Nitrogen supply affects arbuscular mycorrhizal colonization of *Artemisia vulgaris* in a phosphate-polluted field site

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Summary

- Root colonization by arbuscular mycorrhizal fungi (AMF) was investigated in industrially polluted grassland characterized by exceptionally high phosphorus levels (up to 120 g kg^{-1} soil).
- Along a pollution-induced nitrogen gradient, soil and tissue element concentrations of *Artemisia vulgaris* plants and their mycorrhizal status were determined. Additionally, we compared mycorrhization rates and above-ground biomass of *A. vulgaris* at N-fertilized and control plots in the N-poor area.
- Despite high soil and tissue P concentrations, plants from N-deficient plots, which were characterized by low tissue N concentrations and N : P ratios, were strongly colonized by AMF, whereas at a plot with comparable P levels, but higher soil and plant N concentrations and N : P ratios, mycorrhization rates were significantly lower. Correlation analyses revealed a negative relationship between percentage root colonization of *A. vulgaris* by AMF and both tissue N concentration and N : P ratio. Accordingly, in the fertilization experiment, control plants had higher mycorrhization rates than N-fertilized plants, whereas the species attained higher biomass at N-fertilized plots.
- The results suggest that N deficiency stimulates root colonization by AMF in this extraordinarily P-rich field site.

Key words: arbuscular mycorrhiza, *Artemisia vulgaris*, N : P ratio, nitrogen (N), phosphorus (P), root colonization.

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Introduction

One of the most important functions of arbuscular mycorrhizal fungi (AMF) lies in improving the phosphorus nutrition of their host plants. An increased inflow of phosphate into mycorrhizal roots has been demonstrated and associated plants have been found to have higher tissue P concentrations than nonmycorrhizal controls (Sanders & Tinker, 1971). This is because AMF hyphae extend into areas beyond the depletion zone of the plant root (Sanders & Tinker, 1971; Jakobsen *et al.*, 1992a) and have the capacity to absorb poorly mobile phosphate and transport it to the host (Jakobsen *et al.*, 1992b).

Phosphorus fertilization has been proved to reduce the effects of arbuscular mycorrhizal fungi on hosts, and to inhibit root colonization (Daft & Nicolson, 1969; Sanders & Tinker, 1973; Abbott *et al.*, 1984). Some early studies have indicated that it is not the soil P content that determines mycorrhizal colonization, but the P status of the plant (Menge *et al.*, 1978; Ratnayake *et al.*, 1978). Later, De Miranda *et al.* (1989) and Thomson *et al.* (1991) showed that tissue P, as well as soil P, may affect mycorrhization.

Less attention has been paid to the effect of arbuscular mycorrhizas (AM) on the nitrogen nutrition of plants. Although there were early indications that AMF may play a role in N

uptake and transport (Raven *et al.*, 1978; Smith, 1980), N was for a long time believed to be of secondary (Read *et al.*, 1988) or no (Hayman, 1982) importance regarding arbuscular mycorrhizas.

It has been shown, however, that uptake of ammonium and transport of N via AMF hyphae does take place (Ames *et al.*, 1983; Johansen *et al.*, 1992; Frey & Schüepp, 1993; Johansen *et al.*, 1993), and that soil N is effectively depleted by the external mycelium (Johansen *et al.*, 1992; Frey & Schüepp, 1993; Johansen *et al.*, 1993). Despite the ready availability of nitrate for direct uptake by plant roots (Clark & Zeto, 2000), depletion in the soil, absorption and transport of this ion by AMF have been demonstrated (Johansen *et al.*, 1993; Tobar *et al.*, 1994; Bago *et al.*, 1996). The influence of arbuscular mycorrhizas on uptake of organic N compounds has been also discussed (Ames *et al.*, 1983; Hawkins *et al.*, 2000; Hodge *et al.*, 2001).

Nevertheless, evidence about the contribution of arbuscular mycorrhizal fungi to the N nutrition of plants remains contradictory. In some experiments mycorrhizal plants had higher N concentrations than nonmycorrhizal controls (Frey & Schüepp, 1993; Johansen *et al.*, 1994; Tobar *et al.*, 1994); whereas in other studies no differences could be detected in the N status of inoculated and control plants (Johansen *et al.*, 1992; Johansen, 1999; Hawkins *et al.*, 2000).

It is unclear whether colonization of plant roots by AMF and their contribution to N uptake and transport are regulated by both soil N concentration and host N concentration (Azcón *et al.*, 1982), or by tissue N concentration only (Sylvia & Neal, 1990; Johansen *et al.*, 1994). In addition, as in the case of P fertilization, N fertilization has been shown to suppress root colonization (Chambers *et al.*, 1980; Azcón *et al.*, 1982).

Experiments focusing on separate as well as combined effects of P and N have indicated that root colonization was reduced only when both elements were available in sufficient concentrations for the plants (Mosse & Phillips, 1971; Bååth & Spokes, 1989; Sylvia & Neal, 1990; Liu *et al.*, 2000; Corkidiki *et al.*, 2002; Treseder & Allen, 2002; Johnson *et al.*, 2003). Therefore the N : P ratio might be an important factor determining AM development (Liu *et al.*, 2000; Miller *et al.*, 2002; Johnson *et al.*, 2003).

A site with extremely high P concentrations and various levels of N enabled us to study further the influence of P and N on arbuscular mycorrhizal colonization directly in the field.

Recently we revealed most plants at this site to be mycorrhizal despite the high soil P content (Renker *et al.*, 2003, 2005). In molecular analyses of a range of plant species and fungal spores, we found only AMF species also occurring at comparable uncontaminated field sites of the region. This indicates that there are no specialized fungi which are particularly well adapted to the pollution.

Our aim for the present work was to ascertain whether the N level at our field site influences root colonization by AMF.

Materials and Methods

Field site

The field site is located in the Central Saale Valley, 13 km north of Jena, Thuringia, Germany (51°01′ N, 11°41′ E, 140 m above sea level). It is a calcareous, east-facing slope with a thin-layered rendzina soil, a mean annual rainfall of 586 mm, and an average air temperature of 9.3°C (Heinrich *et al.*, 2001).

