

APPENDIX A:

Description of toxicity tests and modeling, as well as additional results

Toxicity Tests

We tested whether nitrogen build-up in the high-nitrogen treatments impaired microbial activity through toxicity effects using an ANOVA with four treatments: no nutrient additions, 900 $\mu\text{gC/g}_{\text{dry mass}}$ only, 180 $\mu\text{gN/g}_{\text{dry mass}}$ only, and 900 $\mu\text{gC/g}_{\text{dry mass}}$ plus 180 $\mu\text{gN/g}_{\text{dry mass}}$ (Fig. A1, squares). We compared carbon mineralization rates in each of these treatments over time using a repeated measures ANOVA. Carbon mineralization was not significantly reduced by N additions over time ($p=0.47$), nor did N addition lead to a decrease in SIR ($p=0.54$).

Model Details

We adapted the latest version of the original Schimel and Weintraub (2003) model, as reported in the appendix of Drake et al. (2013). We found an anomaly in the model reported by Drake et al. (2013), who subtracts enzyme nitrogen allocation rather than adding it to the amount of N required. Our revised equation is:

$$I_N = \frac{\left(UC - Rm - \frac{EPC}{SUE}\right)SUE}{CNm} + EPN - UN$$

We also include boundary conditions on microbial immobilization of N and uptake of DOC and DON, so that uptake never exceeds the size of the pool. Otherwise our model is identical to Drake et al. (2013). Finally, we discard the sensitivity of parameters to temperature by carrying out all our simulations at 20°C and so essentially return to Schimel and Weintraub's (2003) original equations.

Model Spin-up and Simulation

We spun-up the model over 5000 time steps until all pools equilibrated, and we ran our simulations using these starting parameters (Table S2). We chose the spin-up initial conditions based on experimental measurements (Table S3) and when we did not measure an initial level, we relied on the ratios of initial conditions reported by Schimel and Weintraub (2003).

Removing Microbial Control

We removed microbial control by modifying three of the equations from the original model for the uptake of C and N and the creation of enzymes. We created two new parameters: StableB is the size of the active microbial C pool and was set at 373 $\mu\text{g-C}\cdot\text{g dmes}^{-1}$ and StableBN is the size of the active microbial N pool and was set at StableB/CNm= 52 $\mu\text{g-N}\cdot\text{g dmes}^{-1}$.

$$UC = \frac{\text{StableB} \cdot \text{uptake} \cdot \text{DOC}}{K_{\text{uptake}} + \text{DOC}}$$

$$EPC = Ke \cdot \text{StableB}$$

$$UN = \frac{\text{StableBN} \cdot \text{uptake} \cdot \text{DON}}{K_{\text{uptake}} + \text{DON}}$$

These three changes remove microbial biomass control over enzyme production and uptake. The enzyme pool is constant during these simulations, because enzyme synthesis and loss are constant. Decomposition of the SOM pools is controlled by enzyme production, so these changes also remove microbial control over decomposition. Carbon and N cycling are controlled by the uptake rate of C and N and set parameter values. The only aspect that remains under microbial control is maintenance respiration, which is based on the passive microbial biomass pool left in the model. To ensure that maintenance respiration was not driving the microbial response, we set maintenance respiration to a constant value and repeated our analysis. We found that this further model output was no different from either the original or the modified model (N accumulation: $m=1$, $R^2=1$; net N mineralization; $m=1$, $R^2=1$; C mineralization: $m=0.99$, $R^2=0.99$). We chose to leave maintenance respiration as a fraction of the now passive microbial pool, because we wanted to keep the turnover of the microbial pool as consistent with the original simulations as possible to isolate the influence of changing microbial control. To run the simulations we spun-up the model over 500 time steps and started with the new equilibrium values, which were always within $0.5 \mu\text{g} \cdot \text{g} \text{ dmes}^{-1}$ of the original.

