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Nitrogen deposition alters plant–fungal relationships: linking belowground dynamics to aboveground vegetation change

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Abstract

Nitrogen (N) deposition rates are increasing globally due to anthropogenic activities. Plant community responses to N are often attributed to altered competitive interactions between plants, but may also be a result of microbial responses to N, particularly root-associated fungi (RAF), which are known to affect plant fitness. In response to N, *Deschampsia cespitosa*, a codominant plant in the alpine tundra at Niwot Ridge (CO), increases in abundance, while *Geum rossii*, its principal competitor, declines. Importantly, *G. rossii* declines with N even in the absence of its competitor. We examined whether contrasting host responses to N are associated with altered plant–fungal symbioses, and whether the effects of N are distinct from effects of altered plant competition on RAF using 454 pyrosequencing. Host RAF communities were very distinct (only 9.4% of OTUs overlapped). N increased RAF diversity in *G. rossii*, but decreased it in *D. cespitosa*. *D. cespitosa* RAF communities were more responsive to N than *G. rossii* RAF communities, perhaps indicating a flexible microbial community aids host adaptation to nutrient enrichment. Effects of removing *D. cespitosa* were distinct from effects of N on *G. rossii* RAF, and *D. cespitosa* presence reversed RAF diversity response to N. The most dominant *G. rossii* RAF order, Helotiales, was the most affected by N, declining from 83% to 60% of sequences, perhaps indicating a loss of mutualists under N enrichment. These results highlight the potential importance of belowground microbial dynamics in plant responses to N deposition.

Keywords: 454 pyrosequencing, endophytes, Helotiales, ITS, nitrogen deposition, root fungi

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Introduction

Nitrogen (N) emissions are increasing globally due to anthropogenic activities (Vitousek *et al.* 1997; Dentener *et al.* 2006), and N deposition rates in many areas of the world are more than an order of magnitude higher than they would be in the absence of human activity (Galloway *et al.* 2008). Increased N availability can cause a cascade of effects, including alteration of ecosystem function (Carreiro *et al.* 2000; Knorr *et al.* 2005; Treseder 2008; De Vries *et al.* 2010), shifts in dominance between plant species (Pennings *et al.* 2005; Suding *et al.* 2008), reductions in plant species diversity (Strengbom *et al.* 2003; Bobbink 2004; Suding *et al.* 2005; Clark & Tilman 2008) and increased vulnerability of systems to invasion (Bobbink 2004; Cherwin *et al.* 2009).

Traditionally, ecologists have assumed that interspecific competition drives plant community response to N, because species with adaptations for low nutrient availability lose their competitive advantage as nutrient availability increases (Bobbink *et al.* 2010). Recent studies have challenged the traditional assumption, showing

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that plant community response to N is not due solely to altered plant competitive interactions (Roem & Berendse 2000; Johnson *et al.* 2003; Suding *et al.* 2005), and some studies suggest plant–microbe interactions may play a key role (Johnson *et al.* 2008; Suding *et al.* 2008).

Over the past few decades, Niwot Ridge (CO, USA), a long-term ecological research (LTER) site, has experienced a steady increase in N deposition from the cities of Boulder and Denver (Sievering *et al.* 1996; Williams *et al.* 1996). Two codominant plant species, *Geum rossii* (Rosaceae) and *Deschampsia cespitosa* (Poaceae), each cover 30% of moist meadow alpine tundra at Niwot Ridge (Suding *et al.* 2008). Long-term N fertilization, and *G. rossii* and *D. cespitosa* removal plots established in 2001 show that *G. rossii* declines in N plots whether or not it is in competition with *D. cespitosa*, while *D. cespitosa* abundance increases (Suding *et al.* 2008; Farrer *et al.* 2013). These findings suggest that competition is not the only driver of vegetation community response to N in alpine tundra. We suspect that fungal response to N may drive aboveground plant response.

All plants harbour root-associated fungi (RAF), defined here as any fungi within or in contact with plant roots. Mutualist RAF can increase disease resistance and abiotic stress tolerance, aid in nutrient acquisition and/or reduce growth of targeted competitor plants (Rodriguez *et al.* 2008; Porras-Alfaro & Bayman 2011). Parasitic RAF can play a major role in plant–soil feedback processes that affect plant abundance (Klironomos 2002). Therefore, how RAF respond to N, for example, loss or gain of mutualists or parasites, should influence host response to N.

Root-associated fungi respond in a variety of ways to nutrient enrichment. N enrichment can encourage purely parasitic species (Strengbom *et al.* 2002), or mutualistic infection rates may decline (Yesmin *et al.* 1996; Treseder 2004; Morgan *et al.* 2005) or increase with a parallel increase in parasitic tendencies (Johnson *et al.* 1997; Upson *et al.* 2009b). Molecular studies that examine RAF response to N report changes to RAF community composition (Frey *et al.* 2004; Porras-Alfaro *et al.* 2007; Avis *et al.* 2008; Cox *et al.* 2010), but the functional meaning of these community shifts is rarely discussed. Next-generation sequencing, which uncovers more of the microbial communities in environmental samples than traditional methods, has rarely been used to assess microbial response to N.

At Niwot Ridge, soil fungal communities shifted with N and community shifts were accompanied by altered soil conditions, such as increased soil lignin content and altered enzyme activity related to N cycling (Nemergut *et al.* 2008). However, RAF communities are distinct from soil fungal communities due to the unique environment provided by the rhizosphere (Morgan *et al.* 2005; Porras-Alfaro & Bayman 2011), so may respond independently. Although RAF communities have been described in several plant hosts from Niwot (Mullen & Schmidt 1993; Schadt *et al.* 2001; Schmidt *et al.* 2008), the effect of N on RAF has not yet been examined, and the communities of the two moist meadow codominants remain undescribed. Because RAF can directly impact host fitness, their response to N enrichment could be critical to aboveground vegetation dynamics (Klironomos *et al.* 2011).

To determine whether RAF could be associated with plant host response to N, we used barcoded 454 sequencing to characterize the RAF community in *G. rossii* and *D. cespitosa,* and examine community response to N. We hypothesize *D. cespitosa* may benefit from N because it is able to terminate relationships with symbiotic RAF as they become less valuable under nutrient enrichment, resulting in a more flexible RAF community. We predicted that *D. cespitosa* RAF communities would respond more to N addition than *G. rossii* RAF, and that *G. rossii* would be more prone to infection by parasitic and pathogenic species than *D. cespitosa* in N plots. The identity of plant species in a focal individual's neighbourhood can have a significant, though often weak, effect on the RAF community of that individual (Bahram *et al.* 2011; Bogar & Kennedy 2012). Because *D. cespitosa* increases in abundance in N plots, shifts of *G. rossii* RAF in N plots could be due to altered *D. cespitosa* abundance rather than to N itself. By removing *D. cespitosa* from some plots, we examined the effect of releasing *G. rossii* from competition on its RAF. We predicted the presence or absence of a primary plant competitor would have minimal effect on RAF communities compared with the effect of N, and that N would have a distinct effect on RAF from *D. cespitosa* removal. Interactions between N addition and *D. cespitosa* removal would imply that the RAF communities of different host species mediate each other's responses to N enrichment.

Methods

Field

The study was conducted in moist meadow alpine tundra on Niwot Ridge, an LTER site located 35 km west of Boulder, CO, in the Front Range of the Rocky Mountains, elevation 3297–3544 m. Winter and summer mean temperatures are −13 and 8 °C. Soil is under snow pack 9–10 months per year (http://culter.colorado.edu/NWT/site_info/climate/climate.html). The moist meadow is composed of forbes and grasses, dominated by *G. rossii*, a rosaceous forb and *D. cespitosa*, a tillering bunchgrass (May & Webber 1982).