Between 1960 and 1990, a factory south of the study area produced phosphate fertilizer (Heinrich, 1984; Heinrich *et al.*, 2001), and consequent alkaline dust deposition resulted in contamination of areas downwind of the factory. Soil pH was raised and reached values of up to 10; phosphate, fluorine and, for example, sodium, calcium and cadmium levels were exceedingly high (Heinrich, 1984; Metzner *et al.*, 1997). Because of this pollution, areas close to the factory were devoid of vegetation from 1980 to the beginning of the 1990s (Heinrich *et al.*, 2001), which provides an explanation for the low current soil N concentrations (0.1–0.2%) (Metzner *et al.*, 1997).

After closure of the factory, rapid plant succession took place, starting from a near-monospecific vegetation, comprising the salt-tolerant grass *Puccinellia distans* (Jacq.) Parl., and resulting in a diverse ruderal plant community of ≈70 species of higher plants a decade later (Heinrich *et al.*, 2001). Today the soil P content is still very high (up to 120 g kg^{-1} soil), but other elements have been leached out (e.g. sodium, fluorine) or immobilized (e.g. cadmium) caused by the high pH (≈8) (Metzner *et al.*, 1997; Langer & Günther, 2001).

In experiment 1 we examined three plots at the bottom of the slope in a northward (downwind) direction from the previous source of pollution: plots 1–3 situated ≈50, 200 and 800 m, respectively, from the former factory.

Additionally, we performed an N-fertilization experiment (experiment 2) close to the former factory, adjacent to plot 1.

Experiment 1

Soil parameters In 1999, 15 soil samples were taken at each plot, air-dried and analysed according to Deutsches Institut für Normung (DIN) and Technische Güte- und Liefervorschriften (TGL) instructions (VDLUFA, 1991). Soil acidity (pH) was measured; an aqua regia digestion of the soil was carried out to determine total element concentrations of sodium, potassium, magnesium, calcium and cadmium (Na_{tot}, K_{tot}, Mg_{tot}, Ca_{tot} and Cd_{tot}); plant-available phosphorus and potassium (P_{cal} and K_{cal}) were extracted using the method of Schüller (1969); and total nitrogen (N_{tot}) was determined using the method of Hendershot (1985).

Plant material *Artemisia vulgaris* L. plants were grown from seeds of one mother plant from the field site. For the first 3 wk they were grown in walk-in growth chambers (York International GmbH, Mannheim, Germany) with a 14 : 10 h light : dark

photoperiod at 20 : 18°C, 65% relative humidity and ≈1000 μM m^{-2} s^{-1} photosynthetically active radiation.

In April 2000, 120 plants of similar size and morphology were transferred to each field plot, to ensure homogeneous growth conditions. The plots had been cleared of competing ruderal plants. In June 2002, eight plants per plot were harvested randomly to determine element concentrations and mycorrhization rates.

Plant element concentrations Immediately after harvesting, half of the root system, three different old parts of the stem (each ≈3 cm long), and one basal, one intermediate and one apical leaf of each *A. vulgaris* plant were washed with tap water and stored at −80°C. Later, samples were freeze-dried, weighed and finely ground in a pebble mill.

To determine N content, 5 mg samples of the homogenized material were burned at 1000°C in flowing oxygen and analysed with a Leco CHNS-932. The CO_2, H_2O, NO_x and SO_2 combustion gases were passed through a reduction tube with helium as the carrier gas for converting the nitrogen oxides into N_2 and binding the free oxygen. After absorption of CO_2, H_2O and SO_2, the content of the remaining N was determined by thermal conductivity detection.

To determine P, Na, K, Mg, Ca and Cd, inductively coupled plasma with optical emission spectroscopy (ICP-OES) was used. The liquid samples (200 mg of the ground sample material, digested) were introduced into the inductively generated argon plasma through a nebulizer system, and excited. The spectrum emitted was transferred into a spectrometer (Thermo Jarrell Ash IRIS Plasma Spectrometer (TJA-IRIS)) where it was broken down into individual wavelengths and evaluated. The intensities of the spectral lines were measured by charge injection device (CID) semiconductor detectors. Calibration was effected with multi-element solutions mixed from standard solutions.

Root colonization by AMF Parts of the root systems not needed for element content analyses were fixed in formaldehyde–acetic acid (FAA): 6.0% formaldehyde, 2.3% glacial acetic acid, 45.8% ethanol, 45.9% H_2O (v/v). For staining of fungal structures, roots were incubated in 10% KOH for 2 × 15 min at 90°C, rinsed with tap water, acidified with 3.7% HCl for 10 min, and dyed in lactophenol blue solution (Merck 113741) for 90 min. For decolorization of plant cells and storage, roots were washed several times with, and stored in, 50% lactic acid (Phillips & Hayman, 1970, modified after Schmitz *et al.*, 1991).

Percentage colonization of root length was determined with a Zeiss Axioplan light microscope using the line-intersect method (Ambler & Young, 1977, modified after Schmitz *et al.*, 1991). At least 300 segments of each root sample were counted.

Experiment 2

Nitrogen fertilization From 2000 onwards, six pairs of experimental plots close to the former factory, adjacent to plot 1 of the previous experiment, were established. One plot of each pair was fertilized with ammonium nitrate on an annual basis. Every March, plots received 8.5 g N m^{-2}, applied as slow-release pellets (Osmocote™ 23-0-0). The other plot of each pair served as an unfertilized control.

Plant material and root colonization by AMF In June 2003, three *A. vulgaris* plants per plot were harvested to determine mycorrhization rates. Roots were fixed in FAA, stained and checked for mycorrhization as described above.

Above-ground biomass of *Artemisia vulgaris* Parallel to harvesting plants for colonization analyses, vegetation at a subplot of each plot was cut at ground level. To determine *A. vulgaris* biomass, this species was singled out, dried to constant weight at 80°C in a drying chamber, and weighed.

Statistics

Statistical analyses were performed using SPSS 11.0 (SPSS Inc., Chicago, IL, USA).

