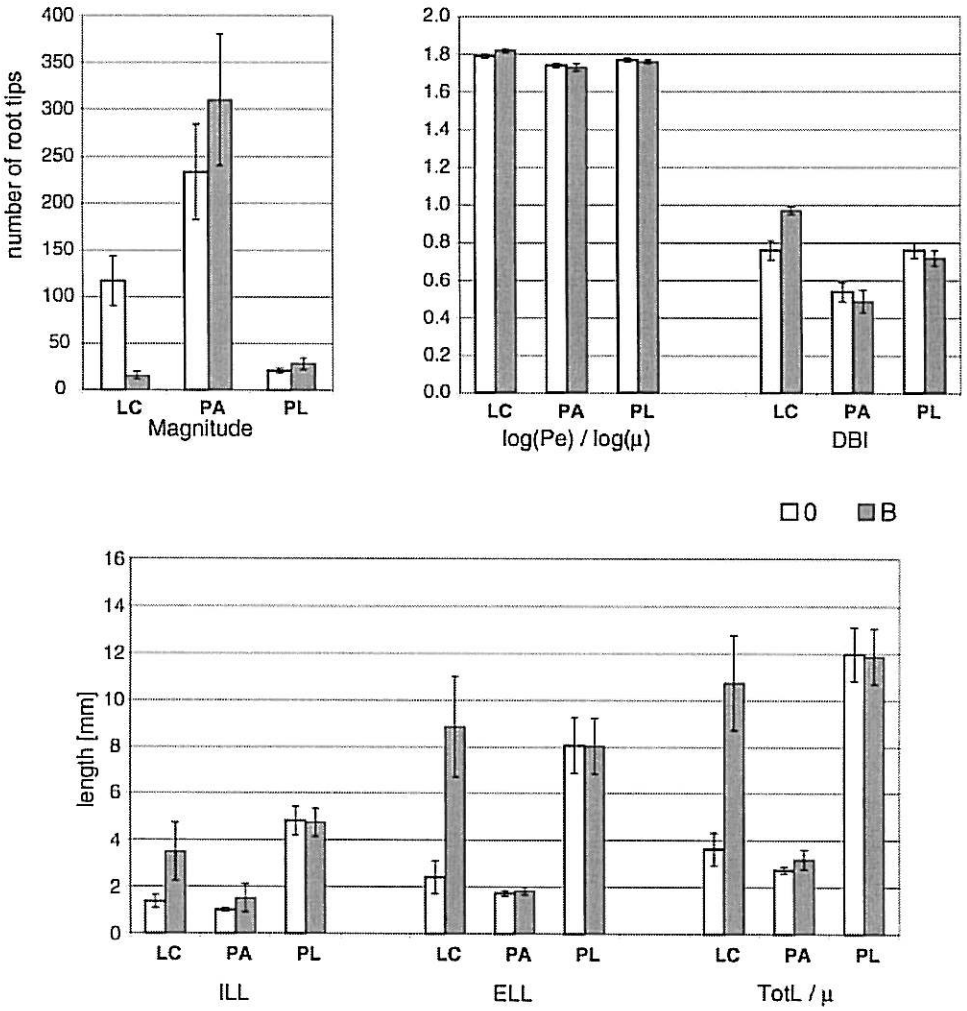

Figure 2: Effect of fungicide application on the measured morphological characteristics of roots that grew into experimental patches. Averages are shown by vertical bars separately for the three studied species (empty bars for patches without added fungicide, filled bars for patches with its addition). Vertical lines indicate 95% confidence intervals. The significance of differences in respect to fungicide application can be found in tables 4, 5, 6 for - respectively - *Luzula campestris* (LC), *Poa angustifolia* (PA), and *Plantago lanceolata* (PL). The averages for the two levels of the **Fungicide** treatment are taken over all levels of the other two experimental factors (**Nutrients** and **Substrate structure**). ILL - the average length of interior links, ELL - the average length of exterior links, μ - the magnitude of the root, TotL - the total length of the root; $\log(P_e)$:$\log(\mu)$ and DBI (dichotomous branching index) are two topological indices (see Methods for DBI definition). For definitions of primary root morphological characteristics see Table 2.

Response variable	ILL	ELL	μ	TotL	TotL:μ	log(p_e):log(μ)	DBI
Nutrients (N)	ns (0.48)	ns (1.09)	ns (0.82)	ns (0.03)	ns (3.02)	ns (2.13)	**0.022 (5.38)**
Fungicide benomyl (B)	**$<10^{-5}$ (22.79)**	**$<10^{-5}$ (39.14)**	**$<10^{-5}$ (111.46)**	**$<10^{-5}$ (77.70)**	**$<10^{-5}$ (73.40)**	**$<10^{-4}$ (16.90)**	**$<10^{-5}$ (24.47)**
Substrate structure (S)	ns (1.51)	ns (2.00)	**0.012 (4.57)**	ns (2.96)	**0.019 (4.11)**	ns (0.88)	ns (0.67)
interaction **N:B:S**	ns (1.92)	**0.017 (4.23)**	ns (2.54)	**0.038 (3.37)**	**0.045 (3.19)**	ns (0.68)	ns (2.54)

