

Carlos Urcelay · M. Sydonia Bret-Harte ·
Sandra Díaz · F. Stuart Chapin

Mycorrhizal colonization mediated by species interactions in arctic tundra

Received: 15 November 2002 / Accepted: 17 June 2003 / Published online: 7 August 2003
© Springer-Verlag 2003

Abstract The Alaskan tussock tundra is a strongly nutrient-limited ecosystem, where almost all vascular plant species are mycorrhizal. We established a long-term removal experiment to document effects of arctic plant species on ecto- and ericoid mycorrhizal fungi and to investigate whether species interactions and/or nutrient availability affect mycorrhizal colonization. The treatments applied were removal of *Betula nana* (*Betulaceae*, dominant deciduous shrub species), removal of *Ledum palustre* (*Ericaceae*, dominant evergreen shrub species), control (no removal), and each of these three treatments with the addition of fertilizer. After 3 years of *Ledum* removal and fertilization, we found that overall ectomycorrhizal colonization in *Betula* was significantly reduced. Changes in ectomycorrhizal morphotype composition in removal and fertilized treatments were also observed. These results suggest that the effect of *Ledum* on *Betula*'s mycorrhizal roots is due to sequestration of nutrients by *Ledum*, leading to reduced nutrient availability in the soil. In contrast, ericoid mycorrhizal colonization was not affected by fertilization, but the removal of *Betula* and to a lower degree of *Ledum* resulted in a reduction of ericoid mycorrhizal colonization suggesting a direct effect of these species on ericoid mycorrhizal colonization. Nutrient availability was only higher in fertilized treatments, but caution should be taken with the interpretation of these data as soil microbes may effectively compete with the ion exchange resins for the nutrients released by plant removal in these nutrient-limited soils.

Keywords Ectomycorrhizas · Ericoid mycorrhizas · Nutrient availability · Mycorrhizal root interactions · Removal experiment

Introduction

Mycorrhizas are the mutualistic symbiosis between plant roots and fungi. Mycorrhizal associations are believed to be most beneficial in habitats where plants face strong nutrient limitations (Allen 1991). In these habitats, mycorrhizal plants tend to be strongly dependent on their fungal partner for nutrient acquisition (Allen and Allen 1991). Accordingly, mycorrhizal colonization is expected to decrease when plant nutrient limitations are ameliorated. This decrease has been documented for arbuscular mycorrhizas (Jasper et al. 1979; Abbott et al. 1984, among others). There is some evidence that nutrient availability, and in particular N, may also affect ectomycorrhizal fungi (Aerts and Bobbink 1999). Nutrient addition experiments often reduce the biomass and diversity of aboveground fruiting bodies of mycorrhizal fungi (Wallenda and Kottke 1998 for a review; Peter et al. 2001). Effects of nutrient addition on below-ground ectomycorrhizal fungal structures are much less known (Peter et al. 2001; Lilleskov and Bruns 2001). Conversely, even though it has been demonstrated that high nutrient availability results in a decline (Moore-Parkhurst and Eglander 1982) or inhibition (Stribley and Read 1976) of ericoid mycorrhizal fungi in laboratory experiments, field studies have failed to confirm this trend (Caporn et al. 1995; Michelsen et al. 1999; Johansson 2000).

Tussock tundra is a strongly nutrient-limited ecosystem co-dominated by four plant functional types: graminoids, mosses, deciduous shrubs, and evergreen shrubs (Chapin et al. 1996). Evergreen and deciduous shrubs exploit the same soil horizon for nutrient uptake (Nadelhoffer et al. 1996; McKane et al. 2002) and are relatively similar in the timing and form of soil N used (McKane et al. 2002). Vegetation changes in tussock tundra are usually attributed to changes in nutrient

C. Urcelay (✉) · S. Díaz
Instituto Multidisciplinario de Biología Vegetal and FCEfyN,
Universidad Nacional de Córdoba—CONICET,
C.C. 495, 5000 Córdoba, Argentina
e-mail: curcelay@imbiv.unc.edu.ar
Fax: +54-351-4332104

M. S. Bret-Harte · F. S. Chapin III
Department of Biology and Wildlife, Institute of Arctic Biology,
University of Alaska—Fairbanks,
Alaska, USA

availability (Hobbie et al. 1999; Bret-Harte et al. 2001) or plant-plant interactions such as competition or facilitation through climatic amelioration (Chapin and Shaver 1985; Chapin et al. 1995; Hobbie and Chapin 1998). Although the strong mycorrhizal dependence of the majority of the vegetation is well known (Read 1990), there is no published laboratory or field study evaluating the effects of plant species interactions that focuses on ecto- and ericoid mycorrhizal communities.

