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MICROBIAL NITROGEN LIMITATION INCREASES DECOMPOSITION

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Abstract. With anthropogenic nutrient inputs to ecosystems increasing globally, there are long-standing, fundamental questions about the role of nutrients in the decomposition of organic matter. We tested the effects of exogenous nitrogen and phosphorus inputs on litter decomposition across a broad suite of litter and soil types. In one experiment, C mineralization was compared across a wide array of plants individually added to a single soil, while in the second, C mineralization from a single substrate was compared across 50 soils. Counter to basic stoichiometric decomposition theory, low N availability can increase litter decomposition as microbes use labile substrates to acquire N from recalcitrant organic matter. This "microbial nitrogen mining" is consistently suppressed by high soil N supply or substrate N concentrations. There is no evidence for phosphorus mining as P fertilization increases short- and long-term mineralization. These results suggest that basic stoichiometric decomposition theory needs to be revised and ecosystem models restructured accordingly in order to predict ecosystem carbon storage responses to anthropogenic changes in nutrient availability.

Key words: carbon sequestration; decomposition; fertilization; Kruger National Park, South Africa; microbial nitrogen mining; stoichiometric theory.

INTRODUCTION

Globally, many ecosystems are experiencing increases in the inputs of anthropogenically derived nitrogen and phosphorous (Vitousek et al. 1997, Bennett et al. 2001). At present, uncertainty regarding how increases in nutrient levels will impact the decomposition of plant litter limits our ability to predict terrestrial-ecosystem responses to global changes in resource availability (Melillo et al. 1996, McMurtrie et al. 2001, Berg and McClaugherty 2003, Luo et al. 2004, Pendall et al. 2004, Knorr et al. 2005). There are two competing hypotheses regarding the impacts of changes in nutrient availability on organic-matter decomposition. On the one hand, decomposition can be considered to be driven by the stoichiometry of substrates and microbial demands for resources (Melillo et al. 1982, Hessen et al. 2004), with maximal rates of decomposition observed when the ratios of supplies of carbon (C), nitrogen (N), and phosphorus (P) to microbes match their demands (Melillo et al. 1982, Sterner and Elser 2002). Although there is a general ignorance regarding P stoichiometry and decomposition (Hobbie and Vitousek 2000, Cleveland et al. 2002), basic stoichiometric decomposition theory predicts that ecosystems will store more C if increasing atmospheric CO_2 leads to greater substrate C:N (Parton et al. 1995, McGuire et al. 2001, Hessen et al. 2004), while increasing N availability would decrease soil C storage (Mack et al. 2004).

The "microbial nitrogen mining" hypothesis (Moorhead and Sinsabaugh 2006) stands in direct contrast to the predictions of basic stoichiometric decomposition theory in that increases in nutrient availability are likely to lead to a net decrease in decomposition rates. Microbial nitrogen mining is the process whereby some microbes use labile C to decompose recalcitrant organic matter in order to acquire N (Berg and McClaugherty 2003, Fontaine and Barot 2005, Moorhead and Sinsabaugh 2006). Since the decomposition of recalcitrant C yields little to no net energy (Couteaux et al. 1995), the ability of a guild of microbes to uniquely access recalcitrant N at a high C cost alters the community-level stoichiometry of microbial decomposition. For example, microbial N mining leads to predictions that ecosystem C storage would increase with greater N availability as mining of recalcitrant C for N was suppressed, which might explain observed declines in decomposition with N addition (Berg and Matzner 1997, Magill and Aber 1998, Neff et al. 2002, Hagedorn et al. 2003, Wang et al. 2004).

Although potentially of major importance to our understanding of ecosystem function, it is unknown whether microbial N mining is a general phenomenon that can be reliably predicted or whether analogous mining occurs for phosphorus. To better address the

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roles of N and P in decomposition and test the competing stoichiometric and mining hypotheses, we examined the roles of substrates, soil properties, and nutrient availability on decomposition in two laboratory experiments. The first experiment compares the mineralization of C from leaf biomass from 108 plants representing 73 species of grass, forbs, and trees on a single soil in an incomplete factorial design with N and P addition, i.e., no treatments with N and P added. Incubations ran for 200 d. The second experiment compares C mineralization over 56 d from a single substrate across 50 soils also in factorial design with N and P addition. These broad, well-replicated surveys increase the power to test the impacts of exogenous nutrient inputs on decomposition across a large range of soil types and litter chemistries. Incubating the soils in the laboratory, as opposed to more standard field litterbags, allows higher resolution of C dynamics and does not confound mass loss due to leaching with C mineralization (Fierer et al. 2005).

