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Interactive effects of defoliation and an AM fungus on plants and soil organisms in experimental legume–grass communities

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We established a 13-week greenhouse experiment based on replicated microcosms to test whether the effects of defoliation on grassland plants and soil organisms depend on plant species composition and the presence of arbuscular mycorrhizal (AM) fungi. The experiment constituted of three treatment factors – plant species composition, inoculation of an AM fungus and defoliation – in a fully factorial design. Plant species composition had three levels: (1) *Trifolium repens* monoculture (T), (2) *Phleum pratense* monoculture (P) and (3) mixture of *T. repens* and *P. pratense* (T+P), while the AM inoculation and the defoliation treatment had two levels: (1) no inoculation of AM fungi and (2) inoculation of the AM fungus *Glomus claroideum* BEG31, and (1) no trimming, and (2) trimming of all plant material to 6 cm above the soil surface three times during the experiment, respectively. At the final harvest, AM colonization rate of plant roots differed between the plant species compositions, being on average 45% in T, 33% in T+P and 4% in P. Defoliation did not affect the colonization rate in T but raised the rate from 1% to 7% in P and from 20% to 45% in T+P. Shoot production and standing shoot and root biomass were 48%, 85% and 68% lower, respectively, in defoliated than in non-defoliated systems, while the AM fungus did not affect shoot production and root mass but reduced harvested shoot mass by 8% in non-defoliated systems. Of the plant quality attributes, defoliation enhanced the N concentration of harvested shoot biomass by 129% and 96% in P and T+P, respectively, but had no effect in T, while the C concentration of shoot biomass was on average 2.7% lower in defoliated than in non-defoliated systems. Moreover, defoliation reduced shoot C yield (the combined C content of defoliated and harvested shoot biomass) on average by 47% across all plant species compositions and shoot N yield by 37% in T only. In contrast to defoliation, the AM fungus did not affect shoot N and C concentrations or shoot N yield, but induced 10% lower C yield in non-defoliated systems and 17% higher C yield in defoliated T. In roots, defoliation led to 56% and 21% higher N concentration in P and T+P, respectively, and 28% higher C concentration in P, while the mycorrhizal fungus lowered root N concentration by 9.7% in defoliated systems and had no effect on root C concentrations. In the soil, the nematode community was dominated by bacterivores and the other trophic groups were found in a few microcosms only. Bacterivores were 45% more abundant in defoliated than in non-defoliated systems, but were not affected by plant species composition or the AM fungus. Soil inorganic N concentration was significantly increased by defoliation in T+P, while the mycorrhizal fungus reduced NH_4–N concentration by 40% in T. The results show that defoliation had widespread effects in our experimental systems, and while the effects on plant growth were invariably negative and those on bacterivorous nematodes invariably positive, most effects on plant C and N content and soil inorganic N concentration varied depending on the plant species present. In contrast, the effects of defoliation did not depend on the presence of the AM fungus, which suggests that while the relative abundance of legumes and grasses is likely to have a significant role in the response of legume–grass communities to defoliation, the role of AM fungi may be less important. In line with this, the AM fungus had only a few significant effects on plant and soil attributes in our systems and each of them was modified by defoliation and/or plant

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species composition. This suggests that the effects of AM fungi in legume–grass communities may largely depend on the plant species present and whether the plants are grazed or not.

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Grazing by mammals can have far-reaching effects in grassland ecosystems. It has been shown that grazing may change species composition (Collins et al. 1998, Olff and Ritchie 1998), primary production (McNaughton 1976, 1985) and nutrient content (Cid et al. 1991, Detling 1998) of grassland plant communities. Studies that have simulated grazing by manual clipping have further shown that defoliation may alter C and N allocation within plants (Briske et al. 1996, Crawford et al. 1997, Thornton and Millard 1997, Louahlia et al. 2000) as well as the release of C from roots into plant rhizospheres (Paterson and Sim 1999, Mikola and Kytöviita 2002, Nguyen and Henry 2002). Changes in root attributes after defoliation may in turn affect belowground organisms, such as root feeders and decomposers, that rely on resources provided by plants (Stanton 1983, Guitian and Bardgett 2000, Mikola et al. 2001a). In relation to the commonness of grazed grasslands among terrestrial ecosystems, however, little is still known of the belowground effects of defoliation in these systems.

Predicting the effects of grazing on plant growth and belowground organisms is complicated by the fact that plant species differ in their response to defoliation. The effects of defoliation on plant resource allocation (Wilsey et al. 1997) and the abundance of soil microbes and animals (Mawdsley and Bardgett 1997, Guitian and Bardgett 2000, Mikola et al. 2001b) may depend on which plant species is defoliated. Moreover, the response of grassland plants to defoliation may be affected by the symbiotic relationships that plants form with arbuscular mycorrhizal (AM) fungi. Since AM fungi improve the nutrient acquisition of plants (especially that of P but also N) and consume a substantial proportion (estimations range from 4 to 20%) of the net photosynthate production (Smith and Read 1997), it is likely that resource allocation and growth after defoliation differ between mycorrhizal and non-mycorrhizal plants. There is little experimental evidence of this (Strauss and Agrawal 1999), however, even though the removal of shoot tissue is known to lead to lower root colonization rate by AM fungi in most plant species (Gehring and Whitham 1994), suggesting that aboveground grazers and mycorrhizal fungi are closely linked through their effects on plant performance.

To further investigate the effects of defoliation in grassland communities, we established a greenhouse trial based on replicated microcosms. Microcosms included *Trifolium repens*, *Phleum pratense* or both species in combination and half of the replicates of each system were inoculated with an AM fungus *Glomus claroideum* BEG31. *Phleum pratense* is an important forage grass in northern pastures (Berg et al. 1996) and *T. repens*, like other legumes, is particularly important in low-input farming (Ledgard 2001). *Glomus claroideum* BEG31 in turn is one of the most common AM fungi in Finnish and other temperate agricultural soils (Vestberg 1995), colonizes a broad range of plant hosts (Mårtensson et al. 1998, Salonen et al. 2001) and has been found to enhance plant growth in soils with low to moderate nutrient content (Vestberg et al. 2000). In the present study, we aimed at specifically testing (1) whether the effects of defoliation on plant and soil attributes in legume-grass communities depend on the plant species composition and the presence of AM fungi, (2) how defoliation affects AM fungi and (3) whether defoliation can modify AM influence on plant and soil attributes.

Materials and methods

Growth conditions

We performed a greenhouse experiment including 120 experimental units; each unit consisted of a plastic pot (height 9 cm, diameter 8 cm), 310 g soil (dry weight equivalent) and two plant individuals. Soil (with a total N content of 0.21% (equivalent to 2.1 g kg^{-1} dry soil), total P content of 0.10%, total C content of 3.5% and a pH (water) of 5.7) was a mixture of grassland soil (collected from Jyväskylä, Central Finland) and sand in a dry weight ratio of 5:1. Before mixing, the grassland soil was passed through a 1-cm mesh sieve and both constituents were autoclaved (121°C for 25 min). In the greenhouse, lighting followed natural summer photoperiod length and was supplemented with eight 400 W daylight lamps for 12 hours each day. The photosynthetic photon flux density varied between 180 and 550 μmol $m^{-2}s^{-1}$ at the height of plant shoots depending on outdoor weather and the position on the greenhouse table (pots were rearranged weekly to equal

the amount of light for each pot). Relative humidity was not controlled and varied between 7 and 47%, while temperature was 17°C at night and varied between 21 and 30°C in the daytime. Plants were watered three times a week and on every occasion pot weights (≈ soil water content) were equalised. Three weeks after planting, transparent plastic cylinders (height 20 cm) were attached to the pots in order to prevent experimental units from shading each other and plants from growing into adjacent pots.