Data were tested for normal distribution with the Kolmogorov–Smirnov test, and for homogeneity of variances with the Levene test. To compare soil and plant element concentrations and mycorrhization rates of *A. vulgaris* across the three plots in experiment 1, one-way ANOVA was performed; for data with heterogeneous variances the Welch statistic was used instead of the *F* statistic. In case of significance, this was followed by a Tukey *post hoc* test (homogeneous variances and equal sample numbers); a Hochberg's GT2 test (homogeneous variances and unequal sample numbers); or a Games–Howell *post hoc* test (heterogeneous variances).

To test for correlations between plant element concentrations and mycorrhization rates, a Spearman's rank correlation was performed. The sequential Bonferroni method was used to adjust the type I error rate in order to limit the overall error rate.

In experiment 2, mycorrhization data from each single plot were averaged to avoid pseudoreplication. To compare percentage root colonization between control and N-fertilized plots, a paired samples *t*-test was performed with mean mycorrhization rates per plot. To test whether N fertilization resulted in an increase in *A. vulgaris* biomass, a one-tailed paired samples *t*-test was carried out. Before analysis, biomass data were square-root transformed to meet the requirement of variance homogeneity.

Results

Experiment 1

Soil parameters Soil data from the three field plots and ANOVA results are shown in Table 1. For means of total Na, Mg and Ca concentrations and plant-available K concentrations,

Table 1 Soil data for the three field plots of experiment 1

Parameter	Plot 1 Mean	Plot 1 SD	Plot 2 Mean	Plot 2 SD	Plot 3 Mean	Plot 3 SD	F or †Welch statistic
Na_{tot} (g kg^{-1})	7.15^{a}	±1.84	9.61^{a}	±7.36	7.73^{a}	±3.57	†0.812
K_{tot} (g kg^{-1})	3.01^{a}	±0.72	9.99^{b}	±3.55	8.01^{b}	±0.95	†142.879***
Mg_{tot} (g kg^{-1})	7.96^{a}	±3.85	8.92^{a}	±3.04	9.58^{a}	±1.65	†1.189
Ca_{tot} (g kg^{-1})	172.5^{a}	±38.6	143.4^{a}	±74.8	166.7^{a}	±16.0	†0.820
Cd_{tot} (mg kg^{-1})	8.60^{b}	±3.60	5.50^{ab}	±3.54	3.46^{a}	±1.34	†13.999***
N_{tot} (%)	0.16^{a}	±0.10	0.18^{a}	±0.19	0.34^{b}	±0.20	9.538***
P_{cal} (mg kg^{-1})	12249^{b}	±6310	6332^{a}	±4506	10549^{b}	±3510	†5.162*
K_{cal} (mg kg^{-1})	772.2^{a}	±319.1	577.4^{a}	±258.5	576.3^{a}	±201.6	2.702
pH	8.38^{b}	±0.32	8.10^{a}	±0.26	8.57^{b}	±0.28	9.817***

Means (n = 15), standard deviations (SD) and F values or Welch statistic (†) from one-way ANOVA. Parameters include total levels measured by aqua regia digestion (Na_{tot}, K_{tot}, Mg_{tot}, Ca_{tot}, Cd_{tot}), total nitrogen concentration (N_{tot}), plant available phosphorus and potassium (P_{cal}, K_{cal}) and soil acidity (pH). Asterisks indicate significance levels: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; superscript letters show significant differences among plots.

no significant differences could be detected. In contrast, total K, Cd, N and plant-available P concentrations and pH differed significantly between plots. Total K was significantly lower at plot 1 compared with plots 2 and 3; total Cd was significantly higher at plot 1 than at plot 3 with an intermediate level at plot 2; total N concentrations were significantly lower at plots 1 and 2 than at plot 3; and plant-available P and pH were significantly higher at plots 1 and 3 compared with plot 2.

Plant element concentrations Table 2 shows element concentrations and N : P ratios in different plant parts of *A. vulgaris*, and ANOVA results. Mean leaf, stem and root concentrations of P, Na and Ca as well as stem Mg did not differ significantly between the three plots, whereas N (Fig. 1) and K concentrations in leaves, stems and roots, N : P ratios in leaves and stems (Fig. 2), and Mg concentrations in leaves and roots were significantly lower at plots 1 and 2 compared with plot 3. The N : P ratio of roots at plot 3 (Fig. 2) was marginally (P = 0.078) higher than at plots 1 and 2.

Root colonization by AMF Table 2 and Fig. 3 show mean mycorrhization rates of *A. vulgaris*. The fractions of root length colonized by internal mycelium, arbuscules and vesicles were significantly higher at plots 1 and 2 compared with plot 3. Element concentrations in leaves, stems and roots showing significant differences among plots (Table 2) were directly opposed to differences in mycorrhization rates. However, out of these only N concentrations (Fig. 1) and N : P ratios (Fig. 2) also corresponded to the respective soil data (N_{tot}, Table 1). Therefore all other elements were not considered further.

Correlations between root colonization by AMF and plant element concentrations Spearman's rank correlation revealed that internal mycelium, arbuscules and vesicles were significantly negatively correlated with the N concentration in leaves, stems and roots, respectively (Table 3; Fig. 4). Regarding the N : P ratios in different plant parts, mycorrhization rates were only significantly correlated with leaf and stem N : P (Table 3; Fig. 5).

Experiment 2

Root colonization by AMF Mean mycorrhization rates of *A. vulgaris* from control plots and N-fertilized plots (N-plots) and results from paired samples t-test are shown in Table 4. Colonized root lengths by internal mycelium, arbuscules and vesicles were significantly lower at N-plots than at control plots (Fig. 6).

Above-ground biomass of *Artemisia vulgaris* *Artemisia vulgaris* biomass per unit area at N-fertilized subplots was several times higher compared with control subplots (Table 4), with the difference bordering on significance (P = 0.06).

Discussion

Experiment 1

In the literature on arbuscular mycorrhiza, available soil P levels are considered high when they exceed 200 mg kg^{-1} soil (Sylvia & Schenck, 1983). Published examples of total P concentrations clearly inhibiting root colonization by arbuscular mycorrhizal fungi range from 228 mg kg^{-1} soil (Graham *et al.*, 1981) to 600 mg kg^{-1} soil (Menge *et al.*, 1978). In our study, available soil P contents were orders of magnitudes higher, ranging from 6332 mg kg^{-1} at plot 2 to 12 249 mg kg^{-1} at plot 1 (Table 1).