Assuming nitrogen mining is an important part of the decomposition of recalcitrant C, we hypothesize that increasing N availability, whether through fertilization, litter N, or soil N supply, would have no effect on the size of the labile-C pool (C_L), but would decrease the rate of decomposition of recalcitrant C (k_R). As organic P is generally not associated with recalcitrant C (McGill and Cole 1981), P should not be mined and therefore it is less likely that C mineralization would decrease with increasing P availability. The effect of P fertilization should be greatest when soil P availability and/or litter P is low.

Methods

Comparison of leaf biomass decomposition

The soil used for the comparison of decomposition of leaf biomass was derived from basaltic parent material and acquired from the top 20 cm of the soil profile from an herbivore exclosure near Makhahlola in Kruger National Park, South Africa (see Plate 1). The soil is a sandy loam with clay, silt, fine sand, medium sand, and coarse sand percentages of 14.0%, 20.0%, 43.3%, 8.3%, and 14.4%, respectively. Soil pH was 5.1 as measured in 1 mol/L KCl. Soil C and N concentrations were 28.3 and 9.5 mg/g, respectively, while available P was 84 mg/kg (Bray II extraction). A total of 108 leaf samples representing 73 species were collected from Kruger National Park in November 2003 and January 2004. These included samples of 33 grasses, 24 forbs, and 51 woody plants representing 22 species of grass, 22 species of forb, and 29 woody species. Leaf C, N, and P concentrations along with wet-chemistry fractions were determined for each sample.

The experimental design for the decomposition survey of leaf biomass included 108 leaf samples in factorial with three nutrient treatment (ambient, N added, P added) for a total of 324 samples. An additional six samples of soil without any leaf biomass added were incubated at each nutrient treatment to serve as controls. For each replicate, 10 g of soil was placed in a 50-mL sterile polypropylene centrifuge tube. For those tubes receiving substrate, the equivalent of 100 mg of ash-free substrate was added to each tube and incorporated into dry soil with a vortex mixer. For those tubes receiving nutrient solution, 100 μL of 1.78 mol/L NH_4NO_3 or 0.323 mol/L KH_2PO_4 were added to appropriate tubes and enough distilled water to reach 35% water holding capacity, which was maintained throughout the entire experiment. Compared to substrate C, nutrient additions lead to mass ratios of approximately C:N of 1 and C:P of 11.

Leaf C and N concentrations were determined via combustion on a Carlo Erba NA 1500 nitrogen analyzer (Carlo Erba Instruments, Milan, Italy). For P concentrations, leaf biomass was first digested in a 10:1:1 mixture of HNO_3, $HClO_4$, and H_2SO_4 for 60 min at 150°C followed by 20 min at 250°C. P concentrations were determined on distilled-water extract of the residual with malachite green colorimetry on a Biotek Powerwave XS microplate spectrophotometer (Biotek Instruments, Winooski, Vermont, USA). Wet-chemistry fractions were determined by sequentially digesting plant material corresponding with soluble cell contents, cellulose, hemicellulose, and lignin on a forage fiber analyzer (ANKOM 200, Macedon, New York, USA). All wet-chemistry fractions are expressed on an ash-free basis using the final ash concentrations after combusting the lignin fraction at 525°C. Nonpolar soluble fraction was determined on the initial biomass for a subset of samples but did not increase explanatory power. Initial ash concentrations were also determined by combusting the original ground plant material at 450°C. Before beginning incubations, all leaves were dried at 45°C and ground in a UDY cyclone mill (UDY Corporation, Fort Collins, Colorado, USA) fitted with a 1-mm screen.

For the incubations, 10 min after moistening soils, tubes were vented, sealed and stored at 25°C. Three tubes without soil were also sealed for later measurement of initial CO_2 concentrations. After approximately two hours, 5 mL of air were removed from each tube with a glass syringe for measurement of CO_2. After addition of solution, CO_2 concentrations in blank tubes and tubes with soil were determined with a LI-COR LI-6262 infrared gas analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA) fitted with a static gas-sampling loop after two hours. After sampling, caps were removed from the tubes and tubes placed in 5.5-L plastic boxes lined with wet paper towels, covered with polyethylene film and stored at 25°C. The procedure was repeated an additional 14 times over an approximately 200-day period (typically 1, 2, 4, 7, 15, 23, 31, 40, 56, 80, 94, 135, 176, and 218 days).

