Conditional Outcomes in the Relationship between Pine and Ectomycorrhizal Fungi in Relation to Biotic and Abiotic Environment

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An increasing number of studies demonstrate the variable and context-dependent nature of mutualistic interactions, the outcome of which may vary in space and time in response to abiotic and biotic factors. In this study we tested whether the intensity of grazing by soil fauna brings about different costs and benefits in the Scots pine–ectomycorrhizal (EM) fungi symbiosis with respect to nitrogen availability for the pine. The experiment was conducted in transparent microcosms in which a soil profile mimicking that of coniferous forest soil with *Pinus sylvestris* seedlings was created. The seedlings were grown either in N-rich or N-poor humus soil. The soils were defaunated, re-inoculated with heterotrophic soil microbes, and one seedling of *P. sylvestris* – either non-infected or infected with 3 species of EM fungi – was planted in the microcosms. Half of the microcosms were thereafter re-faunated with diverse and numerically rich soil fauna typical to coniferous forest soil to represent intensive grazing pressure on EM fungi, while the other half received bacterial feeding protozoans, and microbial feeding nematodes only (representing strongly reduced grazing pressure). The microcosms were incubated in a growth chamber with varying illumination and temperature regimes for two growing seasons of the pine. After 45 weeks ca 10 times more EM fungal biomass was found on pine roots growing in N-poor soils than in N-rich soils. The amount of EM fungi was significantly reduced by the complex and abundant faunal community, particularly in N-poor soils, where the amount of EM fungi was less than 16% of that found in systems with reduced grazing pressure. The shoot and root production were significantly higher in the N-poor systems than in the N-rich systems. Seedlings grown in the N-rich soils had a markedly lower P:N-ratio (0.07) in the needles as compared to the ones in the N-poor soils (0.14). Soil type and soil fauna had a significant interaction on the total pine biomass production; in N-poor soils complex fauna reduced the amount of pine biomass, whereas in N-rich soils the effect of this fauna was negligible. Despite the clear faunal induced changes in the biomass production of the fungal symbiont, and although the effects of grazing on pine growth were distinctly related to the nitrogen status of the soil, the costs and benefits that determine the net effects of the plant–fungus association were not unambiguously related to feeding by fauna on EM fungi. We hypothesize that the divergent influence of soil fauna in relation to pine growth between the two soil types was not associated with reduced costs by the EM fungi for the pine but due to differences in the availability of P for plant uptake in the soils.

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Communities are commonly considered to be organized by three biotic processes: predation, competition and symbiosis. Symbiosis, including mutualism (species interactions of the $++$-type), is known to link a variety of species and affect community organization in a positive way (e.g. Krebs 1978). More precisely, mutualism can be referred to as any interspecific interaction whose benefits usually exceed the costs of both partners (Bronstein 1994). Despite its simple verbal definition, mutualism has proved to be a controversial concept due to the many theoretical constraints involved. In the light of models based on Lotka-Volterra-type interactions, May (1974, 1981) argued that mutualism will inevitably lead to disequilibrium because mathematically stable conditions between the interacting mutualists are difficult to sustain.

Nevertheless, mutualisms are ubiquitous and extremely diverse in nature (Boucher et al. 1982), and the outcomes of such species interactions may be manifold. It is becoming increasingly evident that the costs and benefits that determine net effects of mutualistic associations (Howe 1984, Cushman 1991) vary greatly both in space and time (see the review by Bronstein 1994). Consequently, the type of a particular interrelationship ($++$, $0+$, $-+$, $--$, etc.) is likely to change when the interactors change (due to evolutionary responses), or when the environmental factors constraining the mutualists change. This is suggested to lead to a spatial, ecological, or evolutionary continuum within which mutualism is just one possible interrelationship (Cushman 1991, Cushman and Addicott 1991, Bronstein 1994). When the impact of one species on another changes along this continuum, and when outcomes of interactions are deterministic in relation to the environmental gradient, such interrelationships show conditionality (Cushman and Whitham 1989). Both abiotic (such as resource availability) and biotic (e.g. identity and abundance of other species) settings in which the interaction takes place may influence the outcomes of interrelationships (Bronstein 1994).

Perhaps the most actively studied mutualistic relationship is the one between plants and fungi – mycorrhiza – which is repeatedly reported to improve growth and survival of the plant, e.g., by enhancing nutrient uptake and providing protection from pathogens (see the reviews by Harley and Smith 1983 and Allen 1991). The costs caused by the fungi may be high, estimates ranging between 7 and 60% of the plant's photosynthate production going to support the mycorrhizal fungus (Fogel and Hunt 1979, Stribley et al. 1980, Finlay and Söderström 1992, Rygiewicz and Andersen 1994). This relationship is considered to be especially important in nutrient-poor habitats, such as boreal coniferous forests, where plant survival may not otherwise be possible (Chapin 1980, Allen 1991).

