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Soil Nitrogen Availability Affects Belowground Carbon Allocation and Soil Respiration in Northern Hardwood Forests of New Hampshire

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Abstract

Plant nutrient acquisition in forests requires respiration by roots and mycorrhizae. Belowground carbon allocation and soil respiration should thus reflect plant effort allocated to nutrient uptake, for example in conditions of different nutrient availabilities controlled by site quality or stand history. Soil respiration, belowground C allocation, and fine root biomass were measured in three sites differing in nutrient availability in the northern hardwood forests of the White Mountains of New Hampshire. Annual soil respiration and belowground C allocation measured in two stands at each site were lowest at Jeffers Brook, the site with highest nutrient availability, and higher at Hubbard Brook and Bartlett Experimental Forests. Neither soil respiration nor belowground C allocation differed significantly between mid-aged (31–41 year old) and older stands (>80 year old) within the sites, despite higher fine root (<1 mm) biomass in old stands than mid-aged stands. During the growing season, soil respiration was low where net nitrogen mineralization and net nitrification were high across an extensive sample of thirteen stands and annual belowground C allocation decreased with increasing nitrification across the six intensively studied stands. Available P was not related to soil respiration. The relationships among N availability, belowground C allocation, and soil respiration support the claim that forests allocate more C belowground in ecosystems with low availability of a limiting nutrient.

Key words: calcium; fine root biomass; forest age; litter production; mineralization; nitrification; phosphorus.

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Introduction

Plants deploy their assets to maximize the acquisition of limiting soil resources (Bloom and others 1985; Rastetter and others 2013). In forest ecosystems with low nutrient availability, plants allocate more C belowground to increase nutrient acquisition (Chapin 1991; Phillips and Fahey 2008). One of the key responses to increased soil nutrient

availability is lower C allocation belowground (Haynes and Gower 1995), which allows proportionally higher aboveground production. Belowground C allocation (to roots, rhizosphere, and mycorrhizae) is one of the most important components of forest productivity (Litton and others 2007), but it is difficult to quantify directly. Soil respiration reflects both root and microbial respiration, including the decomposition of above- and belowground litter. Subtracting aboveground litter production from total soil respiration provides an indirect estimate of belowground C allocation, assuming that there is no change in belowground C storage (Raich and Nadelhoffer 1989; Davidson and others 2002).

Most studies of soil respiration in relation to soil nutrient availability have employed fertilization experiments. In forest ecosystems, N addition lowered soil respiration in 75% of more than 200 studies (Janssens and others 2010), even though enhanced productivity could be expected to increase both aboveground litter production and the availability of C for allocation belowground. The possible causes of decreased soil respiration after nutrient addition include reduced fine root biomass (Lee and Jose 2003; Olsson and others 2005; Phillips and Fahey 2007) and the suppression of the decomposition of soil organic matter (Bowden and others 2004), or both. However, it is not clear whether differences in soil respiration in sites with differing native fertility would follow the pattern predicted by nutrient manipulation experiments, and there is only limited evidence in the literature in this regard (Gower and others 1994).

In northern hardwood forests, soil respiration can also vary with stand age (Ryan and others 1997; Tang and others 2008), probably reflecting differences in fine root biomass and aboveground litter production. For example, in a chronosequence of northern hardwoods in the White Mountains in New Hampshire, both litter and root production increased rapidly following forest harvest until canopy closure at about 10 years, and then gradually stabilized (Fahey and others 1998). In a longer northern hardwood chronosequence in the White Mountains, leaf litter production was low in a 13-year old stand compared to those 20–90 years old (Yanai and others 2012) and root biomass was lower in stands about 25 year old compared to those about 65 year old (Yanai and others 2006). Declining nutrient availability during stand development (Vitousek and Farrington 1997) could also contribute to increasing soil respiration with forest age.

The objective of this study was to quantify soil respiration, fine root biomass, and belowground C allocation in northern hardwood stands differing in soil fertility and age. We hypothesized that soil respiration, fine root biomass, and belowground C allocation would be higher in the less fertile sites. We also expected soil respiration, fine root biomass, and belowground C allocation to increase with age from mid-successional to mature forests. Finally, we tested the relative importance of N, P, and Ca availability in explaining variation in soil respiration across stands.

Methods

Study Site

This study was conducted at three sites in the White Mountain National Forest, NH, USA: Bartlett Experimental Forest (BEF: 44° 02–04′N, 71° 16–19′W, 330–570 m elevation), Hubbard Brook Experimental Forest (HBEF: 43° 56′N, 71° 44′W, 500 m elevation), and Jeffers Brook (JB: 44° 02′N, 71° 53′W, 730 m elevation) (Table 1). The soils are predominantly Spodosols developed in glacial drift, but the composition of the till varies. Granite predominates at BEF, whereas till is dominated by a mix of granite and mica schist at HBEF, and amphibolite at JB. Annual precipitation ranges from 1270 to 1400 mm (www.fs.fed.us/ne/durham/4155/bartlett.htm and www.hubbardbrook.org/overview/site_description.htm#Climate). The three sites have inherent differences in soil fertility: JB has the highest and BEF has the lowest N mineralization and Ca availability (Table 2). The BEF site included nine stands (designated C1–C9): three young (19–24 years old; C1–C3), three mid-aged (31–41 years old; C4–C6), and three old (119–126 years old; C7–C9). The HBEF and JB sites each included one mid-aged (35–41 years old) stand and one old (80–98 years old) stand. Four plots were established in each stand making a total of 52 plots in 13 stands. The plots were 20 m × 20 m in the mid-aged stands at HBEF and JB and 30 m × 30 m in all the other stands.

Six stands, one mid-aged and one old stand at each site (Stand C6 and C9 at BEF), were selected for intensive measurements of total soil respiration, fine root biomass, litter production, and soil nutrient availability. The additional seven stands at BEF were included in less frequent measurements of soil respiration, referred to as the ''extensive'' portion of the study.