Experimental design

The experiment constituted of three treatment factors – plant species composition, inoculation of a mycorrhizal fungus and defoliation – in a fully factorial design. The plant species treatment consisted of three levels: 1) *T. repens* monoculture, 2) *P. pratense* monoculture and 3) mixture of *T. repens* and *P. pratense*. The mycorrhizal treatment had two levels: 1) no inoculation of mycorrhizal fungi and 2) inoculation of the AM fungus *G. claroideum* BEG31. Finally, the defoliation treatment consisted of two levels: 1) no trimming and 2) trimming of all plant material to 6 cm above the soil surface. Experimental units were arranged in ten replicate blocks and treatment combinations were randomly allocated within each block.

Plants were raised from seeds sown into vermiculite and at the age of four weeks two seedlings were planted into each pot: one third of pots received two *T. repens* seedlings (*T. repens* monoculture), another third two *P. pratense* seedlings (*P. pratense* monoculture) and the remaining third one seedling of each species (species mixture). The AM fungus was introduced into half of the experimental units during planting by adding 1.5 g of AM inoculum into the rhizosphere of each seedling (the inoculum was produced with *Plantago lanceolata* growing in a 2:1:1 mixture of Terragreen, sand and perlite and consisted of fungal spores, hyphae and colonized root pieces). In non-mycorrhizal pots, seedlings received 1.5 g of sterilized inoculum. In those pots subjected to defoliation, plants were trimmed 5, 8 and 11 weeks after planting and the trimmed material was collected, dried and weighed.

Inoculation of soil organisms

To restore N-fixing bacteria in the autoclaved soil, half a teaspoonful of commercial *Rhizobium* spp. inoculum (10^7 bacteria g^{-1} gamma-radiated peat; Elomestari Oy, Juva, Finland) was added into the rhizosphere of each seedling at planting. Further, to establish a simplified soil community, nematodes and saprophytic microbes were extracted from grassland soil using a wet funnel device (Sohlenius 1979) that was supplied with paper sheets to prevent inoculation of AM fungal spores. Nematodes and microbes were added into each pot in 2 ml of water three weeks after planting, resulting in an inoculation of 44 ± 4 (mean ± 1 SE, $n = 5$) bacterivorous, 29 ± 5 fungivorous, 9 ± 3 herbivorous, 2 ± 2 omnivorous and 1 ± 0.4 predacious nematodes into each pot. Protists were likely also inoculated during this procedure but their abundance was not investigated. Likewise, the biomass of inoculated microbes was not determined.

Harvest procedure

Each pot was destructively harvested 13 weeks after planting. At the harvest, plant shoots were first removed, sorted by species, dried at 70°C and weighed. Dead shoot tissue was weighed separately to discriminate harvested bio- and necromass. For estimating plant root biomass, roots were carefully washed using half of the soil, dried at 70°C and weighed. The C and N concentrations of defoliated shoot material and harvested shoot and root biomass, i.e. the attributes of plant quality, were analysed from five replicates of each treatment combination using a CHNS–O analyser (EA 1110, Fisons Instruments). In the species mixture, root biomass and root C and N concentrations could not be measured separately for each species due to very dense root systems. Shoot biomass and shoot C and N concentrations were analysed separately for each species, but these data are not presented since variable values in the species mixture did not significantly differ from those recorded in the monocultures (suggesting that interspecies competition did not significantly differ from intra-species competition).

For evaluating root colonization rate of the AM fungus, a 0.3 g sub-sample of fresh roots was preserved in 50% alcohol. Samples were later bleached in 10% KOH overnight, acidified in 1% HCl and stained at 90°C for one hour with 0.01% methyl blue (Phillips and Hayman 1970, Grace and Stribley 1991). Root colonization rate of the mycorrhizal fungus was then estimated under a dissecting microscope using the following scale: 0, 1, 5, 10, 20, 30 ... 90, 100% of roots colonized. In the species mixture, the root colonization rate was not determined separately for each species but is an average value of *T. repens* and *P. pratense* roots.

Nematodes were extracted from a 30 g (fresh mass) sub-sample of soil using a wet funnel device (Sohlenius 1979), counted, identified to genus and allocated into trophic groups according to Yeates et al. (1993). The remaining soil was passed through a 4-mm mesh sieve to remove roots and to ensure soil mixing, and concentrations of KCl-extractable NH_4–N and NO_2+NO_3–N were determined from five replicates of each treatment combination using 2 g (fresh mass) sub-samples of soil.

Statistical analyses

The effects of defoliation, AM fungus and plant species composition were statistically analysed with the SPSS statistical package (SPSS 2001) using a three-way analysis of variance (ANOVA). Following ANOVA, Tukey's test was used to test the differences between different plant species compositions. If the three-way ANOVA resulted in significant interactions between the main factors, the main factors were fixed one by one and the effects of other factors were then analysed within the levels of the fixed factor using ANOVA. Homogeneity of variances was tested using Levene's test, and to satisfy the assumption of homogeneity of variances, a logarithmic transformation was applied to dependent variables whenever necessary (the assumption of homogeneity of variances could not be totally satisfied even after transformations in root to shoot ratio). The mycorrhizal root colonisation rate was tested using a two-way non-parametric Kruskal-Wallis test.

Results

The AM fungus

The root colonization rate of the mycorrhizal fungus was highest in the *T. repens* monoculture (on average 45% of roots colonized), intermediate in the species mixture (33% of root colonized) and lowest in the *P. pratense* monoculture (4% of roots colonized; effect of plant species composition, $H = 28.25$, $P < 0.001$, Fig. 1). The effect of defoliation on the mycorrhizal infection rate differed between species compositions (interaction of species composition and defoliation; $H = 5.40$, $P < 0.05$): defoliation did not affect the infection rate in the *T. repens* monoculture ($H = 1.99$, $P > 0.05$) but raised the rate from 1% to 7% in the *P. pratense* monoculture ($H = 8.98$, $P < 0.01$) and from 20% to 45% in the species mixture ($H = 6.15$, $P < 0.05$, Fig. 1). There was also a low infection rate of AM fungi in non-mycorrhizal treatments, presumably emerging from fungal parts introduced by soil fauna or from those surviving autoclaving.