Phosphorus concentrations in roots (0.47, 0.49 and 0.77% at plots 1–3, respectively), stems (0.27, 0.32 and 0.27%) and leaves (0.46, 0.38 and 0.32%) were also high enough to suggest that no, or only slight, colonization by AMF should have occurred. In other studies, root and shoot P concentrations

Table 2 Plant element concentrations and percentage root colonization of *Artemisia vulgaris* by arbuscular mycorrhizal fungi for the three field plots of experiment 1

Parameter	Plot 1		Plot 2		Plot 3		
	Mean	SD	Mean	SD	Mean	SD	*F* or †Welch statistic
N leaves (%)	1.76[a]	±0.28	2.03[a]	±0.34	3.23[b]	±0.17	62.619***
N stems	0.46[a]	±0.06	0.49[a]	±0.10	0.83[b]	±0.22	†9.423**
N roots	0.61[a]	±0.11	0.65[a]	±0.09	1.31[b]	±0.14	86.023***
P leaves (%)	0.46[a]	±0.22	0.38[a]	±0.06	0.32[a]	±0.06	†2.727
P stems	0.27[a]	±0.11	0.32[a]	±0.08	0.27[a]	±0.09	0.864
P roots	0.47[a]	±0.04	0.49[a]	±0.08	0.77[a]	±0.35	†3.044
Na leaves (ppm)	494.2[a]	±450.3	177.4[a]	±110.6	85.0[a]	±29.3	†3.666
Na stems	2284.7[a]	±2453.7	918.2[a]	±787.0	706.8[a]	±448.1	†1.356
Na roots	5375.3[a]	±2013.6	3156.5[a]	±1670.8	5641.9[a]	±2624.1	3.116
K leaves (%)	3.78[a]	±0.61	3.90[a]	±0.36	5.08[b]	±0.53	15.743***
K stems	2.48[a]	±0.52	2.87[a]	±0.77	3.63[b]	±0.87	4.703*
K roots	1.75[a]	±0.76	2.74[a]	±0.36	4.06[b]	±0.96	17.457***
Mg leaves (ppm)	1652.3[a]	±303.3	1557.4[a]	±198.6	2337.4[b]	±619.3	†5.452*
Mg stems	1050.0[a]	±338.8	885.8[a]	±257.0	815.6[a]	±159.1	1.605
Mg roots	1032.3[a]	±286.7	1086.3[a]	±132.9	1913.5[b]	±580.9	12.105***
Ca leaves (%)	1.93[a]	±0.57	1.68[a]	±0.21	1.41[a]	±0.33	3.401
Ca stems	1.35[a]	±0.87	1.29[a]	±0.31	0.68[a]	±0.39	3.313
Ca roots	1.54[a]	±0.18	1.71[a]	±0.23	1.49[a]	±0.70	0.475
Cd leaves (ppm)	<1.5		<1.5		<1.5		
Cd stems	<1.5		<1.5		<1.5		
Cd roots	<1.5		<1.5		<1.5		
N : P leaves	4.29[a]	±1.64	5.46[a]	±1.29	10.40[b]	±1.67	33.202***
N : P stems	2.04[a]	±1.12	1.58[a]	±0.42	3.33[b]	±1.23	6.730**
N : P roots	1.45[a]	±0.41	1.34[a]	±0.26	1.99[a]	±0.82	2.929
I-mycelium (%)	80[b]	±10.2	85[b]	±13.6	17[a]	±16.4	60.926***
Arbuscules	61[b]	±16.3	67[b]	±20.5	11[a]	±9.5	29.595***
Vesicles	43[b]	±16.1	55[b]	±18.0	1[a]	±1.5	†57.807***

Means (n = 8), standard deviations (SD) and F values or Welch statistic (†) from one-way ANOVA. Element concentrations are given for leaves, stems and roots; mycorrhization rates (fraction of root length colonized by AMF) were determined separately for internal mycelium, arbuscules and vesicles. Asterisks indicate significance levels: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; superscript letters show significant differences among plots.

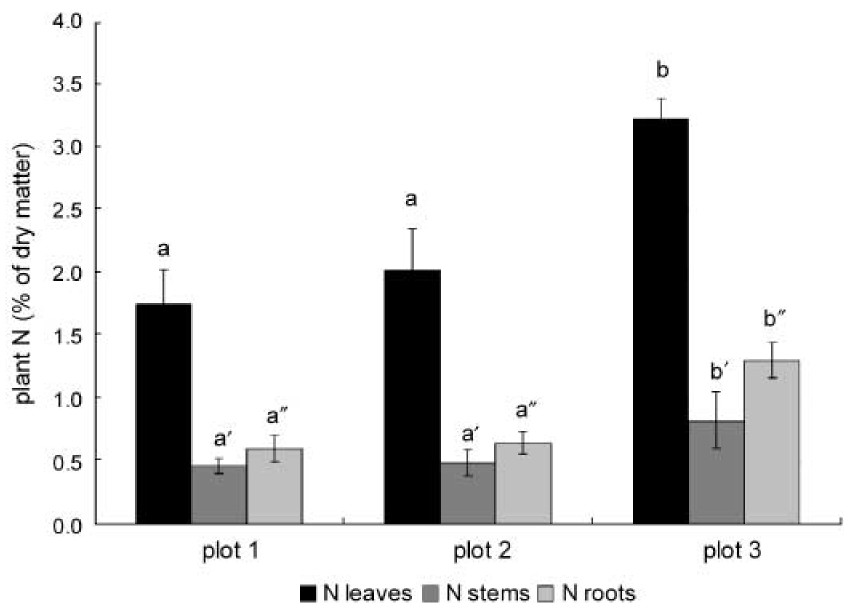

Fig. 1 Nitrogen concentration in leaves, stems and roots of *Artemisia vulgaris* at plots 1–3. Means and SD are shown (n = 8). Letters indicate significant differences between bars of the same shade.

