Letters

Stable isotope probing implicates a species of *Cortinarius* in carbon transfer through ectomycorrhizal fungal mycelial networks in Arctic tundra

Mycorrhizal networks (MNs) occur when the mycelium of one or more mycorrhizal fungus colonizes two or more nearby plants (Molina *et al.*, 1992). MNs can serve as pathways for the transfer of carbon (C), nitrogen (N), phosphorus (P), water, defense signals and allelochemicals among plants (see Simard *et al.*, 2012, for a review) but, to the best of our knowledge, the fungi involved in these resource transfers among green plants in the field have never been directly identified. This information is important because it would reveal the unique roles of mycorrhizal fungal species, shedding light on some of the functions of these complex belowground networks, and potentially providing additional ecological context for the increasingly large body of molecular community data that is accumulating for mycorrhizal fungi (Blaalid *et al.*, 2014; Horn *et al.*, 2014; Morgado *et al.*, 2015). MNs mediate plant–plant interactions with potential implications for plant diversity at local and regional scales (Perry *et al.*, 1989; McGuire, 2007; Deslippe & Simard, 2011) and are likely to have foundational roles in the structure and regeneration of terrestrial ecosystems (Simard, 2009; Simard *et al.*, 2012). Thus, an improved understanding of MNs may lead to more appropriate and effective land conservation and ecological restoration practices.

In recent decades, regional warming associated with anthropogenic climate change has led to increased plant biomass across the Arctic tundra biome (Jia *et al.*, 2003; Chapin *et al.*, 2005; Macias-Fauria *et al.*, 2012). Differences in the relative productivity of plant species have led to altered plant community compositions, with ectomycorrhizal (EM) shrub species increasingly dominating in many regions (Myers-Smith *et al.*, 2011; Bonfils *et al.*, 2012). In the moist-acidic tundra of Arctic Alaska, the EM shrub *Betula nana* has increased most strongly (Sturm *et al.*, 2001) and this effect is further enhanced through experimental warming (Chapin *et al.*, 1995; Sistla *et al.*, 2013). When warmed, the EM fungal (EMF) community associating with *B. nana* shifts from being dominated by members of the Russulaceae to one dominated by *Cortinarius* spp. (Deslippe *et al.*, 2011). *Cortinarius* spp. are C-demanding, rhizomorph-forming basiodimycetes that grow extensive mycelia in soil (Agerer, 2001, 2006). Some *Cortinarius* species produce highly efficient oxidative enzymes (Bodeker *et al.*, 2009, 2014), which they utilize to mobilize growth-limiting N for their host from complex soil organic matter (Lilleskov *et al.*, 2002; Hobbie & Agerer, 2010; Hobbie *et al.*, 2013). Recent work has highlighted the importance of N mobilization by *Cortinarius* spp. in maintaining rates of C-cycling in other high-latitude ecosystems (Clemmensen *et al.*, 2013, 2015; Lindahl & Tunlid, 2015) and similar processes could be involved where shrubs are spreading in Arctic tundra as climate warms. Indeed, the transition from ericaceous tundra heath to EM shrub tundra is accompanied by significantly higher rates of fungal hyphal growth and C turnover and lower soil organic C stocks in Swedish Lapland (Parker *et al.*, 2015).

Studies of C-transfer through EMF mycelial networks have often utilized stable or radiocarbon isotopes and employed 'pulse-chase' methodology to trace photosynthetic C from a labeled 'donor' plant to nearby 'receiver' plants. These studies have revealed that the magnitude of C transfer through EMF mycelial networks is highly variable in nature, ranging from $< 1\%$ to 10% of the donor plant's net photosynthesis (Simard *et al.*, 1997a; Teste *et al.*, 2010). This variability reflects plant physiological factors that affect the magnitude of source–sink gradients for C among networked plants, and fungal factors, such as the extent of fungal colonization of roots, the composition of EMF community and the continuity of the hyphal pathway (see Simard *et al.*, 2012, for a review). Indeed, small but statistically significant C-transfer to receiver plants through soils and a discontinuous hyphal pathway have been observed (Philip *et al.*, 2010; Deslippe & Simard, 2011), suggesting a possible additional role for bacteria, saprotrophic fungi, or other members of the rhizosphere community in C-transfer among plants. Bacterial-mediated C transfer among plants could occur, for example, if a donor plant's rhizodeposit C was acquired by a rhizosphere bacterium which subsequently became an endophyte of the receiver plant (Rosenblueth & Martinez-Romero, 2006).

