Mycorrhizal fungi supply nitrogen to host plants in Arctic tundra and boreal forests: ^{15}N is the key signal[1]

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Abstract: Symbiotic fungi's role in providing nitrogen to host plants is well-studied in tundra at Toolik Lake, Alaska, but little-studied in the adjoining boreal forest ecosystem. Along a 570 km north–south transect from the Yukon River to the North Slope of Alaska, the ^{15}N content was strongly reduced in ectomycorrhizal and ericoid mycorrhizal plants including *Betula*, *Salix*, *Picea mariana* (P. Mill.) B.S.P., *Picea glauca* Moench (Voss), and ericaceous plants. Compared with the ^{15}N content of soil, the foliage of nonmycorrhizal plants (*Carex* and *Eriophorum*) was unchanged, whereas content of the ectomycorrhizal fungi was very much higher (e.g., *Boletaceae*, *Leccinum* and *Cortinarius*). It is hypothesized that similar processes operate in tundra and boreal forest, both nitrogen-limited ecosystems: (*i*) mycorrhizal fungi break down soil polymers and take up amino acids or other nitrogen compounds; (*ii*) mycorrhizal fungi fractionate against ^{15}N during production of transfer compounds; (*iii*) host plants are accordingly depleted in ^{15}N; and (*iv*) mycorrhizal fungi are enriched in ^{15}N. Increased N availability for plant roots or decreased light availability to understory plants may have decreased N allocation to mycorrhizal partners and increased $\delta^{15}N$ by 3‰–4‰ for southern populations of *Vaccinium vitis-idaea* L. and *Salix*. Fungal biomass, measured as ergosterol, correlated strongly with soil organic matter and attained amounts similar to those in temperate forest soils.

Key words: mycorrhizal fungi, ^{15}N, nitrogen cycling, symbiosis, nitrogen isotopes.

Résumé : Le rôle des champignons symbiotiques dans l'apport en azote des hôtes est bien étudié dans la toundra, au lac Toolik, en Alaska, mais peu étudié dans l'écosystème adjacent que constitue la forêt boréale. Le long des 570 km du transect nord–sud allant de la rivière Yukon au versant nord de l'Alaska, le $\delta^{15}N$ était fortement réduit chez les plantes ectomycorhizes et mycorhizes éricoïdes, notamment *Betula*, *Salix*, *Picea mariana* (P. Mill.) B.S.P., *Picea glauca* Moench (Voss) et les éricacées. Le feuillage des plantes non mycorhizes (*Carex* et *Eriophorum*) demeure inchangé en fonction du contenu du sol en ^{15}N, alors que le contenu des champignons ectomycorhizes est beaucoup plus élevé (e.g., *Bolétacées*, *Leccinum* et *Cortinarius*). On a supposé que des processus similaires opèrent dans la toundra et la forêt boréale, deux écosystèmes limités en azote : (*i*) les champignons mycorhizes scindent les polymères du sol et incorporent les acides aminés ou les autres composés azotés; (*ii*) les champignons mycorhizes favorisent le ^{15}N lors du transfert des composés; (*iii*) les plantes hôtes sont conséquemment appauvries en ^{15}N; et (*iv*) les champignons mycorhizes sont enrichis en ^{15}N. La disponibilité accrue de l'azote pour les racines des plantes ou la plus faible disponibilité de lumière pour les plantes en sous-étage peut avoir diminué la portion d'azote allouée aux partenaires des mycorhizes et augmenté le $\delta^{15}N$ de 3‰–4‰ chez les populations de *Vaccinium vitis-idaea* L. et de *Salix* du sud. La biomasse fongique, mesuré sous forme d'ergostérol, est en forte corrélation avec la matière organique du sol et atteint des quantités similaires à celles qui sont retrouvées dans les sols forestiers des régions tempérées.

Mots-clés : champignons mycorhizes, ^{15}N, cycle de l'azote, symbiose, azote isotopique.

[Traduit par la Rédaction]

Received 27 October 2008. Accepted 28 October 2008. Published on the NRC Research Press Web site at cjm.nrc.ca on 3 February 2009.

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[1]This article is one of a selection of papers in the Special Issue on Polar and Arctic Microbiology.
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Introduction

The symbiosis between mycorrhizal fungi and plants is important for plant nutrition in most forests and shrublands, including tundra and boreal forests. In these biomes, nitrogen (N) strongly limits plant growth (Nadelhoffer et al. 1996). As pointed out by Read and Perez-Moreno (2003), it is likely that the large surface area and enzymatic capabilities of ectomycorrhizal (ECM) and ericoid mycorrhizal fungi allow their symbiotic plant hosts to access N from soil pools of amino acids, amino sugars, protein, and chitin. The indirect evidence for this N transfer comes from growth of these fungi on protein-rich media, physiological data on N transfer compounds, and observations that ECM fungi with strong proteolytic capabilities disappear from regions where atmospheric N deposition is high (Taylor et al. 2000; Lilleskov et al. 2002). Additional support for the ability of mycorrhizal fungi to assimilate organic N and supply their host plants with N comes from studies using isotopic tracers (Taylor et al. 2004), studies on the growth of ECM plants on organic N sources (Finlay et al. 1992), and from studies examining the mycorrhizal genes involved in organic N transport and metabolism (Müller et al. 2007).

Another tool for evaluating N transfer is the natural abundance of ^{15}N (expressed as $\delta^{15}N$ values) in soils and plant foliage. A worldwide survey (J. Craine, unpublished data, described in Hobbie and Hobbie (2008)) revealed that the ^{15}N content of foliage and soils was lower in early successional environments, boreal forests, and tundra than in temperate and tropical forests and that foliar $\delta^{15}N$ varied with temperature, precipitation, N concentration, and mycorrhizal type. Site-specific studies have also concluded that mycorrhizal fungi influenced plant $\delta^{15}N$ (Schmidt and Stewart 1997; Michelsen et al. 1998). Amundson et al. (2003) reviewed a number of processes that would result in isotopic discrimination including nitrogen fixation, assimilation of ammonium and nitrate, denitrification, and plant interactions with mycorrhizal fungi. These different processes could result in pools of soil N, each with a different value of $\delta^{15}N$. This was the explanation suggested by Nadelhoffer et al. (1996) who found a large difference among the $\delta^{15}N$ of foliage from plants at a tundra site; these authors and Amundson et al. (2003) postulated that the various types of plants accessed different pools of soil N.

However, Högberg (1990), based on different $\delta^{15}N$ values for arbuscular mycorrhizal and ECM trees in Africa, realized that symbiotic fungi were likely involved in causing the differences in $\delta^{15}N$ among plants, and proposed a direct link between mycorrhizal fungi and differences in $\delta^{15}N$ in plant foliage. He attributed the differences to fractionation during uptake of nitrogen compounds into the fungi. This was an important step in providing a mechanistic explanation for the patterns observed in nature (Schmidt and Stewart 1997). For example, Nadelhoffer et al. (1996) did not include mycorrhizal fungi in their study of $\delta^{15}N$ in tundra plants; for the same tundra site Hobbie and Hobbie (2006) showed that the distribution of observed foliar $\delta^{15}N$ could be completely explained by the type of plant-fungal symbiosis present, i.e., nonmycorrhizal, arbuscular mycorrhizal, ECM, or ericoid mycorrhizal.

