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Fungal but not bacterial soil communities recover after termination of decadal nitrogen additions to boreal forest

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ABSTRACT

The rate at which formerly nitrogen loaded forests will return to their natural nitrogen-limited state is of considerable scientific and societal interest. Yet the sensitivity of soil microorganisms to these putative changes is mainly unknown. We report effects on fungal and bacterial communities caused by two decades of chronic nitrogen fertilization and subsequent changes 14 years after termination of nitrogen load. We compare these changes in community composition with those observed in natural nitrogen supply and pH gradients using DNA fingerprinting methods and Sanger sequencing.

Soil fungal ITS length-heterogeneity profiles correlated equally well to carbon-to-nitrogen ratios and pH. Sequencing results indicated a clear decrease in the relative abundance of amplicons ascribed to known ectomycorrhizal fungi in both natural and experimental high nitrogen conditions, and a recovery of species in the terminated nitrogen treatment. The dominant sequences in low nitrogen soils were identified as members of *Piloderma* spp. Terminal restriction fragment length profiles of the bacterial 16S rRNA gene were linked to carbon-to-nitrogen ratios and pH in the natural locations but to soil nitrogen in the nitrogen addition experiment that had low variability in pH. Sequencing revealed the dominance of *Acidobacteria* and *Proteobacteria* in all soils but also showed a marked increase in *Bacteroidetes* in high nitrogen treatment not evident in the natural high nitrogen and high pH environments. *Proteobacteria* sequences included described strains from high-organic and low-pH systems that are believed be involved in degradation of complex plant material.

There were signs of recovery of fungal but not of bacterial communities in the sense that community's in terminated nitrogen addition plots did not differ significantly from those in control plots or from the low nitrogen stands in the natural nitrogen supply gradient. The need of further examination of the seemingly functionally redundant bacterial communities is stressed.

1. Introduction

The soil microbial community plays a fundamental role in decomposition of organic matter, nutrient cycling and energy flow. Soil microorganisms assimilate carbon from above- and below-ground litter, and photosynthate directly allocated from above-ground structures to the rhizosphere, mycorrhizosphere, and the mycosphere (Nazir et al., 2010). The latter pathway should be of considerable importance in nitrogen poor boreal forests where mycorrhizal fungi dominate both the microbial biomass and necromass (Read and Perez-Moreno, 2003). Increased soil nitrogen causes reduction in ectomycorrhizal sporocarps production, root colonization, and soil-based mycelia. The widely accepted explanation to these frequent observations is that when the plant experiences high nitrogen availability, less carbon is allocated to the roots and associated ectomycorrhizal fungi (Lilleskov et al., 2011, and references therein). The strongest observed correlations appear to be between lower sporocarp counts and increased soil nitrogen (Wallenda and Kottke, 1998), with fewer decreases reported in the rate of root tip colonization. This is a trend that could be predicted, because less photosynthate are needed to support ectomycorrhizal fungi on root tips than actively growing extraradical mycelium producing sporocarps.

The extent to which ectomycorrhizal mycelium decreases with nitrogen addition is hard to quantify, and is still largely unknown (Wallander et al., 2013). A recent $^{13}CO_2$-labeling study estimated

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the decrease to be 60% after high nitrogen additions to a boreal pine forest (Högberg et al., 2010) and nitrogen fertilization reduced plant belowground carbon allocation to ectomycorrhizal growth (Wallander et al., 2011). Previous research in Fennoscandian Norway spruce (*Picea abies* L.) forests reported a decrease in the phospholipid biomarker 18:2ω6, 9 (e.g., Högberg et al., 2003; Nilsson et al., 2005; Demoling et al., 2008). We previously reported a strong correlation between this biomarker and ectomycorrhizal fungi in boreal forest soils associated with tree belowground carbon allocation study (Yarwood et al., 2009). Given these results, we hypothesize that ectomycorrhizal fungi detected in bulk soil samples will decrease under increased soil nitrogen conditions. Furthermore, we expect to see changes in the fungal community dependent upon the sensitivity of individual ectomycorrhizal species (or genus) to changes in nitrogen (e.g., Frey, et al., 2004; Toljander et al., 2006; Cox et al., 2010; Kjøller et al., 2012).

Differences in soil bacterial communities appear to be subtle (Yarwood et al., 2009; Dimitriu and Grayston, 2010; Yarwood et al., 2013) even in forests where nutrient concentrations vary considerably. Although deep sequencing efforts have in some cases shown differences in the rare bacterial biosphere, dominant microbial taxa appear to be similar across locations (Nemergut et al., 2011). Like many other soil ecosystems (Lauber et al., 2008) forest soil pH is the strongest correlating factor in many studies (Fierer and Jackson, 2006; Dimitriu and Grayston, 2010; Yarwood et al., 2010). We hypothesize that the soil bacterial community will differ between the natural forest types in our study location where pH varied by three units (Högberg et al., 2003). Bacterial communities have also been shown to relate to soil nutrient concentrations (Nemergut et al., 2008; Fierer et al., 2012). For example, Nemergut et al. (2008) observed an increase in *Bacteroidetes* in nitrogen amended soils. Based upon previous boreal forest clone libraries (Yarwood et al., 2009; Dimitriu and Grayston, 2010), we hypothesize that bacterial communities will be dominated by *Acidobacteria* and *α-Proteobacteria* in the natural as well as in the long-term nitrogen fertilization experiment, but expect to see an increased abundance of *Bacteroidetes* in forest stands with high inorganic nitrogen concentrations.

