Soil Biology & Biochemistry 107 (2017) 77–84

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Differential sensitivity to climate change of C and N cycling processes across soil horizons in a northern hardwood forest

Jorge Durán [a, *], Jennifer L. Morse [b], Alexandra Rodríguez [a], John L. Campbell [c], Lynn M. Christenson [d], Charles T. Driscoll [e], Timothy J. Fahey [f], Melany C. Fisk [g], Myron J. Mitchell [h], Pamela H. Templer [i], Peter M. Groffman [j, 1]

[a] Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal
[b] Portland State University, Environmental Science and Management, PO Box 751 (ESM) Portland, OR 97207, USA
[c] USDA Forest Service, Northern Research Station, 271 Mast Road, Durham, NH 03824, USA
[d] Vassar College, Biology Department, 124 Raymond Ave., Poughkeepsie, NY 12604, USA
[e] Syracuse University, Department of Civil and Environmental Engineering, 151 Link Hall, Syracuse, NY 13244, USA
[f] Cornell University, Department of Natural Resources, Ithaca, NY 14850, USA
[g] Miami University, Department of Zoology, 501 E High St, Oxford, OH 45056, USA
[h] SUNY-College of Environmental Science and Forestry, Department of Environmental Resources Engineering, 1 Forestry Dr, Syracuse, NY 13210, USA
[i] Boston University, Department of Biology, 5 Cummington Mall, Boston, MA 02215, USA
[j] Cary Institute of Ecosystem Studies, 2801 Sharon Turnpike, Millbrook, NY 12545, USA

ARTICLE INFO

Article history:
Received 5 September 2016
Received in revised form
28 December 2016
Accepted 30 December 2016
Available online 5 January 2017

Keywords:
Carbon
Nitrogen
Forest soil
Global warming
Snow
Frost

ABSTRACT

Climate of the northern hardwood forests of North America will become significantly warmer in the coming decades. Associated increases in soil temperature, decreases in water availability and changes in winter snow pack and soil frost are likely to affect soil carbon (C) and nitrogen (N) cycling. Most studies of the effects of climate change on soil function have focused on the upper-organic part of the soil profile (e.g., forest floor), and little is known about effects on deeper mineral soil horizons. We exploited an elevation/orientation gradient at the Hubbard Brook Experimental Forest (New Hampshire, USA) to evaluate how variation in climate, similar to that projected to occur over the next 50–100 years, affects soil C and N pools and transformation rates in different soil horizons of northern hardwood forests. Lower elevation, south-facing plots with higher soil temperature, less soil moisture and snow, and increased frequency of soil freeze/thaw events had less soil inorganic N content and lower potential net N mineralization rates compared to higher elevation, north facing plots. These differences in N pools and fluxes were consistent for all soil horizons, but sensitivity to climate variation increased with soil depth, confirming that assessments of climate change effects that do not consider variation throughout the soil profile are likely to be incomplete and potentially inaccurate. Nitrogen cycling processes were more sensitive to climate variation than C cycling processes, suggesting a decoupling of C and N cycles in coming decades, with important implications for ecosystem function. Soil processes showed greater sensitivity to climate variation in summer than in spring, and in the warmer and less snowy year of sampling, suggesting that the effects of climate change might become more pronounced as temperatures increase and snow fall and water availability decrease in the coming decades.

1. Introduction

Global climate is projected to become significantly warmer in the coming decades (Intergovernmental Panel on Climate Change, 2013). In northeastern North America, air temperatures are projected to increase 2.1–5.3 °C by 2100, with substantial and statistically significant reductions in the depth and duration of the winter snow pack (Hayhoe et al., 2007). These changes are expected

* Corresponding author.
E-mail address: humia20@gmail.com (J. Durán).
[1] Current address: City University of New York, Advanced Science Research Center and Brooklyn College Department of Earth and Environmental Science, 85 St. Nicholas Terrace, New York, NY 10031, USA.

http://dx.doi.org/10.1016/j.soilbio.2016.12.028
0038-0717/

to be accompanied by colder soils and an increased frequency of soil freeze-thaw events in the winter, due to reductions in the insulating effect of snow, and by warmer and drier soils during the growing season (Brown and DeGaetano, 2011; Campbell et al., 2010; Durán et al., 2014). However, despite the importance of abiotic conditions (e.g. temperature or moisture) as drivers of soil function, we still have an incomplete understanding of the responses of terrestrial ecosystems to climate-driven changes in soils (Rustad et al., 2001; Schlesinger and Bernhardt, 2012; Sternberg et al., 2011).

Temperate forest biomes provide a myriad of ecosystem services, such as the regulation of water regimes and climate, the preservation of soil quality, and the maintenance of nutrient cycling and biodiversity (Jacobs et al., 2013). Also, temperate forests contain approximately 10% of global soil carbon (C) stocks, and are responsible for most of the terrestrial C sequestration at mid-latitudes in the Northern Hemisphere (Monson et al., 2006; Rasmussen et al., 2006). Nitrogen (N) frequently constrain primary production of temperate forests (LeBauer and Treseder, 2008; Vitousek and Howarth, 1991), whose capacity to sequester C depends on soil N availability (Fernández-Martínez et al., 2014). On the other hand, excess N contributes to many environmental problems (e.g. acidification, NO_x emissions, eutrophication; Galloway et al., 2008; Porter et al., 2013).

Experimental and observational studies carried out in forests in northeastern North America have shown that changes in soil climatic conditions can significantly influence plant and microbial functions that alter soil C and N pools and cycling rates during both the winter and the growing season (Brooks et al., 2011; Durán et al., 2014; Groffman et al., 2012; Joseph and Henry, 2008; Morillas et al., 2015). Previous research has shown that higher air temperatures and reductions in soil moisture and in snow depth and duration lead to lower microbial biomass and activity, protease and oxidative enzyme production, microbial respiration and nitrification rates in surface organic horizons of northern hardwood forests (Durán et al., 2014, 2016; Sorensen et al., 2016). However, most of this knowledge comes from studies of the upper-organic portion of the soil profile, where many biogeochemical processes relevant to ecosystem functions are concentrated, and few studies have compared responses across soil horizons, including mineral soil layers (Harrison et al., 2003, 2011; Jandl et al., 2014; Rodríguez et al., 2014). Although biogeochemical processes are greater on a per unit soil mass basis in the organic-rich surface horizons, the much larger mass of soil is associated with deeper soil horizons. As a result, a substantial part of the total soil profile biological activity occurs in lower mineral horizons, which increases their potential role in soil responses to climate change (Buchholz et al., 2014; Jobbágy and Jackson, 2000; Lawrence et al., 2013; Mobley et al., 2015; Morse et al., 2014). Thus, the lack of studies that explicitly include deeper soil layers has likely resulted in an incomplete understanding of how forest soils function and respond to environmental change (Harrison et al., 2003).

In addition to changes in the concentration of organic matter and biological activity, environmental controls over biogeochemical process rates also likely vary with depth. Plant nutrient acquisition in northern forests occurs mostly in the upper layers of soil where the majority of fine roots occur (Tierney et al., 2003). Thus, while the response of surface horizons to climate change is strongly affected by plants, the response of deeper horizons is likely to be less tightly coupled to plant activity and responses and more dominated by abiotic conditions (Dungait et al., 2012). Further, whereas higher rates of biological processes in surface horizons are driven in part by the higher proportion of labile carbon at shallow depths, recent evidence suggests that more recalcitrant soil organic matter pools, which dominate at depth, may be more sensitive to changing soil climate than labile pools (Bauer et al., 2008; Dungait et al., 2012). On the other hand, daily, seasonal and annual fluctuations in soil temperature and moisture are greater for surface than deeper horizons.