In a detailed review, Hobbie and Hobbie (2008) hypothesized that under N-limited conditions the processes involved in the transfer of N from organic compounds in the soil to fungi and plants (Fig. 1) included the following steps: (*i*) fungal enzymes hydrolyze soil protein into amino acids and oligopeptides; (*ii*) subsequent uptake of those compounds into hyphae is without fractionation against ^{15}N (Nadelhoffer and Fry 1994; Goericke et al. 1994; Hobbie and Hobbie 2006); (*iii*) biochemical transformations, such as aminotransferase reactions (Macko et al. 1986), discriminate against ^{15}N by the quantity Δ_f and produce ^{15}N-depleted amino groups within fungal hyphae; (*iv*) ^{15}N-depleted amino groups, either as amino acids or ammonium, are transferred to host plants that are therefore depleted in ^{15}N value relative to the ^{15}N assimilated from soil; (*v*) because an isotopic mass balance must be preserved, nitrogen compounds incorporated into fungal hyphae and fruiting bodies are enriched in ^{15}N relative to N assimilated from the soil. The effects of these processes on $\delta^{15}N$ values can be quantified in the following 2 equations:

$$[1] \qquad \delta^{15}N_{plant} = \delta^{15}N_{available\ nitrogen} - \Delta_f(1 - T_r)f$$

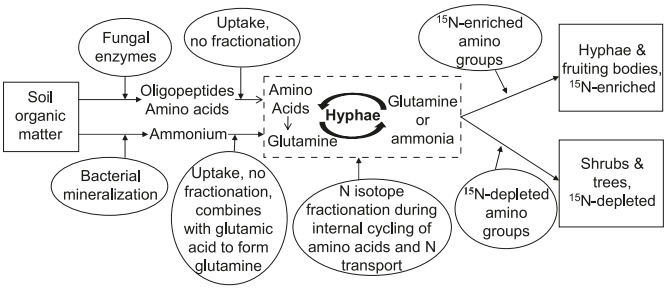
$$[2] \qquad \delta^{15}N_{fungi} = \delta^{15}N_{available\ nitrogen} + \Delta_f T_r$$

where T_r is the fraction of fungally assimilated N that is transferred to host plants and f is the proportion of plant N that is derived from fungal transfer.

Note that the simultaneous enrichment of ^{15}N in fungal fruiting bodies and the depletion of ^{15}N in plant foliage cannot be explained if the fractionation is due to pools of ^{15}N-depleted N available in the soil. These would be produced when gases were lost or a chemical product was produced. However, as noted in Hobbie and Hobbie (2008), it might be possible that the amount of final depletion in the foliage $\delta^{15}N$ would be larger if the source soil protein was depleted and acted upon by fungal fractionation. There is as yet no evidence for this source depletion, and the natural abundance of the different forms of organic N of the soil needs to be determined. Thus, the key for interpretation of the $\delta^{15}N$ signal is to sample the $\delta^{15}N$ of plant foliage, soil, and ECM fungi. The ericoid mycorrhizal fungi do not produce aboveground fruiting bodies, but the $\delta^{15}N$ evidence is that their internal fractionation process is similar to ECM fungi (Hobbie and Hobbie 2006). The only data, however, from northern regions that include mycorrhizal fruiting bodies are a few measurements from Arctic and subarctic sites in Sweden, Greenland, and Siberia reported by Michelsen et al. (1998) and measurements at Toolik Lake, Alaska, reported by Hobbie and Hobbie (2006) and Clemmensen et al. (2006).

We also make use of the data on the $\delta^{15}N$ of plant foliage and the percentage of N in needles collected along the boreal forest portion of the transect by Schulze et al. (1994). At the time of this collection in 1990, it had not been realized that ECM and ericoid mycorrhizal fungi might be involved in the fractionation; therefore, no fungal fruiting bodies were collected. The authors did mention fungi, however, and concluded that plants with different types of fungi, ECM versus ericoid mycorrhizal fungi, were accessing pools of N compounds in the soil with different values for $\delta^{15}N$. The combination of the Schulze et al. (1994) and the later transect data allow us to ask if differences in dependence on the mycor-

Fig. 1. The likely processes that occur as organic N in the soil moves through microbes into fungi and plants; N isotopes are fractionated within the fungal hyphae before amino groups are transferred into plant roots. This scheme applies to ectomycorrhizae and ericoid mycorrhizae.



rhizal fungi for N can explain the variation in foliar $\delta^{15}N$ noted in the transect.

In this paper we extend the $\delta^{15}N$ data in Alaska beyond the Toolik Lake site with samples along a 570 km transect from the Yukon River north to Prudhoe Bay (Fig. 2). The transect crosses the tundra–boreal forest border and includes $\delta^{15}N$ values of soils, vegetation, and fruiting bodies of both ECM fungi and saprotrophic fungi. We include in our figures the $\delta^{15}N$ foliage values from the same transect published by Schulze et al. (1994). The $\delta^{15}N$ data from plants in Denali National Park, south of the transect, provide additional information on $\delta^{15}N$ in canopy versus understorey plants of different mycorrhizal types. Fungal biomass in soil profiles from the transect was estimated using ergosterol, a compound unique to fungi.

We ask the following questions: Is the distribution of $\delta^{15}N$ values in soils, plant foliage, and mycorrhizal fungi in the Arctic similar to those in boreal sites? Does the hypothesis of fractionation against ^{15}N within fungal hyphae appear to explain the transect patterns? Do differences in dependence on mycorrhizal fungi for their N explain differences in plant $\delta^{15}N$? Do estimates of fungal biomass explain some of the shifts in $\delta^{15}N$ among various sites along the transect?