Soil Respiration

Five soil respiration collars were systematically installed in each plot, avoiding tree boles, boulders,

Table 1. Northern Hardwood Stands Used in this Study at Bartlett (BEF), Hubbard Brook (HBEF), and Jeffers Brook (JB)

Site	Stand	Age	Year cut	Elevation (m)	Aspect	Slope (%)	Basal area (m^2 ha^{-1})	Dominant species based on basal area
BEF	C1	Young	1990	570	Flat to SE	5–20	25.2	*Betula papyrifera, Prunus pensylvanica, Fagus grandifolia*
	C2	Young	1988	340	NE	15–30	23.4	*Acer rubrum, F. grandifolia, B. papyrifera*
	C3	Young	1985	590	NNE	8–20	30.5	*P. pensylvanica, F. grandifolia, A. rubrum*
	C4	Mid-aged	1978	410	NE	20–25	32.9	*B. papyrifera, Populus grandidentata, P. pensylvanica*
	C5	Mid-aged	1976	550	NW	20–30	27.2	*B. papyrifera, P. pensylvanica, A. rubrum*
	C6	**Mid-aged**	**1975**	**460**	**NNW**	**13–20**	**30.1**	***A. rubrum, B. papyrifera, F. grandifolia***
	C7	Old	About 1890	440	ENE	5–10	32.1	*F. grandifolia, A. saccharum, Tsuga canadensis*
	C8	Old	1883	330	NE	5–35	35.2	*F. grandifolia, A. saccharum, B. alleghaniensis*
	C9	**Old**	**1890**	**440**	**NE**	**10–35**	**32.7**	***A. saccharum, F. grandifolia, B. alleghaniensis***
HBEF		**Mid-aged**	**1970**	**500**	**S**	**10–25**	**29.5**	***B. alleghaniensis, B. papyrifera, A. rubrum***
		Old	**1911–1913**	**500**	**S**	**25–35**	**33.9**	***B. alleghaniensis, F. grandifolia, A. saccharum***
JB		**Mid-aged**	**About 1974**	**730**	**WNW**	**25–35**	**27.9**	***B. alleghaniensis, B. papyrifera, A. saccharum***
		Old	**1915–1929**	**730**	**WNW**	**30–40**	**35.7**	***A. saccharum, B. alleghaniensis, F. grandifolia***

The stands in bold type included measurements of annual soil respiration and belowground C allocation and are described as intensively studied.

and big roots. The collars were made from 10-cm slices of 20-cm inside-diameter PVC pipe and were inserted 2–4 cm into the soil.

In the six intensively studied stands, soil respiration was measured every 3–4 weeks during the summer (June–August) and every 4–5 weeks during spring (March–May) and fall (September–November) from June 2009 to November 2010, for a total of 15 dates. Most soil respiration measurements were made between 9:30 a.m. and 2:30 p.m. (10% were made between 2:30 and 4:00 p.m.) using an infrared gas analyzer system (LI-8100 survey system; Li-Cor Biosciences, Lincoln, NE, USA). The rate of soil C efflux was calculated based on the increase in chamber CO_2 concentration over 2 min. If a soil respiration measurement differed by more than 50% from other measurements in the stand, that point was re-measured and the second measurement was used. Hereafter, these data are designated ''measured soil respiration'' to distinguish them from estimated annual soil respiration.

To estimate annual soil respiration in the intensively measured stands, we used daily (24 h average) soil temperature monitored near our study sites. At BEF, continuous soil temperature data were taken near a gas exchange tower by the North American Carbon Program (Richardson, unpublished data). At HBEF, soil temperature was monitored by the Soil Climate Analysis Network (www.wcc.nrcs.usda.gov/nwcc/site?sitenum=2069&state=nh). At JB, continuous soil temperature data were not available. Comparison of static measurements indicated that soil temperature was consistently lower at JB than at BEF; this difference averaged 1.5 C from May through October, with slightly smaller differences in summer than spring and fall (Fahey, unpublished data). For simplicity, we subtracted 1.5 C from the BEF continuous soil temperature values to model soil respiration at JB.

To estimate annual soil respiration, we used a composite of regression and linear interpolation (Aulenbach and Hooper 2006). The residuals from the regression are added to the predictions for the dates of observation, and for the dates in between, a linear interpolation of the residuals is added to the predictions, which forces the estimates through the observations while showing the expected relationship to temperature in the intervals between observations. For this approach, soil respiration was modeled as an exponential function of the soil temperature, ae^{bT} (Van't Hoff 1884), where a and b are coefficients and T is daily soil temperature at 10 cm depth (see Supplementary Table S1). This model was applied to the average of the five collars in each plot for the growing seasons in 2009 (n = 5

Table 2. Nutrient Availability (Plot Mean ± Standard Error) in Oe, Oa, and Mineral (0–10 cm) Horizons at Bartlett (BEF), Hubbard Brook (HBEF), and Jeffers Brook (JB)