The objectives in this study were to apply DNA fingerprinting methods (length heterogeneity polymerase chain reaction, LH-PCR and terminal restriction length polymorphism, T-RLFP), along with clone library sequences, to characterize the soil microbial community composition. Bacteria were characterized by targeting a 900 bp region of the 16S that included many variable regions (including V4–V6). Fungi were characterized by targeting a region that includes ITS-1 and ITS-2, regions that are often targeted due to the high sequence variability. Comparisons were made across natural gradients in pH and nitrogen supply and a 34-year-old nitrogen fertilization experiment including a nitrogen treatment terminated 14 years before this study. By analyzing both bacterial and fungal community composition and in the same soil samples, which is rarely done, we could ask the following questions: (i) do fungal and bacterial community compositions show the same sensitivity to nitrogen? (ii) do fungal and bacterial compositions recover after termination of nitrogen loading? For defining recovery of formerly nitrogen loaded plots, our null hypothesis is that the parameters studied will remain altered in the terminated nitrogen treatment, i.e., not significantly different from observations in on-going nitrogen treatments. The null hypotheses will be rejected if data indicate no significant difference with the control plots, i.e., indicating that the studied system has returned to states characteristic of the control.

2. Materials and methods

2.1. Study sites

2.1.1. Natural soil nitrogen supply and pH gradient

The site is located northwest of Betsele in northern Sweden (64°39′N, 18°30′ E, 235 m altitude). We used three previously described 90-m-long transects, 25–70 m apart, through a 130-year-old forest (Högberg et al., 2007, and references therein). These transects encompass the variations in forest productivity, N supply, and soil pH encountered through Fennoscandian boreal forests. The DS (dwarf-shrub) forest is an open low-productivity *Pinus sylvestris* L. forest; the field layer is dominated by ericaceous dwarf shrubs. The intermediate SH (short-herb) forest is a dense *P. abies* L. forest; here, several short herbs are abundant. The highly productive TH (tall-herb) forest, with the highest soil pH and nitrogen supply (Table 1), is dominated by *Picea abies* (up to 36 m in height); the field layer consists mainly of tall herbs. Soils in the entire area are sandy till soils with many boulders, classified as Haplic Podzols (FAO, 1988). The slope is 2%. Mean annual temperature and precipitation are 1 °C and 570 mm, respectively. On average the site is covered by snow from late October until early May.

2.1.2. Long-term nitrogen loading experiment

This experiment is a 50-year-old low productivity Scots pine (*P. sylvestris* L) forest of DS forest type similar to that at Betsele with *Vaccinium vitis-idaea* and *V. myrtillus* as dominant understory species. It is located on a gently (2–5%) sloping till soil at Norrliden (64°21′N, 19°45′ E, 267 m altitude) c. 65 km E of Betsele, with similar climate and soils. This forest was planted in 1953 after prescribed burning in 1952. Ammonium nitrate was applied annually to plots (30 × 30 m) at four rates; N0–N3 with three replicate plots per treatment. N0 is the untreated control, which only receives the background deposition of c. 3 kg N ha^{-1} yr^{-1}, N1 has received annual additions of c. 34 kg N ha^{-1} yr^{-1} from 1971 to 2004, N2 twice the N dose of N1, and N3 received c. 108 kg N ha^{-1} yr^{-1} from 1971 to 1990, and is thus a high nitrogen treatment recovering from the previous high nitrogen load (Högberg et al., 2011). Further details about experimental design, soils, etc. are given by Tamm (1999).

2.2. Soil sampling

Sampling was performed in late August 2004. We used a 0.15-m corer and sampled the combined F + H horizons (0.065 m

Table 1
Soil chemical characteristics of the mor-layer at the study sites including a natural gradient of three forest types at Betsele and a long-term nitrogen fertilization experiment (Norrliden). Means of N = 3. Data are from the same samples as in Oecologia in Högberg et al. (2007). Total nitrogen N, N_{tot}, is from Giesler et al. (1998).

Site	Forest type[a]	Treatment[b]	pH[c]	C/N	N_{tot}[d]	NH_4–N	NO_3–N
					(kg ha^{-1})	(μg g^{-1} o.m.)	
Betsele	DS	–	4.0	38.1	370	4.6	0.9
	SH	–	4.6	22.9	700	5.2	0.7
	TH	–	5.3	14.9	1330	15.9	3.4
Norrliden	DS	N0	4.1	37.5	165	0.5	0.7
	DS	N1	4.1	31.1	316	39.9	1.5
	DS	N2	4.2	27.7	351	88.4	7.3
	DS	N3	4.1	27.2	416	3.3	0.6

[a] Dwarf shrub (DS), short herb (SH), and tall herb (TH) forest type.
[b] N0 is the control and N1, N2, and N3 denote increasing rates of N load. Note that N3 treatment was terminated in 1990.
[c] Soil:water ratio 1:3 (v/v).
[d] Difference between total N in the DS at Betsele and N0 at Norrliden is mainly due to the build-up of the mor-layer during the time elapsed since fire at Betsele (165 years) and prescribed burning at Norrliden (60 years).