Previously, through $^{13}CO_2$ pulse-chase labeling of *B. nana* plants in Arctic tundra we showed significant transfer of C through EMF mycelial networks to aboveground tissues and rhizomes of *B. nana* receivers in conspecific pairs only. We also found low but nonzero C transfer among *B. nana* pairs through soil pathways. Here we report the use of stable isotope probing (SIP) of phospholipid fatty acids (PLFAs) paired with a DNA-SIP-pyrosequencing approach of root samples collected in that study to test the hypotheses that: (1) EMF were the primary conduits for C among *B. nana* individuals, and (2) *Cortinarius* were more important conduits for C than other members of the EMF community associating with *B. nana*. A significant and unique role for one or more species of *Cortinarius* in C-transfer among *B. nana* individuals would constitute evidence that EMF species in mixed communities perform particular functions for their host, one step towards elucidating the functions of these diverse relationships.

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Materials and Methods

This study took place in Low Arctic tussock tundra at Toolik Lake, Alaska, USA (68°38′N, 149°34′W). The plant community at this site is co-dominated by the EM dwarf shrub *B. nana* L. and the nonmycorrhizal sedge *Eriophorum vaginatum*, with an understory mainly of ericaceous plant species and mosses (Supporting Information Table S1). *B. nana* 'donor' plants were sealed in gas-tight chambers and pulsed with 3.2 mmol $^{13}CO_2$ (Fig. 1a). *B. nana* donors were removed from labeling chambers when CO_2 concentrations inside the chamber fell below ambient concentrations (*c.* 4 h; Fig. 1b). After a 7-d chase period, stems, leaves and rhizomes of the *B. nana* donor and all potential 'receiver' plant species present in the 55 cm-diameter study plots were harvested and

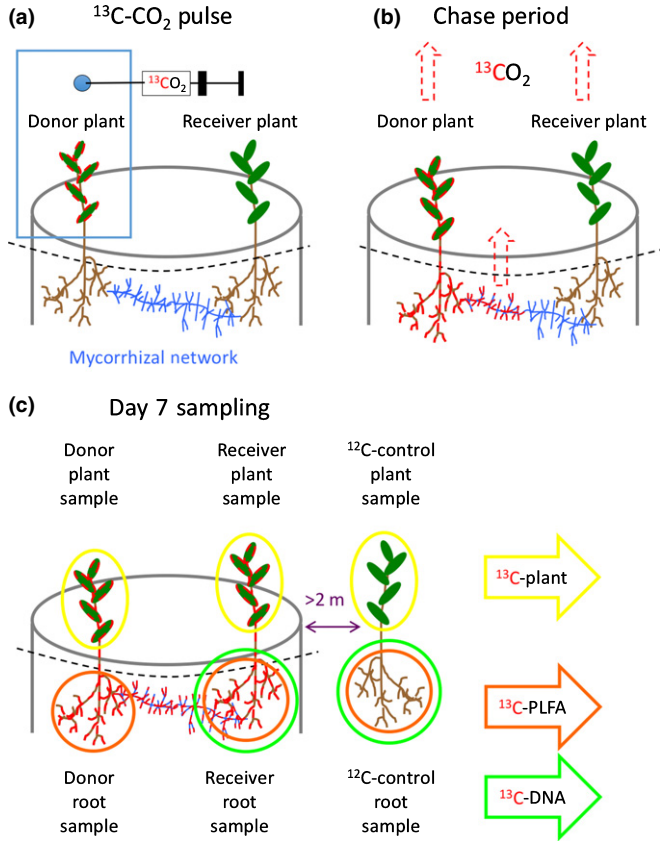

Fig. 1 Schematic of experimental procedures and sampling. (a) Plots contained pairs of *Betula nana* in an intact tussock tundra community. A donor plant was sealed in a gas-tight labeling chamber and pulse-labeled with ^{13}C-CO_2. Plant roots and fungal hyphae are drawn in brown and blue, respectively. (b) During the chase period the ^{13}C fixed in photosynthesis in donor plant leaves was redistributed to linked carbon sinks. A portion of ^{13}C was lost to the atmosphere via plant and soil respiration (red dashed arrows), ^{13}C enrichment of plant and fungal tissues is indicated by red-dashed outlines. (c) Leaf and rhizosphere samples were collected from donor and receiver plants on Day 7. Leaf tissues of donor and receiver plants were analyzed for ^{13}C-content. Donor and receiver rhizosphere samples were analyzed for microbial acquisition of ^{13}C-phospholipid fatty acid (PLFA) and ^{13}C-DNA by DNA stable isotope probing paired with 454-pyrosequencing. ^{13}C-label had been redistributed from the leaves of the donor plant to the roots, the mycorrhizosphere community, and into the leaves of the receiver plant.

