Biogeochemistry
DOI 10.1007/s10533-015-0172-6

Response of forest soil respiration to nutrient addition depends on site fertility

Hongzhang Kang · Timothy J. Fahey · Kikang Bae · Melany Fisk · Ruth E. Sherman · Ruth D. Yanai · Craig R. See

Received: 9 June 2015 / Accepted: 8 December 2015

Abstract Flux of CO_2 from the forest soil surface (F_{CO_2}) reflects the activity of roots and microbes responding to plant and soil properties that are influenced by global changes such as nitrogen deposition and increasing temperature and atmospheric CO_2. We added low levels of N (3 g/m^2-year), P (1 g/m^2-year) or N + P to thirteen northern hardwood stands of different age and soil N cycling and measured soil respiration, microbial respiration and fine root turnover. We hypothesized that soil respiration would decline in response to nutrient addition, but that this response would vary depending on forest age and N cycling rate. Soil respiration was significantly higher in successional (<40-year-old) than mature stands (>90-year-old). Overall, no significant treatment effects or age x treatment interactions were observed. However, on an individual stand basis, significantly lower soil respiration was observed in nutrient addition plots at four of the most infertile sites. Over half of the variation in the response ratio (fertilized-control/control) of soil respiration to fertilization was explained by using pre-treatment N cycling rate as a predictor: i.e., the greatest reduction in soil respiration on N and N + P fertilized plots occurred on the sites with lowest pre-treatment soil N mineralization and litterfall N flux. Nutrient additions did not significantly affect either fine root turnover (minirhizotrons) or microbial respiration (laboratory

Responsible Editor: Jack Brookshire.

H. Kang
School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China
e-mail: kanghz@sjtu.edu.cn

T. J. Fahey (✉) · R. E. Sherman
Department of Natural Resources, Cornell University, G16 Fernow Hall, Ithaca 14853-3001, NY, USA
e-mail: tjf5@cornell.edu

R. E. Sherman
e-mail: res6@cornell.edu

K. Bae · R. D. Yanai · C. R. See
Department of Forest and Natural Resources Management, SUNY College of Environmental Science and Forestry, Syracuse, NY 13210, USA
e-mail: kbae02@gmail.com

R. D. Yanai
e-mail: rdyanai@syr.edu

C. R. See
e-mail: craigrsee@gmail.com

K. Bae
International Cooperation Division, International Affairs Bureau, Korea Forest Service, Daejeon 302-701, Republic of Korea

M. Fisk
Department of Biology, Miami University, Oxford, OH 45056, USA
e-mail: fiskmc@miamioh.edu

Published online: 28 December 2015

incubations). Perhaps responses of fine root biomass or rhizosphere C flux influenced the response of soil respiration to increasing soil fertility.

Keywords Fine root · Microbial · Nitrogen · Northern hardwood · Phosphorus

Introduction

Human activity has profoundly altered the biogeochemistry of N by greatly increasing the amount of reactive forms of N deposited to global ecosystems. The consequences of anthropogenic N deposition for the productivity and health of natural ecosystems are complex, depending on the ecosystem type, its nutrient status and the levels and duration of N deposition. For chronically N-limited forests low-level N addition would be expected to stimulate NPP whereas high N addition could result in detrimental effects associated with N saturation (Aber et al. 2003). Productivity of many temperate zone forests is limited by N availability, and the provision of extra N might switch forests to limitation by another mineral nutrient, often phosphorus (Crowley et al. 2012). The implications of widespread N deposition for global change feedbacks, including the global C cycle, remain highly uncertain (Thomas et al. 2013) in part because of variable ecosystem responses owing to these and other factors such as moisture limitation.

Soil respiration (soil CO_2 efflux, F_{CO_2}) is among the largest flux pathways in the global C cycle (ca. 75 Pg C/year; Schlesinger and Andrews 2000), exceeded only by ocean uptake and fixation by terrestrial vegetation. Hence, a better understanding of the controls of F_{CO_2} is essential for predicting the response of the global C cycle to global change drivers like N deposition. Soil respiration includes two distinct components, respiration directly associated with plant roots ("root-associated" respiration) which includes respiration by root tissues as well as mycorrhizal fungi and other rhizosphere organisms; and heterotrophic respiration mostly by microbial decomposers, bacteria and saprotrophic fungi that utilize dead organic matter. Although direct measurement of F_{CO_2} is straightforward, distinguishing the contributions of these components of the respiration flux process is difficult (Hanson et al. 2000), and their responses to global change drivers may differ (Yan et al. 2010).

Many field studies of F_{CO_2} response to nutrient additions have been conducted in terrestrial ecosystems. The response of F_{CO_2} to fertilization of forest ecosystems is variable with increases (Brumme and Beese 1992; Cleveland and Townsend 2006; Gallardo and Schlesinger 1994), decreases (Bowden et al. 2004; Burton et al. 2004; Lee and Jose 2003) and no change (Allison et al. 2008; Lee and Jose 2003) reported in different experiments. These variable responses can be attributed to several factors including forest composition (Lee and Jose 2003), the identity, rate and duration of nutrient addition (Bowden et al. 2004; Mo et al. 2008); forest composition and age (Lee and Jose 2003); and inherent site fertility. For example, in a meta-analysis Janssens et al. (2010) reported that low-level nitrogen additions (<5 g/m^2-year) usually result in lower F_{CO_2} whereas at higher fertilization levels the responses are less predictable. Low-level N additions more accurately simulate the effects of chronic atmospheric N deposition in polluted regions which is typically less than 2 g N/m^2-year (Galloway1998). In general, N addition can impede microbial decomposition of plant detritus (Hobbie 2008; Janssens et al. 2010; Knorr et al. 2005) by mechanisms often attributed to the production and activity of lignin-degrading soil enzymes (Carreiro et al. 2000). Based on their meta-analysis, Janssens et al. (2010) concluded that this effect on the C cycle could be similar in magnitude on a global basis to the stimulation of NPP by N addition. Root-associated respiration also may be suppressed by nutrient addition (Mo et al. 2008; Olsson et al. 2005; Phillips and Fahey 2007) as fertilization of nutrient-limited forests would be expected to result in reduced proportional C allocation to root processes (Litton et al. 2007). However, if total NPP is stimulated by fertilization, then absolute root C allocation could actually increase (Nadelhoffer 2000). These considerations also suggest that the response of F_{CO_2} to nutrient addition might vary depending upon forest site fertility. On low fertility sites where a higher proportion of total NPP is allocated belowground (Bae et al. 2015), trees would be expected to respond to nutrient additions by decreasing proportional allocation to roots, thereby reducing F_{CO_2}; whereas, on high fertility sites higher total NPP might result in higher F_{CO_2}.

