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Reduced snow cover alters root-microbe interactions and decreases nitrification rates in a northern hardwood forest

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Abstract. Snow cover is projected to decline during the next century in many ecosystems that currently experience a seasonal snowpack. Because snow insulates soils from frigid winter air temperatures, soils are expected to become colder and experience more winter soil freeze-thaw cycles as snow cover continues to decline. Tree roots are adversely affected by snowpack reduction, but whether loss of snow will affect root-microbe interactions remains largely unknown. The objective of this study was to distinguish and attribute direct (e.g., winter snow- and/or soil frost-mediated) vs. indirect (e.g., root-mediated) effects of winter climate change on microbial biomass, the potential activity of microbial exoenzymes, and net N mineralization and nitrification rates. Soil cores were incubated *in situ* in nylon mesh that either allowed roots to grow into the soil core (2 mm pore size) or excluded root ingrowth (50 μm pore size) for up to 29 months along a natural winter climate gradient at Hubbard Brook Experimental Forest, NH (USA). Microbial biomass did not differ among ingrowth or exclusion cores. Across sampling dates, the potential activities of cellobiohydrolase, phenol oxidase, and peroxidase, and net N mineralization rates were more strongly related to soil volumetric water content ($P < 0.05$; $R^2 = 0.25$–0.46) than to root biomass, snow or soil frost, or winter soil temperature ($R^2 < 0.10$). Root ingrowth was positively related to soil frost ($P < 0.01$; $R^2 = 0.28$), suggesting that trees compensate for overwinter root mortality caused by soil freezing by re-allocating resources towards root production. At the sites with the deepest snow cover, root ingrowth reduced nitrification rates by 30% ($P < 0.01$), showing that tree roots exert significant influence over nitrification, which declines with reduced snow cover. If soil freezing intensifies over time, then greater compensatory root growth may reduce nitrification rates directly via plant-microbe N competition and indirectly through a negative feedback on soil moisture, resulting in lower N availability to trees in northern hardwood forests.

Key words: *Hubbard Brook; nitrification; phenol oxidase; root production; snow; soil frost; sugar maple* (Acer saccharum).

Introduction

Tree roots represent a dynamic linkage between aboveground plant production and soil carbon (C) storage (Fontaine et al. 2007). In temperate forests, trees allocate up to 60% of net primary production to roots, which release organic nutrients that fuel soil microbial metabolism and exoenzyme production (Phillips and Fahey 2006, Litton et al. 2007). The breakdown of soil organic matter (SOM) by exoenzymes contributes to soil formation (Six et al. 2006), makes nutrients available for root uptake, and releases CO_2 to the atmosphere (Finzi et al. 2015). As such, root-microbe interactions mediate soil C and nitrogen (N) cycle responses to myriad global change drivers and outcomes, including elevated atmospheric CO_2 (Phillips et al. 2011), soil warming (Zhu and Cheng 2011), and drought (Zhu and Cheng 2013). Yet, the extent to which root-microbe interactions are affected by loss of winter snow remains largely unknown.

Climate warming during the next 100 yr is expected to reduce winter snow depth and duration in the northern hardwood forests of the northeastern U.S. (Hayhoe et al. 2007). Because deep snow cover (>0.5 m) keeps soils above 0°C during winter (Zhang 2005), declines in the depth and duration of snow cover are expected to be accompanied by colder soils and an increased frequency of soil freeze-thaw cycles (Brown and DeGaetano 2011). Soil microbes are metabolically active at soil temperatures below 0°C (McMahon et al. 2009). However, the direct negative effects of soil freezing, including microbial

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mortality and altered resource use (Deluca et al. 1992, Schimel and Mikan 2005), are to likely increase as snow depth and duration declines. Soil N pools decline by up to 25% when soils remain unfrozen during winter due to N uptake by an active subnivean microbial community (Kuhnert et al. 2012, Ueda et al. 2013). Overwinter net N mineralization rates also decline in years with more soil freezing (Contosta et al. 2011).

Winter soil freezing is known to increase overwinter root mortality and decrease root N uptake during the spring following the snowmelt period (Tierney et al. 2001, Campbell et al. 2014). On the one hand, increased root necromass and higher N availability following snowmelt could increase microbial population size, rates of enzyme production and rates of N mineralization in N-rich soil microsites (Allison and Vitousek 2005, Cheng et al. 2014). On the other hand, winter soil freezing can stimulate root production during the following growing season as a result of compensatory fine-root regrowth (Tierney et al. 2001). If compensatory regrowth increases plant demand for N or reduces soil moisture availability, then exoenzyme production could also decline, and partially offset increases in SOM decomposition expected to result from climate warming (Schimel and Weintraub 2003, Kuzyakov and Xu 2013). Hence, understanding the effect of snowpack loss on root-microbial interactions is necessary for developing a predictive understanding of soil C and N cycle responses to climate change.

The objective of this study was to distinguish and attribute direct (e.g., winter snow- and soil frost-mediated) and indirect (e.g., root-mediated) effects of winter climate change on growing season microbial biomass, the potential activity of microbial exoenzymes, and net N mineralization and nitrification rates. Winter snow and soil frost depth are inversely related along the elevation gradient at Hubbard Brook Experimental Forest (HBEF), NH (USA), where high elevation sites experience a greater depth and duration of snow cover compared to low elevation sites (Durán et al. 2014). Consequently, soil frost depth and duration is greater at low elevation compared to high elevation sites, creating a natural winter climate gradient. Snow depth and duration during winter are positively related to microbial biomass, protease and oxidative enzyme production, microbial respiration, and nitrification in the springtime at HBEF (Durán et al. 2014, Sorensen et al. 2016). Additionally, soil N cycling rates and soil respiration co-vary with soil N availability and generally increase from low to high elevation locations (Bohlen et al. 2001, Groffman et al. 2009, Durán et al. 2014, Morse et al. 2015). However, previous studies have not addressed whether such patterns arise mainly from physical or soil properties that co-vary with elevation or if they arise mainly from interactions among winter snow or soil frost, roots, and microbial activity.