Plots used for this study are a subset of those established by Suding *et al.* (2008) (coordinates between 40 03 01 N, 105 34 13 W and 40 03 38 N, 105 36 02 W). Briefly, 1 × 1 m^2 plots were set up at seven replicate sites, between 200 and 800 m apart, in 2001. We used four treatment plot types per site: N addition, *D. cespitosa* removal, *D. cespitosa* removal + N addition and control. N has been added annually to N addition plots in the form of urea (at a maximum rate of 28.8 g $N/m^2/y$, ~40 times natural deposition rates), completely saturating soils. *D. cespitosa* has been removed annually by repeated clipping (hereafter called removal treatment). Clipping succeeded in killing most of the *D. cespitosa* plants in the removal plots; clipped biomass of *D. cespitosa* in 2008 was only 2% of the clipped biomass in 2001.

In 2008, a *G. rossii* individual (defined by a single aboveground rosette) was uprooted at random from each treatment combination at each site. *D. cespitosa* was collected from N and control plots in a subset of sites. This resulted in a total of 28 *G. rossii* and 11 *D. cespitosa* root samples. Two *D. cespitosa* samples were later excluded from analysis due to extremely different RAF composition, indicative of contamination. Plants were sent to University of New Mexico for storage and processing.

Laboratory preparation

Root surfaces were washed aggressively with milliQ-filtered water and stored at −80 °C. Roots were not surface-sterilized because we were interested in both endophytes and fungi associated with the root surface. A mix of small, medium and large healthy looking roots were selected from each plant and combined for DNA extraction. Tissue was lysed with liquid N using a mortar and pestle. DNA was extracted using DNEasy Plant MiniKit (Qiagen). *Geum rossii* tissue is high in phenolics, which inhibit polymerase enzymes. To ensure a good extraction product, we checked that each sample could be successfully amplified using ITS1F-4 primers. Extracts that could not be amplified were diluted 1:10 in milliQ-filtered water to dilute phenolics, which resulted in successful amplification of all samples. Extracts were sent to Research and Testing Laboratories (RTL) in Lubbock, TX for 454 titanium pyrosequencing of the fungal ITS region, which has been identified as the fungal barcode and has been used in multiple environmental studies for its resolution at the species level (Schoch *et al.* 2012), using ITS 1F-4 primers. Fungal libraries were created using a one-step PCR with HotStar Taq master mix (Qiagen) and the following thermocycles: initial denaturation at 95 °C for 5 min, and then 30 cycles of 95 °C for 30 s, 54 °C for 30 s and 72 °C for 1 min, and a final extension at 72 °C for 10 min. Amplification products were pooled to equimolar concentrations and cleaned using Diffinity RapidTip (Diffinity Genomics) and size selected using Agencourt AMPure XP (BeckmanCoulter). Hybridizations, emPCRs and sequencing followed manufacturer's protocols (454 Life Sciences). Samples were sequenced in three runs (on a single region each), each sample within a run had its own 8-nt barcode.

Sequence analysis

We used QIIME 1.7.0 (Caporaso *et al.* 2010) to remove reads with mean quality scores less than 25 and shorter than 150 bp. Because current curated databases fail to encompass much of the diversity of fungal ITS sequences recovered from environmental samples, we used the *de novo* method in UCHIME as implemented in QIIME (Edgar 2010; Edgar *et al.* 2011) to identify putative chimeric ITS sequences. Each query was compared with all sequences in the sequence library to identify potential pairs of parents and chimeric 'offspring' via 3-way alignments. A total of 385 chimeras were removed from 140 561 sequences.

After chimera removal, sequences that were 97% similar to each other were clustered into operational taxonomic units (OTU) representative of distinct species (Nilsson *et al.* 2008) using UCLUST (Edgar 2010) through QIIME. The most common sequence in each cluster was selected as the representative sequence for each OTU. Representative sequences were BLASTed (Altschul *et al.* 1990) in QIIME against the Fungal Metagenomics Project's curated ITS database (University of Alaska, Fairbanks) to assign taxonomy. Our results show that multiple OTUs hit to the same species. If a sequence does not have an identical match in the database, the BLAST method results in hits to the best match available (with *e*-value <0.001), so OTUs that obtained the same taxonomy assignment are closely related to each other, but not necessarily the same species.

Data analysis

In all analyses, we test two models. First, we exclude removal plots and test the effect of host, N addition, and their interaction on various RAF community characteristics, such as diversity, community composition and relative abundance of individual taxa. Second, we exclude *D. cespitosa* samples and test the effect of N, removal and their interaction on *G. rossii* RAF community characteristics. Community characteristics may change at some taxonomic levels but not others. Because ITS cannot be used to build accurate phylogenies across the Fungi, the degree of relatedness of

affected OTUs is not incorporated into any of our analyses. To assess which taxonomic levels were most affected by host and treatment, we performed analyses at all taxonomic levels.

Four hundred and fifty four sequencing poorly resolves the exact length of homopolymers, which occasionally results in sequences that diverge more than 3% from the cluster to which they belong. The resulting singletons (OTUs comprised of only one sequence) are likely to be sequencing artefacts. Because these artefacts are made without site bias, they have no effect on community composition estimates (Kuczynski *et al.* 2010). However, they result in overestimation of species richness (Reeder & Knight 2010). We therefore excluded singletons from our analyses. Doing so also excludes some true members of the rare biosphere, but we are interested in taxa that may impact overall plant fitness so are not concerned with extremely rare taxa.

Alpha diversity was calculated in QIIME, version 1.7.0 (Caporaso *et al.* 2010), using Simpson's diversity index, Simpson's evenness and taxonomic richness. There was large variation in sequencing depth among samples. To control biasing effects of sequencing depth on alpha diversity measures, we rarefied samples by subsampling to the depth of the most shallowly sequenced sample (557 sequences). Rarefaction curves were not saturated at this depth (Fig. S1, Supporting Information). Alpha diversity measures were calculated on each of 100 rarefactions, and averaged. Type III ANOVA (package nlme, Pinheiro *et al.* 2011; in R, R Development Core Team 2011) was used to analyse effect of host and treatments on RAF alpha diversity.

Redundancy analysis (RDA) and Monte Carlo permutation tests based on Euclidean distances of unrarefied data normalized by sample were used to test treatment effects on RAF community composition (vegan package, Oksanen *et al.* 2011; package nlme, Pinheiro *et al.* 2011). One thousand permutations were used to obtain pseudo-F and P statistics. OTUs present in fewer than three plots were removed from analyses, as we were primarily interested in members of the community that are ubiquitous and whose presence or absence could be responsible for *G. rossii* decline across N plots (McCune *et al.* 2002). Euclidean distances were chosen because shared absences reduce distance between communities. This is a useful approach to address hypotheses concerning species disappearance as well as invasion with disturbance (Anderson *et al.* 2011). Our hypothesis is that loss or gain of RAF species could be responsible for *G. rossii* response to N and thus shared losses should be counted as important. Data were log-transformed to improve signal from less abundant OTUs in community distance calculations, and site was included as a cofactor in all analyses. SIMPER (PRIMER, version 6) was used to identify which species contributed most to pairwise distances between host and treatment combinations.

SIMPER can confound mean group distances with within group variability, which causes it to sometimes identify the most variable species rather than the taxa that contribute most to community distances (Warton *et al.* 2012). Additionally, low abundance taxa that contribute little to community distances may be important if they are responsible for disease in the host. We therefore used type III ANOVA (package nlme, Pinheiro *et al.* 2011) on rarefied data to verify SIMPER results and to detect less dominant RAF taxa that shift significantly with host and/or treatment. Taxa in fewer than three plots were removed from the analysis to focus on ubiquitous taxa whose presence or absence could be responsible for *G. rossii* decline, and because two replicates are too few to make statistical comparisons. Due to the large number of comparisons, a false discovery rate (FDR, Yoav & Yosef 1995) correction was applied to *P*-values. Only one taxa was correlated with N amendments after FDR correction, so we also discuss uncorrected results. Overall communities significantly differed between host and treatment plots, suggesting more than a single OTU is affected by N. Our statistical power may be weak given the small number of replicates ($n = 7$ at most per treatment) and disregarding raw *P*-values likely results in discarding many true positives. Several OTUs with identical database hits were significantly correlated with N in the same direction when FDR was not applied, strengthening support for the effect of N on those related taxa.