In this study, we aimed to evaluate, under natural conditions, how projected increases in temperature and decreases in snow accumulation affect soil C and N pools and transformation rates in different soil horizons of northern hardwood forests. To do so, we exploited a natural elevation/orientation gradient at the Hubbard Brook Experimental Forest (HBEF, New Hampshire, USA), which encompasses relatively uniform soil and forest vegetation and produces a difference in mean annual temperature very similar to that projected over the next 50–100 years (Hayhoe et al., 2007) for the northeast U.S. Our study builds on previous analyses of relationships among soil temperature and moisture, snow depth, soil freezing, and N and C cycling at the HBEF based on both experimental snow pack manipulations and observational studies along the elevation gradient (Campbell et al., 2014; Cleavitt et al., 2008; Durán et al., 2014; Fitzhugh et al., 2001; Groffman et al., 2011; Morse et al., 2015; Sorensen et al., 2016; Tierney et al., 2001). We hypothesized that, throughout the soil profile, warmer soil temperatures and shallower winter snow pack in lower elevation, south-facing plots would be linked to smaller C and N pools and lower net mineralization rates compared to higher elevation, north-facing plots. We also hypothesized that deeper soil horizons, with less organic matter but likely more recalcitrant C, and lower biotic control over biogeochemical processes would be more sensitive to climate change than shallower, more organic soil layers.

2. Methods

We conducted this study at the HBEF, in the White Mountain National Forest, New Hampshire, USA (43.56° N, 71.45° W). Elevation within the 3162 ha HBEF ranges from 225 m to 1100 m. The climate is cool, humid, and continental with average maximum air temperature = 19 °C, average minimum air temperature = −9 °C; mean annual temperature has increased by approximately 0.3 °C decade^{-1} over the last 50 years (Hamburg et al., 2013). Average annual precipitation is 1400 mm. The snow pack usually persists from late December until mid-April.

In October 2010, we established 20 independent (separated by > 300 m) 10 m diameter sites within the HBEF; 12 were higher elevation, north-facing plots, and 8 were lower elevation, south-facing plots. This experimental design allowed to use the natural elevation/orientation gradient of HBEF as a surrogate/analog for climate change. For this study, we used data from only the highest 8 north-facing plots, along with all 8 south-facing, low elevation plots to produce an elevation gradient that spanned 375–775 m asl. This plot selection allowed us to maximize the differences in climatic conditions while keeping a balanced design to facilitate statistical analysis. The design produced a range in mean annual air temperature of ~2.5 °C, which is similar to the change that is projected to occur over the next 50–100 years in northeastern North America (Hayhoe et al., 2007). All the plots have similar soils: well to moderately well-drained acidic (pH 3.9) Typic Haplorthods with loamy sand texture and an organic layer consisting of leaf-litter (O_i), a dense root-mat and decomposing organic material (O_e), and a nutrient rich humus layer (O_aA) overlying a soil mineral layer (Bohlen et al., 2001). Vegetation is also similar among the plots and is dominated by sugar maple (*Acer saccharum*; Schwarz et al., 2003).

We measured soil temperature and volumetric water content (WC) at 5 cm depth continuously with Decagon 5TM® combination probes coupled to Decagon EM50® dataloggers. We used a Federal snow sampling tube (Rickly Hydrological Company, Columbus,

Ohio, USA) to measure snow depth and water equivalent, and methylene blue dye-filled frost tubes (Hardy et al., 2001; Ricard et al., 1976) to measure frost depth, at three locations at each site at bi-weekly intervals through the winters of 2010/2011 and 2011/2012. We integrated seasonal time series measurements of snow depth, soil frost depth, and snow water equivalent by converting them to a single continuous variable (area under the curve = AUC) following the procedure of Durán et al. (2014). As a measure of winter soil temperature variability, and as an indicator of the likely occurrence of freeze/thaw events, we calculated the SDL coefficients of variation (standard deviation of log-transformed observations (McArdle and Gaston, 1995); of daily soil winter temperatures (hereafter referred as to 'frzthaw'; Durán et al., 2013).

Ten cylindrical (5 cm diameter, 20 cm depth) soil samples were collected from each plot in May of 2011 and 2012 ('Spring 2011' and 'Spring 2012', respectively) and in August of 2011 and 2012 ('Summer 2011' and 'Summer 2012', respectively). Soil samples, after discarding undecomposed plant litter, were separated into three layers: the surface organic layer consisting of dense root-mat and decomposing organic material (O_e), a nutrient rich humus layer (O_a), and the first 10 cm of the underlying mineral layer (B). Samples were then transported in coolers to the laboratory, hand-sorted to remove roots and rocks and homogenized, and stored at 4 °C until analysis (less than 1 week). To express results on a dry mass basis, soil moisture (SM) was determined by drying at 60 °C until constant mass of soils was obtained (McInnes and Weaver, 1994). Soil organic matter content was determined by loss on ignition at 450 °C for 4 h (Nelson and Summers, 1996). We extracted soil total inorganic N [TIN = ammonium (NH_4^+) + nitrate (NO_3^-)] by mixing 7.5 g of soil with 30 ml of 2 M KCl; measured potential microbial respiration (PMR), net N mineralization (PM) and net nitrification (PN) rates in 10 day laboratory incubations; and measured soil microbial biomass N (MBN) and C (MBC) using the chloroform fumigation-incubation method (Jenkinson and Powlson, 1976), as described by Durán et al. (2014).

To test the effect of elevation/orientation on response variables for each soil layer, a distance-based permutational repeated measures ANOVA was fitted with elevation as the main categorical fixed factor, taking into account time dependencies between samplings (PERMANOVA; Anderson et al., 2008). Relationships between soil biogeochemical measurements and elevation were investigated using an *a posteriori* pairwise comparison with the PERMANOVA t-statistic. The same analyses were carried out to test the effect of soil horizon on response variables and to investigate differences among soil horizons (O_e, O_a, B). To account for spatial dependencies among horizons, the PERMANOVA was fitted with horizon as a categorical random factor. A maximum of 9999 permutations were used to obtain pseudo-F and P-values in each data set.

To estimate the sensitivity of the different soil layers and the different variables to elevation-driven variation in climatic conditions, we calculated the percent change of all analyzed variables using the following equation:

$$\frac{Vlow - Vhigh}{Vhigh} \times 100$$

where: *Vlow* is the average value for all low elevation, south-facing plots and *Vhigh* is the average value for all high elevation, north-facing plots. We also developed and calculated a '*multisensitivity*' index averaging the percent change of a subset of independent variables (MBC, PMR, TIN, MBN, PM and SM). This index allowed us to aggregate information on variation in the sensitivity (estimated through normalized percent changes) of multiple key ecosystem function variables in different soil layers in different sampling seasons.

All statistical analyses were carried out using Primer 6 and Permanova + (PRIMER-E Ltd, Plymouth, UK) statistical package.

3. Results

On average, low elevation, south-facing plots consistently had higher soil temperature, lower soil moisture and snow-AUC, but greater frequency of soil freeze/thaw events ('Frzthaw') than high elevation, north-facing plots (Fig. 1) during the two years of sampling. No significant differences between high elevation, north-facing and low elevation, south-facing plots were found for soil frost-AUC or snow water equivalent-AUC.

There were statistically significant differences ($P < 0.05$) among soil horizons for all studied response variables, with the highest values found in the O_e horizon and the lowest in the mineral horizon, with intermediate values in the O_aA horizon, for all sampling dates and annual averages (Table 1; Fig. S1). There were significant differences in soil NO_3^--N content, total inorganic N content and potential nitrification rates between elevations, with consistently higher values in the high elevation, north-facing plots (Table 1; Fig. S1). No significant differences were found between elevations for the remainder of the studied variables.

The percent change analyses revealed that, on average, the mineral soil was the most sensitive soil horizon to the effects of the elevation on all variables (Fig. 2). Although the sensitivity of the different horizons for the different variables varied among sampling dates (Fig. 3), the percent change between low elevation, south-facing plots and high elevation, north-facing plots tended to be higher in the second year and during summer compared to the first year and spring (Fig. 2). Our multisensitivity index confirmed that the mineral horizon was the most sensitive soil horizon, followed by the O_aA and the O_e horizons (Fig. 2), and that sensitivity to the effects of elevation was higher in Summer than in Spring, and in 2012 compared to 2011 (Fig. 3).