TABLE A1. The parameter values used for both model spin up and simulations. Only parameters Kd and LE were altered from Drake et al. (2013). All parameter identifiers are equivalent to Drake et al. (2013). LE: percentage of inorganic N pool lost each time step.

| Parameter | Units | Description | Value |
|-----------|--|---|----------|
| Kd | $\mu\text{g} \cdot \text{g soil}^{-1} \cdot \text{day}^{-1}$ | Maximum decay rate of exo-enzymes | 10 |
| Kes | $\mu\text{g} \cdot \text{g soil}^{-1}$ | Half saturation of exo-enzymes | 0.3 |
| Ke | unitless* | Fraction of biomass allocated to enzyme production | 0.0005 |
| SUE | unitless | Substrate use efficiency | 0.5 |
| Km | unitless | Microbial maintenance rate | 0.01 |
| Kl | unitless | Decay constant for enzymes | 0.05 |
| CNs | unitless | C:N ratio of soil | 13.9 |
| Kt | unitless | Microbial death rate | 0.012 |
| Kr | unitless | Proportion of microbial biomass that is available to microbes | 0.85 |
| CNm | unitless | C:N ratio of microbial biomass | 7.16 |
| Uptake | $\mu\text{g} \cdot \text{g soil}^{-1} \cdot \text{day}^{-1}$ | Maximum rate of nutrient uptake | 6250 |
| Kuptake | $\mu\text{g} \cdot \text{g soil}^{-1}$ | Half-saturation rate of nutrient uptake | 0.000454 |
| LE | unitless | Inorganic N leaching rate | 0.025 |

*Many parameters are unitless, because they are proportions of a particular pool or element ratios.

TABLE A2. The spin-up starting conditions and equilibrium values for the model. All pools are in $\mu\text{g} \cdot \text{g soil}^{-1}$. Spin-up starting conditions were taken from initial soil data whenever possible (Table S3).

| State Variable | Description | Spin-up Start | Simulation Start |
|----------------|----------------------------|---------------|------------------|
| DOC | Dissolved organic carbon | 50 | 13.0629 |
| BiomC | Microbial biomass carbon | 245.52 | 373.2258 |
| EnzC | Enzyme carbon | 70 | 3.732258 |
| DON | Dissolved organic nitrogen | 3 | 1.19759 |
| BiomN | Microbial biomass nitrogen | 34.29050 | 52.12651 |
| EnzN | Enzyme nitrogen | 23.33333 | 1.244086 |
| Ninorg | Inorganic nitrogen | 20 | 19.92559 |

TABLE A3. Baseline soil properties for the six sites included in our study. Sites were spaced 300 m apart in a grid design. $\mu\text{g-N/g}$: KCl extractable nitrogen, $\mu\text{g-C/g}$: microbially-available carbon*, SIR: substrate induced respiration**, WHC: water holding capacity (g/g), WC: gravimetric water content (g/g).

| Site | $\mu\text{g-N/g}$ | $\mu\text{g-C/g}^*$ | SIR $\mu\text{g-C/g}\cdot\text{hr}^{**}$ | WHC | WC | ^{15}N | ^{13}C | %N | %C | C:N |
|------|-------------------|---------------------|--|------|------|-----------------|-----------------|------|------|-------|
| 1 | 25.24 | 7683 | 1.74 | 0.26 | 0.31 | 9.92 | -25.98 | 0.21 | 2.63 | 12.48 |
| 2 | 25.71 | 4619 | 1.13 | 0.28 | 0.30 | 8.14 | -26.38 | 0.19 | 2.33 | 12.45 |
| 3 | 14.82 | 3651 | 1.76 | 0.27 | 0.32 | 6.33 | -26.22 | 0.19 | 2.51 | 13.07 |
| 4 | 16.59 | 4092 | 2.27 | 0.31 | 0.27 | 5.84 | -26.56 | 0.18 | 2.42 | 13.21 |
| 5 | 16.24 | 3436 | 2.40 | 0.33 | 0.33 | 13.28 | -25.96 | 0.22 | 3.33 | 14.92 |
| 6 | 29.29 | 4106 | 2.52 | 0.44 | 0.36 | 5.05 | -26.59 | 0.29 | 5.14 | 17.48 |

* Microbially-available carbon is the integral under the respiration curve for control treatments.