We incubated soil cores for up to 29 months along this natural winter climate gradient in nylon-mesh that either allowed or excluded root ingrowth. We hypothesized that (1) root ingrowth would be higher at low compared to high elevation sites, consistent with compensatory regrowth associated with winter soil frost; (2) microbial biomass, exoenzyme activity, and net N mineralization and nitrification rates would be greater at high elevation where the snow is deeper and of longer duration; and (3) the stimulatory effect of roots on microbial activity and soil process rates would be greater at higher elevation, due to less influence from winter soil freezing on roots compared to lower elevation sites.

Methods

Field site description

The HBEF is located in the White Mountain National Forest in New Hampshire, USA (43.56° N, 71.45° W). Elevation ranges from 225 to 1,100 m at HBEF. Average maximum-annual air temperature is 19°C while average minimum air temperature is −9°C and the mean annual temperature has increased by approximately 0.3°C decade^{-1} over the last 50 yr (Hamburg et al. 2013). Mean annual precipitation is 1,400 mm, one-third of which occurs as snowfall during winter (Bailey et al. 2003*a*). Winter soil frost depth ranges from 0 to 25 cm depth below the soil surface and a continuous snowpack of 70–100 cm depth typically develops each year by late December and persists until late April (Campbell et al. 2010). Over the last 50 yr, the maximum depth of winter snowpack has declined by 26 cm and the duration of winter snow cover has declined by four days per decade (Hamburg et al. 2013).

The soils at HBEF have traditionally been described as acidic (pH 3.9) Typic Haplorthods with a 6-cm thick organic layer consisting of leaf-litter (O_i), dense root-mat and decomposing organic material (O_e), and a nutrient rich humus layer (O_a) (Bohlen et al. 2001). More recently, soil development has been shown to be mediated by complex interactions between landscape position and hydrology, resulting in several distinct soil types (Bailey et al. 2014). Soils at all the sites in this study are sandy loams derived from mixed glacial till and underlain by the Silurian Rangeley Formation (Bailey et al. 2003*b*). Tree species below 750 m elevation in this northern hardwood forest primarily include American beech (*Fagus grandifolia* Ehrh.), sugar maple (*Acer saccharum* Marsh.), and yellow birch (*Betula alleghaniensis* Britt.).

Winter climate gradient at Hubbard Brook

Twenty sites that spanned the winter climate gradient were established in October 2010, with each site located in sugar maple dominated stands (Durán et al. 2014). In this study, we selected 6 of the 20 sites that maximized the differences in winter snow, frost and soil temperature among sites. Three sites were on north-facing slopes at high elevation (539, 555, and 595 m) and three were on south-facing slopes at low elevation (375, 411, and 511 m). Aspect influences the length of the growing season at HBEF (Richardson et al. 2006) and increases air temperatures on

south-facing slopes. In addition to differences in soil microclimate, soil organic matter content and dissolved NO_3-N are generally greater at high compared to low elevation, but that trend is not statistically significant at our study locations (Morse et al. 2015). Soil temperature and volumetric water content were measured continuously from December 2010 to November 2013 using Decagon 5TM combination probes and Decagon EM50 dataloggers. Snow depth was measured using a Federal Snow Sampling Tube (Rickly Hydrological Company, Columbus, OH, USA) at three locations at bi-weekly intervals at each site from winter 2010/11 through winter 2012/13. Soil frost depth was measured using methylene blue dye-filled frost tubes (Rickard and Brown 1972; Campbell et al. 2014). Snow depth and soil frost depth data collected from 2010 to 2012 are presented in Durán et al. (2014) and are available online (http://hubbardbrook.org/data/dataset.php?id=136).

Experimental plots and root exclusion and ingrowth cores

Three 1 m × 2 m plots, separated by at least 50 m, were established at each of the six sites (18 plots total) in May 2011. Each plot was subdivided into eight 50 cm × 50 cm subplots, and soil cores were installed within three of the eight subplots. The soil cores were 5 cm diameter × 15 cm deep and constructed of nylon mesh. Root exclusion cores were constructed with 50 μm mesh that excluded all roots but not fungal hyphae. The root ingrowth cores were constructed of 2 mm mesh, which allowed fine roots to grow into the soil core.

Native soil was collected adjacent to each of the six sites and was hand-sorted in the field to remove roots, gravel, and coarse woody debris. The site-specific homogenized soil was then used to pack the root exclusion and root ingrowth cores at each site. We did not transplant or mix soils across locations, and therefore did not formally test the relative contributions of local adaption (i.e., home-field advantage or microbial community structure) vs. environmental heterogeneity in explaining differences in soil process rates across sites (Keiser et al. 2014). The bottom 10 cm of each core was packed with homogenized mineral soil (B-horizon), while the top 5 cm was packed with homogenized organic horizon soil (combined Oe and Oa horizons). Cores of each type (root ingrowth and root exclusion) were installed in duplicate in three subplots at all 18 plots along the gradient (6 sites × 3 replicate plots × 3 subplots × 2 core types × 2 duplicate cores = 216 cores total). We harvested cores from one subplot at each plot in July 2012 (14 months after installation), October 2012 (17 months after installation), and October 2013 (29 months after installation; n = 36 exclusion and 36 ingrowth cores at each sampling time).