An additional consideration regarding nutrient addition effects on F_{CO_2} is the identity of the limiting nutrient(s). Vadeboncoeur (2010) presented evidence

that NPP of many temperate deciduous forests is co-limited by multiple nutrients, especially N and P. Stand age class also might be expected to influence F_{CO_2} response to nutrient additions. For example, Rastetter et al. (2013) predicted that early successional northern hardwood forests would be more limited by N whereas mature forests would be limited by P or colimited by N and P together.

Based on these considerations we established a long-term nutrient addition experiment in northern hardwood forest ecosystems in central New Hampshire, USA. We added relatively low-levels of N (3 gN/m^2-year), P (1 gP/m^2-year) or N + P in a factorial design to thirteen northern hardwood forest stands of differing age class and soils for 3 years and measured the response of F_{CO_2} in the field and microbial respiration potential in the laboratory. We also evaluated the response of fine root turnover in seven of these stands using minirhizotrons. We hypothesized that low-level N additions would suppress F_{CO_2} in these stands either by reducing microbial respiration or root associated-respiration, or both. We expected the greatest F_{CO2} suppression in young stands and stands on low N status soils, whereas a higher proportion of NPP is allocated belowground (Bae et al. 2015). We also anticipated interactive effects of N and P addition on F_{CO_2}, reflecting possible colimitation by these essential nutrients.

Methods

Study sites

The study was conducted at three sites in the White Mountain National Forest, NH, USA: Bartlett Experimental Forest (BEF; 44°2–4′N, 71°9–19′W; elevation 250–500 m), Hubbard Brook Experimental Forest (HBEF; 43°56′N, 71°44′W; elevation 500 m) and Jeffers Brook Forest (JBF; 44°02′N, 71°53′W; elevation 730 m). The climate of the study area is cool-temperate, humid continental with mean July and January temperatures of 19 and −9 °C, respectively (at 450 m elevation). Mean annual precipitation is about 140 cm, evenly distributed throughout the year and varying only slightly across the study sites. The soils are mostly Spodosols (Typic and Aquic Haplorthods) derived from glacial till; however, the till composition varies among the three study sites: at BEF granite and gneiss predominate; at HBEF, mica schist and quartz monzonite; and at JBF, amphibolite. These differences were expected to influence the inherent soil fertility across the study sites (Bae et al. 2015). Soils at all the sites are loamy sands overlain by an organic horizon of variable thickness (4–10 cm).

Atmospheric N deposition in this region has been moderately high since the mid-twentieth century, averaging about 8 kg N ha^{-1} $year^{-1}$ (wet + dry) at Hubbard Brook (Aber et al. 1997); recent declines in the nitrate component of N deposition have been observed (Du et al. 2014). Nevertheless, strong ecosystem N retention is observed in the second-growth northern hardwood forests (Yanai et al. 2013), suggesting N limitation of NPP.

All the forest stands used for the present experiment have been subject to human disturbance in the form of logging, but they were never cleared for agriculture. Five of the stands (three at BEF, one each at HBEF and JBF) are old forests (>80 years) dominated by sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh) and yellow birch (*Betula alleghenien-sis* Britt.). These stands have a history of logging in the late nineteenth and early twentieth century but have been disturbed only by natural events (e.g., 1938 hurricane) since that time. Eight of the stands (six at BEF, one each at HBEF and JBF) are younger forests that developed after clearcutting between 1966 and 1990 (Table 1). These successional forests are dominated by a variable mixture of maple-beech-yellow birch plus early successional species, paper birch (*Betula papyrifera* Marsh.), pin cherry (*Prunus pennsylvanica* L.f.), red maple (*Acer rubrum* L.) and aspen (*Populus grandidentata* Michaux).

Experimental design

In each forest stand four 50 × 50 m experimental plots were established. The four plots in each stand were positioned in the rugged topography with the objective of achieving as similar as possible forest composition, topographic position and soils. A 30 × 30 m measurement area was surveyed in the center of each 50 × 50 m treatment plot (i.e. 10 m wide treated buffer for each plot); all measurements reported herein were conducted in the measurement area. The four plots in each stand were assigned randomly to the different treatments: control, N addition, P addition, N + P addition, with the stipulation that control plots were not located directly downslope of fertilized plots.

Table 1 Site characteristics of thirteen northern hardwood forest stands in central New Hampshire, USA used in the present study

Stand	Forest age	Year cut	Elevation (m)	Soil N mineralization ($\mu g\ g^{-1}$ soil d^{-1})	Litterfall N flux (g N m^{-2} $year^{-1}$)	Basal area (m^2 ha^{-1})	Dominant species
Bartlett, C1[a]	Successional	1990	570	0.3	2.82	25.2	*Betula papyrifera, Prunus pensylvanica, Fagus grandifolia*
Bartlett, C2[a]	Successional	1988	340	0.31	2.35	23.4	*Acer rubrum, F. grandifolia, B. papyrifera*
Bartlett, C3	Successional	1985	590	0.46	na	30.5	*P. pensylvanica, F. grandifolia, A. rubrum*
Bartlett, C4	Successional	1979	410	0.38	3.23	32.9	*B. papyrifera, Populus grandidentata, P. pensylvanica*
Bartlett, C5[a]	Successional	1976	550	0.45	na	27.2	*B. papyrifera, P. pensylvanica, A. rubrum*
Bartlett, C6[a]	Successional	1975	460	0.49	2.90	30.1	*A. rubrum, B. papyrifera, F. grandifolia*
Bartlett, C7	Mature	1890	440	0.36	na	32.1	*F. grandifolia, A. saccharum, Tsuga canadensis*
Bartlett, C8[a]	Mature	1883	330	0.27	2.40	35.2	*F. grandifolia, A. saccharum, B. alleghaniensis*
Bartlett, C9[a]	Mature	1890	440	0.38	3.07	32.7	*A. saccharum, F. grandifolia, B. alleghaniensis*
Hubbard Brook	Successional	1970	500	0.58	4.29	29.5	*B. alleghaniensis, B. papyrifera, A. rubrum*
Hubbard Brook	Mature	1911–1913	500	0.71	3.60	33.9	*B. alleghaniensis, F. grandifolia, A. saccharum*
Jeffers Brook[a]	Successional	1974	730	0.59	2.84	27.9	*B. alleghaniensis, B. papyrifera, A. saccharum*
Jeffers Brook[a]	Mature	1915–1929	730	0.54	2.48	35.7	*A. saccharum, B. alleghaniensis, F. grandifolia*

