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Drivers of microbial respiration and net N mineralization at the continental scale

Benjamin P. Colman*, Joshua P. Schimel

Department of Ecology, Evolution, and Marine Biology, Mail Stop 9610, University of California, Santa Barbara, CA 93106-9610, United States

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ABSTRACT

The dominant pools of C and N in the terrestrial biosphere are in soils, and understanding what factors control the rates at which these pools cycle is essential in understanding soil CO_2 production and N availability. Many previous studies have examined large scale patterns in decomposition of C and N in plant litter and organic soils, but few have done so in mineral soils, and fewer have looked beyond ecosystem specific, regional, or gradient-specific drivers. In this study, we examined the rates of microbial respiration and net N mineralization in 84 distinct mineral soils in static laboratory incubations. We examined patterns in C and N pool sizes, microbial biomass, and process rates by vegetation type (grassland, shrubland, coniferous forest, and deciduous/broadleaf forest). We also modeled microbial respiration and net N mineralization in relation to soil and site characteristics using structural equation modeling to identify potential process drivers across soils. While we did not explicitly investigate the influence of soil organic matter quality, microbial community composition, or clay mineralogy on microbial process rates in this study, our models allow us to put boundaries on the unique explanatory power these characteristics could potentially provide in predicting respiration and net N mineralization. Mean annual temperature and precipitation, soil C concentration, microbial biomass, and clay content predicted 78% of the variance in microbial respiration, with 61% explained by microbial biomass alone. For net N mineralization, only 33% of the variance was explained, with mean annual precipitation, soil C and N concentration, and clay content as the potential drivers. We suggest that the high R^2 for respiration suggests that soil organic matter quality, microbial community composition, and clay mineralogy explain at most 22% of the variance in respiration, while they could explain up to 67% of the variance in net N mineralization.

1. Introduction

Mineral soils store over three times as much C and over forty times more N than living biomass in terrestrial ecosystems (Batjes, 1996; Jobbágy and Jackson, 2000). These stores of C and N are turned over by soil microbes through respiration and N mineralization, and understanding the factors that drive these processes has long been a goal of soil scientists (Lipman and Edwards, 1911). This goal remains essential for accurately describing and modeling these processes at a range of spatial and temporal scales. The extent to which the "state factors" (Amundson and Jenny, 1997; Jenny, 1941) of climate, vegetation, parent material, and others ultimately regulate these processes is surprisingly difficult to determine because they so often covary strongly within specific biomes and regions.

Many studies have tried to decipher the factors that define the capacity of soils for C and N cycling, which are then modulated by short-term drivers such as temperature and moisture. Most of these studies, however, focus on the factors that vary the most within the study site, region, vegetation type, or biome. For example, in northern temperate forests, studies mostly focus on the impacts of organic matter quality (Finzi and Canham, 1998; Melillo et al., 1989; Scott and Binkley, 1997), while grassland studies largely focus on climatic gradients and organic matter quantity (Burke et al., 1997, 1989). This is not because organic matter quality is unimportant in grasslands, or climate is unimportant in temperate forests; rather it is because these factors may not vary much within those regions, and so these factors may fail to explain the variation in process rates caused by factors such as landscape position, soil type, plant community composition, etc. Studies often use gradients of age, altitude, climate, or disturbance to try to disentangle

* Corresponding author. Present address: Department of Biology, Duke University, Durham, NC 27708, United States. Tel.: +1 919 599 2058; fax: +1 919 660 7293.
E-mail address: colmanb@gmail.com (B.P. Colman).

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the many factors that potentially drive C and N cycling (Bekku et al., 2004; Burke et al., 1997; Compton et al., 1998; Frank and Groffman, 1998; Santiago et al., 2005). However, oftentimes other factors covary with the primary factor that defines the gradient (*e.g.*, organic matter content and precipitation) making it difficult to discern the flow of influence from the covarying factors to the process of interest. Some studies have looked beyond single gradients and across ecosystem or vegetation boundaries. Even still, these tend to be regional in scale, comparing different forest types (Campbell and Gower, 2000; Compton et al., 1998; Reich et al., 1997), or pairs of vegetation types within a biome (Chen and Stark, 2000; McCulley et al., 2004; McKinley and Blair, 2008). These may reveal patterns that highlight differences, but also lack the ability to discern the relationships between drivers that are controlling the processes.

Are there universal relationships among the drivers of C and N cycling at the continental scale? Or are these patterns of controlling variables specific within biomes, vegetation types, or regions? One approach to answering this question would be to conduct a meta-analysis using published data. Such an approach is constrained by the limited number of parameters reported in individual studies, and/or the small number of soils with a full set of parameters that could be included in the analysis. Individual studies also vary in methods (*e.g.*, incubation time, soil handling, and soil moisture) or focus on ecosystem-specific parameters rather than those that may be important at larger scales. Developing a large-scale understanding of the relationships among the variables controlling C and N mineralization would benefit from a coordinated integrated experiment measuring all the appropriate variables using consistent methods.

We analyzed how environmental drivers that vary at continental-scales regulate soil properties and ultimately microbial activities in soil. We sampled mineral soils across gradients of temperature, rainfall, parent material, vegetation type, and litter chemistry. We focused exclusively on mineral soils since they contain the majority of SOM. We sampled 84 individual soils from across North America, Puerto Rico, and Hawaii. These soils represent a diversity of ecosystems ranging from tropical rainforests to arctic tundra, and high desert to coastal grasslands and forests. We measured microbial biomass, and incubated soils at constant moisture and temperature to assess microbial respiration and net N mineralization potentials. Results were used to discern pattern, and develop statistical models—using structural equation modeling—to study the interplay of soil, vegetation, and climate characteristics with soil microbial community size and processes.

In recent years three research areas have been developing that have argued that they offer the potential to make significant contributions to understanding the overall dynamics of soil C and N cycling: these are advances in organic matter chemistry, molecular microbial methods, and clay mineralogy. Researchers have long argued that "quality" is a critical control over SOM dynamics, but SOM quality has been a poorly defined concept. The most successful large-scale SOM models, such as CENTURY (Parton et al., 1987), have characterized OM pools based on their turnover time rather than their chemical composition, but an emerging understanding suggests that much of SOM may be simple biomolecules that persist (Schmidt et al., 2011). Additionally, with the development of microbial ecology, researchers have suggested that microbial community composition may be important in controlling the dynamics and fate of organic matter (Fontaine and Barot, 2005; Marschner and Kalbitz, 2003). And finally, as our conceptual models of organo-mineral associations have advanced (Kleber et al., 2007), so too has our understanding of the importance of clay mineralogy in regulating the mechanisms by which SOM associates with mineral phases in soils (Kleber et al., 2007; Lützow et al., 2006) and SOM dynamics (Paul, 1984; Sollins et al., 1996; Torn et al., 1997).

While we did not examine these factors directly, we assessed the integrated role of soil chemistry, microbial community composition, and clay mineralogy on soil processes indirectly. We did this by evaluating how much of the variance in soil processes we could explain based on simple aggregate measures (*e.g.*, total SOM content, microbial biomass, clay content, etc.). We also defined how much variance remained unexplained which could possibly result from variations in organic matter chemistry, microbial community composition, and clay mineralogy. This would not explain how much of the residual variation results from any of these other drivers (or how much is truly random variation), but would put bounds on their potential importance. Thus our approach both allowed us to determine what of our measured parameters had direct vs. indirect effects on microbial processes, and allowed for us to put bounds on the importance of other factors, such as soil organic matter chemistry, microbial community composition, and clay mineralogy to these process rates.

2. Materials and methods

2.1. Site characterization and soil collection and processing

Eighty-four soils, each distinct in edaphic factors and site characteristics, were collected across North America and the islands of Puerto Rico and Hawai'i. All soils were 0–5 cm mineral soils, and were collected at the peak of the growing season from sites that were unsaturated during much of the year. Soils were composited from 5 to 10 representative samples within each site. Samples were shipped at field moisture to the University of California, Santa Barbara for processing. For each sampling site, mean annual precipitation (MAP), mean annual temperature (MAT), and actual evapotranspiration (AET) were determined (Fierer et al., 2006). Soils were grouped by the dominant site vegetation and were classified as coniferous forest, deciduous/broadleaf evergreen forest, grassland, or shrubland.

