Preprint

This preprint is a PDF of a manuscript that has been accepted for publication in an ESA journal. It is the final version that was uploaded and approved by the author(s). While the paper has been through the usual rigorous peer review process of ESA journals, it has not been copy-edited, nor have the graphics and tables been modified for final publication. Also note that the paper may refer to online Appendices and/or Supplements that are not yet available. We have posted this preliminary version of the manuscript online in the interest of making the scientific findings available for distribution and citation as quickly as possible following acceptance. However, readers should be aware that the final, published version will look different from this version and may also have some differences in content.

The doi for this manuscript and the correct format for citing the paper are given at the top of the online (html) abstract.

Once the final published version of this paper is posted online, it will replace the preliminary version at the specified doi.
Testing a soil nutrient cycling model

Microbial stoichiometry overrides biomass as a regulator of soil carbon and nitrogen cycling

ROBERT W. BUCHKOWSKI*, OSWALD J. SCHMITZ, MARK A. BRADFORD

School of Forestry and Environmental Studies, Yale University, New Haven, CT 06511, USA

* Corresponding author: robert.buchkowski@yale.edu
ABSTRACT

Understanding the role of soil microbial communities in coupled carbon and nitrogen cycles has become an area of great interest as we strive to understand how global change will influence ecosystem function. In this endeavor microbial-explicit decomposition models are being adopted because they include microbial stoichiometric- and biomass-mediated mechanisms that may be important in shaping ecosystem response to environmental change. Yet there has been a dearth of empirical tests to verify the predictions of these models and hence identify potential improvements. We measured the response of soil microbes to multiple rates of carbon and nitrogen amendment in experimental microcosms, and used the respiration and nitrogen mineralization responses to assess a well-established single-pool microbial decomposition model. The model generally predicted the empirical trends in carbon and nitrogen fluxes, but failed to predict the empirical trends in microbial biomass. Further examination of this discontinuity indicated that the model successfully predicted carbon and nitrogen cycling because stoichiometry overrode microbial biomass as a regulator of cycling rates. Stoichiometric control meant that the addition of carbon generally increased respiration and decreased nitrogen mineralization, whereas nitrogen had the opposite effects. Biomass only assumed importance as a control on cycling rates when stoichiometric ratios of resource inputs were a close match to those of the microbial biomass. Our work highlights the need to advance our understanding of the stoichiometric demands of microbial biomass in order to better understand biogeochemical cycles in the face of changing organic and inorganic matter inputs to terrestrial ecosystems.
Keywords

Microbial decomposition model, carbon mineralization, nitrogen mineralization,
ecological stoichiometry, microbial biomass, soil respiration, soil microbial community, nitrogen accumulation
INTRODUCTION

A cornerstone of the ecosystem concept is the central role that coupled carbon (C) and nitrogen (N) cycling plays in sustaining ecosystem properties and services (Bormann and Likens 1967, Schlesinger et al. 2011, Menge et al. 2012). The amounts of these two elements cycled within ecosystems are being drastically altered by human activities such as the burning of fossil fuels and the production of mineral N fertilizer (Vitousek et al. 1997, van Groenigen et al. 2006). The potential consequences of these activities on coupled cycles can be estimated using mathematical models, many of which rely on theory from ecological stoichiometry (Sterner and Elser 2002) to estimate organismal C versus N demands and hence cycling rates (Parton et al. 1987, Parton et al. 1998, Schimel and Weintraub 2003, Allison et al. 2010, Allison 2012, Drake et al. 2013, Wieder et al. 2014). In particular, the changing elemental demands of soil microbes are of interest given the dominant role these organisms play in coupling the terrestrial cycling of C and N (Deruiter et al. 1993, Hanson et al. 2000, Knops et al. 2002, Bradford et al. 2013).