^{13}C-tissue content analyzed (Fig. 1c). We found statistically significant ^{13}C-transfer only among pairs of *B. nana* plants (Deslippe & Simard, 2011). We sampled the roots of each donor *B. nana* plant as well as one independent receiver *B. nana* plant per plot. Independent receiver *B. nana* plants were not connected to the donor plant via belowground plant tissues (e.g. rhizomes or root grafts). The independence of the receiver plant was determined at the time of destructive sampling of the plot. Where more than one *B. nana* receiver occurred, the largest plant was selected for root sampling. Likewise, we sampled the roots of an unlabeled *B. nana* plant growing a minimum distance of 2 m upwind from each ^{13}C-CO_2 labeling plot (^{12}C control plant; Fig. 1c). Fine roots occurred predominantly in the organic soil horizon. We collected *B. nana* roots by tracing the main stem to belowground rhizomes and these to clusters of root tips, which could then be sampled in an intact state. Considerable effort was made to sample the entire fine root system of each plant, but it is possible that some fine root clusters were missed. Each sample consisted of a minimum of three terminal root clusters and adhering organic soil particles, these were 5–10 cm^3 in volume, and all were visibly EM. Samples were placed immediately on ice and frozen at −80°C within 2 h. They remained frozen during transport to the laboratory.

For PLFA-SIP, we selected root samples from pairs of *B. nana* (one donor, one receiver) in six experimental plots from the Deslippe & Simard (2011) study, selecting the plots where the greatest total ^{13}C-enrichment of the receiver plant tissues (sum of leaf, stem and rhizome ^{13}C-contents) had been observed. We also sampled the roots of ^{12}C control plants adjacent to each plot (Fig. 1c). The purpose of the ^{12}C-control plant root sample was to provide estimates of the natural abundance of ^{13}C in microbial PLFAs. Total lipids were extracted from *c.* 1 g freeze-dried rhizosphere soil, fractionated, and the phospholipid fraction was trans methylated as described by Bengtson *et al.* (2009). Fatty acid methyl esters (FAMEs) were analyzed by capillary GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS) at the Stable Isotope Research Unit in the Department of Crop and Soil Science, Oregon State University, Corvallis, OR, USA. FAMEs were identified as described by Williams *et al.* (2006) and Butler *et al.* (2003) and quantified using 19:0 methyl ester as the internal standard. We identified 21 FAMEs containing 14–20 C atoms. The $\delta^{13}C$ values of individual PLFAs were determined according to Williams *et al.* (2006), where the atomic ^{13}C excess of PLFAs in samples was calculated relative to the mean $\delta^{13}C$ value for the corresponding PLFA for the root samples of the six unlabeled control *B. nana* plants. Values were expressed as ng PLFA ^{13}C incorporation g^{-1} dry weight soil. We used the ^{13}C enrichment of 18:2ω6,9 as an indication of fungal ^{13}C incorporation in each sample. As a measure of bacterial ^{13}C incorporation, we used the sum of the enrichment of the following PLFAs: i15:0, a15:0, 15:0, i16:0, 10Me16:0, i17:0, a17:0, cy17:0, 17:0, 18:1n7, 10Me18:0 and cy19:0 (Frostegard & Baath, 1996). We assessed correlations among the ^{13}C-enrichments of fungal and bacterial PLFAs and dry weight of plant tissue ^{13}C content using Pearson's product-moment correlation in SPSS v.22 (IBM SPSS Statistics, Armonk, NY, USA). For each PLFA, the proportion of ^{13}C-enrichment per

sample was then calculated. Means are reported ± 1 standard error (SE).