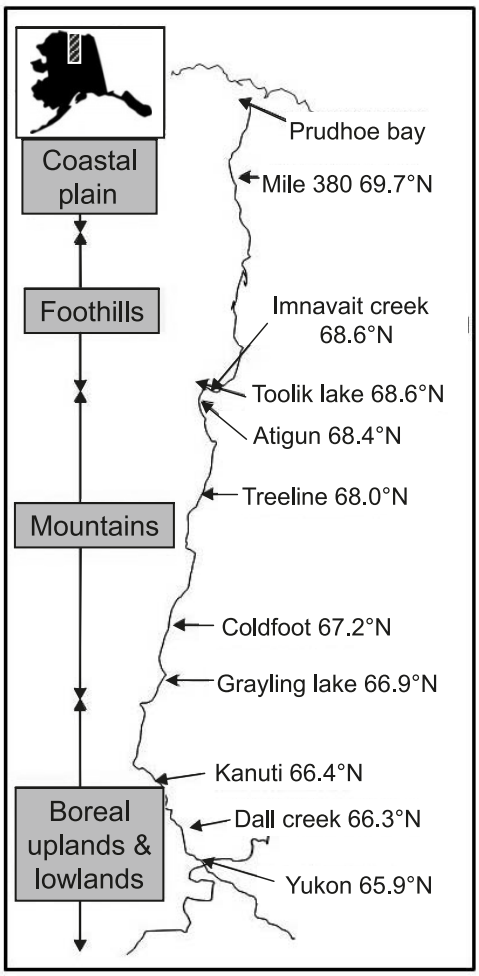
Materials and methods

Locations and protocols for sampling soils, vegetation, and fungi

Collections were primarily made along the Dalton Highway, the pipeline service road that runs from slightly south of the Yukon River north to Prudhoe Bay, Alaska (Fig. 2). Data from 3 Yukon–Arctic transects along the Dalton Highway in Alaska are included in this report, but only the first of these, by Schulze et al. (1994), has been published. Their study, in 1990, measured concentrations of inorganic N in soils, and $\delta^{15}N$ and concentrations of N, P, Mg, K, and Ca in foliage of white spruce (*Picea glauca* Moench (Voss)), black spruce (*Picea mariana* (P. Mill.) B.S.P.), and lingonberry (*Vaccinium vitis-idaea* L.). The second transect study, by M. Conte and J.C. Weber in 2004 (unpublished data), measured $\delta^{15}N$ and $\delta^{13}C$ as well as concentrations of N and C in trees, shrubs, litter, and soils. The third sampling transect, by J.E. Hobbie, E.A. Hobbie, H. Drossman, and J. Shamhart in 2007 (unpublished data), made the same measurements as Conte and Weber but added the $\delta^{15}N$ and $\delta^{13}C$ of the fungal fruiting bodies and the soil ergosterol concentrations. Additional Toolik Lake data for $\delta^{15}N$ in fungal fruiting bodies used here are from Clemmensen et al. (2006) and Hobbie and Hobbie (2006). Exact locations for each sample are given in Supplementary Table S1.[3] In addition, we present a table of $\delta^{15}N$ data from the foliage of 11 plant species in a white spruce–balsam popular stand near Denali National Park sampled by E.A. Hobbie and located at 64.1°N and 148.6°W (Table 1).

Foliage samples at each site were collected in 2004 and 2007 from at least 3 individual plants of each species. Ten leaves per individual were clipped and pooled before drying. Later, samples from each plant type were pooled and ground before isotope analysis.

Fig. 2. Sampling sites along the Dalton Highway, northern Alaska. The highway lies between 149°W and 150°W longitude and runs from 65°N to 70°N at Prudhoe Bay, on the Arctic Ocean.



[3] Supplementary data for this article are available on the journal Web site (http://cjm.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3890. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.

Table 1. Nitrogen isotope values (±SE; $n = 5$) for plant taxa in a white spruce (*Picea glauca*) – balsam poplar (*Populus balsamifera*) forest at Rock Creek watershed, Denali National Park, Alaska.

Plant taxa	$\delta^{15}N \pm SE$ (‰)	Root symbiont
Picea glauca	–4.7±0.5a*	Ectomycorrhizal
Populus neoalaskensis	–4.4±0.2a	Ectomycorrhizal
Geocaulon lividum	–4.1±0.4ab	Hemiparasite†
Arctostaphylos sp.	–2.9±0.7abc	Arbutoid/ectomycorrhizal
Vaccinium vitis-idaea	–1.1±1.0bcd	Ericoid mycorrhizal
Empetrum nigrum	–0.8±0.4cd	Ericoid mycorrhizal
Ledum groenlandicum	–0.7±0.4cd	Ericoid mycorrhizal
Lupinus sp.	0.1±0.1cde	Rhizobial N_2 fixer
Vaccinium uliginosum	1.5±0.3def	Ericoid mycorrhizal
Salix sp.	2.7±0.3ef	Ectomycorrhizal
Pyrola secunda	3.5±1.4f	Arbutoid mycorrhizal

*Values not followed by the same letter are different at $p = 0.05$ according to a Tukey–Kramer post hoc test.
†Hemiparasitic plants tap into the root systems of other plants to capture nutrients from their host.

Soil and litter samples in 2004 were collected from each site with a trowel; intact leaves at the surface were discarded. Two layers were collected: the litter layer consisted of decomposing leaves with recognizable plant parts, while the upper soil layer immediately beneath consisted of dark-colored highly decomposed material (top 5 cm collected). In 2007, a 15–20 cm deep, 6 cm diameter core was collected at a representative site within 0.25 m of an example of the dominant vegetation type at each site. After collection, soil samples were immediately placed on ice and frozen within 6 h. In the laboratory, each core from the 2007 transect was thawed and sectioned into 3 organic layers of approximately equal depth and 2 mineral layers of approximately equal depth for analysis of ergosterol, soil moisture, and soil organic matter. When present, roots were removed by hand from each subsection when the sample was passed through a 2 mm screen.

The tree communities of the Interior Highlands of the boreal forest contain *Betula nana* L. (dwarf birch), *Betula glandulosa* Michx. (resin birch), *Betula papyrifera* Marsh. (paper birch), *Populus tremuloides* Michx. (quaking aspen), *Populus balsamifera* L. (balsam poplar), *Alnus fruticosa* Rupr. (Siberian alder), *Alnus incana* L. Moench ssp. *tenuifolia* (Nutt.) Breitung (thinleaf alder), *Salix* sp. including *Salix alaxensis* Andersson Coville (feltleaf willow), *Salix arbusculoides* Andersson (littletree willow), and *Salix arctica* Pall. (arctic willow) (Viereck and Little 2007). Within the Brooks Range trees become sparser until the northern treeline of spruce is reached at 68°N; farther north the vegetation of the Brooks Range becomes a dry heath tundra of *Carex* and *Eriophorum* (sedges), *Calamagrostis* (grass), ericaceous species such as *Ledum* (Labrador tea), *V. vitis-ideae*, *Vaccinium uliginosum* L. (blueberry), dwarf *Salix* species (willows), and *B. nana*. The vegetation of the Foothills includes tussock tundra composed of graminoids, shrub tundra of dwarf birch and willows, and dry heath tundra of ericaceous species. The Coastal Plain is mostly wet sedge tundra.

Fruiting bodies of ECM fungi and saprotrophs were collected at Toolik Lake in 2002 and 2003 (Clemmensen et al. 2006; Hobbie and Hobbie 2006) and along the entire transect in 2007, one of the driest summers on record. The fruiting bodies were dried at 50 °C. Our identification of these fungi was helped by discussion with G. Laursen of the University of Alaska, Fairbanks. A further analysis utilizing DNA and the growing sequence database at the University of Alaska is underway.