Results

Host RAF communities

After filtering and chimera checking, a total of 104 668 sequences were obtained. 103 169 sequences were from *G. rossii* roots constituting 1499 OTUs (averaging 3685 sequences and 118 OTUs/sample; SE = 403.46 and 6.33, respectively) and 19 210 sequences from *D. cespitosa* making up 479 OTUs (averaging 2134 sequences and 89 OTUs/sample; SE = 279.36 and 8.54, respectively). Only 9.4% of the 785 OTUs found in control plots were shared between hosts.

In control plots, both hosts were dominated by Ascomycota (95% in *G. rossii*, 82% in *D. cespitosa*). In *G. rossii*, the next dominant phylum was Basidiomycota (4%), followed by Glomeromycota (1%) and fungi with no BLAST hits or hits to unclassified fungi (1%). *D. cespitosa* roots obtained more hits to unclassified fungi (12%) than Basidiomycota (6%).

Geum rossii roots were strongly dominated by fungi in the order Helotiales (83%), and more than a third of sequences were unidentified Helotiales. The most abundant orders in *D. cespitosa* were as follows: Helotiales (30%), Mortierellales (16%), Pleosporales (15%), Hypocreales (7%) and Agaricales (6%). Another 9% of the communities were comprised of unknown orders in Ascomycota. We compared alpha diversity measures between hosts from control plots only. *D. cespitosa* communities were more diverse than those from *G. rossii*, mainly due to greater order evenness, and a trend towards greater richness (Table 1, Fig. 1).

Treatment effects on RAF alpha diversity

There were marginal and significant interactions between host and N on Simpson's diversity from family through phylum, in which N decreased or did not change diversity of *D. cespitosa* RAF, but increased diversity of *G. rossii* RAF. N also tended to decrease richness in *D. cespitosa* but increased richness in *G. rossii* (Table 1).

When the effects of N addition and removal were examined on *G. rossii*, removal did not have a significant effect on alpha diversity on its own, but there were marginal and significant interactions between treatments on taxonomic richness at most taxonomic levels, in which N increased richness in the presence of *D. cespitosa* but decreased it in the competitor's absence. Simpson's diversity at the class and order level also exhibited interactive effects: N increased diversity in the presence of *D. cespitosa*, but decreased diversity in its absence. Overall, N and removal treatments had the most significant effects on family through class alpha diversity (Table 2).

Treatment effects on RAF community composition

Geum rossii and *D. cespitosa* RAF community composition were significantly different at all taxonomic levels (as shown by RDA, Fig. 2a, Table 3). This effect was strongest when communities were described by coarser taxonomic groupings such as phylum, where 35.35% of community variation was explained by host (pseudo-F = 11.69,

Table 1 Effect of treatment on alpha diversity measures of RAF in *G. rossii* and *Deschampsia cespitosa* at each taxonomic level

	Means				Model: host + N + host*N			Model: host (control plots only)
	DesC	DesN	GeumC	GeumN	P (Host)	P (N)	P (interaction)	P (host)
OTU								
Simpson's	0.93	0.89	0.94	0.96	*0.05**	0.62	0.23	0.56
Evenness	0.15	0.15	0.26	0.20	*0.06*	0.43	0.45	0.17
Richness	105.30	79.66	112.03	134.45	*0.03**	0.90	*0.08*	0.77
Genus								
Simpson's	0.88	0.80	0.82	0.82	0.46	0.18	0.13	*0.06*
Evenness	0.26	0.22	0.24	0.20	0.52	0.21	0.90	0.61
Richness	34.62	28.18	26.52	33.20	0.68	0.97	*0.09*	0.13
Family								
Simpson's	0.84	0.79	0.69	0.76	*<0.01**	0.84	*0.06*	*<0.01**
Evenness	0.27	0.24	0.21	0.20	*0.08*	0.59	0.82	0.19
Richness	25.72	23.28	18.34	23.26	0.18	0.64	0.18	*0.07*
Order								
Simpson's	0.77	0.78	0.32	0.56	*<0.01**	*<0.01**	*<0.01**	*<0.01**
Evenness	0.29	0.34	0.12	0.15	*<0.01**	0.39	0.89	*<0.01**
Richness	17.39	15.30	13.87	16.79	0.56	0.81	0.16	0.21
Class								
Simpson's	0.73	0.68	0.30	0.49	*<0.01**	0.11	*0.01**	*<0.01**
Evenness	0.42	0.45	0.15	0.2	*<0.01**	0.45	0.82	*0.002**
Richness	9.72	7.56	9.85	10.36	0.12	0.37	0.15	0.92
Phylum								
Simpson's	0.38	0.21	0.08	0.13	*<0.01**	0.25	*0.05**	*<0.01**
Evenness	0.42	0.41	0.34	0.36	0.16	0.92	0.66	0.25
Richness	4.07	3.24	3.45	3.50	0.58	0.23	0.18	0.29

Diversity measure means for each host × treatment combination, as well as *P*-values for effect of each treatment and their interaction on alpha diversity, are displayed. Removal plots are excluded from this analysis.
DesC, *D. cespitosa* control; DesN, *D. cespitosa* N addition; GeumC, *G. rossii* control; GeumN, *Geum rossii* N addition.
Marginal and significant effects are italicized, significant effects include an asterisk.

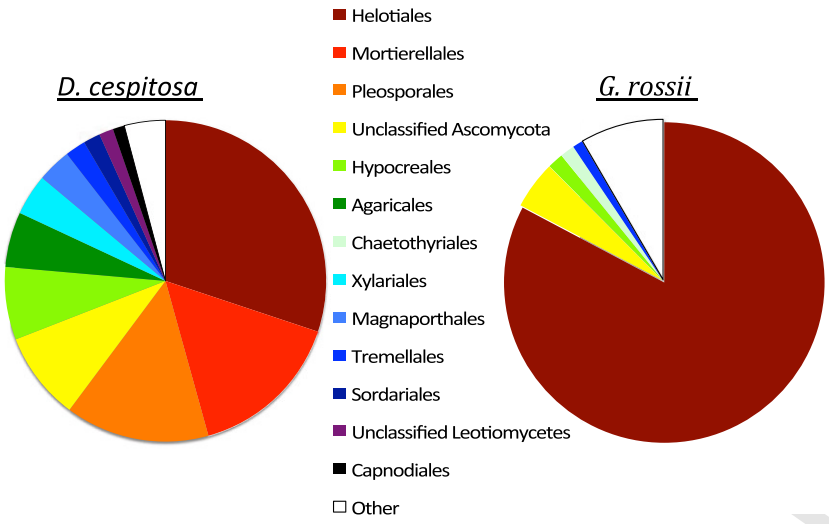

Fig. 1 Order profiles of the two hosts from control plots.

COLOR

P_{MC} = 0.001). There were marginal or significant interactions between host and N at all taxonomic levels: N addition caused greater shifts in *D. cespitosa* than in *G. rossii* community composition (Fig. 2a, Table 3). These patterns were consistent when using NMDS plots and permutation of Bray–Curtis distances to identify between group variation (vegan package in R, Oksanen *et al.* 2011) (Fig S1 and Table S1, Supporting Information). According to SIMPER analyses, the OTUs that contributed most to N-induced community shifts in *G. rossii* were related to *Articulospora tetracladia* (increased), several *Lachnum spp.* (decreased), *Helotiales spp.* (decreased), *Phialocephala spp.* (increased) and a *Phialophora spp.* (increased) (Table S2, Supporting Information). Most OTUs belonged to the order Helotiales. The OTUs that contributed most to community shifts with N in *D. cespitosa* were identified as *Microdochium spp.* (increased), *Geomyces spp.* (increased) and *Herpotrichia juniperi* (decreased) (Table S2, Supporting Information).