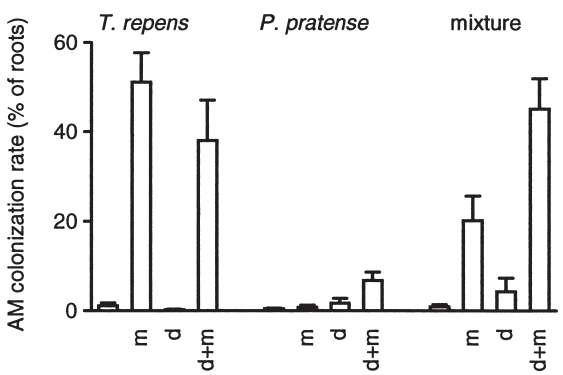

Fig. 1. Root colonization rate of mycorrhizal fungi (mean + 1 SE; n = 10) in experimental grassland communities in relation to plant species composition, defoliation and inoculation of AM fungus. *T. repens* = *Trifolium repens* monoculture, *P. pratense* = *Phleum pratense* monoculture, mixture = mixture of *T. repens* and *P. pratense*, m = inoculation of the arbuscular mycorrhizal fungus, *Glomus claroideum* BEG31, d = defoliation.

Plant growth

Shoot production (composed of defoliated biomass, harvested biomass and harvested necromass) and harvested shoot biomass were on average lower in the *P. pratense* monoculture than in the *T. repens* monoculture and in the species mixture (Table 1, Fig. 2a, b). Defoliation reduced shoot production and harvested shoot biomass on average by 48% and 85%, respectively ($P < 0.001$, Fig. 2a, b), while the mycorrhizal fungus had no effect on shoot production (Table 1, Fig. 2a) but reduced harvested shoot biomass by 8% in non-defoliated systems ($P < 0.01$, Fig. 2b).

Root mass was on average lowest in the *T. repens* monoculture, intermediate in the species mixture and highest in the *P. pratense* monoculture (Table 1, Fig. 2c). Root mass was reduced by defoliation on average by 68% ($P < 0.001$), but was not affected by the mycorrhizal fungus (Table 1, Fig. 2c). The root to shoot ratio was lowest in the *T. repens* monoculture, intermediate in the species mixture and highest in the *P. pratense* monoculture (Table 1, Fig. 2d). Defoliation increased the ratio ($P < 0.001$), while the mycorrhizal fungus had no effect (Table 1, Fig. 2d).

Plant quality

Concentration of N in harvested shoot biomass was on average highest in the *T. repens* monoculture, intermediate in the species mixture and lowest in the *P. pratense* monoculture (Table 1, Fig. 3a), whereas C concentration was not affected by plant species composition (Table 1, Fig. 3b). Defoliation increased N concentration by 129% and 96% in the *P. pratense* monoculture and in the species mixture ($P < 0.001$), respectively, but had no effect in the *T. repens* monoculture ($P > 0.05$, Fig. 3a). Meanwhile, defoliation led to on average 2.7% reduction in C concentration in each system (Table 1, Fig. 3b). The mycorrhizal fungus did not affect N or C concentrations in harvested shoot biomass (Table 1, Fig. 3a, b).

The C to N ratio of harvested shoot biomass was on average highest in the *P. pratense* monoculture, intermediate in the species mixture and lowest in the *T. repens* monoculture (Table 1, Fig. 3g). Defoliation lowered the ratio in the *P. pratense* monoculture and in the species mixture ($P < 0.001$) but had no effect in the

Table 1. F-statistics from three-way ANOVA of the effects of plant species composition, defoliation and inoculation of an AM fungus on plant growth (n = 10), plant quality (n = 5), and soil nematodes (n = 10) and inorganic N (n = 5) in grassland microcosms at the final harvest (shoot production comprises defoliated shoot biomass and harvested bio- and necromass, while shoot N and C yields include N and C contents of defoliated and harvested shoot biomass).

	Source of variation													
	Species composition		Defoliation		AM fungus		Spec × Def		Spec × AM		Def × AM		Spec × Def × AM	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Plant growth														
Shoot production	119.56	< 0.001	1072.04	< 0.001	2.07	0.154	9.08	< 0.001	0.41	0.662	3.93	0.050	0.79	0.458
Shoot biomass	133.87	< 0.001	4931.80	< 0.001	2.97	0.088	8.19	< 0.001	1.60	0.207	5.47	0.021	0.72	0.488
Root mass	385.96	< 0.001	609.36	< 0.001	0.11	0.747	7.72	0.001	0.29	0.752	1.97	0.164	1.29	0.279
Root to shoot ratio	456.27	< 0.001	238.45	< 0.001	0.22	0.643	4.03	0.021	1.31	0.274	0.07	0.796	0.68	0.510
Plant quality														
Shoot N concentration	465.24	< 0.001	128.09	< 0.001	1.41	0.241	33.17	< 0.001	0.33	0.717	0.47	0.497	0.50	0.613
Shoot C concentration	0.45	0.644	63.02	< 0.001	2.32	0.135	0.19	0.832	0.02	0.984	0.26	0.612	1.52	0.230
Shoot N yield	784.67	< 0.001	20.78	< 0.001	0.44	0.508	18.90	< 0.001	0.63	0.539	0.57	0.453	3.29	0.046
Shoot C yield	92.02	< 0.001	488.98	< 0.001	2.90	0.095	5.07	0.010	0.31	0.739	4.13	0.048	4.77	0.013
Root N concentration	671.63	< 0.001	16.32	< 0.001	0.89	0.350	11.32	< 0.001	1.33	0.275	4.38	0.042	1.22	0.306
Root C concentration	28.07	< 0.001	12.94	0.001	2.23	0.142	6.57	0.003	1.56	0.221	1.23	0.272	2.50	0.093
Shoot C to N ratio	587.44	< 0.001	228.80	< 0.001	1.17	0.285	43.91	< 0.001	0.06	0.939	0.09	0.765	0.39	0.682
Root C to N ratio	621.02	< 0.001	10.67	0.002	0.37	0.549	10.28	< 0.001	1.47	0.240	2.78	0.102	< 0.01	0.998
Soil nematodes and inorganic N														
Abundance of bacterivorous nematodes	1.52	0.224	8.78	0.004	0.81	0.369	0.64	0.528	0.28	0.757	0.38	0.537	0.30	0.741
NH_4–N concentration in soil	89.31	< 0.001	13.92	0.001	4.16	0.047	4.07	0.023	4.73	0.013	2.27	0.139	0.85	0.436

T. repens monoculture ($P > 0.05$, Fig. 3g). The mycorrhizal fungus did not affect the ratio (Table 1, Fig. 3g).

Shoot N yield (i.e. the combined N content of defoliated and harvested shoot biomass) was lowest in the *P. pratense* monoculture, intermediate in the species mixture and highest in the *T. repens* monoculture (Table 1, Fig. 3c). Shoot C yield was also smaller in the *P. pratense* monoculture than in the *T. repens* monoculture and in the species mixture, but the latter two did not differ from each other (Table 1, Fig. 3d). Defoliation reduced N yield by 37% in the *T. repens* monoculture ($P < 0.001$) but had no effect in other systems ($P > 0.05$, Fig. 3c). In contrast, defoliation reduced C yield in each system on average by 47% ($P < 0.001$, Fig. 3d). The mycorrhizal fungus had no effect on N yield in any system ($P > 0.05$, Fig. 3c), but decreased C yield by 10% in non-defoliated systems ($P < 0.05$) and increased C yield by 17% in the defoliated *T. repens* monoculture ($P < 0.05$, Fig. 3d).