Table 4: Response of morphological characteristics of *Luzula campestris* roots to experimental factors, based on an ANOVA table. The ANOVA model included a full interaction between the treatment effects, but the table shows all the main effects and only the interaction terms that were significant for at least one response variable (the third-order interaction term in this case). The estimate of the Type I error probability is followed by the F-ratio statistic, on which it is based. The number of residual DFs is equal to 104 for log(Pe)/log(μ) and DBI, 106 for ILL, and 109 for the other response variables. There are further 97, 101, or 130 residual DFs at the "within-core" error level. The test DF is equal to 1 for the N and B factors and 2 for the S effect and the N:B:S interaction term. For the used abbreviations see Table 3.

Plantago roots responded to increased availability of nutrients in most of their dimensional parameters, but no change in root topology was found. In the patches with added nutrients, *Plantago* had shorter exterior as well as interior links and the average root length per root tip decreased with application of nutrients. *Poa* responded to nutrient enrichment only by production of slightly shorter exterior links. The topology (as expressed by DBI) was influenced by nutrient application only in *Luzula* roots, indicating decreased dichotomy of branching.

Response variable	ILL	ELL	μ	TotL	TotL:μ	log(p_e):log(μ)	DBI
Nutrients (N)	ns (1.12)	**0.038 (4.57)**	ns (0.24)	ns (0.45)	ns (0.41)	ns (0.31)	ns (0.26)
Fungicide benomyl (B)	**0.034 (4.78)**	ns (2.43)	ns (0.59)	ns (1.93)	**0.024 (5.46)**	ns (0.96)	ns (1.70)
Substrate structure (S)	ns (0.31)	**0.024 (4.06)**	ns (0.06)	ns (0.04)	ns (2.10)	ns (2.96)	ns (2.09)
interaction **B:S**	ns (1.31)	**0.005 (5.92)**	ns (0.43)	ns (0.42)	ns (0.97)	ns (1.18)	ns (1.40)

Table 5: Response of morphological characteristics of *Poa angustifolia* roots to experimental factors, based on an ANOVA table. The ANOVA model included a full interaction between the treatment effects, but the table shows all the main effects and one of the second-order interaction terms, as the other interaction terms were non-significant for any of the response variables. The estimate of the Type I error probability is followed by the F-ratio statistic, on which it is based. The number of residual DFs is 45 for all the response variables. There are further 140 residual DFs at the "within-core" error level. The test DF is equal to 1 for the N and B factors and 2 for the S effect and the B:S interaction term. For the other abbreviations see Table 3.

Application of benomyl affected all root characteristics of *Luzula*, while both *Poa* and *Plantago* responded only slightly. In patches treated with benomyl *Luzula* produced shorter, less branched roots with longer interior and exterior links. Number of root tips (μ) decreased by a larger extent than the total root length, as indicated by the significant increase of average root length per root tip. Both topological indices indicate a decreased extent of dichotomous branching in roots that grew into the benomyl treated patches. *Poa* had longer interior links and higher root length per root tip in patches treated with benomyl, whereas *Plantago* responded to benomyl application only by slightly changed branching pattern (more dichotomously branched roots), but this was indicated only by the log(p_e):log(μ) index. No interaction was found between the effects of nutrient and benomyl application.

Response variable	ILL	ELL	μ	TotL	TotL:μ	log(p_e):log(μ)	DBI
Nutrients (N)	**$<10^{-5}$ (26.46)**	**$<10^{-3}$ (13.15)**	ns (3.54)	ns (0.09)	**$<10^{-5}$ (22.25)**	ns (1.57)	ns (1.19)
Fungicide benomyl (B)	ns (0.09)	ns (0.01)	ns (1.32)	ns (1.48)	ns (0.07)	**0.044 (4.18)**	ns (0.18)
Substrate structure (S)	ns (1.04)	ns (2.00)	ns (0.35)	ns (0.06)	ns 0.76)	ns (1.80)	ns (0.06)

Table 6: Response of morphological characteristics of *Plantago lanceolata* roots to experimental factors, based on an ANOVA table. The ANOVA model included a full interaction between the treatment effects, but the table shows only the main effects, as the interaction terms were non-significant for all the response variables. The estimate of the Type I error probability is followed by the F-ratio statistic, on which it is based. The number of residual DF is equal to 102 for log(Pe)/log(μ) and DBI, to 104 for ILL, and to 106 for the other response variables. There are further 265, 277, or 294 residual DFs at the "within-core" error level. The test DF is equal to 1 for the N and B factors and 2 for the S factor. For the used abbreviations see Table 3.

Substrate modification influenced some dimensional characteristics of *Poa* and *Luzula* roots, but not their topological indices. *Luzula* had more root tips (higher μ) and shorter root length per root tip in patches with mixture of soil and coarse sand; the response to fine sand was in the opposite direction, and values of both root characteristics from patches with non-amended sieved soil were between these two extremes. The average exterior link length of *Poa* roots was higher in patches with soil mixed with sand than in the sieved soil (the longest links were in patches with fine sand fraction). The effect of benomyl application upon the average exterior link length of *Poa* roots was significantly influenced by manipulation of substrate structure in the patches.