Soil core processing, root biomass, and root production

Root ingrowth and root exclusion cores were processed within approximately 48 h of field collection. The organic horizon soil (0–5 cm depth below surface, see above) and mineral soil (5–15 cm depth below surface, see above) were removed from each core and kept separate during lab processing. Fine roots (<2 mm) were removed from each root ingrowth and exclusion core using forceps. Root biomass in each core was determined after drying the roots for 48 h at 60°C. We did not distinguish live from dead fine-roots. A five gram subsample of soil from each core was oven-dried at 60°C for 48 h to determine gravimetric soil moisture content. Given that each core was initially free of fine-roots, we considered root biomass ingrowth over time as an index of root production along the gradient.

Microbial biomass N, exoenzyme activity, and soil N cycling rates

Microbial biomass N was determined on a subset of soil cores harvested in October 2012 and October 2013 from one plot at each of the six sites (n = 12 exclusion and 12 ingrowth cores at each harvest date) using the chloroform fumigation-extraction method (Brookes et al. 1985). Following alkaline persulfate digestion (Cabrera and Beare 1993), total N in the fumigated and nonfumigated soils was determined by measuring NO_3^- colorimetrically (λ = 540 nm) using a Versamax microplate spectrophotometer (Doane and Horwath 2003). Microbial biomass N (μg N g dry soil^{-1}) was calculated as the difference in NO_3-N concentration between the fumigated and non-fumigated soils. We did not apply an extraction efficiency correction factor.

The potential activity of four hydrolytic microbial exoenzymes - acid phosphatase (AP), β-*N*-acetylglucosaminidase (NAG), β-1,4-glucosidase (BG) and cellobiohydrolase (CBH) were measured in each soil core on each sampling date. AP releases phosphate groups from soil organic matter, NAG hydrolyzes amino sugars and chitin in soil, and both BG and CBH decompose components of cellulose. A soil slurry consisting of 1.5 g field-moist soil in 100 mL 250 mM sodium acetate buffer was mixed on a stir plate for 1 min. Eight 200 μL analytical replicates were aliquoted onto black 96 well flat-bottom microplates along with 50 μL methylumbelliferone (MUB) linked-substrate. We tested for the activity-saturating concentration (AP - 4,000 μM; NAG – 2,000 μM; BG - 2,000 μM; CBH - 1,750 μM) of MUB linked-substrate by incubating a subset of soils across a range of substrate concentrations (German et al. 2011). The microplates were incubated in the dark at 20°C for 2 h before enzyme activity was determined fluorometrically (Gemini XS Spectramax, Molecular Devices; Sunnyvale, CA, USA).

Two additional oxidative exoenzymes, phenol oxidase (PPO) and peroxidase (PERX), were measured on each sampling date. These enzymes are associated with the oxidative breakdown of soil organic C. A soil slurry of 1.5 g soil in 100 mL 50 mM sodium acetate buffer was mixed on a stir plate for 1 min. Eight 200 μL analytical replicates were aliquoted onto 96 well clear, flat-bottom

microplates along with 50 μL 25 mM L-DOPA. Enzyme activity was determined colorimetrically (λ = 460 nm) after a four hour incubation period using a Spectramax microplate spectrophotometer (Molecular Devices).

Rates of net N mineralization and nitrification were measured in short-term (14 d) lab incubations for all soil cores. Because these measurements were made at constant lab temperature (~22.5°C), we considered them as potential rather than absolute rates of net N mineralization or nitrification. Extractions were completed using 5 g field-moist soil and 30 mL 2 M KCl. The concentrations of NH_4^+ and NO_3^- on Day 1 and Day 14 of the incubation were determined colorimetrically using a Versamax microplate spectrophotometer (Molecular Devices; Sims et al. 1995, Doane and Horwath 2003). Potential net nitrification (μg N g $soil^{-1}$ d^{-1}) was calculated as the difference in NO_3^- measured on Day 1 and Day 14, divided by the incubation period. Potential net N mineralization (μg N g $soil^{-1}$ d^{-1}) was calculated as the difference in the sum of NH_4^+ plus NO_3^- measured on Day 1 and Day 14, divided by the incubation period.

Statistical analysis

All statistical analyses were conducted using R v. 3.2.0 (R Development Core Team 2010). Snow or soil frost depth measurements made at each site throughout winter were converted into a single continuous variable (area under the curve =AUC; Durán et al. 2014, Sorensen et al. 2016) by calculating the integral of the time (x-axis = time measured in units "days") vs. snow or frost depth (y-axis = depth measured in units cm) relationship using the R package "pracma" (Borchers 2011). Hereafter, we refer to these variables as either "snow" or "soil frost," which integrates the depth and duration of either snow or soil frost and the units are cm x days. Site differences in soil microclimate ("snow," "soil frost," minimum winter soil temperature, and soil temperature or volumetric water content averaged over 1-d prior to soil core harvesting) were assessed using separate linear mixed-effects models, where site was the fixed effect and year was the random effect in the model. Within each site, volumetric water content averaged 1 d prior to core harvesting was significantly correlated with both volumetric water content averaged 30-d and 10-d prior to harvesting ($P < 0.05$). Additionally, the relationship between moisture and microbial activity did not change by using 1 d prior vs. either 30-d or 10-d averages.