Soils were sieved, and then characterized for water holding capacity, soil texture, C and N content, and pH (Fierer et al., 2006). After sieving, soils were adjusted to 35% of water holding capacity (WHC)—either by adding deionized water or by air drying at 20 °C—because a standard proportion of WHC provided a roughly equivalent water potential (Gulledge and Schimel, 1998) and allowed us to eliminate moisture content as a variable in the analysis. Soils were then allowed to equilibrate at the new water potential for ten days at 20 °C to avoid the pulse in activity caused by the disturbance of sieving and adjusting water content (Fierer and Schimel, 2002). Microbial biomass was then measured using substrate induced respiration (Fierer et al., 2006). Soils were also analyzed for dissolved organic C (DOC) and total dissolved N (TDN) by extracting them with 0.5M K_2SO_4 for 1 h, and analyzing the extracts on a Shimadzu TOC-V CPH (Columbia, USA). Ammonium (NH_4^+), and nitrate + nitrite ($NO_2^- + NO_3^-$) were measured on these same extracts using a Lachat Instruments 2300 Autoanalyzer (Lachat Instruments, Milwaukee, USA) using Lachat methods 31-107-06-5-A and 12-107-04-1-B, respectively. Dissolved organic nitrogen (DON) was then calculated by subtracting $NH_4^+ + (NO_2^- + NO_3^-)$ from TDN. Base cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) were analyzed by extracting fresh soils with ammonium acetate and subsequently analyzing the extracted cations using ion chromatography. Ammonium loaded onto exchange complexes during cation extraction was itself extracted and analyzed to determine cation exchange capacity.

2.2. Microbial respiration and net N mineralization

Microbial respiration and net N mineralization were measured by incubating triplicate samples at 20 °C for 50 days in airtight

tubes with butyl rubber septa. Accumulation of CO_2 was measured periodically using an infrared gas analyzer (LiCor Model LI-6252; LiCor, Lincoln, USA). If the concentration of CO_2 reached 0.5%, samples were vented after measurement. In addition to measuring the average rate of CO_2 accumulation over the first 25 days, we also quantified the respiration kinetics over the course of the whole 50 days of the experiment. Data were tested for consistency with models with one, two, and three C pools, but because of the short duration of our analyses, we were able to assume that little of the old, recalcitrant C would have been lost and so used a simple first order rate model with one carbon pool:

$$\mathrm{Resp_{mic}} = \mathrm{C_0}\left(1 - \mathrm{e}^{-kt}\right),$$

where $\mathrm{Resp_{mic}}$ is the cumulative carbon respired at time t, $\mathrm{C_0}$ is the pool of labile carbon, and k is the kinetic coefficient for decomposition (adapted from Campbell et al., 1993; Stanford and Smith, 1972; Zak et al., 1993). Data was fitted using the JMP® (v. 7, SAS Institute Incorporated) non-linear modeling routine.

We looked at net N mineralization, a commonly used method for determining the net balance of mineralization and immobilization processes. One set of triplicate samples was extracted at day 0 and one at day 25 with 0.5M K_2SO_4 for 1 h and concentrations of NH_4^+ and NO_3^- were measured using a Lachat Autoanalyzer as described above. Net N mineralization was calculated as the difference in $NH_4^+ + NO_3^-$ pool size at the end and beginning of the incubation period, and net nitrification was calculated as the change in NO_3^- pool sizes.

2.3. Statistical analyses

Given the goals of our study, to both describe pattern in data and discern drivers of pattern, we used several different but complimentary statistical approaches. To describe pattern, we used linear regressions and analysis of variance (ANOVA), as well as non-linear regression for C decomposition kinetics. To test for drivers of process rates we used structural equation modeling (SEM).

For all analyses, distributions were analyzed for normality, and non-normal data were log-transformed and rechecked for normality. In the case of non-normal data that included values ≤ 0, data were linear-transformed prior to log-transformation. A correlation matrix was derived for all variables using least-squares fitting with JMP. Net N mineralization and microbial respiration data were then examined for outliers, where outliers were operationally defined as data that were beyond three standard deviations of the mean. In multiple linear regression analyses, residuals were examined for normality and to identify data with leverage and influence inconsistent with the full dataset; such data points were also excluded (Martin, 1992). To compare differences among different vegetation types, data were analyzed using analysis of variance (ANOVA) with Tukey-HSD post-hoc comparisons using the group-wise $\alpha = 0.05$.

We used structural equation modeling to build, test, and optimize models for our first 25 days of respiration and net N mineralization data to examine the importance of different causal associations in our data (Grace, 2006). Structural equation modeling (SEM) is a multivariate statistical tool that uses the covariance among many variables to build models that test pathways of influence among those variables. With SEM it is possible to use knowledge of the system to represent factors in the test as proximal (immediate drivers of the process at hand) or distal (factors that control the immediate drivers of the process). This technique goes beyond standard multiple regression or ordination approaches which indicate variables related to the process of interest, but do not show how the variables interact among each other to produce the overall effects.

Our first step was to create a conceptual model of how the many variables were causally related to one another and to our response variables. Based on bivariate regressions, we included only MAT, MAP, C, N, Ca^{2+}, pH, microbial biomass, clay, and silt in our model, as other components were poorly correlated with both our process based metrics and other characteristics. Since samples were incubated under standard temperature and moisture conditions, we assumed that MAT and MAP only acted on our response variables indirectly through their influence on soil texture, soil C and N, Ca^{2+} and pH. We assumed that soil C and N alone among our variables would influence microbial biomass directly, as bivariate regressions with texture, pH, and base cations showed little direct association with microbial biomass. While clay is thought to directly affect organic matter, due to our wide range of samples, organic matter did not tightly correlate with clay and so we did not include this path in our base model. Finally, we assumed that soil texture, C, N, Ca^{2+}, pH, and microbial biomass could directly influence net N mineralization and microbial respiration.

These assumptions led us to the base model pictured in Fig. 1. The boxes are indicator or response variables, and each arrow represents a causal connection between variables, or a causal path. The direction of the arrow indicates the direction of causation, and two headed arrows represent nondirected covariance between two factors. Arrow length does not indicate the strength of the connection, but rather are based on what is necessary to give as clear of a visual representation of the model as possible. When a model is tested, each path has a coefficient and a p-value, which are the slope of the relationship between variables and the probability that the relationship is significant, respectively. For every dependent response variable there is an R^2 value which indicates how well it is predicted by preceding model variables.

The coefficient for each causal relationship can be expressed as either a standardized or an unstandardized form. Standardized path coefficients are based on variance relationships and are the amount of change in units of standard deviations of the dependent variable per one standard deviation change in the independent variable. Unstandardized coefficients are based on relationships between the variables directly and represent the change in the dependent variable that results with a one unit change in the independent variable. When examining overall model structure and effects, standardized coefficients are used as they are more easily compared across the model structure. For mathematical relationships between variables, unstandardized coefficients are used

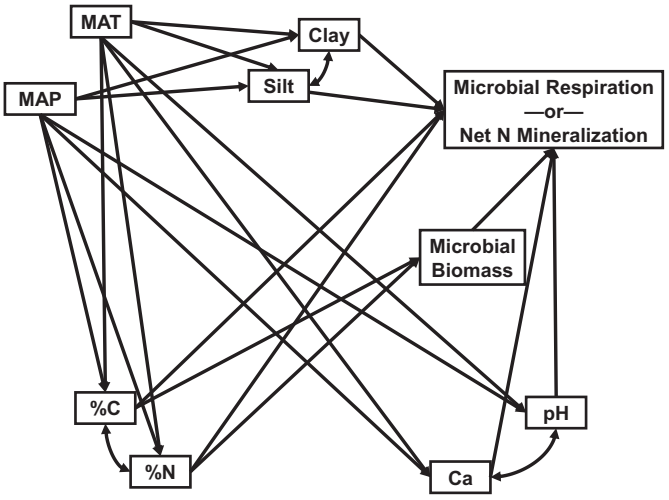

Fig. 1. Base structural equation model with variables (boxes) and potential causal relationships (arrows). Double headed arrows represent covariance between related variables. Arrow direction indicates the hypothesized direction of causation.