To further explore the response of *Luzula* roots to fungicide application, the overall tendency of roots of the target species to produce poorly branched roots (with magnitude $\mu<7$) in patches with different treatments was analysed, using a generalized linear model. For *Poa*, only three roots were poorly branched, each from a patch with a different treatment combination, so this species was not included into this analysis. The analysis showed that *Luzula* produced a significantly larger proportion of poorly branched roots in patches with benomyl application ($F_{1,64}=5.52$, $p=0.022$), while for *Plantago* this proportion was not significantly different among the patches with different treatment ($F_{1,54}=0.09$, ns).

To compare behaviour of roots of the target species with the growth response of neighbouring community, the relation between the root characteristics of the three species and the total root biomass proliferated into the patches was analysed. A model with additive relation between the effects of the target species and of the root biomass was compared with another model, where the biomass effect was nested within the species effect. This comparison showed that the effect of root biomass differed among the target species [$p < 0.001$ for ILL ($F_{2,803}=17.25$), ELL

($F_{2,854}$=8.41), μ ($F_{2,854}$=14.09), and TotL:μ ($F_{2,854}$=18.72), p = 0.002 for log(p_e):log(μ) ($F_{2,783}$=6.16), p = 0.0011 for DBI ($F_{2,783}$=6.89), and p = 0.0016 for TotL ($F_{2,854}$=6.50)]. Table 7 therefore summarises results of separate analyses for the individual target species. After accounting for the effect of experimental manipulation, the effect of total root biomass remained significant only for *Plantago*, in majority of its root characteristics.

	ILL		**ELL**		**μ**		**TotL**		**TotL:μ**		**log(P_e): log(μ)**		**DBI**	
Luzula	▲	**0.03 (4.86)**		ns (1.46)	▼	**0.006 (7.87)**	▼	**0.009 (6.97)**	▲	**0.023 (5.30)**	▲	**0.01 (6.35)**	▲	**0.005 (8.29)**
*Luzula**		ns (0.57)		n. a.		ns (1.67)		ns (2.86)		ns (0.002)		ns (0.78)		ns (0.52)
Poa		ns (0.08)	▼	**0.02 (5.69)**		ns (0.09)		ns (0.02)		ns (2.81)		ns (0.04)		ns (0.01)
*Poa**		n. a.		ns (3.11)		n. a.		n. a.		n. a.		n. a.		n. a.
Plantago	▼	**$<10^{-5}$ (33.9)**	▼	**$<10^{-3}$ (15.0)**	▲	**0.008 (7.38)**		ns (0.16)	▼	**$<10^{-5}$ (28.7)**	▼	**0.02 (5.53)**		ns (2.88)
*Plantago**	▼	**0.015 (6.10)**	▼	**0.044 (4.18)**		ns (3.16)		n. a.	▼	**0.007 (7.63)**	▼	**0.05 (3.84)**		n. a.

Table 7: Regression models of the dependence of measured root characteristics on the total root biomass proliferated into the experimental patches during the experiment. The models were fitted separately for each of the three target species. Rows marked with asterisk (*) correspond to models where also the effects of experimental manipulation were included. Symbol ▲ indicates an increase of the values of the root morphological characteristics with the increasing total root biomass, symbol ▼ indicates the decrease of the root morphological characteristic values. n. a. - not analysed, ns - not significant, for other abbreviations see Table 3. The estimate of the Type I error probability is followed by the F-ratio statistic, on which it is based.

No mycorrhizal colonisation was found in *Luzula* roots from any treatment. Non-mycorrhizal fungi were also very rare in these roots. *Poa* and *Plantago* roots differed in percentage of mycorrhizal colonisation ($F_{1,\ 102}$= 35.37, $p<10^{-8}$): *Plantago* roots in control patches had mycorrhizal colonisation in more than half of their length ($\bar{x}$=51.2%, SE=3.06), while *Poa* roots had less than 10% of their length colonised ($\bar{x}$=9.6%, SE=2.67). Mycorrhizal development was reduced by fungicide only in *Plantago* roots ($F_{1,49}$=38.17, $p<10^{-5}$; Figure 3).