**Average of control treatment tubes at the end of the experiment for each site.

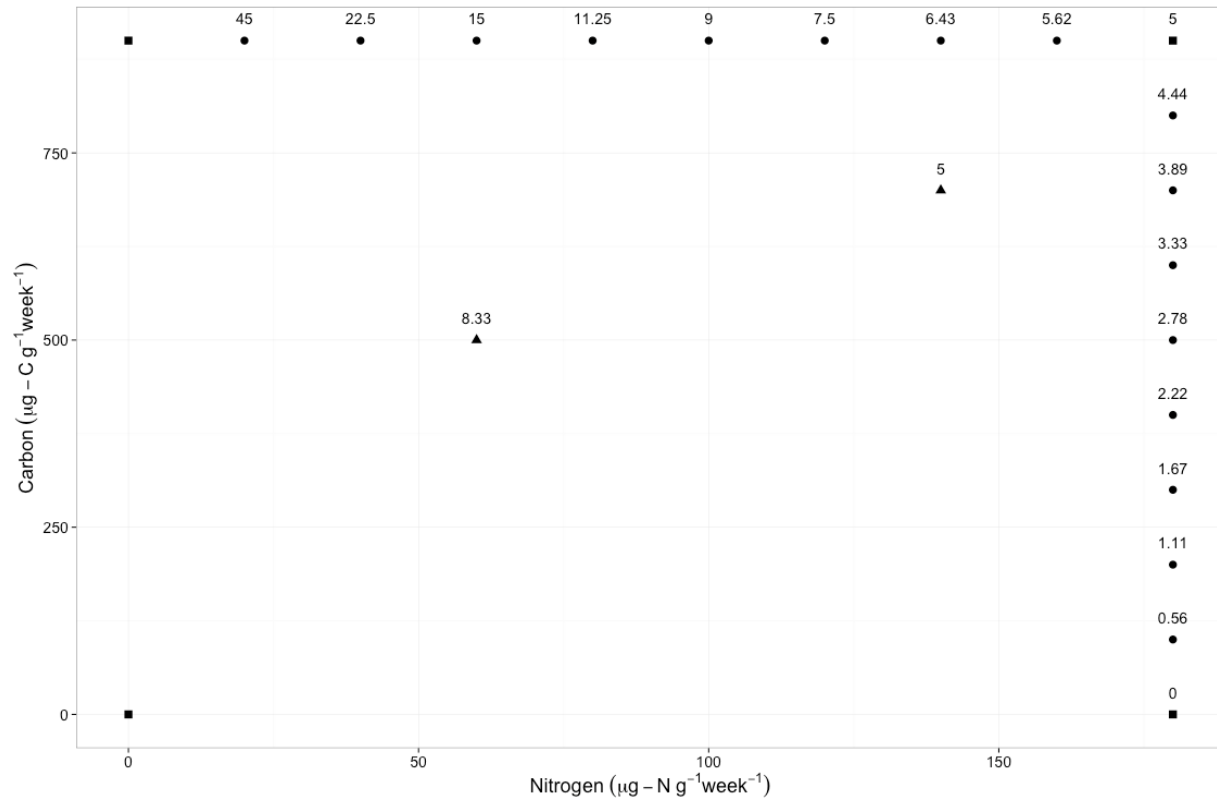


FIG. A1. The treatments included in the experiment. Numbers above the points indicate the C:N ratio (when possible) of the additions and symbols indicate the different categories referred to in the manuscript: squares are points used in the ANOVA design for toxicity tests, triangles are interaction treatments, and diamonds are all other treatments.