Concentrations of N and C in root biomass were on average highest in the *T. repens* monoculture, intermediate in the species mixture and lowest in the *P. pratense* monoculture (Table 1, Fig. 3e, f). Defoliation increased root N concentration by 56% and 21% in the *P. pratense* monoculture and in the species mixture ($P < 0.01$), respectively, but had no effect in the *T. repens* monoculture ($P > 0.05$, Fig. 3e). Defoliation also enhanced root C concentration by 28% in the *P. pratense* monoculture ($P < 0.001$), but had no effects in other systems ($P > 0.05$, Fig. 3f). The mycorrhizal fungus lowered root N concentration on average by 9.7% in defoliated systems ($P < 0.05$), but had no effect in non-defoliated ones ($P > 0.05$, Fig. 3e) and did not affect root C concentration in any system (Table 1, Fig. 3f).

The C to N ratio of root biomass was on average highest in the *P. pratense* monoculture, intermediate in the species mixture and lowest in the *T. repens* monoculture (Table 1, Fig. 3h). Defoliation lowered the ratio in the *P. pratense* monoculture and in the species mixture ($P < 0.001$) but had no effect in the *T. repens* monoculture ($P > 0.05$, Fig. 3h). The AM fungus did not affect the ratio in any system (Table 1, Fig. 3h).

Soil nematodes and inorganic N

Bacterivores dominated the nematode community, and fungivores, herbivores, omnivores and predators were only found in 36, 32, 1 and 4 of the total 120 experimental units, respectively. The abundance of bacterivores was 45% higher in defoliated than in non-defoliated systems but was not affected by plant species composition or the AM fungus (Table 1, Fig. 4a).

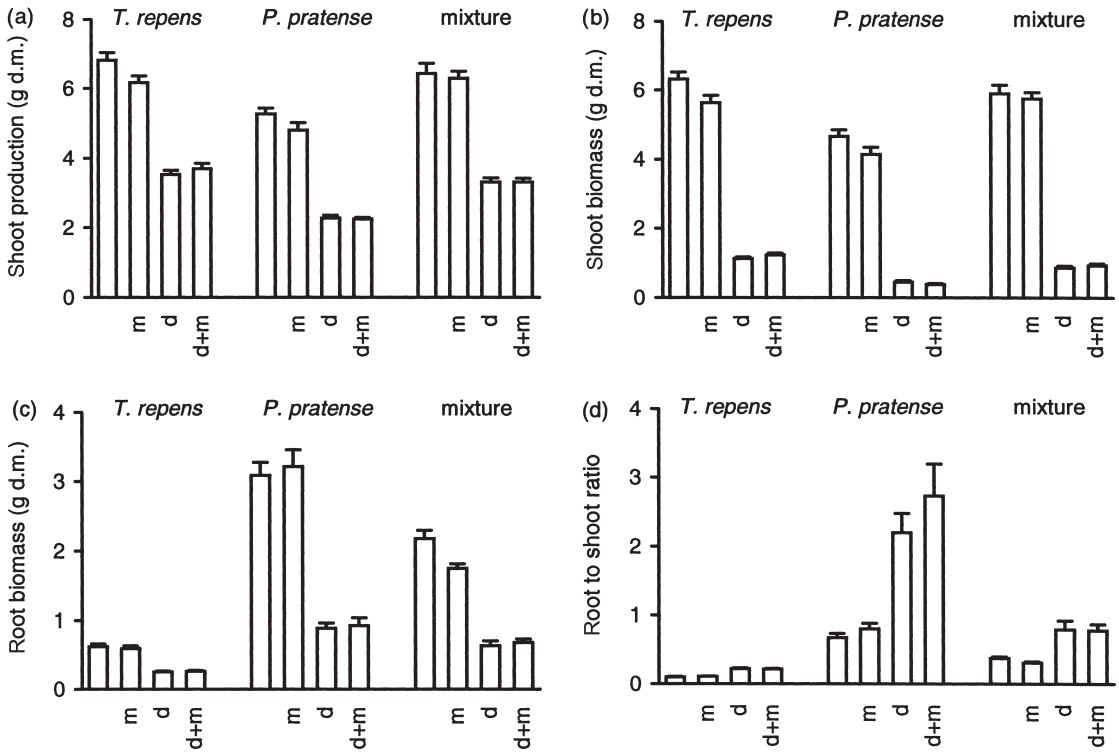

Fig. 2. Plant growth attributes (mean + 1 SE; n = 10) in experimental grassland communities in relation to plant species composition, defoliation and inoculation of AM fungus: (a) shoot production during the experiment (i.e. the sum of defoliated shoot mass, harvested shoot biomass and harvested necromass), (b) shoot biomass at harvest, (c) root biomass at harvest, (d) root to shoot ratio at harvest. Treatment symbols are as for Fig. 1.

Concentration of NH_4–N in soil was higher in the *T. repens* monoculture than in the species mixture or in the *P. pratense* monoculture (Table 1, Fig. 4b). Defoliation increased NH_4–N concentration in the species mixture ($P < 0.001$) but had no effect in the other systems ($P > 0.05$, Fig. 4b). The mycorrhizal fungus reduced NH_4–N concentration on average by 40% in the *T. repens* monoculture ($P < 0.001$), but had no effect in the *P. pratense* monoculture or in the species mixture ($P > 0.05$, Fig. 4b). Concentrations of $NO_2 + NO_3$–N were mostly undetectable.

Discussion

General effects of species composition

In our study, the AM colonization rate of *T. repens* roots was high and that of *P. pratense* roots low, which is consistent with earlier findings that legumes have high mycorrhizal colonization due to the high P demands of N-fixing, while plants with fibrous roots, such as *P. pratense*, benefit less from mycorrhizal associations (Johnson et al. 1997). Moreover, as expected, the quantity and quality of biomass produced by *T. repens* and *P. pratense* differed considerably. Although the non-defoliated *P. pratense* monocultures produced more harvested biomass (on average 7.55 g dry mass per pot) than those of *T. repens* (6.58 g dry mass), the ability of *T. repens* to fix N_2 was clearly illustrated by its low biomass allocation to roots and high concentrations of N in shoot and root biomass. However, despite the substantial differences in root growth and quality between *T. repens* and *P. pratense* monocultures, the abundance of bacterivorous nematodes was not statistically significantly different in the rhizospheres of the two species, suggesting that bacterial biomass production in soil did not significantly differ in the presence of these species.