na not available

[a] Stands with minirhizotron measurements

Treatments were initiated in May 2011. Fertilizer was applied uniformly by hand to each treatment plot in mid-May and early July of 2011–2013. The N-treated plots received 30 kg N/ha year in the form of dry NH_4NO_3 and the P-treated plots received 10 kg P/ha year as dry NaH_2PO_4; the N + P plots received both nutrients. Control plots were subjected to the trampling associated with fertilization of treatment plots, but no fertilizer was applied.

Pre-treatment N and P cycling rate

Two indices of pre-treatment soil N and P cycling were measured: potential net N mineralization, resin extractable P and leaf litterfall N and P flux. In the last week of June 2009 multiple soil cores ($\sim$30.2-cm cores) were collected from each plot to a depth of 10 cm in the mineral soil and Oe, Oa, and mineral soil horizons were separated. Cores were pooled by horizon into one composite sample per plot. Each sample was

homogenized and inorganic N was extracted from an initial subsample. A second subsample was incubated in the laboratory for 21 days at approximately 20 °C and then inorganic N was extracted. Subsamples were shaken in 2 M KCl (approximately 1:5 soil mass:extractant volume) for 1 h and extracts were filtered through Whatman #1 paper after approximately 18 h settling time. We used a phenolate–hypochlorite method to quantify NH_4^+ (method 351.2, US EPA 1983) and a cadmium reduction method to quantify NO_3^- (method 353.2, US EPA 1983) in extracts. Potential net N mineralization was estimated as the difference in KCl-extractable NH_4^+ plus NO_3^- between initial and incubated soil subsamples. Available inorganic P was measured by shaking subsamples in deionized water with anion exchange resin, then extracting resin-based P in 0.5 mol/L HCl.

Leaf litterfall N and P flux was measured in autumn 2010 in ten of the stands (not Bartlett C3, C5, C7) following procedures detailed by See et al. (2015). Fresh leaf litter for N and P analysis was collected periodically during rain-free intervals in mesh traps in each plot and pooled by species. Leaf litter N concentration was determined using a CN elemental analyzer (EA1112 elemental analyzer; Thermo Electric Corp., Waltham, MA, USA) and P by dry-ashing and ICP-OES (See et al. 2015). Five litter baskets (0.23 m^2 each) were positioned systematically in each plot to estimate litter mass. Litter collected from August to November was sorted by species, dried at 50 °C and weighed. Leaf litter N flux was calculated as the sum across species of the product of N concentration and litter mass.

Soil respiration

Flux of CO_2 from the soil (F_{CO_2}) was measured in each plot using a LI-8100 F_{CO2} system (Licor Biosciences, Lincoln, NE). Five PVC collars (20 cm diameter) were installed in each plot prior to measurements that began in May 2010. Soil respiration was measured between 9 AM and 4 PM in each plot during the growing season, May–September. In 2010–2012 four to six measurements were made during the growing season in all the plots except BEF stand C3 and C5, which were measured only twice. In 2013, all the plots were measured on five dates between May and September.

Root turnover

Minirhizotron measurements were conducted in a subset of seven stands (four successional forests, three mature; Table 1). Five minirhizotron access tubes were installed at a 30° angle to a depth of 30 cm plot in fall 2010. Images were collected from each tube at monthly intervals during the growing season in 2011–2013. Images were digitized manually along the surface of each tube and recorded by depth interval (0–10 cm, 10–20 cm, 20–30 cm). For each image and date, we recorded the number of root tips, the number of those tips that were newly grown, and the number of previously recorded root tips that grew (elongated) or died (disappeared) since the previous measurement. We estimated the annual relative turnover of roots (turnover index, $year^{-1}$) as the ratio of the sum of the number of new root tips and roots that grew (i.e. elongated) divided by the average number of root tips observed for each year. Although this root turnover index does not provide a quantitative measurement of root turnover rate, it does indicate treatment effects on root growth and turnover (Cleavitt et al. 2008).

Microbial respiration

The effect of fertilization on microbial respiration was evaluated using laboratory incubations in July 2012 after two years of treatment. Sixteen soil cores (5 cm diameter) were collected in each plot, separated into O_e and O_a horizons based on visual criteria, and pooled within plots. Samples were refrigerated at 2–4 °C and processed for respiration measurements within 1 week. Pooled samples were sorted by hand to remove most roots and coarse fragments and thoroughly mixed. Subsamples equivalent to dry mass of 3–6 g (O_e), 5–10 g (O_a) were incubated in Mason jars at room temperature (approximately 18 °C). Microbial respiration was estimated by quantifying CO_2 evolution in the sealed jars using a NaOH-trap method. In this method glass vials containing 10 mL of 0.1 M NaOH were sealed inside the jars to absorb CO_2 over a 24 h incubation. The vials were replaced for each of two additional 24 h incubations for a total of 72 h. Each vial was titrated with 0.1 M HCl in the presence of 0.3 mL of 2.5 M BaCl to quantify CO_2.