4. Discussion

The elevation/orientation gradient across the HBEF is an effective platform for investigation of climate variation on ecosystem properties and processes (Durán et al., 2016; Morse et al., 2015; Sorensen et al., 2016). The subset of plots used for this study provided a range of soil temperature, snow and frost conditions comparable to that expected with climate change in coming decades. Low elevation, south-facing plots, consistently had higher surface soil temperature and lower soil moisture than high elevation, north-facing plots. These changes are associated with significantly less snow and an increase in the number of soil freeze/thaw cycles on the low elevation plots, but not with significant increases in soil frost depth (Campbell et al., 2010; Durán et al., 2014; Groffman et al., 2011).

Our previous work has shown that climate variation along the HBEF gradient affects a wide range of soil biogeochemical properties, leading to lower amounts of soil inorganic N and lower potential N transformation rates, at least in the upper, more organic layers of the soil (Durán et al., 2014, 2016; Sorensen et al., 2016). Here we show that these effects are also evident in deeper soil horizons, supporting the idea that projected climate warming may result in lower N availability in northern hardwood forests during the next century (Groffman et al., 2009). Several complementary, climate-related mechanistic explanations have been proposed to explain these effects. First, reduced winter snow pack and more freeze/thaw events in warmer low elevation plots could stress and/or kill microbial populations, while the microbial communities and belowground biological processes remain intact under the insulating snow pack in colder, high elevation, north-facing plots

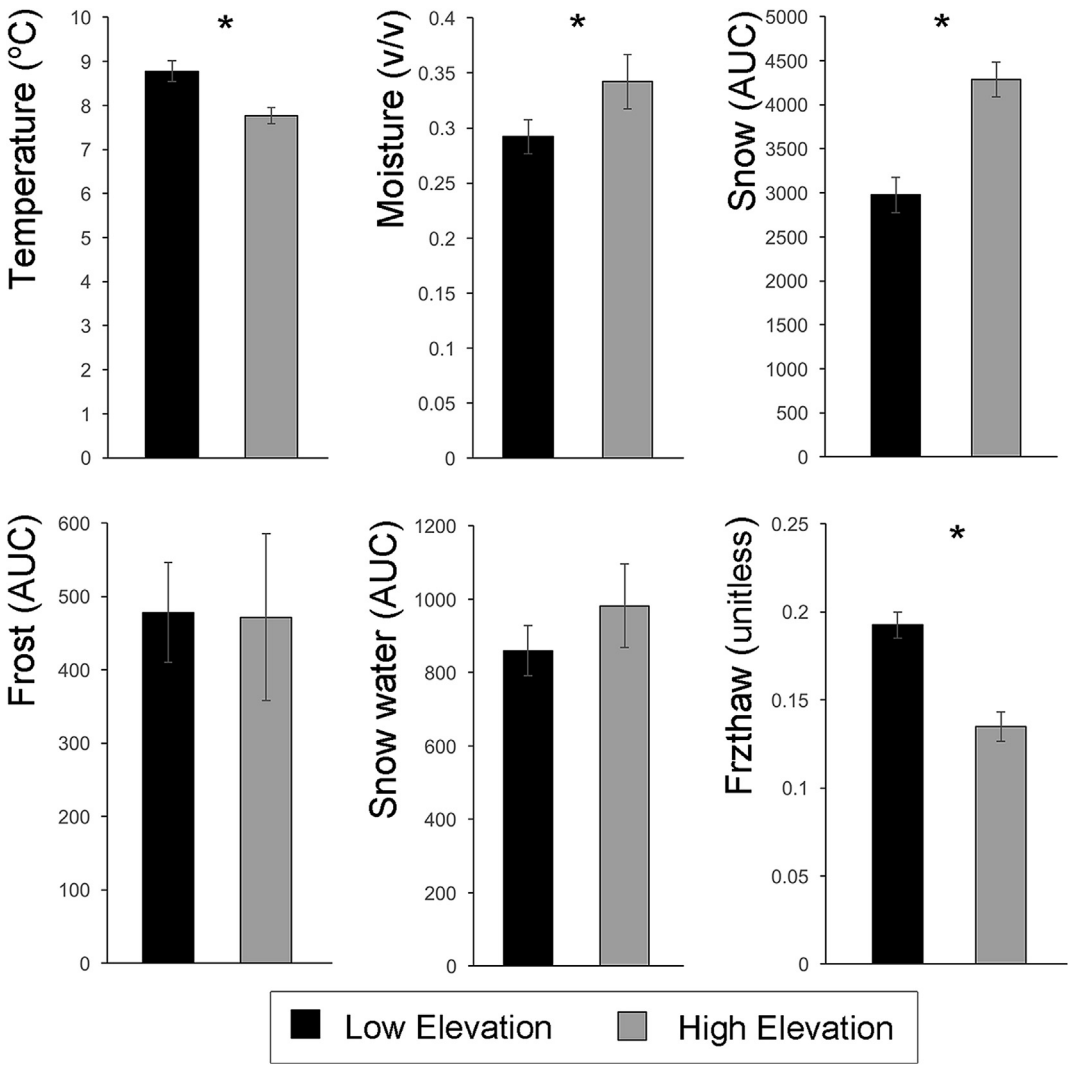

Fig. 1. Soil temperature and moisture, snow, frost and snow water equivalent 'area under the curve' (AUC), and the variability of soil winter temperature (Frzthaw) measured in low elevation-south facing (black bars) and high elevation-north facing (grey bars) plots. Values are mean with standard error of spring and summer sample dates in 2011 and 2012. Asterisks refer to statistically significant ($P < 0.05$; PERMANOVA) differences between low elevation-south facing and high elevation-north facing plots.

(Brooks et al., 1998; Stuanes et al., 2008). These effects on soil microbial populations also likely decrease rates of microbial decomposition and mineralization activity during the following growing season (Durán et al., 2014). Second, increased tree root mortality due to reductions in snow pack during winter could be followed by high compensatory root production and N uptake during the growing season that could reduce microbial N processing (Sorensen et al., 2016; but see Campbell et al., 2014). Finally, reductions in water availability during the growing season may also decrease nitrification rates and soil NO_3^-N pools in the low elevation compared to the high elevation plots (Stark and Firestone, 1995).

As is true for all natural gradient studies, this study has limitations based on concerns that factors other than climatic conditions, such as differences in pH, plant productivity or in the quality of organic matter (OM) inputs, might covary with climatic conditions and influence the observed elevation/orientation effects. However, soils and vegetation are quite similar along the gradient (we focused on maple dominated plots) and past studies have not found significant variation in C and N stocks with elevation or orientation within the hardwood vegetation zones at the HBEF (Bohlen et al., 2001; Johnson et al., 2000). Nevertheless, we acknowledge that a more detailed analysis of the multiple factors that vary with elevation and orientation, as well as more specific measurements of plant, microbial and enzymatic activities, would improve our ability to understand and predict the effects of climate change on ecosystem functioning.

Analyses of percent change and the multisensitivity index clearly show that the sensitivity of these forest soils to climate variation increases with soil depth. This differential sensitivity could be explained by several mechanisms. First, recalcitrant SOM, whose proportion increases with soil depth, is known to be more sensitive to change in soil climatic conditions than labile SOM (Bauer et al., 2008; Dungait et al., 2012). We have not carried out any specific analysis of the recalcitrance of different soil horizons. However, the ratios between the rates of microbial activity and the amount of microbial biomass, consistently higher in surface than in deeper horizons, seem to support the idea that the organic matter in the deep horizons could be indeed more recalcitrant (Fig. S2). Second, deeper soil layers with less organic matter have lower water holding capacity and are therefore more susceptible to temperature-driven moisture losses (Johnson et al., 1991; Morse et al., 2014). Certainly, only the mineral soil samples showed

Table 1
Soil microbial biomass N (MB-N), microbial biomass-C (MB-C), total inorganic N (TIN), nitrate ($NO3^-$-N), ammonium ($NH4^+$-N), potential mineralization (PM), potential nitrification (PM) and potential microbial respiration (PMR) in low elevation-south facing (Low) and high elevation-north facing (High) plots in the O_e, O_aA and Mineral soil horizons in the spring and summer of 2011 and 2012, together with the horizon and sampling averages. Lowercase letters show statistical differences between elevations, whereas capital letters show significant differences among soil horizons ($P < 0.05$; PERMANOVA).