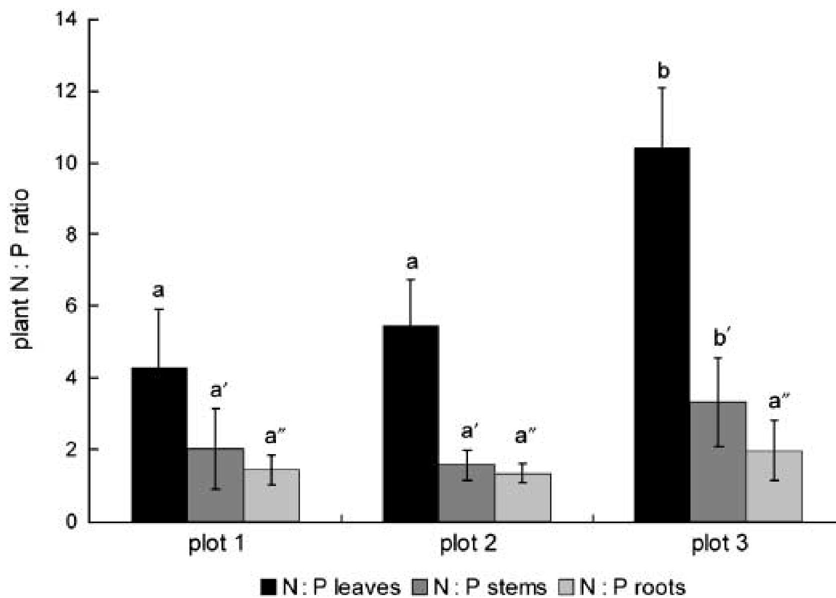

Fig. 2 N : P ratio in leaves, stems and roots of *Artemisia vulgaris* at plots 1–3. Means and SD are shown (n = 8). Letters indicate significant differences between bars of the same shade.

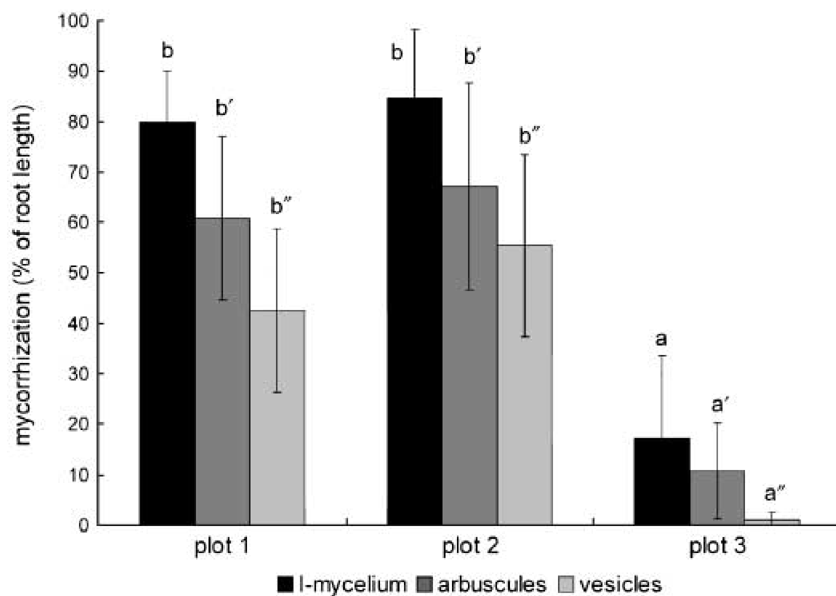

Fig. 3 Fraction of root length colonized by arbuscular mycorrhizal fungi for *Artemisia vulgaris* at the three plots of experiment 1. Mycorrhization rates are separated for internal mycelium (I-mycelium), arbuscules and vesicles. Means and SD are shown (n = 8). Letters indicate significant differences between bars of the same shade.

of ≈0.3% were sufficient to suppress more than sporadic mycorrhization (Graham *et al.*, 1981; De Miranda *et al.*, 1989).

Thus, if P had been the only determining factor for mycorrhization, plants at our field site should have been, at most, slightly mycorrhizal. Nevertheless, *A. vulgaris* was found to have high mean mycorrhization rates of 80 and 85% (internal mycelium), 61 and 67% (arbuscules), and 43 and 55% (vesicles) at plots 1 and 2, respectively. At plot 3 colonization was significantly lower: mean rates were 17% (internal mycelium), 11% (arbuscules), and 1% (vesicles).

The most prominent difference between soils of the three plots was the N level. Whereas plots 1 and 2 had low total N concentrations (0.16 and 0.18%, respectively), N was significantly higher at plot 3 (0.34%), at a greater distance from the former factory. Reports about N-fertilization experiments to define soil N thresholds inhibiting mycorrhizal colonization are scarce, as N has rarely been regarded as an important factor determining root colonization by AMF.

The trend in plant N concentration measured at the three plots paralleled that in soil N. Plants at plots 1 and 2 were characterized by a significantly lower N concentration in leaves (1.76 and 2.03%, respectively), stems (0.46 and 0.49%), and roots (0.61 and 0.65%), compared with plants at plot 3 (leaf N 3.23%, stem N 0.83%, root N 1.31%). Crops are known to be N-limited if their shoot N concentration is

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Table 3 Results of Spearman's rank correlation: correlation coefficients (r_s) and sample size (n) for internal mycelium (I-mycelium), arbuscules and vesicles vs N concentration and N : P ratio of leaves ($_L$), stems ($_S$) and roots ($_R$), respectively, of *Artemisia vulgaris*

		N_L	N_S	N_R	$N : P_L$	$N : P_S$	$N : P_R$
I-mycelium	r_s	−0.683*	−0.595*	−0.676*	−0.766*	−0.609*	−0.287
	n	22	23	22	22	23	22
Arbuscules	r_s	−0.687*	−0.604*	−0.658*	−0.789*	−0.608*	−0.257
	n	22	23	22	22	23	22
Vesicles	r_s	−0.686*	−0.663*	−0.717*	−0.713*	−0.632*	−0.299
	n	22	23	22	22	23	22

*, Significant correlation ($P < 0.05$; two-tailed test adjusted by the sequential Bonferroni method).