Growing *T. repens* and *P. pratense* together had partly unpredictable effects in our communities. While the effects of mixing the species on most community variables could be predicted based on the effects of individual species (i.e. when the variable value in the species mixture was a mean of those in the monocultures), effects on some variables appeared unpredictable: for instance, while shoot biomass in the species mixture followed that in the *T. repens* monoculture, concentration of NH_4–N in soil followed that in the *P. pratense* monoculture. These results support the earlier findings that the effects of plant species combinations – either those of living plants (Wardle and Nicholson 1996) or

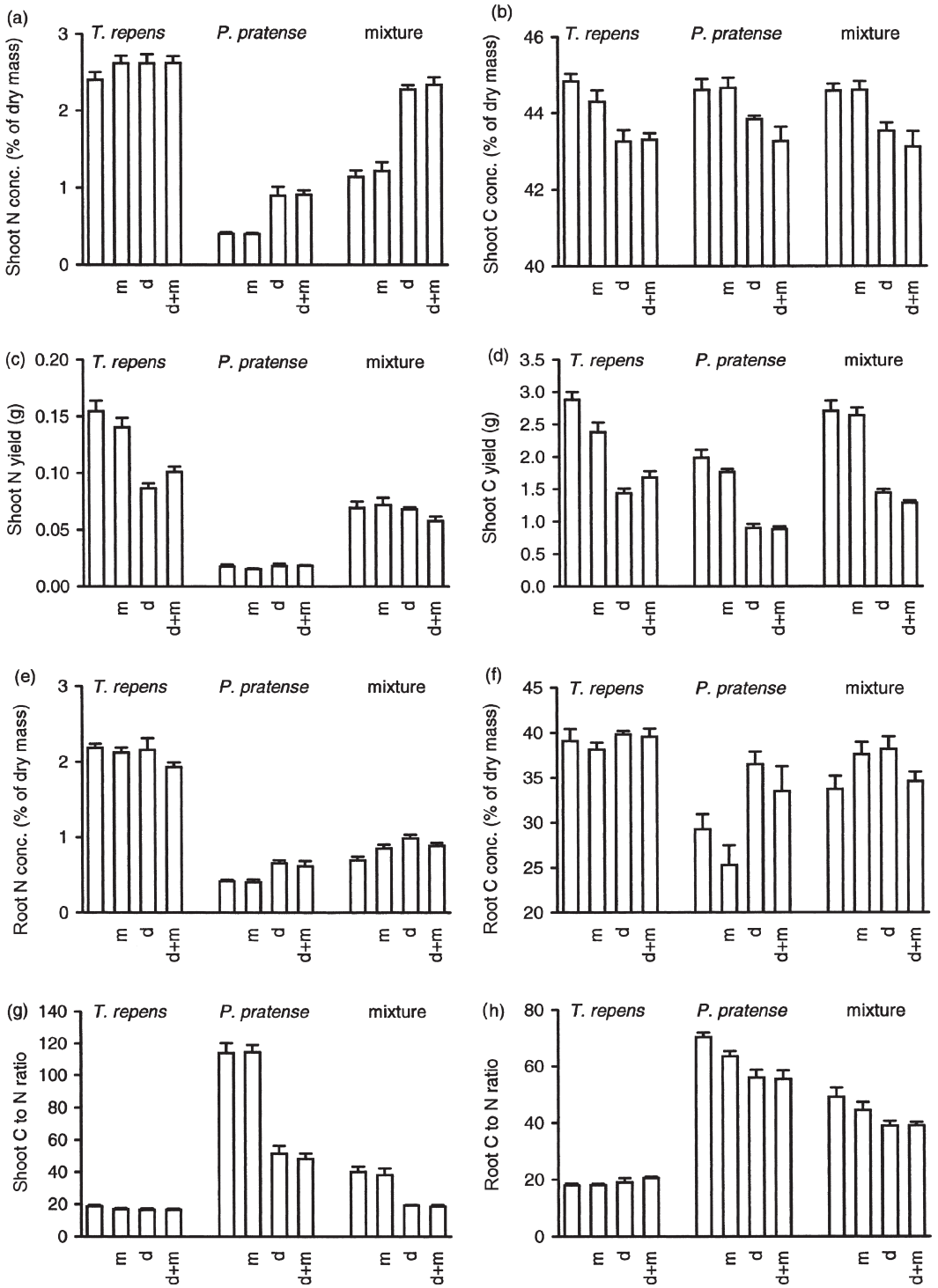

Fig. 3. Plant quality attributes (mean + 1 SE; n = 5) in experimental grassland communities in relation to plant species composition, defoliation and inoculation of AM fungus: (a) concentration of N in shoot biomass at harvest, (b) concentration of C in shoot biomass at harvest, (c) shoot N yield (i.e. total amount of N in defoliated and harvested shoot biomass), (d) shoot C yield, (e) concentration of N in root biomass at harvest, (f) concentration of C in root biomass at harvest, (g) C to N ratio of shoot biomass at harvest, (h) C to N ratio of root biomass at harvest. Treatment symbols are as for Fig. 1.

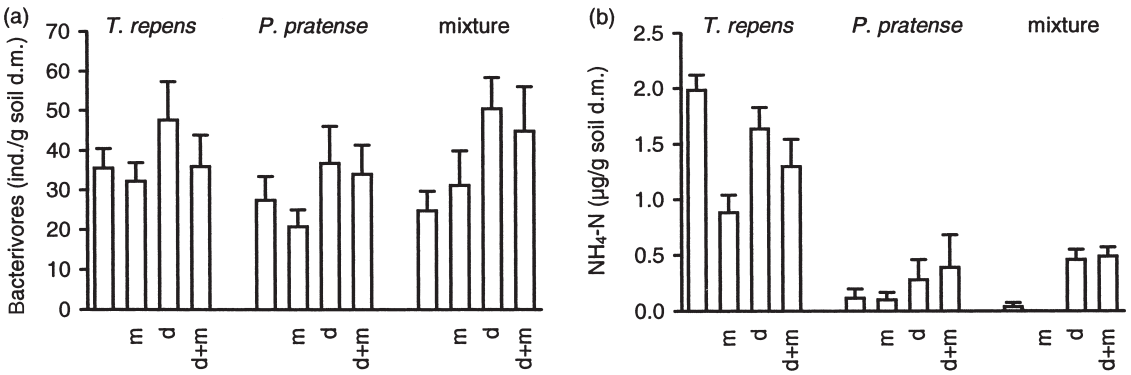

Fig. 4. Soil nematodes (mean+1 SE; n = 10) and inorganic N (mean±1 SE; n = 5) in experimental grassland communities in relation to plant species composition, defoliation and inoculation of AM fungus: (a) abundance of bacterivorous nematodes, (b) concentration of NH_4–N in soil. Treatment symbols are as for Fig. 1.

plant litter (Blair et al. 1990, Wardle et al. 1997, Nilsson et al. 1999) – on community variables may be difficult to predict based on the effects of individual species.

Effects of defoliation

Defoliation had widespread effects in our experimental systems, and while the effects on plant growth were invariably negative and those on bacterivorous nematodes invariably positive, most effects on plant C and N content and soil inorganic N concentration varied depending on the plant species present (Table 2). Meanwhile, the AM fungus did not modify the effects of defoliation (Table 2), which suggests that while the relative abundance of legumes and grasses is likely to have a significant role in the response of legume-grass communities to defoliation, the role of AM fungi may be less important.