Analysis

To evaluate overall treatment effects on F_{CO_2} we utilized a repeated-measures ANOVA using Proc Mixed in SAS (SAS Inc., 2005) and data from 2011, 2012 and 2013. Mean summer respiration values by plot for each year were used in the analysis. Year, treatment and forest age class (successional vs. mature) were fixed variables, the experimental unit and repeated measure was the treatment plot, and the mean of the five collars in each plot was the sample unit. We also evaluated differences among plots on an individual stand basis using RM ANOVA; we acknowledge that this analysis does not allow attribution to treatments which are not replicated at the stand level. For all analyses a log transformation of F_{CO2} values was applied. Treatment effects in laboratory respiration incubations were tested using a mixed effects model with treatment and forest age class as fixed effects, forest stand as a random effect and stands nested within age class (SAS Proc Mixed). Post-hoc separation of means between treatments was conducted using Tukey differences of least square means.

We applied a general linear model to evaluate the effect of soil N cycling rate (measured pre-treatment) on the response of F_{CO_2} to the fertilization treatment. For this purpose response ratios (fertilized-control/control) were calculated for each treatment, using the overall mean growing season F_{CO_2} in each stand and year. Soil N cycling indices included potential net nitrogen mineralization and litterfall N flux.

Finally, we applied a general linear model to evaluate the response of fine root turnover index to the treatments. Treatment, forest age class and soil depth increment were fixed effects. The turnover index was estimated as the average number of root tips divided by the number of new tips plus roots that elongated summed across six monthly sampling dates, May–October 2013; average values for the five tubes in each were used in the ANOVA.

Results

Soil N cycling rate

Prior to treatment, soil N cycling differed markedly among the forest stands. For example, potential N mineralization in the upper 10 cm of the mineral soil and leaf litterfall N flux exhibited nearly a three-fold and two-fold range, respectively across the sites (Table 1), as detailed by Fisk et al. (2014). In general, N recycling rate was higher at the JBF and HBEF stands than at BEF, but there was no consistent effect of stand age class on fertility (Table 1).

Field F_{CO_2}

The effects of nutrient treatments on F_{CO_2} were not consistent across all stands (Table 2). Mature stands had significantly higher F_{CO_2} than the successional stands ($p < 0.0001$). Stand age did not explain patterns in treatment response, as there was not a statistically significant interaction of age × treatment. The lack of overall treatment effects reflected the fact that F_{CO_2} apparently was lowered by fertilization in some stands but increased by fertilization in others.

Analyzed on a stand-by-stand basis, in the first (2011) year of treatment, there were no significant differences among plots in F_{CO_2} in any of the individual stands. However, in the second and third year of treatment, nutrient additions significantly reduced F_{CO_2} in two of the successional stands (BEF C1 and C3) and in two mature stands (BEF C7 and C9; Table 3) among the thirteen stands studied. The opposite pattern, higher F_{CO2} in the treated plots, was observed in some of the stands, but none of these differences was statistically significant. These statistical models at the stand level allow evaluation of differences among plots but not the effects of treatment which was not replicated within stands.

Table 2 Repeated measures analysis of variance of F_{CO2} during the growing seasons of 2011, 2012 and 2013 in treated (N, P and N + P) and control plots of thirteen forest stands of two ages (successional and mature)

Effect	Degrees of freedom	F value	Pr (>F)
Year	2, 85	8.99	<0.001
Trmt	3, 47	0.90	0.45
Age	1, 47	7.74	0.007
Trmt × year	6, 95	0.38	0.89
Age × year	2, 85	1.83	0.17
Age × Trmt× year	9, 247	0.62	0.78

Table 3 Repeated measures analysis of variance of F_{CO2} in treated (N, P, N + P) and control plots in four individual stands and 2 years (2012, 2013)

	Degrees of freedom	F value	Pr(F)
Site C1			
Treatment	3	10.29	<0.001
Year	1	2.53	0.131
Treatment × year	3	0.16	0.921
Site C3			
Treatment	3	3.28	0.049
Year	1	15.16	0.001
Treatment × year	3	0.49	0.691
Site C7			
Treatment	3	10.47	<0.001
Year	1	9.54	0.007
Treatment × year	3	1.67	0.213
Site C9			
Treatment	3	4.40	0.019
Year	1	34.71	<0.001
Treatment × year	3	0.94	0.443

Error degrees of freedom = 16

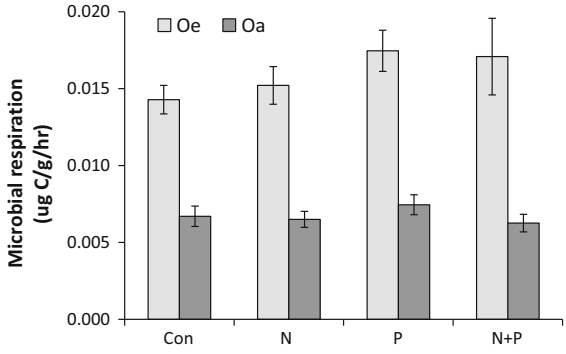

Fig. 1 Microbial respiration in laboratory incubations of forest floor horizons (Oe, Oa) in treated and control plots of thirteen northern hardwood stands in the 2nd year of treatment (2012). *Error bars* indicate standard errors based on 13 stands

Microbial respiration

Laboratory incubations of organic horizons in summer 2012 (2nd year of treatment) suggested a positive effect of P addition on microbial respiration rate (Fig. 1). Respiration rate in O_e horizon soils collected from the P-treated plots exceeded that in controls by an average of 22 % ($F = 3.75$, $p = 0.06$). There were no effects of N, N + P, or forest age class on microbial respiration rate in Oe-horizon soils. No treatment effect on microbial respiration was detected in the O_a horizon. Respiration in the O_a horizon was higher in mature than successional forests ($F = 10.29$, $p = 0.01$; data not shown).

Fine root turnover

Fine root turnover was measured in 2013 for seven of the stands (four successional, three mature) using the minirhizotron method. The root turnover index (Fig. 2) was marginally higher in the successional than the mature stands ($p = 0.06$). However, there was no significant effect of nutrient addition on fine root turnover index ($p = 0.95$, Table 4).