We first calculated cumulative respiration for soils unamended with litter for each fertility treatment. A nonlinear curve was fit to the average cumulative respiration data over time of each of the six combina-

tions of soil and treatment by parameterizing a two-pool model (Alvarez and Alvarez 2000) with nonlinear curvefitting in JMP 5.0 (SAS Institute 2006):

$$C_t = C_L \times \left(1 - e^{k_L t}\right) + k_R t \quad (1)$$

where C_t is the known cumulative amount of C respired at sampling time t, C_L is the size of the labile C pool, k_L is the decay constant for the labile pool, and k_R is the mineralization for the recalcitrant pool. This model assumes an exponential decay of the labile pool, a linear decay of the recalcitrant pool (constant decay rate), and was not constrained for C_t. In our analyses of cumulative respiration, we also tested a three-pool model that assumes an exponential decay of the recalcitrant pool and a passive pool, but many samples did not show an exponential decline in the decomposition of recalcitrant C over time and could not be solved. For those samples that did show an exponential decay rate for the recalcitrant pool, the k_R values of the two- and three-pool models were well correlated, in part since the same amount of C was added in each replicate (data not shown).

Once each of the five variables (C_L, k_L, k_R, and cumulative respiration to 30 d and 200 d) were determined for each replicate, samples with a Mahalanobis distance >4.5 for all combinations of soil and nutrient treatments were considered outliers and removed from subsequent analyses for that parameter. This led to the removal of no more than 5% of the data for any given parameter.

To test the effects of nutrient treatments on each of the five variables, we examined the model II relationships between parameters (Sokal and Rohlf 1994) for each nutrient treatment and the same parameter for the water control for each soil. For each relationship, we tested whether the slopes of each relationship differed significantly from 1 and whether the average of the variable changed with nutrient addition with a matched pair t test. To test whether the responses in decomposition could be predicted from a priori litter measurements, we ran general linear models that included treatment, [C], [N], [P], and all pairwise interactions to predict each of the five variables. [C] was negatively correlated with initial ash content, but results were similar whether using [C] of the initial biomass or the calculated [C] of the ash-free biomass, so the [C] of the initial biomass was used throughout. We do not present any results involving k_L as there were largely no significant effects of nutrient treatments or plant chemistry on k_L and C_L, and k_L were occasionally autocorrelated (data not shown).

After calculating cumulative respiration for each sample, for each sampling point for all samples amended with plant biomass, we calculated the cumulative respiration at that time of the appropriate control. This cumulative respiration of the soil organic matter was then subtracted out of the cumulative respiration of the soil amended with leaf biomass to give the cumulative respiration of the leaf biomass alone, assuming strict additivity. With data on cumulative respiration over time for just leaf biomass, we fit the cumulative respiration to Eq. 1 and calculated C_L, k_L, and k_R for each replicate. Cumulative respiration at 720 h (30 d) and 4800 h (200 d) was determined by linear interpolation of cumulative-respiration data for each replicate between the time points immediately before and after the desired time.

Decomposition of a single substrate across 50 soils

For the cross-soil survey, soil was collected from 50 sites in South Africa representing a broad range of climates from desert to sub-tropical and summer to winter rainfall. Site vegetation ranged from Fynbos to Karoo to savanna to high elevation grasslands. For the cross-soil survey, soils were collected between 5 March 2005 and 6 May 2005, except for two soils that were collected in March 2004. Soil samples for each site were composited from five locations separated by 2 m each with soil obtained from the top 15 cm. Soils were air dried at room temperature within three days of collection. Dry soils were passed through a 2-mm sieve and then stored at room temperature for 2–5 months. Soil organic C and N, particle-size fractions, and water-holding capacity were determined for each soil. For the common substrate, *Themeda triandra* shoots from Pretoriuskop area of Kruger National Park, South Africa, were collected in October 2004, dried at 45°C, and ground in a UDY cyclone mill fitted with a 1-mm mesh. *Themeda triandra* is a dominant grass species throughout a broad range of grasslands and not known to have any constitutive secondary compounds. Grass C and N concentrations were 6 mg N/g and 430 mg C/g.

The experimental design for the cross-soil survey was a three-way factorial among 50 soils, three nutrient treatments (ambient, +N, +P), and two substrates (control, grass shoots) for a total of 300 samples. For substrate-amended samples, 100 mg of ash-free biomass was added to 10 g dry soil. Soil moisture, nutrient treatments, and incubation protocols were the same as above. For this experiment, the determination of respiration rates was repeated 9 times over 56 d (typically 1, 2, 4, 7, 15, 23, 31, 39, and 56 d). At the end of 56 days, soils were extracted with 0.5 K_2SO_4 and extracts analyzed for PO_4^-, NO_3^-, and NH_4^+ colorimetrically. Cumulative respiration was calculated as previously described. With no replicates for the controls for each soil, C_L and k_R for the grass biomass for each soil was determined as the difference between each parameter for the grass and soil combined and the control soils alone. As before, we do not analyze patterns of k_L.