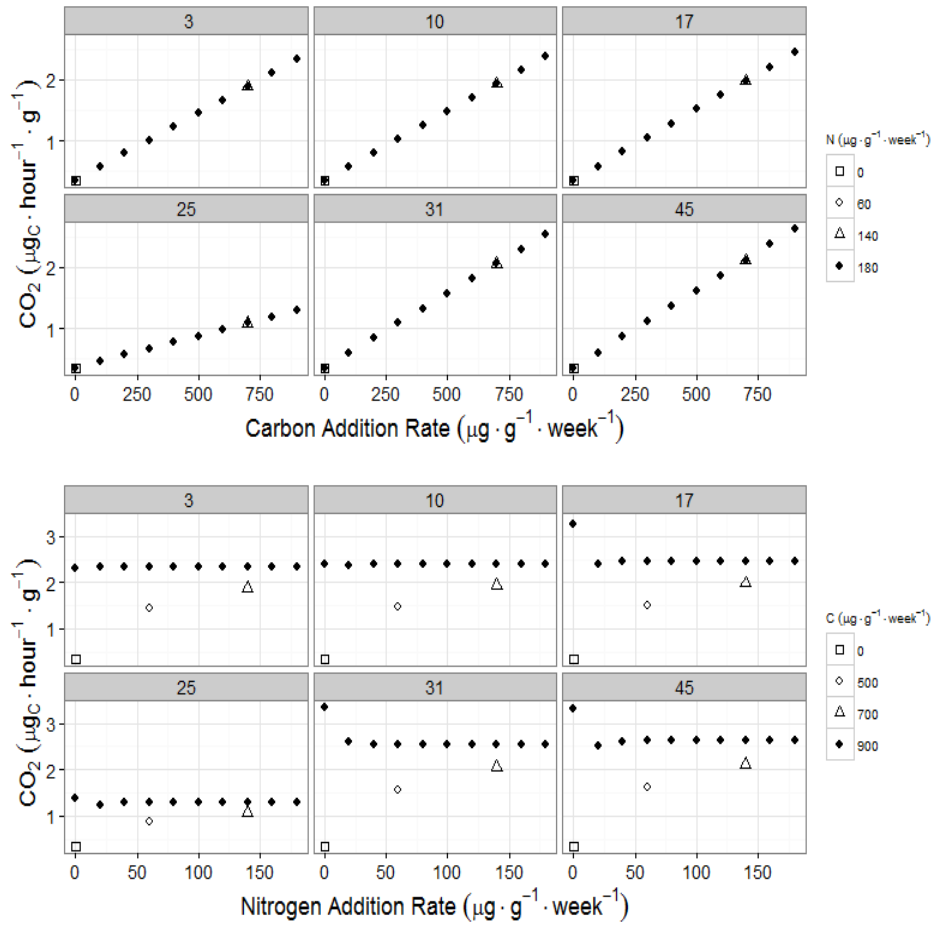


Fig. A2. Predicted soil respiration (C mineralization) rates across N and C addition gradients. Carbon mineralization increases with C addition rate and decreases or remains constant with N addition rate. Each panel summarizes a measurement day ranging from 3 to 44. Symbols indicate the addition rate of either C or N added across the relevant addition gradient (i.e. C or N). All addition rates are based on grams of oven dried equivalent soil.