The aim of our study was to assess the effects of nutrient availability and removal of plant species, belonging to different functional types, on ecto- and ericoid mycorrhizal colonization in tussock tundra vegetation.

Materials and methods

Site description and treatments

This study was conducted in moist tussock tundra near Toolik Lake at the arctic Long Term Ecological Research (LTER) site in the northern foothills of the Brooks Range of arctic Alaska (68°38'N, 149°34'W, elevation 760 m). Vegetation on the site is characterized by approximately equal biomass of graminoids (mainly *Eriophorum vaginatum* and *Carex bigelowii*), deciduous shrubs (mainly *Betula nana*, with less *Salix pulchra*), evergreen shrubs (mainly *Ledum palustre* spp. *decumbens* and *Vaccinium vitis-idaea*), and mosses (mainly *Hylocomium splendens*, *Aulacomnium turgidum*, *Dicranum* spp. and *Sphagnum* spp.) (Shaver and Chapin 1991). Nomenclature follows Hultén (1968).

In 1997, we established six replicate blocks in homogeneous acidic tussock tundra on a gentle (5%) north-facing slope, approximately 100 m uphill from the LTER experimental plots (Bret-Harte et al. 2001, 2002), on the same geologic surface. Each block consisted of a row of seventeen 2×3-m plots separated by 1-m buffer strips. Blocks were oriented perpendicular to the slope, separated from each other by 2-m buffer strips. Boardwalks were constructed in the 1-m buffer strips between the plots and around the perimeter of the experiment, so that all plots could be accessed without trampling. Within each block, three plots were randomly assigned to the following removal treatments: (1) control (no species removed), (2) *Betula* (removal of *B. nana*), (3) *Ledum* (removal of *Ledum palustre* ssp. *decumbens*). An additional three plots were randomly assigned to receive the same removal treatments specified above, with the addition of N and P fertilizers. The remaining 11 plots in each block received other removal treatments (M. S. Bret-Harte, unpublished data), results of which are not included in the present report.

Species were removed by pulling out the aboveground stems and as much as possible of the belowground stems until they broke below ground (*Betula* and *Ledum* removal treatments). *Betula* removal took away approximately 125 g m⁻², *Ledum* removal took away approximately 195 g m⁻². Care was taken to avoid breaking the stems of non-target species, but some damage did occur, particularly to fine roots of species next to stems of the target species. Control and fertilized plots without removals were disturbed to simulate the side effects of removal by pulling on the stems of the vegetation without breaking them and by disrupting the moss layer by grabbing and shaking it. No biomass was removed from these non-removal plots (the total vascular biomass in the disturbed control community was approximately 500 g m⁻² at the time of sampling). After establishment of the removal treatments in 1997, the plots were maintained annually by removing in early June of each year the regrowth of target species from remaining sections of belowground stem and by disturbing the control and fertilized plots. Although removal experiments have some drawbacks, a synthesis of the results of these experiments suggests that they are very useful for understanding complex

interspecific interactions and other ecological questions relevant to the functioning of real communities and ecosystems in the field (Diaz et al. 2003).

For all fertilization treatments, we used the same N and P fertilizers and application methods as in previous work (Shaver and Chapin 1980, 1986; Chapin et al. 1995; Chapin and Shaver 1996; Bret-Harte et al. 2001). N and P were applied to the plots annually in early June, starting in 1997. We applied N (as slow-release granular NH₄NO₃) at 10 g m⁻² year⁻¹ and P (as commercial granular superphosphate, a mixture of calcium phosphates) at 5 g m⁻² year⁻¹.

Root sampling

In the fourth year of nutrient addition and removal treatments, on 6 and 12 July 2000, six cores of 5.5 cm diameter and 10–15 cm length, depending on the mycorrhizal roots' profile depths, were taken in each plot; these six subsamples were combined to constitute one sample per plot. Ectomycorrhizal roots of *B. nana* were easily distinguishable from ericoid roots of *L. palustre*, *V. vitis-idaea* and other much less abundant ericaceous shrubs (e.g., *Vaccinium uliginosum*, *Empetrum nigrum*). Both types of roots were carefully separated and washed with water for examination.