^{15}N analyses

The1990 collection was analyzed as described in Schulze et al. (1994). The Denali 1998 collection was analyzed on a Finnigan Delta-Plus linked to a Carlo Erba NC2500 elemental analyzer (Finnigan MAT GmbH, Bremen, Germany) located at the United States Environmental Protection Agency in Corvallis, Oregon. Laboratory standards for isotopic measurements were pine needles (NIST 1575) and acetanilide. The precision of duplicate samples was ±0.2‰. The 2004 collection was analyzed at the University of California, Davis Stable Isotope Facility with the same methods described in Hobbie and Hobbie (2006). Laboratory standards included peach leaves (NIST 1547) and apple leaves (NIST 1515). The 2007 collections were analyzed at the University of New Hampshire, Durham, New Hampshire, on a Finnigan Delta-Plus isotope ratio mass spectrometer coupled to a Costech CHN analyzer. Precision on duplicate samples was ±0.2‰. Concurrently run standards included acetanilide, pine needles (NIST 1575a), and apple leaves (NIST 1515). To express variations in the $^{15}N/^{14}N$ ratio for samples in a tractable form, they are referenced against a universal standard and calculated as $\delta^{15}N$ values, defined as $\delta^{15}N_{sample} = [(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{standard} - 1]1000$, with the standard the $^{15}N/^{14}N$ value of atmospheric N_2, which, therefore, has a $\delta^{15}N$ value of 0‰.

Ergosterol

This fungal-specific sterol, a component of the cell membrane, estimates living fungal biomass in soils. We modified the saponification methods described by Bååth (2001) and de Ridder-Duine et al. (2006) for HPLC quantification of ergosterol. Duplicate or triplicate subsamples (0.5 g) of the sieved soil fractions were saponified by heating for 90 min at 70 °C in 2 mL of methanol and 0.5 mL of 2 mol/L NaOH after brief vortex mixing. After cooling, 1 mL of

methanol and 3 mL of pentane were added and samples vortex-mixed for 20 s. Samples were centrifuged briefly to eliminate emulsions and the upper pentane phase, containing ergosterol, was collected. The pentane addition, extraction, and centrifugation was repeated twice more, but with 2 mL of pentane per extraction step. The pentane (upper) phase was collected and combined with previous extracts. The combined pentane extracts were dried under nitrogen flow, dissolved in 1.000 mL methanol, filtered through a 0.45 μm pore size PTFE syringe filter (Millipore Corporation, Billerica, Massachusetts) and injected into a Waters Acquity ultra performance liquid chromatography (UPLC) system with a binary solvent manager, a sample manager, and a photodiode array detector (PDA) (Waters Corporation, Milford, Massachusetts). Separation was on an Acquity UPLC BEH C18 1.7 μm particle size, 2.1 × 50 mm reverse phase column maintained at 40 °C. A 5 μL injection of each extract was eluted with 92% methanol – 8% water at a flow rate of 0.5 mL·min^{-1}. Using Millenium software (Waters) the UV spectrum of the ergosterol peak at ~2 min was compared with standard ergosterol (Fluka Company, St. Louis, Missouri) and was quantified at 282 nm with calibration standards in the range of 0.05–20 ppm. Calibration provided linear fit (r^2 > 0.999) with standards in all runs with a limit of detection (S/N = 3:1) of ~30 ppb. Spike recoveries determined by the method of de Ridder-Duine et al. (2006) are 82% ± 4%, in agreement with prior literature values (Bååth 2001). Variance for duplicate or triplicate samples resulting from sample inhomogeneity, extraction, and instrumental sources were generally <5% and are calculated as standard deviation for each sample.

The amount of ergosterol per g of dry soil was calculated as the concentration of ergosterol (from the UPLC peak area) times the final methanol volume of the ergosterol solution per g of dry mass of soil. The percentage of the soil organic matter made up of ergosterol is the μg of ergosterol per g of soil organic matter on a dry mass basis.

Several conversion factors have been used to calculate fungal biomass from ergosterol. Montgomery et al. (2000) proposed a factor of 4 mg fungal biomass per μg ergosterol based on a calibration with 6 species of cultured saprotrophic fungi, whereas Salmanowicz and Nylund (1988) proposed a factor of 3 mg of fungal biomass per μg of ergosterol based on cultures of 3 different species of ECM fungi. We followed Clemmensen et al. (2006) and used 3 mg·μg^{-1} as this reflects the fungal taxa most commonly observed at our sampling sites.

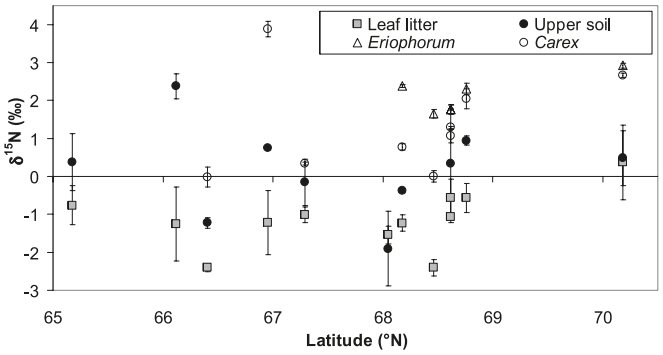
Soil moisture was determined gravimetrically on a separate subsample by drying to constant mass. Soil organic matter was calculated from the loss in mass of the dry soil after heating at 500 °C for 12 h.

Results

^{15}N of leaf litter and upper soil

Most samples of soil along the transect had a δ^{15}N slightly greater than the 0‰ value assigned to atmospheric N_2 (Fig. 3 and Table S1).[3] The values are similar to those of Nadelhoffer et al. (1996) for the Toolik Lake region (δ^{15}N of –3‰ to 1‰) and those of Hobbie and Hobbie (2006) (δ^{15}N of 1‰ to 2‰).

Fig. 3. Measurements of δ^{15}N in single samples of leaf litter, soil, *Eriophorum*, and *Carex* along the 2004 transect. Standard deviation represents the analytical error and sample heterogeneity for duplicate subsamples of single field samples.



^{15}N of plant foliage

Most plant species analyzed for δ^{15}N were symbiotic with ECM or ericoid mycorrhizal fungi. Exceptions along the transect included the nonmycorrhizal sedges *Carex* and *Eriophorum* (Chapin et al. 1988, 1993) and the arbuscular mycorrhizal *Rubus*. In Fig. 4 the *Rubus* samples stand out as the only plants with positive foliar δ^{15}N (2‰–4‰).

Ericoid mycorrhizal plants and ECM plants had negative δ^{15}N values along the gradient from the Yukon River to the North Slope. Only one of these plants (a *Vaccinium*) north of the treeline (~68°N) had a δ^{15}N more positive than –3‰. In contrast, many plants collected south of the treeline had δ^{15}N values between 0‰ and –3‰. With data from Schulze et al. (1994) on *Vaccinium vitis-idaea,* foliar δ^{15}N is significantly different (p = 0.001, 1-tailed t test) between northern locations (67.76°N–68.03°N) characterized by open boreal forest (mean = –5.6 ± 0.5‰, n = 10) and southern locations (66.33°N–66.81°N) characterized by more closed forests (mean = –2.5 ± 0.7‰, n = 8). A significant difference (p = 0.005, 1-tailed t test) also exists between *Salix* collected north of 67.76°N (mean = –4.7 ± 0.5‰, n = 7) or south of 67.76° (mean = –1.3‰, n = 4).