Our results show that although defoliation reduced shoot production and root mass, i.e. the total amount of resources available to aboveground and belowground consumers, the quality of resources improved in the *P. pratense* monoculture and in the species mixture in terms of increasing N concentration and decreasing C to N ratio. This is in line with earlier investigations that have reported reduced growth (Ferraro and Oesterheld 2002) and elevated shoot N concentrations (Wilsey et al. 1997, Fahnestock and Detling 1999, Green and Detling 2000) in graminoids after defoliation. It has been

Table 2. Summary of the effects of defoliation and the AM fungus on plant growth, plant quality and soil nematodes and inorganic N in grassland communities (−, + and 0 mean negative, positive and neutral effects, respectively) and the dependence of these effects on other treatment factors (spec = plant species composition, def = defoliation, AM = inoculation of an AM fungus). Combination of symbols (−/0, 0/+, −/+, or −/0/+) indicates that the effect is modified by other treatment factors (e.g. '−/+' together with 'spec' indicates that the effect is either negative or positive depending on plant species composition).

Variable	Defoliation		AM fungus	
	Effect	Depending on	Effect	Depending on
The AM fungus				
Root colonization rate	0/+	spec		
Plant growth				
Total shoot production	−		0	
Shoot biomass at harvest	−		−/0	def
Root biomass at harvest	−		0	
Root to shoot ratio at harvest	+		0	
Plant quality				
Shoot N concentration at harvest	0/+	spec	0	
Shoot C concentration at harvest	−		0	
Shoot N yield	−/0	spec	0	
Shoot C yield	−		−/0/+	spec & def
Root N concentration at harvest	0/+	spec	−/0	def
Root C concentration at harvest	0/+	spec	0	
Shoot C to N ratio	−/0	spec	0	
Root C to N ratio	−/0	spec	0	
Soil nematodes and inorganic N				
Abundance of bacterivorous nematodes	+		0	
NH_4–N concentration in soil	0/+	spec	−/0	spec

hypothesized that the higher shoot N concentration in defoliated plants is a result of higher relative allocation of N to shoots after defoliation (Ruess 1988, Louahlia et al. 2000). Our results support this, since defoliation did not reduce *P. pratense* shoot N yield despite reducing its root N content (root N content was 5.8 mg per pot in defoliated and 12.9 mg per pot in non-defoliated systems).

However, enhanced shoot N concentrations did not appear to arise completely at the expense of roots, since defoliation also enhanced root N concentrations in systems including *P. pratense*. This supports the hypothesis that aboveground herbivory enhances root quality by increasing root N concentrations (Seastedt 1985, Seastedt et al. 1988). Moreover, the positive association between soil NH_4–N concentrations and plant N concentrations in the *P. pratense* monoculture and species mixture supports the hypothesis that increased N concentrations in plant tissues after defoliation may result from improved availability of inorganic N in soil (Holland and Detling 1990). However, our results show that these typical graminoid responses to defoliation may not occur among leguminous species, such as *T. repens.* In our study, defoliation did not enhance plant N concentrations or soil inorganic N concentrations in the *T. repens* monoculture, which eventually resulted in a negative effect of defoliation on shoot N yield. This suggests that the relative abundance of grasses and legumes in grasslands may determine how community- and ecosystem-level parameters, such as plant shoot N yield, root quality and mineral N availability in soil, are affected by defoliation.

Defoliation reduced shoot C concentration in each of our plant species compositions, which indicates that defoliation reduced the ability of plants to assimilate C. However, lower shoot C concentrations did not result in lower root C concentrations in any system, which contradicts the common view that plants tolerate defoliation by increasing C allocation to growing shoots at the expense of roots after defoliation (Caldwell et al. 1981, Briske et al. 1996, Strauss and Agrawal 1999). In fact, defoliation increased root C concentration in the *P. pratense* monoculture, which is consistent with the hypothesis that plants allocate photosynthate to roots relative to shoots after defoliation to create C storages less accessible to aboveground herbivores (Dyer et al. 1991, Holland et al. 1996). In an earlier study, defoliation was not found to increase allocation of recently assimilated C to *P. pratense* roots (Mikola and Kytöviita 2002), but the present results show that total C concentration of *P. pratense* roots can still be elevated by defoliation.

Although negative effects of defoliation and mammal grazing on AM colonization rate dominate in earlier studies (reviewed by Gehring and Whitham 1994), both positive (Hartley and Amos 1999) and negative (Allsopp 1998) effects are possible. In our study, defoliation did not affect AM colonization rate in the *T. repens* monoculture and increased the colonization rate in the *P. pratense* monoculture and in the species mixture. Negative effects of defoliation on AM fungi are usually attributed to photosynthate limitation after defoliation (Gehring and Whitham 1994, Gange et al. 2002). In our experiment, defoliation reduced shoot production and shoot C concentration in each plant species composition, thus implying declined photosynthate production in defoliated systems. However, this did not lead to reduced root C concentration in any system, which may explain the non-negative effects of defoliation on AM colonization rate in our study. That root C concentration may have controlled root AM colonization rate in our study is further supported by the fact that the increase in root C concentration in defoliated *P. pratense* monocultures, relative to non-defoliated systems, was accompanied by a 9.6-fold increase in AM infection rate of *P. pratense* roots.

In our study, the abundance of bacterivorous nematodes was 45% higher in defoliated than in non-defoliated systems, suggesting that bacterial growth was significantly enhanced by defoliation. This may result from higher root mortality in defoliated systems (Mikola et al. 2001a) and is in agreement with earlier studies in which the abundance of bacteria was found to be higher in the rhizospheres of defoliated than in the rhizospheres of non-defoliated plants (Mawdsley and Bardgett 1997) and in which defoliation led to increased numbers of bacterivorous nematodes (Todd et al. 1992, Mikola et al. 2001a). In the present study, we were not able to test the response of fungivorous nematodes to defoliation due to their low abundance in the experimental systems, but there is earlier evidence that defoliation also increases the number of fungivores (Mikola et al. 2001a, b). This suggests that defoliation may have a general positive influence on soil decomposers in grassland communities despite concurrently reducing plant growth. Finally, it is remarkable that defoliation had a very uniform positive effect on bacterivores in our study even though the effects on root quality attributes varied a lot between the plant species compositions.

Effects of the AM fungus

In our study, the AM fungus had only a few significant effects on plant and soil variables and each of them, including the root colonization rate by the fungus, were modified by defoliation and/or plant species composition (Table 2). This suggests that the effects of AM fungi in legume–grass communities may depend largely on the plant species present and whether the plants are grazed or not.

In our study, neither *T. repens* nor *P. pratense* clearly benefited from the symbiosis in terms of enhanced growth or elevated N concentration. Using separate soil compartments for plant roots and AM fungal hyphae, it has been shown that AM fungi may substantially contribute to N uptake by plants (Mäder et al. 2000). However, the contribution of AM fungi to N uptake becomes negligible when roots and hyphae exploit a common soil space (Hodge et al. 2000, Mäder et al. 2000) which undeniably is the case in pot experiments. Even though the fungus did not affect N concentration or N yield in aboveground plant parts in our systems, it decreased root N concentration in defoliated plants. This effect is probably indirect, mediated by soil organisms and soil inorganic N content, rather than a consequence of direct consumption of root N by the fungus since AM fungi are known to take most of their N directly from soil (Nakano et al. 2001a, b).