For each soil, we determined soil N via combustion on a Leco FP-528 nitrogen analyzer (Leco, Saint Joseph, Michigan, USA) and organic C was determined as loss on ignition. Soil was fractionated into five particle-size classes using the hydrometer method after dispersion

PLATE 1. View from within Makhahlola exclosure in Kruger National Park, South Africa. The exclosure, located on a basalt-derived soil, is the location where soil was acquired for the comparison of leaf biomass decomposition in a common soil for this paper. Photo credit: J. M. Craine.

with sodium hexametaphosphate. In statistical analyses of C_L, k_R, and cumulative respiration, values with a Mahalanobis distance >4.5 were again considered outliers and removed from comparisons of treatments.

RESULTS

Adding nitrogen to soils amended with leaf biomass of different grass species reveals that the decomposition of labile-C pools follows the predictions of simple stoichiometric theory. Averaged across the suite of leaves, fertilization with N significantly increased C_L (the size of the labile-C pool) by 6% (Fig. 1a), indicating an increase in the mineralization of extant labile C with an increased supply of exogenous N. The increases in respiration with additional N were greater for samples with lower C_L under ambient nutrient availability. Increases were as high as 49% for leaves that had a C_L of 5.0 mg C/g ash-free biomass (Fig. 1a). In concordance with stoichiometric theory, the greatest response to N addition came from leaf samples with the highest C:N, both due to high leaf-C concentrations ([C]) and low leaf-N concentrations ([N]) (Fig. 2). Although C_L increasing with [N] might be interpreted as evidence for N limitation of labile-C decomposition, C_L was still positively correlated with [N] for samples fertilized with N (Fig. 2b), suggesting the quality of carbon in the labile-C pool is responsible for the apparent correlation between [N] and C_L.

As opposed to the effects of N on labile-C respiration, the effects of N on recalcitrant-C mineralization

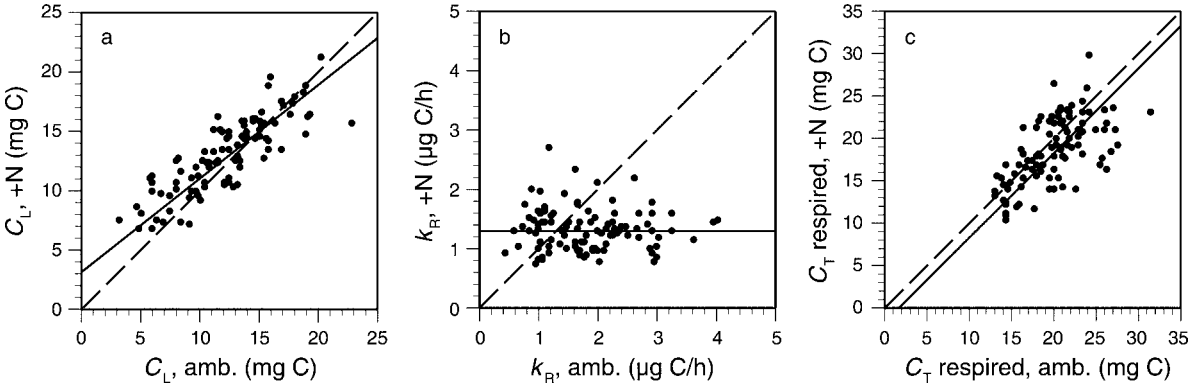

FIG. 1. Effects of increasing N availability on decomposition of C: (a) labile C (C_L), (b) mineralization rate of recalcitrant C (k_R), and (c) total C (C_T) respired over 200 days across a wide variety of leaves, under conditions of increased N (+N, *y*-axes) vs. ambient conditions (amb., *x*-axes). Dashed lines indicate 1:1, and solid lines show RMA (reduced major axis) regression, except for (b) which is the mean k_R at elevated N since there was no significant relationship between the two variables. Respired C represents substrate C after background soil C respiration was removed.

