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Short Communication

Nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied

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ABSTRACT

We tested how amendments of different forms of nitrogen (N) affect microbial respiration rates by adding six different forms of N (NH_4NO_3, $(NH_2)_2CO$ (urea), KNO_3, NH_4Cl, $(NH_4)_2SO_4$, $Ca(NO_3)_2$) to three distinct soils. All inorganic N forms led to a net reduction in microbial respiration, and the magnitude of the observed response (up to 60 % reduction) was consistent across all soils and negatively correlated with N concentration. Urea also reduced respiration rates in nearly all cases, but the effect was attenuated by the associated input of labile organic carbon. We observed decreases in respiration regardless of soil type, the specific N counter ion, N added as NH_4^+ or NO_3^-, or the effects of N form on soil pH, suggesting that decreases in respiration rates were mainly a direct result of the increase in soil N availability, rather than indirect effects caused by the form of N added.

A large number of published field and laboratory studies have examined how nitrogen (N) affects belowground communities and processes by adding N to soils and then measuring how microbial respiration, biomass and microbial community composition respond (e.g. Söderström et al., 1983; Fog, 1988; Waldrop et al., 2004; Treseder, 2008; Janssens et al., 2010). The form of N added varies across studies, yet we know little about how different N forms may impact microbial processes. N additions to soil can affect microbial activity, yet it is not clear if these impacts are a direct function of the increase in N availability or a result of indirect effects of the fertilizer inputs on other soil chemical characteristics (e.g. changes in pH or concentrations of cations/anions other than N). Specifically, N addition frequently decrease microbial respiration (Kowalenko et al., 1978; Söderström et al., 1983; Thirukkumaran & Parkinson, 2000; Bowden et al., 2004; Craine et al., 2007 Treseder, 2008), but it is not known whether this microbial response is a direct effect of the increase in N availability and if adding different forms of N would yield a similar response. Resolution of this question can provide a better understanding of soil responses to N additions (Treseder, 2008; Janssens et al., 2010).

Here we describe a laboratory experiment designed to determine how the form of N and the rate of N application influences soil microbial respiration. We added N at different concentrations to three soil types, using six different N forms that varied in the oxidation state of N, the ion paired with N, and the presence of organic C. Comparing treatments allowed us to examined how the effects of N on microbial respiration are influenced by 1) the amount of N added; 2) N added as NH_4^+ or as NO_3^-, or N counter ion type; 3) the indirect effects of fertilizer additions on soil pH; and 4) the co-addition of reduced C when adding urea.

Soils were collected in May 2009 from the top 5 cm of the profile of three sites within the Front Range of the Rocky Mountains. These soils were selected because they represent multiple ecosystem and vegetation types ('aspen', 'pine', and 'grassland') with distinct soil edaphic characteristics (Table 1). Soils were sieved on site to 2 mm, thoroughly homogenized, and then stored at 4 °C for one week. Subsamples of soil (5 g each) were weighed into 60 mL glass vials. Each sub-sample was treated with one of six N-fertilizer forms most commonly used in field or laboratory N-amendment experiments: NH_4NO_3, $(NH_2)_2CO$ (urea), KNO_3, NH_4Cl, $(NH_4)_2SO_4$, $Ca(NO_3)_2$, in one of four concentrations (10, 50, 200, 500 $\mu g\ N^{-1}\ g\ soil^{-1}$). Each soil was subsequently adjusted to 50% of water holding capacity (WHC). Control soils received only water. Each treatment was replicated 3 times, yielding 90 sub-samples per soil type for a total of 270 individual vials. Soils were incubated for 45 days at 21 °C. Soil respiration

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Table 1
A description of the three soils used in this study. Mean values for measured soil properties with standard error of the mean (SEM) indicated in parentheses when available.

Soil	Latitude	Longitude	Dominant species	C:N	Total C ($mg\,g^{-1}$ soil)	Total N ($mg\,g^{-1}$ soil)	Extractable N ($mg\,g^{-1}$ soil)	pH
Aspen	40.02	−105.48	Aspen (*Populus tremuloides*)	16.55 (0.56)	19.39 (0.61)	1.17 (0.00)	0.009 (NA)	5.66 (0.12)
Pine	40.02	−105.48	Ponderosa pine (*Pinus ponderosa*), Lodgepole pine (*Pinus contorta*)	27.21 (0.51)	15.23 (0.69)	0.56 (0.01)	0.001 (NA)	6.10 (0.17)
Grassland	39.13	−105.72	Blue grama (*Bouteloua gracilis*)	10.08 (0.16)	9.40 (0.27)	0.93 (0.01)	0.014 (NA)	5.91 (0.24)

rates were measured 9 times throughout the incubation period using the method described in Fierer et al. (2003). Net CO_2 production was calculated by measuring the increase in headspace CO_2 concentrations relative to the controls over time. Statistical analyses were conducted with R, version 2.10.1 (http://www.r-project.org).