commonly considered to be a positive function of the active microbial biomass (e.g. Blagodatsky et al. 2010), which is in turn a product of absolute and relative C and N availabilities (Schimel and Weintraub 2003, Drake et al. 2013). Therefore, decomposition rates in microbial-explicit decomposition models emerge from underlying stoichiometric assumptions. These principles underlie models with both single and multiple microbial pools, the only difference being that the relative biomass of different microbial groups, as well as total biomass, regulates decomposition in the multi-pool models (Moorhead and Sinsabaugh 2006, Waring et al. 2013, Wieder et al. 2014). A central assumption in microbial-explicit models then is that relative and absolute C and N availabilities determine the size of the microbial biomass pool(s), and hence decomposition rates. Consequently, to have confidence in the projections of microbial-explicit models and to advance general understanding of links between microbial communities and biogeochemical cycling, it is necessary to evaluate the assumption that stoichiometric control on the size of the microbial biomass is a key mechanism regulating soil C and N cycling rates.

Few experimental tests exist of the mechanisms assumed to underpin microbial-explicit decomposition models (Blagodatsky et al. 2010, Allison 2012, Drake et al. 2013). Yet one argument for the fact that they can explain empirical observations better than traditional models is the explicit representation of key mechanisms (Lawrence et al. 2009, Todd-Brown et al. 2012, Wieder et al., 2014). We combined empirical observations of C and N mineralization rates with evaluation of a widely applied microbial-explicit model (Schimel & Weintraub 2003, Drake et al. 2013, Waring et al. 2013), to test the biomass and stoichiometry mechanisms assumed to underpin soil biogeochemical cycling rates. We applied an experimental array of nutrient additions, using a response surface experimental design (as opposed to ANOVA design: [Inouye 2001]) to capture broad variation in relative and absolute C and N availabilities, and asked two
general questions: (1) How effective are microbial-explicit models at capturing empirical responses of C and N mineralization rates? (2) Are empirical responses consistent with the underlying theory that differences in mineralization rates arise from differences in microbial biomass, the size of which is determined by relative nutrient availability?

METHODS

Model Adaptation

We implement a widely-cited, single microbial-pool model aimed at predicting microbial biomass and C and N mineralization rates (Schimel and Weintraub 2003, Drake et al. 2013). The model describes the flow of C and N between five soil pools (Schimel and Weintraub 2003). Those five soil pools with C and N sub-pools are soil organic matter (SOM), dissolved organic matter (DOM), microbial biomass, exoenzymes, and inorganic nutrients.

The model treats the microbial community as a homogeneous functional group characterized as sub-pools of C and N and assumes that the microbial pool has a relative demand for C and N based on one set of stoichiometric constraints (van Veen et al. 1984, Schimel and Weintraub 2003, Drake et al. 2013). The nutrient in excess of microbial demand is mineralized via waste respiration (for C) or N mineralization (Sterner and Elser 2002).

The model structure requires characterization of three key processes: (i) exoenzyme kinetics and the associated rate of SOM degradation, (ii) the ability of the microbial community to uptake dissolved or mineral nutrients, and (iii) the partitioning of these nutrients within the microbial cell. By using the established model we assume that the rates of the first two processes follow reverse and forward Michaelis-Menten kinetics, respectively, whereas fixed proportions of acquired nutrients determine partitioning to biomass synthesis and maintenance (Schimel and
Enzyme synthesis is calculated as a fixed proportion of standing microbial biomass rather than C uptake (Drake et al. 2013).

Weintraub 2003, Drake et al. 2013). With three exceptions, we used parameters from earlier versions of the model because microbial life history parameters were not available for our system (sensu Drake et al. 2013). We increased the parameter governing enzyme decomposition rate (Kd) by a factor of 10 during the spin-up phase to bring equilibrium microbial biomass into the range measured in our microcosms (0.1-0.25 mg C•g oven dry mass equivalent soil (dmes)^{-1}). We present simulation results that retain this parameter change; however results are qualitatively similar if the original parameter value is used (Tables A1 and A3). Furthermore, we reduced the parameter governing soil N leaching and loss (LE) from 40% loss to 2.5% loss to match inorganic N levels measured in our system (~20 μg N•g dmes^{-1}, Table A1, A3) and account for the fact that N leaching did not occur in our experiment (see Experimental Methods). Changing the magnitude of LE shifts the intercepts of our predicted results, but does not change qualitative trends. Finally, we changed the C:N ratio of the soil to 13.9 to match the C:N ratio measured in our soil. Our use (in general) of the original model parameters, developed for soil microbes in Alaskan soils (Vance and Chapin 2001, Schimel and Weintraub 2003), means we restrict our comparison to qualitative trends and effect sizes. We chose initial conditions for the state variables to match our experimental system, but started experimental simulations using the steady state values determined by the model following the spin-up phase (Table A2).