The fact that the plant–fungus interrelationship comes in various shapes and degrees, together with observations suggesting the importance of this association affecting processes high up at the ecosystem level (Harley 1971, Perry et al. 1989, Allen 1991, O'Neill et al. 1991), has recently inspired several authors to explore the mechanisms by which this mathematically unstable relationship (sensu May 1974, 1981) is regulated. Several field and laboratory investigations with coniferous trees and their ectomycorrhizal (EM) symbionts have shown high nitrogen availability to reduce, and in some cases inhibit mycorrhizal development (Harley and Smith 1983, Ohenoja 1988, Wallander and Nylund 1991, 1992, Arnebrant 1994). It has been suggested that mycorrhizal fungi become detrimental to their hosts in fertile soils (Fitter 1977, Bowen 1980), potentially shifting the relationship from mutualism to parasitism (Harley 1968). On the other hand, the view that plants are not prisoners to fungi but may actively regulate (Molina and Trappe 1982, Gemma and Koske 1988, Allen et al. 1989) or passively affect (Harley and Smith 1983, Nylund 1988, Perry et al. 1992, Wallander 1992) the allocation of photosynthates to their fungal partner, has recently gained support. These findings indicate that the quality and quantity of a plant–fungus interaction may not be pre-determined, but could vary along an environmental gradient, therefore potentially showing conditionality in relation to soil fertility.

Heithaus et al. (1980) and Addicott and Freedman (1984) suggested that a third factor, external to the two mutualists, is needed to constrain the growth of the mutualists, thereby allowing the development of the symbiosis. Soil fauna may exert such a control over the development of mycorrhizal fungi; in their laboratory studies Harris and Boerner (1990), Ek et al. (1994), and Setälä (1995) showed that by grazing on mycorrhizal fungi, soil fauna can either directly or indirectly induce changes in the plant biomass. The intensity of grazing on mycorrhizal fungi seems to vary in relation to the abundance (Harris and Boerner 1990, Ek et al. 1994) and complexity (Setälä 1995) of the faunal community, suggesting the outcomes of the plant–fungi association to be conditional with respect to properties attributed to soil fauna.

Considering the plant–EM association, nutrient availability (an abiotic factor) is likely to induce, and intensity of grazing by soil fauna on EM fungi (a biotic factor) has a potential (e.g. Warnock et al. 1982, Harris and Boerner 1990, Ek et al. 1994, Setälä 1995) to result in conditionality. The main objective of the present work was to test whether the intensity of grazing by soil fauna brings about different costs and benefits, i.e. outcomes in the pine–EM fungi association with respect to nitrogen availability for the plant. We posed two hypotheses on the conditionality of the plant–fungus relationship. We expected (1) the relationship between the plant and the fungi to differ between two soil types in a following manner: when N is limiting, the pine should invest more to mycorrhizal production to

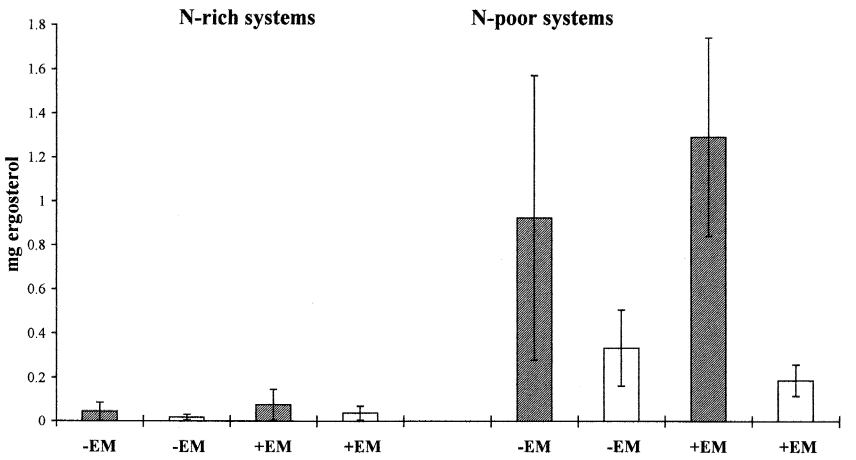

Fig. 1. The amount of ectomycorrhizal fungi (expressed as mg ergosterol per microcosm, mean ± s.d.) on pine roots in nitrogen rich and nitrogen poor microcosms at the end of the experiment (Week 45). Hatched columns = microcosms with a simple bacterial feeding fauna; open columns = microcosms with a complex, natural set of soil fauna of the coniferous forest floor. +EM = pine seedlings initially infected with three species of mycorrhizal fungi; −EM = initially uninfected pine seedlings. $n = 5$.

attain the best benefit from the association, whereas in N-rich environment pines should renounce/minimize the costs of EM by producing as little of reward as necessary to obtain some service. Under such conditions (2), the impact of grazing by an abundant fauna on EM fungi should be more negative to the plant–fungus association in N-poor environment than in systems where N is not limiting plant growth. Consequently, in N-rich soils where the costs of mutualism may exceed the benefits, grazing by fauna on EM fungi should not diminish pine growth, but, on the contrary, may exert a positive influence on pine growth by reducing the costs the EM fungi induces to its host.