(Grace and Bollen, 2005). We present both coefficients to show the quantitative relationships between variables, and the relative importance of the variables within the model.

Our metric of the overall model goodness of fit was the maximum-likelihood generated chi-squared (χ^2) statistic and corresponding whole-model p-value. A low χ^2 and a high p-value (>0.05) suggest that there is little difference between the model and the data, which in modeling is the desired goal. The model was therefore optimized to increase the whole-model p-value. If the p-value was low (≤ 0.05), this indicated that the model fit the data poorly, and so paths or even variables needed to be added or subtracted to make the model consistent with the data. While in linear regression analyses or ANOVA tests it is common for p to be greater than 0.05—making this seem like a low bar to clear—in reality for a χ^2 test of goodness of fit of a SEM, it is quite difficult and requires that no significant path be left out of the model (Grace, 2006).

Model optimization was an iterative process analogous to stepwise multiple regression. First, modification indices—model output based on projected improvements to goodness of fit if suggested changes are enacted—were examined in order to ensure that no important paths were left out of the model. Second, the p-value of each causal path was evaluated, and the evaluation of path p-values followed the "normal" statistical pattern for correlations (*i.e.*, a low value meant a high level of confidence in the association). Paths with coefficients that were not significant at $p \leq 0.05$ were removed and the model was rerun to see if the overall model fit was improved. This iterative process continued until model fit was consistent with the data.

Once we had achieved a model that was consistent with the data, we generated a set of alternative models by eliminating predictor variables and assessing changes in model fit. While χ^2 and p statistics give insight into the overall goodness of fit of the models, they do not correct for the increased likelihood of acceptance for models with higher numbers of predictive variables and causal paths. Thus, to more rigorously compare alternative models, we used Akaike's Information Criterion (AIC) to determine the model that was most parsimonious with the data (Burnham and Anderson, 2002). When considering two models, both of which are consistent with the data, if the difference in AIC between the two models is >7, then this indicates strong support for the model with the lower AIC value. With models that had similar AIC values, further inference could be gained from examining Hoelter's critical N for the significance level of 0.05 (Hoelter, 1983). This number gives the number of samples yielding the same χ^2 and p value that would be required to show the data are not consistent with the model with larger numbers signifying a model less likely to be shown to be inconsistent with the data with a higher sample size.

3. Results

3.1. Patterns in soil and site characteristics

Soils encompassed a range of physical, chemical, and climate characteristics (Table 1). Mean annual temperature ranged from −9.3 to 22.8 °C, while mean annual precipitation ranged from 150 to 5000 mm. The pH of soils sampled ranged from 3.56 in a hemlock forest (Site 15) to 9.86 in desert pavement shrubland (Site 58), which represents a difference in hydrogen ion concentration of over six orders of magnitude. Soil carbon ranged from a low of 0.08% in desert shrubland (Site 55) to a high of 18.2% in a Hawaiian grassland soil (Site 38), while nitrogen ranged from 0.018 to 1.6% in the same two soils. Across all soils, C and N contents were highly correlated ($r^2 = 0.84$; Fig. 2), though C:N ratio ranged from 4.4 in a desert shrubland (Site 55), to 38 in a Southeastern coniferous forest (Site 25). Similarly, DOC and DON were also correlated ($r^2 = 0.67$), and DOC:DON had a similar range from 3.9 in a desert shrub site (Site 56) to 32.6 in a semi-arid coastal shrub site (Site 74). Not only was C correlated with N in both DOM and SOM, DOC correlated with C ($r^2 = 0.29$) and DON correlated with N ($r^2 = 0.44$), indicating a strong connection between DOM and SOM.

The overall trends in levels of C, N, DOC, and DON paralleled each other across vegetation types. Post-hoc tests (Tukey-HSD) showed that deciduous/broadleaf forests were always among the highest in all C and N characteristics (Fig. 3). The mean C concentration in forest and grassland soils was not different, and all were higher in C concentration compared to shrublands. The mean N concentration of coniferous forest soils was not different from deciduous/broadleaf and grasslands soils (which both had the highest N concentration), or shrublands (which had the lowest N concentration). The trends for DOC and DON were similar to those observed for C and N, respectively. DOC concentrations of coniferous forest and grassland soils were not different from either deciduous/broadleaf forest, or from shrubland. Forests had the highest DON, while grasslands were not different from forests or shrublands.

3.2. Microbial respiration, microbial biomass, net N mineralization, and net nitrification

Microbial respiration spanned from 0.18 to 23 μg C g^{-1} soil day^{-1} in a desert soil (Site 58) and Hawaiian grassland soil (Site 39), respectively. Microbial respiration differed by vegetation type ($p = 0.001$; Fig. 4), and the pattern was similar to that of organic C pools, where forests and grasslands were not significantly different from one another, and were all consistently higher than shrubland soils. Microbial biomass ranged from 0.5 to 18.4 μg C g^{-1} dry soil h^{-1} (Sites # 55 and 39, respectively), and like microbial respiration, it also differed among vegetation types ($p = 0.003$), with grassland and forest soils having significantly higher microbial biomass than shrublands (Fig. 4).

Fitting the respiration data with the first order kinetic model, the model succeeded for 75 of the 84 soils. For the other nine, the data were linear, and were best fit with a straight line, suggesting either a very large bioavailable C pool with no sign of depletion, or that some resource other than C supply constrains respiration rates in those soils. The k values varied from 0.013 wk^{-1} in a New York grassland soil (Site #45) to 0.49 wk^{-1} in a desert soil (Site #58); estimates of C_0 ranged from 0.008 mg C g^{-1} soil in a Hawaiian grassland soil (Site #36) to 7.4 mg C g^{-1} soil in a grassland in New York (Site #45).

Net N mineralization ranged from net immobilization of −0.22 μg N g^{-1} soil day^{-1} in a Midwestern hardwood forest (Site #47), to net mineralization of 3.6 μg N g^{-1} soil day^{-1} in a Southwestern spruce forest (Site #83). Even though N pool size differed between vegetation types, net N mineralization had no such pattern by vegetation type (Fig. 4, $p = 0.98$). Net nitrification was on average 88% of net N mineralization, but there was no relationship between net nitrification and pH (Fig. 5), with high values spread across the pH range.

3.3. Modeling drivers of respiration

The base structural equation model was designed to include all likely causal linkages, including climate, soil C and N, soil chemistry, and soil texture to determine whether there is a universal large-scale pattern. Our base structural equation model for microbial respiration showed that many of the variables proved redundant or unnecessary, which gave an overall poor model fit. The χ^2 for the base model was 135 and the p value was <0.0001 (AIC = 208, H05 = 18), which indicates that the model was not consistent with

Table 1
Site and soil characteristics for samples used in this study.