In a closed canopy white spruce – balsam poplar stand at Denali National Park, foliage from 11 different plant species were sampled. The 2 canopy dominants and the root parasite *Geocaulon lividum* Fern. (false toadflax) were lowest in δ^{15}N (<–4‰), ericoid mycorrhizal plants ranged from –1‰ for *Vaccinium vitis-idaea* to 1.5‰ for *Vaccinium uliginosum*, the N-fixing plant *Lupinus* was 0‰, ECM *Salix* was 3‰, and the arbutoid mycorrhizal plant *Pyrola* averaged 3.5‰ (Table 1).

δ^{15}N of fungi

Both ECM and saprotrophic fungi were analyzed for δ^{15}N. At Toolik Lake, δ^{15}N values for taxa of ECM fungi ranged from 2.5‰ for *Laccaria* to 7.4‰ for *Leccinum* (Fig. 5). Saprotrophic fungi collected at Toolik Lake varied from –3.1‰ for *Marasmius* to 6.9‰ for a single specimen of *Hypholoma* (Fig. 5). Specimens of ECM fungi were generally enriched in ^{15}N relative to the soil across the gradient (Figs. 3–5).

Fig. 4. The $\delta^{15}N$ of vegetation and ectomycorrhizal (ECM) fungi collected along the transect in 1990, 2004, and 2007. The data from 1990, for black spruce (*Picea mariana*), white spruce (*Picea glauca*), and *Vaccinium*, are from Schulze et al. 1994. Foliage data from 2004 and 2007 include the same species as well as *Ledum*, *Empetrum*, *Cassiope*, *Rubus*, *Betula*, and *Salix*. The fruiting bodies of ECM fungi were collected in 2007 and are reported as average values for each taxa at each collection site.

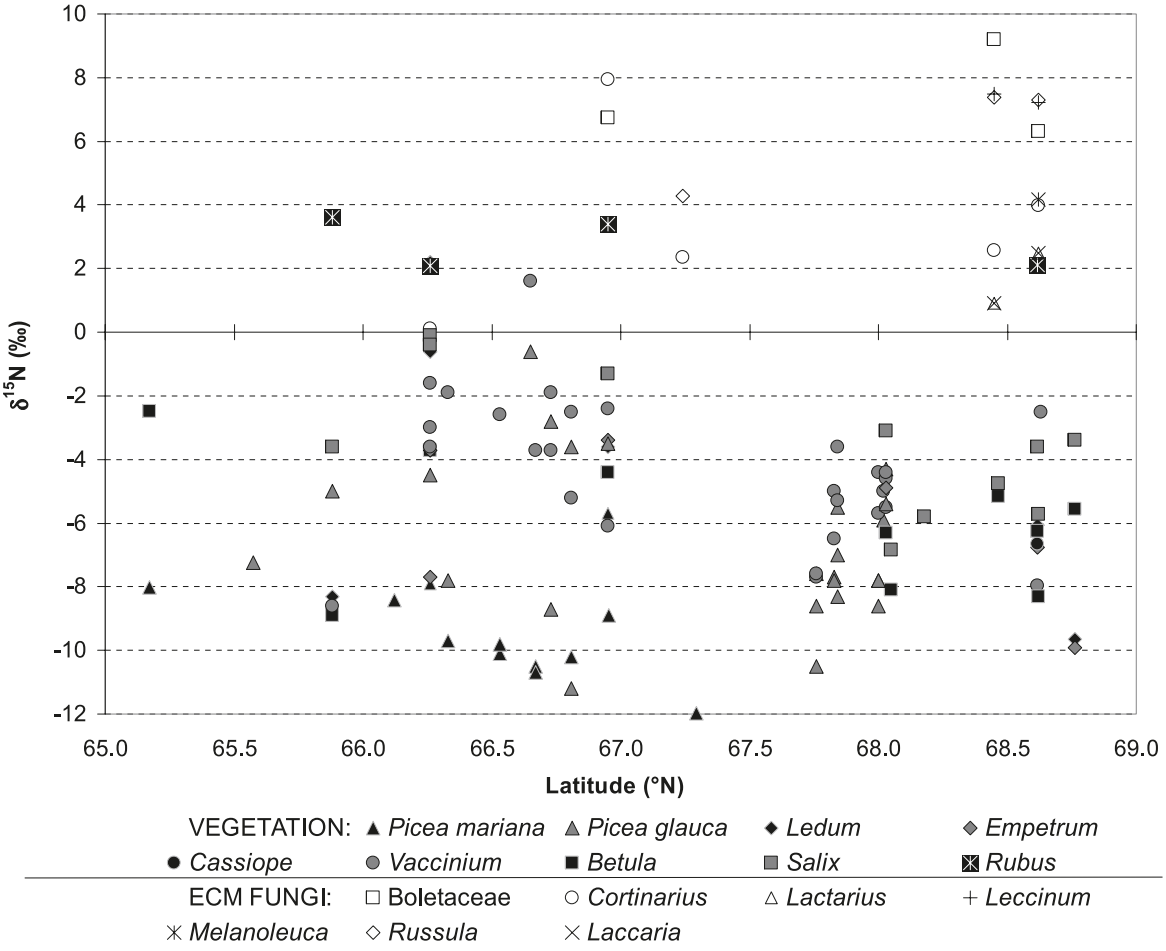
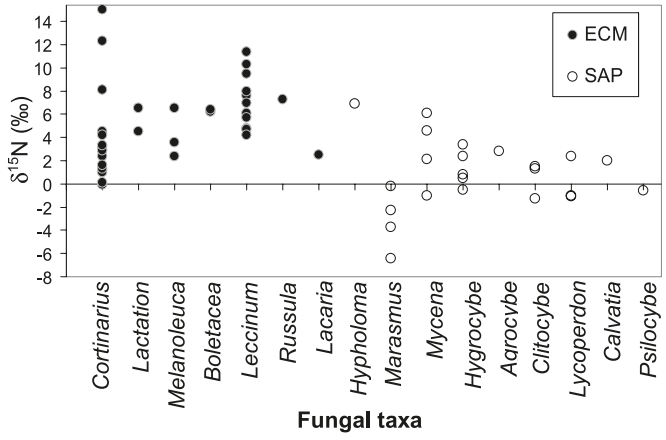


Fig. 5. The $\delta^{15}N$ for individual ectomycorrhizal (ECM) and saprotrophic (SAP) fungi collected at Toolik Lake, Alaska. Fungi were collected in 2002 (Clemmensen et al. 2006), 2003 (Hobbie and Hobbie 2006), and 2007.



