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Long-term reactive nitrogen loading alters soil carbon and microbial community properties in a subalpine forest ecosystem

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ABSTRACT

Elevated nitrogen (N) deposition due to increased fossil fuel combustion and agricultural practices has altered global carbon (C) cycling. Additions of reactive N to N-limited environments are typically accompanied by increases in plant biomass. Soil C dynamics, however, have shown a range of different responses to the addition of reactive N that seem to be ecosystem dependent. We evaluated the effect of N amendments on biogeochemical characteristics and microbial responses of subalpine forest organic soils in order to develop a mechanistic understanding of how soils are affected by N amendments in subalpine ecosystems. We measured a suite of responses across three years (2011–2013) during two seasons (spring and fall). Following 17 years of N amendments, fertilized soils were more acidic (control mean 5.09, fertilized mean 4.68), and had lower %C (control mean 33.7% C, fertilized mean 29.8% C) and microbial biomass C by 22% relative to control plots. Shifts in biogeochemical properties in fertilized plots were associated with an altered microbial community driven by reduced arbuscular mycorrhizal (control mean 3.2 mol%, fertilized mean 2.5 mol%) and saprotrophic fungal groups (control mean 17.0 mol%, fertilized mean 15.2 mol%), as well as a decrease in N degrading microbial enzyme activity. Our results suggest that decreases in soil C in subalpine forests were in part driven by increased microbial degradation of soil organic matter and reduced inputs to soil organic matter in the form of microbial biomass.

1. Introduction

Atmospheric N deposition is a chronic stress that has already altered ecosystem dynamics in even the most remote locations (Pardo et al., 2011; Kim et al., 2014). Reactive N deposition has increased approximately fourfold over the last century (IPCC, 2013) and is expected to continue growing in the coming decades (Galloway et al., 2008). Human sources of reactive N exceed all natural sources combined, including biological fixation, biomass burning, and lightning (Erisman et al., 2013). While this drastic alteration of global N cycling has been documented to drive a wide range of environmental stress (Galloway et al., 2013), we are only beginning to understand the potential impact of reactive N on belowground C cycling (Liu and Greaver, 2010).

Understanding the net response of terrestrial C cycling to reactive N requires integration of a wide-range of potential above and belowground responses. These include C inputs (e.g. litterfall, root production, root exudation), C losses (e.g. DOC leaching, soil respiration), and additional characteristics that act as gatekeepers of C reservoirs (e.g. extracellular enzyme activity, microbial biomass). While aboveground responses to N amendments are fairly universal, including enhanced primary production and C storage in biomass (Frey et al., 2014), several recent meta-analyses conclude that the effects of reactive N deposition on belowground C cycling depend on the ecosystem and length of exposure to reactive N (Janssens et al., 2010; Liu and Greaver, 2010; Lu et al., 2011; Geisseler and Scow, 2014; Zhou et al., 2014). Forests and croplands consistently store more C both above and belowground in response to N amendments, with the boreal forest showing the greatest C gains (Janssens et al., 2010; Liu and Greaver, 2010; Geisseler and Scow, 2014). In contrast, grasslands have shown no net change in C pools following N fertilization (Liu and Greaver, 2010) and tundra soils and peatlands have lost C as a result of N amendments (Mack et al., 2004; Bragazza et al., 2006). Over time, the effect of N amendments on response variables has been shown to diminish as ecosystems adjust to enhanced N levels (Zhou et al.,

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2014). While the effect of reactive N has been evaluated in a wide range of ecosystems, few studies have looked at long-term effects of reactive N on alpine or subalpine ecosystems, demonstrated to be sentinels of global change (Vinebrooke et al., 2014).

Microbial processing is challenging to incorporate into ecosystem models contributing to the poorly constrained predictions for belowground C cycling responses to reactive N amendments. A suite of microbial properties, including the amount of microbial biomass, community composition, extracellular enzyme activities, carbon use efficiency, and rates of biomass turnover, influence microbial processing of available C inputs. Recent work illustrates that the addition of microbial mechanisms including biomass pool sizes and community diversity improve predictions over those without microbial information when modeling belowground C cycling under elevated N content (Wieder et al., 2015). However, more empirical data are required to validate and describe the relationships that are central to this process. A better understanding of the underlying microbial variables that drive belowground C-cycling may provide a more mechanistic understanding and better predictive capabilities to describe ecosystem C cycling response to reactive N.

Here we describe the net result of elevated reactive N amendments on features of belowground C cycling and associated microbial characteristics in a subalpine forest. We evaluated differences in C and N content between fertilized and control plots which have undergone 17 years of experimental N amendments, and examined a range of microbial properties and processes including microbial biomass, bacterial:fungal ratios, and extracellular enzyme activity in the organic soil horizon during spring and fall over three consecutive years (2011–2013). We expected the subalpine forest would respond to high levels of N amendments with gains in soil carbon content similar to other mature coniferous forests (Liu and Greaver, 2010). We also expected to find reduced microbial biomass (Treseder, 2008; Ramirez et al., 2012), a decrease in mycorrhizal fungi (Treseder et al., 2007; Zheng et al., 2014), and increased cellulose degrading enzyme activities (Saiya-Cork et al., 2002; Waldrop et al., 2004a). We evaluated responses in the subalpine forests in context with those previously reported for other systems to identify common responses useful for mechanism-based parameterization of earth system models.