Soil ID	Name of area	Vegetation type	MAT °C	MAP mm	%C	%N	pH	Ca mEq/ 100 g dry soil	Silt %	Clay %	Microbial respiration μg C or N g^{-1} soil day^{-1}	Net N mineralization μg C or N g^{-1} soil day^{-1}	Net nitrification μg C or N g^{-1} soil day^{-1}	SIR biomass μg C g^{-1} soil h^{-1}	C_0 μg C g^{-1} soil	k Wk^{-1}
1	Bear Brook Watershed, ME	Coniferous	6.1	1200	12.8	0.63	4.25	0.2	34	7	6.5	0.14	0.20	4.1	121	0.11
2	Bear Brook Watershed, ME	Deciduous/Broadleaf	6.1	1200	5.2	0.24	4.6	0.5	36	8	2.6	0.02	0.07	2.2	48	0.11
3	Bousson Experimental Forest, PA	Deciduous/Broadleaf	7.8	1000	6.4	0.47	4.05	1.4	50	10	6.5	1.1	1.04	5.5		
4	Bousson Experimental Forest, PA	Deciduous/Broadleaf	7.8	1000	9.5	0.63	3.61	1.4	46	10	7.9	1.06	0.75	5.5		
[a]5	Badlands National Park, SD	Grassland	6.6	450	3.1	0.21	7.53	31.3	53	12	10.7	1.07[a]	1.23	5.3	457	0.26
6	Bonanza Creek LTER, AK	Coniferous	−2.9	260	3.0	0.16	5.12	4.6	68	12	5.6	−0.03	0.08	3.1	262	0.22
7	Bonanza Creek LTER, AK	Coniferous	−2.9	260	3.0	0.15	5.16	7.2	69	10	10.8	0.39	0.50	5.2	506	0.22
8	Bonanza Creek LTER, AK	Coniferous	−2.9	260	3.7	0.18	5.36	5.3	68	12	13.2	0.01	0.02	5.0	663	0.20
9	Cedar Mountain, AZ	Coniferous	10.3	400	1.7	0.13	7.27	13.1	51	22	3	0.38	0.38	3.0	171	0.16
10	Cedar Mountain, AZ	Shrubland	10.3	400	2.2	0.16	8.02	20	49	25	2.3	0.11	0.11	2.0	105	0.22
11	Cedar Mountain, AZ	Shrubland	10.3	400	1.5	0.12	7.02	14.3	51	24	0.7	0.09	0.09	2.0	30	0.24
12	Cedar Creek LTER, MN	Grassland	5.8	720	1.9	0.13	6.06	2.5	6	5	5.1	0.18	0.29	2.9	80	0.14
13	Catskills, NY	Deciduous/Broadleaf	5.3	1300	2.6	0.19	3.92	0.5	37	12	1.9	0.59	0.50	2.4		
14	Catskills, NY	Deciduous/Broadleaf	5.3	1300	4.1	0.21	3.63	0.1	19	9	3.1	0.6	0.22	1.9		
15	Catskills, NY	Coniferous	5.3	1300	4.3	0.26	3.56	0.2	57	20	2.7	0.43	0.20	2.3		
16	Calhoun Experimental Forest, SC	Deciduous/Broadleaf	15.9	1250	2.3	0.12	5.68	2.7	23	12	6.2	0.48	0.25	3.1	268	0.25
17	Calhoun Experimental Forest, SC	Grassland	15.9	1250	2.3	0.18	5.57	1.9	16	9	5.4	0.26	0.26	3.5	283	0.18
18	Calhoun Experimental Forest, SC	Coniferous	15.9	1250	1.2	0.05	4.89	1.4	12	7	5.5	0.12	0.00	2.0	312	0.16
19	Calhoun Experimental Forest, SC	Grassland	15.9	1250	1.7	0.13	5.03	1.5	19	17	2.9	0.31	0.32	2.5	173	0.15
20	Clymer Meadow Preserve, TX	Grassland	18.5	850	3.0	0.24	7.85	39.8	40	42	2.5	0.07	0.07	6.7	191	0.11
21	Front Range near Ft. Collins, CO	Grassland	−3	600	1.6	0.08	6.13	3.4	9	5	6.5	0.72	0.78	2.8	235	0.32
22	Front Range near Ft. Collins, CO	Coniferous	6.1	350	1.8	0.06	5.68	4.5	18	6	11.9	0.08	0.00	3.0	704	0.15
23	Shortgrass Steppe LTER, CO	Grassland	9.3	322	0.82	0.07	6.02	4.8	16	8	3.4	0.68	0.70	1.9	151	0.24
24	Coffey Ranch, TX	Grassland	18.4	850	2.8	0.27	8	34.5	49	15	3.7	0.01	0.02	3.1	207	0.17
25	Duke Forest, NC	Coniferous	14.6	1100	2.8	0.07	5.37	2.4	37	6	15	−0.02	0.00	3.8	1100	0.12
26	Duke Forest, NC	Deciduous/Broadleaf	14.6	1100	5.5	0.30	6.84	9.2	44	9	24.4	0.92	1.11	13.8	1433	0.17
27	Duke Forest, NC	Deciduous/Broadleaf	14.6	1100	1.7	0.07	5.05	0.3	15	5	5.8	0.61	0.26	3.5	243	0.26
28	Great Basin Experimental Range, UT	Grassland	2	400	2.8	0.23	6.84	14.8	44	33	7.3	0.08	0.08	9.5	112	0.14
29	Great Basin Experimental Range, UT	Deciduous/Broadleaf	2	400	6.9	0.53	7.57	34.4	43	22	7	0.2	0.20	5.6	82	0.21
30	Great Basin Experimental Range, UT	Coniferous	2	400	5.7	0.40	7.18	25.6	48	28	7.1	0.1	0.11	6.7	172	0.08
31	Great Basin Experimental Range, UT	Grassland	2	400	3.6	0.29	6.85	19.9	24	38	5.6	0.18	0.18	11.2	93	0.13
32	Great Basin Experimental Range, UT	Shrubland	4.8	400	1.7	0.14	8.22	28.1	55	15	7.4	0.36	0.36	3.5	154	0.09
33	Great Basin Experimental Range, UT	Grassland	2	400	2.2	0.20	7.23	16.2	45	34	4.3	0.02	0.02	7.8	49	0.22
34	Harvard Forest LTER, MA	Deciduous/Broadleaf	7	1100	12.8	0.57	4.25	0.4	24	9	18.4	0.37	0.00	12.5	1127	0.15
35	Harvard Forest LTER, MA	Coniferous	7	1100	9.6	0.46	3.98	1.3	30	8	12.5	0.93	0.91	9.3	851	0.13
36	Hawai'i, HI	Grassland	22.8	250	1.1	0.10	6.45	4.1	43	15	0.7	0.03	0.16	0.7	8	0.23
[a]37	Hawai'i, HI	Grassland	22.8	750	15.9	1.44	6.32	20.7	32	4	14.3	0.45[a]	0.36	13.5	204	0.16
[a]38	Hawai'i, HI	Grassland	22.8	1000	18.2	1.62	6.53	25.6	21	4	14.6	0[a]	0.00	11.2	423	0.06
39	Hawai'i, HI	Grassland	22.8	1500	10.8	0.71	4.92	2.2	34	18	24.4	0	0.06	18.4	983	0.05
40	HJ Andrews LTER, OR	Coniferous	9.4	2000	7.0	0.19	5.41	1.6	28	13	6.5	0	0.00	5.7	358	0.17
41	HJ Andrews LTER, OR	Deciduous/Broadleaf	9.4	2000	7.6	0.29	5.36	6	32	15	6	0.44	0.57	5.7	294	0.20
42	Institute for Ecosystem Studies, NY	Grassland	8.6	1200	2.7	0.23	5.27	2.4	38	11	7.9	0.04	0.35	4.4	1821	0.04
43	Institute for Ecosystem Studies, NY	Grassland	8.6	1200	4.1	0.31	5.52	2.9	38	11	11.6	0.41	0.32	7.5	4570	0.02
44	Institute for Ecosystem Studies, NY	Grassland	8.6	1200	6.4	0.53	5.72	6.7	33	8	16	1.18	1.19	10.2		
45	Institute for Ecosystem Studies, NY	Grassland	8.6	1200	3.3	0.28	6.29	5.4	36	10	11.3	0.11	0.14	6.8	7381	0.01
46	Institute for Ecosystem Studies, NY	Grassland	8.6	1200	5.3	0.41	5.57	4.4	32	10	25.2	0	−0.09	11.2	2299	0.10
[a]47	Itasca State Park, MN	Deciduous/Broadleaf	3	750	6.3	0.28	5.78	8	22	5	18.8	−0.22[a]	0.07	7.5	357	0.11
48	Itasca State Park, MN	Coniferous	3	750	3.9	0.17	5.42	4.3	14	5	19	0.16	0.00	4.5	349	0.11
49	Konza Prairie LTER, KS	Grassland	12.5	835	6.1	0.45	6.37	17.3	63	15	5.5	0.25	0.25	8.2	360	0.13
50	Konza Prairie LTER, KS	Grassland	12.5	835	4.6	0.35	6.5	15.3	60	17	4.8	0.13	0.13	5.2	251	0.18
51	Konza Prairie LTER, KS	Shrubland	12.5	835	6.9	0.39	7.92	35	51	25	2.3	0.14	0.14	4.7	175	0.10
52	Luquillo LTER, PR	Deciduous/Broadleaf	19.3	5000	14.0	0.57	4.89	1	51	13	5.4	−0.1	0.06	3.5		
53	Luquillo LTER, PR	Deciduous/Broadleaf	21.5	3500	4.1	0.32	5.03	1.5	45	39	7.2	0.08	−0.08	8.0		

