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Decoupled stoichiometric, isotopic, and fungal responses of an ectomycorrhizal black spruce forest to nitrogen and phosphorus additions

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ABSTRACT

Many northern forests are limited by nitrogen (N) availability, slight changes in which can have profound effects on ecosystem function and the activity of ectomycorrhizal (EcM) fungi. Increasing N and phosphorus (P) availability, an analog to accelerated soil organic matter decomposition in a warming climate, could decrease plant dependency on EcM fungi and increase plant productivity as a result of greater carbon use efficiency. However, the impact of altered N and P availability on the growth and activity of EcM fungi in boreal forests remains poorly understood despite recognition of their importance to host plant nutrition and soil carbon sequestration. To address such uncertainty we examined above and belowground ecosystem properties in a boreal black spruce forest following five years of factorial N and P additions. By combining detailed soil, fungal, and plant $\delta^{15}N$ measurements with *in situ* metrics of fungal biomass, growth, and activity, we found both expected and unexpected patterns. Soil nitrate isotope values became ^{15}N enriched in response to both N and P additions; fungal biomass was repressed by N yet both biomass and growth were stimulated by P; and, black spruce dependency on EcM derived N increased slightly when N and P were added alone yet significantly declined when added in combination. These findings contradict predictions that N fertilization would increase plant P demands and P fertilization would further exacerbate plant N demands. As a result, the prediction that EcM fungi predictably respond to plant N limitation was not supported. These findings highlight P as an under appreciated mediator of the activity of denitrifying bacteria, EcM fungi, and the dynamics of N cycles in boreal forests. Further, use of $\delta^{15}N$ values from bulk soils, plants, and fungi to understand how EcM systems respond to changing nutrient availabilities will often require additional ecological information.

1. Introduction

Increased terrestrial N availability is a global issue with impacts extending beyond industrialized regions (Matson et al., 2002; Galloway et al., 2008). In most N-limited boreal forests anthropogenic deposition is less pronounced, but landscape modification and accelerated decomposition resulting from climatic warming can increase *in situ* N mineralization and profoundly alter above and belowground ecosystem responses (Nadelhoffer et al., 1991; Hyvönen et al., 2007; Allison and Treseder, 2008; Aerts, 2010). Boreal ecosystems subjected to increased N availability may respond with greater carbon (C) fixation (Högberg et al., 2003), altered C and nutrient allocation patterns (Nadelhoffer, 2000; Mack et al., 2004; Vogel et al., 2008), and shifts in plant and fungal diversity, biomass, and elemental stoichiometry with uncertain functional consequences (Shaver et al., 2001; Nordin et al., 2005; Clemmensen et al., 2006; Treseder, 2008; Janssens et al., 2010; Wardle and Lindahl, 2014). Understanding how impacts of altered N availabilities will influence the function of boreal ecosystems requires assessment of multiple N cycling processes integrated through time. As such, stable isotope ratios of N (^{15}N:^{14}N represented as $\delta^{15}N$), as key integrative signals of the N cycle (Robinson, 2001), appear promising.

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Measurements of soil and plant $\delta^{15}N$ have been used to detect variations in N cycling due to climate (Amundson et al., 2003), disturbance (Pardo et al., 2002), reforestation (Davidson et al., 2007), deposition (Elliot et al., 2007), and the activity of ectomycorrhizal (EcM) fungi. This is due to key fractionation steps related to N loss-to-production ratios or shifts in the source or demand for N by host plants (Hobbie and Högberg, 2012; Mayor et al., 2015). A major limitation to interpreting plant $\delta^{15}N$ values as an indicator of altered N cycling arises from uncertainty in $\delta^{15}N$ values among forms of available N because bulk soil $\delta^{15}N$ is commonly the only pool measured (Craine et al., 2009; Pardo and Nadelhoffer, 2010). Measuring $\delta^{15}N$ of individual soil N forms permits assignment of plant $\delta^{15}N$ values as tracers of available N after accounting for intermediate sources of biological fractionation (Pardo et al., 2006; Templer et al., 2007; Kahmen et al., 2008; Mayor et al., 2012). For instance, once baseline ecosystem $\delta^{15}N$ values are established, both the source and proportional amount of EcM derived N can be better constrained (Hobbie and Hobbie, 2008; Yano et al., 2010) and the individual and interactive effects of N and P fertilization independently assessed (Mayor et al., 2014).

In order to assess the alteration of $\delta^{15}N$ sources and the activity of EcM fungi, we conducted a five year factorial N and P addition experiment in a mature black spruce forest of central Alaska. By combining detailed measurements of soil, plant, and fungal $\delta^{15}N$ with estimates of fungal biomass, growth, and activity, we sought to evaluate several hypothesized relationships governing plant and fungal nutrient limitations. By explicitly targeting the response of EcM fungi to altered soil fertility we aimed to better inform global change predictions regarding plant–soil functional interactions (Johnson et al., 2013; Deckmyn et al., 2014) and to elucidate understudied interactions with P availability in a putatively N-limited ecosystem.