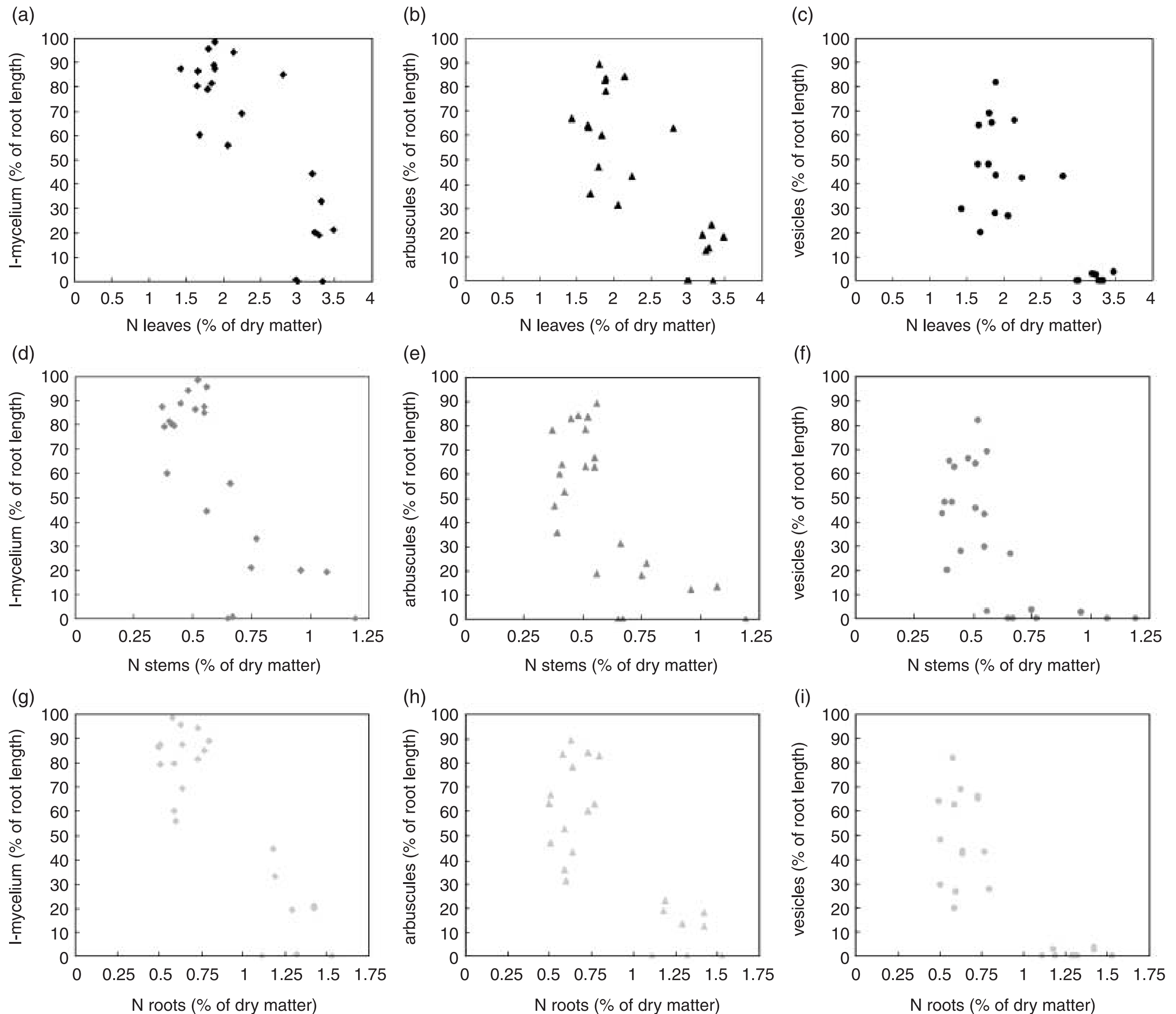

Fig. 4 Fraction of root length colonized by internal mycelium (I-mycelium), arbuscules and vesicles, plotted against N concentration in leaves, stems and roots of *Artemisia vulgaris*. Data for all three plots were pooled.

below 1.4% (Verhoeven *et al.*, 1996), which applies to plots 1 and 2 when considering mean values of leaves and stems. Azcón *et al.* (1982) found a reduction of mycorrhizal colonization at a shoot N >1.9%, which would apply to plot 3. Other clear data on tissue N concentrations at which a distinct reduction of mycorrhization occurs are not available in the literature, but there are a number of studies concerning mycorrhizal colonization dealing with the proportion of P and N.