(*continued on next page*)

Table 1 (*continued*)

Soil ID	Name of area	Vegetation type	MAT °C	MAP mm	%C	%N	pH	Ca mEq/ 100 g dry soil	Silt %	Clay %	Microbial respiration μg C or N g^{-1} soil day^{-1}	Net N mineralization μg C or N g^{-1} soil day^{-1}	Net nitrification μg C or N g^{-1} soil day^{-1}	SIR biomass μg C g^{-1} soil h^{-1}	C_0 μg C g^{-1} soil	k Wk^{-1}
54	Luquillo LTER, PR	Deciduous/Broadleaf	20.5	4500	6.4	0.28	4.67	0.7	17	18	3.5	0.04	0.12	3.6		
55	Mojave Desert, CA	Shrubland	21	150	0.08	0.02	8.83	32.3	25	17	0.2	0	0.06	0.5	10	0.30
56	Mojave Desert, CA	Shrubland	21	150	0.42	0.05	7.65	9.4	15	11	3	0.48	0.45	2.0	100	0.42
57	Mojave Desert, CA	Shrubland	21	150	0.12	0.02	7.9	4.3	14	5	1.3	0.12	0.11	1.2	50	0.33
58	Mojave Desert, CA	Shrubland	21	150	0.12	0.02	9.86	25	34	13	0.3	0	0.01	1.1	9	0.49
59	Mojave Desert, CA	Shrubland	21	150	0.57	0.06	8.07	16.2	13	7	8.1	0.97	1.00	2.4	312	0.32
60	Mary's Peak, OR	Grassland	8.8	2200	10.7	0.73	4.56	1.5	32	9	7.4	0.52	0.61	12.3	364	0.20
61	Mary's Peak, OR	Coniferous	8.8	2200	9.9	0.54	4.38	0.3	33	9	8.8	0.93	0.44	11.6	501	0.17
62	USDA Grassland Research Center, TX	Shrubland	18.1	840	3.9	0.32	7.92	41.2	44	38	2.4	0.31	0.30	5.6	122	0.20
63	USDA Grassland Research Center, TX	Grassland	18.1	840	3.8	0.31	8.07	42.6	45	35	2.2	−0.18	−0.13	5.9	107	0.20
64	Sunset Crater, AZ	Coniferous	10.3	400	2.3	0.13	6.9	4.8	5	4	5	0.36	0.37	2.2	286	0.16
65	Sunset Crater, AZ	Shrubland	10.3	400	2.5	0.15	8.1	14.4	3	5	7.5	0.75	0.75	2.1	726	0.08
[a]66	Sunset Crater, AZ	Shrubland	10.3	400	2.3	0.13	7.25	2.5	1	3	1.1	0.03[a]	0.03	0.7	68	0.14
67	Santa Barbara, CA	Shrubland	15	550	2.7	0.16	7.92	24.3	39	15	9.3	0.18	0.20	3.5	428	0.23
[a]68	Sierra Nevada Mts., CA	Coniferous	3.6	600	4.3	0.19	4.95	2.6	13	7	14.6	1.64[a]	1.40	4.4	1246	0.10
69	Sierra Nevada Mts., CA	Grassland	3.6	600	0.39	0.03	5.58	0.2	6	4	0.8	0.1	0.06	0.9	36	0.24
70	Sierra Nevada Mts., CA	Shrubland	3.6	600	1.7	0.12	5.74	1.9	14	6	8.5	0.91	0.88	4.5	341	0.30
71	Sequoia National Park, CA	Shrubland	12.7	650	1.7	0.09	6.25	7.5	24	12	10.1	0.05	0.06	8.1	528	0.18
[a]72	Sequoia National Park, CA	Grassland	3.6	750	8.1	0.51	5.13	0.9	18	6	8.2	−0.65	−0.59	10.2	475	0.16
73	Sedgwick Reserve, CA	Deciduous/Broadleaf	17.2	500	4.6	0.41	6.84	18.1	37	18	7.8	0.45	0.50	6.4	369	0.23
74	Sedgwick Reserve, CA	Shrubland	17.2	500	1.5	0.13	8	27.4	28	20	3.3	0	0.03	2.6	139	0.24
75	Sedgwick Reserve, CA	Grassland	17.2	500	3.3	0.30	6.95	10.6	40	21	9.7	0.36	0.45	10.9	392	0.29
76	Sevilleta LTER, NM	Shrubland	13.5	210	0.30	0.06	8.31	20.3	15	7	2.9	0.62	0.62	1.2	115	0.27
77	Sevilleta LTER, NM	Grassland	13.5	210	0.23	0.05	8.44	24.2	12	8	2	0.45	0.45	1.1	88	0.23
78	Sevilleta LTER, NM	Shrubland	13.5	210	0.30	0.06	8.31	22.4	17	7	3.3	0.69	0.69	1.2	193	0.15
79	Sevilleta LTER, NM	Grassland	13.5	210	0.27	0.05	8.29	20.6	17	7	2.8	0.52	0.52	1.2	118	0.26
80	Toolik Lake LTER, AK	Grassland	−9.3	400	7.0	0.38	4.58	0.8	37	20	11.7	−0.09	−0.01	5.3	656	0.17
81	Toolik Lake LTER, AK	Shrubland	−9.3	400	15.8	0.96	6.47	37.8	55	6	15.2	0.5	0.57	8.0	870	0.16
82	Toolik Lake LTER, AK	Shrubland	−9.3	400	5.4	0.22	4.23	2	42	10	20.7	0	0.00	8.8	1191	0.16
[a]83	Valles Caldera National Preserve, NM	Coniferous	2.5	500	5.7	0.33	5.55	8.9	32	9	16.9	3.59[a]	2.91	7.3	2050	0.07
84	Valles Caldera National Preserve, NM	Grassland	2.5	500	3.4	0.30	5.99	9.7	49	10	6.6	0.71	0.72	4.4	1538	0.04

[a] Samples excluded from net N mineralization model due to excessive leverage or inference.

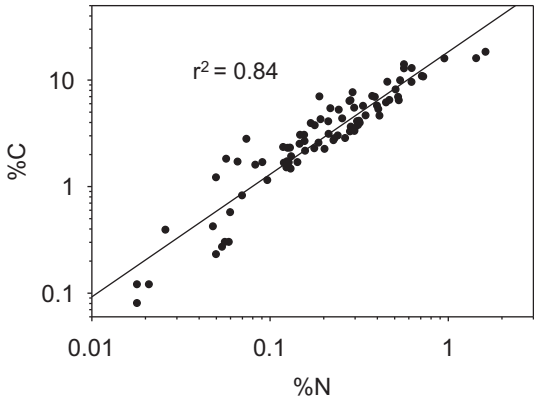

Fig. 2. Percent organic nitrogen in soils compared to percent organic carbon in all soil samples collected for this study ($n = 84$), with best fit line ($r^2 = 0.84$).

the data, and thus needed to be revised. The $R^2 = 0.80$ for microbial respiration, which shows that the factors in the base model predicted the amount of respiration well despite the fact that the model was inconsistent with the data due to unnecessary pathways.

To improve model fit, we first looked at modification indices. There were two paths missing that were added into the revised model (Fig. 6a). The first represented an influence of clay content on microbial biomass, while the second accounted for covariance in the data between MAT and microbial respiration (directed path from MAT to Microbial respiration). By explicitly representing these correlations, the overall model fit improved. Then, we removed paths whose p-values were not significant at $p \leq 0.05$. After eliminating many paths and adding the two new paths, the model was consistent with our data ($\chi^2 = 5.2$, $p = 0.74$, AIC = 31.2, H05 = 249). This revised model (Fig. 6) contained MAT, MAP, %C, microbial biomass, % clay, and explained 78% of the variance in microbial respiration, indicating that little predictive power was lost when the model was revised, while at the same time it more accurately represented the causal associations and drivers of microbial respiration.