The effect of soil core type (i.e.,, root exclusion vs. root ingrowth) on root biomass was tested by using a generalized linear model (GLM) with a Gaussian-error distribution and a log link function. Linear-mixed effects models were used to test whether snow or frost depth AUC (fixed-effects) were related to root biomass in the ingrowth cores. Field plot was the random-effect in the model. The amount of variation in root biomass ingrowth explained by snow or frost depth AUC was determined by calculating the R^2_{marginal} of the mixed-effect model. The R^2_{marginal} is the variance attributed to the fixed-effect(s), in this case either snow or frost depth AUC, divided by the total model variance (Nakagawa and Schielzeth 2013).

GLMs were also used to test for the effect of the presence or absence of roots (i.e., root ingrowth) on microbial biomass N, exoenzyme activity, net nitrification, and net N mineralization for cores that were collected in October 2013. We anticipated that the effect of root ingrowth would be greatest in October 2013 because the cores had been in the field for the longest duration (i.e., 29 months) at that time. Microbial biomass N and potential exoenzyme activity were modelled using a gamma error distribution and an inverse link function, with soil core type as the main effect in the model. A Gaussian-error distribution and a log link function were used to determine the effect of root ingrowth on potential net N mineralization and nitrification. We also calculated Akaike Information Criteria scores using the R package "AICcmodavg" (Mazerolle 2011) to test whether root ingrowth, winter soil microclimate, growing season microclimate, or their interaction could best explain patterns in microbial activity across sites (see Appendix S1: Table S2).

Results

We observed a natural gradient in snow and soil frost depth x duration (hereafter "snow" or "soil frost," respectively) across the six field sites. Snow increased while soil frost decreased at high compared to low elevation sites ($P \leq 0.05$, Table 1). The site located at 555 m elevation had the highest average soil volumetric water content, while the two sites located at the lowest elevations (375 m and 401 m) were the driest (Table 1). Mean soil temperature differed only between the lowest (375 m) and highest elevation sites (595 m; ~1.3°C; Table 1). Soil C:N ratio ranged from approximately 23–16 and declined from high compared to low elevation in both soil horizons (Appendix S1: Table S1). There were no differences in total soil mass, mass C or mass N in the organic soil horizon, although soil mass N did vary among sites in the mineral soil (Appendix S1: Table S1).

Root biomass was 14× greater in root ingrowth compared to root exclusion cores ($P \leq 0.01$; Fig. 1a). The difference in root biomass between exclusion vs. ingrowth cores increased with the duration of core incubation (Fig. 1b). Root biomass in the ingrowth cores did not systematically differ by elevation ($P > 0.05$, Table 1). Similarly, root biomass in ingrowth cores was not related to snow (Fig. 3c). By contrast, root ingrowth was positively related to soil frost ($R^2_{\text{marginal}} = 0.28$, $P \leq 0.01$) and was about 40% greater at the low elevation sites compared to the high elevation sites which experienced little soil frost (Fig. 1d, Table 1).

Microbial biomass N did not differ among root ingrowth and exclusion cores ($P > 0.05$, Table 2). In addition, microbial biomass did not vary by elevation

TABLE 1. Soil microclimate in winter and growing season 2012 and 2013. Mean soil temperature (Soil Temp) and volumetric water content (Vol. Water Content) were calculated from the daily mean at each site 1 d prior to the date the soil cores were harvested from the gradient (July and October 2012, October 2013). Root biomass is for ingrowth cores only. Site means (± SEM) were compared post hoc and statistical differences are indicated by different letters within each column ($\alpha = 0.05$).

Elevation	Frost	Snow	Min. Soil Temp	Soil Temp	Vol. Water Content	Root biomass
375	$394^{a} \pm 20$	$1026^{c} \pm 302$	$-1.1^{d} \pm 0.3$	$13.4^{a} \pm 2.2$	$0.27^{b,c} \pm 0.03$	$3.8^{a} \pm 0.4$
401	$287^{b} \pm 3$	$1364^{b,c} \pm 363$	$-0.8^{c,d} \pm 0.2$	$13.0^{a,b} \pm 2.3$	$0.26^{c} \pm 0.05$	$3.4^{a,b} \pm 0.6$
511	$158^{c} \pm 10$	$1727^{b} \pm 421$	$-0.3^{c,b} \pm 0.2$	$12.7^{a,b} \pm 2.1$	$0.32^{b} \pm 0.02$	$1.8^{b} \pm 0.2$
539	$65^{d} \pm 16$	$3062^{a} \pm 679$	$0.6^{a} \pm 0.2$	$12.6^{a,b} \pm 1.7$	$0.32^{b} \pm 0.02$	$2.6^{a,b} \pm 0.3$
555	$135^{c,d} \pm 42$	$3000^{a} \pm 669$	$-0.1^{b} \pm 0.1$	$12.6^{a,b} \pm 1.8$	$0.38^{a} \pm 0.01$	$2.1^{b} \pm 0.2$
595	$108^{c,d} \pm 10$	$3149^{a} \pm 669$	$0.1^{c,b} \pm 0.1$	$12.1^{b} \pm 1.9$	$0.30^{b} \pm 0.01$	$3.2^{a,b} \pm 0.3$

Units of measurement: Elevation (m), AUC, area under the curve (cm × days), temperature (°C), volumetric water content (cm^3 H_2O cm^{-3} $soil^{-1}$), root biomass (mg dry mass cm^{-3} soil); Snow AUC, Frost AUC, and Minimum Soil Temperature data collected in 2012 are also summarized in Durán et al. (2014), soil volumetric water content data collected in 2012 are also presented in Morse et al. (2015).

and the interaction between elevation and soil core type was not a significant predictor of microbial biomass N ($P > 0.05$).