Soil horizon	Site	Stand	Age	Soil pH	Forest floor mass (kg m^{-2})	Net N mineralization (μg g^{-1} day^{-1})	Net nitrification (μg g^{-1} day^{-1})	Available P (μg g^{-1})	Exchangeable Ca (μg g^{-1})
A. Forest floor horizons									
Oe	BEF	C1	Young	4.4 ± .06	2.14 ± 0.16	15.96 ± 1.24	−1.83 ± 0.31	15.53 ± 1.34	5744 ± 234
		C2	Young	4.4 ± .13	1.83 ± 0.16	16.83 ± 0.75	−1.72 ± 0.61	11.75 ± 0.34	5152 ± 525
		C3	Young	4.1 ± .05	3.50 ± 0.12	30.69 ± 4.67	0.98 ± 1.04	16.67 ± 3.78	4285 ± 553
		C4	Mid-aged	4.1 ± .02	2.23 ± 0.14	18.08 ± 1.67	−0.91 ± 0.78	17.68 ± 1.93	4480 ± 408
		C5	Mid-aged	4.5 ± .09	5.15 ± 0.14	23.11 ± 3.00	−0.24 ± 0.48	16.55 ± 1.14	5223 ± 517
		C6	**Mid-aged**	**4.2 ± .04**	**1.92 ± 0.26**	**36.90 ± 10.08**	**0.24 ± 1.80**	**14.97 ± 2.66**	**4323 ± 159**
		C7	Old	4.1 ± .05	2.82 ± 0.68	10.16 ± 2.21	−0.87 ± 0.32	17.58 ± 2.12	2839 ± 223
		C8	Old	4.1 ± .11	2.55 ± 0.22	8.91 ± 1.69	−1.64 ± 0.51	21.42 ± 4.65	3582 ± 534
		C9	**Old**	4.1 ± .04	**3.80 ± 0.35**	**17.45 ± 3.21**	**−0.46 ± 1.37**	**14.75 ± 3.47**	**3717 ± 474**
	HBEF		**Mid-aged**	4.3 ± .06	**2.48 ± 0.17**	**21.55 ± 2.56**	**4.79 ± 1.05**	**16.75 ± 3.24**	**3086 ± 90**
			Old	4.1 ± .08	**2.54 ± 0.20**	**17.48 ± 2.70**	**1.73 ± 0.58**	**19.07 ± 3.17**	**3594 ± 190**
	JB		**Mid-aged**	4.7 ± .14	**1.60 ± 0.19**	**18.52 ± 2.30**	**6.95 ± 1.75**	**15.16 ± 2.76**	**5870 ± 584**
			Old	4.4 ± .09	**2.39 ± 0.36**	**23.39 ± 3.17**	**5.55 ± 1.65**	**17.06 ± 3.07**	**4807 ± 867**
Oa	BEF	C1	Young	4.0 ± .08	12.64 ± 0.14	9.92 ± 2.11	0.41 ± 0.14	9.88 ± 0.96	1569 ± 380
		C2	Young	3.8 ± .12	7.69 ± 0.56	6.17 ± 1.53	0.49 ± 0.08	9.03 ± 1.21	1956 ± 283
		C3	Young	4.0 ± .16	13.21 ± 0.23	9.11 ± 1.01	2.72 ± 0.55	10.22 ± 1.82	1149 ± 196
		C4	Mid-aged	3.8 ± .07	8.52 ± 0.90	7.80 ± 0.94	0.35 ± 0.04	13.36 ± 1.97	1526 ± 355
		C5	Mid-aged	4.3 ± .14	9.32 ± 0.98	8.84 ± 0.97	1.26 ± 0.20	9.24 ± 0.95	1322 ± 197
		C6	**Mid-aged**	3.9 ± .05	**11.84 ± 0.71**	**7.89 ± 0.43**	**3.23 ± 0.20**	**13.77 ± 1.15**	**898 ± 83**
		C7	Old	3.9 ± .14	7.01 ± 0.79	4.55 ± 0.48	0.71 ± 0.11	12.75 ± 1.91	737 ± 83
		C8	Old	4.0 ± .08	7.33 ± 0.42	4.10 ± 0.25	0.47 ± 0.03	14.83 ± 1.92	1602 ± 509
		C9	**Old**	4.2 ± .07	**11.09 ± 2.16**	**7.81 ± 1.09**	**0.92 ± 1.28**	**13.09 ± 2.11**	**1176 ± 158**
	HBEF		**Mid-aged**	3.9 ± .04	**8.18 ± 0.62**	**7.38 ± 1.14**	**4.00 ± 0.83**	**11.57 ± 0.72**	**625 ± 149**
			Old	3.7 ± .04	**10.69 ± 0.79**	**8.01 ± 1.21**	**2.88 ± 0.83**	**16.57 ± 0.95**	**899 ± 142**
	JB		**Mid-aged**	4.4 ± .17	**6.48 ± 0.38**	**5.57 ± 0.89**	**4.43 ± 0.57**	**10.13 ± 1.33**	**1661 ± 170**
			Old	4.4 ± .11	**8.08 ± 0.40**	**7.36 ± 0.79**	**5.80 ± 1.13**	**9.44 ± 1.48**	**1495 ± 399**

Table 2. continued

Site	Stand	Age	Soil pH	Soil texture		Net N mineralization ($\mu g\ g^{-1}\ day^{-1}$)	Net nitrification ($\mu g\ g^{-1}\ day^{-1}$)	Available P ($\mu g\ g^{-1}$)	Exchangeable Ca ($\mu g\ g^{-1}$)
				Sand (%)	Clay (%)				
B. Upper mineral soil (0–10 cm)									
BEF	C1	Young	4.6 ± 0.0	67 ± 5	15 ± 2	0.34 ± 0.05	0.17 ± 0.03	2.52 ± 0.21	139 ± 16
	C2	Young	4.5 ± 0.1	64 ± 3	16 ± 2	0.31 ± 0.04	0.16 ± 0.01	1.38 ± 0.17	80 ± 13
	C3	Young	4.5 ± 0.1	66 ± 2	17 ± 3	0.47 ± 0.04	0.38 ± 0.04	2.51 ± 0.28	82 ± 10
	C4	Mid-aged	4.1 ± 0.1	67 ± 2	16 ± 3	0.38 ± 0.08	0.24 ± 0.04	1.70 ± 0.18	56 ± 9
	C5	Mid-aged	4.7 ± 0.0	62 ± 2	18 ± 2	0.42 ± 0.07	0.30 ± 0.04	1.83 ± 0.17	118 ± 18
	C6	**Mid-aged**	**4.5** ± 0.0	**52 ± 3**	**15 ± 1**	**0.49 ± 0.04**	**0.36 ± 0.02**	**1.53 ± 0.23**	**65 ± 6**
	C7	Old	5.2 ± 0.1	57 ± 4	16 ± 3	0.36 ± 0.03	0.18 ± 0.02	2.65 ± 0.27	76 ± 8
	C8	Old	4.7 ± 0.1	48 ± 7	16 ± 4	0.27 ± 0.04	0.19 ± 0.03	1.80 ± 0.16	88 ± 28
	C9	**Old**	**5.6 ± 0.0**	**57 ± 2**	**15 ± 1**	**0.38 ± 0.05**	**0.31 ± 0.03**	**2.37 ± 1.03**	**67 ± 3**
HBEF		**Mid-aged**	**3.8 ± 0.1**	**59 ± 3**	**18 ± 1**	**0.59 ± 0.05**	**0.57 ± 0.05**	**3.94 ± 0.08**	**185 ± 102**
		Old	**3.8 ± 0.0**	**53 ± 3**	**22 ± 2**	**0.54 ± 0.06**	**0.45 ± 0.07**	**2.46 ± 0.23**	**84 ± 14**
JB		**Mid-aged**	**4.9 ± 0.1**	**55 ± 1**	**15 ± 1**	**0.58 ± 0.09**	**0.66 ± 0.07**	**2.46 ± 0.24**	**312 ± 28**
		Old	**4.9 ± 0.1**	**56 ± 3**	**15 ± 2**	**0.71 ± 0.06**	**0.71 ± 0.04**	**3.73 ± 0.32**	**258 ± 84**