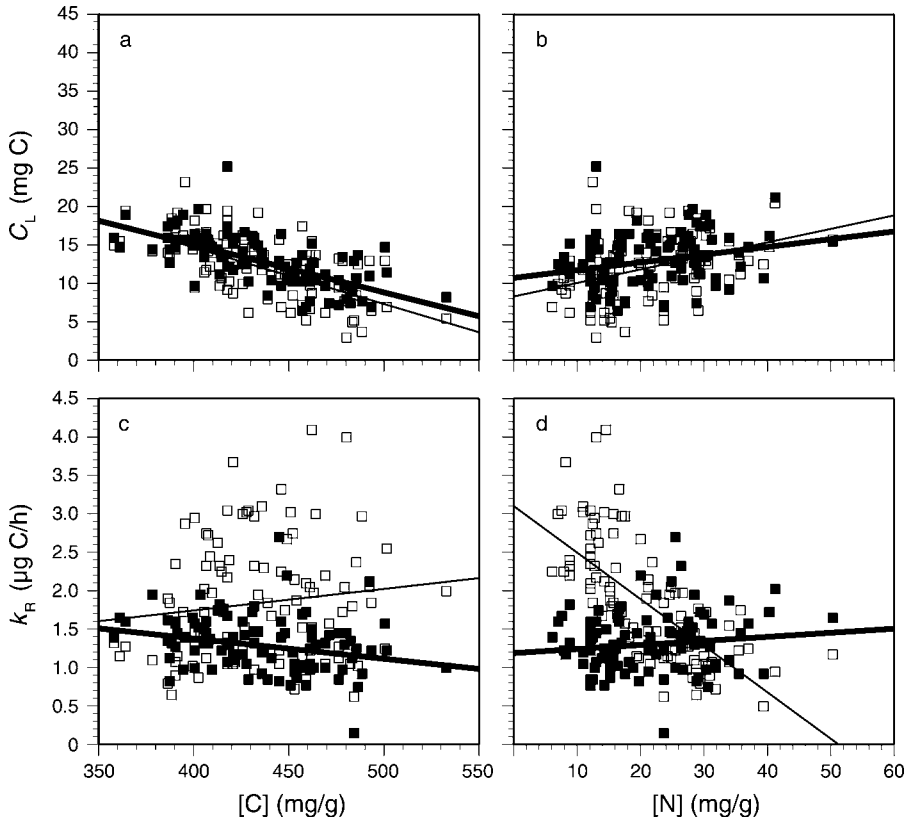

FIG. 2. Relationships between (a and b) labile-C pool size (C_L) vs. substrate [C] and (c and d) recalcitrant-C decay rate (k_R) vs. substrate [N] for leaves with no nutrients added (thin line) and N added (thick line).

followed N-mining theory with N fertilization decreasing k_R (decay rate of the recalcitrant-C pool) by 29% on average (Fig. 1b). Declines in k_R with N fertilization were greater for substrates with higher k_R under ambient nutrient conditions, so that adding N collapsed k_R to a narrow range for all substrates. The substrates that showed the greatest declines in k_R had low N concentrations (Fig. 2). Elemental concentrations and wet-chemistry fractions could explain little of the variation in k_R under N addition (data not shown). For substrates not fertilized with N, increasing substrate [N] from 10 to 30 mg/g decreased k_R by ~50% (Fig. 2b). For substrates with 32 mg N/g, which was approximately a C:N of 15, k_R under ambient nutrient supplies was equivalent to the average k_R of substrates after N addition. Overall, the addition of N decreased decomposition rates over the course of the 200-d incubation. Although we observed a 6% increase in C_L with N addition (see above), the decrease in k_R with N addition led to a 9% average decrease in cumulative respiration rates for samples amended with N (Fig. 1c). Samples that had 10 mg N/g showed a 20% decline in cumulative respiration over 200 d with fertilization, while samples with 30 mg N/g increased by only 1% (Fig. 3).

As predicted from stoichiometric and microbial nitrogen-mining theories, N supply to microbes was

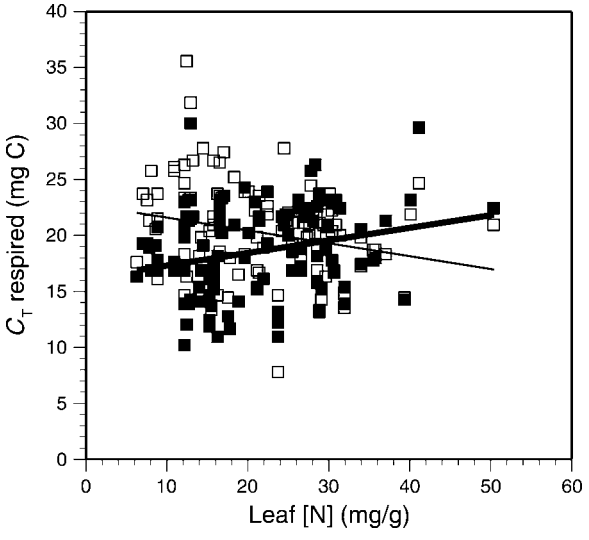

FIG. 3. Relationship between cumulative respiration over 200 d and leaf-N concentrations for leaves added to soil along with N (open squares, thin line) and leaves added to soil without any supplemental N (solid squares, thick line).