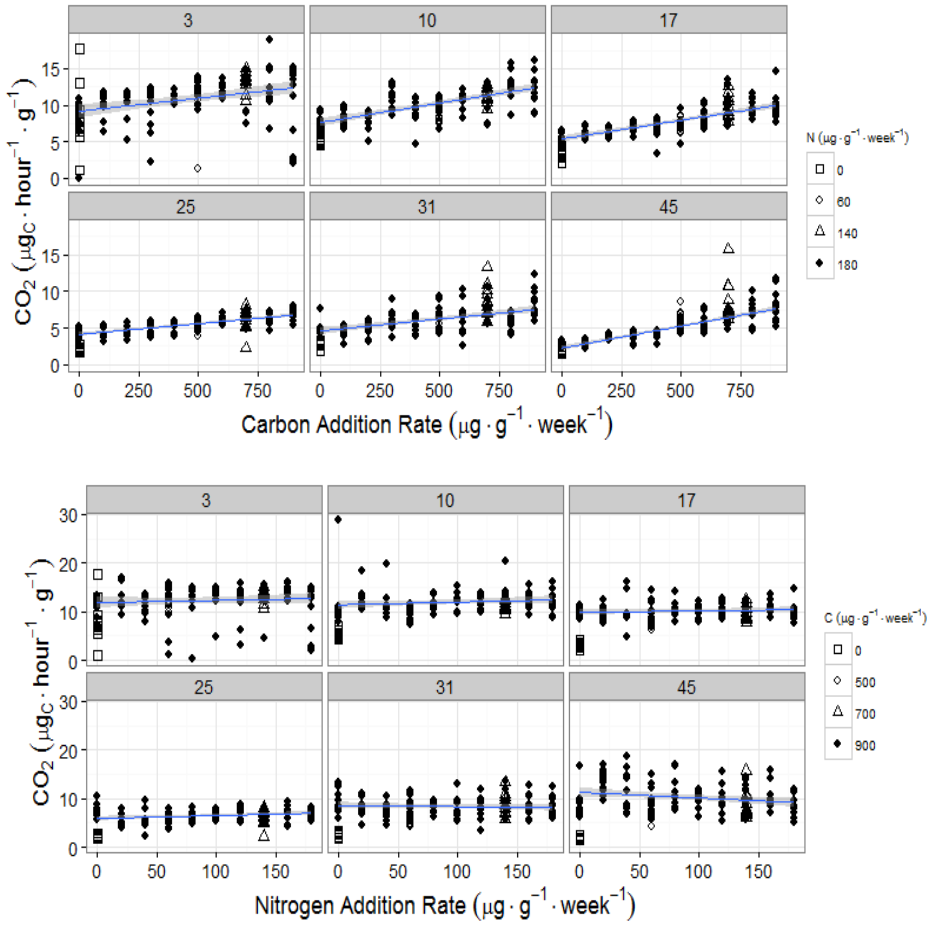


Fig. A3. Empirical soil respiration (C mineralization rate) across N and C addition gradients with each panel summarizing a measurement day ranging from 3 to 44. Carbon mineralization increased with C addition rate irrespective of N addition. Symbols indicate variations in the other element's addition rate and lines are linear fits with 95% confidence intervals ($n = 12$). All addition rates are based on grams of oven dried equivalent soil.

