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Legumes affect alpine tundra community composition via multiple biotic interactions

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Abstract. The soil engineering function of legumes in natural ecosystems is paramount but associated solely with soil nitrogen (N) subsidies, ignoring concomitant biotic interactions such as competitive or inhibitory effects and exchange between mycorrhizas and rhizobia. We aim to (1) disentangle legume effects on plant community composition, and plant and soil N and phosphorus (P) concentrations, separating the effects of N subsidies from other legume effects; (2) estimate effects of mycorrhizal-rhizobial interactions on nutrient acquisition modes of plants co-existing with legumes.

We compared plant community structure and plant nutrition modes in micro-sites in a Caucasian alpine tundra ecosystem that were either: (1) dominated by legumes in symbiosis with N-fixing rhizobia ('N-fixing legumes'), (2) dominated by legumes without symbiosis with rhizobia ('not N-fixing legumes'), or dominated by non-legumes and either (3) unfertilized ('controls') or (4) experimentally fertilized.

Fertilization and the presence of N-fixing legumes affected the ecosystem similarly: soil was enriched with plant-available N compared to controls and sites dominated by a not N-fixing legume. Also, N turnover pathways and plant nutrition modes were strongly affected by the latter site types, as indicated by 5–10% higher plant tissue N concentration, altered soil and plant $\delta^{15}N$, more than 4-fold reduced lichen amounts, 2.5-fold increased litter accumulation and doubling of aboveground biomass of non-legume plants.

Vascular plant community composition was affected by the presence of legumes in a similar way regardless of whether they fixed N, suggesting that other factors overrode the N subsidy effects. Shading and microclimate changes in sites dominated by both types of legumes are possible explanatory factors. Both tissue N and $\delta^{15}N$ of non-legume plants near legumes were affected by interactions of mycorrhizal type and site type (without legumes, dominated by N-fixing, or not N-fixing legume), suggesting an important role of plant mycorrhizal status for adjusting nutrition mode to the legume presence.

We conclude that N-fixing legumes play an engineering role in natural plant communities, but their role goes much beyond N fixation. Plant mycorrhizal status defines the way plants adjust their nutrition mode to the presence of legumes.

Key words: biomass; fertilization; *Leguminosae*; lichens; litter; mycorrhiza; nitrogen availability; nitrogen fixation; plant nutrition; plant-soil interactions; phytomass.

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Introduction

In nutrient-poor environments, plants hosting symbiotic nitrogen (N) fixing rhizobial bacteria are often considered keystone species (Munzbergova and Ward 2002, Anderson et al. 2004) based on their ability to enrich soil with N. Especially in the early successional stages, symbiotic N fixation can be a primary source of soil N (Vitousek and Howarth 1991, Chapin et al. 1994). However, in later-successional plant communities where N may be recycled within plants or bound up in recalcitrant organic compounds, symbiotic N fixation could also contribute substantially to N availability in the soil (Cleveland et al. 1999). Several vascular plant families possess the ability to fix N, particularly legumes (*Leguminosae* s.l., including *Fabaceae*, *Mimosaceae* and *Caesalpiniaceae*). Although this role as natural N fertilizers is widely recognised, especially in agricultural systems (Ledgard and Steele 1992, Zahran 1999, Carlsson and Huss-Danell 2003), the effects of the presence of native legumes on plant community structure and composition have rarely been directly compared to N fertilization effects in a natural ecosystem.

The role of legume species in an ecosystem goes beyond N subsidies to soil. Legumes can reduce soil phosphorus (P) availability (Thomas and Bowman 1998) due to inherent high P demand (Vitousek et al. 2002, Power et al. 2010), suppress growth of co-occurring species via allelopathic effects (Newman and Rovira 1975, Carlsen and Fomsgaard 2008, Power et al. 2010), and inhibit germination (Walker and Vitousek 1991) and seedling establishment (Morris and Wood 1989). Moreover, cushion-forming herbaceous legumes can reduce light and water availability to co-occurring plants (Jacot et al. 2005). However, the effects of each mechanism of legume control on plant community composition remain unclear because it is difficult to disentangle facilitation due to extra N input from inhibitory effects that cushion-forming legumes can impose.

Mycorrhizal symbiosis also plays an important role in plant nutrition (i.e., nutrient availability to plants) in nutrient-poor environments (Michelsen et al. 1996, Aerts 1997). In arctic tundra, 61–86% of N can be supplied to plants via various forms of mycorrhiza, of which the most prominent was the ericoid (Hobbie and Hobbie 2006). Arbuscular mycorrhizal fungi (AMF) are capable of N transfer from diverse sources to plants (Hawkins et al. 2000, Azcon et al. 2001, Govindarajulu et al. 2005, Leigh et al. 2009) as well as between plants (Morris and Wood 1989, Johansen and Jensen 1996, He et al. 2003, Moyer-Henry et al. 2006). Moreover, substantial amounts of N may be transferred from N-fixing plants to not N-fixing plants via AMF (Haystead et al. 1988, Bethlenfalvay et al. 1991, Jalonen et al. 2009). Nevertheless, the role of interactions between mycorrhizal and rhizobial N availability in shaping plant community composition and structure of co-occurring species is poorly understood.

Alpine tundra ecosystems are cold, dry, and N-poor environments where positive interactions and competition between plants play an essential role in community structure (Callaway et al. 2002). Symbiotic N fixation by herbaceous legumes can be an essential source of N (Thomas and Bowman 1998, Jacot et al. 2000). Estimates of legume-associated N fixation in alpine tundra and grasslands range from 100 to 500 mg N m^{-2} yr^{-1}, comparable to the rate of net N mineralization in alpine communities (Bowman et al. 1996, Jacot et al. 2000). N-fixing legumes have a strong influence on alpine tundra plant community structure and composition through positive and negative effects on co-occurring species (Thomas and Bowman 1998, Gigon 1999, Jacot et al. 2005).

Here we aim to: (1) unravel how the presence of legumes affects the plant species composition, and (2) distinguish between effects of extra N input from other possible biotic effects. We address our objectives by analyzing plant community composition as well as soil and plant nutrition in areas inhabited by two taxonomically close legume species: *Oxytropis kubanensis* that has a symbiotic relationship with N-fixing rhizobia bacteria, and *Trifolium polyphyllum* that does not have this relationship (Onipchenko

2004). Hereafter we refer to these species as "N-fixing" and "not N-fixing". Both species are found in the same plant community of alpine lichen tundra on soils of similar depth (Batchaeva et al. 2003). Both species create patches of similar density and size (30–100 cm diameter) with similar maximum rooting depth (to 50–80 cm) (Onipchenko 1987, Batchaeva et al. 2003). The level of arbuscular mycorrhizal infection is similar: 40% infection rate for *O. kubanensis* and 52% for *T. polyphyllum* (Onipchenko 2004). Thus, differences in effects of these species on their host plant communities are probably to a considerable extent due to differences in their ability to fix N. This analysis in combination with ^{15}N isotope tracking approach and comparison with experimental N fertilization effects on the same community provides a way to reveal the potential mechanisms of legume effects on plant community composition, structure, and chemistry.