When both treatments were considered, we found N affected *G. rossii* communities at finer taxonomic levels (species through order) and explained up to 10.34% of community variation (at the genus level). Removal also had an effect on community composition, but only at the species and genus level (in the latter it explained 5.62% of community variation, Table 3, Fig. 2b). Significant interactions between treatments were found on Bray–Curtis distances at taxonomic levels family, order and class but not on Euclidean distances at any taxonomic level (Table S1, Supporting Information). Species that contributed most to community shifts with N addition according to SIMPER were again, *Articulospora tetracladia*, several *Lachnum*, Helotiales and *Phialocephala spp* and a *Phialophora spp.* Species that contributed most to community shifts with removal also belonged to *Articulospora tetracladia*, *Lachnum* and unidentified Helotiales *spp*, and *Phialocephala europa* (Table S3, Supporting Information).

Treatment effects on individual RAF taxa

ANOVA found only four OTUs to be significantly affected by N in *G. rossii* and four to be significantly affected by N in *D. cespitosa* (Table 4). In *G. rossii*, these were identified as close relatives of a *Rhizoscyphus ericae* aggregate spp., *Meliniomyces bicolor* and two unidentified Helotiales. All declined with N except one unidentified Helotiales. In *D. cespitosa*, affected species belonged to the genera *Geomyces* (positively affected by N), *Gyoerffyella*, *Gibberella*, and *Mortierella* (negatively affected). All *G. rossii* OTUs that responded belonged to the order Helotiales. When tested, the order Helotiales as a whole declined significantly with N, from 83% to 60% of community composition. This was also the only taxonomic group to shift significantly with N after FDR correction. No OTUs related to known pathogens responded significantly to N in *G. rossii*, and one putative pathogen responded negatively to N in *D. cespitosa* (Table 4).

When the effects of both treatments on *G. rossii* RAF were examined, many more taxa were found to be significantly affected by N, likely due to inclusion of more N vs. no N replicates crossed with removal (Table 5). Most taxa affected were also identified as important using SIMPER (Table S3, Supporting Information). Several putative saprobes increased with N, but these were of low abundance and were not found to be important to community shifts using SIMPER. Dark septate endophytes (DSE) increased with N, whereas several species identified as ericoid mycorrhizal fungi (ERM) decreased with N. Eight OTUs were affected by the interaction between N and removal, responding to N differently depending on the presence or absence of *D. cespitosa*. These were

Table 2 Effect of treatment on alpha diversity measures of RAF in *Geum rossii* at each taxonomic level

	Means				Model: N + removal + N*removal		
	CC	CN	DC	DN	P (N)	P (removal)	P (interaction)
OTU							
Simpson's	0.94	0.96	0.94	0.90	0.43	0.12	0.11
Evenness	0.26	0.20	0.16	0.15	0.22	*0.02**	0.51
Richness	112.03	134.45	121.51	100.87	0.94	0.31	*0.08*
Genus							
Simpson's	0.82	0.82	0.77	0.79	0.59	*0.07*	0.74
Evenness	0.24	0.20	0.15	0.21	0.72	0.11	*0.06*
Richness	26.52	33.20	32.99	24.70	0.82	0.77	*0.04**
Family							
Simpson's	0.69	0.76	0.69	0.73	*0.05**	0.67	0.68
Evenness	0.21	0.20	0.16	0.23	0.11	0.84	*0.04**
Richness	18.34	23.26	22.01	17.77	0.89	0.72	*0.08*
Order							
Simpson's	0.32	0.56	0.42	0.40	*0.02**	0.50	*<0.01**
Evenness	0.12	0.15	0.11	0.14	*0.02**	0.38	0.86
Richness	13.87	16.79	17.04	13.25	0.80	0.91	*0.05**
Class							
Simpson's	0.30	0.49	0.40	0.37	*0.08*	0.85	*0.02**
Evenness	0.15	0.2	0.16	0.19	*<0.01**	0.85	0.51
Richness	9.85	10.36	11.09	8.71	0.27	0.81	*0.09*
Phylum							
Simpson's	0.08	0.13	0.10	0.07	0.77	0.58	0.29
Evenness	0.34	0.36	0.29	0.36	0.23	0.47	0.56
Richness	3.45	3.50	4.28	3.29	0.21	0.40	0.17

Diversity measure means for each host × treatment combination, as well as *P*-values for effect of each treatment and their interaction on alpha diversity, are displayed.
Deschampsia cespitosa is excluded from this analysis. CC, control; CN, N addition; DC, *D. cespitosa* removal; DN, *D. cespitosa* removal + N addition.
Marginal and significant effects are italicized, significant effects include an asterisk.

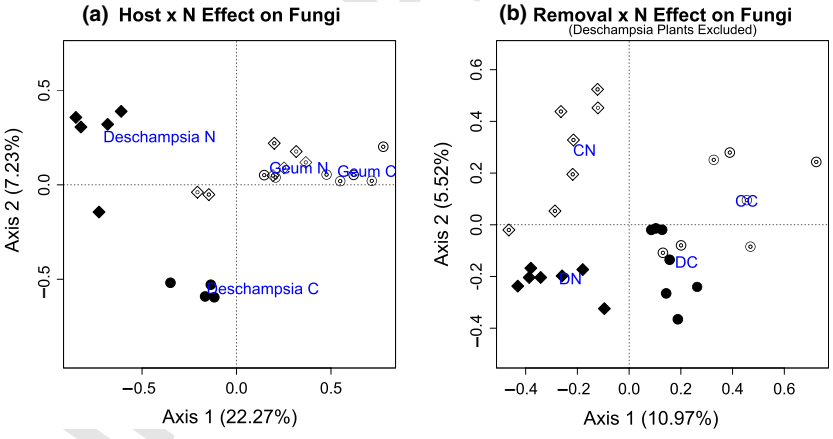

Fig. 2 RDA plots of host and treatment effects on RAF communities at the genus level, the taxonomic level at which effects were strongest. (a) Axes are constrained by host and N addition. Diamonds indicate N plots, circles indicate no N plots. Dark symbols indicate *D. cespitosa*, open circles indicate *G. rossii*. Significant interaction (pseudo-F = 2.4081, Monte Carlo permutation test $P_{MC} = 0.009$). (b) Axes are constrained by N and removal. Diamonds indicate N plots, circles indicate no N plots. Dark symbols indicate *D. cespitosa* removal plots, open circles received no removal.

COLOR

mostly unknown Helotiales or *Lachnum spp*, one was related to a common soil fungi in cold soils, one to an ERM and one to a potential pathogen.

A number of OTUs related to known ERM decreased with removal. Other than this, no consistent trend could be found between ecological function and removal. Though several OTUs affected by removal had BLAST hits to the same genus or species as those affected by N, several genera were affected by only one treatment type.