Materials and methods

General description

We conducted a laboratory experiment with seedlings of Scots pine (*Pinus silvestris* L.) growing in microcosms simulating the coniferous forest floor. The seedlings were grown either in nitrogen-rich or in nitrogen-poor soils, either in the presence or absence of qualitatively and quantitatively abundant soil fauna. A complete description of the experimental design, composing of 3 factors and 8 treatments, is presented in Table 1. The experiment was established in transparent acrylic cylinders (area 55.2 cm^2, height 30 cm) with a hole (1 cm diameter) in the transparent lid covered with 25 μm mesh to provide ventilation in the systems, yet preventing mesofauna from entering/escaping the microcosms. A *Myrtillus*-type forest soil was created in the microcosms: a 4-cm thick layer of mineral soil (collected from a *Pinus* stand, sieved through a 5-mm sieve and thoroughly washed in hot tap water to remove organic debris) was spread on the bottom of each microcosm. On the mineral soil a layer of organic material, including humus (ca 4 cm thick, 43 g in dry mass) and litter (ca 0.5 cm thick, 3 g in d.m., consisting mainly of needle litter and mosses), was created. Two materials with different nitrogen content were applied: half of the replicates ($n = 20$) received humus collected from a spruce stand in Kannonkoski, central Finland, repeatedly fertilized with ammoniumsulphate, -nitrate and urea (cumulative amount of N added since 1959 equalling 950 kg/ha; see Smolander et al. 1994 for more details). Humus soil applied to the rest of the replicates was collected in the same forest but from a non-fertilized control plot. Characteristics of the N-rich and N-poor humus are presented in Table 2. Before being added to the microcosms the humus was sieved using a 10-mm sieve. The somewhat lower initial pH of the N-rich humus (pH 3.9) was raised to the same as the N-poor humus (pH 4.5) by gradually adding $CaCO_3$ dissolved in tap water. Fine grained mineral soil was added to the N-rich humus with an initial organic matter content of 56% to adjust its mineral content to be the same as in the N-poor humus (% O.M. 47). Before being added to the microcosms, all test materials were defaunated twice, one week inbetween the procedures, in liquid nitrogen. Amount of KCl-extractable NH_4^+ in the humus was analysed (according to the SFS standard 3032) before and after defaunation (Table 2). Total N concentration in the humus was analysed using the Kjeldahl method, and concentrations of total P, K, Ca, and Mg were analysed by standard methods (Association of Official Analytical Chemists, AOAC) with plasma emission spectrometry (Table 2).

Two days before the start of the experiment (Day −2) the microcosms were inoculated with soil heterotrophic microbes and Protozoa using 5 ml of soil-water suspension, filtered through a 10-μm nylon mesh. Half of the microcosms were refaunated using soil collected from a mixed pine and spruce stand as follows: microarthropods were extracted with large Tullgren funnels from soil samples, corresponding to an area roughly 5 times larger than that of the microcosms, into bottles with moist filter paper. After the extraction macrofauna

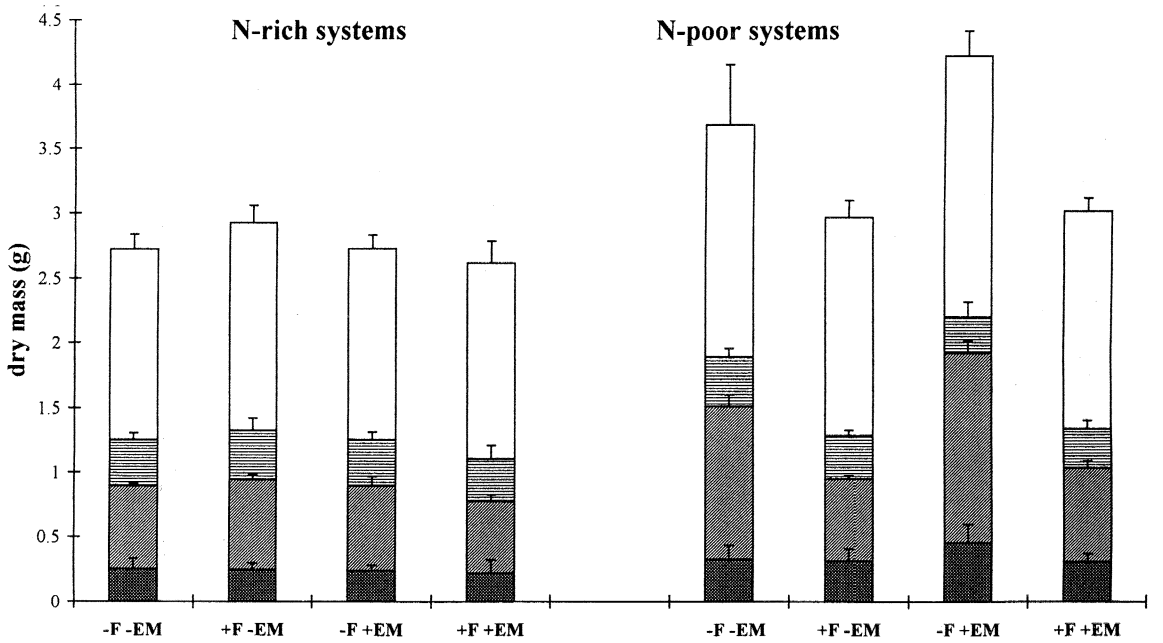

Fig. 2. Biomass production (g dry mass, mean + s.d.) of pine needles (open bars), stems (horizontally hatched parts), roots in the organic layer of the soil (diagonally hatched parts), and roots in the mineral soil (bars closest to the *X*-axis) in nitrogen rich and nitrogen poor microcosms at the end of the experiment (Week 45). +EM = pine seedlings initially infected with three species of mycorrhizal fungi; −EM = initially uninfected pine seedlings; −F = microcosms with a simple bacterial feeding fauna; +F = microcosms with a complex, natural set of soil fauna of the coniferous forest floor. $n = 5$.