The consumption of photosynthate by the AM fungus was illustrated by a negative effect of the fungus on harvested shoot biomass and C yield in non-defoliated systems. However, in defoliated systems, no negative effects on shoot growth or C yield were observed, and in fact the fungus enhanced C yield in the defoliated *T. repens* monoculture. This might be explained by the lower root biomass in defoliated than in non-defoliated systems, since the positive impact of AM inoculation on plant growth is known to decline with increasing root density in soil (Bååth and Hayman 1984). Together with the effects on root N concentration, effects on shoot growth and C yield imply that the effects of AM fungi on host plants may significantly depend on whether the hosts are grazed or not. However, this interaction between mycorrhizal infection and defoliation is likely to be extremely complex, as illustrated by an earlier grassland study, in which defoliation and mycorrhizal infection were found either to strengthen or nullify each other's positive and negative effects on plant growth, with the direction of the original effect and its modification both depending on the species of plant subjected to the treatments (Grime et al. 1987).

When considering the relevance of our data in terms of AM benefits to plant performance, it is important to note that greenhouse conditions may promote a negative balance between mycorrhizal costs and benefits that may not necessarily occur in the field. This is because AM fungi may not offer improvement in nutrient capture in the limited soil volume of experimental pots despite consuming photosynthate, and sub-optimal light intensity may restrict photosynthesis and lead to allocation of photosynthate of limited supply to the fungi. Autoclaving may also release nutrients from soil pools otherwise not available to roots and thus diminish the importance of AM fungi in plant nutrient uptake. Moreover, since we did not measure plant P concentrations, we do not know whether our plants benefited from AM infection in terms of enhanced P acquisition. This has been shown to be possible even when the AM fungus reduces plant growth if soil P supply is low enough (Graham and Abbott 2000). Generalisations from greenhouse studies using single isolates of fungus may also be limited as the effects of AM fungi on plant growth may vary a lot among fungal isolates (Graham and Abbott 2000) and as plant roots also in agricultural soils may host a considerable number of AM fungal species (Kjøller and Rosendahl 2001).

Earlier studies have shown that both AM (Burke et al. 2002) and ectomycorrhizal (EM) fungi (Bonkowski et al. 2001) are able to reduce total microbial biomass and especially the number of bacteria in plant rhizospheres. In our study, bacterivorous nematodes were not affected by the AM fungus, which suggests that bacterial growth was not significantly different in the presence and absence of the fungus. It has also been shown that the presence of EM fungi increases the abundance of fungivorous nematodes in soil (Setälä et al. 1999), but the low abundance of fungivores in our study prevented us from testing whether AM fungi have similar effects on fungal grazers. Nevertheless, our results suggest that the presence of the AM fungus, in comparison to defoliation, had a minor role in determining soil decomposer abundance.

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References

Allsopp, N. 1998. Effect of defoliation on the arbuscular mycorrhizas of three perennial pasture and rangeland grasses. – Plant Soil 202: 117–124.

Berg, C. C., McElroy, A. R. and Kunelius, H. T. 1996. Timothy. – In: Moser, L. E., Buxton, D. R. and Casler, M. D. (eds), Cool season forage grasses. Am. Soc. Agron., Madison, pp. 643–664.

Blair, J. M., Parmelee, R. W. and Beare, M. H. 1990. Decay rates, nitrogen fluxes, and decomposer communities of single- and mixed-species foliar litter. – Ecology 71: 1976–1985.

Bonkowski, M., Jentschke, G. and Scheu, S. 2001. Contrasting effects of microbial partners in the rhizosphere: interactions between Norway spruce seedlings (*Picea abies* Karst.), mycorrhiza (*Paxillus involutus* (Batsch) Fr.) and naked amoebae (protozoa). – Appl. Soil Ecol. 18: 193–204.

Briske, D. D., Boutton, T. W. and Wang, Z. 1996. Contribution of flexible allocation priorities to herbivory tolerance in C_4 perennial grasses: an evaluation with ^{13}C labeling. – Oecologia 105: 151–159.

Burke, D. J., Hamerlynck, E. P. and Hahn, D. 2002. Effect of arbuscular mycorrhizae on soil microbial populations and associated plant performance of the salt marsh grass *Spartina patens*. – Plant Soil 239: 141–154.

Bååth, E. and Hayman, D. 1984. Effect of soil volume and plant density on mycorrhizal infection and growth response. – Plant Soil 77: 373–376.

Caldwell, M. M., Richards, J. H., Johnson, D. A. et al. 1981. Coping with herbivory: photosynthetic capacity and re-