Table 3 Results of permutation tests on Euclidean distances. 'F' indicate pseudo-F

Taxonomic Level	Host (F)	Host (P)	N (F)	N (P)	Host × N (F)	Host × N (P)
Host + N + Host × N						
Phylum	12.252	*0.001**	1.184	0.295	2.523	*0.093*
Class	9.782	*0.001**	2.502	*0.023**	1.815	0.105
Order	7.247	*0.001**	2.920	*0.011**	2.129	*0.030**
Family	5.584	*0.001**	2.825	*0.003**	2.178	*0.011**
Genus	5.766	*0.001**	2.871	*0.001**	2.252	*0.009**
OTU	3.657	*0.001**	1.919	*0.005**	1.587	*0.033**

Taxonomic Level	N (F)	N (P)	Removal (F)	Removal (P)	N × removal (F)	N × removal (P)
N + removal + N × removal						
Phylum	0.887	0.428	0.812	0.474	0.708	0.549
Class	1.710	*0.092*	1.865	*0.085*	0.815	0.572
Order	2.215	*0.014**	1.296	0.213	1.140	0.320
Family	1.722	*0.051*	1.085	0.379	1.349	0.164
Genus	3.299	*0.001**	1.786	*0.026**	1.067	0.376
OTU	2.003	*0.001**	1.564	*0.019**	1.068	0.387

The first model tests the effects of host plant and N on RAF community composition. The second model tests the effect of N and removal on *Geum rossii* RAF.
Marginal and significant effects are italicized, significant effects include an asterisk.

Table 4 *Geum rossii* and *Deschampsia cespitosa* RAF taxa significantly correlated with N addition with type III ANOVA

	Taxon	Effect direction	P	Relative abundance (C) (%)	Relative abundance (N) (%)	Ecological function
Geum rossii						
Class	Leotiomycetes	−	0.014	83.87	66.65	
Order	Helotiales*	−	<0.001	83.24	60.27	
Genus	*Geomyces*	+	0.014	0.11	2.40	
	Meliniomyces	−	0.018	7.03	0.15	
	Papulaspora	+	0.017	0.00	0.54	
	Rhizoscyphus	−	0.023	7.18	0.57	
	Unknown Helotiales	+	0.038	4.29	0.91	
OTU	Helotiales sp. B1	+	0.002	0.05	1.67	Ubiquitous and diverse order
	Helotiales sp .16 MV-2011	−	0.044	6.10	0.90	Ubiquitous and diverse order
	Rhizoscyphus ericae aggregate	−	0.039	1.63	0.05	Ericoid mycorrhizae
	Meliniomyces bicolor	−	0.024	3.62	0.00	Ericoid mycorrhizae
Deschampsia cespitosa						
Phylum	Ascomycota	+	0.003	75.13	87.29	
Order	Mortierellales	−	0.046	15.63	8.48	
Genus	*Mortierella*	−	0.046	18.86	9.01	
	Unknown Helotiales	−	0.023	5.78	1.33	
OTU	*Geomyces sp.* FMCC-3	+	0.018	0.07	1.00	Psychrotolerant soil fungi, Saprobe
	Gibberella sp. PPn9-A Fr	−	0.043	2.51	0.46	Potential pathogen
	Gyoerffyella sp. PB1-R3-D Fr.3	−	0.007	0.39	0.30	Dark septate fungi
	Mortierella sp. W161	−	0.047	1.19	0.10	Saprobic

These data exclude removal plots from *G. rossii* samples. Taxa present in fewer than three plots were removed from analysis.
Uncorrected *P*-values, direction of N effect and relative abundance of the taxa are listed.
Taxa significantly correlated with treatment after FDR correction are indicated by an asterisk. Putative ecological functions of OTUs correlated with N are included.

Discussion

Here, we report substantial host-specific effects of N enrichment on RAF, suggesting that RAF communities may be important drivers of response to N in two dominant alpine tundra plants.

Plant species host different RAF communities

There were substantial differences between the RAF communities of the two hosts under natural conditions, perhaps accounting for their divergent responses to N. *G. rossii*'s RAF community was strongly dominated by the order Helotiales. Helotiales are common root fungi, and include many ERM and DSE (Zijlstra *et al.* 2005; Newsham *et al.* 2009; Tedersoo *et al.* 2009). ERM are common mutualists in nutrient poor soils (Read 1996), and can provide hosts with resistance to plant-produced phytotoxic tannins and other environmental stresses (Cairney & Ashford 2002; Cairney & Meharg 2003). DSE from the Helotiales are common mutualists in cold-stressed habitats, particularly of Rosaceae plants (Newsham *et al.* 2009; Upson *et al.* 2009a; Newsham 2011). ERM and DSE have both been shown to harvest and provide their hosts with nutrients immobilized in organic matter (which is high in cold soils due to slow decomposition rates) (Read 1996; Caldwell *et al.* 2000; Upson *et al.* 2009a).

In this study, many of *G. rossii*'s most abundant OTUs match known DSE and ERM, and more are unidentified Helotiales. Inoculation experiments deducing the function of unidentified Helotiales root isolates from cold or heathland soils suggest many are mutualists, especially when supplied with an organic N source, and are likely DSE or ERM (Zijlstra *et al.* 2005; Upson *et al.* 2009b; Newsham 2011). A large abundance of this taxonomic group might suggest *G. rossii* RAF specialize in harvesting immobilized nutrients from these high organic matter soils and/or influence *G. rossii* tolerance to environmental stresses associated with cold climates. Interestingly, *G. rossii* immobilizes N in the biomass of associated microbes and phenolic exudates (Bowman *et al.* 2004; Meier *et al.* 2009). Perhaps *G. rossii*'s RAF community improves uptake of these sequestered nutrients.

Deschampsia espitosa RAF were more diverse than those of *G. rossii*. *D. cespitosa* covers a much broader geographic and environmental range than *G. rossii* (biodiversity occurrence data accessed through GBIF data portal, data.gbif.org), which may be facilitated by its diversified symbiont community. Diverse RAF may provide versatility via resistance to a wider variety of stresses, and access to nutrients from a wider variety of sources.

That the RAF communities of the two hosts differ is not surprising, as other research has found unique fungal communities associated with different host species residing in the same habitat (Upson *et al.* 2009a). More interesting is that the RAF communities from the two hosts differed in their response to N, perhaps due to differences in RAF communities under ambient conditions.

N and plant competition differentially impact RAF communities in two codominant plant hosts

Nitrogen had opposing effects on the RAF from the two hosts, increasing Simpson's diversity in *G. rossii* but decreasing or not changing it in *D. cespitosa*. *D. cespitosa* lost RAF taxonomic richness under N, while *G. rossii* gained richness. N also caused a massive reduction in the dominant order, Helotiales, in *G. rossii*, contributing to the positive effect of N on *G. rossii* diversity. These results contrast with other studies, which have shown a decrease in fungal biodiversity under elevated N (Frey *et al.* 2004; Avis *et al.* 2008; Lilleskov *et al.* 2008). However, these studies have primarily examined aboveground fruiting bodies rather than DNA from root tissue. Observed aboveground fungal diversity may not correlate with belowground diversity (Gardes & Bruns 1996). Porras-Alfaro *et al.* (2007) found N increased arbuscular mycorrhizal fungal (AMF) diversity using molecular methods and also attributed this to loss of the dominant AMF species.

When *D. cespitosa* was removed, the alpha diversity trends in *G. rossii* were reversed. The ecological or biological implications of these interactions are difficult to determine. As far as we know, no other study has described such interactions between N and neighbour identity on RAF diversity. These data show that presence of certain plant species can influence the effect of N on a focal individual's RAF diversity.

It has been shown that initial evenness in microbial communities provides resilience and preserves functional stability in the face of environmental stress, because uneven communities depend heavily upon the functional role of the most dominant taxa, which may decline without replacement (Wittebolle *et al.* 2009). Thus, the extreme dominance and potential functional importance of Helotiales in *G. rossii* may make *G. rossii* RAF vulnerable to functional disturbance. *D. cespitosa* RAF diversity may provide functional stability in the face of environmental change due to greater functional redundancy.