In this study, we compared plant communities developed on patches: (1) dominated by the N-fixing legume *O. kubanensis* or (2) dominated by the not N-fixing legume *T. polyphyllum* with (3) N-fertilized plots and (4) "control" plots without legumes, not fertilized. We compared communities for both non-legume phytomass structure and non-legume vascular plant community structure. We hypothesized that: (1) soil N and P availability are related to the presence of a N-fixing legume; (2) patches dominated by N-fixing and not N-fixing legumes differ from each other in both non-legume aboveground phytomass structure and non-legume vascular plant community structure, while patches dominated by a N-fixing legume resemble communities fertilized with N, in terms of aboveground non-legume phytomass and biomass structures. Further, we hypothesized that: (3) the aboveground biomass N tissue concentrations and 15N signatures of non-legume plant species are related to the presence of neighbouring legumes, and this relationship is affected by the mycorrhizal status of the species.

Materials and Methods

Site description

The investigation was conducted in the Teberda Natural Reserve (43°27′ N, 41°42′ E, NW Caucasus, Russia) in the alpine zone of Mt. Malaya Khatipara (2800 m a.s.l.) on a southern slope. Soils are Umbric Leptosols (FAO Classification) with many stones, mostly biotite schists. Mean annual temperature is −1.2°C, mean July temperature is 7.9°C (Grishina et al. 1986). Annual precipitation (1400 mm) mostly falls as snow that is blown away by strong winds. The growing season lasts from about mid May to September.

The study was conducted in a lichen-dominated tundra where >50% of the soil surface area is covered by lichens. The vascular plant community does not have a clear dominant species. The most abundant species are graminoids (*Festuca ovina* L., *Carex sempervirens* Vill., *Carex umbrosa* Host), forbs (*Anemone speciosa* Adams ex G.Pritz., *Antennaria dioica* [L.] Gaertn., *Campanula tridentata* Schreb., *T. polyphyllum*, *O. kubanensis* Leskov), and the dwarf shrub *Vaccinium vitis-idaea* L. (nomenclature after Vorob'eva and Onipchenko 2001). For a more detailed description of the plant community see (Onipchenko 2004).

Previous investigation showed that *T. polyphyllum* does not possess root nodules and therefore does not fix N (Onipchenko et al. 2001), while *O. kubanensis* has nodules (Onipchenko 2004). We verified this difference in N-fixation for the study area in two ways. First, we collected live roots (30–70 mg dry weight) of *T. polyphyllum* (Fig. 1a) and *O. kubanensis* (Fig. 1b) and tested them for N fixation with the acetylene reduction method based on the ability of the nitrogenase enzyme, responsible for N_2-fixation, to reduce C_2H_2 to C_2H_4 (Dilworth 1966). We used 5 replications for each species and 5 vials with C_2H_2 without roots as controls. Roots were removed from soil particles in the field by washing with water, and then placed into 15 ml glass vials that were hermetically sealed. We added 1 ml of C_2H_2 to each vial and then incubated them for 24 hours at field temperature (9–14°C). A 10 ml gas sample was analyzed by measuring the concentration of C_2H_4 on a Chrom-5 gas chromatograph with a flame ionization detector. The yield of C_2H_4 by *O. kubanensis* roots was 28 times that of *T. polyphyllum,* the latter similar to control measurements.

Second, we analyzed *T. polyphyllum* and *O. kubanensis* above-ground plant material (above-

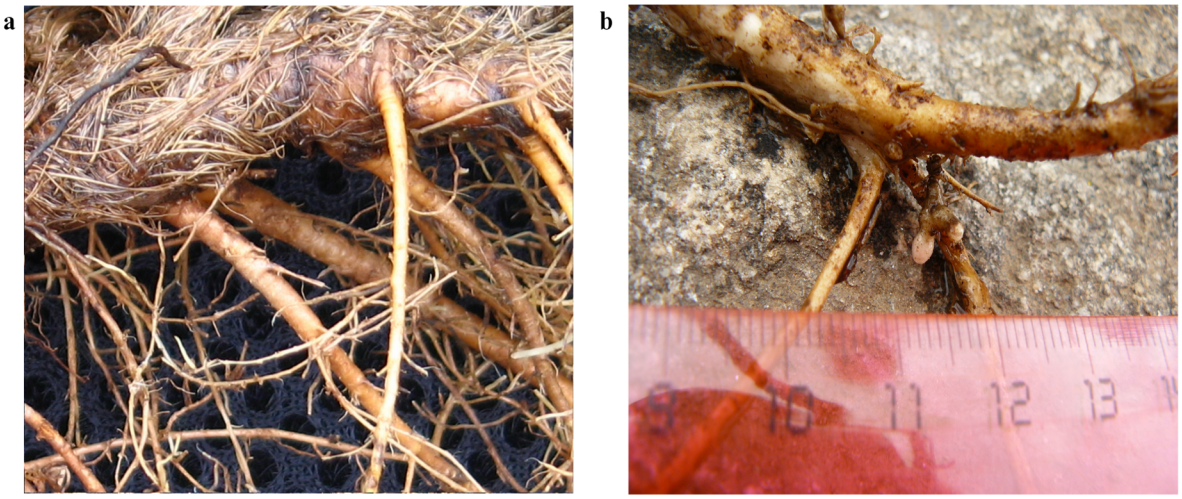

Fig. 1. Roots of the alpine Fabaceae plants (a) *Trifolium polyphyllum* C.A.Mey, which does not possess symbiotic N-fixing nodules, and (b) *Oxytropis kubanensis* Leskov, which fixes N (see nodules in the middle of the figure). Photo by the authors.

ground biomass sampling below) for N isotopic composition ($\delta^{15}N$). Mean $\delta^{15}N$ of *T. polyphyllum* was −3.6 (range of −2.5 to −4.0) is typical for not N-fixing plants of this community with similar rooting depth (Makarov and Onipchenko, *unpublished data*), while mean $\delta^{15}N$ of *O. kubanensis* was −0.9 (range −1.5 to −0.3).