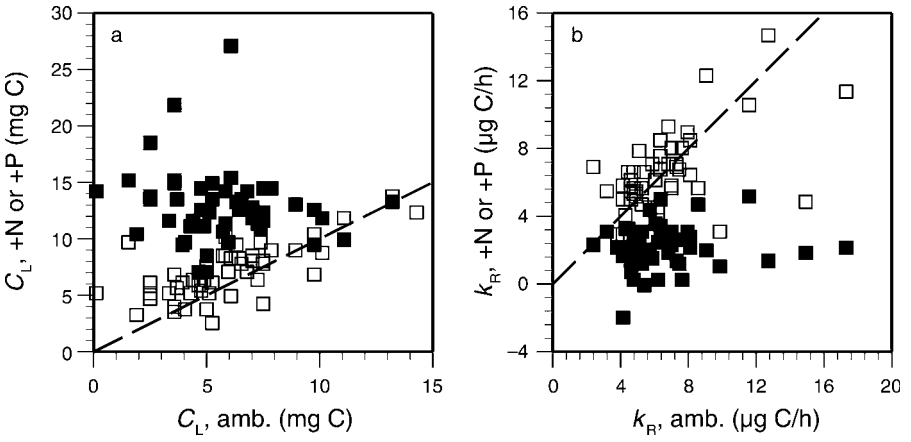

FIG. 4. Across 50 soils, comparison of decomposition of a common substrate between soils with no nutrients added (amb.) and those with additions of either N (solid squares) or P (open squares). Data are shown for (a) labile-C decomposition and (b) recalcitrant-C decomposition. The dashed lines show a 1:1 relationship.

also key in determining the effect of N addition on the decomposition dynamics of the common substrate across the 50 distinct soils. Parallel to the responses seen across litters, averaged across soils, adding N increased the C_L of the decomposing grass by 110% and decreased k_R by 68% (Fig. 4). For soils that had high soil-N availability, there was little effect of N addition on C_L (Fig. 5). In contrast, soils with low soil organic-matter (SOM) concentrations showed large increases in substrate C_L with N addition. The decline in k_R was invariant with soil N content and potential N mineralization (Fig. 5; $P > 0.15$), which would be expected with the large C additions from the grass producing net immobilization of N by microbes across all soils (data not shown). With N addition increasing short-term C mineralization, but decreasing it long-term, cumulative respiration was increased by 5% over 56 d, but would have been 40% lower over 200 d if the declines in k_R were extended beyond the initial 56-d incubation.

While microbial N mining factors importantly in the responses of litter decomposition to N addition, stoichiometric theory predicts the responses of labile-C and recalcitrant-C mineralization to P addition, with no evidence of P mining. Across the suite of leaves, fertilization with P increased C_L by 9% and k_R by 7% (Fig. 6). The increases in respiration of labile C with additional P were greater for high-quality samples, which had lower [C], higher [N] and [P], and slightly lower N:P (data not shown), implying that the high-P demands of fast-growing bacteria cause the microbial community to be more P limited when decomposing high-quality C. Across soils, soils with low P availability showed the greatest increases in respiration of the common grass biomass with P addition (Fig. 7).

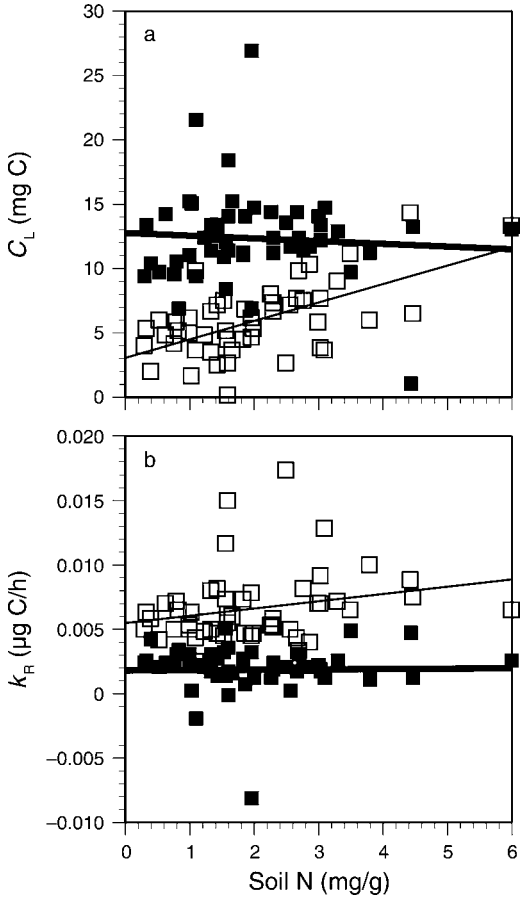

FIG. 5. Across 50 soils, relationship between soil N content and (a) labile-C pool size (C_L) and (b) recalcitrant-C decomposition rate (k_R). Data are from soils with ambient nutrient availability (solid squares, thick line) and elevated nitrogen availability (+N, open squares, thin line).