Deschampsia cespitosa communities were more sensitive to N than were *G. rossii* communities. Perhaps this flexible RAF response contributes to *D. cespitosa*'s ability to adapt to N enrichment. Research suggests hosts that

Table 5 *Geum rossii* RAF that are significantly correlated with treatment

Taxa that with N	Species name	Effect	CC	CN	DC	DN	P	Ecological function
Ascomycota								
Leotiomycetes Helotiales Helotiaceae	*Articulospora tetracladia*	–	0.18	0.00	0.00	0.00	0.036	Saprobic
	Meliniomyces bicolor	–	2.03	0.00	0.23	0.06	0.028	Ericoid mycorrhizae
	Meliniomyces bicolor	–	3.14	0.00	0.31	0.10	0.002	Ericoid mycorrhizae
	Rhizoscyphus ericae	–	0.97	0.00	0.03	0.03	0.021	Ericoid mycorrhizae
	Rhizoscyphus ericae	–	1.04	0.04	0.16	0.07	0.031	Ericoid mycorrhizae
	Rhizoscyphus ericae aggregate	–	1.41	0.03	0.63	0.11	0.004	Ericoid mycorrhizae
	Rhizoscyphus ericae aggregate	–	1.22	0.00	0.06	0.03	0.038	Ericoid mycorrhizae
Leotiomycetes Helotiales Hyaloscyphaceae	***Lachnum sp. YM272***	–/+	0.07	0.00	0.00	0.03	0.049	Mostly saprobic, commonly root associated
	Lachnum sp. 252	–	0.08	0.00	0.00	0.03	0.037	Mostly saprobic, commonly root associated
Leotiomycetes Helotiales unknown	***Helotiales sp. 16 MV-2011***	–/+	5.32	0.70	0.95	1.85	0.005	Ubiquitous and diverse order
Leotiomycetes Helotiales incertae sedis	*Phialocephala fortinii*	+	0.00	0.27	0.07	0.04	0.028	Dark septate endophyte
	Phialocephala turiciensis	+	0.00	1.25	0.40	0.54	0.033	Dark septate endophyte
Leotiomycetes incertae sedis Myxotrichaceae	*Geomyces pannorum*	+	0.00	0.18	0.00	0.00	0.009	Psychrotolerant soil fungi, saprobe
	Geomyces sp. FFI 30	+	0.06	0.99	0.18	0.55	0.025	Psychrotolerant soil fungi, saprobe
	Geomyces sp. FMCC-2	+	0.00	0.11	0.00	0.00	0.008	Psychrotolerant soil fungi, saprobe
	Geomyces sp. FMCC-2	+	0.00	0.25	0.00	0.00	0.013	Psychrotolerant soil fungi, saprobe
Sordariomycetes Hypocreales Hypocreaceae	*Hypocrea rufa*	+	0.00	0.17	0.00	0.04	0.032	Mostly saprobic
Incertae sedis	*Papulaspora sp. MTFD02*	+	0.00	0.40	0.00	0.04	0.012	Potential plant and fungal pathogens
	Papulaspora sp. MTFD02	+/–	0.00	0.16	0.09	0.00	0.049	Potential plant and fungal pathogens
	Spirosphaera beverwijkiana	–	0.57	0.00	0.34	0.00	0.021	Aquatic hyphomycete
	Tetracladium furcatum	+	0.14	0.89	0.13	0.00	0.028	Saprobic
Incertae sedis								
Incertae sedis Mortierellales Mortierellaceae	*Mortierella alpine*	+	0.00	0.14	0.00	0.00	0.009	Saprobic

Taxa that shift with *Deschampsia cespitosa* removal	Species Name	Effect direction	CC	CN	DC	DN	P	Ecological function
Ascomycota								
Eurotiomycetes Chaetothyriales Herpotrichiellaceae	*Capronia sp. UBCTRA*	+	0.09	0.00	0.53	0.05	0.030	Root-associated saprobes
	Capronia sp. UBCTRA	+	0.00	0.10	0.24	0.03	0.037	Root-associated saprobes
Leotiomycetes Helotiales Helotiaceae	*Articulospora tetracladia*	–	0.18	0.00	0.00	0.00	0.036	Saprobic
	Meliniomyces bicolor	–	0.22	0.03	0.00	0.04	0.028	Ericoid mycorrhizae
	Meliniomyces bicolor	–	2.03	0.00	0.23	0.06	0.049	Ericoid mycorrhizae
	Meliniomyces bicolor	+/–	3.13	0.00	0.31	0.10	0.002	Ericoid mycorrhizae
	Rhizoscyphys ericae	–	0.97	0.00	0.03	0.03	0.021	Ericoid mycorrhizae
Leotiomycetes Helotiales Hyaloscyphaceae	***Lachnum sp. 252***	+/–	0.08	0.00	0.00	0.03	0.045	Mostly saprobic, commonly root associated
	Lachnum sp. YM272	–	0.07	0.00	0.00	0.03	0.048	Mostly saprobic, commonly root associated
	Lachnum sp. YM272	+	0.00	0.03	0.54	0.00	0.048	Mostly saprobic, commonly root associated
	Lachnum sp. YM272	+	0.55	0.09	1.98	0.16	0.030	Mostly saprobic, commonly root associated
	Lachnum sp. YM272	+	0.00	0.00	0.66	0.00	0.011	Mostly saprobic, commonly root associated
	Lachnum sp. YM272	+	0.00	0.05	0.56	0.00	0.047	Mostly saprobic, commonly root associated

Table 5 *Continued*

Taxa that shift with *Deschampsia cespitosa* removal	Species Name	Effect direction	CC	CN	DC	DN	P	Ecological function
Leotiomycetes Helotiales incertae sedis	*Phialocephala europaea*	–	0.36	0.10	0.00	0.03	0.029	Dark septate endophyte
	Phialocephala europaea	–	0.53	0.05	0.03	0.00	0.042	Dark septate endophyte
	Phialocephala fortinii	–	0.14	0.03	0.00	0.03	0.044	Dark septate endophyte
Leotiomycetes Helotiales unknown	***Helotiales sp. 16 MV-2011***	+/–	5.32	0.70	0.95	1.85	0.008	Ubiquitous and diverse order
	Helotiales sp. 17 MV-2011	-	0.22	0.07	0.00	0.00	0.040	Ubiquitous and diverse order
	Helotiales sp. SC1-1	+	1.74	1.32	7.45	1.84	0.006	Ubiquitous and diverse order
	Helotiales sp. SC1-1	+	0.00	0.00	0.10	0.06	0.046	Ubiquitous and diverse order
Sordariomycetes Diaporthales unknown	*Diaporthales sp. E6927e*	+	0.00	0.03	0.34	0.00	0.039	Includes plant pathogens
Incertae sedis	*Gyoerffyella sp. PB1-R3-D Fr*	+	0.00	0.05	0.17	0.00	0.046	Dark septate endophyte
	Gyoerffyella sp. PB1-R3-D Fr	+	0.00	0.05	0.16	0.03	0.046	Dark septate endophyte
	Gyoerffyella sp. PB1-R3-D Fr	+	0.00	0.00	0.23	0.10	0.043	Dark septate endophyte
	Leptodontidium orchidicola	+	0.00	0.00	0.53	0.03	0.038	Mutualist or parasite

Taxa present in fewer than three plots were removed from analysis. The direction of treatment effect on each taxon, average relative abundance in each treatment, uncorrected *P*-values and putative ecological function are listed.
Species showing significant interactions between N addition and *Deschampsia cespitosa* removal are in bold.

reduce or eliminate infection by mutualists under nutrient elevation avoid parasitism, because hosts that cannot control mutualist infection rates run the risk of being parasitized by their once mutualists (Johnson & Oelmüller 2009). Reduction of mutualists could also explain loss of *D. cespitosa* RAF diversity under N.

That the effect of N was stronger and caused shifts across more distantly related species in comparison with removal suggests N has an effect on *G. rossii* RAF communities that is distinct from the effect of competitor presence. Additionally, RAF communities under removal treatment diverged from communities under N addition. Significant interactions between treatments were found on Bray–Curtis (Table S1, Supporting Information) but not Euclidean distances (Table 3) between RAF communities, but treatment had similar patterns of effect in both ordinations (data not shown). Shared absences reduce Euclidean distances but are not included in Bray–Curtis distances, so these results may indicate that there was a stronger interactive effect on relative abundance of present taxa rather than shared losses.