Sampling and use of data obtained in other studies

We selected 20 patches dominated by *T. polyphyllum* (hereafter referred as Legume N−), 20 patches dominated by *O. kubanensis* (hereafter referred as Legume N+), and 20 Control patches where both species were absent. All legume-dominated patches had >30% of total aboveground biomass attributed to the legume. All patches were similar in size (>60 cm diameter) and located within a 400 × 400 m area at the same elevation, slope (approx. 2–3°), and snow regime. Distances between patches were at least 3 m. To minimize downhill effects between patches, we avoided patches located directly below each other, and randomized the location of patch types with respect to elevation. In each patch, we sampled all aboveground phytomass in 40 cm-diameter circular located in the center. The phytomass was sorted into lichens, litter, and vascular plants by species, air-dried and later oven-dried (70°C, 48 hours), weighed, and stored for chemical analysis. For each plot we calculated non-legume aboveground phytomass structure based on its relative contribution to total non-legume aboveground phytomass, and non-legume vascular plant community structure based on its relative contribution to the total aboveground biomass of non-legume vascular plants. All sampling was done in the middle of growing season at peak aboveground biomass (end July).

From 10 plots of each patch type, a soil sample (15 cm diameter to 15 cm depth; A horizon) was collected for chemical analysis and stoniness analysis. For analysis of total soil and plant N and ^{15}N, samples were air-dried and later oven-dried (70°C, 48 hours), sieved to <2 mm and ground to powder. Nitrogen concentration and ^{15}N abundance were determined by dry combustion on a NC 2500 elemental analyser (Carlo Erba, Rondana, Italy) coupled with a Deltaplus continuous-flow isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany). We calculated $\delta^{15}N‰$ as: [(atom% ^{15}Nsample − atom% ^{15}Nstandard)/atom% ^{15}Nstandard] × 1000. Total soil P content was analyzed by acid digestion followed by the ammonium molybdate method (Murphy and Riley 1962). Standard reference material with known N and P concentrations was used. To quantify soil nutrient availability, N-NH_4 and N-NO_3 were extracted from the sieved samples (<2 mm) with 1.0 M KCl at a soil to

solution ratio of 1:5 and determined by colorimetric reactions on a continuous-flow autoanalyser (Skalar SANplus). For stoniness analysis, soil samples were air-dried and later oven-dried (100°C, 48 hours), weighed and sieved to <2 mm. Stones left on the sieve were washed, oven dried, and the stone/soil mass ratio was calculated.

In 1999–2003, a five-year N fertilization experiment was conducted in the same plant community, but located 300m away on a similar slope and elevation (details in Soudzilovskaia and Onipchenko 2005, Soudzilovskaia et al. 2005). In the current analysis, only data from 10 plots where legumes were absent or had <1% of total aboveground biomass were used. To ensure that the fertilization experiment and our current sampling were conducted in a similar plant community, and the plant community was not affected by yearly fluctuations in some species, we compared the aboveground phytomass structure and vascular plant community structure in Control plots of the fertilization experiment to those of the current investigation by canonical correspondence analysis, CCA. In both analyses, we log-transformed the species data and used plot type as environmental data. The legume aboveground biomass was omitted from the Legume N+ and Legume N– plots in order to detect differences among the non-legume aboveground biomass. We found no significant differences between communities.

Rate and type of mycorrhizal infection of vascular plants growing in our study site were analysed microscopically using thin roots (<2 mm in diameter) from five well-developed plants of each species, and reported previously (Onipchenko 2004). Here we use species-level data of type of mycorrhizal infection (i.e., ericoid mycorrhiza, AMF mycorrhiza, non-mycorrhizal). To minimize bias associated with variability in infection rate with nutrient availability and soil nutrient stoichiometry, we included only species with >20% mycorrhizal infection, which we considered unlikely to lose their infection completely under different environmental conditions.

Statistical analysis

Soil nutrients as affected by legumes.—Data on soil total N, ratio of N to P, $\delta^{15}N$, N-NH_4, N-NO_3, stoniness, and N concentration in litter were analysed by one-way ANOVA with plot type (Legume N+, Legume N–, Control) as the independent factor and a soil parameter as a response variable. Significance of differences between particular types of plots was detected with Tukey test. Due to heterogeneity of variance, the ANOVA on soil P was not possible, and Kruskal-Wallis followed by Mann-Whitney test was conducted instead.

Plant community structure as affected by presence of legumes and by fertilization.—We compared legume aboveground biomasses on Legume N+ versus Legume N– plots with one-way ANOVA on log-transformed data to meet assumptions of normality.

We used canonical correspondence analyses (CCA) to compare non-legume aboveground phytomass structures and non-legume vascular plant community structures among Legume N+, Legume N–, Control, and Fertilized plots. In both analyses we log-transformed the species data, using the plot type as environmental data. The legume aboveground biomass was omitted from the Legume N+ and Legume N– plots, aiming to detect differences among the non-legume aboveground phytomass/biomass elements in the communities.

The discriminatory power of the analysis was tested by the non-parametric Monte-Carlo permutation test with 9999 permutations. When overall significant differences among plot types were detected, we ran the following five pairwise comparisons: Legume N+ vs. Fertilization, Legume N+ vs. Control, Legume N+ vs. Legume N–, Legume N– vs. Fertilization, Legume N– vs. Control. To compensate for a higher type 1 error probability due to multiple comparisons, we applied Bonferroni correction (Quinn and Keough 2002) and reduced the level of acceptable statistical significance five times: $\alpha = 0.05/5 = 0.01$. The same analysis was used for comparisons between aboveground phytomass structure and vascular plant community structure in non-fertilized (i.e., Control) plots of the fertilization experiment and the Control plots of the current investigation.

Combined effects of legume presence in the plant community and type of mycorrhizal infection possessed by plants.—To assess effects of legume presence and type of mycorrhizal infection on aboveground biomass of non-legume plants, we (1) tested the overall difference between Legume

N+, Legume N– and Control plots with CCA on log-transformed total per-plot aboveground biomasses of non-legume plants for non-mycorrhizal species, species with AMF mycorrhiza, and species with ericoid mycorrhiza, using plot type as environmental data; (2) calculated fractions of total aboveground biomass of non-legume plants for non-mycorrhizal species, species with AMF mycorrhiza, and with ericoid mycorrhiza, and ran one-way ANOVAs, followed by Tukey post hoc tests for each mycorrhizal type separately with plot type as independent factor and the fraction values as a dependent variable.

We tested effects of legume presence and type of mycorrhizal infection on plant N and $\delta^{15}N$ of non-legume species with two-way nested ANOVAs with plot type (Legume N+, Legume N–, Control) and mycorrhizal type as independent factors, and species nested within mycorrhizal type. We used sum of squares type 1 for this analysis. Although the Levene's test detected variance heterogeneity in $\delta^{15}N$ data, residuals plot did not show any specific pattern of variance with mean values. As absence of such a pattern is the most crucial assumption of ANOVA in respect to variance distribution, and because the transformation of negative values of $\delta^{15}N$ would complicate the interpretation of the results, we conducted the ANOVA on non-transformed $\delta^{15}N$ data.