TSR treatment response versus soil N cycling

Prior to treatment (2010) no relationship was observed across the 13 stands between soil N mineralization and the response ratio of F_{CO_2} ([fertilized- control]/control) for any of the planned treatments ($r^2 = 0.01$, $p = 0.99$). In the first year of treatment (2011) there also was no statistically significant relationship between the pre-treatment response ratio and soil N mineralization. However, responses of F_{CO_2} to nutrient addition were observed in the second and third year of treatment. Using the average response ratio for the second and third year of treatment for each stand and treatment, we observed that two indices of soil N cycling could explain variation in the response ratio: N mineralization in the mineral soil (Fig. 3a) and litterfall N flux (Fig. 3b). In general, the response ratio of F_{CO_2} to the nutrient additions increased with site N cycling; that is, F_{CO_2} decreased on N-poor sites in response to nutrient addition whereas it marginally increased on more N-rich sites. The slope of the correlations between the response ratio and soil N cycling were nearly identical for the N and N + P plots (Fig. 2). The response ratio in the P addition plots was not correlated with litterfall N flux but it was positively correlated with soil N mineralization ($p = 0.04$). Pre-treatment measurements of P availability (resin extracts, litterfall P) did not provide significant predictors of F_{CO_2} response to P or N additions (data not shown).

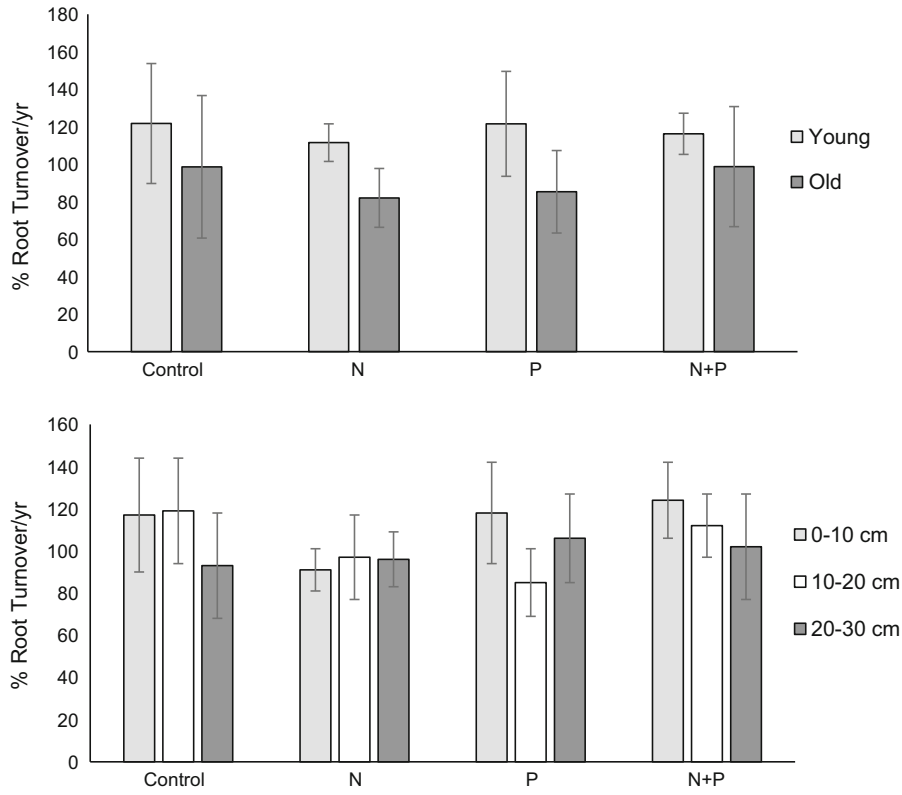

Fig. 2 Fine root turnover index (year 2013) in control and nutrient addition plots as a function of **a** Stand age, and **b** soil depth interval. *Error bars* indicate standard errors

Table 4 Analysis of variance of fine root turnover index during the third year of treatment (2013) in seven northern hardwood forest stands (see Table 1) in New Hampshire, USA treated with N, P or N + P

Source	Degrees of freedom	Type III SS	Mean square	F value	Pr > F
AGE	1	11,146	11,146	3.63	0.061
TRMT	3	2256	752	0.24	0.865
DEPTH	2	3509	1754	0.57	0.568
AGE × TRMT	3	822	274	0.09	0.966
AGE × DEPTH	2	1439	719	0.23	0.792
TRMT × DEPTH	6	8608	1435	0.47	0.83
Error	66	202,811	3073		

Discussion

Our observation of the effects of low-level nutrient addition on F_{CO_2} of northern hardwood forests supported our overall hypothesis: nitrogen addition resulted in lower F_{CO_2} in the more N poor sites whereas in the more N rich stands F_{CO_2} either did not differ or was marginally higher in the nutrient addition plots (Fig. 3). No evidence of an effect of forest age class on this relationship was observed. The nutrient additions in the present study were low (N = 3 g/m^2 year; P = 1 g/m^2 year), which would minimize artifacts associated with large, abrupt changes in nutrient availability that can confound interpretation of high-level fertilization treatments (Magill et al. 2004). Despite the low levels of addition, resin-available nutrients (N and P) were significantly elevated in the treatment plots by the 2nd year of fertilization (Fisk et al. 2014) indicating that our treatments were successful at exceeding the chemical immobilization capacity of these soils and the short-term bio logical demand of microbes and plants. Notably, the