A. Forest floor horizons, B. Upper mineral soil (0–10 cm). The stands in bold type included measurements of annual soil respiration and belowground C allocation and are described as intensively studied. Different superscript letters are significantly different from one another at $\alpha = 0.05$.

dates) and 2010 ($n = 6$ dates) and the dormant season of 2009–2010 ($n = 5$–7 dates), because the relationships differed significantly by year ($p = 0.03$) and season ($p < 0.01$). Each plot was modeled separately to give four independent estimates of annual respiration for each stand. Finally, we evaluated the sensitivity of annual soil respiration to the lower soil temperatures at JB by estimating annual respiration at JB using actual soil temperature values from BEF.

The composite method gave similar results to estimating annual respiration from the mean measured soil respiration in each of three seasons (winter, growing, and spring and fall seasons) and from linear interpolation between all pairs of measurements. The three methods varied by at most 4% (up to 28 g C m^{-2} y^{-1}) of estimated annual respiration (Bae 2013). The area occupied by rocks and tree root crowns was measured in each plot using a line intercept method (Bae 2013) and used to scale respiration measurements per unit area to the plot scale.

Respiration was measured in seven additional stands during summer 2010, and we used all thirteen stands to explore the relationship between soil respiration and nutrient availability. Soil respiration was measured twice (C3 and C5), three times (C4 and C8), or four times (C1, C2, C7) between June and August. To test for a bias in respiration due to the dates of sampling in the stands that were measured only twice, we compared respiration for the mean of the two dates at which all stands were measured to the mean of all three or four sampling dates for 2010, using a paired t test on the other 11 stands ($p = 0.63$). The mean of all respiration measurements taken in summer 2010 was used in the comparisons; the results did not differ substantially from those based on only two dates for all the stands or based on only 11 stands for all the dates.

Fine Root Biomass

Root biomass was measured in the six intensive stands. Ten 5-cm diameter soil cores were taken to 30 cm depth (measured from the top of the Oe horizon) in each plot at HBEF and JB in July 2008 and at BEF in August 2010. Root samples were frozen until they could be processed. Soil samples were thawed and roots less than 5 mm diameter (divided visually into 0–1 and 1–5 mm classes) were manually separated from soil, cleaned with tap water, oven-dried at 60°C, and weighed. Dead roots and herbaceous roots were excluded, distinguished by their color, brittleness, and resiliency.

Litter Production

Leaf litterfall was collected in five baskets measuring 0.23 m^2 in each plot in 11 stands (not BEF C3 and C5). We collected litter in November, May, and August, beginning in August 2009 to August 2011. Woody litter production was not included in this study; leaf litter constituted greater than 80% of total litter production at HBEF (Fahey and others 2005a). Carbon was assumed to be 50% of the leaf litter mass.

Soil Properties

Soil samples were collected in late June, 2009, to evaluate soil pH, texture, and fertility in all 13 stands (Table 2). Approximately 30 soil cores (2 cm diameter) were collected in each plot and separated into Oe, Oa, and the upper 10 cm of the mineral soil; the latter included a varying mixture of E and B horizon soil as is typical in spodosols. Cores were pooled to give one composite sample per depth increment per plot. We concentrated on these depths because they effectively discriminate site differences in soil fertility and because biological activity is concentrated there; for example, fine roots are highly concentrated in surface soils in these forests: at BEF over half of the less than 1 mm roots in the entire soil profile are found at these depths (Park and others 2007). Composite samples were thoroughly mixed, coarse fragments (>2 mm) were removed by hand and samples were stored for up to 1 week at 4 C prior to analysis.

Soil pH in the forest floor horizons and upper mineral soil was measured electrometrically using 10 g of air-dried soil in a 2:1 mixture of water: soil (Robertson and others 1999). Texture of mineral soil was quantified using the hydrometer method (Sheldrick and Wang 1993).

Net N mineralization was estimated from laboratory incubations in sealed Mason jars for 21 days at 20°C. Subsamples were extracted before and after incubation by shaking in 40 mL of 2 M KCl for 1 h, waiting for 18 h, and filtering through Whatman #1 filter paper. Concentrations of NH_4^+ and NO_3^- in extracts were measured on a Lachat flow-injection analyzer (Lachat Instruments, Loveland, CO) using a phenolate-hypochlorite method (351.2, US EPA 1983) and a cadmium reduction method (353.2, US EPA 1983), respectively. Net N mineralization was calculated as the difference in $NH_4^+ + NO_3^-$ between the initial and final extracts and net nitrification was calculated as the difference in NO_3^- between initial and final extracts.

Resin-extractable P was measured by shaking soil subsamples for 18 h in 100 mL of distilled water with nylon mesh bags containing bicarbonate-form anion exchange resins (JT Baker Anion Exchange Resin, 325 NA-38, OH-Form, Type I, 16–50 Mesh). The bags were washed in distilled water to remove soil particles and resin-extractable P was recovered from ion-exchange resins by shaking bags for 1 h in 100 mL 0.5 M HCl. Inorganic P in HCl extracts was analyzed using the ammonium molybdate-ascorbic acid method (Murphy and Riley 1962) and quantifying absorbance at 660 nm on a BioTek plate reader (Winooski, VT).

Soil extractable Ca was determined by shaking 10-g soil samples for 30 min with 100 mL of 1 M NH_4Cl. After waiting 18 h, soil samples were shaken again for 45 min and the extract was filtered through Whatman #1 paper. The Ca concentration was analyzed using a Varian Spectra Atomic Absorption Spectrometer (Mulgrave, Victoria, Australia) with a 10,000 ppm (1%) lanthanum chloride solution.