DISCUSSION

In light of the results of these decomposition experiments, it is important to revisit whether the greater decomposition under low N availability com-

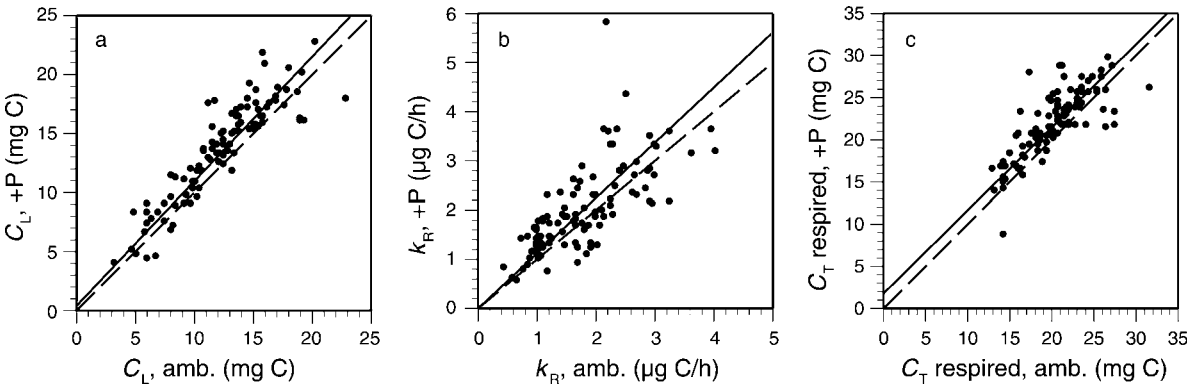

FIG. 6. Effects of increasing P availability on (a) decomposition of labile C (C_L), (b) recalcitrant C (k_R), and cumulative respiration over 200 days of a wide variety of leaves. Dashed lines show 1:1 relationships; solid lines are RMA (reduced major axis) regressions.

pared to high N availability is unequivocally a consequence of microbial N mining. In contrast to N mining, the declines in decomposition with greater N availability could also be a consequence of increased condensation reactions and decreases in the degradability of recalcitrant C. Alternatively, greater microbial assimilation rates of C that lead to less respiration, but similar decomposition, or changes in the food-web structure of the soil ecosystem that increases microbial grazing, could also lower decomposition rates. Although possible, evidence for these alternative hypotheses is scarce. For example, Clinton et al. (1995) added ^{15}N-labeled inorganic N to a forest floor and with CPMAS (cross polarization magic angle spinning) ^{15}N NMR (nuclear magnetic resonance) spectroscopy found little of the added N had been incorporated into recalcitrant organic matter. Transformation of N into heterocyclic structures appears to be rare in most soils (Knicker 2004) and would be unlikely to explain the rapid declines in respiration that we observed. In contrast, other experiments have shown that increasing N availability decreases the quantity of enzymes that are responsible for recalcitrant-C decomposition (Sinsabaugh et al. 2005), and fertilization with N actually decreases the degradation of heterocyclic N (Sims 2006).

Recognizing the lack of direct evaluation of mechanisms behind the observed patterns of respiration, the most conservative statement that can be made is that the patterns of decomposition observed across the featured contrasts are consistent with expected changes in N mining. In lieu of more mechanistic data such as enzyme activity or NMR analyses of SOM, we cannot definitively state that changes in N mining are the mechanism that underlies these patterns. Instead, the results are consistent with predictions of conceptual models that incorporate N mining, which seems to be the most parsimonious explanation at this time.

If future research confirms the importance of N mining over alternative mechanisms, the need to incorporate the unique stoichiometry of microbial nitrogen mining becomes clear. The key shift in modeling organic-matter decomposition to incorporate mining is that labile-C and recalcitrant-C pools cannot be considered to decompose independently of one another as available N determines the assimilatory fate of labile C. With N mining, recalcitrant-C decomposition should be greatest when labile C is available and N is in short supply, which is likely greatest for substrates with low [N] on soils with low N mineralization in the absence of fertilization.

The mechanisms behind a decline in N mining could occur through different, but not necessarily exclusive, mechanisms. On the one hand, the increased N availability could induce individual microbes to decrease their production of the enzymes responsible for recalci-

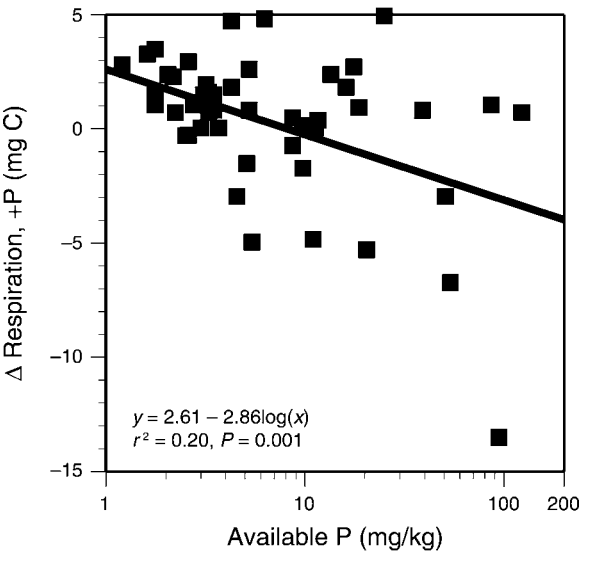

FIG. 7. Across 50 soils, relationship between Bray II available P and the change in respiration of a common grass biomass with P addition. Note that the *x*-axis scale is logarithmic.