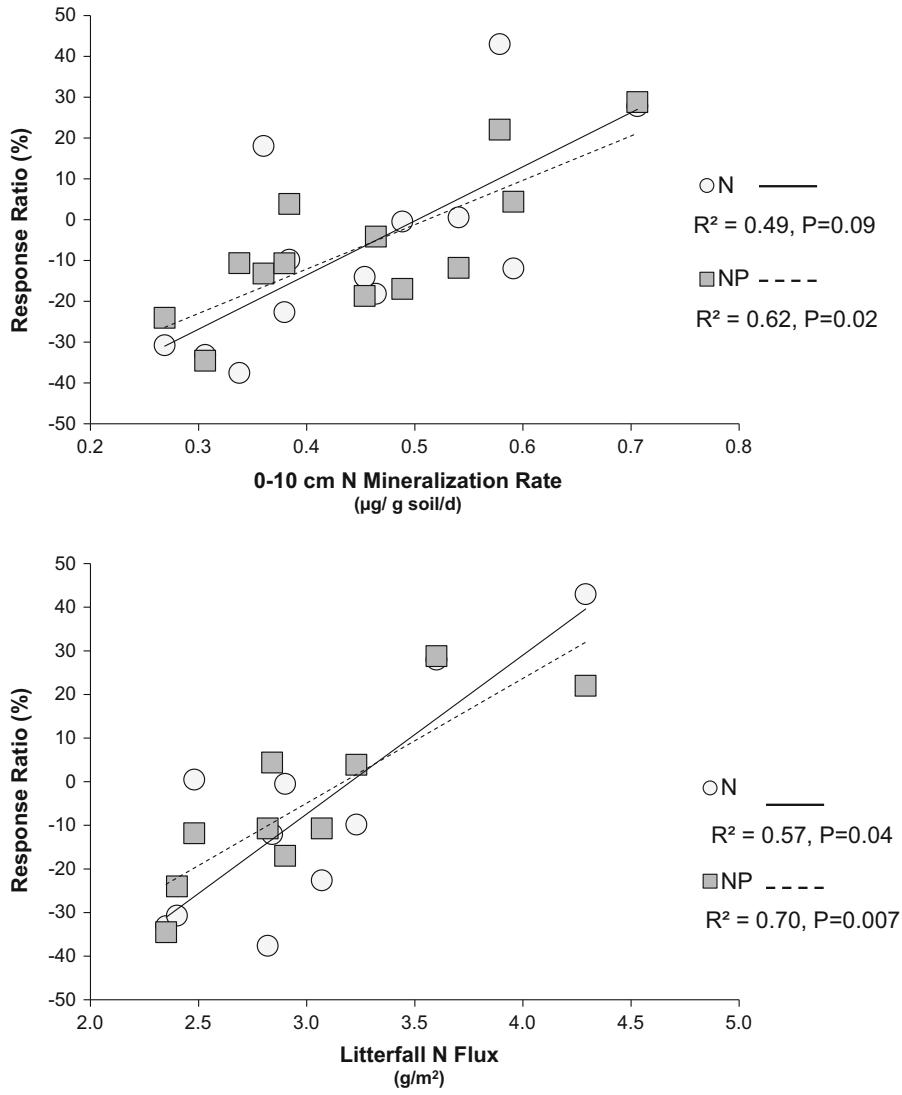

Fig. 3 Response ratio of growing-season (May–September) soil respiration (i.e. (fertilized-control)/control) based on average values for the 2nd and 3rd year of treatment as predicted by pre-treatment N cycling rate. n = 13 stands. Site nitrogen cycling: **a** Soil N mineralization rate, **b** litter N flux

development of treatment responses of F_{CO2} to nutrient additions was gradual, with no effects observed in the first year of treatment. A similar delay in F_{CO_2} response to fertilization has been reported previously in northern hardwood forests receiving higher levels of nutrient addition (Phillips and Fahey 2007).

Soil respiration is a multi-faceted process that includes both heterotrophic and autotrophic components, and either or both of these components could decrease in response to addition of limiting nutrients (Lee and Jose 2003; Olsson et al. 2005; Yan et al. 2010). The observed response of F_{CO_2} to nutrient addition might be attributed to any of the following mechanisms: (1) changes in heterotrophic activity in litter decay or soil organic matter processing (Hobbie 2008); (2) changes in root respiration either because of changes in root growth, root biomass or metabolism (Ryan et al. 1996); or (3) changes in rhizosphere C flux including allocation to mycorrhizal fungi and consequent respiratory responses (Phillips and Fahey 2007). Although we cannot conclusively assign the F_{CO2} responses in the present study to these mechanisms, in light of literature some of our observations are suggestive, as discussed below.

Numerous N fertilization studies have demonstrated that elevated N can suppress microbial respiration. Microbial decay of plant detritus can be

suppressed by high N availability (Janssens et al. 2010; Knorr et al. 2005), although the mechanisms explaining this effect remain controversial and the effect varies among sites and litter types (Hobbie 2008). Adding N may shift substrate use by microorganisms from less to more labile C sources (Craine et al. 2007; Hagedorn et al. 2012; Ramirez et al. 2010), and it can also suppress microbial respiration by increasing the efficiency of microbial assimilation of C (Schimel and Weintraub 2003). Thus, it seems likely that N suppression of microbial activity could have contributed to reduced F_{CO_2} in some of the N and N + P plots. Laboratory assays of microbial respiration in O_e and O_a horizons from our plots in 2014 suggested slight suppression (ca. 10 %) in N addition treatments (M. Fisk, unpublished data), although assays of microbial respiration in 2012 indicated no clear N addition effects (Fig. 1). Increased microbial respiration in incubated soils from P addition plots in most of our stands (Fig. 1) is consistent with a number of earlier studies (Bradford et al. 2008; Cleveland and Townsend 2006; Craine et al. 2007). Thus, some of the variation in the response of F_{CO_2} to N and P addition could be associated with differential effects of N and P addition on microbial respiration.

In theory, the alleviation of chronic soil resource limitation by fertilization would be expected to result in decreased C allocation to root systems (Bloom et al. 1985; Haynes and Gower 1995). Decreased root-associated respiration in fertilized plots could result from reduced fine root biomass, root turnover, specific root respiration rate or rhizosphere C flux. Several notable examples of decreased fine root biomass in fertilized forests have been reported (Helmisaari and Hallbäcken 1999; Maier and Kress 2000; Olsson et al. 2005). We have not yet tested for changes in fine root biomass, because high spatial variability prevents the detection of small changes. We saw no evidence that fine root turnover was affected by nutrient addition in our stands (Fig. 2), but we caution that high variability limited our ability to detect change. Previous research indicates that N addition can either lower (Burton et al. 2000) or increase (Aber et al. 1985) root turnover in northern hardwood forests. Decreased fine root turnover would be expected to result in decreased root respiration, because of lower growth and maintenance respiration rates of older roots (Veen 1981). Specific root respiration typically increases with tissue N concentration (Ryan et al. 1996; Burton et al. 2002); thus nutrient additions would not be expected to cause decreases. Finally, decreased rhizosphere C flux in nutrient addition plots is possible, as Phillips and Fahey (2007) observed suppressed rhizosphere microbial activity in fertilized northern hardwood forest. Similarly, decreased C allocation to mycorrhizal fungi would be expected in response to N and P addition (Treseder 2004). In sum, although it is likely that responses of both heterogrophic and root-associated respiration contributed to treatment effects, additional process-level research is needed to clarify the mechanisms contributing to the observed response of F_{CO_2} to N and P additions in this study.