Assessment of mycorrhizal colonization

B. nana roots

The roots in each sample were divided into two subsamples; the roots of each subsample were spread over a grid-lined Petri dish and all the root intersections with the gridline were assessed for mycorrhizal colonization. Only fine roots that can be potentially colonized were considered. Four hundred to 600 root intersections were scored per sample, depending on sample size. Percentage of root colonization was assessed as the proportion of total root intersections that were colonized (Bundrett et al. 1996).

Morphotypes of different ectomycorrhizas were distinguished by macroscopic characteristics of the fungal mantle, such as color, surface appearance, presence of emanating hyphae and hyphal strands, as well as microscopic features such as mantle type and hyphal connections (Agerer 1987–1993). This classification was intended to differentiate ectomycorrhizal types, but it is not adequate for taxonomic purposes. We assigned a designator letter to each distinct type.

Ericoid roots

Ericoid roots were easily recognizable by their small diameter, the presence of "hair roots" and a characteristic white or brown color depending on their age. No mantle or Hartig net were observed in ericoid roots. Therefore we assumed that all structures measured belonged to the ericoid mycorrhizal type. The roots were obtained from soil cores, therefore we were not able to distinguish between roots of individual species. They were separated from non-ericoid roots and soil, washed with water; and stored in formaldehyde-acetic acid-alcohol until processed. All dead and damaged roots were discarded. All thin roots (<1 mm without apparent suberin) were cleared and stained following Grace and Stribley (1991). They were then mounted on semi-permanent slides in polyvinyl-lactic acid-glycerol (Omar et al. 1979); three slides per sample were prepared. The root endophyte quantification was made using the magnified intersection method (McGonigle et al. 1990) using a compound microscope (Kyowa optical, Model LSCB-VC-2B-L), magnification ×150. Fifty intersections per slide were scored (150 intersections/sample). No ectomycorrhizal colonization was observed in ericoid roots.

Assessment of nutrient availability

We measured the accumulation of NH_4^+ , NO_3^- , and PO_4^{3-} on ion exchange resins incubated in the soil to compare the relative availability of N and P in the different treatments (Giblin et al. 1991). Resin bags were made from nylon stocking material, which was soaked in 0.1 M HCl overnight before filling with ion exchange resins. Each bag contained 9 g fresh weight mixed-bed ion exchange resins (IONAC nm-60 H^+/OH^- form, type I beads 16–50 mesh; Baker, Phillipsburg, N.J.). Resin bags (one per plot) were placed at approximately 5 cm depth in the soil on 22 June, and were removed on 14 August 1999. Resin bags were washed free of soil using distilled water, then extracted in 100 ml of 2 M NaCl in 0.1 M HCl overnight. Extracts were frozen until analysis. For analysis, extracts were thawed in a refrigerator, brought to neutral pH by the addition of NaOH and analyzed colorimetrically for NH_4^+ , NO_3^- , and PO_4^{3-} (Whitledge et al. 1981) on a modified Technicon autoanalyzer (Tarrytown, New York).

Statistical analysis

Data from subsamples were combined for a given plot prior to statistical analysis. After testing for the assumptions of normality, homogeneity of variances and independence of errors, data on ericoid and ectomycorrhizal roots were analyzed by a 2-way ANOVA (General Linear Model, general factorial procedure with species removal, fertilization and block as main effects, and with a species removal \times fertilization interaction effect) (SPSS System for Windows, Norussis 1997). To test the effects of removal of individual species (*Betula* and *Ledum*) with and without fertilization on mycorrhizal colonization, the main question addressed by this research, Tukey honestly significant difference tests were applied a posteriori to locate the differences among treatment means (SPSS system for Windows, Norussis 1997). Data on nutrient availability had inhomogeneous variance because of high variation in the fertilized plots. Because log-transformation failed to produce homogenous variance (Cochran's test; Winer et al. 1991), data were rank-transformed and 2-way ANOVAs (same model as above) were run on the rank data (Zar 1999). This non-parametric analysis yielded the same conclusions as parametric ANOVAs run on the untransformed data, suggesting that it had sufficient power (Zar 1999).