For DNA-SIP, we selected root samples from the receiver *B. nana* in three experimental plots together with root samples from each adjacent unlabeled ^{12}C-control plant (Fig. 1c). DNA was extracted twice from 0.5 g of fresh-frozen root sample using a FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH, USA). The replicate DNA extractions were then pooled and quantified using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). DNA-SIP followed the 'fractionation' method without EtBr described by Neufeld *et al.* (2007). Gradients were formed using a Beckman Coulter Ultracentrifuge fitted with a Vti 65.2 rotor (Beckman Coulter Canada LP, Mississauga, ON, Canada). Following Gallagher *et al.* (2005), we included 10 μg '^{13}C-carrier DNA' within each ultracentrifuge tube. ^{13}C-DNA was generated by extracting DNA from *Escherichia coli* grown in Minimal Media liquid culture, with ^{13}C-glucose as a sole C source. Gradients were displaced and separated into 12 fractions with water colored with toludine blue using a Razel Clinical Syringe Pump (Razel Scientific Instruments Inc., Georgia, VT, USA). The total DNA content of each fraction was quantified by agarose gel electrophoresis through comparison to a known quantity of 1 kb Trackit™ quantification ladder (Life Technologies, Waltham, MN, USA) using AlphaView imaging software (Protein Simple, Toronto, ON, Canada). ^{13}C-enriched 'heavy' DNA was contained in fractions 7 and 8, while fraction 10 contained 'light' ^{12}C-DNA. We pooled fractions 7 and 8 from each receiver or control plant for a single 'heavy' ^{13}C DNA fraction per plant. Eukaryotic ribosomal internal transcribed spacers (ITS2) were amplified from the heavy and light fractions using the primers ITS3/ITS4 (White *et al.*, 1990) and sequenced using 454-pyrosequencing GS-FLX Titanium technology (Roche 454 Life Sciences, Branford, CT, USA) at the Genome Quebec Innovation Centre, Montreal, Canada. We pyrosequenced heavy and light fractions of the receiver plants from the three experimental plots individually, but pooled the heavy fractions (7 and 8) of all three unlabeled control plants for a total of seven pyrosequencing samples (i.e. 'Biosamples'). The fungal community of the biosample derived from the pooled heavy fractions of the unlabeled control plant roots was compared to that derived from the heavy fractions of receiver plants from experimental plots. This served to confirm that the fungi identified as being enriched in the heavy fractions of receivers from experimental plots did not also occur in significantly higher abundances in samples with only natural abundance of ^{13}C. This situation could arise, for example, for taxa with higher than average G + C contents as the lower buoyant densities of high G + C content sequences could cause them to migrate to the heavier fractions. Details of the PCR and sequencing methodologies are described by Hartmann *et al.* (2012). Our DNA-SIP pyrosequencing approach yielded 82 599 raw sequence reads, which were submitted to the European Nucleotide Sequence Read Archive under the study accession no. PRJEB8276.

Procedures for pyrotag processing, operational taxonomic unit (OTU) delimitation, and assigning taxonomic affiliations to OTUs are described by Hartmann *et al.* (2014). Briefly, curated sequences were clustered into OTUs at an identity threshold of 97% using the unsupervised Bayesian clustering algorithm CROP (Hao *et al.*, 2011). OTUs were queried against the UNITE database (Abarenkov *et al.*, 2010) and assigned taxonomy using the naive Bayesian classifier (Wang *et al.*, 2007) and a minimum bootstrap support of 60% in MOTHUR (Schloss *et al.*, 2009). These taxonomic assignments were used for all statistical analyses, although the taxonomic resolution achieved through analysis of the ITS2 amplicon differed among OTUs. We then used manual GenBank-BLASTn searches of sequences to gain additional insight to the taxonomic affiliations of OTUs that differed in abundance among the heavy and light fractions. Additionally, we aligned all unique *Cortinarius*-affiliated sequences with closely related INSD sequences that were derived from vouchered *Cortinarius* specimens and created a phylogenetic tree using GENEIOUS v.8.0.5 (Kearse *et al.*, 2012) (Fig. S1).

We used indicator species analysis to determine the fidelity of OTUs to the heavy and light fractions. Indicator values were calculated with the method of Dufrene & Legendre (1997) using Monte Carlo tests of significance with 5000 permutations, as implemented in PC-ORD v.6.19 (McCune & Mefford, 1999). In addition, we used indicator species analysis to examine the fidelity of the sum of all Russulaceae-affiliated sequences to heavy or light fractions. Similarly, we examined the fidelity of the sum of all *Cortinarius*-affiliated sequences that did not belong to the dominant *Cortinarius* OTU, to heavy and light fractions. We focused on members of the Russulaceae and on *Cortinarius* spp. because they are common members of the EMF community associating with *B. nana* at this site (Deslippe *et al.*, 2011) and because the identification of several Russulaceae-affiliated, and *Cortinarius* sp. OTUs could have reduced our ability to detect significant ^{13}C-enrichment of these groups overall. All means are reported ± 1 SE.