		Spring 2011		Summer 2011		Spring 2012		Summer 2012		Average	
		Low	High	Low	High	Low	High	Low	High	Low	High
MBN (μg N g soil^{-1})	O_e	686.11^{A}	688.96^{A}	761.56^{A}	755.65^{A}	648.17^{A}	658.7^{A}	583.2^{A}	501.31^{A}	669.76^{A}	651.15^{A}
	O_aA	296.59^{B}	257.48^{B}	340.53^{B}	363.86^{B}	312.05^{B}	288.11^{B}	282.74^{B}	365.2^{A}	307.97^{B}	318.66^{B}
	Mineral	93.03^{C}	88.12^{C}	79.36^{C}	100.91^{C}	67.09^{C}	96.6^{C}	68.17^{a,C}	112.44^{b,B}	76.91^{C}	99.52^{C}
	Average	358.56	344.85	393.82	406.81	342.44	347.80	311.37	326.32	351.55	356.44
MBC (μg C g soil^{-1})	O_e	7180.6^{A}	5438.2^{A}	7894.7^{A}	7635.8^{A}	7626.1^{A}	10038.4^{A}	6189.9^{A}	4106.6^{A}	7222.8^{A}	6804.8^{A}
	O_aA	2867.4^{B}	2476.0^{B}	3013.5^{B}	3022.9^{B}	3627.1^{B}	2828.5^{B}	2795.8^{B}	2642.5A	3075.9^{B}	2742.5^{B}
	Mineral	1031.8^{C}	902.9^{C}	698.4^{C}	827.3^{C}	781.4^{C}	1116.9^{C}	496.55^{a,C}	859.6^{b,B}	752.0^{C}	926.7^{C}
	Average	3693.3	2939.0	3868.9	3828.6	4011.6	4661.3	3160.7	2536.2	3683.6	3491.3
TIN (μg N g soil^{-1})	O_e	109.48^{A}	120.01^{A}	101.5^{A}	115.25^{A}	96.89^{A}	96.18^{A}	32.07^{A}	40.7^{A}	84.99^{A}	93.04^{A}
	O_aA	27.23^{B}	28.54^{B}	25.17^{B}	35.02^{B}	19.92^{B}	22.66^{B}	16.25B	30.4^{A}	22.14^{B}	29.16^{B}
	Mineral	7.25^{C}	8.25^{C}	7.05^{a,C}	10.14^{b,C}	5.56^{C}	8.33^{C}	4.18^{a,C}	8.22^{b,B}	6.01^{a,C}	8.73^{b,C}
	Average	47.99	52.26	44.57	53.47	40.79	42.39	17.5^{a}	26.44^{b}	37.71^{a}	43.64^{b}
NO_3-N (μg N g soil^{-1})	O_e	12.24^{A}	21.53^{A}	7.75^{A}	19.06^{A}	6.3^{AB}	11.21^{A}	5.7^{AB}	9.47^{AB}	8.00^{a,A}	15.32^{b,A}
	O_aA	11.10^{A}	14.39^{A}	10.18^{A}	20.18^{A}	7.7^{A}	13^{A}	8.78^{A}	14.39^{A}	9.44^{a,A}	15.49^{b,A}
	Mineral	3.38^{B}	3.76^{B}	2.43^{B}	5.36^{B}	1.28^{a,B}	3.81^{b,B}	1.09^{a,B}	4.35^{b,B}	2.05^{a,B}	4.32^{b,B}
	Average	8.91	13.23	6.79	14.87	5.11	9.34	5.19	9.40	6.5^{a}	11.71^{b}
NH_4-N (μg N g soil^{-1})	O_e	97.24^{A}	98.48^{A}	93.75^{A}	96.19^{A}	90.55^{A}	84.97^{A}	26.37^{A}	31.22^{A}	76.98^{A}	77.72^{A}
	O_aA	16.13^{B}	14.15^{B}	14.99^{B}	14.84^{B}	12.22^{B}	9.66^{B}	7.46^{B}	16.02^{A}	12.70^{B}	13.67^{B}
	Mineral	3.87^{C}	4.48^{B}	4.61^{C}	4.78^{B}	4.27^{C}	4.52^{B}	3.08^{C}	3.87^{B}	3.96^{C}	4.41^{C}
	Average	39.08	39.04	37.79	38.60	35.68	33.05	12.31	17.04	31.21	31.93
PM (μg N g soil^{-1} day^{-1})	O_e	97.24^{A}	98.48^{A}	93.75^{A}	96.19^{A}	90.55^{A}	84.97^{A}	26.37^{A}	31.22^{A}	13.58^{A}	14.70^{A}
	O_aA	16.13^{B}	14.15^{B}	14.99^{B}	14.84^{B}	12.22^{B}	9.66^{B}	7.46^{B}	16.02^{A}	3.98B^{a}	4.71^{b,A}
	Mineral	3.87^{C}	4.48^{C}	4.61^{C}	4.78^{C}	4.27^{C}	4.52^{C}	3.08^{a,C}	3.87^{b,B}	0.67^{C}	1.02^{C}
	Average	39.08	39.04	37.79	38.60	35.68	33.05	12.31a	17.04b	6.08	6.81
PN (μg N g soil^{-1} day^{-1})	O_e	1.77^{AB}	3.89^{A}	0.94^{a,A}	3.41^{b,A}	1.53^{AB}	2.59^{A}	1.71^{A}	2.47^{A}	1.49a,A	3.09b,A
	O_aA	1.5^{A}	2.23^{A}	1.72^{A}	2.95^{A}	1.78^{A}	3.02^{A}	2.04^{A}	3.28^{A}	1.76a,A	2.87b,A
	Mineral	0.48^{B}	0.57^{B}	0.37^{B}	0.82^{B}	0.19^{a,B}	0.53^{b,B}	0.26^{a,B}	0.75^{b,B}	0.33^{a,B}	0.67^{b,B}
	Average	1.25	2.23	1.01	2.39	1.17	2.05	1.34	2.17	1.19a	2.21b
PMR (μg C g soil^{-1} day^{-1})	O_e	197.23^{A}	194.47^{A}	174.21^{A}	186.83^{A}	242.54^{A}	348.91^{A}	168.2^{A}	148.55^{A}	195.54^{A}	219.69^{A}
	O_aA	54.94^{B}	46.19^{B}	44.05^{B}	44.51^{B}	65.54^{B}	64.14^{B}	34.03^{B}	54.55^{B}	49.64^{B}	52.35^{B}
	Mineral	16.21^{C}	16.19^{C}	9.26^{C}	11.76^{C}	16.92^{C}	15.98^{C}	8.49^{C}	11.55^{C}	12.72^{C}	13.87C
	Average	89.46	85.62	75.84	81.04	108.33	143.01	70.24	71.55	85.97	95.30
SM (v/v)	O_e	0.75^{A}	0.72^{A}	0.67^{A}	0.67^{A}	0.73^{A}	0.76^{A}	0.71^{A}	0.68^{A}	0.72^{A}	0.71A
	O_aA	0.62^{B}	0.58^{B}	0.52^{B}	0.55^{B}	0.62^{B}	0.62^{B}	0.57^{B}	0.61^{A}	0.58^{B}	0.59^{B}
	Mineral	0.39^{C}	0.42^{C}	0.24^{a,C}	0.34^{b,C}	0.33^{a,C}	0.44^{b,C}	0.29^{a,C}	0.41^{b,B}	0.31^{a,C}	0.4^{b,C}
	Average	0.59	0.57	0.47	0.52	0.56	0.61	0.52	0.57	0.54	0.57