Results

Ectomycorrhizas

After 3 years of treatment, we found that fertilization and *Ledum* removal each promoted a significant reduction ($P=0.042$ and $P=0.043$ respectively) in ectomycorrhizal colonization of *Betula* (Table 1, Fig. 1). There was no significant effect of fertilization under species removal treatment, and no significant effect of removal under

Table 1 Results of two-way ANOVA on ecto- and ericoid mycorrhizal colonization. *Block* Main effect of block treatment, *Fertilization* main effect of fertilization treatment, *Species removal*

Variables	Source of variation											
	Block			Fertilization			Species removal			F \times SR		
Mycorrhizal type	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
Ectomycorrhizal Colonization	0.46	5, 23	0.803	4.94	1, 23	0.042	4.89	1, 23	0.043	8.28	1, 23	0.012
Ericoid mycorrhizal colonization	0.78	5, 34	0.612	1.16	1, 34	0.309	7.80	2, 34	0.003	0.1	2, 34	0.891

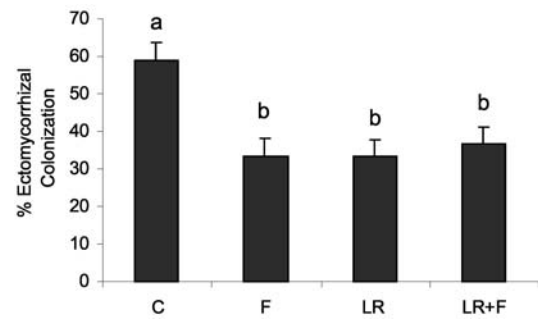


Fig. 1 Percentage of ectomycorrhizal colonization of *Betula* roots in the tussock tundra, Alaska, under different treatments: control (C), fertilizer addition (F), *Ledum* removal (LR), *Ledum* removal+fertilizer addition (LR+F). Error bars indicate +1 SE ($n=6$ blocks). Bars with the same letters are not significantly different [Tukey's honestly significant difference (HSD) test, $P < 0.05$]

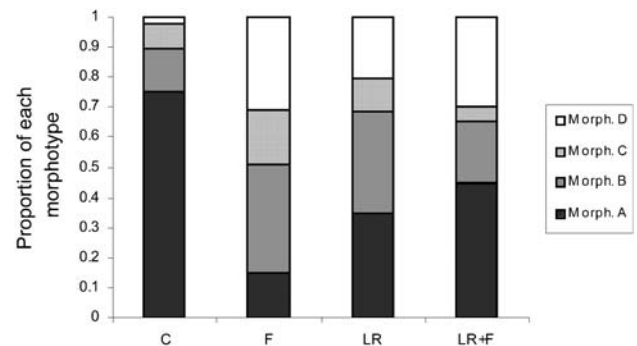


Fig. 2 Proportion of each mycorrhizal morphotype (*Morph.*) in *Betula* roots in the tussock tundra, Alaska under different treatments: C, F, LR, LR+F. For other abbreviations, see Fig. 1

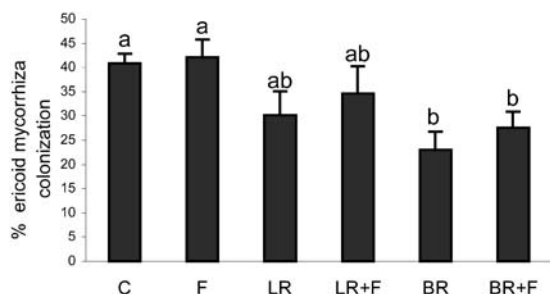
fertilization, but we found a significant interaction ($P=0.012$) between fertilization and *Ledum* removal treatments. These results indicate that the effects of *Ledum* removal and fertilization were not additive (Fig. 1).