Many of the OTUs responsible for community shifts with N belonged to the same genera as those responsible for shifts with removal, indicating these genera are generally sensitive to disturbance, but the two treatments sometimes elicited opposite responses from these sensitive genera, and some genera were affected by only one of the treatments.

Description of RAF taxa affected by N

Most taxa affected by N were putative mutualists and commensals. Many *G. rossii* OTUs that significantly declined with N were assigned to the *Rhizoscyphus ericae* aggregate and one of its subclades, *Meliniomyces bicolor* (Hampleton & Sigler 2005). The *Rhizoscyphus ericae* aggregate are mainly ERM (Grelet *et al.* 2009). Yesmin *et al.* (1996) also found N reduced ERM infection rates in a greenhouse experiment. A couple putative DSE from *G. rossii* roots increased with N. Upson *et al.* (2009b) found that DSE in the Helotiales behave as mutualists when supplied with an organic N source, but could become parasitic when supplied with an inorganic N source. Most OTUs affected belonged to the order Helotiales. Indeed, the order Helotiales as a whole declined dramatically with N in *G. rossii* roots. Because Helotiales from roots in cold climates seem important to N uptake (Caldwell *et al.* 2000; Upson *et al.* 2009b; Newsham 2011), it is perhaps not surprising that this group is highly sensitive to inorganic N enrichment. Interestingly, few putative saprobic taxa responded to N, suggesting that the plant species turnover that occurs in N fertilized plots does not trigger increases in RAF decomposers. Despite some taxonomic

overlap, several RAF species responded only to N or to removal, again highlighting the distinct effects of N vs. competitor presence.

Deschampsia cespitosa OTUs found to be affected by N did not share a common phylogeny or known ecological function. The order Mortierellales declined with N, which contains mostly saprobes, and includes many genera with the ability of complex organic substrate transformations (Wagner *et al.* 2013), but members of this order were rare.

RAF and host response to N

Geum rossii are asymptomatic in N addition plots (Farrer, *pers. comm.*), they simply do not return after a 4-year lag from the start of N addition (Suding *et al.* 2008). Parasites may be depleting C resources required for overwintering. There is substantial evidence that *G. rossii* plants are C limited in N plots, having very reduced C/N ratios, lower nonstructural carbohydrate levels in rhizomes and fewer preformed leaves (necessary for resprouting in the spring) compared with those in unfertilized conditions (Farrer *et al.* 2013). However, Farrer *et al.* (2013) also shows that parasitism is not occurring in the summer. Schadt *et al.* (2003) found that microbial activity peaks in winter in Niwot alpine tundra soils and that most of winter microbial biomass is fungal. These fungi would require substantial C sources during the 9-month dormant season when plants are not photosynthesizing.

Dark septate endophytes from the Helotiales can become parasitic when supplied with inorganic N (Upson *et al.* 2009b). *Geum rossii* DSE may parasitize their host as inorganic N rises. If this were true, however, we would expect Helotiales relative abundance to increase rather than decline in N plots. Some OTUs related to known DSE did increase with N, but given that other related groups primarily declined, evidence for parasitism is weak. One potentially pathogenic genus increased with N, *Papulaspora spp*. Species in this genus can be either plant or fungal pathogens, making its role in *G. rossii* fitness unclear here. Winter sampling and fungal quantification methods may provide different insights in this regard.

Members of the Helotiales could also simply be beneficial to *G. rossii*, and their lessened dominance corresponds to reduced *G. rossii* fitness due to loss of associated benefits, such as access to organically bound nutrients (Michelsen *et al.* 1996; Caldwell *et al.* 2000) and resistance to phytotoxic tannins and other harsh environmental conditions (Cairney & Ashford 2002; Cairney & Meharg 2003), services provided by DSE and ERM. This hypothesis is bolstered by Schmidt *et al.* (2004) findings that N amendments selected against microbes that break down phenolics and complex organic matter at Niwot Ridge. We found that putative ERM declined with N. Greenhouse experiments are needed to further assess the role of Helotiales *spp.* in host fitness, but given the extreme dominance of this order in *G. rossii* and research on similar taxa in similar ecosystems, their presence likely influences *G. rossii* fitness, and they are likely involved in N uptake and/or tolerance to stress associated with cold ecosystems.

Summary

Few studies have employed DNA sequencing to examine the effect of N on RAF community composition. Culture- and microscopy-based techniques have shown repeatedly that soil fertility drives fungal symbiont abundance, richness and community composition (Peter *et al.* 2000; Frey *et al.* 2004; Edgerton-Warburton *et al.* 2007; Avis *et al.* 2008; Lilleskov *et al.* 2008; Cox *et al.* 2010). We compared RAF response to altered plant competition and to N to assess whether similar taxa were affected by both.

We found that N affects RAF differently than does altering competitor abundance. We show for the first time that a host plant that thrives under N enrichment harbours a diverse fungal community that is highly responsive to N relative to the fungal community of a host plant that responds negatively to N. Perhaps a flexible RAF community is key to adapting to nutrient enrichment. We confirm that Helotiales are dominant root symbionts in cold soils and find they are particularly abundant and sensitive to N in a host plant that is negatively impacted by N enrichment, but less abundant and sensitive to N in a plant that is unaffected by N enrichment. RAF are known to affect plant fitness, so these N-induced shifts in RAF community could affect plant fitness. If they do, belowground microbial dynamics are implicated in aboveground plant response to abiotic change. Future research should be directed to better describing these alpine RAF and their interactions with alpine vegetation.

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S.L. Hicks conducted laboratory work, bioinformatics, statistical analyses, and wrote the paper. A. Porras-Alfaro did initial sample processing. E.C. Farrer conducted field work and helped with the statistical analyses. D.L. Taylor contributed to bioinformatics. A. Porras-Alfaro, K.N. Suding and R.L. Sinsabaugh developed project design and acquired funding. All authors edited and participated in discussions related with this manuscript.

Data accessibility

Raw DNA sequences, R code, and OTU tables and other R input files are available on Dryad (doi:10.5061/dryad.sv33f).

Supporting information

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

Fig. S1 Alpha rarefaction curves showing species richness for all host × treatment groups.

Fig. S2 NMDS plot of Bray-Curtis distances between RAF communities from different hosts and treatments.

Table S1 Results of permutation tests on Bray Curtis distance matrices.

Table S2 SIMPER results indicating which OTUs are most responsible for RAF community shifts with N for each plant host, and their taxonomic assignments.

Table S3 SIMPER results indicating which *G. rossii* RAF OTUs are responsible for community shifts with N and removal, and their taxonomic assignments.