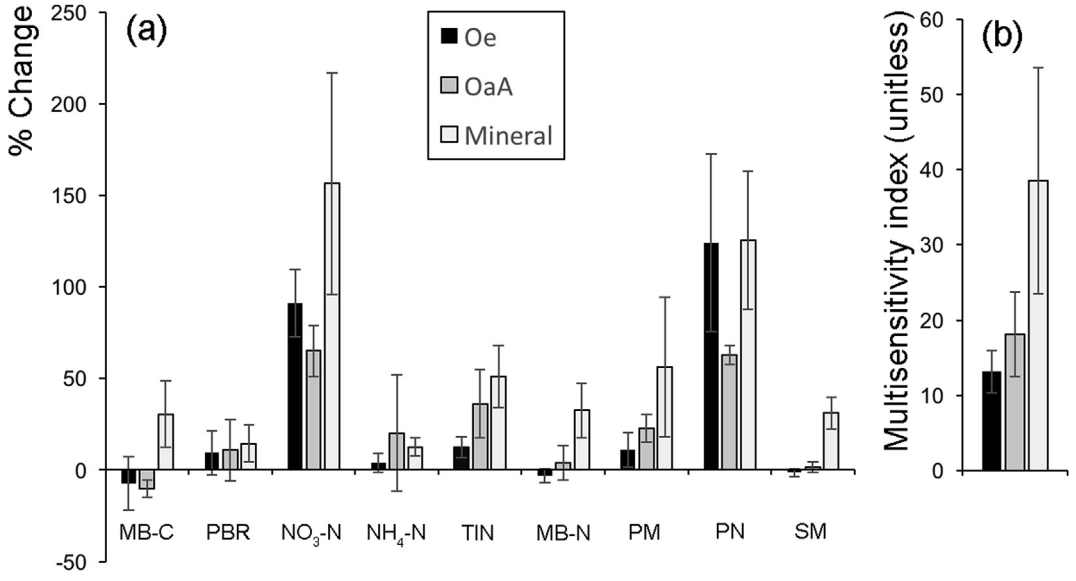

Fig. 2. (a) Percent change between low elevation-south facing and high elevation-north facing plots in soil microbial biomass C (MB-C), potential microbial respiration (PMR), nitrate ($NO3^-$-N), ammonium ($NH4^+$-N), total inorganic N, microbial biomass-N (MB-N), potential mineralization (PM), potential nitrification (PN) and moisture (SM) in the O_e (black bars), O_aA (grey gars) and Mineral (white bars) soil horizons. (b) Multisensitivity index estimated for the O_e, O_aA and Mineral soil horizons. For both plots, values are means and standard errors of spring and summer sample dates in 2011 and 2012 (n = 4).

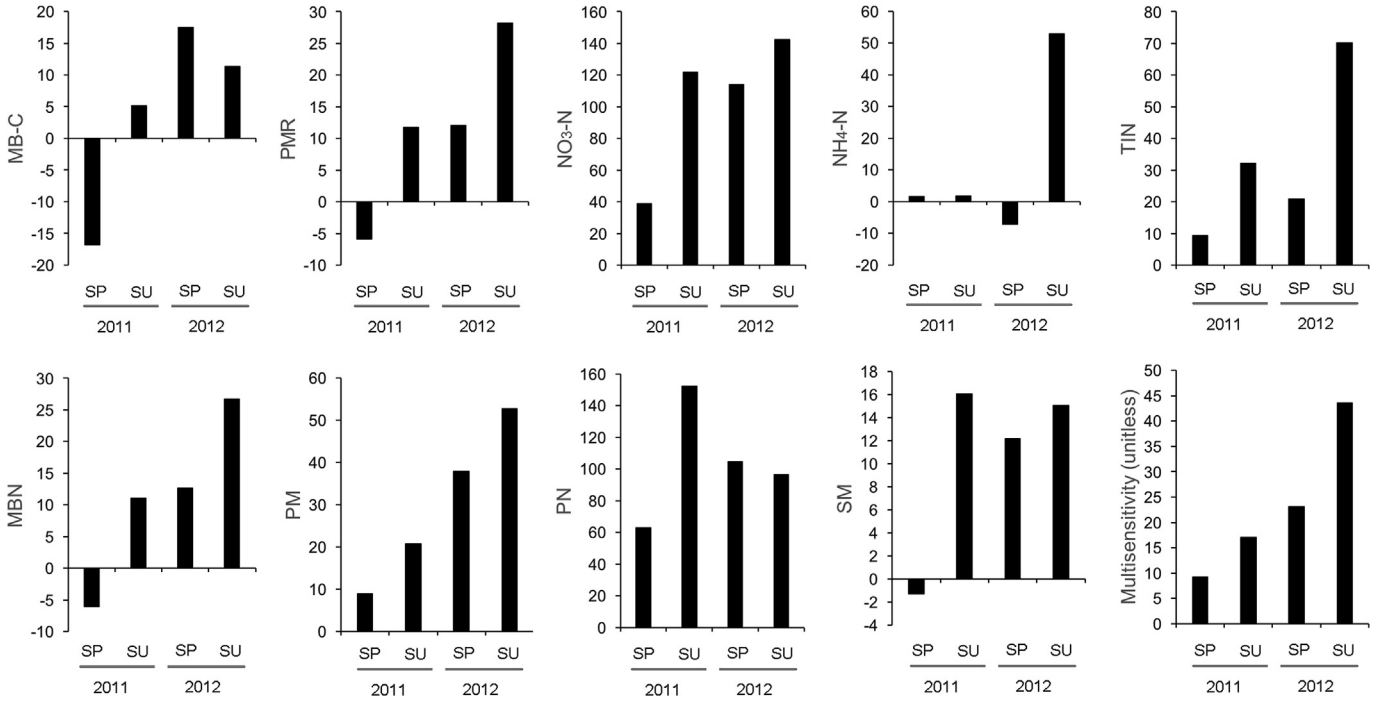

Fig. 3. Percent change between low elevation-south facing and high elevation-north facing plots estimated in soil microbial biomass C (MB-C), potential microbial respiration (PMR), nitrate ($NO3^-$-N), ammonium ($NH4^+$-N), total inorganic N, microbial biomass-N (MB-N), potential mineralization (PM), potential nitrification (PN) and moisture (SM), and *multisensitivity* index in spring (SP) and summer (SU) sample dates in 2011 and 2012.

significant differences in gravimetric water content between low and high elevation plots (Table 1). Third, the strong control of nutrient cycling typically exerted by plants and microbes in the surface organic soil horizons might have dampened the effects of changing conditions on soil function, whereas in deeper layers the controls over activity might have been more dominated, and therefore more affected, by physical conditions (Dungait et al., 2012). Finally, surface soil layers with a greater organic component have significantly more microbial biomass (Fig. 2) and likely higher diversity than deeper soil horizons (Delgado-Baquerizo et al., 2016). Recent studies clearly show that decreasing organic matter, aeration and/or fertility as soil depth increases results in significant decreases in microbial biomass and taxonomic and functional diversity (e.g. Baldrian et al., 2012; Delgado-Baquerizo et al., 2016; Eilers et al., 2012; Zhang et al., 2015). Thus, the larger, more diverse microbial communities in the shallower organic horizons may be less sensitive to climate variation due to the presence of functionally redundant microbial species (Nielsen et al., 2011).

Much of the research studying the effect of climate change on forest soils has focused on the surface and organic rich soil horizon, which likely leads to limited understanding of how deeper soil horizons function and how they will be affected by ongoing climate change (Buchholz et al., 2014; Harrison et al., 2003, 2011). Here we show that the O_aA and mineral soil horizons, which can account for up to 90% of total soil profile depth in northern hardwood forests (Johnson et al., 1991), are particularly sensitive to climate change in terms of N cycling and pools. These results indicate that failure to consider deeper horizons in forest climate change research can result in an incomplete perspective and overly conservative assessment of the effects of climate change on forest soil functioning.