Net nitrification in the organic horizon (i.e., 0–5 cm below the surface) was related positively to snow in both root ingrowth and exclusion cores (Fig. 2a). Soil C:N ratio did not explain patterns in net nitrification in the organic horizon ($R^2 = 0.07$, $P > 0.10$). Rather, the interaction between snow and root ingrowth best explained patterns in net nitrification in the organic horizon (Appendix S1: Table S2). Compared to net nitrification rates in the exclusion cores, root ingrowth reduced rates of net nitrification by 30% at the three north-facing, high elevation sites which experienced the deepest and longest duration of snow cover ($R^2_{marginal} = 0.47$, $P \leq 0.01$, Fig. 2a). Conversely, root ingrowth did not affect net nitrification rates at the south-facing low elevation sites. In the organic soil horizon, net nitrification was positively related to net N mineralization in root ingrowth cores ($R^2_{marginal} = 0.43$, $P \leq 0.01$), but not in root exclusion

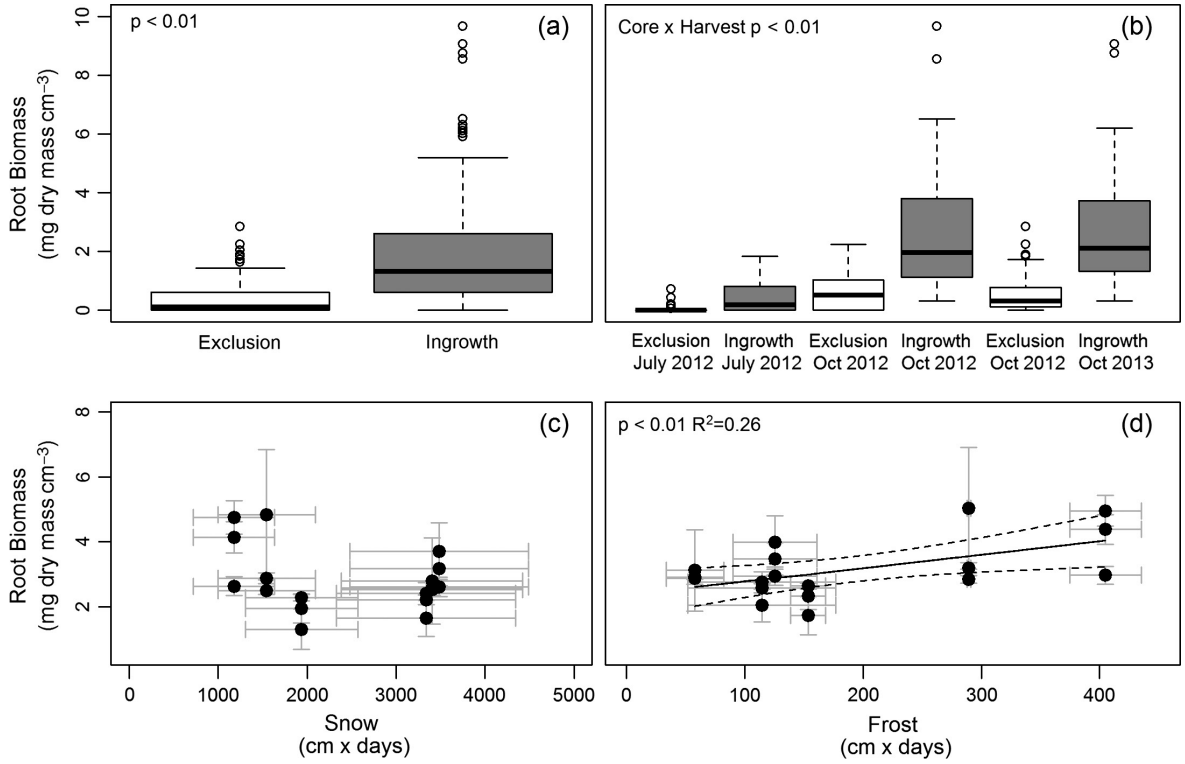

FIG. 1. Differences in root biomass (a) in ingrowth and exclusion cores across all dates or (b) on each harvest date. Root biomass in ingrowth cores was not (c) related to winter snow, but was (d) related positively to soil frost.

TABLE 2. Median and interquartile range (IQR) of microbial biomass N, potential enzyme activity, net nitrification, and net N mineralization rates of soils incubated either in the absence (root exclusion) or the presence (root ingrowth) of roots. Data are presented for cores harvested in October 2013 only.