3.4. Modeling N mineralization

After removing two outliers with unusually high net N mineralization rates (Sites #68 and #83), and six other soils (Sites #5, 37, 38, 47, 66, and 72) that had disproportionate influence and leverage on the residuals of multiple linear regression analyses, we then tested our base model on this remaining set of 76 soils. Much like for microbial respiration, the base model for net N mineralization failed, with a $\chi^2 = 92.1$ and $p < 0.0001$, suggesting that the model was not consistent with the data.

After adding in pathways suggested by the modification indices to yield improvements in model fit, and after removing path coefficients that were not significant at $p < 0.05$ as well as variables that were not directly or indirectly related to N mineralization, we were left with the following predictive variables: MAT, MAP, Clay, %C, and % N. Paths were added to reflect the covariance in %C and %N explained by clay content, yielding a reduced model with a $\chi^2 = 3.4$ and $p = 0.64$, indicating the model was consistent with the data. To test the importance of both climate variables to the proper functioning of the model, we removed either MAT or MAP and examined changes in model fit by first examining changes in the Akaike Information Criterion (AIC) values (see Section 2.3). The model with just MAP gave a decrease in AIC value from 35.3 to 26.6, while the model with just MAT had an AIC value of 28.1. These two AIC values were not different enough to discriminate between these two models based on AIC alone, but both were improvements over the model with both climate variables included. To discriminate between these two simplifications of our model, we next turned to Hoelter's critical N for the significance level of 0.05 (H05; see Section 2.3). The H05 values for our two models with individual climate factors were 214 and 696 for the model containing only MAT or MAP, respectively, suggesting that the model with MAP is a more robust model. This reduced model (Fig. 6b) has $R^2 = 0.33$, $\chi^2 = 0.65$, and $p = 0.72$.

4. Discussion

4.1. Microbial respiration

From fitting our respiration data to the kinetic model two patterns arise, the first a positive correlation between C_0 and total C

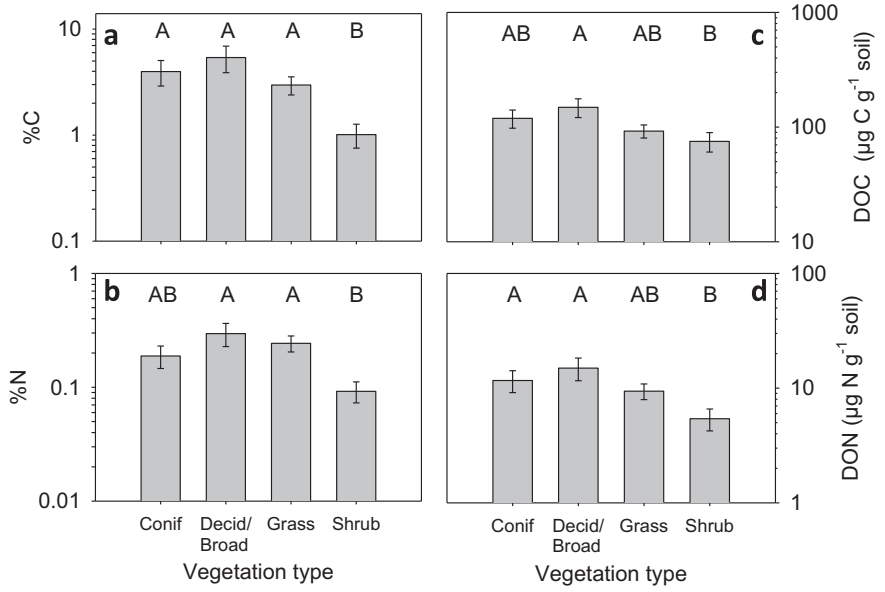

Fig. 3. Average **a**. %C, **b**. %N, **c**. DOC, and **d**. DON by vegetation type (Coniferous, Deciduous/Broadleaf, Grassland, and Shrubland, $n = 17$, 16, 31, and 20 respectively). Error bars are 95% confidence interval. Shared letters denote no significant difference at $p < 0.05$ between treatments (ANOVA, Tukey-HSD post-hoc test).

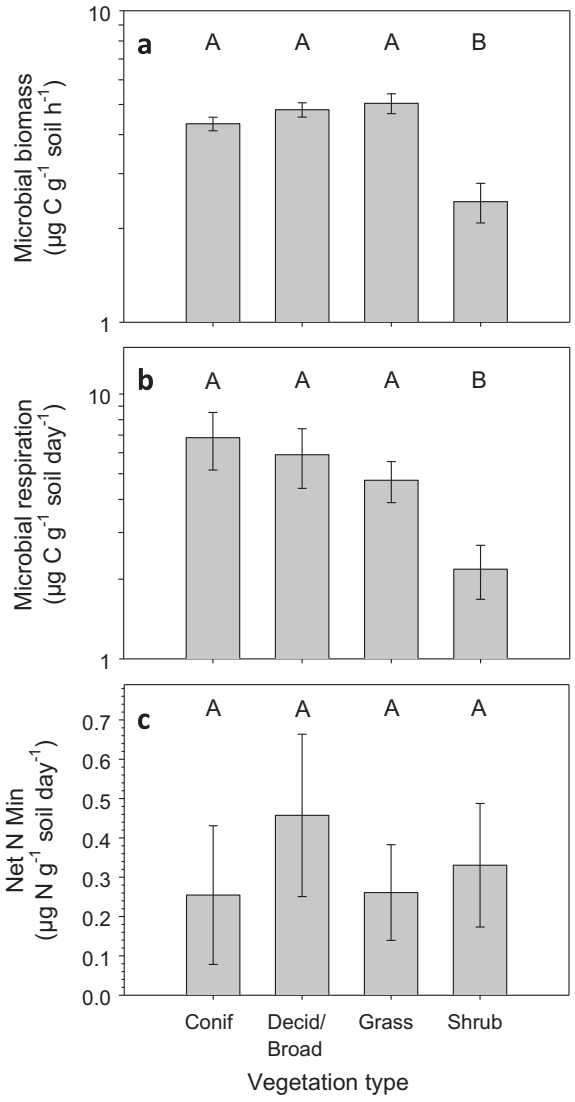

Fig. 4. Average **a**. microbial biomass, **b**. microbial respiration, and **c**. net N mineralization. Error bars are 95% confidence interval. Shared letters denote no significant difference at $p < 0.05$ between treatments (ANOVA, Tukey-HSD post-hoc test).

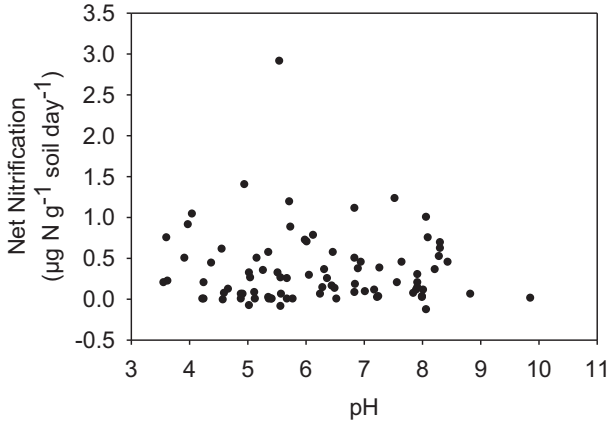

Fig. 5. Net nitrification over 25 days plotted against soil pH.