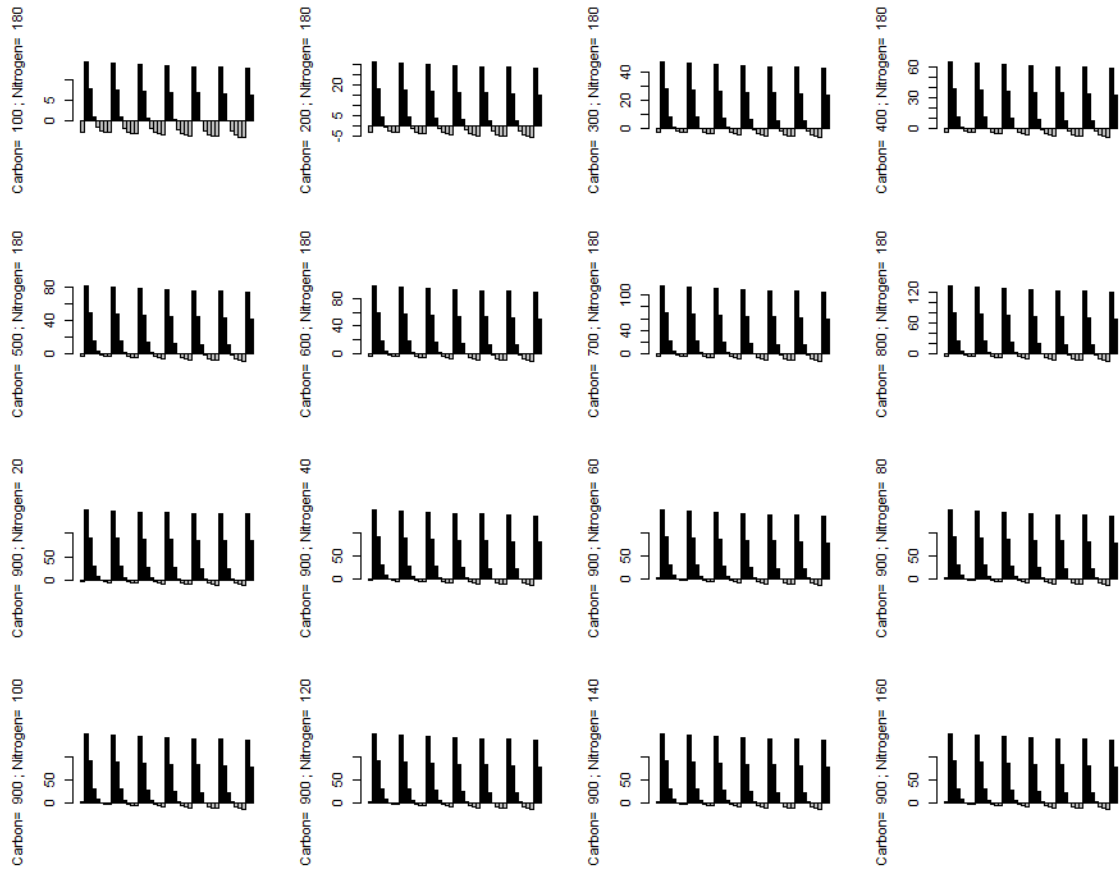


FIG. A4. The limitation profiles of treatments, excluding the interaction treatments, which are not presented in Fig. 2. The top eight have increasing C, whereas the bottom eight have increasing N. The treatment identity is on the y-axis and the y-axis and x-axis titles are suppressed for clarity.