In all 5 inorganic N treatments, N-fertilizer additions significantly decreased microbial CO_2 production (Fig. 1). At the highest N concentrations ($500\,\mu g\,N^{-1}\,g\,soil^{-1}$) average CO_2 production over the course of the incubation period decreased by approximately 60% in aspen soil, 60% in the pine soil and 30% in grassland soil relative to the control (no added N) ($P < 0.01$ in all cases as determined by ANOVA) (Fig. 1). All soils experienced similar rates of decline in respiration over the 45 d incubation ($P > 0.05$ in all cases) (Fig. 2). For the 5 inorganic N-fertilizers, the inhibition of respiration persisted throughout the duration of the incubation. By day 45, control soils still produced significantly more CO_2 than soils receiving the highest N concentrations ($500\,\mu g\,N^{-1}\,g\,soil^{-1}$) ($P < 0.01$ in all cases). As determined by multiple linear regression (MLR), for these five forms of N, respiration was significantly influenced by concentration of N ($P < 0.01$) and soil type did not affect this relationship ($P > 0.05$). When examining possible indirect effects, only pH had an observable impact on respiration ($P < 0.05$), and other likely contributors such as N as NH_4^+ or NO_3^- and N counter ion types did not influence respiration ($P > 0.05$, as determined by linear regression). Further examination of pH and N concentration for each soil type shows that two of the three soils experience inconsistent or insignificant effects of N additions on soil pH (Table 2), yet all three soils exhibited decreases in microbial activity. This evidence strongly suggests that pH changes resulting from the N additions are not responsible for the consistent decreases in respiration rates observed here.

In contrast to the C-free forms of N, urea addition decreased respiration in the aspen and pine soils by only 27% and 11%, respectively, but increased respiration rates by 20% in the grassland soil, which had the lowest C:N ratio ($P < 0.05$) (Table 1). While urea produced substantially more CO_2 in the initial days relative to the control ($P < 0.05$; ANOVA) (Fig. 2D), by day 17 this effect was negligible ($P > 0.05$ in all soils; ANOVA). With the N inhibition of microbial respiration evident by the end of the incubation in two of the three soils, these results suggest that the additional C provided by urea was readily labile and quickly consumed.

All inorganic N forms applied to the soils decreased microbial respiration rates, with the magnitude of the decrease varying across the soil types but not across the N forms. Any indirect effects (e.g. pH) of N additions on respiration were inconsistent between treatments and therefore cannot explain the consistent decrease in

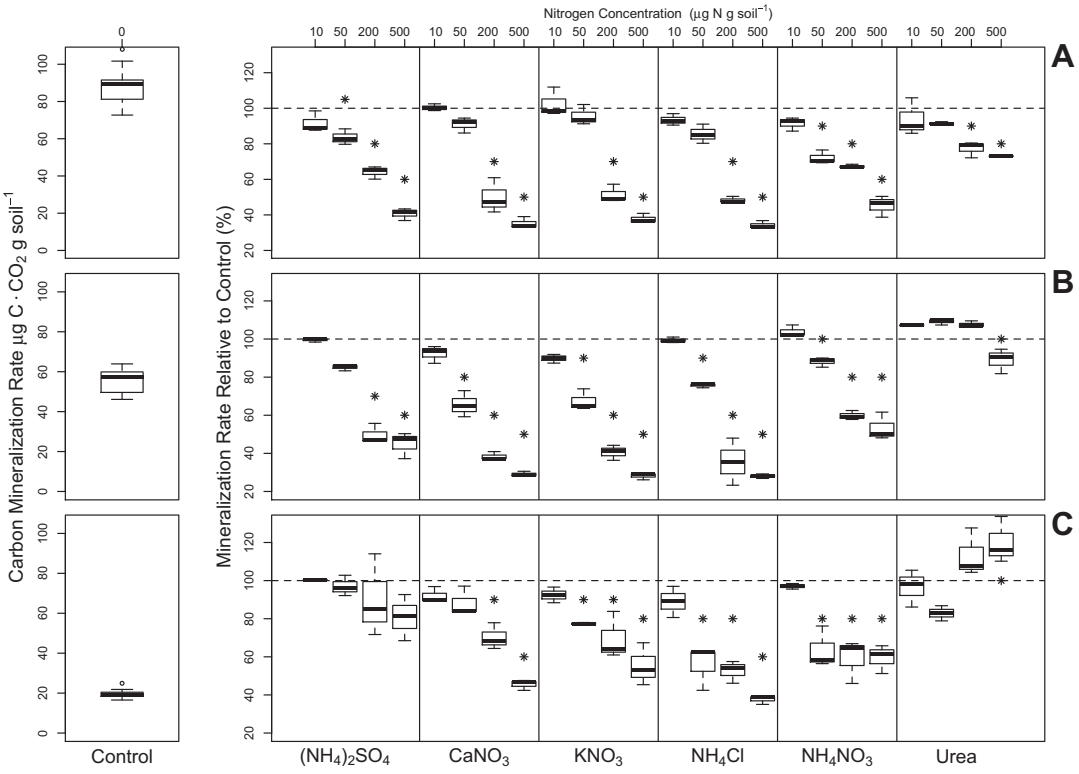

Fig. 1. Box plots (showing the median, surrounded by a 50% quantile box and 100% quantile whiskers) of total respiration from control samples (no N added) (left panels), and percent difference in total respiration, relative to the control, for each N form, concentration ($\mu g\,N\,g\,soil^{-1}$) and soil type aspen (A), pine (B), and grassland (C) (right panels). * Indicate significant difference ($P < 0.05$) from control (ANOVA, TukeyHSD).