All analyses were performed by SPSS Statistics v. 17, except for the CCA, which was conducted by Canoco v 4.5. Significance level $P < 0.05$ was used in all analyses.

Results

Soil nutrients as affected by legumes

Soil N content showed a trend among plot types ($F_{2,28} = 3.3$; $P = 0.052$; Fig. 2a), being highest in the Legume N+ and lowest in Legume N– plots (10.2 ± 0.6 mg g^{-1} and 8.2 ± 0.5 mg g^{-1}, respectively). Soil P was not significantly different in treated compared to Control plots ($\chi_{2,28}{}^2 = 4.0$; $P = 0.12$, data not shown). Soil N/P was higher in Legume N+ plots whereas values for Control and Legume N– were similar ($F_{2,28} = 5.2$; $P = 0.012$; Fig. 2b). Soil $\delta^{15}N$ was lowest in Legume N+ plots, highest in the Control, and intermediate in Legume N– plots ($F_{2,28} = 4.5$; $P = 0.021$; Fig. 2c).

Extractable soil N-NH_4 and N-NO_3 in Legume N+ plots were 20% and 30% higher, respectively, than in Control and Legume N– plots ($F_{2,28} = 11.0$, $P < 0.001$ and $F_{2,28} = 5.0$; $P = 0.014$; Fig. 2d, e). Litter N concentration in Legume N+ plots (1.55%) was 60% higher than the concentration in Control and Legume N– plots ($F_{2,27} = 40$, $P < 0.001$). Soil stoniness did not differ among communities ($F_{2,28} = 0.59$, $P = 0.56$; data not shown).

Plant community structure as affected by presence of legumes and by fertilization

Both the non-legume aboveground phytomass structure ($F_{2,57} = 16.8$, $P < 0.001$) and non-legume vascular plant community structure ($F_{2,57} = 2.99$, $P < 0.001$) differed significantly among all four communities (Table 1). Pair-wise analyses indicated that non-legume aboveground phytomass structures (Fig. 3) of the fertilized and Legume N+ plots were similar ($F_{1,38} = 0.24$, $P = 0.924$) with small amount of lichens, and large fractions of both litter and non-legume vascular plants. Phytomass structures of fertilized and Legume N+ plots were significantly different from these of Legume N– and Control plots ($P < 0.001$, in both cases). Legume N– plots showed high similarity to Control plots ($F_{1,38} = 2.7$, $P = 0.073$) due to the high percentage of lichens, and low percentage of litter.

Aboveground biomass of legumes was 245 ± 18 and 84 ± 5.4 g/m^2 (mean ± SE) on Legume N+ and Legume N– plots, respectively ($F_{1,24} = 95.6$, $P < 0.001$), which constituted 37% and 15% of total aboveground phytomass on these plots. In spite of this doubled aboveground biomass fraction of non-legume vascular plants in Legume N+ compared to Legume N– plots (32% and 17%, respectively; Fig. 3), the structure of non-legume vascular plant community was similar ($F_{1,38} = 1.42$; $P = 0.106$) whereas both plot types differed from fertilized plots ($F_{1,38} = 2.26$, $P = 0.002$ and $F_{1,38} = 2.12$, $P = 0.01$, respectively) and even more from Controls ($P < 0.001$). Fig. 4 shows relative contribution of each non-legume vascular plant species to the non-legume community aboveground biomass at different plots, and Table 2 shows the raw aboveground biomass values of vascular plants.

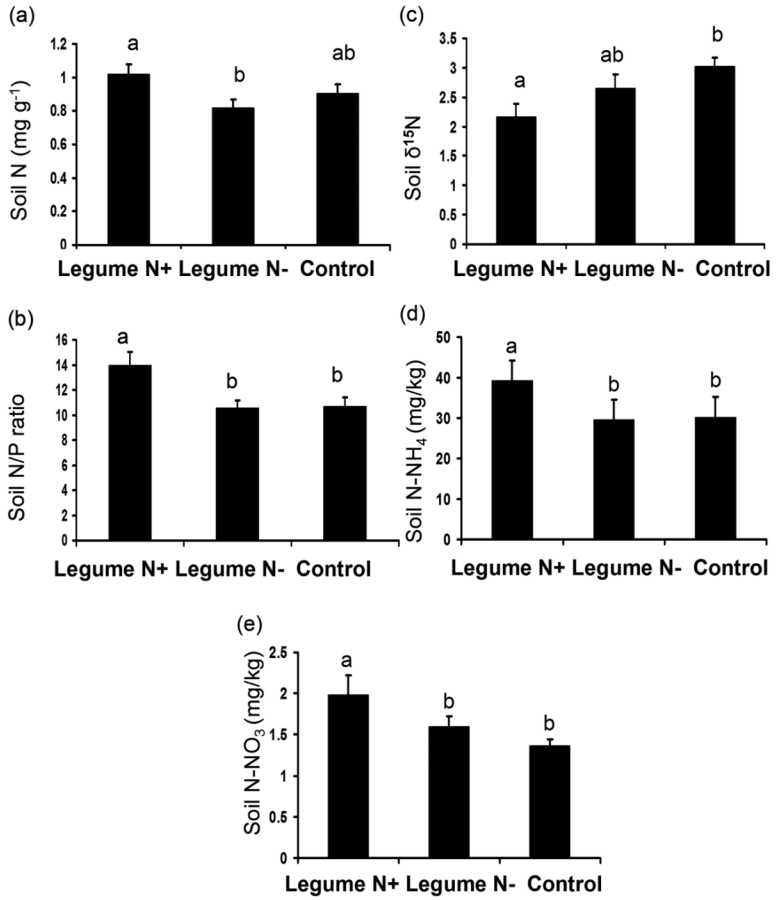

Fig. 2. Soil nutrient content: (a) N content, (b) N/P ratio, (c) $\delta^{15}N$, (d) soil N available as ammonium and (e) as nitrate, on plots dominated by the N-fixing legume *Oxytropis kubanensis* (Legume N+), the not N-fixing legume *Trifolium polyphyllum* (Legume N−) and plots without legumes (Control) in alpine tundra plant community. Mean values and standard errors (n = 10) are shown. Different letters indicate significant differences ($P < 0.05$).