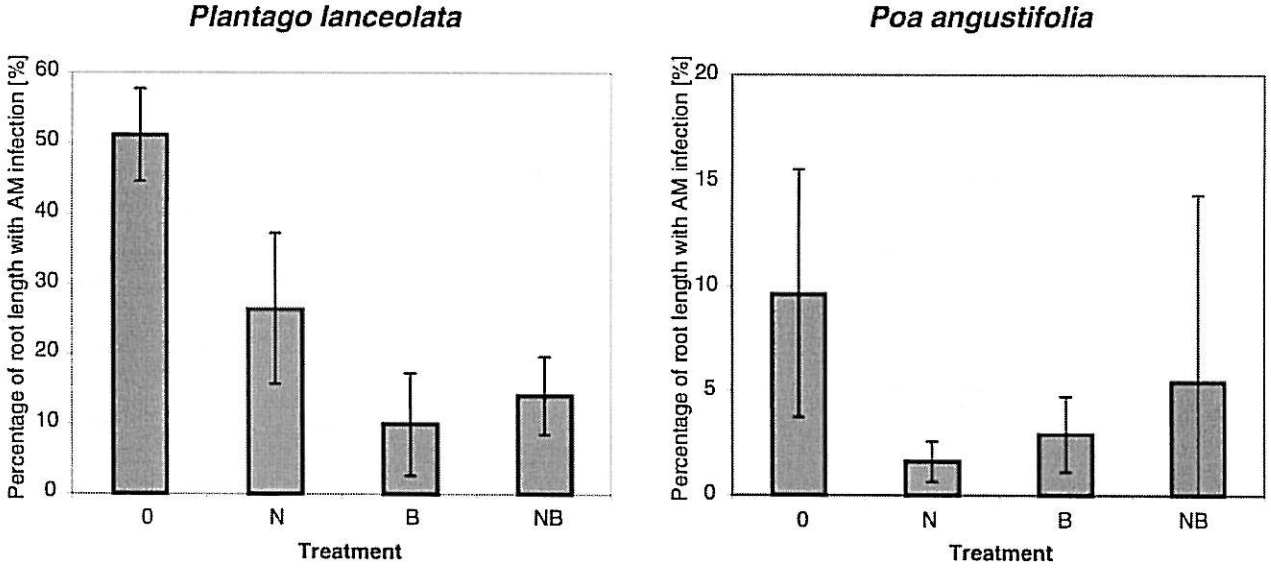

Figure 3: Percentage of root length with developed arbuscular mycorrhizal symbiosis in roots of two target species (*Plantago lanceolata* and *Poa angustifolia*) in individual combinations of two experimental factors (application of **Nutrients** (N) and application of **Fungicide** (B)). Treatment **0** corresponds to cores where neither nutrients nor fungicide were added, cores with treatment **NB** were supplemented with both nutrients and fungicide solution. The measurements are averaged over all three levels of the third experimental factor (**Substrate structure**) because the mycorrhizal infection level was not significantly different among the substrate types.

Discussion

Presented results show that the three studied grassland plant species differed significantly in their response to experimentally induced soil heterogeneity. It seems that each of these species uses a different strategy for exploitation of the heterogeneous soil environment.

Luzula with its slow growth, short links and the most herringbone topology (see Table 3) is a typical species of nutrient-poor sites (Fitter *et al.*, 1991). There are several possible explanations of its low sensitivity to nutrient application. The first explanation is that the duration of experiment was too short for a significant response of a slowly growing species. Nevertheless, the exploitation of nutrients from patches by micro-organisms and plants can be quite fast (Fransen *et al.* 1998, Hodge *et al.* 1998) and therefore the response to a newly established patch has to be sufficiently speedy so that the expenses do not exceed the profit. Small non-significant changes of all measured characteristics indicate some "hesitation" to branch. This can represent an economical strategy, when in the nutrient-enriched patches the amount of nutrients sufficient for species adapted to nutrient-poor sites can be acquired by the less branched roots. The more herringbone systems are considered to be more efficient in nutrient acquisition but more expensive to construct and maintain (Fitter, 1991). The alternative explanation may be the possibly higher importance of physiological plasticity in comparison with the morphological plasticity during the resource acquisition in *Luzula*.

Poa angustifolia was able to produce the longest roots in the newly created patches with most dichotomous branching and very short links (see Table 3). The ability of this species to create very fine, amply branched roots was only slightly influenced by nutrients or fungicide application. It seems that *Poa* roots grow so fast that they are able to occupy the empty soil space in a short time and to acquire nutrients without any essential change of root morphological properties. The low topological plasticity of *Poa* roots found in this study agrees with the results of Taub & Goldberg (1996) for grasses, although they found grasses to have a maximum herringbone topology. Perhaps the seedling roots behave differently from the proliferating roots of adult individuals.

Plantago had the least branched roots (with a typical magnitude value of 16), but with the longest exterior and interior links. The growth rate of its roots was slower - if expressed by the total root length - than the growth rate of *Poa* roots. *Plantago* responded to addition of nutrients by production of more compact roots (with shorter links and lower average root length per one root tip) which accords with results of study of Fitter *et al.* (1988), but not with those shown in Fitter (1994), where exterior links were longer in treatments with higher nutrient availability. Glimskar (2000) also found that a grassland forb *Polygala vulgaris* responded to low nitrogen supply by markedly increasing the length of root links. The increased compactness of *Plantago* roots from nutrient-rich patches, observed in our experiment, may contribute to increased competition among roots of the same plant and its profitability would depend on the patch properties (Fitter, 1991, 1994).