Ergosterol

The concentration of ergosterol along the transect ranged from 3 to 303 μg ergosterol/g soil organic matter (SOM) with highest concentrations generally at the soil surface (Fig. 6). Along the transect, ergosterol concentrations correlated strongly with percent SOM (r^2 = 0.76) (Fig. 7). Ergosterol concentrations measured in tussock tundra at Toolik Lake (81–243 μg/g SOM in Fig. 6) overlapped with the 50–160 μg/g SOM reported by Clemmensen et al. (2006) for control plots at the same location. Three soil cores were analyzed at the Toolik Lake site to provide an idea of the in-site variance. The data for all 3 Toolik Lake sites are included in Fig. 6 and Fig. 7.

Discussion

^{15}N in soils, plants, and fungal fruiting bodies

There was no apparent pattern in the soil and litter $\delta^{15}N$ values along the transect (Fig. 3). The soil $\delta^{15}N$ varied by 2‰ around 0‰ in response to low $\delta^{15}N$ from leaf litter, litter with slightly positive $\delta^{15}N$ from the nonmycorrhizal plants (*Carex* and *Eriophorum*) shown in Fig. 3, and mycorrhizal hyphae with positive $\delta^{15}N$. As also found in many forests, soil $\delta^{15}N$ values increased with depth by 1.5‰ within the or-

Fig. 6. Ergosterol content of soils at sites ordered from south to north along the transect. Standard deviation on 2–3 analytical subsamples is shown. The horizontal line in each site diagram is the depth of the transition from organic to mineral soil. Three separate sample sites at the Toolik Lake, Alaska, site are shown.

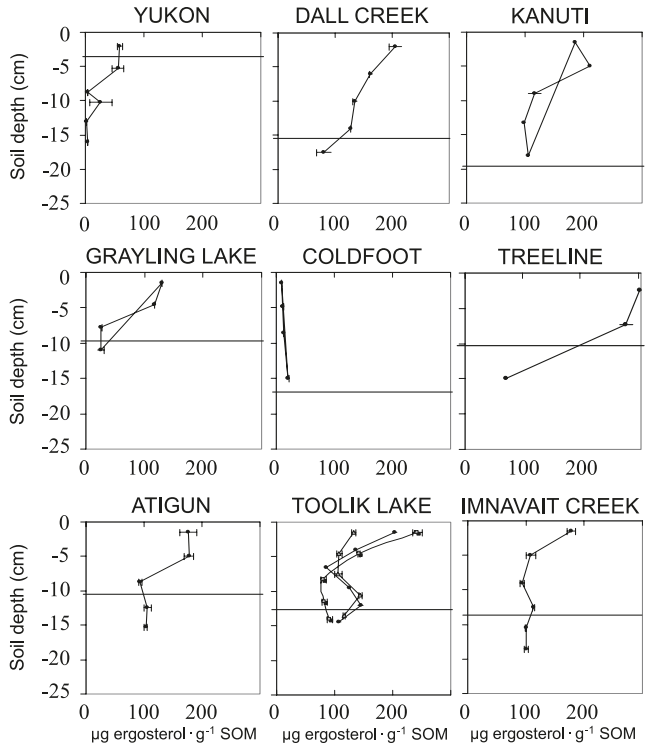
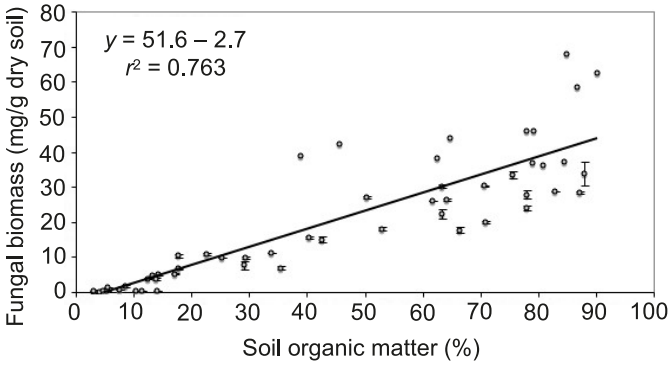


Fig. 7. Regression of soil organic matter versus fungal biomass estimated from ergosterol. Error bars represent standard deviations owing to sample inhomogeneity, extractions, and instrumental analysis (n = 2–3). Data are from 2007 transect.



ganic layer at Toolik Lake and by 2‰–6‰ with depth across various ecosystems at 1 nearby site (Nadelhoffer et al. 1996). Lindahl et al. (2007) suggested that the widely recognized increases with depth in soils were due to hyphae.

The positive values for *Rubus* (Fig. 4) throughout the transect were similar to those found by Hobbie and Hobbie (2006) and Nadelhoffer et al. (1996) who measured, respectively, values of 2.2‰ and 1.5‰ for *Rubus* at Toolik Lake. Similarly positive $\delta^{15}N$ values were recorded for the nonmycorrhizal *Carex* and *Eriophorum* (Fig. 3), and the arbuscular mycorrhizal grass *Calamagrostis canadensis* (Michx.) Beauv. had an average $\delta^{15}N$ value of 1‰ along the transect of Schulze et al. (1994) (data not shown). Arbuscular mycorrhizal fungi lack the proteolytic capabilities (Chalot and Brun 1998) present in many ECM and ericoid mycorrhizal fungi (Read and Perez-Moreno 2003). Although direct measures of carbon allocation to arbuscular mycorrhizal fungi are few (Johnson et al. 2002; Gavito and Olsson 2003), the available evidence and the much smaller spatial extent of arbuscular mycorrhizal fungal hyphae relative to ECM fungal hyphae indicate that allocation to arbuscular mycorrhizal fungi is less than to ECM fungi. For example, arbuscular mycorrhizal hyphae extend up to 6–10 cm from roots, whereas ECM hyphae extend up to several meters from roots (Coleman et al. 2004). Both of these factors may influence the apparently weak ability of arbuscular mycorrhizal fungi to alter the $\delta^{15}N$ signature of N that they may take up and then transfer to their host plants.

The $\delta^{15}N$ values of plants and ECM fungi along the transect (Fig. 4) are quite similar to values predicted from the Toolik Lake data (Nadelhoffer et al. 1996; Hobbie and Hobbie 2006). With the exception of *Rubus chamaemorus* L., trees and shrubs are depleted in ^{15}N, whereas the ECM fungi are enriched. Note that the averages of the various plants and fungi are plotted and do not reflect the abundance of the forms. *Leccinum* and other *Boletaceae*, for example, were very abundant. The individual ECM and saprotrophic (SAP) fungi collected at Toolik Lake (Fig. 5) varied considerably in ^{15}N for each taxa, with ECM forms generally more enriched than SAP forms.

The similarity in the distribution of $\delta^{15}N$ values in plants, soils, and ECM fungi along the entire transect and in a range of other ecosystems agrees with the hypothesis about the processes at work (Fig. 1). It is likely that fungi facilitate the transfer of N from soil organic matter to plants and appear to influence $\delta^{15}N$ values as much in the boreal forest as in the Arctic tundra.

Do light levels or canopy position influence $\delta^{15}N$ of mycorrhizal plants?