Statistical Analysis

We used a randomized block design with four replicate plots blocked by stand. The number of stands depended on the analysis. There were six intensive stands in which all variables were measured. In all 13 stands, respiration was measured at least during the growing season (see above), and we measured soil nutrient availability in the Oe, Oa, and 0–10 cm mineral soil layers. In the intensive stands, we analyzed measured soil respiration as a function of stand age (mid-aged and old) and site (BEF, HBEF, and JB) using repeated-measures analysis of variance (ANOVA) in SAS (SAS Inc 2003). We also used ANOVA to analyze the effects of stand age and site on estimated annual soil respiration, litter production, fine, and coarse root biomass (0–1, 1–5 mm, respectively), belowground C allocation, and soil nutrient availability in the Oe, Oa, and 0–10 cm mineral soil horizons, using the GLM procedure in Minitab v.10.

In the intensive plus the extensive stands (13 stands for respiration and nutrient availability and 11 stands for litter production), Pearson's product moment correlations based on individual plot values blocked by stand were calculated between observed soil respiration rate in summer (mean of June to August 2010) and leaf litter production and soil nutrient availability, using SAS (SAS Inc 2003). In the six intensively studied stands where annual soil respiration was estimated, correlations were calculated between belowground C allocation and fine root biomass and soil nutrient availability. Belowground C allocation was estimated as the difference between C flux in annual soil respiration and annual litterfall; note that this approach overestimates belowground C allocation in our study because we did not include woody litter in our litterfall sampling.

Results

Soil Respiration, Fine Root Biomass, and Belowground C Allocation in the Intensively Studied Stands

Measured soil respiration was lower at JB than at BEF or HBEF ($p < 0.01$; Figure 1), consistent with expectations based on generally higher fertility at JB (Table 2). Mid-aged stands at BEF and JB had significantly lower measured soil respiration than old stands ($p < 0.05$), but the difference with stand age was not significant at HBEF ($p = 0.28$).

Measured soil respiration increased with temperature; soil temperature (24 h average) measured at HBEF and at BEF explained 72–97% of the variation in soil respiration measurements within plots over time. However, soil temperature did not explain differences between years (2009 and 2010) or sites (BEF and HBEF) in annual soil respiration. The average soil temperature was 0.7°C higher in 2010 than in 2009 at both BEF and HBEF, but annual soil respiration in 2009 was 35–58% higher than in 2010 (Figure 1). Soil moisture also was similar in the 2 years.

Fine root biomass (<1 mm) for 0–30 cm depth was significantly higher at BEF than at JB ($p < 0.01$), but coarse root biomass (1–5 mm) did not differ among sites ($p = 0.21$) (Table 3). Fine root biomass differed by stand age ($p < 0.01$) with about one-third higher fine root biomass in old stands than mid-aged stands (Table 3). The mean annual soil respiration per unit fine root biomass in the mid-aged stands was 10–49% higher than in the old stands. In contrast, leaf litter production did not differ significantly with stand age ($p = 0.20$) or site ($p = 0.11$; Table 3).

Estimated annual soil respiration, interpolated between measurement dates using the composite method, differed among sites ($p < 0.01$), being lower at JB than at BEF or HBEF. Some of this difference can be attributed to the lower soil temperatures at JB as estimated annual respiration was 2% higher for JB when interpolated using the BEF soil temperatures. This temperature effect is relatively small compared with the 13.7–21% differences in annual soil respiration between JB and the

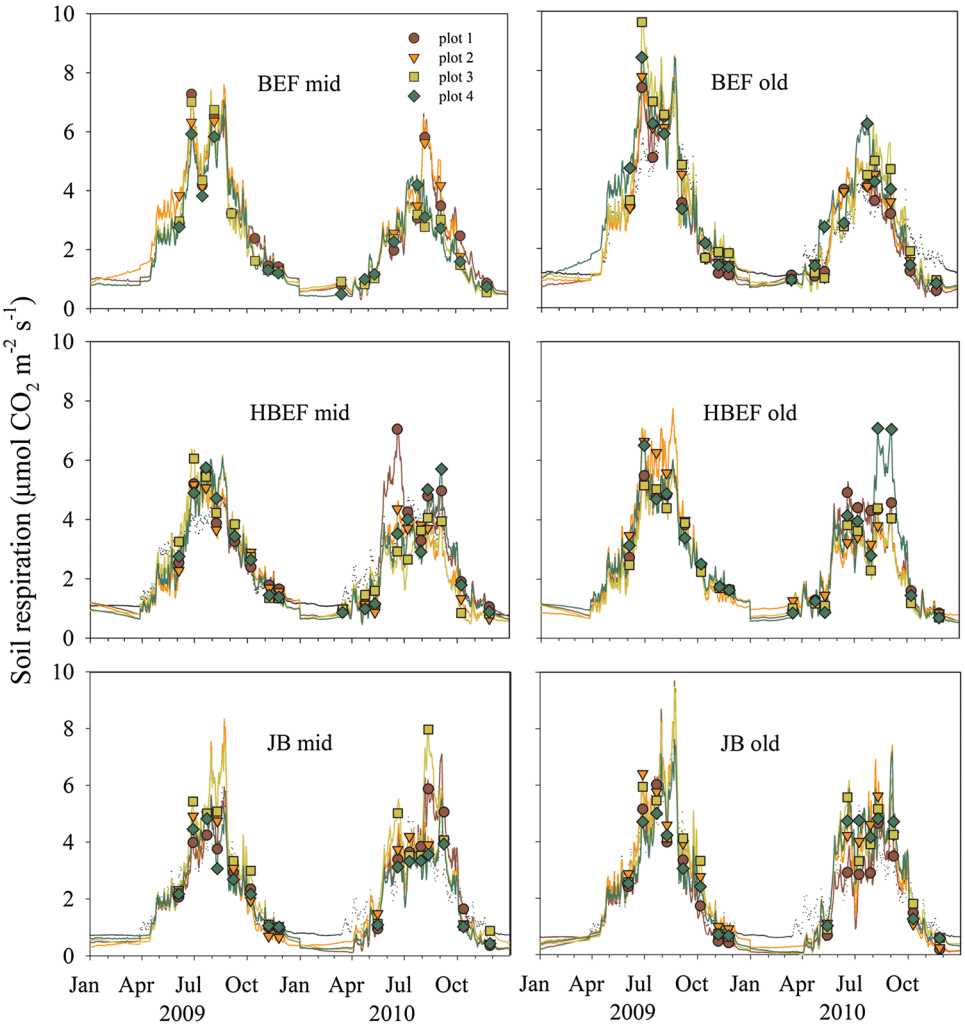

Figure 1. Soil respiration measured in four plots in mid-aged and old stands at Bartlett (BEF), Hubbard Brook (HBEF), and Jeffers Brook (JB). Respiration between measurement dates was estimated based on measured soil temperature from nearby locations and regression relationships between temperature and measured respiration in each plot.