Insensitivity of morphological properties of *Luzula* roots to nutrients addition strongly contrasts with its extensive negative response to the application of fungicide. Studies dealing with the plant response to fungicide application measured the fungicide effect on roots as the effect on the root biomass (Borowicz, 1993, Newsham *et al.*, 1994) or on the root length per individual plant (Carey *et al.*, 1992, Sukarno *et al.*, 1993), but not on the root morphology. Response of non-mycorrhizal plants to benomyl application has been mentioned only by Fitter & Nichols (1988) for *Sinapis alba* and by Borowicz (1993) for *Brassica napus.* No effect of this fungicide on P inflow (Fitter & Nichols, 1988) or on root mass (Borowicz, 1993) was found. Several authors studied the influence of benomyl on other soil micro-organisms and some metabolic processes. Beside the suppression of non-mycorrhizal fungi (e.g. Carey *et al.*, 1992, West *et al.*, 1993), an interference with the nitrogen cycle was found (Chen *et al.*, 1995, Cademenun & Berch, 1997). Neither Fitter & Nichols (1988) nor Merryweather & Fitter (1996) found any substantial increase in soil phosphate concentration as a consequence of benomyl application. The whole community response to benomyl application expressed by total root biomass grown into the patches was positive (Šmilauerová, 2001). Response of *Luzula* roots to benomyl application was, after removal of effects of experimental manipulation, independent of the total root biomass grown into the patches (see Table 7). Because the response of *Luzula* differed from the response of the whole community, at least in its tendency not to proliferate into benomyl-treated patches, we consider the benomyl effect upon *Luzula* to be specific for this species. Although the root morphological response to benomyl application was much more pronounced than the response to nutrient enrichment, the direction of their effects was identical for each of the measured characteristics

(see Figures 1 and 2). It is therefore possible that "conservative" *Luzula*, adapted to nutrient-poor soils, is more sensitive to fluctuation of environmental characteristics with amplitude outside the usual conditions. Encountering a soil microsite with increased nutrient concentration, slightly exceeding the standard variability under natural conditions, could cause just a small delay in root growth, whereas a rapid decline in microorganismal activity after benomyl application or occurrence of some by-products of Bavistin decomposition could be so far from the standard conditions, that such a strict negative response was invoked. Nevertheless, the negative response of *Luzula* to benomyl had probably just a short duration because the long-term restriction of root growth and branching would lead to a suppression of *Luzula* by more competitive species. However, such effect was not recorded in a long-term experiment performed on this site (Šmilauer & Šmilauerová, 2000, unpublished results for *Luzula* abundance).

Although the growth of *Poa* roots in new patches was very fast, the experiment duration seems to be too short for the development of mycorrhizal symbiosis to the extent commonly seen in *Poa* roots at this site (average of colonised root length is 25%, unpublished data). For this reason, the response of *Poa* roots to fungicide application was probably not a matter of mycorrhizal development restriction (in fact, mycorrhizal development was not significantly suppressed by fungicide application), but rather a side effect of the fungicide application. Wilson & Hartnett (1997, 1998) found that the cool-season grasses from tallgrass prairie, including *Poa pratensis*, are insensitive to mycorrhizal colonisation or even benefiting from reduction of mycorrhizal root colonisation by benomyl. This indicates facultative mycotrophy of these grass species, although their mycorrhizal status in North American prairie can differ from that in European grasslands. The fact that the mycorrhizal colonisation in *Poa* roots was suppressed more effectively by nutrient addition then by fungicide application (see Figure 3), supports the idea of facultative mycotrophy of this grass species.

Plantago lanceolata has been used in numerous experiments as a typical mycorrhizal host species possessing a broad spectrum of mycorrhizal fungal taxa (e.g. Sanders & Fitter, 1992, Bever *et al.*, 1996, Gange *et al.*, 1999). Surprising is then the low response of *Plantago* roots to suppression of mycorrhizal colonisation in present experiment. Its roots at this study site have always well developed mycorrhizal structures (average colonisation is 85% of root length, unpublished data) and the mycorrhizal development was successfully restricted by benomyl in this experiment (see Figure 3) as well as in another, long term experiment (Šmilauer & Šmilauerová, 2000). It is possible that in *Plantago* roots the morphological changes induced by mycorrhizal fungi are not as marked as in some other species (Hetrick *et al.*, 1988, Hetrick, 1991, Fusconi *et al.*, 2000).

Substrate structure modification had only a slight effect and only on some of the dimensional parameters of *Poa* and *Luzula* roots. Higher magnitude of *Luzula* roots and its shorter average root length per a root tip in patches with coarse sand mixture can be a result of higher branching frequency caused by contact of growing root tips with sand particles of sufficient size. Nevertheless, similar response was not found either in fine roots of *Poa* or in thicker roots of *Plantago*. *Poa* had longer exterior links in patches with sand. The response had an opposite

direction to the response of this root parameter to nutrients addition. It can be explained by a higher sensitivity of this parameter in *Poa* to changes in nutrient availability in patches combined with a dilution of soil nutrients in the mixture with sand. It is surprising that *Plantago* with its highest sensitivity to nutrients addition did not respond at all to the substrate structure modifications.