In all soil layers, N-cycle related variables were found to be more sensitive to elevation-induced climate variation than C-cycle related variables. This greater sensitivity of N- compared to C-cycle variables has been observed in recent studies of the effect of disturbances on biogeochemical cycles (Durán et al., 2013; Evans and Burke, 2012; Morillas et al., 2015; Nave et al., 2011; Rodríguez et al., 2016), but the causes of these differences are not clear. A differential sensitivity to winter freezing and summer drought of components of the microbial community intimately related to N transformations, and other components of the microbial community more related to C transformations, could be contributing to the greater sensitivity of N- comparted to C-related variables associated with climate variation (Asner et al., 1997; Isobe and Ohte, 2014). While our results do not shed light on these potential mechanisms, they do suggest that climate change could lead to a decoupling between the C and N cycles in northern hardwood forests (Li et al., 2007; Schimel and Bennett, 2004), with important but difficult to anticipate effects on plant and microbial activity and diversity, organic matter decomposition and primary productivity (Delgado-Baquerizo et al., 2013; Finzi et al., 2011; Peñuelas et al., 2012; Schimel, 2010; Schimel and Bennett, 2004).

The results of this two-year observational study along a natural gradient should be taken with caution. However, the fact that we observed consistent differences in sensitivity to climate in two very different years provides strong support for our interpretation of the results. Interestingly, both the percent change and the multisensitivity index suggest that there is also seasonal and annual variation in the response of these forest soils to climate change. We observed greater variability in the second than in the first year, and in summer than in spring. These differences in vulnerability, again more evident in the mineral than in the shallower soil horizons, were likely driven by differences in key climatic conditions. Lower snowfall and cover in 2012 compared to 2011 (Durán et al., 2014), together with higher temperatures and lower water availability in summer than in spring and in 2012 than in 2011 (Table 1; Durán et al., 2014) likely exacerbated the differences between low and high elevation plots and their respective climatic differences. These

results suggest that the effects of climate change on soil processes could become more marked as temperatures increase, and snow fall and water availability decreases in coming decades.

5. Conclusions

Our results confirm that projected changes in climate will affect a wide range of soil biogeochemical properties in organic and mineral soil horizons of northern hardwood forests, leading to lower amounts of soil inorganic N, lower N transformation rates, and likely lower N availability. However, we demonstrated that sensitivity of these forest soils to climate change increases with soil depth. We propose that a shift form primarily biotic to physical controls with depth alters climatic responses, likely exacerbating the climate change-driven decoupling of C and N cycles. This differential sensitivity indicates that failure to consider deeper soil horizons in forest climate change research can result in an incomplete perspective and overly conservative assessment of the effects of climate change on forest soil and ecosystem functioning. Finally, we show that there is also seasonal and annual variation in the response of these forest soils to climate variation, and anticipate effects of climate change on soil processes could become more marked as temperatures increase, and snow fall and water availability decreases in coming decades.

Acknowledgements

This research was supported by the US National Science Foundation (Grants DEB 0949664 - Ecosystem Studies; and DEB 1114804 – Long-Term Ecological Research). J.D. was supported by a Fulbright fellowship of the Spanish Ministry of Education (FU2009-0708) and by the Portuguese FCT Research Programme (IF/00950/2014).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.12.028.

References

Anderson, M., Gorley, R., Clarke, K., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. Primer-E Ltd., Plymouth, UK.

Asner, G.P., Seastedt, T.R., Townsend, A.R., 1997. The decoupling of terrestrial carbon and nitrogen cycles. BioScience 47, 226–234.

Baldrian, P., Kolařík, M., Štursová, M., Kopecký, J., Valášková, V., Větrovský, T., Žifčáková, L., Šnajdr, J., Rídl, J., Vlček, C., Voříšková, J., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. The ISME Journal 6, 248–258. http://dx.doi.org/10.1038/ismej.2011.95.

Bauer, J., Kirschbaum, M.U.F., Weihermüller, L., Huisman, J.A., Herbst, M., Vereecken, H., 2008. Temperature response of wheat decomposition is more complex than the common approaches of most multi-pool models. Soil Biology and Biochemistry 40, 2780–2786. http://dx.doi.org/10.1016/j.soilbio.2008.07.024.

Bohlen, P.J., Groffman, P.M., Driscoll, C.T., Fahey, T.J., Siccama, T.G., 2001. Plant-soil-microbial interactions in a northern hardwood forest. Ecology 82, 965–978. doi:10.1890/0012-9658(2001)082[0965:PSMIIA]2.0.CO;2.

Brooks, P.D., Grogan, P., Templer, P.H., Groffman, P., Öquist, M.G., Schimel, J., 2011. Carbon and nitrogen cycling in snow-covered environments. Geography Compass 5, 682–699. http://dx.doi.org/10.1111/j.1749-8198.2011.00420.x.

Brooks, P.D., Williams, M.W., Schmidt, S.K., 1998. Inorganic nitrogen and microbial biomass dynamics before and during spring snowmelt. Biogeochemistry 43, 1–15. http://dx.doi.org/10.1023/A:1005947511910.

Brown, P.J., DeGaetano, A.T., 2011. A paradox of cooling winter soil surface temperatures in a warming northeastern United States. Agricultural and Forest Meteorology 151, 947–956. http://dx.doi.org/10.1016/j.agrformet.2011.02.014.

Buchholz, T., Friedland, A.J., Hornig, C.E., Keeton, W.S., Zanchi, G., Nunery, J., 2014. Mineral soil carbon fluxes in forests and implications for carbon balance assessments. GCB Bioenergy 6, 305–311. http://dx.doi.org/10.1111/gcbb.12044.

Campbell, J.L., Socci, A.M., Templer, P.H., 2014. Increased nitrogen leaching following soil freezing is due to decreased root uptake in a northern hardwood forest. Global Change Biology 20, 2663–2673. http://dx.doi.org/10.1111/gcb.12532.

Campbell, Ollinger S.V., Flerchinger, G.N., Wicklein, H., Hayhoe, K., Bailey, A.S., 2010. Past and projected future changes in snowpack and soil frost at the Hubbard Brook Experimental Forest, New Hampshire, USA. Hydrological Processes 24, 2465–2480.

Cleavitt, N.L., Fahey, T.J., Groffman, P.M., Hardy, J.P., Henry, K.S., Driscoll, C.T., 2008. Effects of soil freezing on fine roots in a northern hardwood forest. Canadian Journal of Forest Research 38, 82–91.

Delgado-Baquerizo, M., Maestre, F.T., Gallardo, A., Bowker, M.A., Wallenstein, M.D., Quero, J.L., Ochoa, V., Gozalo, B., García-Gómez, M., Soliveres, S., García-Palacios, P., Berdugo, M., Valencia, E., Escolar, C., Arredondo, T., Barraza-Zepeda, C., Bran, D., Carreira, J.A., Chaieb, M., Conceição, A.A., Derak, M., Eldridge, D.J., Escudero, A., Espinosa, C.I., Gaitán, J., Gatica, M.G., Gómez-González, S., Guzman, E., Gutiérrez, J.R., Florentino, A., Hepper, E., Hernández, R.M., Huber-Sannwald, E., Jankju, M., Liu, J., Mau, R.L., Miriti, M., Monerris, J., Naseri, K., Noumi, Z., Polo, V., Prina, A., Pucheta, E., Ramírez, E., Ramírez-Collantes, D.A., Romão, R., Tighe, M., Torres, D., Torres-Díaz, C., Ungar, E.D., Val, J., Wamiti, W., Wang, D., Zaady, E., 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature 502, 672–676. http://dx.doi.org/10.1038/nature12670.

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Trivedi, P., Osanai, Y., Liu, Y.-R., Hamonts, K., Jeffries, T.C., Singh, B.K., 2016. Carbon content and climate variability drive global soil bacterial diversity patterns. Ecological Monographs 86, 373–390. http://dx.doi.org/10.1002/ecm.1216.

Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biology 18, 1781–1796. http://dx.doi.org/10.1111/j.1365-2486.2012.02665.x.