Our experimental design incorporated different nutrient addition treatments (N, P, N + P) in northern hardwoods stands of different age class (successional and mature) across an anticipated soil fertility gradient. Differences in mineralogy of soil parent materials across our study area are reflected in leaf litter P concentrations (See et al. 2015), but differences in P availability were not as important as those in N cycling rate as indicated by litter N flux and soil N mineralization potential (Table 1). The causes of these parent material effects on soil N pools and fluxes are not known. We observed significantly higher F_{CO2} in mature than successional stands (Table 2), reflecting higher pre-treatment fine root biomass (Bae et al. 2015). However, we did not observe stand age class effects on the response of F_{CO2} to nutrient additions. Rastetter et al. (2013) hypothesized greater N limitation in successional than mature northern hardwood forests, but the between-site differences in N availability apparently superseded any age class effects. An influence of tree species composition on F_{CO2} responses also is possible. Although all the stands were composed of northern hardwoods the relative importance of different species varied (Table 1), and growth responses of northern hardwood tree species to nutrient additions may differ (Fahey et al. 1998; Vadeboncoeur 2010).

We anticipated interactive effects of N and P addition on F_{CO2} in part because of evidence for colimitation of NPP by these nutrients in northeastern deciduous forests (Vadeboncouer 2010). Evidence for true synergistic effects (Harpole et al. 2011) was not observed as the response of F_{CO_2} to N and N + P treatments was similar across the N availability

gradient (Fig. 3). Moreover, the independent effect of P addition on the F_{CO_2} response was marginally correlated with soil N mineralization but not with litter N flux. A more complex synergism including heterotrophic activity also is possible. For example, in the first year of treatment of the present study, Fisk et al. (2014) observed that N + P addition increased the availability of N much more than N addition alone. The mechanism responsible for this pattern was not clear, but some effects on heterotrophic activity seem likely, including stimulation of microbial N immobilization. It will be important to establish the mechanisms of the coupling among C, N and P cycles influencing biogeochemical responses to global change drivers like climate change, N deposition and CO_2 fertilization (Finzi et al. 2011). Long-term experiments on N and P co-limitation like the one reported here will help discern the mechanisms of C, N and P interaction.

Acknowledgments For their contributions to this research the authors thank S. Hamburg and M. Vadeboncouer who helped establish the study; many student assistants in the field and laboratory; and F. Vermeulen for statistical consulting. This research was supported by grants from the National Science Foundation (DEB 1114804; DEB 0949854).

References

Aber JD, Melillo JM, Nadelhoffer KJ, McClaugherty CA, Pastor J (1985) Fine root turnover in forest ecosystems in relation to the quantity and form of nitrogen availability: a comparison of two methods. Oecologia 66:317–321

Aber JD, Ollinger SV, Driscoll CT (1997) Modeling nitrogen saturation in forest ecosystems in response to land use and atmospheric deposition. Ecol Model 101:61–78

Aber JD, Goodale CL, Ollinger SV, Smith M-L, Magill AH, Martin ME, Hallett RA, Stoddard JL (2003) Is nitrogen deposition altering the nitrogen status of northeastern forests? Bioscience 53:375–389

Allison SD, Czimczik CI, Treseder KK (2008) Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Glob Chang Biol 14:1156–1168

Bae B, Fahey TJ, Yanai RD, Fisk M (2015) Soil nitrogen availability affects belowground carbon allocation and soil respiration in northern hardwood forests of New Hampshire. Ecosystems 18:1179–1191

Bloom AJ, Chapin FS, Mooney HA (1985) Resource limitation in plants: an economic analogy. Annu Rev Ecol Syst 16:363–392

Bowden RD, Davidson E, Savage K, Arabia C, Steudler P (2004) Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. For Ecol Manag 196:43–56

Bradford MA, Fierer N, Reynolds JF (2008) Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. Funct Ecol 22:964–974

Brumme R, Beese F (1992) Effects of liming and nitrogen fertilization on emissions of CO_2 and N_2O from a temperate forest. J Geophys Res 97:12851–12858

Burton AJ, Pregitzer KS, Hendrick RL (2000) Relationships between fine root dynamics and nitrogen availability in Michigan northern hardwood forests. Oecologia 125(3):389–399

Burton AJ, Pregitzer KS, Ruess RW, Hendrick RL, Allen MF (2002) Root respiration in North American forests: effects of nitrogen concentration and temperature across biomes. Oecologia 131:559–568

Burton AJ, Pregitzer KS, Crawford JN, Zogg GP, Zak DR (2004) Simulated chronic NO_3^- deposition reduces soil respiration in northern hardwood forests. Glob Chang Biol 10:1080–1091

Carreiro MM, Sinsabaugh RL, Repert DA, Parkhurst DF (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81:2359–2365

Cleavitt NL, Fahey TJ, Groffman PM, Hardy JP, Henry KS, Driscoll CT (2008) Effects of soil freezing on fine roots in a northern hardwood forest. Can J of For Res 38:82–91

Cleveland CC, Townsend AR (2006) Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. Proc Natl Acad Sci USA 103:10316–10321

Craine JM, Morrow C, Fierer N (2007) Microbial nitrogen limitation increases decomposition. Ecology 88:2105–2113

Crowley K, McNeil B, Lovett G, Canham C, Driscoll CT, Rustad L, Denny E, Hallett R, Arthur MA, Boggs J (2012) Do nutrient limitation patterns shift from nitrogen toward phosphorus with increasing nitrogen deposition across the northeastern United States? Ecosystems 15:940–957