We assessed the degree to which microbial biomass was driving the model output by removing microbial control over two key processes: microbial uptake of DOM and enzyme production. We replaced the biomass term in these equations with the constant value equal to the equilibrium biomass, but allowed the actual microbial biomass pool to vary across treatments.
thereby retaining as much of the original model structure as possible (Appendix A). Without control over uptake and enzyme production this modeled microbial pool was analogous to a donor-controlled C pool (sensu Lawrence et al. 2009). We examined the consequences of removing microbial biomass control over other potential modifications because our empirical results (see Results) suggest that microbial biomass does not generally control C and N cycling.

We simulated C and N additions, once the model was at steady-state, over a 45-day period with additions occurring once every 7 days. We chose our maximum C addition rate (900 $\mu$g C g dmes$^{-1}$) following Bradford et al. (2010), who found that adding 840 $\mu$g C g dmes$^{-1}$ week$^{-1}$ sustained constant soil microbial respiration for at least 77 days. We calculated the maximum N addition rate by converting our maximum C addition rate to N using the C:N ratio measured in our old-field plant litter (see Experimental Methods) following the approach of Clarholm (1985). Nitrogen additions ranged from 0 to 180 $\mu$g N g dmes$^{-1}$, so that variation in C:N ratios of our additions fell within the natural range experienced by old-field microbes (C:N=0-45, Fig. S1, Hawlena and Schmitz 2010). We simulated the addition of C to the DOC pool and N to the inorganic N pool, respectively. We created two gradients of C and N addition rates by varying the concentration of one element, while holding the other constant at the highest addition rate. We therefore captured the “edges” of a response surface (Fig. A1). We added two treatments with intermediate levels of C and N to quantify potential interactions within the middle of the response surface and also included a control treatment that received no C or N additions.

We sampled the inorganic N pool at the beginning and end of the simulation and measured the C mineralization rate every 7 days (with two changes to match experimental conditions: see Experimental Methods). An important challenge facing effective C and N
addition experiments is separating added from mineralized nutrients. Carbon additions are easily separated, because mineralized C is released in gaseous form and C can be added as an organic compound (see Bradford et al. 2008 for methodology). Nitrogen is difficult to separate, because inorganic N additions are necessarily made to the same inorganic pools that receive mineralized N. Here we use two N cycling metrics to capture the distinction between dynamics driven by N mineralized in situ from the SOM and exogenously added inorganic N. First, N accumulation is the rate at which inorganic N builds up in or is removed from the soil; making it the sum of N addition, N mineralization, and N immobilization. Therefore, negative N accumulation implies that the microbial community immobilizes more N than was added and mineralized. Second, net N mineralization is the sum of N mineralization and immobilization (Schimel and Bennett 2004). When inorganic N is added exogenously to the soil, net N mineralization becomes the difference between N accumulation and N addition.

The model equations were transcribed into R language and simulations were run using 4th order Runge-Kutta iterations (Soetaert et al. 2010). Sensitivity analyses for the model have been reported previously (Drake et al. 2013).

**Experimental Methods**

We tested model predictions by supplementing C and N to experimental microcosms containing surface mineral soil and litter layers. Effects of larger-bodied soil fauna and plant roots were deliberately excluded to focus on the mechanisms underpinning the functioning of the microbial community in C and N cycling.