trant-C decomposition associated with a reduction in the relative benefits of using the labile C to produce enzymes, such as phenol oxidase and peroxidase, for nitrogen mining as opposed to using the labile C for growth (Waldrop et al. 2004, Sinsabaugh et al. 2005). Alternatively, the decline in N mining could be manifested through competitive effects that are driven by the relative availability of N and labile C. Increasing litter substrate N or increasing the external N supply could decrease the availability of labile C to those microbes responsible for decomposing recalcitrant C (Fontaine and Barot 2005, Moorhead and Sinsabaugh 2006). If these microbes are competitively inferior for labile C when N availability is relatively high, then this would cause a decline in their abundance as well as a decline in N mining at the community level. With N fertilization, often there are changes in microbial community structure that would reflect a shift away from microorganisms that undertake N mining (Waldrop et al. 2004) Although we were unable to test whether variation in the supply of N from soil organic matter had similar effects on k_R, a similar experiment that added proportionally smaller amounts of C or longer incubations would reduce microbial immobilization of C and better test whether soil-N mineralization affects the decomposition response to N addition.

The increase in C_L (the size of the labile-C pool) with N fertilization was not predicted to occur. With the assumptions of the two pool model of decomposition, there should be a rapid exponential decline in the availability of labile C. Nutrient availability might affect the rate at which labile C is utilized by microbes, but not the amount that is respired. The increase in C_L with N fertilization implies that with low N availability either labile C is utilized but not respired (i.e., changes in microbial assimilation rates) or it is inappropriate to assume an exponential decay to labile C under low N-availability conditions. In a similarly structured experiment where multiple biomass samples were ground and added to replicates of a single soil, the addition of exogenous inorganic N also increased C_L (Wang et al. 2004), suggesting that the decrease in effective labile-C pool size with low N availability might be a consistent, although poorly understood, phenomenon.

Although our results are consistent with another laboratory incubation survey that varied N availability (Wang et al. 2004), the most comprehensive field survey of decomposition to investigate the effects of litter and exogenous N on decomposition suggested inconsistent responses of decomposition to the two sources of N (Hobbie 2005). Yet, reconciling the positive relationship between litter N and field decomposition rates, the lack of average response of decomposition rates to N addition in the field, and the greater response of decomposition to N addition in sites with lower N availability all observed by Hobbie (2005) does not necessitate a novel hypothesis regarding differential response of microbial assemblages to organic and inorganic N. It appears more parsimonious to suggest that the positive relationship between litter N and decomposition rates observed by Hobbie (2005) can be explained if (1) relatively labile-C decomposition was N limited, (2) biomass N was correlated with the quality of the labile-C pool, which might not be best measured by standard wet-chemistry fractionation techniques, and/or (3) if biomass N is correlated with soluble C, which includes more than just amino acids, and litters with high biomass experienced more soluble-C loss. The lack of response to exogenous inorganic N observed by Hobbie (2005) does not require microbes to respond differently to inorganic N than to organic N, since the effects of N likely have offsetting effects on labile-C and recalcitrant-C utilization by microbes. In our laboratory incubations, both presumably organic N supplied from leaves and exogenously supplied inorganic N were associated with greater labile-C utilization and suppressed recalcitrant-C decomposition. Laboratory decomposition experiments where substrates were synthesized from constitutive fractions such as starch, cellulose, and lignin in order to control for any correlation between labile C and organic N would likely clear up any remaining questions about the effects of litter N and decomposition.

The differential response of labile and recalcitrant C to N and the importance of P limitation for decomposition in some soils will require restructuring C-cycling models. With labile C (along with N) affecting recalcitrant-C decomposition rates, microbial nitrogen mining has strong consequences for our predictions of ecosystem function under altered resource availability, and a key link for understanding ecosystem C storage will be quantifying changes in labile C and N availability. Microbial nitrogen mining produces predictions for ecosystem C storage that are opposite of those of basic stoichiometric decomposition theory. For example, a link between elevated atmospheric CO_2 concentrations and N mining via increased labile C and/or decreased N availability could explain why soil C has been declining in some regions over the past few decades (Bellamy et al. 2005) and could negate progressive nitrogen limitation from litter quality feedbacks (Luo et al. 2004).

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