Removal and fertilization also resulted in changes in the relative abundance of fungal morphotypes colonizing the roots of *Betula* (Fig. 2). There was both a relative and an absolute decrease in morphotype A, which was the dominant in the control, and an increase in morphotype D. That fertilizer addition and *Ledum* removal had similar effects on ectomycorrhizal colonization and morphotype composition suggests that the interactions between *Ledum*

main effect of species removal treatment, $F \times SP$ fertilizer-by-species removal interaction term in the ANOVA

Table 2 Results of two-way ANOVAs on the effects of treatments and blocks on relative nutrient availability, as measured by ion accumulation on resin bags. For explanation of terms, see Table 1

Variable	Source of variation											
	Block			Fertilization			Species removal			F×SR		
	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
NH ₄ ⁺ -N	1.938	5, 17	0.140	67.775	1, 17	<0.0001	0.124	2, 17	0.884	1.126	2, 17	0.347
NO ₃ ⁻ -N	0.364	5, 17	0.866	40.184	1, 17	<0.0001	1.745	2, 17	0.205	0.802	2, 17	0.465
PO ₄ ³⁻ -P	0.286	5, 16	0.914	37.599	1, 16	<0.0001	0.311	2, 16	0.737	0.374	2, 16	0.694

**Fig. 3** Percentage of ericoid mycorrhizal colonization of ericaceous plant roots in the tussock tundra, Alaska, under different treatments: C, F, LR, LR+F, *Betula* removal (BR), *Betula* removal+fertilizer addition (BR+F). Error bars indicate +1 SE (*n*=6 blocks). Bars with the same letters are not significantly different (Tukey's HSD test, *P*<0.05). For other abbreviations, see Fig. 1

and *Betula* mycorrhizal roots may be mediated by nutrient availability.

Ericoid mycorrhizas

Fertilization treatments did not affect ericoid mycorrhizal colonization significantly (Table 1, Fig. 3). In contrast, the removal treatments resulted in a significant reduction (*P* = 0.003) of ericoid mycorrhizal colonization, primarily due to the reduction in ericoid mycorrhizas caused by *Betula* removal (Table 1, Fig. 3). In other words, high levels of colonization of mycorrhizal fungi in ericoid roots may have been directly linked to the presence of *Betula* mycorrhizal roots. The presence of *Ledum* seems to have a similar, but less strong effect on the remaining ericoid roots (Fig. 3). In contrast to ectomycorrhizas, changes in ericoid mycorrhizal colonization may be related to factors different from nutrient enhancement.

Nutrient availability

Fertilization had a significantly positive effect (*P*<0.0001) on the accumulation of NH₄⁺, NO₃⁻, and PO₄³⁻ by resins (Table 2). Fertilization caused a two- to ninefold increase in accumulation of available inorganic N and P by resins where no species were removed. For both NO₃⁻ and NH₄⁺, there was an increase when fertilization was

combined with species removal than under fertilization alone suggesting an additive effect. However, species removal did not significantly affect resin accumulation of N or P, and the interaction between species removal and fertilization was also not significant for either N or P (Table 2). NO₃⁻ made up approximately 10% of the available inorganic N accumulated by the resins in control plots, a bit less than what has been seen previously in resins incubated in tussock tundra soil (Giblin et al. 1991). NO₃⁻ made up a higher proportion of the available inorganic N in fertilized plots, as expected.

Discussion

The percentages of root colonization by both ecto- and ericoid mycorrhizal fungi found in our study were similar to those reported for other cold, nutrient-limited areas of the world, mainly boreal temperate forests and heathlands (Helm et al. 1996, 1999; Johansson 2000). It has been proposed that root colonization by mycorrhizal fungi decreases with increasing nutrient availability in the soil, because plants can meet their nutrient requirements through direct uptake by roots without the extra C expenditure required to support mycorrhizal fungi (Aerts and Chapin 2000). The lower rates of ectomycorrhizal colonization that we observed in *Betula* roots under fertilization are consistent with this idea and with the results of previous studies (Wallenda and Kottke 1998; Peter et al. 2001).