Table 1. Results of canonical correspondence analysis for non-legume aboveground phytomass structure (Phytomass structure) and non-legume vascular plant community structure (Plant community structure) of alpine tundra plots dominated by the N-fixing legume *O. kubanensis* (LN+), the not N-fixing legume *T. polyphyllum* (LN−), plots without legumes (C), and plots fertilized with N for 5 years (F). Column "Overall test" shows the results of Monte Carlo tests for significance of difference within all four communities. Other columns show results of planned pair-wise comparisons. Stat., statistical parameters; F, Fisher F statistics; P, significance of differences.

Variables tested	Stat.	Overall test	LN+ vs. F	LN− vs. F	LN− vs. LN+	LN− vs. C	LN+ vs C
Phytomass structure	F	16.78	0.24	21.47	26.25	2.70	27.47
Phytomass structure	*P*	<0.001	0.924	<0.001	<0.001	0.073	<0.001
Plant community structure	F	2.99	2.26	2.12	1.42	2.94	3.81
Plant community structure	*P*	<0.001	0.002	0.001	0.106	<0.001	<0.001

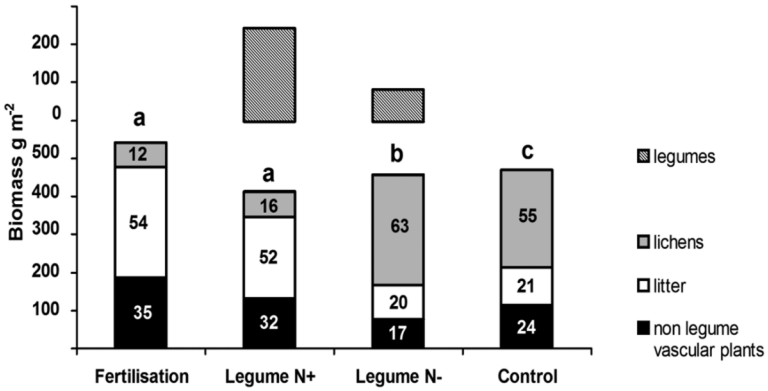

Fig. 3. Non-legume aboveground phytomass structure of plant communities with different N inputs: N fertilized, dominated by the N fixing legume *Oxytropis kubanensis* (Legume N+), the not N-fixing legume *Trifolium polyphyllum* (Legume N–), and unfertilized plots without legumes (Control). Legume aboveground biomasses are shown separately and were not included in the analysis. Numbers show the participation of each group expressed as percentage of the total non-legume aboveground phytomass. Different letters indicate significant differences between communities in terms of relative contributions of lichens, litter and non-legume vascular plants into total non-legume aboveground phytomass, as revealed by canonical correspondence analysis. Note that the figure does not directly reflect the CCA conducted, which takes into account non-legume aboveground phytomass fractions (log-transformed) rather than the absolute amount of each component. The data of aboveground phytomass on N fertilized plots correspond to previous data (Soudzilovskaia et al. 2005).

Combined effects of legume presence in the plant community and type of mycorrhizal infection possessed by plants

Per-plot aboveground biomass of non-mycorrhizal non-legume species was significantly different among Legume N+, Legume N– and Control plots ($F_{2,57} = 5.302$; $P = 0.003$) as a result of pair-wise differences between Legume-dominated plots and Control ($P < 0.001$), while Legume N+ and Legume N– plots did not differ ($F_{1,38} = 0.365$; $P = 0.7$). Aboveground biomass fractions of non-legume species of all mycorrhizal types were affected by plot type (Fig. 5). Ericoid species aboveground biomass fraction in Control plots was half that in Legume N+ or Legume N– plots ($F_{2,57} = 5.2$; $P = 0.008$). Non-mycorrhizal species had higher aboveground biomass fraction in Legume N+ or Legume N– than in Control plots. Species with AMF mycorrhiza had a higher aboveground biomass fraction in Control (74%) as compared to Legume N+ (53%) or Legume N– plots (56%) ($F_{2,57} = 9.8$; $P < 0.001$). Raw biomass data for plants with each mycorrhizal type are shown in Table 3.

Plant tissue N concentration of non-legume plants was affected by both mycorrhizal type ($F_{2,292} = 146$; $P < 0.001$) and plot type ($F_{2,57} = 78$; $P < 0.001$) as well as by their interaction ($F_{4,292} = 4.2$; $P = 0.003$; Fig. 6a). Both AMF and non-mycorrhizal plants had higher N on legume N+ plots, while species with ericoid mycorrhiza did not show a significant pattern. Separate analysis conducted for groups of plants with distinct mycorrhizal status revealed that AMF species tissue N in Legume N+ plots was 14% higher than in Control and Legume N– plots (overall ANOVA $F_{2,171} = 80$, Tukey test $P < 0.001$), while in non-mycorrhizal species, the difference was much smaller (7%) and only marginally significant (overall ANOVA: $F_{2,57} = 4.4$; $P = 0.017$; Tukey test for Legume N+ vs. control: $P = 0.042$, for Legume N+ vs. Legume N–: $P = 0.038$).

Plant $\delta^{15}N$ of non-legume plant species was affected by both mycorrhizal type ($F_{2,292} = 5.1$, $P < 0.007$) and plot type ($F_{2,292} = 141$; $P < 0.001$) as well as their interaction ($F_{4,292} = 3.1$; $P = 0.016$; Fig. 6b). Species with AMF mycorrhiza had 50% more negative $\delta^{15}N$ than non-mycorrhizal ones, and 22% more negative $\delta^{15}N$ than species with ericoid mycorrhiza. Surprisingly, non-mycorrhizal species had $\delta^{15}N$ on Legume N+ 28% more negative than on Control plots (overall ANOVA:

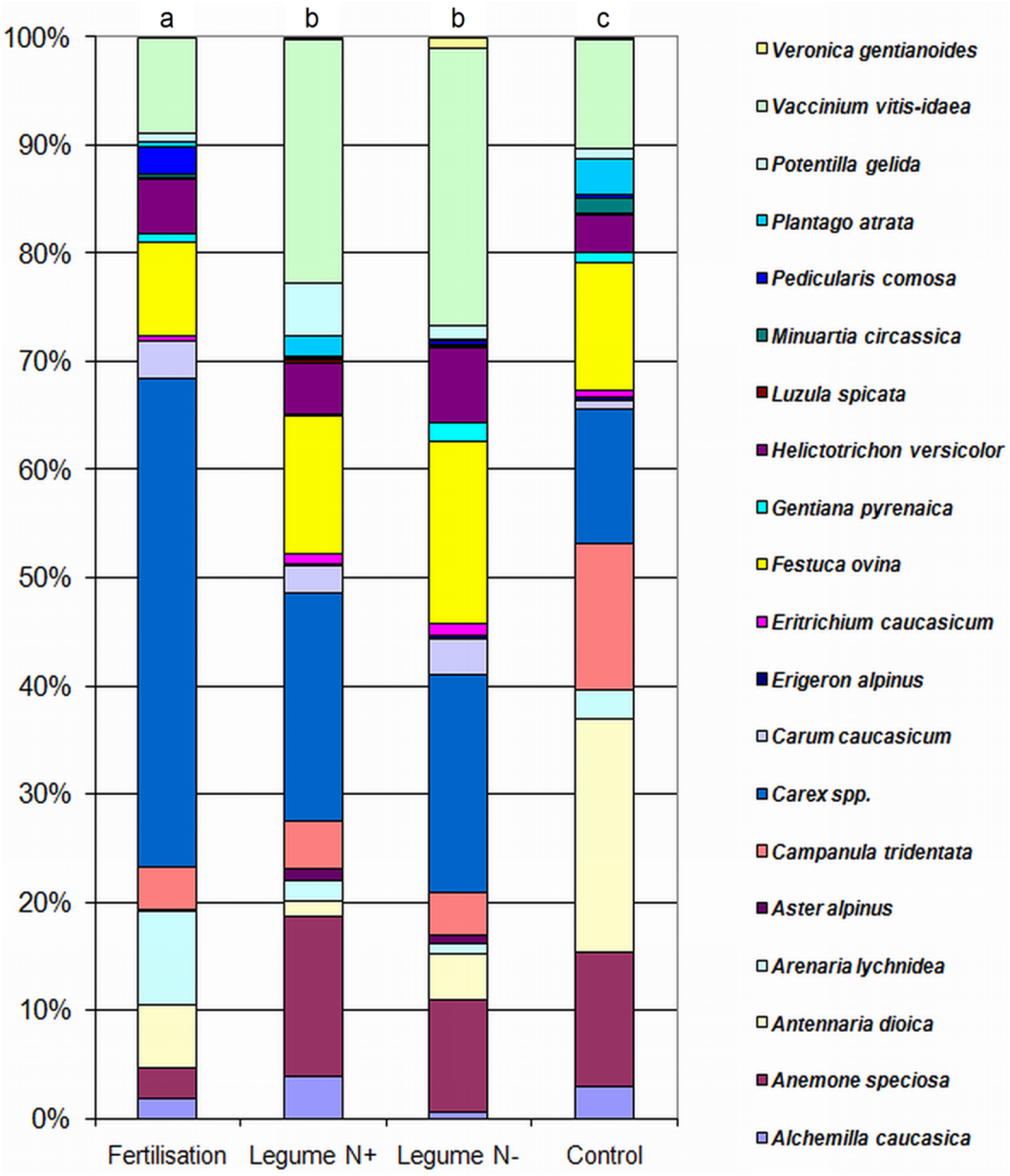

Fig. 4. Non-legume vascular plant community structure of the plant communities with different nitrogen inputs: nitrogen fertilized, dominated by the nitrogen fixing legume *Oxytropis kubanensis* (Legume N+), the non-fixing legume *Trifolium polyphyllum* (Legume N–) and unfertilized plots without legumes (Control). Different letters indicate significant differences between communities in terms of relative contributions of distinct vascular plants, as revealed by canonical correspondence analysis. *Carex* spp refers to the mixture of *Carex sempervirens* Vill. and *Carex umbrosa* Host, which are difficult to distinguish in the field when they do not have flowers.

$F_{2,57} = 4.4$, $P = 0.016$, Tukey test Legume N+ vs. control: $P = 0.013$), while AMF-associated species showed the opposite pattern with $\delta^{15}N$ on Legume N+ being 20% less negative than on Control plots (overall ANOVA: $F_{2,171} = 24$, $P < 0.001$, Tukey test Legume N+ vs. control: $P < 0.001$). Legume N– plots had an intermediate position. Species with ericoid mycorrhiza did not

Table 2. Mycorrhizal status of non-legume vascular plants used in the study (AMF, plant with arbuscular mycorrhiza; ER, plant with ericoid mycorrhiza; no, non-mycorrhizal plant); and biomass (g/m^2) of non-legume vascular plants in the plant communities with different nitrogen inputs: nitrogen fertilized, dominated by the nitrogen fixing legume *O. kubanensis* (Legume N+), the non-fixing legume *T. polyphyllum* (Legume N–) and unfertilized plots without legumes (Control). *Carex* spp refers to the mixture of *Carex sempervirens* Vill. and *Carex umbrosa* Host, which are difficult to distinguish in the field when they do not have flowers.

Species	Myc. st.	Fertilization	Legume N+	Legume N–	Control
Alchemilla caucasica Buser	AMF	3.3 ± 1.8	4.6 ± 1.3	0.4 ± 0.4	3.3 ± 1.1
Anemone speciosa Adams ex G.Pritz.	AMF	4.9 ± 1.5	17.9 ± 6.3	7.6 ± 2.1	14.5 ±0.4
Antennaria dioica (L.) Gaertn.	AMF	10.5 ± 6.3	1.6 ± 0.4	3.2 ± 0.9	25.2 ± 3.1
Arenaria lychnidea Bieb.	no	15.8 ± 8.0	2.4 ± 1.1	0.7 ± 0.6	3.1 ± 1.7
Aster alpinus L.	AMF	0.2 ± 0.2	1.2 ± 1.0	0.6 ± 0.4	0.0 ± 0.0
Campanula tridentata Schreb.	AMF	7.1 ± 3.0	5.4 ± 2.4	2.9 ± 0.9	15.7 ± 3.0
Carex spp.	no	81.1 ± 13.4	25.4 ± 4.3	15.0 ± 3.1	14.4 ± 3.2
Carum caucasicum (Bieb.)Boiss.	AMF	6.2 ± 2.6	3.1 ± 1.0	2.4 ± 1.6	1.0 ± 0.4
Erigeron alpinus L.	AMF	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.1
Eritrichium caucasicum (Albov) Grossh.	no	0.9 ± 0.5	1.1 ± 0.5	0.9 ± 0.3	0.7 ± 0.3
Festuca ovina L.	AMF	15.4 ± 2.7	15.3 ± 1.9	12.5 ± 2.6	13.8 ± 2.9
Gentiana pyrenaica L.	AMF	1.6 ± 0.5	0.2 ± 0.1	1.3 ± 1.1	1.1 ± 0.5
Helictotrichon versicolor (Vill.)Pilger	AMF	9.1 ± 3.6	5.8 ± 1.4	5.2 ± 1.8	3.9 ± 0.9
Luzula spicata (L.) DC.	no	0.2 ± 0.2	0.4 ± 0.3	0.1 ± 0.1	0.2 ± 0.2
Minuartia circassica (Albov) Woronow	no	0.5 ± 0.5	0.2 ± 0.2	0.1 ± 0.1	1.7 ± 0.6
Pedicularis comosa L.	no	4.4 ± 1.7	0.1 ± 0.1	0.3 ± 0.3	0.3 ± 0.2
Plantago atrata Hoppe	AMF	1.0 ± 0.8	2.4 ± 1.3	0.0 ± 0.0	3.8 ± 1.7
Potentilla gelida C.A.Mey.	AMF	1.4 ± 1.4	5.9 ± 1.9	1.0 ± 0.4	1.1 ± 0.4
Vaccinium vitis-idaea L.	ER	16.0 ± 5.3	27.0 ± 4.4	19.1 ± 3.9	11.9 ± 3.3
Veronica gentianoides Vahl.	AMF	0.0 ± 0.0	0.3 ± 0.3	0.7 ± 0.4	0.2 ± 0.1