(earthworms, beetles, etc.) were removed, and the filter papers with the fauna were gently placed in the microcosms. The procedure was repeated twice, at Day 0 and Day 7. Each complex system became hereby refaunated with 4000 individuals (or 724 000 ind./m^2) of Collembola, and 7000 (1 104100/m^2) specimens of Acari. Enchytraeids (*Cognettia sphagnetorum* Vejd.) were extracted using the wet funnel technique by O'Connor (1962), and at Day −1, 20 individuals were introduced into the refaunated systems. Nematodes, rotifers and tardigrades were extracted using the wet funnel method by Sohlenius (1979). At Day −1, all microcosms received 20 nematode specimens (including bacterial feeding *Acrobeloides* spp. and *Plectus* spp., and fungal feeding *Aphelenchoides* spp., Tylenchida, and occasional omnivorous dorylaimids), and an unquantified number of other microfauna in 5 ml tap water.

While constructing the microcosms and pre-treating the soils, pine seedlings were grown from surface-sterilized seeds collected from a single tree in aseptic conditions in a sand:perlite:peat-mixture. Three weeks after germination the seedlings were infected with 3 species of ectomycorrhizal fungi (*Cenococcum geophilum* and *Piloderma croceum*; cultures obtained from Henry Väre, Univ. of Oulu, and *Suillus bovinus* culture from Robin Sen, Univ. of Helsinki) as follows: before the infection took place the 3 mycorrhizal species were separately grown on filter paper on agar (Modified Melin-Norkran's) plates. After being colonized by the fungus the filter papers from each culture were transferred to a new agar plate without sugar, and a pine seedling was placed in the Petri dish so that the root was placed on the filter paper. The shoot of the pine seedling was allowed to grow out of the Petri dish through a hole in the lid. Seedlings to be placed into microcosms without mycorrhiza were treated similarly as described above, except that the filter papers on which the roots were growing did not contain mycorrhizal fungi. After 30 d the seedlings were removed from the agar plates and placed into the microcosms (Day 0).

The microcosms were incubated in randomized order in a climate chamber (Kryo-Service, Helsinki, Finland: volume 900 l) for 45 weeks. A daily cycle of 20 h of daylight (metal halide lamps, model HQI-T; illumination 80 W/m^2 measured from the top of the microcosms) and temperature of +18°C, and 4 h darkness, with a temperature of +12°C was maintained in the climate chamber for most of the growing periods. Artificial winter with constant darkness was created by gradually lowering the temperature to +1°C (see Table 3 for the temperature and illumination adjustments). The water lost due to evapotranspiration was replaced at regular intervals by adding tap water to the systems.

Analyses

The length of the pine seedlings (from the base to the terminal bud) was measured at Week 11. At the end of

Table 1. Experimental design with 3 factors: soil type; nitrogen rich vs nitrogen poor, ectomycorrhiza (EM); present (+) or absent (−), and soil fauna; complex (+) vs simple (−) in structure.

Treatment								
Soil type	N-rich	N-rich	N-rich	N-rich	N-poor	N-poor	N-poor	N-poor
EM	−	−	+	+	−	−	+	+
Soil fauna	−	+	−	+	−	+	−	+

the second growth period (Week 45) all the microcosms were destructively sampled for measuring the total biomass of the seedlings. For estimating root biomass the soil was halved, and one half was submerged in tap water to thoroughly wash the roots free of soil. After a 0.5-g sample of roots (fresh mass) had been taken for mycorrhizal analyses, roots were dried for 48 h in +70°C and weighed.

The biomass of ectomycorrhizal fungi on the roots was measured using the ergosterol method of Nylund and Wallander (1992) with slight technical modifications in the extraction procedure and HPLC analysis. After determination of dry matter content of the shoots (48 h at +70°C), accumulation of total Kjeldahl N in the needles was analysed separately for each seedling, whereas concentrations of total P, K, Ca and Mg were analysed with plasma emission (see above) from pooled needle material so as to form one sample per treatment. No needles had abscised during the experiment.

The remaining soil was used for analyses of soil fauna, water content, water holding capacity (WHC), organic matter content (5 h at 550°C), pH (H_2O) of the organic and the mineral soil, and concentration of NH_4^+ and content of total N in the organic soil were analysed as described above.

Statistical analyses

To test differences between treatments a General factorial ANOVA (SPSS for Windows v.6.1.) was used. The three factors used were: soil type (N-rich/N-poor), mycorrhiza (presence/absence), and soil fauna (complex/simple). Data which were not normally distributed were transformed to $\log_{10}(n+1)$. The number of replicates in each treatment combination was five.

Results

Characteristics of the soil

The most marked differences in the soil characteristics at the end of the experiment took place between the two soil types (N-rich treatment vs N-poor treatment), while the effects of soil fauna and mycorrhiza on soil properties were less consistent (Table 4). Organic matter content ($F = 322.8$, $P < 0.001$), water content ($F = 8.6$, $P = 0.006$), and pH (humus: $F = 250.1$, $P < 0.001$; mineral soil: $F = 51.9$, $P < 0.001$) were significantly higher in N-rich soils than in N-poor soils. Similarly, more total N (humus: $F = 46.3$, $P = 0.001$), and NH_4^+-N (humus: $F = 654.4$, $P < 0.001$; mineral soil: $F = 124.8$, $P < 0.001$) were found in the N-rich soils, and more water (higher WHC; $F = 235.8$, $P < 0.001$) was retained in N-rich soils (Table 4).