Table 3. Fine Root Biomass (Plot Mean ± Standard Error), Leaf Litter Production, Average Annual Soil Respiration, and Belowground C Allocation at Mid-Aged and Old in Intensively Studied Stands at Bartlett (C6 and C9, BEF), Hubbard Brook (HBEF), and Jeffers Brook (JB)

Forest age	Site	Fine root biomass (g m^{-2})		Annual Leaf litterfall (gC m^{-2})	Average annual soil respiration (gC m^{-2})	Belowground C allocation (gC m^{-2})
		0–1 mm	0–5 mm			
Mid-aged	BEF (C6)	361 ± 24	563 ± 37	170 ± 9	790 ± 36	620 ± 36
	HBEF	299 ± 43	451 ± 34	197 ± 5	790 ± 23	593 ± 16
	JB	227 ± 36	443 ± 28	136 ± 26	670 ± 32	534 ± 28
Old	BEF (C9)	519 ± 57	732 ± 65	183 ± 5	864 ± 19	681 ± 18
	HBEF	411 ± 29	783 ± 53	178 ± 9	812 ± 26	634 ± 16
	JB	381 ± 16	706 ± 22	141 ± 4	708 ± 41	567 ± 38

BEF and HB stands. Estimated annual soil respiration in mid-aged stands was lower than that in old stands by 9% at BEF, 3% at HBEF, and 6% at JB ($p = 0.10$).

Belowground C allocation was calculated as the difference between estimated annual soil respiration minus annual aboveground leaf litter production, assuming no change in belowground C storage. Like estimated annual soil respiration, belowground C allocation varied more by site ($p = 0.01$) than by stand age ($p = 0.07$), being lower at JB than at BEF or HBEF (Table 3). Annual soil respiration and belowground C allocation showed similar patterns, because litterfall was more consistent than soil respiration

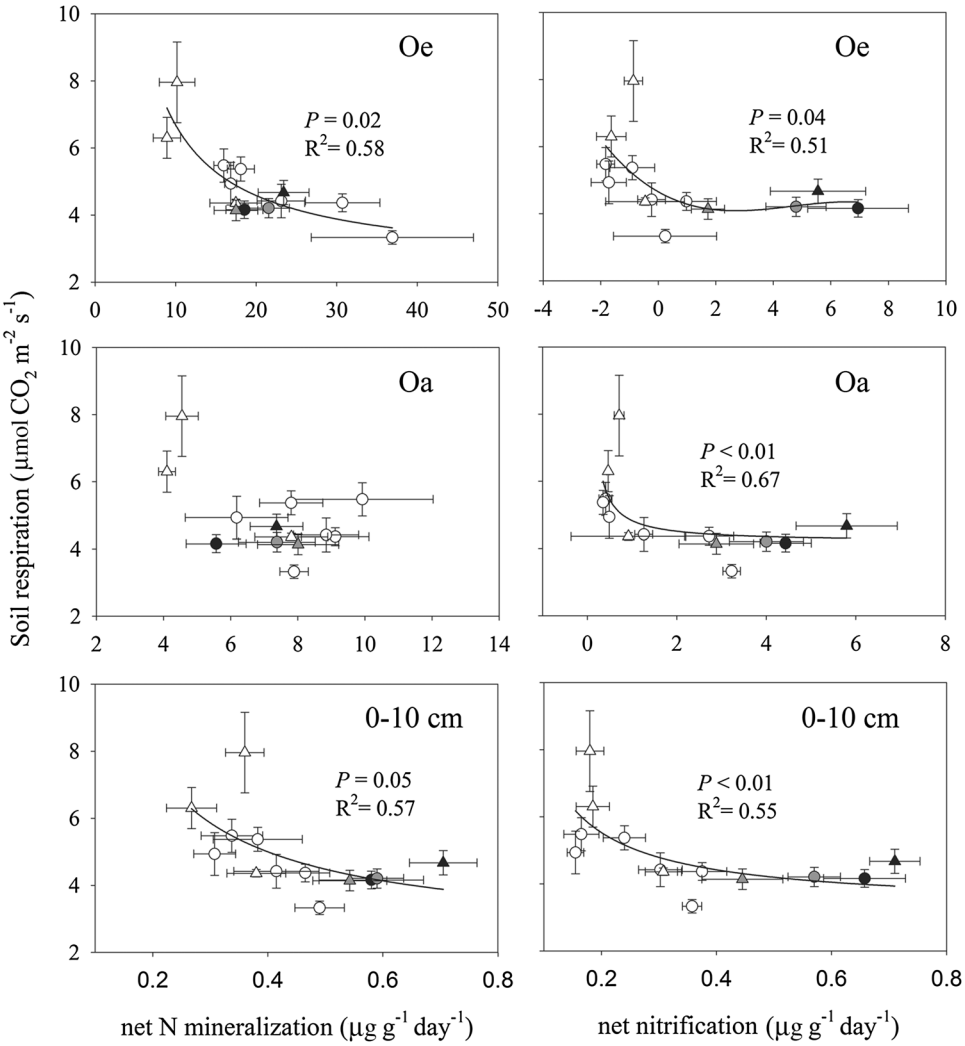

Figure 2. Relationship between soil respiration in summer (mean of the 11 dates between Jun and Aug in 2009 and 2010) and N availability in young and mid-aged (*circles*) and old (*triangles*) stands at Bartlett (*white*), Hubbard Brook (*gray*), and Jeffers Brook (*black*). The same respiration values are graphed in all the panels; the *x* axes show N mineralization or nitrification in the Oe, Oa, and 0–10 cm depth in the mineral soil. Points represent the mean and bars the standard error of the mean (n = 4 plots per stand), and log regression lines show relationships significant at $\alpha = 0.05$.

across stands and made a relatively small contribution (20–22% of annual soil respiration) to the estimates of belowground C allocation. Belowground C allocation was positively related to fine root biomass ($p < 0.01$), but not to coarse root biomass ($p = 0.18$). As noted in Methods, belowground C allocation is underestimated in Table 3 because woody litter was not included; assuming woody litter is about 20% of total litter these values would underestimate belowground C flux by about 6%.