**Soil and Litter Collection.**–We collected soil and litter from an old-field ecosystem (Lat: 41.956724, Long: -72.115549) at the Yale-Myers Forest in northeastern Connecticut in the fall of 2012. We sampled soil from six sites spaced evenly within an 18-ha section of the field by
collecting the top 10 cm of soil below the organic layer (Robertson et al. 1999). Five soil cores (dia. 8 cm) from each site were homogenized before soil was passed through a 4-mm sieve. We froze the soil twice to -20°C to kill larger-bodied soil fauna, while minimizing damage to the microbial biomass and functional capacity (Kandeler et al. 1994, Pesaro et al. 2003, Koponen et al. 2006). We determined the water holding capacity and gravimetric moisture of the soil using standard methods (Bradford et al. 2008) and measured the C:N ratio of each soil using an elemental analyzer (ESC 4010, Costech Analytical Technologies, Valencia, CA, USA).

We harvested litter from the two dominant plant species in the field: goldenrod (Solidago rugosa) and grass (Poa spp.). We dried the litter at 60°C for 48 h, ground it with a coffee grinder, passed it through a 4-mm sieve, and sterilized it in an autoclave. The litter was mixed at a ratio by mass of goldenrod: grass of 7:3 to replicate field densities (Hawlena and Schmitz 2010; Supplemental Material). We determined the water holding capacity of the litter using standard methods (Bradford et al. 2008).

**Microcosms.**–We used 50-mL centrifuge tubes as our microcosms, and filled these with 10 g (oven dry weight equivalent) of soil and 0.5 g of air-dried litter. The day of litter addition was considered the first of the experiment, and we began nutrient additions the next day (see *Nutrient Additions*). Throughout the experiment, microcosms were maintained in an incubator at 20°C and 60% water holding capacity. Water content was adjusted after nutrient additions and preserved between sampling periods by storing microcosms in loosely sealed plastic bags that permitted gas exchange but limited water loss.

**Nutrient Additions.**–We added nutrients each Monday for 7 weeks. Nutrient addition rates followed the same response surface experimental design described for the model. We replicated each treatment twice for each of the six sites giving a total of 264 microcosms. Labile
C was delivered as glucose (Clarholm 1985), and labile N as ammonium sulfate. We choose sulfate as a counter-ion to minimize the effects of a pH change on the soil microbial community (Gulledge et al. 1997, Bradford et al. 2008).

**Carbon Mineralization.**—We monitored C mineralization on days 3, 10, 17, 25, 31, and 45 of the experiment by measuring CO₂ production within the headspace of each microcosm using an infrared gas analysis (IRGA) method (Bradford et al. 2008). Incubation time was ~4 h, except on Day 3 when incubation lasted 24 h. CO₂ production rate was calculated as the product of CO₂ concentration in the headspace and the headspace volume divided by incubation time (Bradford et al. 2008).

**Nitrogen Accumulation and Net Nitrogen Mineralization.**—We measured N accumulation and net N mineralization rates using a KCl extraction (Robertson et al. 1999). Soil was shaken in 2M KCl solution and allowed to settle overnight before the supernatant was decanted and frozen until analysis. We thawed the supernatant for ammonium and nitrate analysis and measured concentrations using a flow analyzer with an atomic absorption apparatus capable of measuring NH₄⁺-N and NO₃⁻-N (Astoria 2, Astoria-Pacific Inc., Clackamas, OR, USA). Rates of N accumulation and net mineralization were calculated using the difference in extractable inorganic N at the start (Day 2) and end (Day 45) of the experiment. As detailed under Model Adaptation, net N mineralization rate was the difference between N accumulation and N addition rate.

**Microbial Biomass.**—Microbial biomass was estimated using substrate-induced-respiration (SIR), which measures the size of the active microbial pool (Anderson and Domsch 1978). We followed the methods of Bradford et al. (2010) using ~8 g oven dry weight equivalent soil in each SIR incubation and a 5 h incubation time. We used yeast extract rather than glucose as our substrate to ensure that microbial communities already receiving glucose, and potentially
more adept at metabolizing it, did not bias our measurements. We converted SIR measurements to active microbial biomass following Anderson and Domsch (1978) to facilitate comparison with model results.