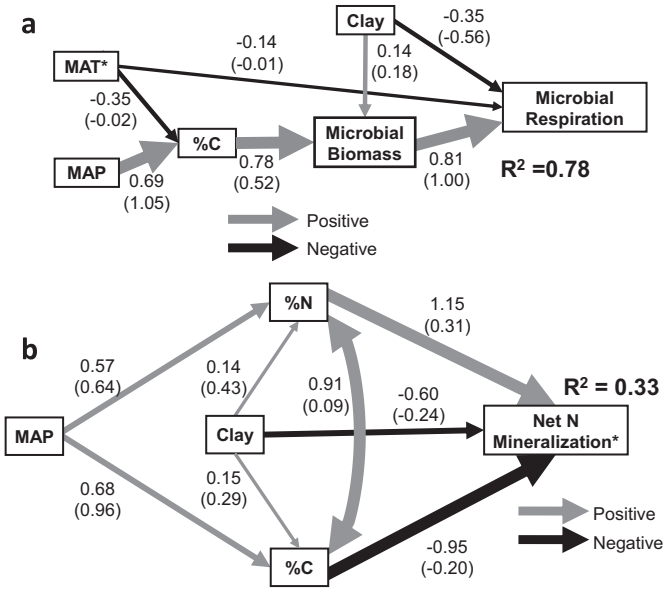

Fig. 6. Revised model for **a**. microbial respiration, and **b**. net N mineralization. Arrow width indicates effect strength, and arrow color denotes either positive (gray) or negative (black) relationships. Numbers are standardized path coefficients with non-standardized coefficients in parentheses. Model for microbial respiration (A) had a $\chi^2 = 5.2$, $p = 0.74$, and AIC = 31, while the model for Net N mineralization (B) had a $\chi^2 = 3.8$, $p = 0.29$, and AIC = 38. All variables log transformed for normality with the exception of MAT and Net N mineralization (indicated by *).

pool ($r^2 = 0.3$), and the second an inverse correlation between k and C (either expressed as C_0, $r^2 = 0.33$; or C, $r^2 = 0.37$). Thus, soils with larger C pools tended to have more labile C (C_0), but these same soils also tended to have lower decay constants (k) indicating slower respiration per unit C.

While the direction of causality is not readily discerned between k, C, and C_0 we postulate that this result occurs because as total C increases, the fraction that is protected by soil physicochemical mechanisms increases—giving a positive correlation between C and C_0—but the physicochemically protected fraction increases to a greater degree than does the labile fraction, giving a negative correlation between the overall decay constant, k, and our metrics of C pool size. Thus, even if the chemical quality of each specific pool were the same across all ecosystems, such a pattern would produce a lower overall k value as total soil C increased. It makes intuitive sense that SOM that is bioavailable—both with regard to physicochemical protection and chemical lability—has a much more finite accumulation capacity and will get consumed. This may suggest that as the SOM pool increases, microbes and their extracellular enzymes are less capable of breaking down the SOM, even though there may be a larger pool of potentially labile C. This would be consistent with predictions that a spatial separation of microbes and their enzymes from SOM would lower the ability of the microbial community to degrade the SOM (Schimel and Weintraub, 2003) and yield a lower k value. However, it may also/instead be that the inherent decomposability of SOM as reflected by k is driving the size of the C pool.

Turning from kinetics to modeling the relationship between potential predictors and microbial respiration, the revised structural equation model (Fig. 6) shows that three factors directly influenced microbial respiration: microbial biomass had a large positive effect, mean annual temperature appeared to have a slight positive effect, and clay content had a small negative effect on respiration (Fig. 6). There were also four soil and site characteristics that indirectly influenced microbial respiration: mean

annual temperature, mean annual precipitation, clay content, and organic C concentration.

Microbial biomass has often been found to be correlated positively with respiration (Bekku et al., 2004; Parfitt et al., 2005; Pinzari et al., 1999; Wang et al., 2003), and in our study we found that microbial biomass at the beginning of our incubation was correlated with respiration over the course of the entire 25 day incubation. Indeed, it is intuitive that there should be an overall correlation between microbial biomass and respiration, because they are both sensitive to total soil C as a master variable. What is less intuitive is that, while the model allowed for both a direct and indirect influence of soil C on respiration, only the indirect influence mediated by soil microbial biomass remained in the model. This is surprising given that, in mineral soils, it has long been argued that the bulk of the microbial community is in a passive or dormant starvation state, though physiologically ready to act (Brookes et al., 1987). Part of this may be that the main processes regulating substrate consumption and microbial respiration appear to be the physical and chemical processes of disruption, desorption, and diffusion (Navarro-Garcia et al., 2011; Schimel et al., 2011), not just C concentration. Regardless, soil C had only an indirect influence on soil respiration as mediated by microbial biomass.

Clay had a direct negative effect on microbial respiration across vegetation and ecosystem types, which is consistent with clays protecting SOM from microbes (Dilustro et al., 2005; Franzluebbers, 1999; Hassink, 1992; Zak et al., 1994). This estimate of clay effects on respiration is likely conservative since our sample pretreatment (sieving and adding water) broke down macroaggregates, which are one way clays contribute to protecting SOM.

To examine the magnitude of the indirect effects of climate on respiration, all the path coefficients connecting them to our response variable were multiplied (Grace, 2006; Fig. 5, standardized values are shown in Table 2). The indirect effect of MAT was stronger than its direct effect, but MAP had a positive albeit indirect influence that was stronger still. For both climate factors, organic C mediated their effect on microbial biomass, which then directly influenced microbial respiration. The importance of the indirect effects of climate in the revised microbial respiration model (Fig. 6) fits with our conceptual model of how climate drives soil formation (Jenny, 1941). As MAT increases, so does *in-situ* decomposition of SOM and plant litter, leading to smaller C pools. Thus from our model, we show evidence that soils from climates with higher MAT, all other things being equal, will tend to have less C, microbial biomass, and less microbial respiration (Amundson, 2001; Barrett and Burke, 2000; Jenny, 1941). It also is consistent with reports that as MAP increases, plant productivity increases, which in conjunction with inhibition of microbial respiration as soils spend more time saturated and thus anaerobic can lead to higher C pools. Together, these can lead productivity to outpace decomposition, thus causing SOM to accumulate (Amundson, 2001; Jenny, 1941). A larger pool of SOM can sustain a larger microbial biomass, and under aerobic conditions such as those used in our laboratory incubations these factors would lead to higher respiration. Thus temperature and precipitation—by driving SOM pool size in contrasting ways—indirectly drive microbial respiration.

Table 2
Standardized direct, indirect, and total effects on microbial respiration (A) and net N mineralization (B) of climate and soil characteristics from the revised models.

(A)	MAP	MAT	%C	Clay	Biomass
Direct	0	−0.14	0	−0.35	0.81
Indirect	0.44	−0.22	0.64	0.11	0
Total	0.44	−0.36	0.64	−0.24	0.81

(B)	MAP	%C	%N	Clay
Direct	0	−0.95	1.15	−0.60
Indirect	0.015	0	0	0.18
Total	0.015	−0.95	1.15	−0.42

Neither pH nor Ca^{2+} were included in the final model, despite their role in stimulating microbial activity (Anderson, 1998; Baath and Arnebrant, 1994; Badalucco et al., 1992) or the suggested role of Ca^{2+} in cation bridging and protecting organic matter (Muneer and Oades, 1989). We hypothesize that it was possible to remove Ca^{2+} and pH as explicit variables because they are subsumed to some degree into the more global variables (MAT, MAP, clay). Temperature and moisture regulate weathering by stimulating dissolution and leaching and thus control Ca^{2+} and pH, while clay plays an important role in retaining base cations.

The revised model also showed that 60% of the variance in soil organic carbon content could be predicted by MAP and MAT. Furthermore 63% of the variance in microbial biomass could be predicted, and a structural equation model for microbial biomass using only MAT, MAP, clay, and C is consistent with the data ($\chi^2 = 3.3$, $p = 0.19$). This further illustrates the importance of climate as not only an immediate driver of *in situ* microbial process rates through the direct effects of temperature and moisture, but also reminds us that climate is also a key driver of soil formation (Jenny, 1941). Through the legacy of climate on soil characteristics, climate drove microbial biomass and microbial respiration in this study.

While advances in analyzing and understanding microbial community composition, soil organic matter chemistry, and clay mineralogy are all pushing our understanding of soil microbial ecology in new directions, our present analysis omitted all three and yet it still explains 78% of the variance in respiration. Our model thus suggests that when it comes to microbial respiration, quantity—of microbial biomass, clay, and C—trumps quality. Quality may covary with the quantity, but the most unique variance that quality could explain is the 22% of variance unexplained by the factors in our revised model. This should not be seen as undermining the potential importance of any of these three promising frontiers, but rather it puts a bound on the extent that these factors can provide additional unique inference into microbial respiration.