Results and Discussion

PLFA-SIP analysis of the root samples of six *B. nana* pairs indicated greatest enrichment of the saturated fatty acid 16:0, which is common to both prokaryotes and eukaryotes (Fig. 2), and thus reflects ^{13}C-incorporation by plant roots as well as by soil organisms. The fungal biomarker 18:2ω6,9 showed the next greatest enrichment, with the roots of receiver plants containing on average, one-third of the absolute ^{13}C-enrichment ($0.016 \pm 1.2 \times 10^{-3}$ ng ^{13}C-PLFA g^{-1} dry weight (DW) soil) as 18:2ω6,9 in the roots of donor plants ($0.058 \pm 4.2 \times 10^{-3}$ ng ^{13}C-PLFA g^{-1} DW soil). For all samples, 18:2ω6,9 showed much greater ^{13}C-enrichment than did the sum of ^{13}C-enrichment of all bacterial PLFAs. Mean proportional ^{13}C-enrichment of the fungal biomarker was nearly seven-times that of the bacterial biomarkers, and these means did not differ significantly between root samples of donor and receiver plants (Fig. 2). These results suggest a prominent role for fungi relative to bacteria in ^{13}C-transfer among pairs of *B. nana* in the field, providing strong support for our first hypothesis.

Plant tissue ^{13}C enrichment of donors and receivers was significantly positively correlated to the ^{13}C enrichment of 18:2ω6,9 (Kendall's tau = 0.455, $P = 0.04$), but not significantly correlated to the sum of ^{13}C-enrichment of all bacterial PLFAs, or

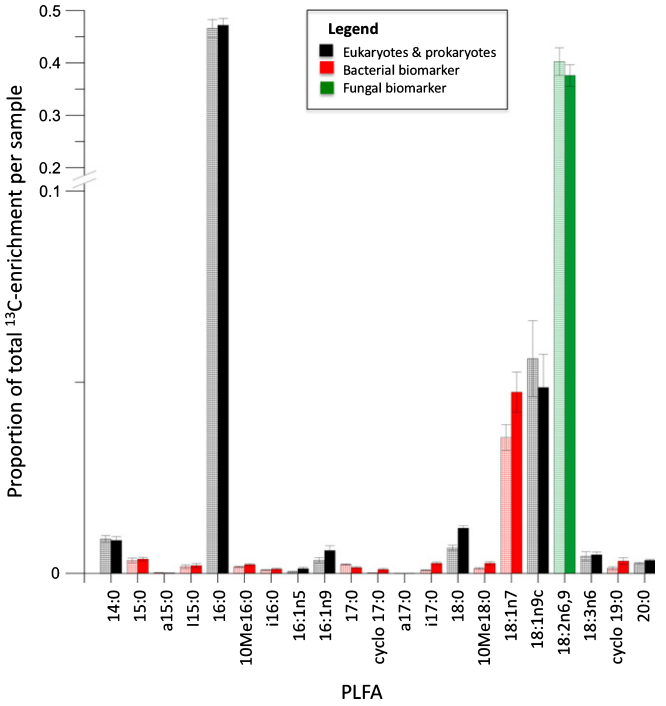

Fig. 2 Mean ± standard error (SE) proportion of total ^{13}C-enrichment per sample ($n = 6$) for phospholipid fatty acids (PLFAs) from the rhizospheres of paired ^{13}C-labeled donor (hatched bars) and unlabeled receiver (closed bars) *Betula nana* plants. Bar color indicates the taxonomic biomarker of each PLFA. Note that the *y*-axis is broken between 0.1 and 0.2.