2. Materials and methods

2.1. Site description

Loch Vale watershed (LVWS) is located in Rocky Mountain National Park (RMNP) on the eastern edge of the Colorado Front Range. The climate is high-mountain continental with 105 cm of mean annual precipitation in LVWS (1984–2012), about 20 cm of which falls during the summer months and a mean annual temperature of 1.4 °C (Heath and Baron, 2013). Wet N deposition to LVWS ranges between 3 and 4 kg N ha^{-1} yr^{-1}, an amount that, while low, is more than sufficient to affect ecosystem processes (Baron et al., 2000). Three, 30 × 30 m, northeast facing, old-growth closed canopy Engelmann spruce-subalpine fir stands at approx. 3200 m elevation, fertilization plots were established in 1996, have been fertilized from 1996 until the present and were paired with unfertilized control stands (Rueth et al., 2003). Ammonium nitrate (NH_4NO_3) has been applied at a rate of 25 kg ha^{-1} yr^{-1}; 2.5 kg N ha^{-1} were applied monthly from June–October, and once a snowpack had accumulated 7.5 kg N ha^{-1} was applied as an overwinter application (Rueth et al., 2003). Between the initiation of the experiment and 2011, when the first data for the current analyses were collected, 400 kg N ha^{-1} was applied to each LVWS fertilization plot. This quantity of nitrogen was selected based on global maximum deposition rates at the time the experiment began. Our intent was not to simulate a future trend for Colorado; rather to evaluate what would happen if we pushed the subalpine forest into N saturation as experienced in other ecosystems.

2.2. Soil collection and analyses

After brushing away the litter layer, organic horizon soils ($O_e + O_a$) were collected with a tulip bulb corer in spring and fall in 2011, 2012 and spring 2013. Regional flooding and road closure prevented soil collection in fall 2013. O horizon thickness varied across samples ranging from approximately 2 to 11 cm. Soils (n = 150, 5 cores per plot × 3 plots × 2 treatments × 5 sampling events) were homogenized by hand through sealed zip-locked bags, subsampled for nucleic acid extraction, and then sieved through a standard 8 mm soil sieve. Gravimetric water content (GWC) was calculated by drying subsamples to a constant weight in a 105 °C oven. Samples dried at 60 °C were ground for soil organic C and N analysis with a LECO Tru-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA). After sieving, soils were partitioned for extraction of inorganic N (NH_4^+ and NO_3^-), extractable organic C (EOC), extractable N (EN), microbial biomass C (MBC) and microbial biomass N (MBN). For inorganic N analyses, soils were extracted within 8 h of sampling with 2 M KCl (1:10, soil weight:extraction volume). Samples were shaken for 1 h and stored at 10 °C overnight then filtered. KCl extracts were analyzed for NH_4^+ using segmented flow analysis and for NO_3^- using flow injection analysis on an Alpkem Flow Solution IV Automated wet chemistry system (O.I. Analytical, College Station TX). The EOC and EN soils were extracted with H_2O (1:10, soil weight:extraction volume); and to solubilize microbial biomass, soils were extracted with 1% $CHCl_3$ (1:10 soil weight:extraction volume). EOC was measured using a Total Organic Carbon analyzer with an N measuring unit (Shimadzu TOC-V_{CPN}; Shimadzu Scientific Instruments, Wood Dale, IL, USA). The MBC was calculated as the difference between 1% chloroform slurry carbon and the EOC concentration, and was not corrected for extraction efficiency.

2.3. DNA extraction and qPCR

DNA was extracted from spring and fall, 2011 and 2012 samples using a MoBio Power Soil DNA extraction kit (MoBio Laboratories, Carlsbad CA). DNA was then quantified using a Quant-iT DNA BR kit (Life Technologies, Grand Island, NY). Real-time PCR (qPCR) was used to determine the relative abundance of bacterial 16S and fungal ITS genes in each of the sampled plots with the respective primers (Eub 338 ACT CCT ACG GGA GGC AGC AG and Eub 518 ATT ACC GCG GCT GCT GG, ITS1F TCC GTA GGT GAA CCT GCG GITS and 5.8S CGC TGC GTT CTT CAT CG) using Absolute SYBER Fluorescein Mix (Thermo Scientific, Grand Island, NY) in a Bio-Rad MyiQ Real-Time PCR Detection System (Hercules, CA).

2.4. PLFA extraction and analysis

Phospholipid fatty acids (PFLAs) were extracted from soil samples using conventional methods (Denef et al., 2007). In brief, subsamples of frozen soil composited at the plot level, from spring and fall 2011, and 2012, was 2 mm sieved to remove coarse roots, and then lyophilized. 1.5 g of freeze-dried soil was mixed with a 0.1 M phosphate buffer: chloroform: methanol solution (0.8:1:2 volume (mL) ratios per gram of soil). Neutral, glyco- and phospholipids were separated over a silicon hydroxide column eluting with chloroform, acetone and methanol, respectively. Phospholipids were saponified to obtain free fatty acids, and then methylated to form fatty acid methyl esters (FAMEs). FAMEs were

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