Soil type	Response variable	Root exclusion		Root ingrowth	
		Median	IQR	Median	IQR
Organic soil	Microbial biomass N	309	278–354	343	269–395
	Acid phosphatase **	7.50	4.3–8.6	5.25	4.1–6.2
	N-acetylglucosaminidase	0.68	0.5–1.0	0.65	0.5–1.3
	Beta glucosidase *	1.30	0.7–1.4	0.90	0.2–1.4
	Cellobiohydrolase	0.27	0.2–0.4	0.30	0.2–0.4
	Phenol oxidase	0.84	0.6–1.1	0.82	0.6–1.0
	Peroxidase	1.22	1.0–1.5	1.30	1.0–1.7
	Net N mineralization ***	5.17	3.0–6.5	2.98	1.3–4.0
	Nitrification ***	1.24	0.6–3.1	0.37	0.3–1.1
Mineral soil	Microbial biomass N	111	90–140	124	91–149
	Acid phosphatase ***	2.60	2.1–3.4	1.53	1.2–2.0
	N-acetylglucosaminidase	0.20	0.1–0.3	0.15	0.1–0.2
	Beta glucosidase	0.33	0.2–0.4	0.21	0.2–0.3
	Cellobiohydrolase **	0.15	0.04–0.2	0.04	0.03–0.06
	Phenol oxidase ***	0.50	0.4–0.7	0.37	0.3–0.5
	Peroxidase **	1.32	1.1–1.6	1.09	0.7–1.5
	Net N mineralization	0.57	0.4–0.8	0.52	0.3–0.7
	Nitrification	0.42	0.3–0.6	0.37	0.2–0.5

*$P \leq 0.10$, **$P \leq 0.05$, ***$P \leq 0.01$. Microbial biomass N and enzyme activity data were fit to a gamma distribution, while nitrification and net N mineralization data were fit to a logistic distribution to estimate medians and the interquartile range. Units of measurement - microbial biomass N (μg N g soil^{-1}), AP, NAG, BG, CBH (μmol MUB g soil^{-1} h^{-1}), phenol oxidase and peroxidase (μmol L-DOPA g soil^{-1} h^{-1}), net N mineralization and nitrification (μg N g soil^{-1} d^{-1}).

cores (Fig. 2b; $P > 0.05$). Unlike in the organic horizon (i.e., Fig. 2a), we did not observe an interactive effect of snow and root ingrowth on net nitrification in the mineral soil, where rates in the ingrowth cores were about 2-fold lower compared to the organic soil.

Across sampling dates, soil volumetric water content was related positively to net N mineralization and the potential activity of cellobiohydrolase, and phenol oxidase in organic soils ($P \leq 0.01$, $R^2_{\text{marginal}} = 0.28$–$0.36$; Fig. 3). Similarly, soil volumetric water content was related positively to exoenzyme activity and net N mineralization in the mineral soil. We observed only weak relationships between microbial exoenzyme activity and either snow, soil frost, or minimum winter soil temperature in the organic soil across sampling dates (i.e., $R^2_{\text{marginal}} < 0.1$; Appendix S1: Table S3). Similar patterns were observed in the mineral soil (Appendix S1: Table S4).

Root ingrowth reduced net N mineralization rates and the potential activity of acid phosphatase, and β-glucosidase, in organic horizon soils in October 2013 when root density was at a maximum in the ingrowth cores ($P \leq 0.05$, Table 2). Root ingrowth also reduced gravimetric water content in the organic soil horizon (Fig. 4). In mineral soils, the potential activity of acid phosphatase, cellobiohydrolase, phenol oxidase, and peroxidase were also lower in root ingrowth compared to root exclusion cores ($P \leq 0.05$, Table 2).

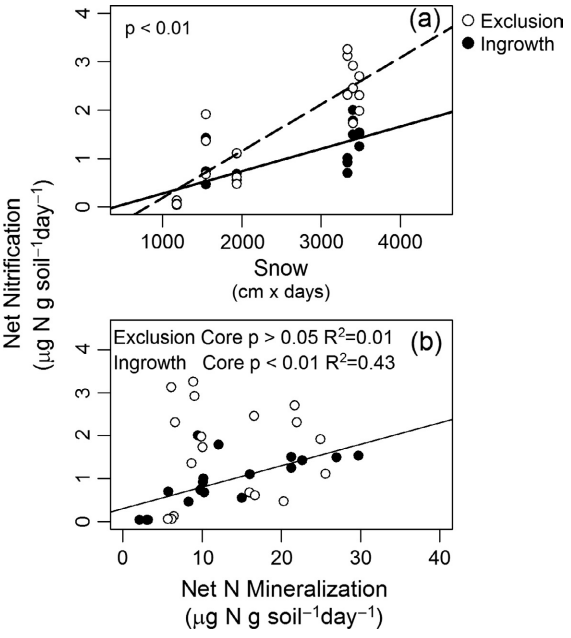

FIG. 2. Net nitrification in the organic soil horizon was (a) positively related to snow. Nitrification rates and N mineralization rates were (b) positively related in root ingrowth cores only. Points in both figures are means averaged across plots for cores collected in October 2012 and 2013.