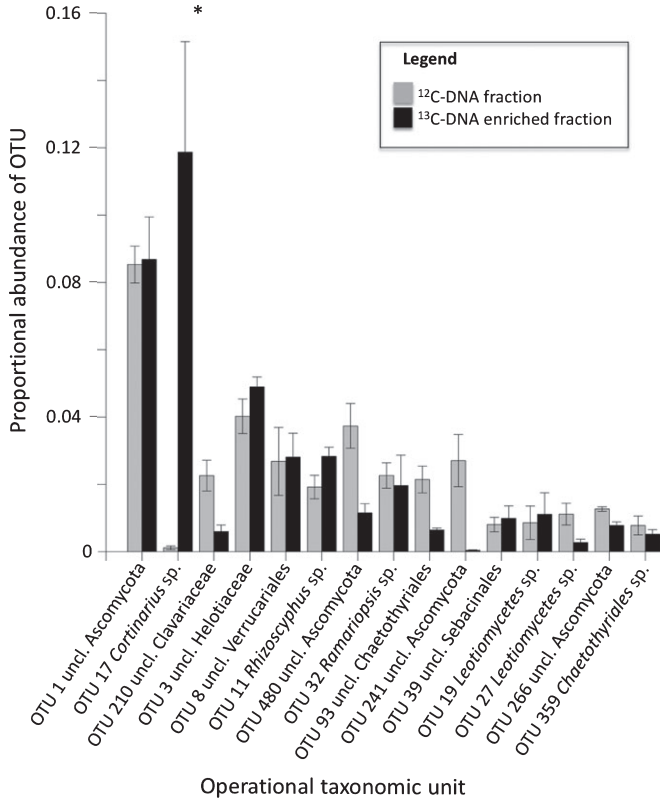

Fig. 3 Mean ± standard error (SE) proportional abundance per sample ($n = 3$) for the fungal internal transcribed spacer (ITS)-operational taxonomic units (OTUs) that comprised > 1% of the entire community. Asterisk indicates the taxa that was a significant indicator of the ^{13}C-fractions ($\alpha = 0.1$).

to the sum of total PLFA ^{13}C-enrichment. These findings suggest that fungal, rather than bacterial ^{13}C enrichment is more closely linked to plant ^{13}C tissue content. In the case of donor plants, this is consistent with the observation that plant C is acquired first by mycorrhizal fungi before being dissipated through the saprotrophic fungal and bacterial community (Treonis *et al.*, 2004; Drigo *et al.*, 2010; Churchland *et al.*, 2012; Kaiser *et al.*, 2015). In the case of receiver plants, this observation provides support for a direct hyphal link as a pathway for C-transfer among *B. nana* pairs.

The ^{13}C enrichment of 18:2ω6,9 was also significantly positively correlated to both total and bacterial PLFA ^{13}C-enrichment (Kendall's tau = 0.818, $P < 0.001$; Kendall's tau = 0.545, $P = 0.01$, respectively), indicating that fungi dominated acquisition of ^{13}C from plants and suggesting that they may have mediated subsequent acquisition of ^{13}C by the bacterial community, a finding that agrees with other recent work (Kaiser *et al.*, 2015), and provides additional support for our first hypothesis.

Our DNA-SIP pyrosequencing approach yielded 31 996 fungal sequences that clustered into 640 OTUs at a 97% sequence identity threshold (Table S2). A curated file in FASTA format of all fungal ITS sequences generated in this study is appended to this manuscript (Methods S1). Overall, the rhizosphere fungal community was moderately even (Pielou's $J' = 0.56$), with the most abundant OTU comprising only 15% (4734) of all fungal sequences, while the seven next most abundant OTUs comprised between 5% and 9% each (Table S2).

The proportional abundances of most OTUs were similar in the heavy and light fractions of samples. The most abundant OTU, an unclassified member of the Ascomycota with 100% identity to the uncultured fungus from boreal Alaska (INSD accession no. JN889866), was a prominent example of this (Fig. 3; Table S3). However, the second most abundant OTU17 (11.2%, 3017) was on average two orders of magnitude more abundant in the heavy than light fractions (0.12 ± 0.033 vs 0.0012 ± 0.00057; Fig. 3; Table S3), while it was absent from the heavy fractions derived from the unlabeled ^{12}C-control plant rhizospheres (Fig. S2). OTU17 was detected as the sole significant indicator of the heavy fraction at $\alpha = 0.1$ ($P = 0.093$; Fig. 3; Table S4). This finding provides support for our second hypothesis that *Cortinarius* sp. was an important conduit for C among *B. nana* plants relative to other members of the root-associated fungal community.

The 3017 sequences that clustered into OTU17 represented 45 different ITS2 sequences, which had 98–100% sequence identity to ITS2 amplicons derived from vouchered specimens of *Cortinarius collinitus*, *C. favrei*, *C. fennoscandicus*, *C. muscigenus*, *C. septentrionalis* and *C. stillatitius*, with *C. collinitus* returned as the closest match for 41 of 45 sequences. Phylogenetic analysis indicated that ITS2 is conserved among these morphological species (Fig. S1) and we are therefore unable to provide more taxonomic detail. However, *C. fennoscandicus* and *C. septentrionalis* are considered to be exclusively associated with *Betula* (Bendiksen *et al.*, 1993). We observed numerous

Cortinarius spp. fruiting at the study site. One of these, which had a very slimy cap, a character consistent with *Cortinarius* section Colliniti (Bendiksen *et al.*, 1993), was much more common than the others (Fig. S3). This *Cortinarius* sp. occurred in very high abundance from early August until snowfall each year.