On the community level, the total belowground biomass that proliferated into the patches was 1.6 times higher in patches with added nutrients, and 1.14 times higher in patches treated with benomyl, when compared with the control patches (Šmilauerová, 2001). None of the three studied species responded to nutrient addition by an increased total root length of the ingrown roots. Therefore, we should ask which root parameter changed so that the total belowground biomass in treated patches increased. One possibility would be an increase of the diameter of proliferating roots but this is contradicted by most of the recent findings (e.g. Hetrick *et al.*, 1988, Bilbrough & Caldwell, 1995, Hodge *et al.*, 1998, Arredondo & Johnson, 1999). These studies found significant increase of specific root length (SRL) in nutrient patches or in roots without mycorrhizal infection, while Fransen *et al.* (1999a) found a significant increase of root biomass in nutrient patches without any change in specific root length. The second root characteristic that could be responsible for the increase of total root biomass in the patches is the number of proliferating roots. As far as we know, nobody noticed this characteristic in studies investigating root proliferation into nutrient patches or into soil microsites with restricted development of mycorrhizal symbiosis. A third possibility is that the total biomass increase was caused primarily by the other grassland species, which behave differently from the three species we have studied.

For root characteristics of *Luzula* (as well as for ELL in *Poa* and for magnitude in *Plantago*), it is not possible to separate the effect of total root biomass in the patches upon the measured root characteristics from the effect of experimental treatment (see Table 7). *Plantago* was the only one among the studied species, which responded significantly to differences in total root biomass, after accounting for the treatment effects. Its response to increasing total root biomass in patches (shorter links and shorter root length per one root tip) was similar to its response to nutrients addition, but the set of responding parameters was extended by an increased dichotomy of branching. This suggests that this response can be a result of competitive suppression, rather than an economical tactic.

In summary, we found considerable differences in root response of three grassland species to nutrient microsites and restriction of mycorrhizal colonisation in the patches. Topology of roots was insensitive to nutrient addition. Two mycorrhizal species were less affected by fungicide benomyl application then the non-mycorrhizal *Luzula campestris*. We found a negative impact of total root biomass proliferated into patches on the measured root characteristics (after accounting for treatment effects) only for *Plantago lanceolata*.

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References

Arredondo JT, Johnson DA. 1999. Root architecture and biomass allocation of three range grasses in response to nonuniform supply of nutrients and shoot defoliation. *New Phytologist* **143**: 373-385.

Bever JD, Morton JB, Antonovics J, Schultz PA. 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. *Journal of Ecology* **84**: 71-82.

Bilbrough CJ, Caldwell MC. 1995. The effect of shading and N status on root proliferation in nutrient patches by the perennial grass *Agropyron desertorum* in the field. *Oecologia* **103:** 10-16.

Borowicz VA. 1993. Effects of benomyl, clipping, and competition on growth of prereproductive *Lotus corniculatus*. *Canadian Journal of Botany* **38**:1169-1175.

Cademenun BJ, Berch SM. 1997. Response of mycorrhizal western red cedar to organic phosphorus sources and benomyl. *Canadian Journal of Botany* **75**: 1226-1235.

Cahill JF, Casper BB. 1999. Growth consequences of soil nutrient heterogeneity for two old-field herbs, Ambrosia artemisiifolia and Phytolacca americana, grown individually and in combination. *Annals of Botany* **83**: 471-478.

Caldwell MM, Manwaring JH, Jackson RB. 1991a. Exploatation of phosphate from fertile soil microsites by three Great Basin perennials when in competition. *Functional Ecology* **5**: 757-764.

Caldwell MM, Manwaring JH, Durham SL. 1991b. The microscale distribution of neighbouring plant roots in fertile soil microsites. *Functional Ecology* **5**: 765-772.

Caldwell MM, Dudley LM, Lilieholm B. 1992. Soil solution phosphate, root uptake kinetics and nutrient acquisition: implications for a patchy soil environment. *Oecologia* **89**: 305-309.

Caldwell MM, Manwaring JH, Durham SL. 1996. Species interaction at the level of fine roots in the field: Influence of soil nutrient heterogeneity and plant size. *Oecologia* **106**: 440-447.

Campbell BD, Grime JP, Mackey JML. 1991. A trade-off between scale and precision in resource foraging. *Oecologia* **87**: 532-538.

Carey PD, Fitter AH, Watkinson AR. 1992. A field study using the fungicide benomyl to investigate the effect of mycorrhizal fungi on plant fitness. *Oecologia* **90**: 550-555.

Chen SK, Subler S, Edwards CA. 1995. A comparison of soil microcosm and laboratory incubation methods for determining the effects of benomyl on soil microbial activity and N dynamics. *80th annual ESA Meeting. Program and Abstracts*: 46

Cui M, Caldwell MM. 1996. Facilitation of plant arbuscular mycorrhizas from enriched soil patches. I. Roots and hyphae exploiting the same soil volume. *New Phytologist* **133**: 453-460.

Derner JD, Briske DD. 1999. Does a tradeoff exist between morphological and physiological root plasticity? A comparison of grass growth forms. *Acta Oecologica* **20**: 519-526.

Einsmann JC, Jones RH, Pu M, Mitchell RJ. 1999. Nutrient foraging traits in 10 cooccurring plant species of contrasting life forms. *Journal of Ecology* **87**: 609-619.