In the presence of a complex animal community pH of both humus soil and mineral soil was slightly higher ($F = 4.6$, $P = 0.04$) as compared to systems with simple fauna. Also, WHC ($F = 4.2$, $P = 0.048$) and concentration of NH_4^+-N ($F = 31.3$, $P < 0.001$) were higher in microcosms with complex fauna (Table 4).

Soil type and soil fauna had a significant interaction effect on soil pH ($F = 30.2$, $P < 0.001$); in N-poor systems, in the presence of complex fauna, pH of the soil was higher than in systems with simple fauna, whereas no faunal effects on soil pH were detected in N-rich soil. Similarly, soil fauna and soil type exerted a significant interaction effect on NH_4^+-N concentration in the soil ($F = 41.5$, $P < 0.001$); N-poor systems had more ammonium when complex fauna was involved, but in N-rich soils the influence of complex fauna was insignificant (Table 4).

The systems in which the pine seedlings were initially infected with mycorrhiza retained slightly less water ($F = 6.6$, $P = 0.015$), and had more NH_4^+-N in the soil than did the systems with initially non-infected seedlings. Moreover, mycorrhiza and soil fauna had a significant interaction on the WHC of the humus ($F = 5.5$; $P = 0.026$); soil fauna had a positive effect on soil WHC in systems initially non-infected with mycorrhiza,

Table 2. Characteristics of the humus of the two soil types at the beginning of the experiment. NH_4^{+1} = KCl-extractable ammonium before defaunation with liquid nitrogen, and after defaunation (NH_4^{+2}); % = concentration of total N, P, K Ca and Mg in the dry humus. $n = 5$.

		N-rich	N-poor
tot. N	(%)	1.01	0.66
NH_4^{+1}	(µg/g dry soil)	75.9	0.002
NH_4^{+2}	(µg/g dry soil)	328.69	115.28
P	(%)	0.06	0.07
K	(%)	0.06	0.07
Ca	(%)	0.31	0.18
Mg	(%)	0.04	0.03
pH (H_2O)		4.4	4.5

Table 3. Adjustments of temperature and illumination in the growth chamber.

	Weeks	Temperature (°C) day/night	Illumination (h)
Summer	0–14	18/12	20
Autumn a	15–17	13/8	12
Autumn b	18–20	10/5	9
Autumn c	21–23	5/5	6
Winter	24–28	1–2/1–2	0
Spring a	29–31	6/4	7
Spring b	32–34	12/8	13
Summer	35–45	18/12	20

whereas this effect was insignificant in the presence of mycorrhiza (Table 4).

Soil fauna

The fauna in the complex systems remained diverse and numerically abundant till the end of the experiment. Some differences in the abundance of animal taxa existed between the soil types: Nematoda (mostly bacterial feeders) existed in much lower numbers in N-rich soils ($\bar{X} = 720$ ind./m^2, s.e. ± 330) than in N-poor soils ($\bar{X} = 2\,079\,720$ ind./m^2, s.e. $\pm 1\,623\,600$) ($F = 5.6$, $P = 0.024$). Also the enchytraeids were less abundant in N-rich soils ($F = 90.4$, $P < 0.001$). Of the micro-arthropods oribatid mites were more numerous in N-rich soils ($F = 5.4$, $P = 0.033$), and Poduridae (Collembola) were somewhat more abundant in systems initially inoculated with mycorrhizal seedlings than in systems without mycorrhiza ($F = 6.0$, $P = 0.026$) (Table 5). Only bacterial feeding nematodes and protozoans were found in the simple systems.

Mycorrhiza

Visual observations through the transparent wall of the microcosm revealed that during the first growing period only the roots that were initially infected with EM fungi showed clear signs (dichotomous short root branches and fungal mantles) of mycorrhizal development. Based on ergosterol analysis all of the pine seedlings, including the ones initially non-inoculated with ectomycorrhiza, had become infected with mycorrhiza by the end of the experiment. At this time there was no difference in the amount of ergosterol on the roots between the infected and non-infected treatments (Fig. 1). About 10 times more ergosterol was found on the pine roots growing in the N-poor than in the N-rich soils ($F = 100.7$, $P < 0.001$). Of the three initially inoculated mycorrhizal types the *Suillus*-type mycorrhizal formation seemed to dominate in each microcosm. *Piloderma* sp. and *Cenococcum geophilum* were frequently found in the microcosms but in small quantities.

In the complex systems significantly less ergosterol was detected on the pine roots as compared to the simple systems – this was particularly the case in nitrogen-poor soils ($F = 17.1$, $P < 0.001$) (Fig. 1).

Production and nutrient content of pine seedlings

By Week 11 neither soil type nor faunal complexity influenced the height of the seedlings. The growth of the seedlings was, however, significantly affected by mycorrhiza; the seedlings inoculated with mycorrhiza were significantly ($F = 13.8$, $P = 0.001$) smaller than the non-inoculated ones in both soil types (Table 6). However, mycorrhizal treatment had no influence on pine growth at the end of the experiment (Fig. 2).

By the final harvesting significantly more biomass (including needles, stems and roots) ($F = 17.5$, $P < 0.001$) was produced in N-poor systems than in systems rich in N. The enhanced ($F = 9.4$, $P = 0.004$) above-ground production was due to improved needle biomass production – soil type did not affect accumulation of stem biomass. The amount of fine roots (diameter < 1 mm) in the humus layer ($F = 26.7$, $P < 0.001$) and in the mineral soil ($F = 9.6$, $P = 0.004$) were significantly higher in systems with N-poor soil (Fig. 2).