**Statistics.**—Carbon mineralization and SIR data were analyzed using linear mixed effects (LME) models with C, N and, when appropriate, day as fixed effects (Pinheiro et al. 2011), and the best linear model was selected using Akaike Information Criterion (Burnham and Anderson 2002). Site of soil harvest was included as a random effect. Nitrogen cycling was explored using LME models chosen based on conservative $p$-value estimates (Bates et al. 2011, Tremblay 2012). Model fit was determined using $R^2$ values following Nakagawa and Schielzeth (2013). All statistical analyses were conducted in R (version 3.1.1).

**RESULTS**

**Carbon Mineralization.**—The model predicts an approximately 10-fold increase in C mineralization with increasing C additions (Fig. 1, Fig. A2). Experimental results capture the same qualitative trend, but produce a smaller increase (~1.5 fold) in C mineralization (Fig. 1, Fig. A3). Modeled nitrogen additions decreased C mineralization up to a threshold of 40-60 $\mu$g-N g\textsuperscript{-1} dmes\textsuperscript{-1} week\textsuperscript{-1}, above which they had no effect (Fig. 2, Fig. A2). Experimental, N additions first promoted and later reduced C mineralization rates generating a significant N $\times$ day interaction ($p<0.0001$, Fig. 2, Fig. A3). In the sole experimental treatment where N additions were made in the absence of C (C=0; N=180), the addition of nitrogen decreased C mineralization across all replicates and all measurement days as compared to the control treatment (C=0; N=0, $p=0.0004$). As such, the model generally captures the qualitative trends in C mineralization measured experimentally, but fails to estimate their magnitude or the shifting impact of N addition across time (Fig. 1, Fig. 2).
Nitrogen Accumulation and Net Nitrogen Mineralization.—Simulated and experimental N addition resulted in significant N accumulation (Fig. 2). The model and experiment predict the same threshold addition rate of 40-60 μg-N·g dmes⁻¹·week⁻¹, above which N accumulates in the inorganic pool (Fig. 2). Carbon additions decreased N accumulation in the model and the experiment, but had a weaker effect than N (Fig. 1). Although the model predicted consistently slower rates of N accumulation than measured in the experiment, the trends and effect sizes were relatively similar.

The model predicted net N immobilization in all treatments across all additions, with both C and N increasing the amount of N immobilized (Fig. 1, Fig. 2). Nitrogen immobilization in the model matched the model stoichiometry where the microbes were generally N limited whenever C was added (Fig. 3, Fig. A4). The threshold, which existed in the N accumulation data, was not present in the relationship between N addition rate and N mineralization (Fig. 2). In the experiment, net N mineralization decreased with increased C addition and followed a cubic form as N additions increased (Fig. 1, Fig. 2). Increasing N addition rates led to increasing net N immobilization until the threshold at which N began to accumulate in the soil. Thereafter, N additions reduced net N immobilization and resulted in some net N mineralization under high N addition (Fig. 2).

Microbial Biomass.—Modeled microbial biomass responded positively to both C and N additions up to 40-60 μg-N·g dmes⁻¹·week⁻¹, after which additional N had no positive impact (Fig. 1, Fig. 2). Conversely, microbial biomass at the end of the experiment was largely unaffected by nutrient additions (Fig. 1, Fig. 2), meaning that the model did not accurately predict the effect of C and N additions on active microbial biomass.
Nutrient Limitation of Microbial Biomass.—We determined the nutrient limitation status of the microbial community using the model output (we do not have comparable data from our experimental work). Nutrient treatments translated into different C and N limitation profiles for the microbial community over the 45-day period that we simulated (Fig. 3, Fig. A4). Whenever C was added, the model predicted periodic nutrient limitation wherein N became strongly limiting after each C and N addition followed by a phase of C limitation before the next nutrient addition (Fig. 3). Adding different amounts of C and N shifted the length and severity of these periods of C and N limitation, such that increases in C addition rate increased the duration of N limitation (Fig. A4). Notably, the limitation profile was identical for all simulations receiving more than 60 μg-N•g dmes⁻¹•week⁻¹, so that increasing the N addition rate beyond that point did not help alleviate N limitation (Fig. A4).