Soil Properties

Mineral soil pH varied across stands from 3.8 to 5.6 (Table 2). Soils were mostly sandy loams with sand contents from 48 to 67% and clay contents from 14 to 22% (Table 2). Soil nutrient availability differed significantly among sites. In the mineral soil, net N mineralization, nitrification, and exchangeable Ca were highest at JB and lowest at BEF ($p < 0.01$) (Table 2). In the forest floor horizons (Oe and Oa), nitrification and exchangeable Ca were highest at JB ($p < 0.01$) (Table 2).

Across the 13 stands, soil respiration during summer was negatively related to net N mineralization in Oe and 0–10 cm mineral soil and to nitrification in Oe, Oa, and 0–10 cm mineral soil ($p < 0.05$) (Figure 2). In contrast, soil respiration was not related to available P ($p = 0.34$) or to exchangeable Ca ($p = 0.19$). In the six intensively studied stands, belowground C allocation was negatively related to net nitrification in forest floor and mineral soil and to Ca availability in mineral soil (Figure 3), but it was not significantly related to N mineralization. Net N mineralization, net nitrification, available P, or exchangeable Ca did not show strong correlations with fine root biomass or leaf litter production.

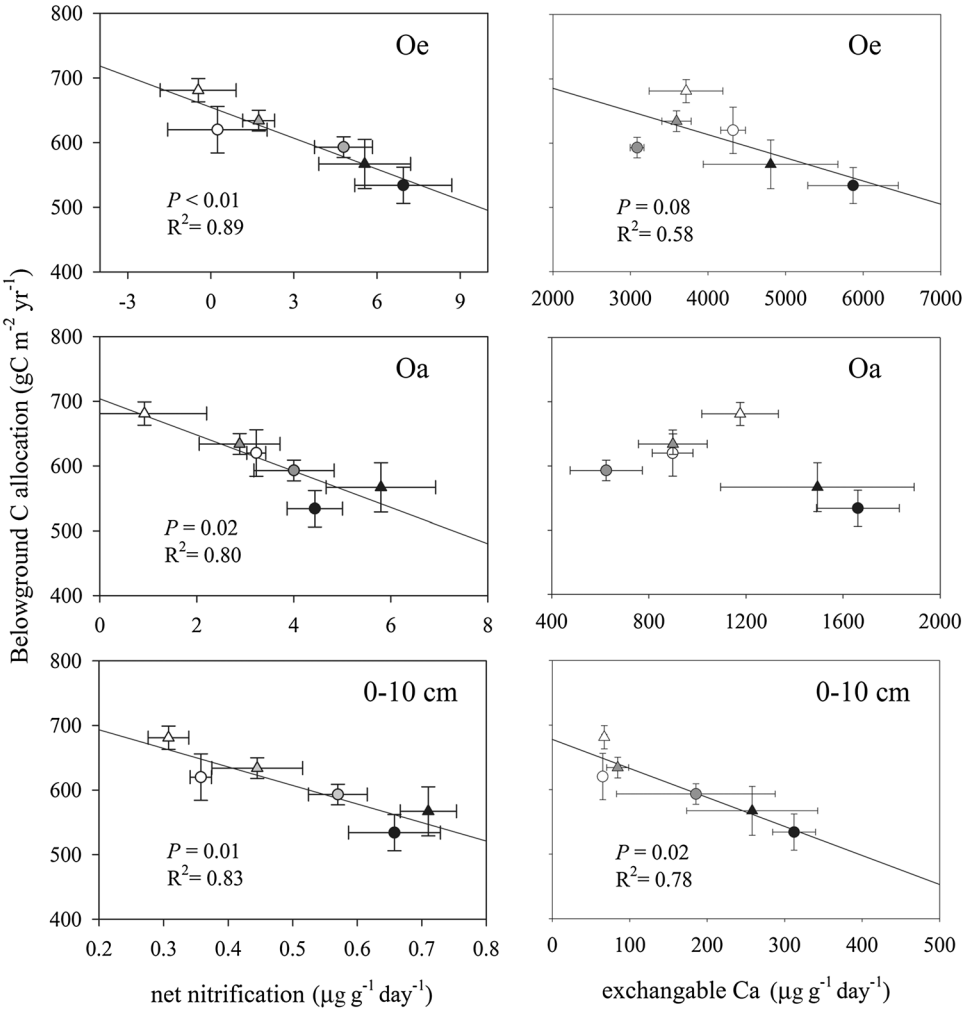

Figure 3. Relationship between belowground C allocation and N and Ca availability in the six intensive stands in mid-aged (*circle*) and old (*triangle*) stands at Bartlett (*white*), Hubbard Brook (*gray*), and Jeffers Brook (*black*). The same belowground C allocation values are graphed in all the panels; the *x* axes show net nitrification or exchangeable Ca in the Oe, Oa, and 0–10 cm depth in the mineral soil. Points represent the mean and bars the standard error of the mean ($n = 4$ plots per stand) and linear regression lines show relationships significant at $\alpha = 0.05$.