Du E, de Vries W, Galloway JN, Hu X, Fang J (2014) Changes in wet nitrogen deposition in the United States between 1985 and 2012. Environ Res Lett 9:095004

Fahey TJ, Battles JJ, Wilson GF (1998) Responses of early successional northern hardwood forests to changes in nutrient availability. Ecol Monograph 68(2):183–212

Finzi AC, Doney SC, Jackson RB, Holland EA, Cole J (2011) Research frontiers in the analysis of coupled biogeochemical cycles. Front Ecol Environ 9:74–80

Fisk MC, Ratliff TJ, Goswami S, Yanai RD (2014) Synergistic soil response to nitrogen plus phosphorus fertilization in hardwood forests. Biogeochemistry 118:195–204

Gallardo A, Schlesinger WH (1994) Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. Soil Biol Biochem 26:1409–1415

Galloway JN (1998) The global nitrogen cycle: changes and consequences. Environ Pollut 102:15–24

Hagedorn F, Kammer A, Schmidt MWI, Goodale CL (2012) Nitrogen addition alters mineralization dynamics of ^{13}C-depleted leaf and twig litter and reduces leaching of older DOC from mineral soil. Glob Chang Biol 18:1412–1427

Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial contributions to soil

respiration: a review of methods and observations. Biogeochemistry 48:115–146

Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken MES, Elser JJ, Gruner DS, Hillebrand H, Shurin JB, Smith JE (2011) Nutrient co-limitation of primary producer communities. Ecol Lett 14:852–862

Haynes BE, Gower ST (1995) Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. Tree Phys 15:317–325

Helmisaari H-S, Hallbäcken L (1999) Fine-root biomass and necromass in limed and fertilized Norway spruce (*Picea abies* (L.) Karst.) stands. Forest Ecol Manag 119:99–110

Hobbie SE (2008) Nitrogen effects on litter decomposition: a five-year experiment in eight temperate sites. Ecology 89:2633–2644

Janssens IA, Dieleman W, Luyssaert S, Subke JA, Reichstein M, Ceulemans R, Ciais P, Dolman AJ, Grace J, Matteucci G, Papale D, Piao SL, Schulze ED, Tang J, Law BE (2010) Reduction of forest soil respiration in response to nitrogen deposition. Nat Geosci 3:315–322

Knorr M, Frey S, Curtis P (2005) Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86:3252–3257

Lee KH, Jose S (2003) Soil respiration, fine root production and microbial biomass in cottonwood and loblolly pine plantations along a soil nitrogen gradient. Forest Ecol Manag 185:263–273

Litton CM, Raich JW, Ryan MG (2007) Carbon allocation in forest ecosystems. Glob Chang Biol 13:2089–2109

Magill AH, Aber JD, Currie WS, Nadelhoffer KJ, Martin ME, McDowell WH, Melillo JM, Steudler PA (2004) Ecosystem response to 15 years of chronic nitrogen additions at the Harvard Forest LTER, Massachusetts, USA. Forest Ecol Manag 196:7–28

Maier CA, Kress LW (2000) Soil CO_2 evolution and root respiration in 11 year-old loblolly pine (*Pinus taeda*) plantations as affected by moisture and nutrient availability. Can J For Res 30:347–359

Mo J, Zhang W, Zhu W, Gundersen P, Fang Y, Li D, Wang H (2008) Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Glob Chang Biol 14:403–412

Nadelhoffer KJ (2000) The potential effects of nitrogen deposition on fine-root production in forest ecosystems. New Phytol 147:131–139

Olsson P, Linder S, Giesler R, Hogberg P (2005) Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. Glob Chang Biol 11:1745–1753

Phillips RP, Fahey TJ (2007) Fertilization effects on fineroot biomass, rhizosphere microbes and respiratory fluxes in hardwood forest soils. New Phytol 176:655–664

Ramirez KS, Lauber CL, Knight R, Bradford MA, Fierer N (2010) Consistent effects of nitrogen fertilization on the phylogenetic composition of soil bacterial communities in contrasting systems. Ecology 91:3463–3470

Rastetter EB, Yanai RD, Thomas RQ, Vadeboncoeur MA, Fahey TJ, Fisk MC, Kwiatkowski BL, Hamburg SP (2013) Recovery from disturbance requires resynchronization of ecosystem nutrient cycles. Ecol Appl 23:621–642

Ryan GR, Hubbard RM, Pongracic S, Raison RJ, McMurtrie RE (1996) Foliage, fine-root, woody tissue and stand respiration in *Pinus radiata* in relation to nitrogen status. Tree Physiol 16:333–343

Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35:549–563

Schlesinger WH, Andrews JA (2000) Soil respiration and the global carbon cycle. Biogeochemistry 48(1):7–20

See CR, Yanai RD, Fisk MC, Vadeboncoeur MA, Quintero BA, Fahey TJ (2015) Soil nitrogen affects phosphorus recycling: foliar resorption and plant–soil feedbacks in a northern hardwood forest. Ecology 96:2488–2498

Thomas RG, Zaehle S, Templer PH, Goodale CL (2013) Global patterns of nitrogen limitation: confronting two global biogeochemical models with observations. Glob Chang Biol 19:2986–2998

Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. New Phytol 164:347–355

Vadeboncoeur MA (2010) Meta-analysis of fertilization experiments indicates multiple limiting nutrients in northeastern deciduous forests. Can J For Res 40:1766–1780

Veen BW (1981) Relation between root respiration and root activity. Plant Soil 63:73–76

Yan LM, Chen SP, Huang JH, Lin GH (2010) Differential responses of auto- and heterotrophic soil respiration to water and nitrogen addition in a semiarid temperate steppe. Glob Chang Biol 16:2345–2357

Yanai RD, Vadeboncoeur MA, Hamburg SP, Arthur MA, Fuss CB, Groffman PM, Siccama TG, Driscoll CT (2013) From missing source to missing sink: long-term changes in the Nitrogen Budget of a northern hardwood forest. Environ Sci Technol 47(20):11440–11448