Removing Microbial Control.—Removing microbial control of key processes in the model did not significantly alter the results. The modified model output was qualitatively identical to the results obtained from the original model (Fig. A6 and A7). Linear models comparing N accumulation, net N mineralization, and microbial biomass demonstrated that the outputs were also quantitatively similar (slope≈1, $R^2$≈1). The modified model predicted slightly higher N accumulation and net N immobilization rates; these values were most different from the original model in treatments where the C:N ratio of the additions was close to that of the microbial biomass (Fig. A6). Such differences diminished once N addition rates exceeded the microbial saturation point of 60 μg-N•g dmes⁻¹•week⁻¹ (C:N > 15). One exception was microbial biomass (which functions as a passive pool once microbial control is removed), which varied most at higher C:N addition ratios (Fig. A6). Carbon mineralization tended to be higher in the original
Single-pool microbial models are prefaced on the assumption that changes in both microbial biomass and stoichiometric constraints drive differences in C and N mineralization rates (Schimel and Weintraub 2003, Blagodatsky et al. 2010). Empirical work to date has attributed the improved predictive power of these models to the inclusion of these key mechanisms (Lawrence et al. 2009, Allison et al. 2010, Blagodatsky et al. 2010, Wieder et al. 2013). For example in the aforementioned models, microbial biomass controls the size of the exoenzyme pool and therefore the rate of SOM degradation. We report empirical evidence supporting previous claims that single-pool microbial models can predict C and N mineralization rates with reasonable accuracy. However, our results highlight the importance of stoichiometric controls rather than microbial biomass as the mechanism underpinning the accuracy of these predictions.

The importance of stoichiometry is evident in our empirical results because microbial biomass remained relatively constant across all the nutrient treatments. Indeed, our empirical results are broadly consistent with predictions from stoichiometric theory where, for a two nutrient system, adding one nutrient increases its mineralization rate, while simultaneously decreasing the other’s net mineralization rate (Sterner and Elser 2002, Schimel and Weintraub 2003). In our case, the N accumulation threshold is particularly noteworthy, because it unambiguously denotes a change in the microbial demand for N somewhere between 40-60 μg-N•g dmes⁻¹•week⁻¹. The model’s prediction of this same threshold suggests the mechanism underlying the empirical data because the model analysis provides the stoichiometric limitations...
of the microbial pool (e.g. Drake et al. 2013). In the model, the microbial C limitation was
temporarily relieved each time glucose was added. At low doses of N addition, N accumulation
remains low because the microbial community is sufficiently N limited between glucose
additions to uptake the majority of the added N. The switching point occurs when the microbes
no longer require all of the added N to offset the elevated C availability and therefore N
accumulates in the soil (Schimel and Weintraub 2003). As such, the specific threshold in any
system is likely to result from both the magnitude and frequency of C supply to the microbial
biomass.

To test the extent to which the modeled processes were driven by the suggested
stoichiometric mechanism, we removed the microbial control over enzyme synthesis and nutrient
uptake, while leaving the stoichiometric equations untouched. Removing microbial control from
the model provided further evidence that stoichiometric mechanisms dominated the model
output; however, comparing the modified and original models provided a more nuanced
assessment of the importance of microbial biomass. We found that the influence of biomass on C
and N cycling was dependent on how strongly it could influence the relationship between
nutrient supply and demand. Specifically, the microbial biomass had the largest influence where
the C:N ratios of the added substrate were close to the C:N ratio of the microbes. Such “optimal”
nutrient additions increased microbial biomass above equilibrium levels (Krumins 2014). In such
instances, the large microbial biomass changed the relative C and N availability enough to
influence the dominant stoichiometric mechanism – the homeostatic constraints of the microbial
community – defining mineralization rates (Schimel and Weintraub 2003). Even though the
changes in microbial biomass were small in our case, more variable environmental conditions
impacted for example by differences in temperature, moisture or soil type, could change
microbial biomass enough to have more important effects on the availability of C and N (Lawrence et al. 2009, Allison et al. 2010).