Discussion

Soil Respiration, Belowground C Allocation, and Soil Properties

We observed that both soil respiration and belowground C allocation decreased with increasing soil N availability across a set of northern hardwood forests in New Hampshire (Figures. 2, 3), patterns that superseded any stand age effects. The negative relationship between soil respiration and net N mineralization (Figure 2) supports the general hypothesis that allocation belowground declines with increasing fertility. We did not detect a relationship between belowground C allocation and N mineralization, probably because of the smaller number of stands for this estimate. However, the highly significant relationship between belowground C allocation and nitrification, an important process indicating differences in N economy across stands, also supports the idea that belowground allocation is lower with more rapid N recycling. Lower soil respiration under high N conditions is common in temperate forests and can be attributed to lower heterotrophic respiration or root-associated respiration (Janssens and others 2010). Our results provide evidence that reductions in belowground carbon allocation resulting from nutrient additions (Janssens and others 2010) also apply to spatial variations in soil fertility across northern hardwood forests, as previously noted for pine forest ecosystems (Gower and others 1994).

Lower soil respiration in fertile soil is consistent with theories of plant resource allocation (Bloom and others 1985), if N is limiting forest productivity in the northeastern US (Finzi 2009; Vadeboncoeur 2010). Because root respiration and associated microbial activity are two major sources of soil respiration, the low fine root (<1 mm) biomass at high N availability is not surprising. However, it is difficult to estimate how much the low fine root biomass contributes to the low soil respiration in fertile soil without measuring root production and

mortality. The relationship between fine root dynamics and N availability can be complex and many factors could contribute to this finding (Nave and others 2014). Like this study, decreased fine root biomass after fertilization has been reported in several studies (Olsson and others 2005; Phillips and Fahey 2007; Burton and others 2011). However, decreased fine root biomass may be offset by increased root tissue N concentration leading to higher respiration per unit mass of root (Burton and others 2002). Further study is needed to clarify these complex belowground mechanisms.

The relationship between soil respiration and N availability was similar for different indices of N availability (net N mineralization and nitrification), whereas for belowground C allocation strong relationships were only observed for nitrification. Thus, it is possible that the negative relationship between soil respiration and N availability resulted from known suppressive effects of N on decomposition (Janssens and others 2010). Moreover, the relationships between N availability and soil respiration varied among soil layers, especially in organic horizons (Figure 2). The development and properties of surface organic horizons in these forests vary markedly for reasons that are not well understood (Lützow and others 2006), and this source of variation certainly contributes to differences in the measurements of N availability and respiratory activity. Because a high proportion of soil respiration in northern hardwood forests has been attributed to forest floor organic horizons (for example, 58% by Fahey and others 2005b), separating soil respiration of organic and mineral soils would help to understand the relationship between soil respiration and soil nutrient availability.

There was no significant relationship between soil respiration and P or Ca availability across sites. Some declines in forest health and productivity have been attributed to acidic deposition, because decreased soil pH causes lower P and Ca availability (Paré and Bernier 1989; Likens and others 1998). If P or Ca were limiting in the stands we studied, then one might expect to see greater investment belowground, and hence greater soil respiration, in stands with low P or Ca. Instead, we found lower soil respiration where N availability was high, suggesting that C is allocated belowground in response to the demand for N more than P or Ca. Across a much larger (30 fold) Ca availability gradient, Park and others (2008) observed that root production was higher where Ca was high. In the present study, availability of N and Ca were significantly correlated (R^2 = 0.53 for N mineralization and Ca availability; R^2 = 0.67 for nitrification and Ca availability in mineral soil), so it is difficult to distinguish the effect on belowground C allocation of soil Ca from that of soil N.

The assumptions underlying our estimates of belowground C allocation require some justification. Estimating belowground C allocation as the difference between annual soil respiration flux and leaf litter C flux depends on the assumption that the C contents of the forest floor, mineral soil, and living and dead roots are at steady state (Raich and Nadelhoffer 1989). Measuring changes in these pools is difficult because of high spatial variability and imperfect sampling methods (Yanai and others 1999; Yanai and others 2003; Ryzhova and Podvezennaya 2008). The uncertainty in change over time of forest floor C at Hubbard Brook has been estimated at 83 g C m^{-2} y^{-1}, and uncertainty in measurements of mineral soil C stocks are much larger (Yanai and others 2012). The differences observed in belowground C allocation among sites are probably not primarily due to differences in C accumulation rates in these pools. Changes in forest floor C content over 15 years in several young and mid-aged northern hardwood stands in and around BEF were up to around 30 g C m^{-2} y^{-1} (Yanai and others 1999) which is relatively small compared to the 59–114 g C m^{-2} y^{-1} difference we observed in belowground C allocation between JB and the other sites. Nevertheless, some of the between-stand differences in belowground C allocation in this study might be attributed to deviations from the assumption of constancy in the belowground C pools.

Soil Respiration and Fine Root Biomass Across Stand Age

Estimated annual soil respiration (670–864 g C m^{-2} y^{-1}) across the six intensive stands was similar to or slightly higher than nearby hardwood forests at Hubbard Brook (80 years old; 541–801 g C m^{-2} y^{-1}) (Fahey and others 2005b) and at Harvard Forest (60–100 years old; 530–850 g C m^{-2} y^{-1}) (Davidson and others 1998). In this study, annual soil respiration did not differ significantly between mid-aged and old stands even though fine root biomass was about 50% higher in the older stands (Table 3). Fine roots usually account for nearly half of the total soil respiration (Hanson and others 2000), so the lack of a difference in soil respiration between forest ages was surprising given the difference in fine root biomass. Other chronosequence studies have noted coincident changes in soil respiration and fine root biomass with forest development (Wiseman and Seiler 2004; Saiz and others 2006), although the proportion of root and

heterotrophic respiration also can change with stand development (Ewel and others 1987; Gough and others 2007) Our observations suggest higher C flux per unit root biomass in mid-aged than older forests. For example, in this study, mean annual soil respiration rate per unit fine root biomass was 10–49% higher in mid-aged than in old stands. Perhaps higher specific root respiration rates, root turnover, or rhizosphere C flux in mid-aged than in old stands can explain this result (Giardina and Ryan 2002).

In conclusion, our observations of soil respiration and belowground C allocation in northern hardwood forests of differing site fertility suggest that responses observed in experimental fertilization studies also apply to natural variation in nutrient availability: soil respiration and belowground C allocation tend to decline with increasing N availability. Studies to evaluate how soil respiration and belowground C allocation respond to experimental nutrient additions would further inform predictions of N deposition effects on belowground C dynamics on sites with differing inherent fertility.

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