Durán, J., Morse, J.L., Groffman, P.M., Campbell, J.L., Christenson, L.M., Driscoll, C.T., Fahey, T.J., Fisk, M.C., Likens, G.E., Melillo, J.M., Mitchell, M.J., Templer, P.H., Vadeboncoeur, M.A., 2016. Climate change decreases nitrogen pools and mineralization rates in northern hardwood forests. Ecosphere 7. http://dx.doi.org/10.1002/ecs2.1251.

Durán, J., Morse, J.L., Groffman, P.M., Campbell, J.L., Christenson, L.M., Driscoll, C.T., Fahey, T.J., Fisk, M.C., Mitchell, M.J., Templer, P.H., 2014. Winter climate change affects growing-season soil microbial biomass and activity in northern hardwood forests. Global Change Biology 20, 3568–3577. http://dx.doi.org/10.1111/gcb.12624.

Durán, J., Rodríguez, A., Morse, J.L., Groffman, P.M., 2013. Winter climate change effects on soil C and N cycles in urban grasslands. Global Change Biology 19, 2826–2837. http://dx.doi.org/10.1111/gcb.12238.

Eilers, K.G., Debenport, S., Anderson, S., Fierer, N., 2012. Digging deeper to find unique microbial communities: the strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biology and Biochemistry 50, 58–65. http://dx.doi.org/10.1016/j.soilbio.2012.03.011.

Evans, S.E., Burke, I.C., 2012. Carbon and nitrogen decoupling under an 11-year drought in the shortgrass steppe. Ecosystems 16, 20–33. http://dx.doi.org/10.1007/s10021-012-9593-4.

Fernández-Martínez, M., Vicca, S., Janssens, I.A., Sardans, J., Luyssaert, S., Campioli, M., Chapin Iii, F.S., Ciais, P., Malhi, Y., Obersteiner, M., Papale, D., Piao, S.L., Reichstein, M., Rodà, F., Peñuelas, J., 2014. Nutrient availability as the key regulator of global forest carbon balance. Nature Climate Change 4, 471–476. http://dx.doi.org/10.1038/nclimate2177.

Finzi, A.C., Austin, A.T., Cleland, E.E., Frey, S.D., Houlton, B.Z., Wallenstein, M.D., 2011. Responses and feedbacks of coupled biogeochemical cycles to climate change: examples from terrestrial ecosystems. Frontiers in Ecology and the Environment 9, 61–67. http://dx.doi.org/10.1890/100001.

Fitzhugh, R., Driscoll, C., Groffman, P., Tierney, G., Fahey, T., Hardy, J., 2001. Effects of soil freezing disturbance on soil solution nitrogen, phosphorus, and carbon chemistry in a northern hardwood ecosystem. Biogeochemistry 56, 215–238. http://dx.doi.org/10.1023/A:1013076609950.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320, 889–892. http://dx.doi.org/10.1126/science.1136674.

Groffman, P., Hardy, J., Fashu-Kanu, S., Driscoll, C., Cleavitt, N., Fahey, T., Fisk, M., 2011. Snow depth, soil freezing and nitrogen cycling in a northern hardwood forest landscape. Biogeochemistry 102, 223–238. http://dx.doi.org/10.1007/s10533-010-9436-3.

Groffman, P., Hardy, J., Fisk, M., Fahey, T., Driscoll, C., 2009. Climate variation and soil carbon and nitrogen cycling processes in a northern hardwood forest. Ecosystems 12, 927–943. http://dx.doi.org/10.1007/s10021-009-9268-y.

Groffman, P.M., Rustad, L.E., Templer, P.H., Campbell, J.L., Christenson, L.M., Lany, N.K., Socci, A.M., Vadeboncoeur, M.A., Schaberg, P.G., Wilson, G.F., Driscoll, C.T., Fahey, T.J., Fisk, M.C., Goodale, C.L., Green, M.B., Hamburg, S.P., Johnson, C.E., Mitchell, M.J., Morse, J.L., Pardo, L.H., Rodenhouse, N.L., 2012. Long-term integrated studies show complex and surprising effects of climate change in the northern hardwood forest. BioScience 62, 1056–1066. http://dx.doi.org/10.1525/bio.2012.62.12.7.

Hamburg, S.P., Vadeboncoeur, M.A., Richardson, A.D., Bailey, A.S., 2013. Climate change at the ecosystem scale: a 50-year record in New Hampshire. Climatic Change 116, 457–477. http://dx.doi.org/10.1007/s10584-012-0517-2.

Hardy, J.P., Groffman, P.M., Fitzhugh, R.D., Henry, K.S., Welman, A.T., Demers, J.D., Fahey, T.J., Driscoll, C.T., Tierney, G.L., Nolan, S., 2001. Snow depth manipulation and its influence on soil frost and water dynamics in a northern hardwood forest. Biogeochemistry 56, 151–174. http://dx.doi.org/10.1023/A:1013036803050.

Harrison, R.B., Adams, A.B., Licata, C., Flaming, B., Wagoner, G.L., Carpenter, P., Vance, E.D., 2003. Quantifying deep-soil and coarse-soil fractions. Soil Science Society of America Journal 67, 1602–1606. http://dx.doi.org/10.2136/sssaj2003.1602.

Harrison, R.B., Footen, P.W., Strahm, B.D., 2011. Deep soil horizons: contribution and importance to soil carbon pools and in assessing whole-ecosystem response to management and global change. Forest Science 57, 67–76.

Hayhoe, K., Wake, C.P., Huntington, T.G., Luo, L., Schwartz, M.D., Sheffield, J., Wood, E., Anderson, B., Bradbury, J., DeGaetano, A., Troy, T.J., Wolfe, D., 2007. Past and future changes in climate and hydrological indicators in the US Northeast. Climate Dynamics 28, 381–407. http://dx.doi.org/10.1007/s00382-006-0187-8.

Intergovernmental Panel on Climate Change, 2013. Climate Change 2013-The Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press.

Isobe, K., Ohte, N., 2014. Ecological perspectives on microbes involved in N-cycling. Microbes and Environments/JSME 29, 4–16.

Jacobs, S., Dendoncker, N., Keune, H. (Eds.), 2013. Ecosystem Services. Global Issues, Local Practices. Elsevier, Boston.

Jandl, R., Rodeghiero, M., Martinez, C., Cotrufo, M.F., Bampa, F., van Wesemael, B., Harrison, R.B., Guerrini, I.A., Richter Jr., D., deB Rustad, L., Lorenz, K., Chabbi, A., Miglietta, F., 2014. Current status, uncertainty and future needs in soil organic carbon monitoring. Science of The Total Environment 468–469, 376–383. http://dx.doi.org/10.1016/j.scitotenv.2013.08.026.

Jenkinson, D.S., Powlson, D.S., 1976. The effects of biocidal treatments on metabolism in soil—V: a method for measuring soil biomass. Soil Biology and Biochemistry 8, 209–213. http://dx.doi.org/10.1016/0038-0717(76)90005-5.

Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications 10, 423–436. doi: 10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2.

Johnson, C.E., Driscoll, C.T., Siccama, T.G., Likens, G.E., 2000. Element fluxes and landscape position in a northern hardwood forest watershed ecosystem. Ecosystems 3, 159–184. http://dx.doi.org/10.1007/s100210000017.

Johnson, C.E., Johnson, A.H., Huntington, T.G., Siccama, T.G., 1991. Whole-tree clear-cutting effects on soil horizons and organic-matter pools. Soil Science Society of America Journal 55, 497–502. http://dx.doi.org/10.2136/sssaj1991.03615995005500020034x.

Joseph, G., Henry, H.A.L., 2008. Soil nitrogen leaching losses in response to freeze–thaw cycles and pulsed warming in a temperate old field. Soil Biology and Biochemistry 40, 1947–1953. http://dx.doi.org/10.1016/j.soilbio.2008.04.007.

Lawrence, G.B., Fernandez, I.J., Richter, D.D., Ross, D.S., Hazlett, P.W., Bailey, S.W., Ouimet, R., Warby, R.A.F., Johnson, A.H., Lin, H., Kaste, J.M., Lapenis, A.G., Sullivan, T.J., 2013. Measuring environmental change in forest ecosystems by repeated soil sampling: a north american perspective. Journal of Environmental Quality 42, 623–639. http://dx.doi.org/10.2134/jeq2012.0378.

LeBauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globaly distributed. Ecology 89, 371–379. http://dx.doi.org/10.1890/06-2057.1.

Li, J., Okin, G.S., Alvarez, L., Epstein, H., 2007. Quantitative effects of vegetation cover on wind erosion and soil nutrient loss in a desert grassland of southern New Mexico, USA. Biogeochemistry 85, 317–332. http://dx.doi.org/10.1007/s10533-007-9142-y.

McArdle, B.H., Gaston, K.J., 1995. The temporal variability of densities: back to basics. Oikos 74, 165–171. http://dx.doi.org/10.2307/3545687.

McInnes, K., Weaver, R., 1994. Soil water potential. In: Weaver, R. (Ed.), Methods of Soil Analysis, Part 2—microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, pp. 53–58.

Mobley, M.L., Lajtha, K., Kramer, M.G., Bacon, A.R., Heine, P.R., Richter, D.D., 2015. Surficial gains and subsoil losses of soil carbon and nitrogen during secondary forest development. Global Change Biology 21, 986–996. http://dx.doi.org/10.1111/gcb.12715.

Monson, R.K., Lipson, D.L., Burns, S.P., Turnipseed, A.A., Delany, A.C., Williams, M.W., Schmidt, S.K., 2006. Winter forest soil respiration controlled by climate and microbial community composition. Nature 439, 711–714. http://dx.doi.org/10.1038/nature04555.

Morillas, L., Durán, J., Rodríguez, A., Roales, J., Gallardo, A., Lovett, G.M., Groffman, P.M., 2015. Nitrogen supply modulates the effect of changes in drying-rewetting frequency on soil C and N cycling and greenhouse gas exchange. Global Change Biology 21, 3854–3863. http://dx.doi.org/10.1111/gcb.12956.

Morse, J.L., Durán, J., Groffman, P.M., 2015. Soil denitrification fluxes in a northern hardwood forest: the importance of snowmelt and implications for ecosystem N budgets. Ecosystems 2015 (18), 520–532. http://dx.doi.org/10.1007/s10021-015-9844-2.

Morse, J.L., Werner, S.F., Gillin, C.P., Goodale, C.L., Bailey, S.W., McGuire, K.J., Groffman, P.M., 2014. Searching for biogeochemical hot spots in three dimensions: soil C and N cycling in hydropedologic settings in a northern hardwood forest. Journal of Geophysical Research: Biogeosciences 119, 1596–1607.

Nave, L.E., Gough, C.M., Maurer, K.D., Bohrer, G., Hardiman, B.S., Le Moine, J., Munoz, A.B., Nadelhoffer, K.J., Sparks, J.P., Strahm, B.D., Vogel, C.S., Curtis, P.S., 2011. Disturbance and the resilience of coupled carbon and nitrogen cycling in a north temperate forest. Journal of Geophysical Research: Biogeosciences 116, G04016. http://dx.doi.org/10.1029/2011JG001758.

Nelson, D., Summers, L., 1996. Total carbon, organic carbon and organic matter. In: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3. Chemical Methods. American Society of Agronomy-Soil Science Society of America.

Nielsen, U.N., Ayres, E., Wall, D.H., Bardgett, R.D., 2011. Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity–function relationships. European Journal of Soil Science 62, 105–116. http://dx.doi.org/10.1111/j.1365-2389.2010.01314.x.

Peñuelas, J., Sardans, J., Rivas-ubach, A., Janssens, I.A., 2012. The human-induced imbalance between C, N and P in Earth's life system. Global Change Biology 18, 3–6. http://dx.doi.org/10.1111/j.1365-2486.2011.02568.x.

Porter, E.M., Bowman, W.D., Clark, C.M., Compton, J.E., Pardo, L.H., Soong, J.L., 2013. Interactive effects of anthropogenic nitrogen enrichment and climate change on terrestrial and aquatic biodiversity. Biogeochemistry 114, 93–120. http://dx.doi.org/10.1007/s10533-012-9803-3.

Rasmussen, C., Southard, R.J., Horwath, W.R., 2006. Mineral control of organic carbon mineralization in a range of temperate conifer forest soils. Global Change Biology 12, 834–847. http://dx.doi.org/10.1111/j.1365-2486.2006.01132.x.

Ricard, J., Tobiasson, W., Greatorex, A., 1976. The field-assembled frost gage. U.S.A. CRREL Technical Note. New Hampshire, Hanover.

Rodríguez, A., Curiel Yuste, J., Rey, A., Durán, J., García-Camacho, R., Gallardo, A., Valladares, F., 2016. Holm oak decline triggers changes in herbaceous and microbial communities with implications for ecosystem C and N cycling. Plant and Soil. http://dx.doi.org/10.1007/s11104-016-3118-4.

Rodríguez, A., Lovett, G.M., Weathers, K.C., Arthur, M.A., Templer, P.H., Goodale, C.L., Christenson, L.M., 2014. Lability of C in temperate forest soils: assessing the role of nitrogen addition and tree species composition. Soil Biology and Biochemistry 77, 129–140. http://dx.doi.org/10.1016/j.soilbio.2014.06.025.

Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J.H.C., Gurevitch, J., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126, 543–562.

Schimel, D.S., 2010. Drylands in the Earth system. Science 327, 418–419. http://dx.doi.org/10.1126/science.1184946.

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591–602. http://dx.doi.org/10.1890/03-8002.

Schlesinger, W.H., Bernhardt, E.S., 2012. Biogeochemistry an Analysis of Global Change.

Schwarz, P.A., Fahey, T.J., McCulloch, C.E., 2003. Factors controlling spatial variation of tree species abundance in a forested landscape. Ecology 84, 1862–1878. http://dx.doi.org/10.2307/3450005.

Sorensen, P.O., Templer, P.H., Christenson, L., Duran, J., Fahey, T., Fisk, M.C., Groffman, P.M., Morse, J.L., Finzi, A.C., 2016. Reduced snow cover alters root-microbe interactions and decreases nitrification rates in a northern hardwood forest. Ecology. http://dx.doi.org/10.1002/ecy.1599.

Stark, J.M., Firestone, M.K., 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. Applied and Environmental Microbiology 61, 218–221.

Sternberg, M., Holzapfel, C., Tielborger, K., Sarah, P., Kigel, J., Lavee, H., Fleischer, A., Jeltsch, F., Kochy, M., 2011. The use and misuse of climatic gradients for evaluating climate impact on dryland ecosystems - an example for the solution of conceptual problems. In: Blanco, J.A. (Ed.), Climate Change - Geophysical Foundations and Ecological Effects (InTech).

Stuanes, A.O., de Wit, H.A., Hole, L.R., Kaste, O., Mulder, J., Riise, G., Wright, R.F., 2008. Effect of climate change on flux of N and C: air-land-freshwater-marine links: synthesis. Ambio 37, 2–8.

Tierney, G.L., Fahey, T.J., Groffman, P.M., Hardy, J.P., Fitzhugh, R.D., Driscoll, C.T., 2001. Soil freezing alters fine root dynamics in a northern hardwood forest. Biogeochemistry 56, 175–190. http://dx.doi.org/10.1023/A:1013072519889.

Tierney, G.L., Fahey, T.J., Groffman, P.M., Hardy, J.P., Fitzhugh, R.D., Driscoll, C.T., Yavitt, J.B., 2003. Environmental control of fine root dynamics in a northern hardwood forest. Global Change Biology 9, 670–679. http://dx.doi.org/10.1046/j.1365-2486.2003.00622.x.

Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13, 87–115. http://dx.doi.org/10.1007/BF00002772.

Zhang, B., Chen, S.Y., Zhang, J.F., He, X.Y., Liu, W.J., Zhao, Q., Zhao, L., Tian, C.J., 2015. Depth-related responses of soil microbial communities to experimental warming in an alpine meadow on the Qinghai-Tibet Plateau. European Journal of Soil Science 66, 496–504. http://dx.doi.org/10.1111/ejss.12240.