Proposed mechanism – C as a vehicle for N

Here we show that one or more species of *Cortinarius* was very likely the pathway for C-transfer among *B. nana* plants in Arctic tundra. While C-transfer through EM networks to receiver plant tissues has been well documented for nearly two decades (Simard *et al.*, 1997a,b; Wu *et al.*, 2001; Bingham & Simard, 2011; Deslippe & Simard, 2011), its ecological relevance has been the subject of much debate (Fitter *et al.*, 1999; Simard *et al.*, 2002; van der Heijden & Horton, 2009). The crux of this debate has hinged on the counterintuitive notion that a heterotrophic soil fungus would relinquish growth-limiting C to its photoautotrophic host plant (Simard *et al.*, 2012). While this situation is the norm for fungi and plants in mycoheterotrophic relationships, green plants are typically strong carbohydrate sources relative to their symbiotic fungi and the movement of C against this concentration gradient is expected to be minute (Simard *et al.*, 2012). By contrast, EMF are often N sources relative to their host plants, and significant C-transfer among green plants through MNs would be expected if organic N was a dominant form in which C moves. We propose that C-transfer among *B. nana* through EM networks may reflect the movement of amino acid N from *Cortinarius* to *B. nana*.

In the moist acidic tussock tundra near Toolik Lake Alaska soil N in inorganic and free amino acid forms is typically $< 4\ \mu g\ g^{-1}$ DW soil over the growing season (Weintraub & Schimel, 2005a) and surface soil layers contain > 72% organic matter (Marion *et al.*, 1997). In these soils, seasonal peaks in proteolysis coincide with periods of maximal root growth, when soil NH_4 and free amino acid concentrations are at their lowest values (Weintraub & Schimel, 2005b). EMF oxidative exoenzymes (Bodeker *et al.*, 2009, 2014) are likely to contribute significantly to these cycles, securing a high proportion of the 61–86% of foliar N that EMF supply to plants (Hobbie & Hobbie, 2006). Once taken up by plant and EMF transporters, amino acids undergo rapid metabolic conversions involving a wide range of compounds within roots (Nasholm *et al.*, 2009), and presumably hyphae. These metabolic conversions may include deamination via the GS/GOGAT (Glutamine synthetase/Glutamate-2-oxoglutarate aminotransferase) cycle, yielding NH_4. In plant roots, most NH_4 is incorporated to new amino acids before transport occurs (Nasholm *et al.*, 2009, and references cited therein). If similar conversions occur within the EMF mycelium, it seems plausible that an EMF with proteolytic activity could generate relatively high mycelial concentrations of newly formed amino acids, that is, amino acids containing soil-derived N and recent plant photosynthate C. These new amino acids could then move down concentration gradients to be acquired by plants connected in the MN. While studies to elucidate the forms of N transferred from EMF to their host plants are few, glutamine, which contains five C atoms for every two N atoms, was found to be the primary molecule through which N is transferred from members of the Russulaceae to *Fagus sylvatica* (Martin *et al.*, 1986). Moreover, amino acid transporters are abundant in plant genomes (Rentsch *et al.*, 2007) and strongly upregulated during symbioses with EMF (Martin *et al.*, 2010). Thus, while the high ^{13}C enrichment of *Cortinarius* spp. DNA we observed in the roots of receiver *B. nana* plants constitutes evidence that these plants are linked to donors through *Cortinarius* MNs, the transfer of C through these MNs into plant tissues (Deslippe & Simard, 2011) likely reflects mycelial N uptake, conversions and the transfer of newly formed glutamate, to *B. nana* receivers. This situation contrasts with that of arbuscular mycorrhizal fungi (AMF), which take up inorganic N and incorporate it to arginine, but transfer it to the plant without C (Govindarajulu *et al.*, 2005). If amino acids are the dominant form of N transferred from EMF to their host plants, then differences in N-transfer at the symbiotic interface among EMF and AMF may actually underpin the controversy around C-transfer through MNs, as studies that have found large and significant C-transfer through MNs to plant tissues have been exclusively in EM systems (Simard *et al.*, 2002, 2012).