The similarity of *Betula* ectomycorrhizal response to nutrient addition and *Ledum* removal, in terms of both reduced colonization and changes in morphotype composition, suggests that the effect of *Ledum* removal on *Betula* mycorrhizas could be explained by increased nutrient availability. This hypothesis is supported by ¹⁵N addition experiments at Toolik lake, which show that *Ledum* and *Betula* are more similar than any two other tussock tundra species in the soil depth, and form of N (NH₄⁺) exploited, but that *Ledum* exploits these pools earlier in the season than *Betula*, leaving less NH₄⁺ available to *Betula* in late season (McKane et al. 2002). Our data on nutrient availability as measured by accumulation on resins the year before our measurements, show that species removal did not have a significant effect on nutrient availability. Nevertheless, caution should be taken at interpreting these data, because ion

capture by resin bags is sensitive to a number of factors including competition from microbes and plants (Binkley 1984), soil moisture (Lajtha 1988), and rates of mineralization (Giblin et al. 1991). It is possible that, in the extremely nutrient-limited soils of the tussock tundra, uptake by *Betula* and soil microbes competes effectively with the ion exchange resins for the nutrients released by *Ledum* removal and could have prevented accumulation on the resin bags. Also, because we had to use non-parametric statistics to analyze the resin bag data, our power to detect subtle changes is low.

Although aboveground *Betula* biomass responds strongly to nutrient addition (Bret-Harte et al. 2001), it did not show a biomass response to *Ledum* removal in the absence of fertilization in our experiment (M. S. Bret-Harte, unpublished data). It seems possible that within the duration of this experiment, nutrients released by *Ledum* removal are enough for *Betula* to stop supporting high rates of mycorrhizal colonization but insufficient for *Betula* to generate significantly greater aboveground biomass. The effects of fertilizer addition and *Ledum* removal were not additive, perhaps because they both increase nutrient availability to the point where nutrients are no longer limiting and the cost to the plant of supporting low levels of mycorrhizal colonization is small compared to the cost of regulatory mechanisms to exclude the fungi (Johansson 2000).

Changes in mycorrhizal fungal composition in response to plant species manipulation and nutrient addition have been previously observed mainly for aboveground structures (i.e., sporocarps) (Wallenda and Kottke 1998) and recently for belowground structures (Peter et al. 2001). In the present study, which focuses on belowground structures (i.e., morphotypes in colonized roots), morphotype A decreased in the absence of *Ledum* and under nutrient addition, whereas morphotype B, which we believe to be *Cenococcum geophilum*, and morphotype D, increased their rates of colonization. These findings suggest that the different fungal symbionts differ in their competitive advantage, depending on community composition and nutrient availability (Deacon and Fleming 1992).

The lack of response of ericoid mycorrhizal colonization to nutrient addition is consistent with other field experiments (Caporn et al. 1995; Michelsen et al. 1999; Johanson 2000). Together these results demonstrate that ericoid mycorrhizas differ from other mycorrhizal types in their lack of response to nutrient addition. The unexpected decline in the proportion of ericoid roots colonized by mycorrhizal fungi in response to *Betula* and *Ledum* removal, therefore, cannot be explained as a release from competition for nutrients, because fertilization had no effect on ericoid mycorrhizas. Michelsen et al. (1995) suggested two possible mechanisms by which *Betula pubescens* may negatively affect two ericoid species: a negative (perhaps allelopathic) effect of *Betula* on ericoid species or a greater availability of labile C when *Betula* is present that promotes microbial activity and uptake, depleting available N and P pools for ericoid

species. Regardless of the mechanism, it seems that the ericoid mycorrhizal colonization responded to the presence of *Betula* rather than the direct effect of nutrient availability.

In summary, our results suggest that ecto- and ericoid mycorrhizas respond to nutrient addition and species interactions in different ways. Thus, they may play an important role in mediating competitive interactions among plants in this strongly nutrient-limited arctic ecosystem (Chapin and Shaver 1985; Chapin et al. 1995; Hobbie and Chapin 1998; Hobbie et al. 1999; Shaver et al. 2001). Ectomycorrhizas of *Betula* act as an extension of the root system, changing in abundance and composition in response to *Ledum* removal in the same way as they respond to changes in nutrient availability. Ericoid mycorrhizas, however, respond to changes in community composition in ways that are best explained as a direct response to *Betula* removal, rather than to changes in nutrient supply. Mycorrhizas therefore might influence the competitive balance among plant species through different interacting mechanisms that must be considered in any mechanistic approach to understanding the current or future composition of arctic plant communities.

Acknowledgements We thank Joanna Wagner, Suzanne Lippert, Kristen Laughridge, Sonia Nosratinia, Laura Gold, Cameron Walker, Joshua Whorley, Erica Garcia, Kristin Lebacqz, Vincianne Sacré, David Cook, Stacey Young, Theresa McHugh, Jesse Burns, Peter Ray, and Gus Shaver for help in establishing and maintaining this experiment. We gratefully acknowledge the U.S. National Science Foundation (grant no. OPP-9896302, OPP-ATLAS 9896302), IAI (grant no. ISP III-3018), and Fundación Antorchas for their financial support.