Farley RA, Fitter AH. 1999. The responses of 7 co-occurring herbaceous perennials to localized nutrient-rich patches. *Journal of Ecology* **87**: 849-859.

Fitter AH. 1991. Characteristics and functions of root systems. In: Waisel Y, Eshel A, Kafkafi U, eds. *Plant roots: the hidden half.* New York, USA: Marcel Dekker, Inc., 3-25.

Fitter AH. 1994. Architecture and biomass allocation as components of the plastic response of root system to soil heterogeneity. In: Caldwell MM, Pearcy RW, eds. *Exploatation of Environmental Heterogeneity by Plants*. San Diego, USA: Academic Press, Inc., 305-323.

Fitter AH, Nichols R. 1988. The use of benomyl to control infection by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **110:** 201-206.

Fitter AH, Nichols R. 1988. The use of benomyl to control infection by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **110:** 201-206.

Fitter AH, Nichols R, Harvey ML. 1988. Root system architecture in relation to life history and nutrient supply. *Functional Ecology* **2**: 345-351.

Fitter AH, Stickland TR. 1991. Architectural analysis of plant root systems 2. Influence of nutrient supply on architecture in contrasting plant species. *New Phytologist* **118**: 383-389.

Fitter AH, Stickland TR, Harvey ML, Wilson GW. 1991. Architectural analysis of plant root systems. 1. Architectural correlates of exploitation efficiency. *New Phytologist* **118**: 375-382.

Fransen B, de Kroon H, Berendse F. 1998. Root morphological plasticity and nutrient acquisition of perennial grass species from habitats of different nutrient availability. *Oecologia* **115**: 351-358.

Fransen B, Blijjenberg J, de Kroon H. 1999a. Root morphological plasticity of perennial grass species and the exploatation of spatial and temporal heterogeneous nutrient patches. *Plant and Soil* **211:**179-189.

Fransen B, de Kroon H, Berendse F. 1999b. Nutrient heterogeneity changes competition between populations of *Festuca rubra* L. and *Anthoxanthum odoratum* L. In: Fransen B. *Root foraging. The consequences for nutrient acquisition and competition in heterogeneous environments.* PhD thesis, Wageningen University, Wageningen, The Netherlands.

Fusconi A, Tagliasacchi AM, Berta G, Trotta A, Brazzaventre S, Ruberti F, Scannerini S. 2000. Root apical meristems of *Allium porrum* L. as affected by arbuscular mycorrhizae and phosphorus. *Protoplasma* **214**: 219-226.

Gange AC, Bower E, Stagg PG, Aplin DM, Gillam AE, Bracken M. 1999. A comparison of visualization techniques for recording arbuscular mycorrhizal colonization. *New Phytologist* **142**: 123-132.

Glimskar A. 2000. Estimates of root system topology of five plant species grown at steady-state nutrition. *Plant and Soil* **227**: 249-256.

Grime JP. 1994. The role of plasticity in exploiting environmental heterogeneity. In: Caldwell MM, Pearcy RW, eds. *Exploatation of environmental heterogeneity by plants: ecophysiological processes above- and below-ground.* San Diego, USA: Academic Press, Inc., 1-19.

Hartnett DC, Hetric AD, Wilson GWT, Gigson DJ. 1993. Mycorrhizal influence on intra- and interspecific neighbour interactions among co-occuring prairie grasses. *Journal of Ecology* **81**: 787-795.

Hetrick BAD. 1991. Mycorrhizas and root architecture. *Experientia* **47**: 355-362.

Hetrick BAD, Leslie JF, Wilson GT, Kitt DG. 1988. Physical and topological assessment of effects of a vesicular-arbuscular mycorrhizal fungus on root architecture of big bluestem. *New Phytologist* **110**: 85-96.

Hetrick BAD, Hartnett DC, Wilson GWT, Gibson DJ. 1994a. Effects of mycorrhizae, phosphorus availability, and plant density on field relationships among competing tallgrass prairie grasses. *Canadian Journal of Botany* **72**: 168-176.

Hetrick BAD, Wilson GWT, Schwab AP. 1994b. Mycorrhizal activity in warm- and cool-season grasses: variation in nutrient-uptake strategies. *Canadian Journal of Botany* **72**: 1002-1008.

Hodge A, Stewart J, Robinson D, Griffiths BS, Fitter AH. 1998. Root proliferation, soil fauna and plant nitrogen capture from nutrient-rich patches in soil. *New Phytologist* **139**: 479-494.

Huber-Sannwald E, Pyke DA, Caldwell MM, Durham S. 1998. Effects of nutrient patches and root systems on the clonal plasticity of a rhizomatous grass. *Ecology* **79**: 2267-2280.

Jackson RB, Caldwell MM. 1993. Geostatistical patterns of soil heterogeneity around individual perennial plants. *Journal of Ecology* **81**: 683-692.

Koide RT. 1991. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytologist* **117**: 365-386.