Soil type and soil fauna had a significant interaction on the total biomass production ($F = 10.0$, $P = 0.003$); in N-poor soils a complex fauna reduced the amount of pine biomass, whereas in N-rich soils the complex fauna had no effect on total pine biomass (Fig. 2). Complex soil fauna had a negative influence ($F = 17.6$, $P < 0.001$) on fine root biomass, this influence being clear in N-poor soil only (Fig. 2).

The concentrations of total N in the needles were significantly ($F = 107.6$, $P < 0.001$) higher in seedlings grown in N-rich soils than in seedlings in the N-poor systems (Table 6). Consequently, the amount of N taken up by the plants in N-rich microcosms was almost double compared with the seedlings grown in N-poor soils. A complex soil fauna had a slight positive influence ($F = 6.0$, $P = 0.02$) on the total N concentration of pine needles in both soil types (Table 6). Mycorrhiza and soil type showed a small but significant interaction ($F = 6.0$, $P = 0.02$); seedlings initially inoculated with mycorrhiza growing in N-poor soils took up more N than non-inoculated seedlings in the same soil. In N-rich soil no differences in the uptake of N with respect to mycorrhiza treatment was found.

The concentrations of P in the pine seedlings grown in N-rich soil in the presence of complex fauna were slightly smaller, and concentrations of K, Ca and Mg somewhat higher than the ones in the seedlings grown in N-poor soils (Table 6).

Table 4. Characteristics (mean ± s.d., $n = 5$) of the soil in the microcosms with two different soil types at the end of the experiment. pH humus = pH (H_2O) of the humus, pH mineral = pH (H_2O) of the mineral soil, % d.m. = proportion of drymass of the humus of its wet mass, % o.m. = proportion organic material of the dry humus, WHC = water holding capacity (% of dry mass of the humus), NH_4^{+a} = KCl-extractable ammonium (μg/g d.m.) from the humus and mineral soil (NH_4^{+b}), and concentration of total-N in the humus ($n = 1$; pooled from 5 samples within a treatment). +EM = microcosms initially infected with ectomycorrhizal fungi, −EM = microcosms without initial inoculation of ectomycorrhizal fungi, +F = microcosms with a complex soil animal community, −F = microcosms with a simple soil animal community.

	N-rich −EM −F	N-rich −EM +F	N-rich +EM −F	N-rich +EM +F	N-poor −EM −F	N-poor −EM +F	N-poor +EM −F	N-poor +EM +F
pH humus	4.6 ± 0.15	4.4 ± 0.17	4.5 ± 0.15	4.6 ± 0.19	3.6 ± 0.24	4.0 ± 0.08	3.5 ± 0.07	4.0 ± 0.11
pH mineral	5.0 ± 0.16	5.1 ± 0.17	5.0 ± 0.23	5.2 ± 0.26	4.7 ± 0.18	4.8 ± 0.07	4.6 ± 0.14	4.7 ± 0.13
% d.m.	39.5 ± 5.4	40.4 ± 1.0	41.2 ± 2.9	40.5 ± 2.9	44.2 ± 2.3	41.4 ± 0.8	43.6 ± 1.5	42.3 ± 1.5
% o.m.	51.0 ± 1.8	51.2 ± 1.5	52.2 ± 2.3	51.3 ± 1.7	41.5 ± 0.8	40.2 ± 3.6	39.2 ± 1.1	39.3 ± 1.7
WHC	357.1 ± 27.1	365.1 ± 26.7	357.5 ± 21.0	353.6 ± 30.0	218.2 ± 24.4	282.4 ± 12.0	214.0 ± 24.8	213.3 ± 35.8
NH_4^{+a}	0.16 ± 0.07	0.10 ± 0.04	0.15 ± 0.04	0.17 ± 0.06	0.0004 ± 0.0002	0.003 ± 0.003	0.0008 ± 0.0002	0.01 ± 0.006
NH_4^{+b}	0.003 ± 0.001	0.003 ± 0.008	0.003 ± 0.001	0.004 ± 0.002	0.0006 ± 0.0002	0.0006 ± 0.0002	0.0006 ± 0.0002	0.0008 ± 0.0004
% tot-N	0.62	0.65	0.64	0.71	0.48	0.40	0.45	0.35

Table 5. Numbers of soil fauna (10^3 ind./m^2; mean ± s.e., $n = 5$) in the microcosms with complex fauna in N-rich and in N-poor soils; +EM = mycorrhiza inoculated, −EM = mycorrhiza not inoculated while establishing the experiment.