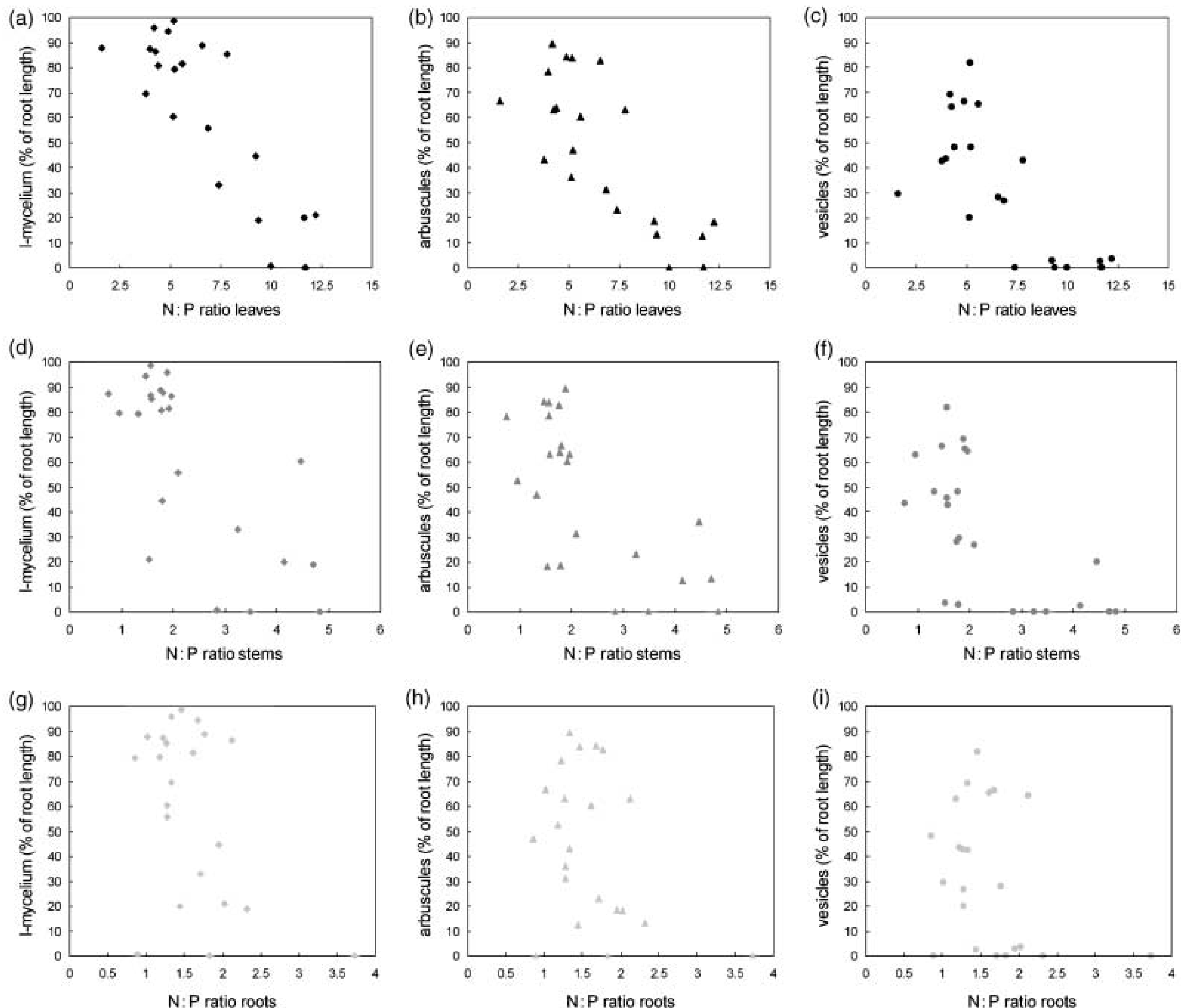

Fig. 5 Fraction of root length colonized by internal mycelium (I-mycelium), arbuscules and vesicles, plotted against N : P ratio in leaves, stems and roots of *Artemisia vulgaris*. Data for all three plots were pooled.

Mosse & Phillips (1971) found that, at high P concentrations, roots were colonized only when the medium lacked N. Nevertheless, they did not consider that AMF may be needed for N nutrition. A later connection between P and N availability was established by Sylvia & Neal (1990), who showed that under N-limiting conditions, P addition had no effect on mycorrhizal colonization whereas, when a sufficient amount of N was supplied, colonization was suppressed by the addition of P. Also Bååth & Spokes (1989) found that only a combination of high P and N concentrations led to reduced mycorrhization.

Whether a plant profits from being mycorrhizal is a question of carbon cost vs benefit through improvement of nutrient uptake by AMF (Corkidiki *et al.*, 2002). If plants are nutrient-limited, they profit from allocating carbohydrates to the fungi because, in turn, the mycobionts provide them with soil nutrients. If all required elements are available without mycorrhiza, the carbon cost exceeds the benefit and plants stop allocating C to the symbionts, which become C-limited (Treseder & Allen, 2002).

Sylvia & Neal (1990) found no relationship between tissue N : P ratios and root colonization rates, but attributed mycorrhization at high P levels to N deficiency in general. In contrast, at least at intermediate N levels, Liu *et al.* (2000) obtained a negative correlation between shoot N : P and percentage colonization by AMF.

Like the soil and plant N concentrations, plant N : P ratios found in our study were significantly (marginally in the case of root N : P) lower in plants growing at plots 1 and 2, which had respective ratios of 4.29 and 5.46 in leaves, 2.04 and 1.58 in stems, and 1.45 and 1.34 in roots, compared with plants at plot 3 (10.4 in leaves, 3.33 in stems, 1.99 in roots).

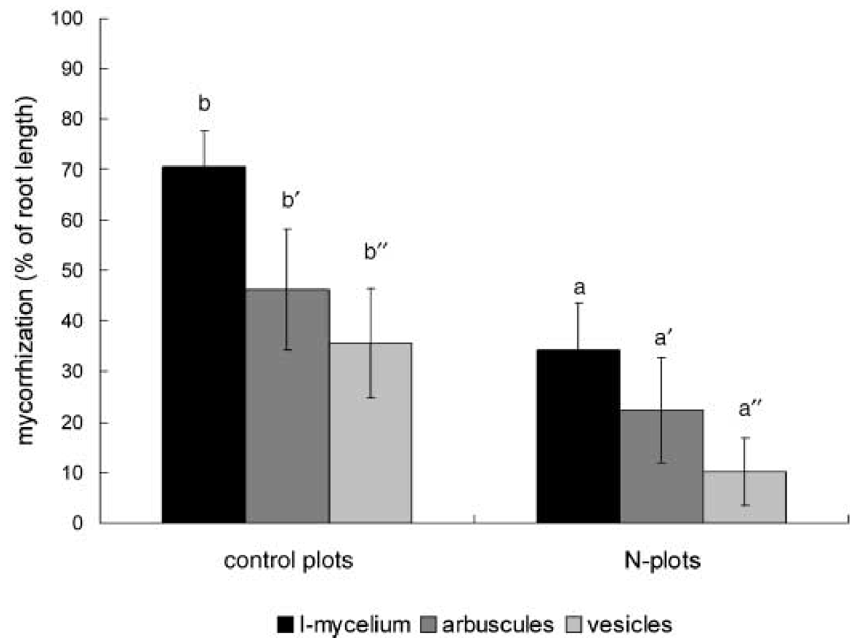

Fig. 6 Fraction of root length colonized by arbuscular mycorrhizal fungi for *Artemisia vulgaris* at control plots and nitrogen-fertilized plots (N-plots) of experiment 2. Mycorrhization rates are separated for internal mycelium (I-mycelium), arbuscules and vesicles. Means and SD are shown ($n = 6$). Letters indicate significant differences between bars of the same shade.