Lapointe L, Molard J. 1997. Costs and benefits of mycorrhizal infection in a spring ephemeral, *Erythronium americanum. New Phytologist* **135**: 491-500.

MathSoft 1999. *S-PLUS 2000, Guide to Statistics, Volume 1.* Seattle, USA: Data Analysis Products Division, MathSoft.

Merryweather J, Fitter AH. 1996: Phosphorus nutrition of an obligately mycorrhizal plant treated with the fungicide benomyl in the field. *New Phytologist* **132**: 307-311.

McConnaughay KDM, Bazzaz FA. 1992. The occupation and fragmentation of space: consequences of neighbouring roots. *Functional Ecology* **6**: 704-710.

McCullagh P, Nelder JA. 1989. *Generalized Linear Models.* Second Edition. - London, UK: Chapman and Hall.

Mullen RB, Schmidt SK. 1993. Mycorrhizal infection, phosphorus uptake, and phenology in *Ranunculus adoneus*: implication for the functioning of mycorrhizae in alpine systems. *Oecologia* **94**: 229-234.

Newsham KK, Fitter AH, Watkinson AR. 1994. Root pathogenic and arbuscular mycorrhizal fungi determine fecundity of asymptotic plants in the field. *Journal of Ecology* **116**: 805-814.

Phillip JM, Hayman DS. 1975. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions British Mycological Society* **55**: 159-161.

Read DJ, Koucheki HK, Hodgson J. 1976. Vesicular-arbuscular mycorrhiza in natural vegetation systems I. The occurrence of infection. *New Phytologist* **77**: 641-653.

Robinson D, Van Vuuren MMI. 1998. Responses of wild plants to nutrient patches in relation to growth rate and life-form. In: Lambers H, Poorter H, Van Vuuren MMI, eds. *Inherent variation in plant growth. Physiological mechanisms and ecological consequences.* Leiden, The Netherlands: Backhuys Publishers, 237-257.

Robinson D, Hodge A, Griffiths BS, Fitter AH. 1999. Plant root proliferation in nitrogen-rich patches confers competitive advantage. *Proceedings of the Royal Society of London, Series B-Biological sciences* **266**: 431-435.

Rothmaler W. 1976. *Exkursionsflora für die Gebiete der DDR und der BRD. Kritischer Band.* Volk und Wissen Volkseigener Verlag, Berlin.

Ryel RJ, Caldwell MM, Manwaring JH. 1996. Temporal dynamics of soil spatial heterogeneity in sagebrush-wheatgrass steppe during a growing season. *Plant and Soil* **184**: 299-309.

Ryser P, Eek L. 2000. Consequences of phenotypic plasticity vs. interspecific differences in leaf and root traits for acquisition of aboveground and belowground resources. *American Journal of Botany* **87**: 402-411.

Sanders IR, Fitter AH. 1992. The ecology and functioning of vesicular-arbuscular mycorrhizas in co-existing grassland species I. Seasonal patterns of mycorrhizal occurrence and morphology. *New Phytologist* **120**: 517-524.

St John TV, Coleman DC, Reid CPP. 1983a. Association of vesicular-arbuscular mycorrhizal hyphae with soil organic particles. *Ecology* **64**: 957-959.

St John TV, Coleman DC, Reid CPP. 1983b. Growth and spatial distribution of nutrient-absorbing organs: selective exploatation of soil heterogeneity. *Plant and Soil* **71**: 487-493.

Sukarno N, Smith SE, Scott ES. 1993. The effect of fungicides on vesicular-arbuscular mycorrhizal symbiosis. *New Phytologist* **25**: 139-147.

Šmilauer P. 1996. *Modern approaches to analysis of ecological data.* PhD thesis, University of South Bohemia, Ceské Budejovice, Czech Republic.

Šmilauer P, Šmilauerová M. 2000. Effect of AM symbiosis exclusion on grassland community composition. *Folia Geobotanica* **35**: 13-25.

Šmilauerová M. 2001. Plant roots response to heterogeneity of soil resources: effects of nutrient patches, AM symbiosis, and species composition. Folia Geobotanica. *in press*

Taub DR, Goldberg D. 1996. Root system topology of plants from habitats differing in soil resource availability. *Functional Ecology* **10**: 258-264.

Tibbett M. 2000. Roots, foraging and the exploitation of soil nutrient patches: the role of mycorrhizal symbiosis. *Functional Ecology* **14**: 397-399.

West HM, Fitter AH, Watkinson AR. 1993. The influence of three biocides on the fungal associates of roots of *Vulpia ciliata* ssp. *ambigua* under natural conditions. *Journal of Ecology* **81**: 345-350.

Wilcox HE. 1991. Mycorrhizae. In: Waisel Y, Eshel A, Kafkafi U, eds. *Plant roots: the hidden half*. New York, USA: Marcel Dekker, Inc., 731-765.

Wilson GWT, Hartnet DC. 1997. Effects of mycorrhizae on plant growth and dynamics in experimental tallgrass prairie microcosms. *American Journal of Botany* **84**: 478-482.

Wilson GWT, Hartnet DC. 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. *American Journal of Botany* **85**: 1732-1738.