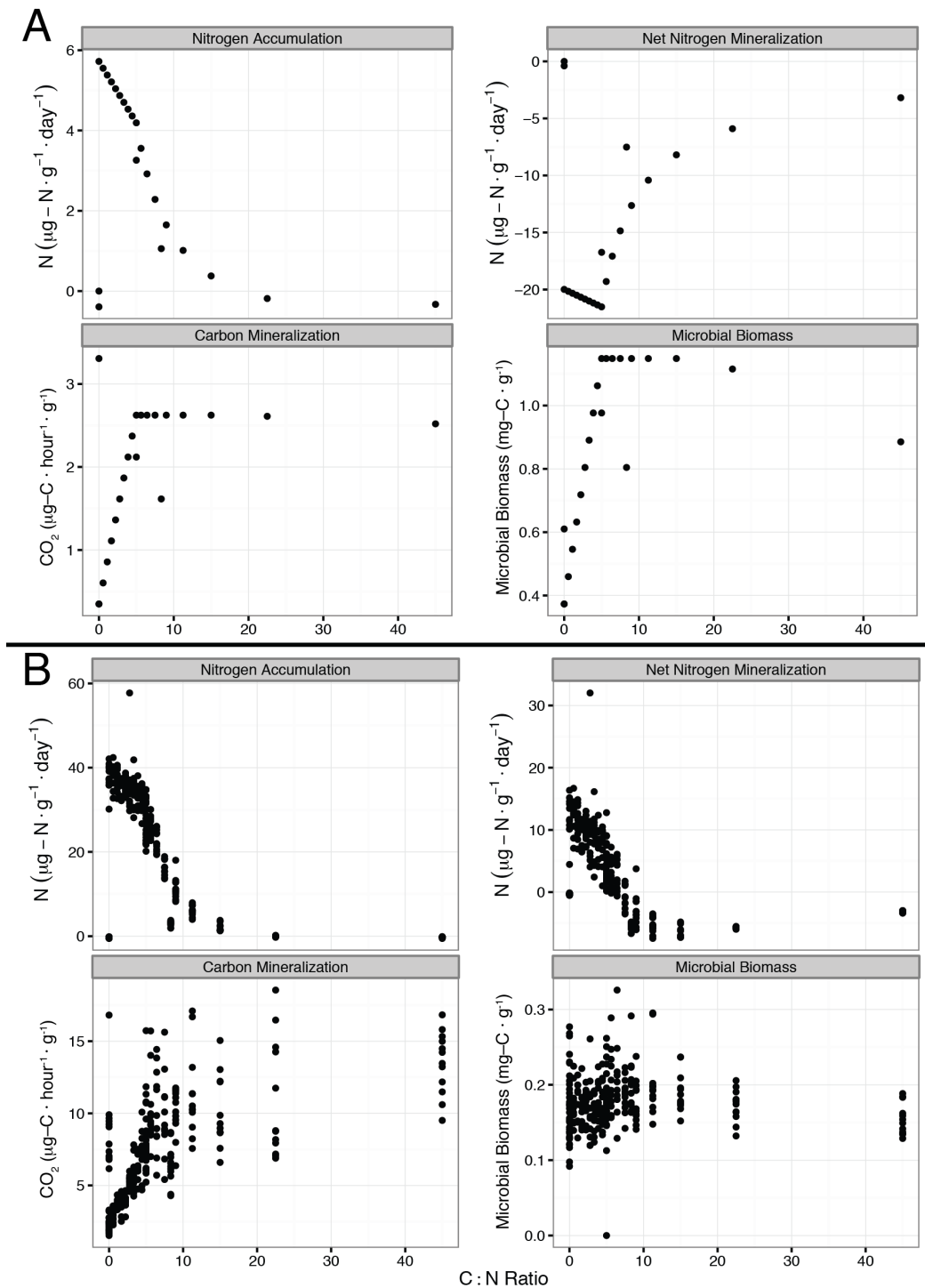


Fig. A5. The main results of our model (A) and experiment (B) plotted as a function of C:N ratio of the addition rate. For treatments with an undefined C:N ratio (0:0 and 0:180), we set the C:N ratio at 0 and are spread vertically along the y-axis because differences in the amounts of C and N added lead to different nutrient cycling and microbial biomass. These data match those in Fig. 1.