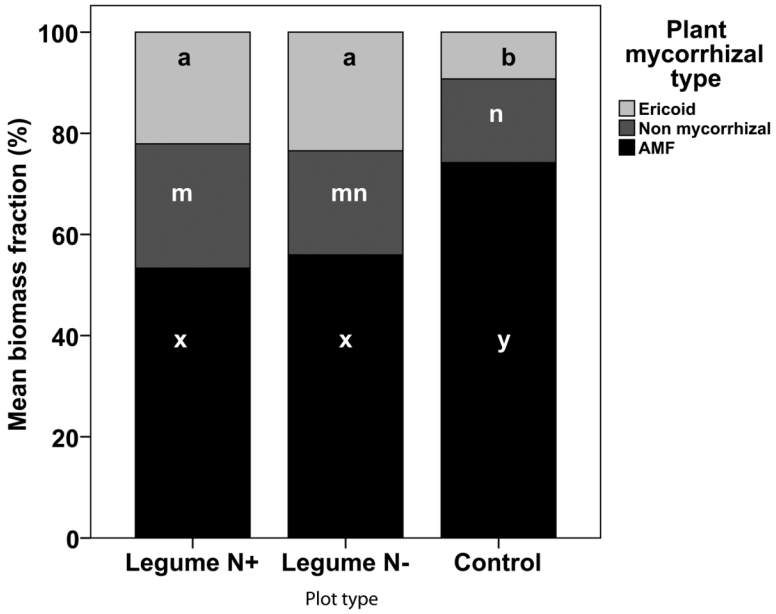

Fig. 5. Aboveground biomass fractions of non-legume species with different mycorrhizal status on plots dominated by the N-fixing legume *Oxytropis kubanensis* (Legume N+), the not N-fixing legume *Trifolium polyphyllum* (Legume N–) and plots without legumes (Control). Within each myccorhizal type, different letters indicate biomass fractions differing significantly ($P > 0.05$) among plot types, as revealed by Tukey post hoc tests conducted within each fraction after per fraction one-way ANOVAs.

Table 3. Aboveground biomass (g/m^2) of non-legume species with different mycorrhizal status on plots dominated by the nitrogen-fixing legume *O. kubanensis* (Legume N+), the non-fixing legume *T. polyphyllum* (Legume N−) and plots without legumes (Control).

	Mycorrhizal type		
Plot type	Ericoid	Non-mycorrhizal	Arbuscular mycorrhizal
Control	11.9 ± 3.4	20.4 ± 3.2	90.4 ± 6.3
Legume N−	20.3 ± 4.3	16.6 ± 3.0	44.1 ± 4.8
Legume N+	27.0 ± 4.5	29.6 ± 4.3	67.1 ± 7.9

show a significant pattern.

Discussion

Legumes as ecosystem engineers

Our study compared natural plant communities dominated by N-fixing rhizobial legumes with communities dominated by not N-fixing legumes and without legumes (fertilized and unfertilized). We found similar non-legume aboveground phytomass structures (i.e., low lichen and high litter mass) compared to vascular plants in N-fertilized plots and unfertilized plots dominated by the N-fixing legume *O. kubanensis*. In contrast, plots without N subsidies, both Controls and plots dominated by the not N-fixing *T. polyphyllum*, had similar litter mass and a much higher proportion of lichen mass compared to living vascular plant mass. Our soil N availability results confirm that legumes with symbiotic N-fixing bacteria form an important source of N in natural plant communities, similar to that in agricultural landscapes (Ledgard and Steele 1992, Zahran 1999). Moreover, the remarkable differences in amounts of litter accumulated in fertilized and Legume N+ plots compared to the Control and Legume N− plots imply differences in nutrient turnover rates and pathways between the two groups of plots. These results confirm our hypotheses 1 and 2 about the ecosystem engineering role (Lawton 1994) of N-fixing legumes in vascular plant communities.

With higher N availability, we expected similar non-legume vascular plant community structure on Legume N+ and fertilized plots. However, the plots of the two types turned out to be very different. Despite differences in N availability and in legume aboveground biomass, per-species fractions of non-legume vascular plant species were similar in Legume N+ and Legume N− plots (Table 1). Thus, hypothesis 2 that predicted a distinct role for not N-fixing legumes compared to N-fixing legumes is only partly supported. Although differences in non-legume above-

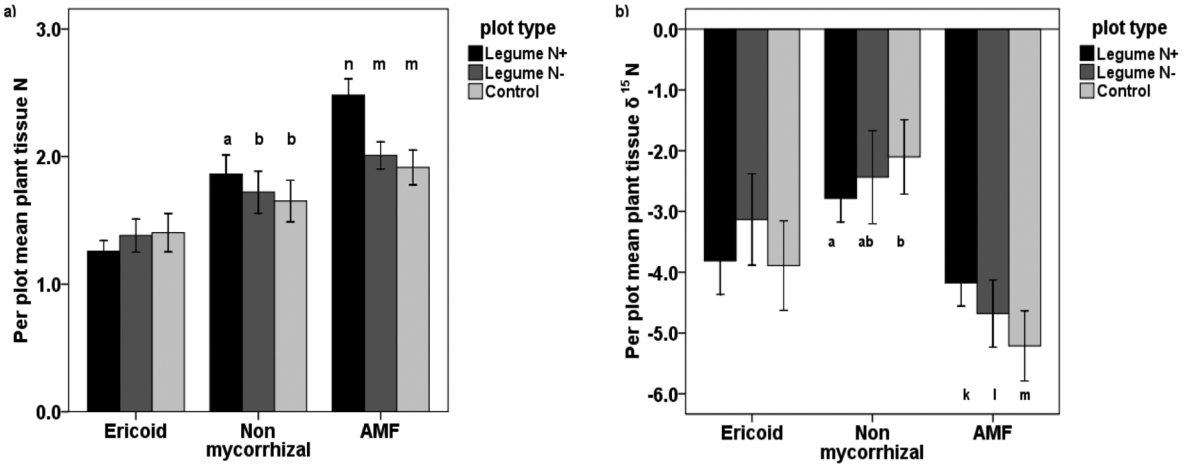

Fig. 6. Vascular plant tissue N concentration (a) and vascular plant tissue $\delta^{15}N$ concentration (b) as a function of mycorrhizal status of the plant and plot type: dominated by the N-fixing legume *Oxytropis kubanensis* (Legume N+), the not N-fixing legume *Trifolium polyphyllum* (Legume N−), or without legumes (Control). Per plot type, means calculated over all plants on a per-plot basis, and their standard errors, are shown. Different letters indicate differences ($P < 0.05$).