The dominance of stoichiometric control in our system explains why the model accurately predicted C and N cycling even though it failed to predict microbial biomass. Across the range of available C and N we tested, stoichiometry rather than biomass appears to define C and N mineralization rates (Schimel and Weintraub 2003). Our combined empirical and model analysis suggests that microbial biomass is likely to be the most important driver of C and N cycling only under certain circumstances (i.e. when C:N ratios of inputs are close to those of the biomass), with stoichiometric demands instead being the primary driver of C and N mineralization rates. However, it is noteworthy that a consequence of using reverse Michaelis-Menten kinetics for decomposition is that the return on investment in enzymes saturates as the enzyme pool grows in size (e.g. Drake et al. 2013). As a result, our model structure includes a strong negative feedback on microbial biomass that does not exist in models using forward Michaelis-Menten decomposition dynamics, where the decomposition correlates positively with pool size (e.g. Allison et al. 2010, Wieder et al. 2013). Both forward (Allison et al. 2010, Wieder et al. 2013, Hagerty et al. 2014, Wieder et al. 2014) and reverse (Schimel and Weintraub 2003, Lawrence et al. 2009, Drake et al. 2013) Michaelis-Menten dynamics are applied to models of the decomposition process, suggesting that an empirical investigation of a forward kinetic model, similar to the approach taken here, would help determine the circumstances under which reverse versus forward kinetics best approximate the rate of exoenzyme-mediated decomposition.

Our model successfully predicted N accumulation, but failed to predict the influence of N addition on net N mineralization. There are two potential explanations for these divergent trends that both act by causing the model to consistently under-predict the rate of N accumulation. First,
it is possible that increases in microbial biomass in the model increased gross N immobilization, thereby consistently reducing N accumulation across nutrient treatments (Schimel and Weintraub 2003). Alternatively, the difference in magnitude between empirical and predicted results may have arisen because we did not parameterize the model for our system (but see Drake et al. 2013 who used the same parameters). The exact magnitude of N accumulation matters because net N mineralization was calculated as the difference between N addition rate and N accumulation. N accumulation was approximately six times smaller in the model. It follows that if the model accurately predicted the magnitude of N accumulation then it would have correctly predicted net N mineralization rate as well. The discrepancy calls for further empirical work to develop microbial life history parameters (Todd-Brown et al. 2012) to update model parameter values (Schimel and Weintraub 2003, Drake et al. 2013). It also suggests a need to develop a clearer understanding of when and why microbial biomass increases under joint C and N additions.

We suspect that another limiting factor might explain why microbial biomass did not increase in response to joint C and N additions in our microcosms. First, another important limiting resource such as P or high quality SOM may have capped the microbial population size in our experiment (van Veen et al. 1984, Sterner and Elser 2002, Cleveland and Liptzen 2007). Alternatively, different nutrient regimes may have facilitated a turnover in microbial community composition that favored fast growing species, such as copiotrophs or bacteria in general, that are capable of using labile C pulses (Bardgett and McAlister 1999, Fierer et al. 2007, Keiser et al. 2014). Competitive costs are generally high amongst microbes unless different groups are limited by different nutrients, and these costs take nutrients and energy resources away from growth (Waring et al. 2013). For example, Vance and Chapin (2001) attributed the lack of microbial growth they observed with joint C and N additions to the transfer of metabolic
expenditures away from growth and towards a stress response. Including such limitations or
unaccounted-for costs in future models would help to increase model ability to predict microbial
biomass in scenarios where neither C nor N is primarily limiting.