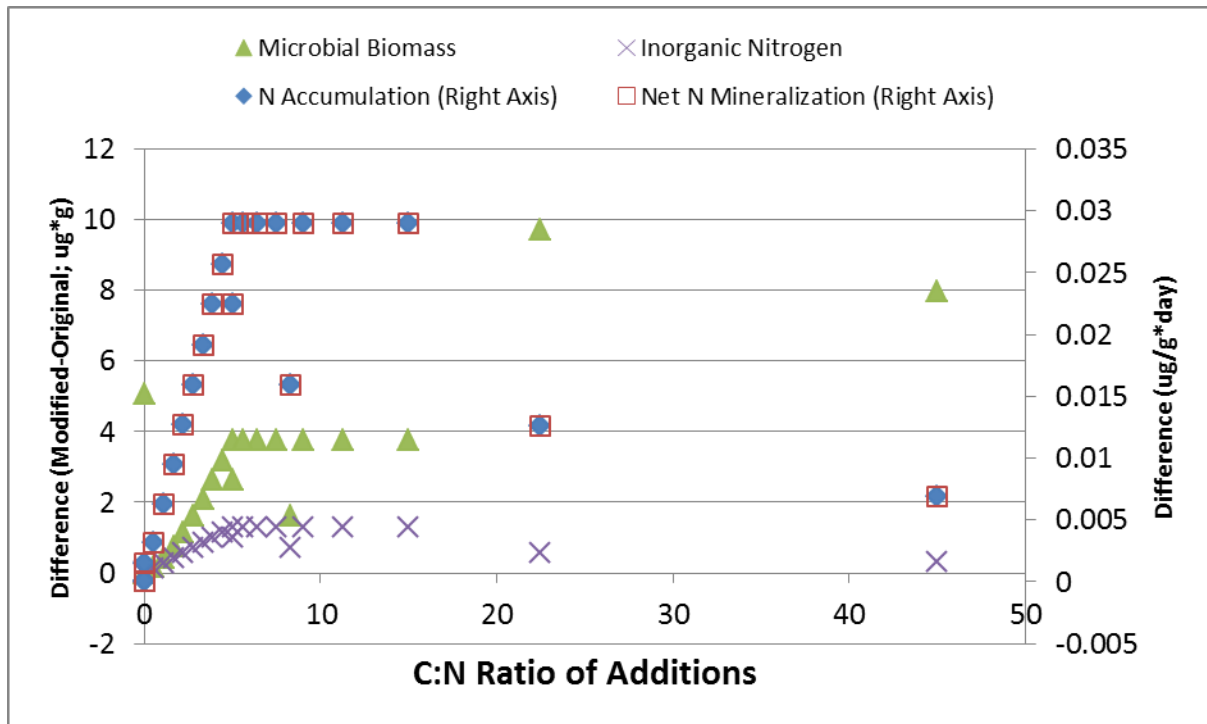


FIG A6. The difference between the original model and the modified model where microbial biomass no longer controls DOM uptake or enzyme production. Nitrogen accumulation and net N mineralization are plotted on the right axis. The magnitude of these changes is small compared to the actual pool sizes or fluxes (Fig. 1).

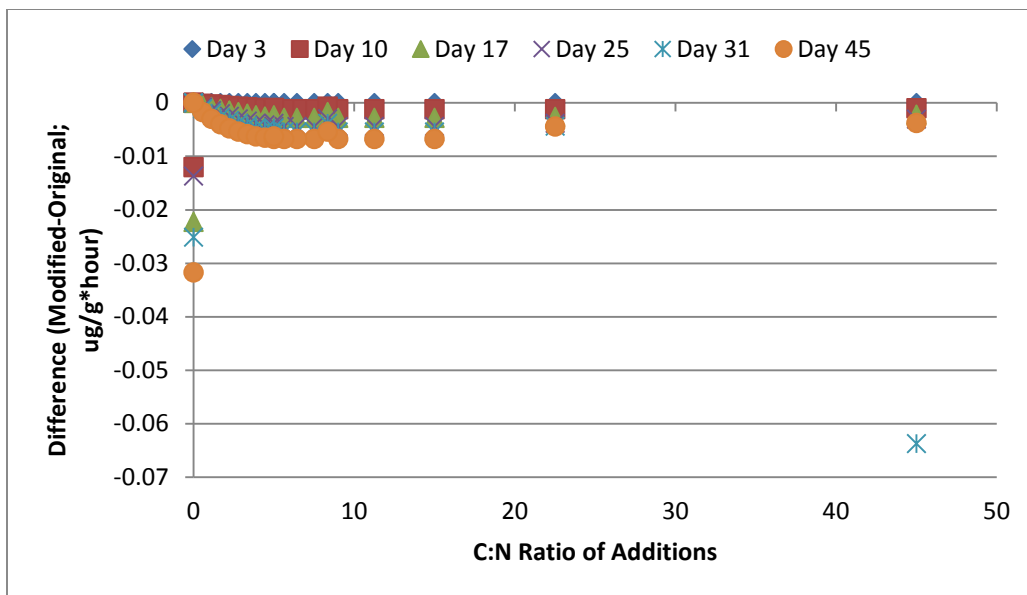


FIG A7. The difference between the original model and the modified model for C mineralization over time. The magnitude of these changes is small compared to the actual C mineralization rate (Fig. S2). The points at C:N=0 that deviate most from zero are from the 900:0 treatment.

LITERATURE CITED

- Drake, J. E., B. A. Darby, M. A. Giasson, M. A. Kramer, R. P. Phillips, and A. C. Finzi. 2013. Stoichiometry constrains microbial response to root exudation—insights from a model and a field experiment in a temperate forest. *Biogeosciences* 10:821–838.
- Schimel, J. P. and M. N. Weintraub. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: A theoretical model. *Soil Biology & Biochemistry* 35:549–563.