	N-rich −EM	N-rich +EM	N-poor −EM	N-poor +EM
Enchytraeidae	39.2 ± 15.2	22.1 ± 3.6	198.0 ± 19.5	194.6 ± 29.0
Acari				
Oribatida	156.8 ± 21.4	193.9 ± 22.7	133.6 ± 20.4	118.6 ± 20.1
Mesostigmata	7.0 ± 3.6	0.2 ± 0.2	4.1 ± 3.0	1.4 ± 0.7
Astigmata	9.7 ± 4.7	11.5 ± 9.3	9.9 ± 5.5	7.2 ± 4.0
Others	17.5 ± 8.8	13.3 ± 3.9	9.0 ± 3.9	22.1 ± 3.9
Collembola				
Isotomidae	774.0 ± 390.0	1158.0 ± 255.0	241.2 ± 68.9	692.0 ± 182.0
Onychiuridae	27.2 ± 16.2	43.9 ± 14.4	12.1 ± 6.4	14.8 ± 4.1
Poduridae	0.7 ± 0.4	3.2 ± 1.5	1.3 ± 1.3	8.6 ± 4.3
Others	0 ± 0	21.6 ± 21.6	0 ± 0	2.5 ± 2.3

Discussion

Since most mycorrhizal fungi are obligately mutualistic, whereas plants may be obligately or facultatively mycotrophic (Allen 1991), the factors potentially affecting the outcomes of the plant–fungus relationship are reasonable to explore from the plant's perspective. Consequently, we will evaluate the costs and benefits on the basis of pine biomass production, even though the costs and benefits provided by the fungus may not fully be evaluated until a more comprehensive set of life history parameters of the plant is considered (see Harper 1977).

Abiotic environment and conditionality

A prerequisite for examining the hypothesis suggesting differential grazing impacts of the fauna on the plant–fungus association in the two soil types is that the outcomes of the plant–fungus symbiosis should differ in relation to N availability for the plant. In this respect the results of the experiment met the requirement: under N-poor conditions the benefits the EM fungi provided the pine outweighed its possible costs, resulting in highest pine biomasses in microcosms with the most extensive EM development. In N-rich soils, however, there was a negative relationship between pine biomass and EM biomass. These results showing a negative relationship between mycorrhizal biomass and nitrogen availability are in good accordance with many previous investigations (see Harley and Smith 1983, Allen 1991, and Wallander 1992, for reviews). However, since the pine seedlings originally intended to serve as non-mycorrhizal controls became infected by EM fungi during the second growing season it remains open whether the EM fungi acted as true parasites. In N-poor soils uninfected seedlings should – according to our hypothesis – have suffered from the lack of the fungal association. Indeed, that the growth of non-infected birch seedlings can almost completely cease after one growing season was shown by Setälä and Huhta (1991) under experimental conditions similar to those in the N-poor systems of the present study.

Outcomes of grazing with respect to conditionality

We aimed to establish an abundant community of soil fauna having a potential to over-graze the EM fungi in the microcosms. By the end of the experiment the fauna, mostly composed of typical representatives of fungal feeders, and being 5 to 10 times more abundant than the one found in similar soils in the field (e.g. Huhta et al. 1986), evidently exerted a remarkable grazing pressure on microbes, including EM fungi. The grazer community in the simple systems was dominated by bacterial feeding microfauna and thus represented a community with a greatly reduced capacity to influence fungal dynamics in the microcosms.

Our hypothesis, according to which the abiotic environment has some control over the biotic factors affecting the plant–fungus symbiosis, proved to be correct; depending on the availability of N for the pine, grazing activity by soil fauna caused different outcomes in the plant–fungus interaction. In N-poor systems, where EM fungi is beneficial for the pine, heavy grazing pressure on EM fungi was accompanied with reduced pine growth. In other words, it seems as if the fungi had provided more benefits for the host when ungrazed. Under conditions of high levels of N, however, EM fungi behaved differently, and in many cases it seemed as if the fungi had become a carbon/energy drain for the pine. Since herbivorous fauna, including root feeders, were absent in the microcosms, it is evident that the rate of primary production was not associated with faunal induced direct trophic effects on the pine. At this point it seems as if substantial grazing by soil fauna had the potential to affect the costs and benefits in the plant–EM fungi relationship, thereby meeting the requirements set for conditionality (see Bronstein 1994).

The mechanisms behind this biotically induced conditionality may not, however, be solely explained by the cost-benefit approach. As a matter of fact, focusing on quantitative analyses on the biomass production of the EM fungi and the host may mask other, perhaps more important, factors determining the outcomes of these interactions. There is good evidence that nutrients other than nitrogen were of crucial importance affecting the

Table 6. Shoot height (cm; mean ± s.d.) of pine seedlings at Week 11, and nutrient concentrations (% of dry mass) in the pine needles grown in microcosms with nitrogen-rich or nitrogen-poor soil at the end of the experiment (Week 45). $n = 5$ for shoot height and concentration of N; concentrations of P, K, Ca and Mg are analysed from composite samples (pooled from five separate samples). See Table 4 for treatment abbreviations.

	N-rich −EM −F	N-rich −EM +F	N-rich +EM −F	N-rich +EM +F	N-poor −EM −F	N-poor −EM +F	N-poor +EM −F	N-poor +EM +F
Shoot height	6.5 ± 0.5	7.0 ± 1.5	5.6 ± 0.6	6.7 ± 0.9	6.6 ± 0.2	6.4 ± 0.9	5.2 ± 0.3	5.4 ± 1.4
N	1.75 ± 0.33	2.08 ± 0.16	1.77 ± 0.34	1.87 ± 0.34	0.91 ± 0.18	1.09 ± 0.11	1.15 ± 0.07	1.26 ± 0.16
P	0.14	0.14	0.14	0.13	0.13	0.16	0.14	0.16
K	1.01	0.95	0.92	0.85	0.52	0.73	0.49	0.73
Ca	0.36	0.3	0.33	0.28	0.24	0.28	0.32	0.38
Mg	0.15	0.13	0.14	0.12	0.06	0.11	0.08	0.12

results. Emphasizing on the availability of phosphorus in the soil, we suggest the following.