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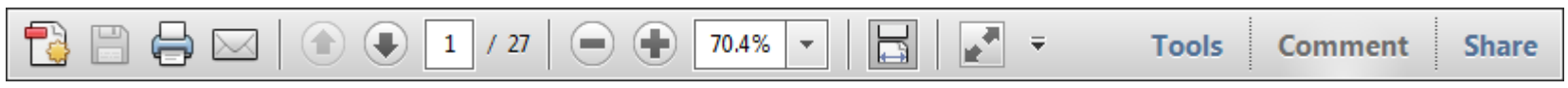

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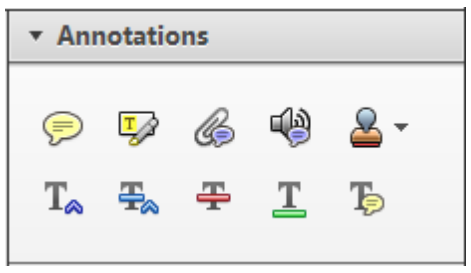

1. Replace (Ins) Tool – for replacing text.

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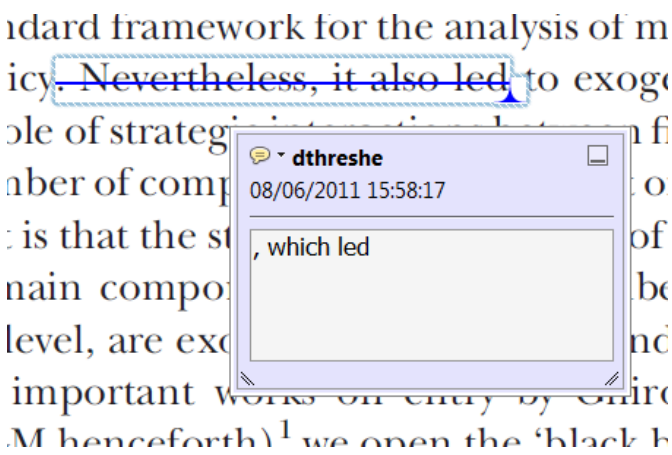

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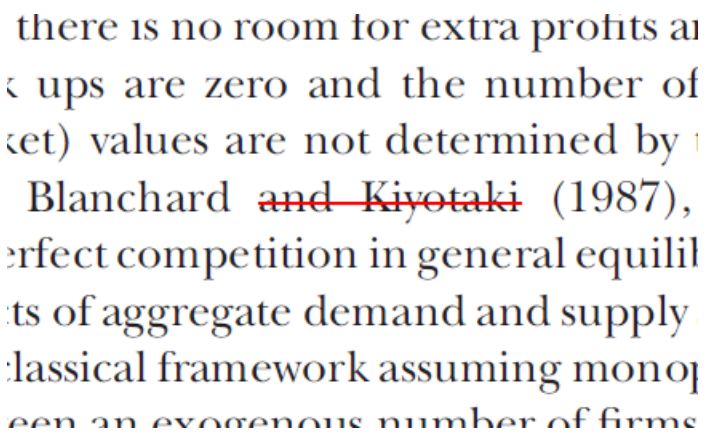

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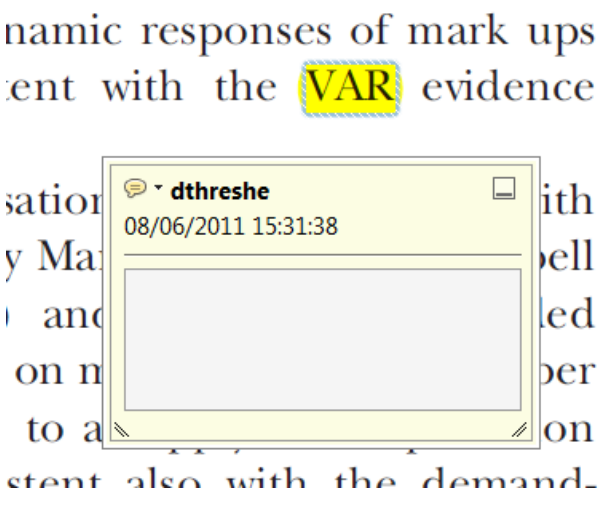

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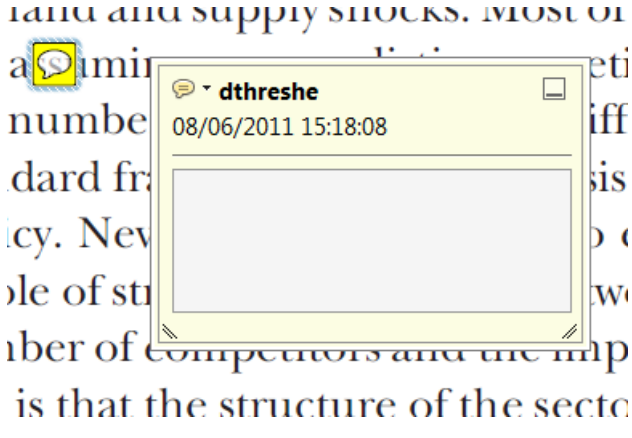

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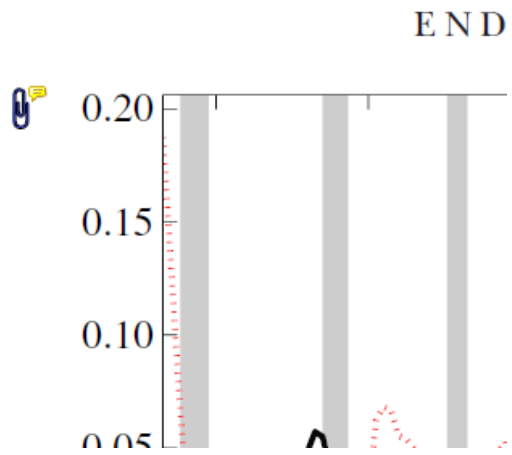

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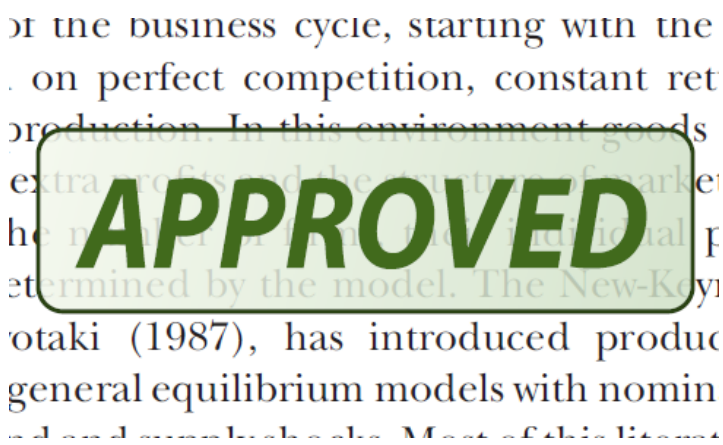

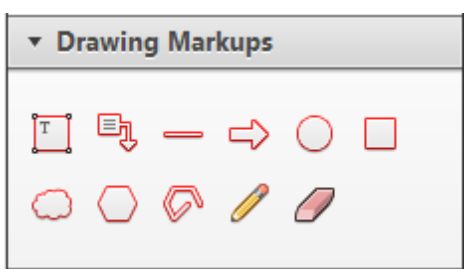

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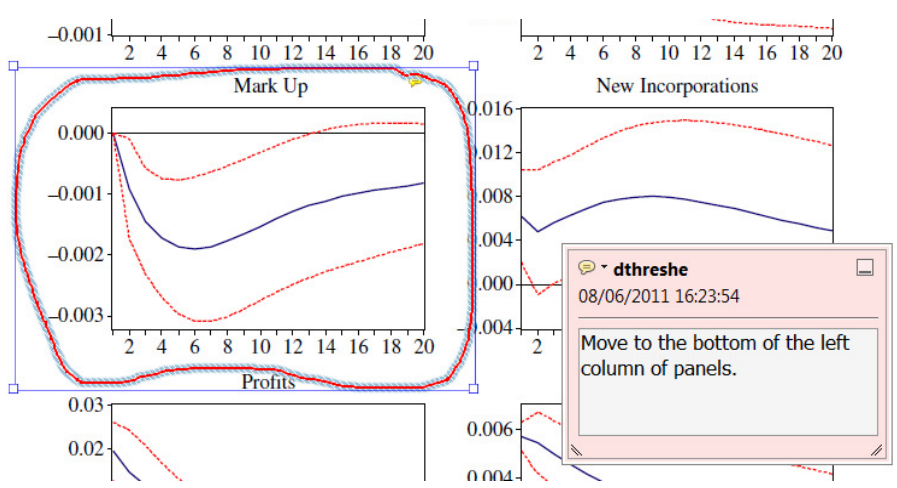

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