source allocation in two semiarid *Agropyron* bunchgrasses. – Oecologia 50: 14–24.
Cid, M. S., Detling, J. K., Whicker, A. D. et al. 1991. Vegetational responses of a mixed-grass prairie site following exclusion of prairie dogs and bison. – J. Range Manage. 44: 100–105.
Collins, S. L., Knapp, A. K., Briggs, J. M. et al. 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. – Science 280: 745–747.
Crawford, M. C., Grace, P. R. and Oades, J. M. 1997. Effect of defoliation of medic pastures on below ground carbon allocation and root production. – In: Lal, R., Kimble, J. M., Follett, R. F. et al. (eds), Management of carbon sequestration in soil. CRC Press, pp. 381–389.
Detling, J. K. 1998. Mammalian herbivores: ecosystem-level effects in two grassland national parks. – Wildlife Soc. B. 26: 438–448.
Dyer, M. I., Acra, M. A., Wang, G. M. et al. 1991. Source-sink carbon relations in two *Panicum coloratum* ecotypes in response to herbivory. – Ecology 72: 1472–1483.
Fahnestock, J. T. and Detling, J. K. 1999. Plant responses to defoliation and resource supplementation in the Pryor Mountains. – J. Range Manage. 52: 263–270.
Ferraro, D. O. and Oesterheld, M. 2002. Effect of defoliation on grass growth. A quantitative review. – Oikos 98: 125–133.
Gange, A. C., Bower, E. and Brown, V. K. 2002. Differential effects of insect herbivory on arbuscular mycorrhizal colonization. – Oecologia 131: 103–112.
Gehring, C. A. and Whitham, T. G. 1994. Interactions between aboveground herbivores and mycorrhizal mutualists of plants. – Trends Ecol. Evol. 9: 251–255.
Grace, C. and Stribley, D. P. 1991. A safer procedure for routine staining of vesicular-arbuscular mycorrhizal fungi. – Mycol. Res. 95: 1160–1162.
Graham, J. H. and Abbott, L. K. 2000. Wheat responses to aggressive and non-aggressive arbuscular mycorrhizal fungi. – Plant Soil 220: 207–218.
Green, R. A. and Detling, J. K. 2000. Defoliation-induced enhancement of total aboveground nitrogen yield of grasses. – Oikos 91: 280–284.
Grime, J. P., Mackey, J. M. L., Hillier, S. H. et al. 1987. Floristic diversity in a model system using experimental microcosms. – Nature 328: 420–422.
Guitian, R. and Bardgett, R. D. 2000. Plant and soil microbial responses to defoliation in temperate semi-natural grassland. – Plant Soil 220: 271–277.
Hartley, S. E. and Amos, L. 1999. Competitive interactions between *Nardus stricta* L. and *Calluna vulgaris* (L.) Hull: the effect of fertilizer and defoliation on above- and belowground performance. – J. Ecol. 87: 330–340.
Hodge, A., Robinson, D. and Fitter, A. H. 2000. An arbuscular mycorrhizal inoculum enhances root proliferation in, but not nitrogen capture from, nutrient-rich patches in soil. – New Phytol. 145: 575–584.
Holland, E. A. and Detling, J. K. 1990. Plant response to herbivory and belowground nitrogen cycling. – Ecology 71: 1040–1049.
Holland, J. N., Cheng, W. and Crossley, D. A. J. 1996. Herbivore-induced changes in plant carbon allocation: assessment of below ground C fluxes using carbon-14. – Oecologia 107: 87–94.
Johnson, N. C., Graham, J. H. and Smith, F. A. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. – New Phytol. 135: 575–585.
Kjøller, R. and Rosendahl, S. 2001. Molecular diversity of glomalean (arbuscular mycorrhizal) fungi determined as distinct *Glomus* specific DNA sequences from roots of field grown peas. – Mycol. Res. 105: 1027–1032.
Ledgard, S. F. 2001. Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures. – Plant Soil 228: 43–59.
Louahlia, S., Lainé, P., Thornton, B. et al. 2000. The role of N-remobilisation and the uptake of $NH4^+$ and NO_3^- by *Lolium perenne* L. in laminae growth following defoliation under field conditions. – Plant Soil 220: 175–187.
Mawdsley, J. L. and Bardgett, R. D. 1997. Continuous defoliation of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the composition and activity of the microbial population of an upland grassland soil. – Biol. Fertil. Soils 24: 52–58.
McNaughton, S. J. 1976. Serengeti migratory wildebeest: facilitation of energy flow by grazing. – Science 191: 92–94.
McNaughton, S. J. 1985. Ecology of a grazing ecosystem: the Serengeti. – Ecol. Monogr. 55: 259–294.
Mikola, J. and Kytöviita, M.-M. 2002. Defoliation and the availability of currently assimilated carbon in the *Phleum pratense* rhizosphere. – Soil Biol. Biochem. 34: 1869–1874.
Mikola, J., Yeates, G. W., Barker, G. M. et al. 2001a. Effects of defoliation intensity on soil food-web properties in an experimental grassland community. – Oikos 92: 333–343.
Mikola, J., Yeates, G. W., Wardle, D. A. et al. 2001b. Response of soil food-web structure to defoliation of different plant species combinations in an experimental grassland community. – Soil Biol. Biochem. 33: 205–214.
Mårtensson, A. M., Rydberg, I. and Vestberg, M. 1998. Potential to improve transfer of N in intercropped systems by optimising host-endophyte combinations. – Plant Soil 205: 57–66.
Mäder, P., Vierheilig, H., Streitwolf-Engel, R. et al. 2000. Transport of ^{15}N from a soil compartment separated by a polytetrafluoroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. – New Phytol. 146: 155–161.
Nakano, A., Takahashi, K. and Kimura, M. 2001a. Effect of host shoot clipping on carbon and nitrogen sources for arbuscular mycorrhizal fungi. – Mycorrhiza 10: 287–293.
Nakano, A., Takahashi, K., Koide, R. T. et al. 2001b. Determination of the nitrogen source for arbuscular mycorrhizal fungi by ^{15}N application to soil and plants. – Mycorrhiza 10: 267–273.
Nguyen, C. and Henry, F. 2002. A carbon-14-glucose assay to compare microbial activity between rhizosphere samples. – Biol. Fertil. Soils 35: 270–276.
Nilsson, M. C., Wardle, D. A. and Dahlberg, A. 1999. Effects of plant litter species composition and diversity on the boreal forest plant-soil system. – Oikos 86: 16–26.
Olff, H. and Ritchie, M. E. 1998. Effects of herbivores on grassland plant diversity. – Trends Ecol. Evol. 13: 261–265.
Paterson, E. and Sim, A. 1999. Rhizodeposition and C-partitioning of *Lolium perenne* in axenic culture affected by nitrogen supply and defoliation. – Plant Soil 216: 155–164.
Phillips, J. M. and Hayman, D. S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. – Brit. Mycol. Soc. Trans. 55: 158–161.
Ruess, R. W. 1988. The interaction of defoliation and nutrient uptake in *Sporobolus kentrophyllus*, a short-grass species from the Serengeti Plains. – Oecologia 77: 550–556.
Salonen, V., Vestberg, M. and Vauhkonen, M. 2001. The effect of host mycorrhizal status on host–parasitic plant interactions. – Mycorrhiza 11: 95–100.
Seastedt, T. R. 1985. Maximization of primary and secondary productivity by grazers. – Am. Nat. 126: 559–564.
Seastedt, T. R., Ramundo, R. A. and Hayes, D. C. 1988. Maximization of densities of soil animals by foliage herbivory: empirical evidence, graphical and conceptual models. – Oikos 51: 243–248.
Setälä, H., Kulmala, P., Mikola, J. et al. 1999. Influence of ectomycorrhiza on the structure of detrital food webs in pine rhizosphere. – Oikos 87: 113–122.
Smith, S. E. and Read, D. J. 1997. Mycorrhizal symbiosis. – Academic Press.

Sohlenius, B. 1979. A carbon budget for nematodes, rotifers and tardigrades in a Swedish coniferous forest soil. – Holarct. Ecol. 2: 30–40.
SPSS 2001. SPSS for Windows, release 10.1.3. – SPSS, Chicago.
Stanton, N. L. 1983. The effect of clipping and phytophagous nematodes on net primary production of blue grama, *Bouteloua gracilis*. – Oikos 40: 249–257.
Strauss, S. Y. and Agrawal, A. A. 1999. The ecology and evolution of plant tolerance to herbivory. – Trends Ecol. Evol. 14: 179–185.
Thornton, B. and Millard, P. 1997. Increased defoliation frequency depletes remobilization of nitrogen for leaf growth in grasses. – Ann. Bot. 80: 89–95.
Todd, T. C., James, S. W. and Seastedt, T. R. 1992. Soil invertebrate and plant responses to mowing and carbofuran application in a North American tallgrass prairie. – Plant Soil 144: 117–124.
Vestberg, M. 1995. Occurrence of some glomales in Finland. – Mycorrhiza 5: 329–336.
Vestberg, M., Kukkonen, S., Neuvonen, E.-L. et al. 2000. Mycorrhizal inoculation of micropropagated strawberry – case studies on mineral soil and on a mined peat bog. – Acta Horticult. 530: 297–304.
Wardle, D. A. and Nicholson, K. S. 1996. Synergistic effects of grassland plant species on soil microbial biomass and activity: implications for ecosystem-level effects of enriched plant diversity. – Funct. Ecol. 10: 410–416.
Wardle, D. A., Bonner, K. I. and Nicholson, K. S. 1997. Biodiversity and plant litter: experimental evidence which does not support the view that enhanced species richness improves ecosystem function. – Oikos 79: 247–258.
Wilsey, B. J., Coleman, J. S. and McNaughton, S. J. 1997. Effects of elevated CO_2 and defoliation on grasses: a comparative ecosystem approach. – Ecol. Appl. 7: 844–853.
Yeates, G. W., Bongers, T., de Goede, R. G. M. et al. 1993. Feeding habits in soil nematode families and genera – an outline for soil ecologists. – J. Nematol. 25: 315–331.