ground phytomass structure and higher tissue N of non-legume plants in Legume N+ plots (Fig. 6a), indicate distinct nutrient turnover pathways in the two types of plots, the non-legume vascular plant community structure did not differ in the Legume N+ and Legume N– plots.

Differences between Legume N+ and fertilized plots and similarities between Legume N+ and Legume N– plots found in non-legume vascular plant community structure can not be attributed to soil N availability because concentrations of plant available N (nitrate and ammonium) were higher on Legume N+ plots as compared to Legume N– and Control plots. We speculate that the effects of N subsidies are overridden by: (1) effects of each of the dominant legumes on light availability by shading co-occurring plants (Jacot et al. 2005) that protects against wind or moisture retention (Callaway et al. 2002), and/or (2) possibly similar allelopatic effects of legumes (Newman and Rovira 1975, Carlsen and Fomsgaard 2008, Power et al. 2010) and slightly lower soil P pool observed on both legume dominated plots.

Plant nutrition in presence of legumes and the role of mycorrhiza

We detected higher concentrations of N in both AMF and in non-mycorrhizal plants (Fig. 6a) and higher total aboveground biomass of non-legume plants (Fig. 3) growing together with the N-fixing legume compared to Legume N– and Control plots. These results contradict the findings of Van der Heijden et al. (2006) who reported no difference in biomass and N concentration for plants growing together with N-fixing legumes compared to those growing without legumes. Van der Heijden et al. (2006) studied plants growing in pots for a short period such that no nutrient transfer via litter occurred. In our study conducted in natural conditions, litter in Legume N+ plots had higher N content than litter on Legume N– plots and Control plots, suggesting that N made available during litter decomposition is utilized by non-legume vascular plants. Higher N availability to plants in Legume N+ plots, as confirmed by higher concentrations of inorganic N in the soil, could also be a consequence of root N exudation of legumes (Jalonen et al. 2009).The setup of our study does not allow distinguishing between these two mechanisms, but the contrast with the results of Van der Heijden et al. (2006), in whose study the root exudation was not excluded but the litter was, provides indirect evidence that the prevailing N transfer path was via litter.

Mycorrhizal fungi are able to transfer N from organic material to host plants (Johansen et al. 1992, Leigh et al. 2009). Also mycorrhizal networks can transfer N directly from N-fixing legumes to plants that do not possess N fixation (Johansen and Jensen 1996). The amount of N transported depends on soil N availability, and is reduced in more fertile conditions (Azcon et al. 2001). In line with these findings, we demonstrated that the N nutrition pathway in the presence of legumes is underpinned by the mycorrhizal status of the species (hypothesis 3). We detected differences in $\delta^{15}N$ for AMF and non-mycorrhizal plant species growing on different types of plots, and a significant interaction between mycorrhizal type and plot type resulting in opposite shifts in $\delta^{15}N$ for these two groups depending on the presence of legumes. Together these findings suggest that AMF and non-mycorrhizal plants not only use distinct sources of N, but also show shifts in preferences for N sources in response to presence/absence of legumes. In Legume N+ plots, plant competition for available N decreased due to higher N availability, a significant ^{15}N enrichment of NH_4^+-N, and ^{15}N depletion of NO_3^--N as a result of nitrification (Koopmans et al. 1997, Koba et al. 1998). Probably, in these conditions AMF-associated species consumed mainly ^{15}N enriched NH_4^+-N, whereas non-mycorrhizal species (mostly several *Carex* species in this study) primarily consumed ^{15}N depleted NO_3-N. Primarily consumption of NO_3^--N by non mycorrhizal *Carex* species was demonstrated previously (e.g., Sorensen et al. 2008).

The only plant species with ericoid mycorrhiza, *Vaccinium vitis-idaea*, is associated with plots dominated by legumes, whether N-fixing or not. Its nutrition pattern was unaffected by the presence of legumes, suggesting that other factors, probably reduced light availability, resulting in growth of elongated shoots (Onipchenko et al. 2001, Hansen et al. 2006) are responsible for the increase of *Vaccinium* aboveground biomass in these plots.

Methodological issues

In this study, we considered differences in soil and plant N, and P content, plot non-legume aboveground phytomass, and vascular plant community structures to be attributable to the presence of legumes. In theory, the possibility exists that legumes establish themselves at patches with specific soil conditions and plant community, or that legume-dominated plots represent a later successional stage compared to controls. Although this possibility can never be completely excluded, we consider it unlikely in our study, because: (1) the tectonic structure underlying the Malaya Khatipara mountain is known to be uniform siliceous parent rock (Vertelina et al. 1996), (2) we selected plots that did not differ in slope neither in micro- topography, and are known to have equal snow regime due to their position close to each other, (3) previous investigations (Batchaeva et al. 2003) showed that soil depths (i.e., distances from the soil surface to the first stone) are equal under *O. kubanensis* and *T. polyphyllum,* and do not deviate from the average soil depth of alpine lichen tundra, (4) soil stoniness did not differ between plots, and (5) patches of *O. kubanensis* and *T. polyphyllum* are distributed randomly through the alpine tundra. All together these considerations suggest that establishment of legumes within the studied site is stochastic, and the observed patterns are due to the presence of legumes and not the other way around.

Conclusions

N-fixing legumes can constitute an important source of N in natural plant communities. These plants enrich soil with N and affect the pathways of soil N turnover, as accompanied by increases in N concentration in plant tissue, greater litter accumulation, and reduction of cryptogam participation in community aboveground biomass. However, our comparison of N-fixing and not N-fixing legumes revealed that, at least in the alpine tundra, the community structure of vascular plants is not considerably affected by the N fixation of legumes. This pattern suggests that other factors, such as soil nutrient availability, allelopathy, light availability, and microclimate may interact with effects of N addition. We conclude that plant mycorrhizal status is an important factor adjusting plant nutrition mode to the presence or absence of legumes.

Acknowledgments

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