Low plant $\delta^{15}N$ values in ECM and ericoid mycorrhizal plants in Alaska result from the transfer of ^{15}N-depleted N from mycorrhizal fungi to host plants and the retention by mycorrhizal fungi of a significant proportion of assimilated N (eq. 1). The functioning of mycorrhizal fungi in these processes depends on the plant supply of labile C to their fungal partners. This suggests that mycorrhizal sequestration of N depends on the supply or allocation of labile C from the plant host. Accordingly, plants in low-light environments may have less active mycorrhizal symbioses and send smaller amounts of labile C to their fungal symbionts. Not surprisingly, restricting plant allocation belowground by other processes, such as defoliation (Markkola et al. 2004) or N fertilization (Wallenda and Kottke 1998) also results in decreased C allocation to mycorrhizal fungi and shifts in species composition (Peter et al. 2001; Lilleskov et al. 2002). The fungal symbionts supported by these plants may shift to taxa less demanding of C, and therefore both fungal N sequestration ($1 - T_r$) and the proportion of plant N supplied by these fungi (f) may diminish (see eq. 1 in the Introduction).

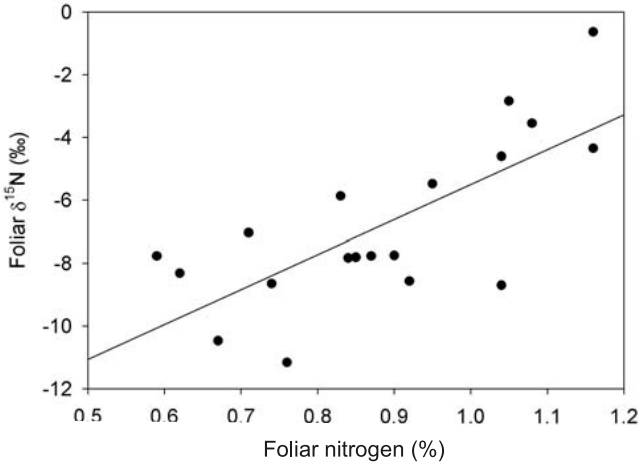
Under conditions of reduced photosynthesis, the quantity $(1 - T_r)\ f$ (eq. 1) may decrease. We therefore predict that

understory plants should be higher in $\delta^{15}N$ than overstory plants of equivalent mycorrhizal status when light limits understory photosynthesis. This mechanism can plausibly explain the high $\delta^{15}N$ values in most understory plants in Denali National Park (Table 1) and many of the understory *Vaccinium* and *Salix* sampled south of the Brooks Range. For example, understory ericoid mycorrhizal plants at Denali National Park averaged 0‰, or 4.5‰ enriched in ^{15}N relative to the overstory dominants. Along the transect south of the Brooks Range (Fig. 4 and Table S1),[3] *Salix* and *Vaccinium* averaged 3‰–4‰ enriched in ^{15}N relative to the dominant *Picea glauca*. Schulze et al. (1994) suggested that the *Vaccinium* in the transect was accessing sources enriched in ^{15}N. In contrast, *Salix* and *Vaccinium* in brightly lit tundra environments had $\delta^{15}N$ values similar to other ECM and ericoid mycorrhizal plants. Parallel patterns were reported from southeast Alaska in Hobbie et al. (2000), with *Salix* and *Populus* on newly colonized substrate (leaf area index (LAI) <1) averaging –7‰ and under a dense spruce–fir canopy (LAI ~ 5) averaging –1‰; the foliage of the overstory spruce and fir ranged from –5‰ to –8‰. At first glance the low $\delta^{15}N$ recorded for the understory plant *Geocaulon* in Denali National Park is an obvious counter-example. However, this root hemiparasite taps directly into plant xylem or phloem (Warrington 1970) and therefore shares the isotopic signature of the overstory plants that dominate the site. *Geocaulon* is known to parasitize spruce, but has not been recorded as parasitizing *Populus*, the other canopy dominant at this site.

Why does the $\delta^{15}N$ of white spruce vary so greatly over the transect?

In the boreal forest north of the Yukon River, white spruce is mostly found along rivers where soils are well-drained and the thawed layer of soil is relatively deep. In the Brooks Range, white spruce occupies well-drained hill slopes and forms the altitudinal tree line. Along the transect, the $\delta^{15}N$ of white spruce varied from –1‰ to –11‰. One possible cause of the isotopic differences could be the availability of N to the trees. If N is limiting, then the proportion of the N supply of a tree that enters through the fungal pathway is high, the proportion of assimilated N retained by mycorrhizal fungi is high, and the $\delta^{15}N$ of foliage is low. If soil N availability is high, then only a small proportion of plant N supply enters through the fungal pathway. These relationships agree with the results of a laboratory culture of a pine seedling and mycorrhizal fungi (Hobbie and Colpaert 2003). In addition, the proportion of assimilated N retained by mycorrhizal fungi should be lower (high transfer ratio) than under low N availability. Foliar N concentrations correlate with N availability (Ingestad and Ågren 1992) and can accordingly be used as an integrated measure of the N environment perceived by a plant. In Fig. 8, %N and $\delta^{15}N$ are correlated in foliage from white spruce collected by Schulze et al. (1994) along the Dalton Highway, strongly suggesting that lower N availability correlates with increased importance of ECM fungi for spruce N supply. For the same reason, foliar %N and $\delta^{15}N$ were positively correlated (r^2 = 0.73) in *Picea sitchensis* (Bong.) Carrière (Sitka spruce) from southeast Alaska along a natural N availability gradient (Hobbie et al. 2000).

Fig. 8. Foliar %N and $\delta^{15}N$ in year 1 needles of *Picea glauca* (white spruce) collected along the pipeline road (n = 19, r^2 = 0.53, p = 0.0002). Data are taken from the report by Schulze et al. (1994).



We conclude that differences in $\delta^{15}N$ of the foliage of the white spruce likely reflect the availability of N and the degree of dependence of the trees on the fungal supply of organic N. Similar conclusions were reached from fieldwork and modeling by Hobbie et al. (1999, 2000) to account for declines in $\delta^{15}N$ of Sitka spruce foliage at Glacier Bay, Alaska. In that marine boreal system, spruce $\delta^{15}N$ declined and N became increasingly limiting as forests developed along a 250-year chronosequence.

Why is black spruce so depleted in ^{15}N?

In the region extending from south of the Brooks Range to the Yukon River, black spruce occupies hill slopes and bogs, whereas black spruce is restricted to bogs along valley bottoms in the Brooks Range. The consistently low $\delta^{15}N$ values of black spruce foliage (generally –8‰ to –10‰) are quite unusual relative to typical values for ECM plants. What could account for these low $\delta^{15}N$ values?