Nutrient balance in the soil and conditionality

Lack of available nitrogen has been the factor that has most strongly limited stand growth in the boreal coniferous forest zone (Melin 1953). Availability of P has not been a problem for the vegetation in young and unweathered boreal forest soils (Clarholm and Rosengren-Brinck 1995). However, when N-availability increases – as has been the case in many areas with a high deposition of N (Tamm 1991) – the situation with respect to the growth regulating nutrient may change. Wallander (1992) suggested the well-known reduction in EM mycelia under an excess supply of N (see Harley and Smith 1983, Ohenoja 1988, Wallander and Nylund 1991, 1992, Arnebrant 1994) to be a consequence of increased allocation of energy by the fungi to the process of N assimilation. Since various EM fungi, including the species applied in the current study, are known to produce phosphatases (e.g. Ho and Zah 1979, Dighton 1991), reduced amount of EM fungi due to high N content in the soil evidently leads to decreased excretion of phosphatases (see Clarholm and Rosengren-Brinck 1995). This will, at least potentially, induce P deficiency in the plant. Assuming this reasoning to be valid in the present study, the smaller pine biomass in the N-rich microcosms than in N-poor systems may not result from the costs of the fungi surpassing the benefits by it. It is more likely that the pines suffered from P deficiency, which was a direct, inevitable consequence of the dramatically reduced EM biomass in N-rich soils.

The much lower P:N ratio (0.071) in the needles growing in the N-rich systems as compared with those in the N-poor microcosms (0.135) gives strong evidence for the former systems to be P-limited. According to Ingestad (1979) the ratio e.g. between P and N in the photosynthesizing apparatus is a more valuable indicator to interpret nutrient status of the soil than are concentrations separately given to each nutrient. The optimal P:N ratio in the foliage of conifers is reported to be close to 0.16; values less than 0.10–0.12 are considered to show P deficiency (Nihlgård 1990, Anonymous 1992). Thus, the lower biomass of the pine seedlings in the N-rich microcosms than in the N-poor systems probably has nothing to do with high costs of the fungi in the former systems. Rather, we conclude that the smaller pine biomass is brought about by the reduced amount of EM fungi, which is reflected in the substantially reduced potential of the plants to acquire P from the soil.

Now, soil fauna seems to play a dual role in respect to the growth limiting nutrient in the artificial nutrient gradient we created. The negative influence of grazing on pine growth in N-poor soil is best explained by the "right", i.e. normal ratio of N to P in the soil, to which the plant–EM fungi association has adapted during its long evolution (see Pirozynski 1981). Any external factor disturbing this association is likely to affect negatively one or both participants of the relationship. Under such conditions – from the plant's point of view – fungal feeding fauna may indeed be interpreted to "eat the benefit". When the limiting factor, N in this case, becomes non-limiting, the long adaptive history of the mutualism ceases to function. Under such conditions an "external" factor, such as excessive soil fauna grazing upon EM fungi, truly is external to the plant–fungus association posing minor influence on the symbiosis itself. Under conditions where N is not limiting plant growth, some other main nutrient, like P in this case, starts to regulate plant production, and the grazing-induced patterns in plant production will therefore differ among soil types.

Concluding remarks on the conditionality of the plant–fungus association

Results of the present study emphasize the difficulty in applying the concept of conditionality for the symbiotic association between plants and their ectomycorrhizal fungi. Unlike situations in which the association is composed of two species only (the "ant protection mutualisms" representing a typical case; see Cushman and Whitham 1989, Cushman and Addicott 1991), the

plant–fungus association is most often composed of several species of fungi (Trappe 1962, Allen 1991). Moreover, conditionality is commonly described under conditions in which a single resource determines the outcome of the symbiotic interaction (Bronstein 1994). In the case of plant–fungus association, however, costs and benefits of the association seem to depend on an array of resources – such as various nutrients. For example, when a particular nutrient, such as N in the present study, is in excess for plant needs, another nutrient will limit plant growth (The "Law of minimum" of J. Liebig). It is important to notice that many of the EM fungi differ in their capacity to absorb nutrients from the soil (Bledsoe 1992), and that nutrient status in the soil affects the species distribution of EM fungi (Laiho et al. 1987, Arnebrant 1995). Consequently, from the plant's perspective there is a constant need for various types of EM fungi to give the best profit. It is therefore obvious that the conventional "two-species approach" of the concept "conditionality" is bound to be of limited operational value when associated with the plant–EM fungi symbiosis.

The role of soil fauna in modifying the costs and benefits in the plant–EM fungi association seems also to be difficult to determine. That the impact of grazing on pine growth was clearly related to nutritional status of the soil emphasizes the complex nature of interactions in below-ground food webs. What comes to the factors controlling the costs and benefits in the interactions between plants, EM fungi, and soil fauna it seems irrelevant to choose one environmental factor at a time, and apply the cost-benefit approach to seek for conditionality in respect to that particular factor. Thus, because soil fauna and nutrient dynamics are so highly interconnected with plant production (Bengtsson et al. 1995) and development of EM fungi in the soils (Setälä 1995), we conclude that the degree of context-dependency in the pine–fungus association in respect to the above-mentioned variables is bound to remain indefinite.

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