Given that productivity of high latitude black spruce forests are considered N-limited, and N limitation of host plants is closely tied to belowground C allocation to EcM fungi (Högberg et al., 2010), we constructed the following hypotheses regarding expected responses of black spruce and associated EcM fungi to factorial N and P fertilization: (H1) N fertilization would increase black spruce [N] and ^{15}N content due to relief of growth limitation and uptake of ^{15}N enriched mineral N resulting from induced fractionation under N saturated conditions (Pardo et al., 2006); and, this would lead to (H2) a reduction in estimated plant dependency on EcM derived N. As such, lowered delivery of fungal N would lead to (H3) reduced relative $\delta^{15}N$ differences between EcM sporocarps and black spruce ($\delta^{15}N_{fungi-plant}$) because of less ^{15}N-retention by associated EcM fungi and less transfer of ^{14}N to host trees (Hobbie and Hobbie, 2008). Furthermore, we hypothesized that relief of tree N limitation through N fertilization would (H4) reduce fungal biomass and mycelial growth due to a reduction of belowground C allocation. In contrast, we expected P addition would only influence ecosystem properties when added with N due to an induced N/P co-limitation. As such, +N + P additions would: (H5) further decrease plant dependency on EcM derived N resulting in (H6) even smaller $\delta^{15}N_{fungi-plant}$ magnitudes relative to the addition of N, and (H7) the largest reduction in standing biomass and mycelial growth of EcM fungi.

2. Methods

2.1. Site description and experimental design

Boreal forest is the second largest terrestrial biome in the world and black spruce (*Picea mariana* [Mill.] BSP) dominated forest is the most abundant forest type in boreal North America (Viereck and Johnston, 1990). Its success in the landscape is attributed to extreme freezing tolerance, the ability to grow in shallow permafrost soils with impeded drainage, as well as the ability to grow on well-drained and more productive upland sites (Chapin et al., 2006).

The experimental site is located approximately 15 km south of Delta Junction AK, and consists of 16 plots arrayed in a factorial N × P design consisting of four blocks of four 10 × 10 m^2. Each treatment plot was fertilized annually in the early spring for 5 years prior to the 2007 growing season when sampling for this study was conducted. In 2002, each plot received single broadcast doses of pelletized $NH_4^+NO_3^-$ (+N), ortho-PO_4^- (+P), both together (+N + P), or none, at an initial level of 200 kg N and 100 kg P ha^{-1} in year 1 and 100 kg N and 50 kg P ha^{-1} yr^{-1} in subsequent years. Although these amounts of added N and P are unlikely to occur under natural conditions, they are of comparable magnitude to other boreal forest fertilization experiments that seek to relieve nutrient limitations (Högberg et al., 2006).

The forest is a mature (~80 years old) dry nonacidic black spruce forest (Hollingsworth et al., 2006) formed under low rainfall (~300 mm yr^{-1} MAP) and cold conditions (−2 °C MAT) with a relatively shallow organic layer (6.3 cm O horizon). Soils are gelisols dominated by silt loams as described elsewhere (Treseder et al., 2004b). The forest canopy is dominated by black spruce with minor components of *Populus tremuloides* in two of the blocks. The understory vegetation consists of minor contributions from *Betula glandulosa*, *Salix spp.*, *Vaccinium vitis-idaea*, *V. uliginosum*, and *Rhododendron groendlandicum* shrubs, with a 30–50% ground cover comprised of feather moss (mainly *Pleurozium schreberi* or *Hylocomium splendens*) and lichen (*Cladina*, *Cladonia*, and *Cetraria* spp.) (Treseder et al., 2004b; Mack et al., 2008).

2.2. Plant and fungal sampling

Needles from five, terminal, full sun branches were collected from the tops of five *P. mariana* trees in the canopy of each plot at peak of needle expansion, August 29–30, 2007, dried at 60 °C, and composited by plot. Fine roots (<2 mm) were carefully excavated from three of these trees in each plot by tracing from trunk to terminal roots within the upper 6 cm of soil, composited by plot, and refrigerated until processing. The thin layer of secondary root tissue was carefully removed to prevent potential inclusion of fungal biomass in subsequent isotopic analyses although a minor component of EcM hyphae could be present as a Hartig net in the remaining root cortex (Högberg et al., 1996). Needle and root tissue were dried at 60 °C for 24 h, ground to a fine powder, and analyzed on a ThermoFinnigan continuous flow isotope ratio mass spectrometer coupled to a Costech C/N elemental analyzer at the University of Florida. Stable isotope abundances are reported as $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000$, where $R = {}^{15}N/{}^{14}N$ and refers to the ratio of the sample and reference standard of atmospheric N_2. Run standard error rates were typically less than 0.2‰. Foliar P concentration (mg g^{-1}) was determined by combustion (1 h at 550 °C) and dissolution of the ash in 10 mL of 1 M H_2SO_4 shaken for 16 h, filtered, and analyzed by automated colorimetry. Tree cores were obtained prior to and in year five of the experiment from all mature trees in each plot and growth rings quantified using WinDendro software. Growth responses were calculated as the change in ring width prior to and in the fifth year of fertilization.

Sporocarps were opportunistically collected during the 2005-07 growing seasons and as a result individual sporocarps may have experienced 3–5 years of nutrient enrichment. Sample sizes varied from 12 to 29 across treatments (individual plots represented by 3–11 sporocarps) with the fewest collected from the +N + P and +N treatments ($N = 12$ and 13, respectively), and the most in the +P and control treatments ($N = 22$ and 29, respectively).

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