Broadly, our empirical evaluation confirmed the general predictive capacity of the single-
pool microbial model proposed by Schimel & Weintraub (2003). However, the accuracy was
driven by a precise portrayal of stoichiometric mechanisms rather than those based on biomass
(e.g. priming, Blagodatsky et al. 2010). Although microbial biomass has been found to be a
dominant mechanism shaping mineralization dynamics when climate factors, such as soil
temperature and moisture, have been considered (Lawrence et al. 2009, Allison et al. 2010), our
data suggest the need to develop and implement similar model evaluations including more
detailed stoichiometric mechanisms. For example, recently developed multiple-pool microbial
models, wherein different functional groups have different stoichiometric constraints, may be a
useful means of introducing more realism into future models (Moorhead and Sinsabaugh 2006,
Allison 2012, Waring et al. 2013, Wieder et al. 2014). One thing seems certain, microbial-
explicit decomposition models are a powerful tool for advancing understanding of how microbial
physiology will shape C and N cycling responses to environmental change. Our work highlights
the need to advance our understanding of the stoichiometric demands of the microbial biomass if
we are to understand biogeochemical cycles in the face of changing organic and inorganic matter
inputs to soils.

ACKNOWLEDGEMENTS

We thank E. Oldfield and A. Keiser for advice on laboratory methods and D. Maynard
for statistical consultation. Funding was from the Schiff Fund and the Edna Bailey Sussman
Fund, as well as to R.B from the Natural Science and Engineering Research Council of Canada.


Kandeler, E., B. Winter, C. Kampichler, and A. Brunckner. 1994. Effects of mesofaunal exclusion on microbial biomass and enzymatic activities in field mesocosms. in K. Ritz,


Tremblay, A. 2012. Lmer convenience functions: A suite of functions to back-fit fixed effects and forward-fit random reffects, as well as other miscellaneous functions. R package version 1.6.8.2.


Appendix A: A detailed description of nitrogen toxicity tests and model modification, as well as additional empirical and model results not shown in the main text.
FIGURE CAPTIONS

Fig. 1: The modeled and experimental response of soils to variations in carbon (C) addition rate. Symbols indicate variability in the rate of addition of nitrogen (N). Note that the majority of treatments include the other element at the highest dose. N accumulation, N mineralization, C mineralization on day 45, and microbial biomass are reported for both the model (left) and the experiment (right). Negative values of net nitrogen mineralization indicates net nitrogen immobilization. The model output predicted experimental trends in C mineralization and N accumulation, but failed to predict net N mineralization or microbial biomass. All addition rates and response variables are reported per gram of oven-dried equivalent soil. A replicated version of our results as a function of C:N ratio can be found in Appendix A (Fig. A5). Note that the relative supply of nitrogen in this figure is higher than in Fig. 2 (e.g. C:N ratio varies from 1-5 across the C gradient and 5-45 across the N gradient).

Fig. 2: The modeled and experimental response of soils to variations in nitrogen (N) addition rate. Symbols indicate variability in the rate of addition of carbon (C). The model output predicted experimental trends in C mineralization and N accumulation, but failed to predict net N mineralization or microbial biomass. See the Fig. 1 caption for details.

Fig. 3: The degree of nutrient limitation experienced by the microbial community in four representative model simulations. Each bar represents the nutrient (C or N) limitation state on a single day of the simulation from 0-45 running left to right. Carbon and/or nitrogen were added every 7 days in the simulations, starting on day 2. Negative values (grey bars) on the y-axis indicate C limitation and positive values (black bars) indicate N limitation. An element limitation of zero would indicate that C and N are available in
exactly the ratio demanded by the microbes. For the two plots on the left where no C was
added, C was limiting each day and to the same extent. For the two plots on the right
where C was added, the microbial community shifted to N limitation following nutrient
additions (Days 2, 9, 16, 23, 30, 37, and 44), but then returned briefly to C limitation
before the next addition. Note the different y-axes scales. The title of each plot notes the
C and N addition rate in $\mu g \cdot g \text{ dmes}^{-1} \cdot \text{week}^{-1}$, where dmes is oven dry-mass equivalent
soil.