High variability of C-transfer through MNs

Previously, we found that on average 4.1% of a donor plant's net photosynthetic C was transferred to receiver *B. nana* tissues through MNs (Deslippe & Simard, 2011). Significant C-transfer occurred only within pairs of *B. nana* and not between *B. nana* and other plant species, including the other EM plants (*Salix pulchra*, *S. reticulata*). However, we observed large variability in C-transfer through the MN pathway relative to other belowground pathways (rhizomes, root grafts and the soil solution). Given that *Cortinarius* sp. was exceptional among root-associated fungi in being highly enriched in ^{13}C, and that the proportional abundance of *Cortinarius*-affiliated EMF on *B. nana* can vary from 4% to 58% of the population in response to warming treatment at this site (Deslippe *et al.*, 2011), it seems reasonable that differences in the relative abundance of *Cortinarius* sp. on *B. nana* roots may account for at least some proportion of the high variability in C-transfer that we observed. Indeed the number of hyphal links among EM plants is significantly positively correlated to P-transfer through MNs (Li *et al.*, 2004).

To further assess support for this notion we used indicator species analysis to independently examine the ^{13}C-enrichment of all Russulaceae-affiliated sequences and of all *Cortinarius*-affiliated sequences that did not cluster into OTU17. However, even when the proportional abundances of these OTUs were summed they did not display higher fidelity for the heavy fractions. This indicates no significant ^{13}C-enrichment among members of the Russulaceae or among members of other species of *Cortinarius* (Table S5). Thus, in addition to the more common and abundant EMF on *B. nana* receivers that were not enriched in ^{13}C in this study (e.g. *Rhizoscyphus ericae*) it appears that members of the Russulaceae and other *Cortinarius* species were not active conduits of C-transfer among *B. nana*. The

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unique enrichment of OTU17, which is likely a single *Cortinarius* sp. supports the notion that MNs dominated by rhizomorph-forming fungal species are better facilitators of resource transfer than those dominated by shorter-distance exploration types such as those we identified in the Russulaceae.

Plant–microbial interactions and ecosystem function

Here we show that a *Cortinarius* sp. was the likely pathway for C-transfer among *B. nana* plants in Arctic tundra. Our data suggest that a *Cortinarius* sp. performs a particular function for its host that other EMF do not do to any significant extent. While the mechanism for C-transfer through MNs has yet to be elucidated, one possible explanation is that C-transfer among *B. nana* individuals through MNs reflects the movement of amino acid N from *Cortinarius* to *B. nana*. This view is consistent with the evidence that some *Cortinarius* species (Bodeker *et al.*, 2009, 2014) are important in mobilizing growth-limiting N for their host from complex soil organic matter (Lilleskov *et al.*, 2002; Hobbie & Agerer, 2010; Hobbie *et al.*, 2013), which in turn is important in maintaining relatively high rates of C-cycling (Clemmensen *et al.*, 2013, 2015; Lindahl & Tunlid, 2015). Thus C-transfer among *B. nana* individuals through mycelial networks of *Cortinarius* sp. may add to the evidence linking certain EMF to the maintenance of C-turnover in high latitude ecosystems (Clemmensen *et al.*, 2015). Given the profound response of Arctic EM shrubs (Callaghan *et al.*, 2011; Myers-Smith *et al.*, 2011; Elmendorf *et al.*, 2012) and EMF communities (Deslippe *et al.*, 2011, 2012; Morgado *et al.*, 2015) to climate warming, it is possible that these unique activities of *Cortinarius* sp. are influencing ecosystem-scale processes.

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Author contributions

J.R.D., S.J.G., S.W.S. and W.W.M. designed the research. J.R.D. conducted the fieldwork and laboratory analyses. J.R.D. and M.H. analyzed the data, J.R.D. wrote the manuscript with input from M.H., S.J.G., S.W.S. and W.W.M.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Phylogenetic tree containing OTU17 and other *Cortinarius*-affiliated ITS2 sequences.

Fig. S2 Proportional abundances of the fungal ITS-OTUs in ^{12}C-control plant root samples.

Fig. S3 *Cortinarius* sp. sporocarp fruiting at the study site at the time of sampling.

Table S1 List of all vascular plant species in study plots

Table S2 Raw and proportional abundances and taxonomic affiliations of all fungal-OTUs generated in this study

Table S3 Proportional abundances and taxonomic affiliations of fungal ITS-OTUs derived from samples

Table S4 Indicator species analysis for fungal OTUs

Table S5 Proportional abundances and taxonomic affiliations of Russulaceae and non-OTU17-*Cortinarius*-affiliated fungal ITS-OTUs

Methods S1 A curated file of the 31 996 fungal ITS sequences generated in this study in FASTA format.

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Key words: Arctic tundra, *Betula nana*, DNA-stable isotope probing (DNA-SIP), ectomycorrhizal fungi, mycorrhizal network (MN), phospholipid fatty acid (PLFA), shrub expansion, stable isotope probing.