References

- Abbott LK, Robson AD, De Boer G (1984) The effect of phosphorus on the formation of hyphae in soil by the fungus, *Glomus fasciculatum*. *New Phytol* 97:437–446
- Aerts R, Bobbink R (1999) The impact of atmospheric nitrogen deposition on vegetation processes in terrestrial non-forest ecosystems. In: Langan S (ed) *The impact of nitrogen deposition on natural and semi-natural ecosystems*. Kluwer, Dordrecht, pp 85–122
- Aerts R, Chapin III FS (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Adv Ecol Res* 30:1–67
- Agerer R (1987–1996) *Colour atlas of ectomycorrhizae*. Einhorn, Schwäbisch Gmünd, Germany
- Allen MF (1991) *The ecology of mycorrhizae*. Cambridge University Press, Cambridge
- Allen E, Allen MF (1991) The mediation of competition by mycorrhizae in successional and patchy environments. In Grace JB, Tilman D (eds) *Perspectives on plant competition*. Academic Press, San Diego, Calif., pp 367–389
- Binkley D (1984) Ion exchange resin bags: factors affecting estimates of nitrogen availability. *Soil Sci Soc Am J* 48:1181–1184
- Bret-Harte MS, Shaver GR, Zoerner JP, Johnstone JF, Wagner JL, Chavez AS, Gunkelman IV RF, Lippert SC, Laundre JA (2001) Developmental plasticity allows *Betula nana* to dominate tundra subjected to an altered environment. *Ecology* 82:18–32