One possibility is that black spruce mycorrhizae follow the same process of fractionation against ^{15}N that is laid out in Fig. 1. In this scenario the foliage could reach such low $\delta^{15}N$ values if N sources in the soil were depleted in ^{15}N relative to the bulk soil. This could occur if a ^{15}N-depleted litter layer builds up slowly in the soil with continued inputs of N from ^{15}N-depleted black spruce foliage. Because black spruce favors the growth of a moss layer and increasingly wet conditions, as well as a rise in the permafrost level, N cycling by spruce mycorrhizae is increasingly restricted to the surface litter layer as the system ages. Under these conditions, N processed during decomposition may be frozen in permafrost or increasingly locked up in cold, wet, deeper soil layers as the system ages (Heilman 1966). This sequestered N should be enriched in ^{15}N. This suggests that the extremely low $\delta^{15}N$ values for black spruce in some systems (e.g., 1 value at Coldfoot was –12‰) probably reflect systems that have been dominated by black spruce for many years with unusually high degrees of recycling between surface litter layers and trees and considerable sequestering of N in inaccessible soil layers.

Fungal biomass

The ergosterol method measures this sterol in cell membranes of most saprotrophic fungi, ECM fungi, and ericoid mycorrhizal fungi, but the measurement is not useful for arbuscular mycorrhizal fungi (Weete 1989; Olsson et al. 2003). While there has been some debate about whether ergosterol measures only living fungal biomass (Mille-Lindblom et al. 2004), the method has been used extensively to measure fungal biomass in soil since the initial report by Grant and West (1986). One difficulty we encountered was in separating recently fallen litter, which was not included in the analysis reported here, from the decomposing litter just below. This intact litter is preferred by saprotrophic fungi and contains high amounts of biomass (Lindahl et al. 2007). Our samples with high ergosterol values, therefore, may contain some surface litter.

Ergosterol concentrations in the humus layer at Toolik Lake (Fig. 6) ranged from 36 to 81 mg fungal biomass per g of SOM. These values are close to the 45–63 mg fungal biomass per g of SOM found in the humus layer of forest soils by Wallander et al. (2004) and the 30–60 mg fungal biomass per g of SOM found by Clemmensen et al. (2006) at their tussock tundra control site at Toolik Lake. Based on measurements at Toolik Lake and Abisko, Sweden, of ergosterol and hyphal growth rates, Clemmensen et al. (2006) suggested that fungal hyphae in boreal forests and Arctic tundra are mostly from ECM and ericoid mycorrhizal fungi.

Fungal biomass is about 40% C (Henn et al. 2002). Fungal C in the humus layer at Toolik Lake averages 17 ± 6 mg C/g SOM. Total microbial C in tussock soils at Toolik Lake has been estimated as 18.5 mg C/g SOM using the chloroform fumigation method (Schmidt et al. 2002) and 17 ± 3 mg C/g SOM at Toolik Lake in the upper 10 cm of tussock tundra using the ergosterol method (Clemmensen et al. 2006).

We note that the fumigation method has not been extensively tested for the efficiency of C extraction from mycorrhizal hyphae during the procedure. However, Ingham et al. (1991) believe that microbial C measured in the standard fumigation method is likely to underestimate the mycorrhizal component of the microbial C. Thus, fungi appear to account for >90% of microbial C at Toolik Lake. For this reason, in these northern systems the biomass of the mycorrhizal fungal is probably greater than that of bacteria.

The regression between fungal biomass (as estimated from ergosterol measurements) and the fraction of organic matter in the soil is shown in Fig. 7 with a slope of 52 mg of fungal biomass per g of organic matter and a nonzero x intercept. We replotted organic matter as the y axis and fungal biomass as the x axis to examine the dependence of fungal biomass on organic matter. On this plot, the y intercept is significantly different than zero ($p = 2 \times 10^{-6}$) and predicts that soil fungal biomass is negligible at an organic matter percentage <3.7%.

Several possible implications follow from these results:

(1) One interpretation is that Arctic plants do not support their mycorrhizal symbionts when the potential return of nutrients derived from organic matter drops below a certain level. Plants are therefore unlikely to derive nutrition from their fungal symbionts below the soil depth where this concentration of organic matter occurs.

(2) Alternatively, the energy that can be extracted from soil organic matter by fungi to fuel new growth of plants is minimal if the organic matter is dispersed approximately 27:1 in the soil mineral matrix.

(3) Although the average C quality of litter declines with decomposition (Ågren and Bosatta 1996), our results indicate that fungal biomass is relatively constant throughout the soil profile as a proportion of soil organic matter. This suggests that labile C inputs to support mycorrhizal fungi must increase as decomposition proceeds to account for a declining biomass of saprotrophic fungi per unit C (Lindahl et al. 2007). These labile C inputs are necessary to support the extraction of nutrients by mycorrhizal fungi from soil organic matter of low C quality. For example, fungi are quite important in moving N into decomposing litter from lower soil horizons (Frey et al. 2003; Caner et al. 2004).

Although the lower soil horizons in the transect contain low amounts of ergosterol, this does not mean that the fungal hyphae are unimportant to the nutrition of their host plants. One possibility, reviewed by Landeweert et al. (2001), is that ECM hyphae excrete organic acids and make available inorganic compounds such as phosphate from the mineral particles that are abundant in the lower soil horizons.

Finally, we suggest that hyphae, probably of mycorrhizal fungi, are as abundant in all the soils of the boreal forest–Arctic tundra transect as in temperate forests. Although variability of saprotrophic versus mycorrhizal fungi could not be estimated using ergosterol, differences in total fungal biomass did not affect shifts in $\delta^{15}N$ in plants along the transect.

Conclusions

Transect data on $\delta^{15}N$ values for ECM fungi, plants, and soils show similar ^{15}N distribution in the Arctic and in boreal forest regions of northern Alaska. The hypothesis that mycorrhizal fungi alter $\delta^{15}N$ values when they supply N to plants appears reasonable and results in a pattern that applies to both tundra and boreal forest across a wide range of ecosystems. The actual values of $\delta^{15}N$ in the plants depend on how much plant N is derived from fungi; the amount derived from fungi depends mainly upon the amount of plant-available N in the soil. Understory plants are often higher in $\delta^{15}N$ than canopy plants, most likely caused by a decrease in C allocation belowground when plants become light limited. That is, the quantity $(1 - T_r) f$ is smaller in shaded plants than in canopy plants. The foliage of black spruce is unusually depleted in ^{15}N, mostly likely because permafrost limits the fungal hyphae to shallow depths of the soil where the already ^{15}N-depleted plant litter is a primary source of N. Based on the observed concentration of ergosterol in soils, fungal biomass in these Arctic and boreal soils is as high as in temperate forests; changes in fungal biomass along the transect play little role in shifts in the $\delta^{15}N$. The $\delta^{15}N$ values for both foliage and fungal fruiting bodies are useful in indicating the activity of the mycorrhizal symbiosis in the N cycle.

Acknowledgements

This work was supported by the National Science Foundation (NSF OPP-0612598 and NSF DEB-0614266). J. Deslippe of the University of British Columbia gave ex-

pert help with sampling and identification in 2007, M. Reuer and A. Ouimette helped with the analyses, the staff of the Toolik Field Station supported field operations, and K. Clemmensen provided unpublished data.

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