Table 4 Percentage root colonization of *Artemisia vulgaris* by arbuscular mycorrhizal fungi (AMF) and above-ground biomass dry weight of *A. vulgaris* for control plots and nitrogen-fertilized plots (N-plots) of experiment 2

	Control plots		N-plots		
	Mean	SD	Mean	SD	*t*
I-mycelium (%)	71	±7.1	34	±9.3	9.412***
Arbuscules	46	±11.9	22	±10.4	3.841*
Vesicles	36	±10.8	10	±6.7	5.244**
Biomass (g m^{-2})	3.5	±6.7	19.3	±19.7	−1.891

Means ($n = 6$), standard deviations (SD) and *t* values from paired samples *t*-test. Mycorrhization rates (fraction of root length colonized by AMF) were determined separately for internal mycelium, arbuscules and vesicles. Asterisks indicate significance levels: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Koerselman & Meuleman (1996) assessed the suitability of vegetation N : P ratio as an indicator for nutrient limitation, and found that a shoot N : P ratio <14 at a community level indicates N limitation. Unfortunately their study, like most others dealing with this subject, was restricted to wetland ecosystems. However, a recent literature survey by Tessier & Raynal (2003) revealed thresholds for N limitation from different ecosystems, ranging from N : P <6.7 to 16, and the authors suggested that these ratios may as well be used to indicate nutrient limitation in individual species. Aerts & Chapin (2000) assumed that a leaf N : P ratio of ≈10 indicates optimal conditions for plant growth, and Güsewell (2004) concluded that a vegetation N : P ratio <10 indicates N limitation. In reference to all these values, *A. vulgaris* appears to be N-limited at plots 1 and 2 of our field site; accordingly, the larger N : P ratio of plants at plot 3 indicates a minor, or no, N limitation.

With the exception of root N : P, both N and N : P in different plant parts were significantly negatively correlated with mycorrhization rates. Spearman's rank correlation coefficients (r_s) for leaves and stems did not differ much between N and N : P, whereas in the case of roots r_s was distinctly higher for the correlation of percentage colonization with N than with N : P. Given the high P availability at our field site, leaf and stem N concentration and N : P ratio both were good indicators of the mycorrhizal status of a plant, whereas in roots N concentration was a better indicator than N : P ratio. These results suggest that N deficiency, as well as N : P imbalances in plants, have similar effects to P deficiency in stimulating root colonization by AMF.

In some cases the relationship between the parameters appeared to be more-or-less linear, for example for internal mycelium vs N concentration of leaves (Fig. 4a); internal mycelium vs N concentration of roots (Fig. 4g); and internal mycelium vs N : P ratios of leaves (Fig. 5a). In other cases, an N or N : P threshold appeared to limit the development of mycorrhizal structures. This is most evident when looking at arbuscules and particularly vesicles, for example in the event of arbuscules vs stem N (Fig. 4e); vesicles vs stem N (Fig. 4f); vesicles vs root N (Fig. 4i); arbuscules vs stem N : P (Fig. 5e); and vesicles vs stem N : P (Fig. 5f), and may well indicate specific reactions of these different fungal structures to changed N levels or N : P ratios in host plants. According to this hypothesis, internal mycelium would directly react proportionally to changes in plant N or N : P level, whereas the

production of arbuscules, and especially of vesicles, would depend rather on the crossing of threshold levels.

To our knowledge there are no previous studies specifically relating the development of different AMF structures to changes in nutrient availability, with the exception of internal vs external mycelium. For example, Abbott *et al.* (1984) and Thomson *et al.* (1986) found that P fertilization first reduced structures inside the root, and Johnson *et al.* (2003) concluded that external structures are more responsive to changes in N availability. In our study we did not determine the length of external mycelium because, under field conditions, it is almost impossible to distinguish AMF hyphae from those of other soil fungi.

Soil and plant element concentrations other than P and N were never related to both each other and mycorrhization rate at the same time, and for this reason were not further considered.

Experiment 2

The results of the N-fertilization experiment confirm the findings of the previous experiment. Because of the proximity to plot 1 of experiment 1, plants at the control plots were exposed to very similar nutrient conditions, i.e. they were well provided with P but presumably deficient in N.

Above-ground biomass data of *A. vulgaris* support this assumption. Although the results just bordered on significance, plant dry weight per square metre was several times higher at N-fertilized than at control plots (Table 4).

Like the plants at plots 1 and 2 of experiment 1, *A. vulgaris* at control plots had high mean mycorrhization rates (71% internal mycelium, 46% arbuscules, 36% vesicles), which probably are a consequence of N limitation. In accordance with plot 3 of experiment 1, plants at N-fertilized plots had significantly lower mycorrhization rates (34% internal mycelium, 22% arbuscules, 10% vesicles). These values are higher than those found at plot 3, which may be caused by the N limitation only being partially relieved at the applied N-fertilization levels. Nevertheless, the N-fertilization experiment clearly shows that increasing the N provision of *A. vulgaris* decreases mycorrhization rates.

Conclusions

The present study suggests that the mycorrhizal status of *A. vulgaris* in the P-rich field site we examined depends on the N level.

Assuming that arbuscular mycorrhiza is able to improve the N uptake of host plants, we can hypothesize that, at plots 1 and 2 of experiment 1 and at the control plots of experiment 2, plants well supplied with P but deficient in N presumably allocate more photosynthates to the mycobionts because they benefit from improved N uptake by the mycorrhiza. This, in turn, results in high colonization rates. In contrast, at plot 3 and at the N-fertilized plots, plants have a better supply of N. Therefore they would benefit less from the symbiosis and thus allocate less C to the fungi. The overall result is a low level of colonization by AMF.

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