- Bret-Harte MS, Shaver GR, Chapin FS III (2002) Primary and secondary growth in arctic shrubs: implications for community response to environmental change. *J Ecol* 90:251–267
- Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture. *ACIAR Monogr* 32
- Caporn SJ, Song MW, Read DJ, Lee JA (1995) The effect of repeated fertilization on mycorrhizal infection in heather [*Calluna vulgaris* (L.) Hull]. *New Phytol* 129:605–609
- Chapin FS III, Shaver GR (1985) Individualistic growth response of tundra plant species to environmental manipulations in the field. *Ecology* 66:564–576
- Chapin FS III, Shaver GR (1996) Physiological and growth responses of arctic plants to a field experiment simulating climatic change. *Ecology* 77:822–840
- Chapin FS III, Shaver GR, Giblin AE, Nadelhoffer KJ, Laundre JA (1995) Response of arctic tundra to experimental and observed changes in climate. *Ecology* 76:694–711
- Chapin FS III, Bret-Harte MS, Hobbie SE, Zhong H (1996) Plant functional types as predictors of transient responses of arctic vegetation to global change. *J Veg Sci* 7:347–358
- Deacon JW, Fleming LV (1992) Interactions of ectomycorrhizal fungi. In: Allen M (ed) *Mycorrhizal functioning*. Chapman & Hall, New York, pp 249–300
- Díaz S, Symstad AJ, Chapin FS III, Wardle DA, Huenneke LF (2003) Functional diversity revealed by removal experiments. *Trends Ecol Evol* 18:140–146
- Giblin AE, Nadelhoffer KJ, Shaver GR, Laundre JA, McKerron AJ (1991) Biogeochemical diversity along a riverside toposequence in arctic Alaska. *Ecol Monogr* 61:415–435
- Grace C, Stribley DP (1991) A safer procedure for routine staining of vesicular-arbuscular mycorrhizal fungi. *Mycol Res* 95:1160–1162
- Helm DJ, Allen EB, Trappe JM (1996) Mycorrhizal chronosequence near Exit Glacier, Alaska. *Can J Bot* 74:1496–1506
- Helm DJ, Allen EB, Trappe JM (1999) Plant growth and ectomycorrhizae formation by transplants on deglaciated land near Exit Glacier, Alaska. *Mycorrhiza* 8:297–304
- Hobbie SE, Chapin III FS (1998) The response of tundra plant biomass, aboveground production, nitrogen, and CO₂ flux to experimental warming. *Ecology* 79:1526–1544
- Hobbie SE, Shevtsova A, Chapin III FS (1999) Plant responses to species removal and removal warming in Alaskan tussock tundra. *Oikos* 84:417–434
- Hultén E (1968) *Flora of Alaska and neighboring territories*. Stanford University Press, Stanford
- Jasper, DA, Robson AD, Abbott LK (1979) Phosphorus and the formation of vesicular-arbuscular mycorrhizas. *Soil Biol Biochem* 11:501–505
- Johansson M (2000) The influence of ammonium nitrate on the root growth and ericoid mycorrhizal colonization of *Calluna vulgaris* (L.) Hull from a Danish heathland. *Oecologia* 123:418–424
- Lajtha K (1988) The use of ion exchange resin bags for measuring nutrient availability in an arid ecosystem. *Plant Soil* 105:105–111
- Lilleskov EA, Bruns T (2001) Nitrogen and ectomycorrhizal fungal communities: what we know, what we need to know. *New Phytol* 149:154–158
- McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol* 115:495–501
- McKane RB, Johnson LC, Shaver GR, Nadelhoffer KJ, Rastetter EB, Fry B, Giblin AE, Kielland K, Kwiatkowski B, Laundre JA, Murray G (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature* 415:68–71
- Michelsen A, Schmidt IK, Jonasson S, Dighton J, Jones HE, Callaghan T (1995) Inhibition of growth, and effects on nutrient uptake of arctic graminoids by leaf extracts—allelopathy or resource competition between plants and microbes? *Oecologia* 103:407–418
- Michelsen A, Graglia E, Schmidt I, Jonasson S, Sleep D, Quarmby C (1999) Differential responses of grass and dwarf shrub to long-term changes in soil microbial biomass C, N and P following factorial addition of NPK fertilizer, fungicide and labile carbon to a heath. *New Phytol* 143:523–538
- Moore-Parkhurst S, Eglander L (1982) Mycorrhizal status of *Rhododendron* spp. in commercial nurseries in Rhode Island. *Can J Bot* 60:2342–2344
- Nadelhoffer KL, Shaver GR, Fry B, Giblin AE, Johnson LC, McKane RB (1996) ¹⁵N natural abundance and N use by plants. *Oecologia* 107:386–394
- Norusis MJ (1997) *SPSS for Windows. Advanced statistics, Release 5*
- Omar MB, Bolland L, Heather WA (1979) A permanent mounting medium for fungi. *Bull Br Mycol Soc* 13:31–32
- Peter M, Ayer F, Egli S (2001) Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. *New Phytol* 149:311–325
- Read DJ (1991) Mycorrhizas in ecosystems. *Experientia* 47:376–391
- Shaver GR, Chapin III FS (1980) Response to fertilization by various plant growth forms in an Alaskan tundra: nutrient accumulation and growth. *Ecology* 61:662–675
- Shaver GR, Chapin III FS (1986) Effect of fertilizer on production and biomass of tussock tundra, Alaska, USA. *Arct Alp Res* 18:261–268
- Shaver GR, Chapin III FS (1991) Production: biomass relationships and element cycling in contrasting arctic vegetation types. *Ecol Monogr* 61:1–31
- Shaver GR, Bret-Harte MS, Jones MH, Johnstone JF, Gough L, Chapin III FS (2001) Species composition interacts with fertilizer to control long-term change in tundra productivity. *Ecology* 82:3163–3181
- Stribley DP, Read DJ (1976) The biology of mycorrhiza in the Ericaceae. VI. The effects of mycorrhizal infection and concentration of ammonium nitrogen on growth of cranberry (*Vaccinium macrocarpon* Ait.) in sand culture. *New Phytol* 77:63–72
- Wallenda T, Kottke I (1998) Nitrogen deposition and ectomycorrhizas. *New Phytol* 139:169–187
- Whitledge TE, Mallow SC, Patton CJ, Wirick CD (1981) Automated nutrient analysis in seawater. Technical report. Ocean Science Division, Brookhaven National Laboratory, Upton, N.Y.
- Winer BJ, Brown DR, Michels KM (1991) *Statistical principles in experimental design*, 3rd edn. McGraw-Hill, New York
- Zar JH (1999) *Biostatistical analysis*, 4th edn. Prentice Hall, Upper Saddle River, N.J.