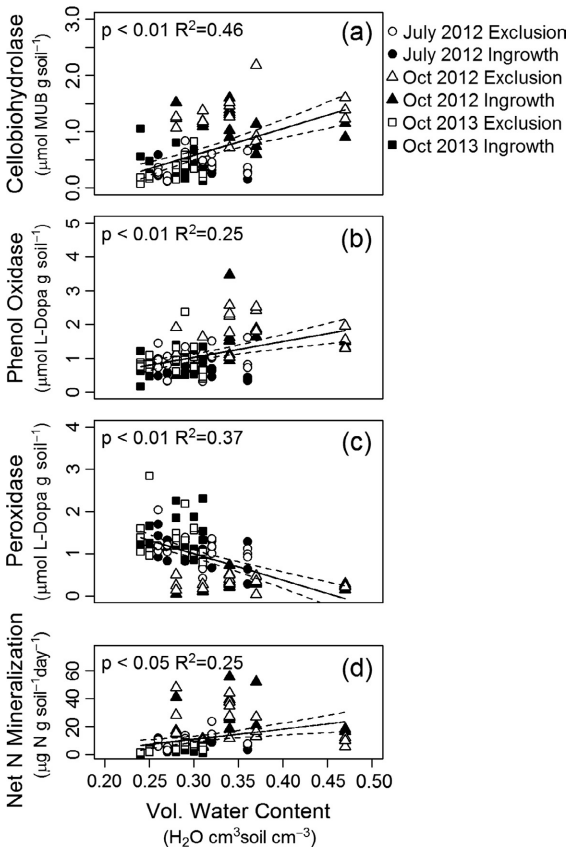

FIG. 3. Volumetric water content in organic soils vs. (a) cellobiohydrolase, (b) phenol oxidase, and (c) peroxidase activity, and (d) net N mineralization. Dashed lines are the 95% confidence interval. Points are plot averages of root ingrowth and exclusion cores (n = 18).

DISCUSSION

Alterations to root-microbe interactions may be viewed as a sentinel of forthcoming changes to forest nutrient retention, plant C allocation, and soil C storage (Rillig and Mummey 2006, Iversen 2010, McCormack et al. 2014). In addition, a decoupling between root-microbial processes due to loss of winter snow cover is frequently implied, yet has rarely been tested, in ecosystems with seasonal snow cover (Fitzhugh et al. 2001, Malyshev and Henry 2012, Campbell et al. 2014, Wipf et al. 2015). In an effort to address this gap in research, we examined microbial biomass, exoenzyme activity, and potential net N mineralization and nitrification in soil cores that were incubated *in situ* in either the presence or the absence of roots for up to 29 months along a natural winter climate gradient.

Consistent with our first hypothesis, root biomass production was related positively to soil frost depth and duration across the winter climate gradient (Fig. 1d, Table 1); with greater ingrowth occurring at the lower elevation sites where soil frost depth and duration were greater. It is unlikely that the relationship between soil frost and root ingrowth simply reflects site-level differences in annual net primary production (ANPP). Increased soil freezing due to snow-removal similarly increased root production during the subsequent growing season in plot-scale studies at HBEF (Tierney et al. 2001). In addition, locations with the highest ANPP do not systematically co-vary with locations with the most winter soil freezing at HBEF (Fahey et al. 2005); or to sites with the highest root ingrowth in our study. However, it is possible that other differences in microclimate (e.g., growing season length, Richardson et al. 2006), or soil properties among sites (e.g., microbial community structure, bulk density, organic matter content, Bohlen et al. 2001, Keiser et al. 2014, Morse et al. 2015) also partially contribute to higher root production at low vs. high elevation locations.

Supporting our second hypothesis, net nitrification increased with winter snow depth and duration (Fig. 2a, see further discussion below). However, potential microbial exoenzyme activity and net N mineralization rates were only weakly related to elevation, snow or soil frost, or minimum winter soil temperature (Appendix S1: Tables S2 and S3). Rather, potential microbial exoenzyme activity and net N mineralization rates were positively correlated with soil moisture (Fig. 3). Thus, drier conditions due to climate warming can be expected to reduce microbial exoenzyme activity and net N mineralization rates at HBEF (Groffman et al. 2009, Durán et al. 2014, Morse et al. 2015), as has similarly been observed in other seasonally snow-covered ecosystems (Fisk and Schmidt 1995). By contrast, soil nitrification rates will be more strongly driven by the interaction between loss of winter snowpack and its' attending effect on tree root dynamics.

The rhizosphere is characterized by elevated rates of microbial activity that contribute significantly to soil C and N fluxes at the ecosystem scale (Finzi et al. 2015). In our third hypothesis, we predicted that the presence of roots would increase microbial activity and that the stimulatory

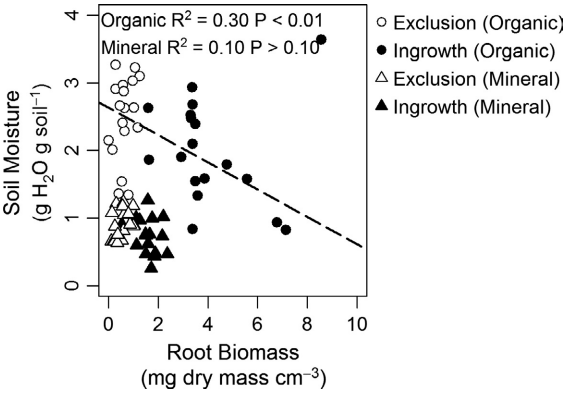

FIG. 4. Gravimetric soil moisture content in root exclusion and ingrowth cores. Points are plot-level means (n = 18) averaged across sampling dates (July or October 2012 and October 2013). Trend line shows relationship in organic soils, excluding one outlier data point.