4.2. N mineralization and nitrification

Soil %N varied by two orders of magnitude, with clear differences between vegetation types (Fig. 3b), while net N mineralization ranged from net immobilization of −0.19 μg g^{-1} soil day^{-1} to net mineralization of 1.1 μg g^{-1} soil day^{-1} but did not differ consistently by vegetation type (Fig. 4c). Given the large range of %N, one approach for comparing net N mineralization would be to "normalize" the rates of N mineralization by N pool sizes (sometimes referred to as representing N turnover). While this N turnover would have led to strong univariate correlations between net N mineralization and a whole host of soil and site characteristics, these statistical relationships would result from spurious correlations (Dunlap et al., 1997; Jasieński and Bazzaz, 1999) driven completely by the denominator, N pool size. Soil N was correlated to many other soil and site characteristics (*e.g.*, C concentration; Fig. 2), and having it in the denominator would have led to correlations between this normalized N mineralization with those same factors that are correlated to N pools. Similarly, an approach we could have taken in our modeling efforts would have been to look at a finer scale (*e.g.*, grouping by vegetation type). This too would have generated strong relationships between site/soil characteristics and net N mineralization, but would violate the assumption of multivariate statistics that the number of replicates should be much larger than the number of descriptive parameters.

Across all sites Net N mineralization correlated strongly with net nitrification ($r^2 = 0.88$), consistent with many reports which show a strong relationship between these two N cycle measurements

(Santiago et al., 2005; Schade and Hobbie, 2005; Venterea et al., 2003). However, in contrast to work on nitrification from wastewater and pure culture studies (Gerardi, 2003), as well as some work from soils (Sahrawat, 2008; Ste-Marie and Pare, 1999), we saw no effect of pH on net nitrification, with some of the highest rates of nitrification occurring in soils with the lowest pH. This is in line with studies that have shown high rates of nitrification can occur in acidic soils when NH_4^+ availability is high enough (Booth et al., 2005; Hart et al., 1994).

There are at least three possible explanations for the lack of a pH effect on nitrification in these data. The first is that when there is adequate NH_4^+ supply, and hence energy for autotrophic nitrifiers, they are able to tolerate and function well in acidic soils (Likens et al., 1969; Schimel et al., 2007). The second possibility is that nitrification in the more acidic soils in our study might be due to heterotrophic nitrifiers (Brierley and Wood, 2001; Lang and Jagnow, 1986), which might be less sensitive than autotrophic nitrifiers to low pH. The third possibility is that archaeal nitrifiers alone or in concert with heterotrophs may drive nitrification in low nutrient and low pH systems (Nicol et al., 2008). This is consistent with reports identifying archaeal nitrifiers as being more numerous than bacterial nitrifiers in many systems (Adair and Schwartz, 2008; Leininger et al., 2006; Nicol et al., 2008; Nicol and Schleper, 2006; Prosser and Nicol, 2008), and specializing in conditions of low N availability and low pH (Valentine, 2007). It remains unclear which mechanism is most responsible for high nitrification in some acid soils, and it is quite possible that all three play a role to some degree.

Our revised model of net N mineralization suggests that C, N, and clay concentration were direct drivers of net N mineralization, while mean annual precipitation had an indirect effect mediated by C and N concentration. The positive effect of %N and negative effect of %C on net N mineralization produce the overall pattern that as soil C:N ratio increases net mineralization decreases. The importance of C and N in this model is consistent with our understanding of microbial energy and N requirements: when C availability is high, N mineralization tends to decrease, whereas when N availability is high, N mineralization tends to increase (Schimel and Bennett, 2004). We could have used the C:N ratio in our model, however it would not have been as clear how these two elements were regulating the process. Our work is consistent with other work that shows that the stoichiometry between C and N correlates with N cycling (Manzoni et al., 2008), though by separating out the two factors and explicitly testing them as separate entities, we see specifically how they affect net N mineralization individually.

Some portion of the unexplained variance in net N mineralization may be explained by the fact that this process is really the balance of many competing gross process rates. The extent to which we see pattern in net N mineralization suggests that there is some degree of coherence in the way these process rates relate to drivers such as MAP, C, N, and clay. However, the many inorganic N producing, transforming, and consuming processes, are all likely to have unique drivers. Given that the drivers of these component processes are what ultimately determine the extent of the net rate, understanding the drivers of each of these component processes may be essential to better understand large scale patterns of net N mineralization.

While quantity trumped quality in modeling microbial respiration, quality may have been relatively more important in determining net N mineralization (given that our model $R^2 = 0.33$). Organic nitrogen in soils can range in chemical composition from amines and amides to heterocyclic nitrogen, and different N-forms may interact with soil minerals differently (Kleber et al., 2007). It is likely that the different soils in this study had different percentages of different chemical forms of N. Not only might chemical composition have affected net N mineralization, but these different forms of N in SOM will interact differently with soil minerals, thus influencing the accessibility of organic matter to microbial enzymatic attack (Kleber et al., 2007). Clay mineralogy might also influence the nature of organo-mineral associations (Kleber et al., 2007) and rates of N cycling, as 2:1 clays can fix NH_4^+ in a non-extractable pool (Allison et al., 1951). In addition, while clay and organic C concentration were not tightly correlated, it is likely that the amount of SOM sorbed on clay surfaces as opposed to associated with SOM would be higher for 2:1 clays, which have a higher reactive surface area than 1:1 clays (Six et al., 2002). This intimately associated organic matter is increasingly recognized as being largely protein based (Kleber et al., 2007), and this association with mineral matter likely makes it more resistant to enzymatic attack. Microbial community composition may further aid in explaining the observed patterns in net N mineralization, making it an interesting focus for future work.

5. Conclusions

While microbial respiration and net N mineralization may be tightly and predictably linked to one another in litter and organic soils, our work shows at the continental scale that the two become decoupled in mineral soils on short time scales. Microbial respiration was well explained ($R^2 = 0.78$) by a model with climate, organic C, microbial biomass and clay, while net N mineralization was not as well explained ($R^2 = 0.33$) and was sensitive to MAP, organic C and N, and clay. Net N mineralization is a common, easy to measure, and useful metric when examining soil N cycling, but comparing net N mineralization across a wide range of ecosystems is inherently difficult, similar to predicting net ecosystem exchange of carbon across a range of ecosystems. Both net N mineralization and net ecosystem exchange are controlled by multiple competing processes, each with their own multitude of drivers. If these processes are closely matched, the net measurements are relatively small compared to the sheer magnitude of the element cycled. It is not that the net measurement is unimportant, but rather that it can be a small amount of noise on a very large signal, and understanding the drivers of the component processes would improve our knowledge of the net effect of those processes.

Looking at decomposition as a whole, the traditional view suggests that even after organic matter has passed from fresh plant litter into more processed forms in mineral soils, and despite having been transformed by enzymatic degradation, humification, and condensation, the quality of SOM should still be important. However, in this study of 84 mineral soils, SOM quality did not drive the rate of microbial respiration. Rather, it was the amount of microbial biomass that was the most direct and most important driver of respiration, and this was not driven by SOM quality, but by SOM quantity. The quantity of SOM itself was driven by MAT and MAP, likely because of their effects on the balance between *in situ* primary productivity and consumption.

It may be that much of the organic matter that persists in soil is actually in chemically labile forms (Kleber et al., 2007; Lützow et al., 2006, 2008), but due to the association of labile compounds with the rest of the soil organic matter and directly or indirectly with mineral surfaces, much of the SOM is insoluble and spatially inaccessible to microbes and their enzymes thereby rendering this labile organic matter effectively refractory. This is consistent with our kinetic model results which showed that in soils with larger pools of organic carbon, the pool of relatively labile C was larger as was microbial biomass, but the decay constant decreased. This inverse relationship between decay constant and pool size suggests that microbes are not limited by the inherent chemical recalcitrance of SOM, but rather decomposition gets saturated as SOM

pools increase, perhaps because microbes may be limited by limited access of their enzymes to reactive sites on the organic matter (Schimel and Weintraub, 2003).

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