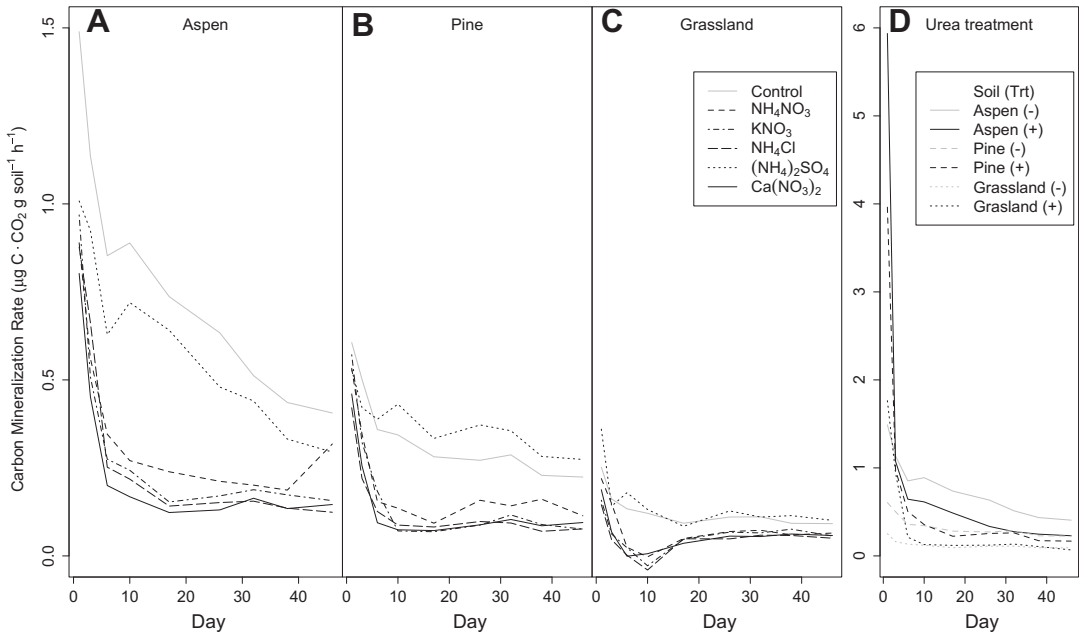

Fig. 2. Panels A–C show average respiration rates (µg C-CO_2 h^{-1} $sample^{-1}$) in the aspen (A), pine (B) and grassland (C) soils for control and inorganic N treatments (500 µg N g $soil^{-1}$). Panel D shows respiration rates (µg C-CO_2 h^{-1} $sample^{-1}$) for control (−) and urea treatments (+) (500 µg N g $soil^{-1}$) for all three soils.

Table 2
Correlation values between pH and N concentrations for each soil and nitrogen type. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, not significant (N.S.). A negative correlation indicates that pH decreased as N concentration increased.

Nitrogen	Aspen	Pine	Grassland
NH_4NO_3	+0.64*	−0.91***	N.S.
Urea	−0.91***	N.S.	N.S.
KNO_3	+0.58*	−0.82***	−0.61**
NH_4Cl	+0.55*	−0.87***	N.S.
$(NH_4)_2SO_4$	N.S.	−0.87***	N.S.
$Ca(NO_3)_2$	−0.60*	−0.84***	N.S.

respiration across soils and treatments. Instead, direct N addition and the concentration of that addition provide the best explanation for the observed decreases in soil respiration. Our study highlights that comparisons among experiments that add different inorganic forms of N can be made reliably, and comparisons that include urea amendments are still appropriate in longer-term studies. While we demonstrate that a range of fertilizer types have similar impacts on soil respiration, future work is still needed to identify the mechanism, or set of mechanisms, responsible for the apparent suppression of microbial respiration.

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References

Bowden, R.D., et al., 2004. Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. Forest Ecology and Management 196, 43–56.
Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105–2113.
Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology and Biochemistry 35, 167–176.
Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Reviews 63, 433–462.
Janssens, I.A., et al., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nature Geoscience 3, 315–322.
Kowalenko, C., Ivarson, K., Cameron, D., 1978. Effect of moisture content, temperature and nitrogen fertilization on carbon dioxide evolution from field soils. Soil Biology and Biochemistry 10, 417–423.
Söderström, B., Baath, E., Lundgren, B., 1983. Decrease in soil microbial activity and biomasses owing to nitrogen amendments. Revue Canadienne de Microbiologie. (Canadian Journal of Microbiology) 29, 1500–1506.
Thirukkumaran, C.M., Parkinson, D., 2000. Microbial respiration, biomass, metabolic quotient and litter decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorous fertilizers. Soil Biology and Biochemistry 32, 59–66.
Treseder, K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecology Letters 11, 1111–1120.
Waldrop, M., Zak, D., Sinsabaugh, R., 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology and Biochemistry 36, 1443–1451.