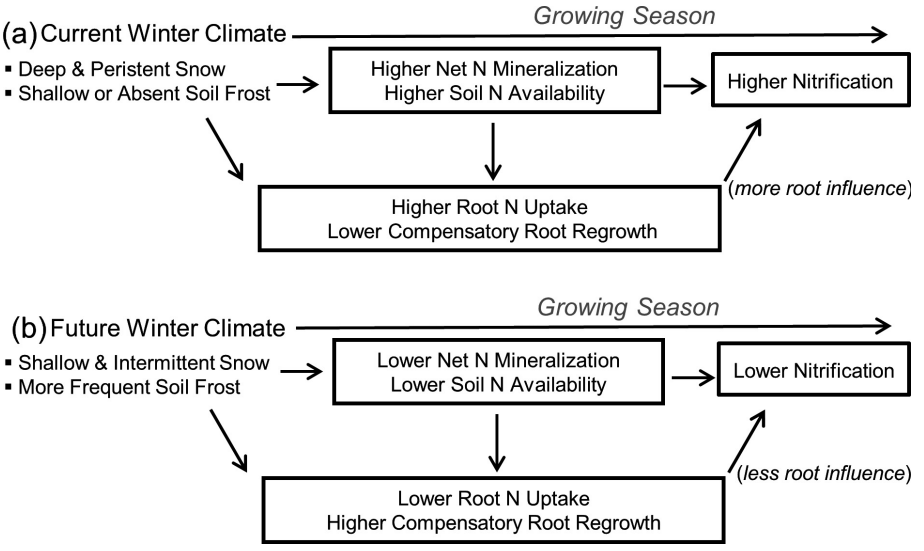

FIG. 5. Deep and persistent snow cover (a) currently leads to higher net N mineralization and net nitrification rates. Higher soil N availability increases relative root N uptake, partially inhibiting nitrification. Loss of snow cover or drier soils in the future are likely to (b) decrease net N mineralization and nitrification rates and reduce N availability to trees, in spite of increased compensatory root regrowth associated with more frequent winter soil freeze-thaw events that cause root damage.

effect of roots would be greater at high compared to low elevation sites due to increased soil freezing at low elevation. However, the presence of plant roots depressed rather than stimulated microbial activity (Table 2). And while rates of exoenzyme activity, N mineralization, and nitrification were generally greater at the higher elevation sites (Figs. 2 and 3; Appendix S1: Tables S3 and S4), there too the presence of tree roots decreased microbial activity.

We suggest three possible reasons, which are not mutually exclusive, for why there was no stimulation of microbial activity in the root ingrowth compared to exclusion cores. First, rhizosphere microbial activity is not as strongly stimulated by arbuscular mycorrhizal-associated tree species, like sugar maple, compared to ectomycorrhizal-associated tree species (Phillips and Fahey 2006, Brzostek et al. 2015). Thus, the root-ingrowth effect that we observed is likely to be more muted than if a similar study were conducted in ectomycorrhizal-associated conifer forest at HBEF. Second, live fine root biomass in the organic horizon at HBEF averages 203 ± 24 g m^{-2} (Fahey and Hughes 1994) or about two-fold greater than the highest root biomass (~105 g m^{-2}) observed in any of our root ingrowth cores, suggesting much lower root-derived C supply in the root ingrowth cores compared to native soils. Third, soil trenching increases soil moisture content by reducing water uptake by roots, leading to higher rates of net N mineralization and soil respiration compared to untrenched soils (Kuzyakov 2006). We observed higher microbial exoenzyme activity and net N mineralization rates in the root exclusion compared to the root ingrowth cores (Table 2), supporting the hypothesis that roots exert an indirect control over microbial exoenzyme activity and net N mineralization that is mediated via water uptake.

Net nitrification was related positively to winter snowpack depth and duration, consistent with previous studies conducted in temperate forests (Durán et al. 2014), temperate grasslands (Vankoughnett and Henry 2013), as well as alpine and arctic tundra (Brooks et al. 1998, Schimel et al. 2004). More notably, in this study root ingrowth reduced net nitrification rates by 30% at the three highest elevation sites with the deepest snow cover. Because microbial biomass N did not vary by elevation nor soil core type, the interactive response to snow depth and root ingrowth is unlikely to be driven by differences in the size of the microbial population. By contrast, the availability of NH_4^+ as a substrate is a dominant control of nitrification. Winter soil freezing reduces root uptake of NH_4^+ by sugar maple (Campbell et al. 2014) and plant N uptake is similarly reduced with decreasing snow depth in the arctic tundra (Welker et al. 2005) and in temperate grasslands (Malyshev and Henry 2012). Thus, the decline in nitrification with root ingrowth at high elevation suggests greater plant-microbe competition for NH_4^+ (Fig. 5a); which does not occur in the absence of roots (Fig. 2b) and is weaker at low elevation where soil freezing effects on roots are greater (Fig. 1). Low soil moisture availability also reduces exoenzyme activity, rates of net N mineralization, and decreases soil N availability at low compared to high elevation sites (Fig. 3), which are conditions that may become more common during summer at HBEF (Fig. 5b).

The results of this study have several important implications. We observed that roots exert significant control over nitrification rates, but the root influence declines with reductions in depth and duration of winter snowpack. If winter soil freezing intensifies over time, then roots may exert less control over ecosystem NO_3^- loss,

particularly during the transition between winter and spring, which is a critical time for soil N cycling in ecosystems with a seasonal snowpack (Brooks and Williams 1999, Campbell et al. 2014, Wipf et al. 2015). However, because nitrification rates declined at locations with less snow in our study, gaseous N losses and N in soil leachate may also decline along with continued loss of winter snow cover. Lastly, greater compensatory root growth during the spring and summer may combine to reduce nitrification rates directly through plant-microbial competition for N and indirectly through a negative feedback on soil moisture, exoenzyme activity, and N mineralization. Thus, understanding the effects that loss of winter snow has on root-microbial interactions is necessary for developing a predictive understanding of soil C and N biogeochemical transformations in the northern hardwood forest and in other seasonally snow-covered ecosystems.

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