



## Review

# Mineral nitrogen input decreases microbial biomass in soils under grasslands but not annual crops



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## ABSTRACT

Anthropogenic inputs of reactive nitrogen (N) to terrestrial ecosystems have increased considerably over the past several decades. Soil microorganisms provide numerous ecosystem services and are closely linked to the global carbon cycle through decomposition of organic material. The objectives of this study were to quantify the long- and short-term effects of mineral N input (ammonium, nitrate or urea) on soil microorganisms in permanent grassland and annual cropping systems. Conducting a *meta*-analysis, we found that in grassland studies ( $n = 54$ ), soil microbial biomass (SMB) decreased significantly by 12% with N addition, while SMB increased significantly by 13.6% in annual crops ( $n = 150$ ). Pronounced shifts among major microbial groups from N additions were not evident in our analysis, even though individual studies sometimes found significant effects of N input on PLFA profiles. We found the best support for the hypothesis that higher crop productivity in annual cropping systems brought on by fertilization increases plant residue inputs, which in turn increases SMB in the long term. In the short term, N inputs may also increase primary production in permanent grassland. However, N inputs gradually reduce grassland plant species richness which likely leads to the observed decrease in SMB. Over time, the effect of plant species richness on SMB becomes more pronounced. Our analysis reveals that management is an important consideration in predicting how ecosystems respond to environmental change and highlights the complexity and diversity of plant-microbial interactions, and underscores the immense value of long-term field trials.

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**Abbreviations:** AM, arbuscular mycorrhizal fungi;  $C_{org}$ , soil organic carbon; CV, coefficient of variation;  $N_t$ , total soil N; PLFA, phospholipid fatty acid;  $qCO_2$ , specific respiration (respiration per unit microbial biomass); RR, response ratio; SMB, soil microbial biomass.

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## 1. Introduction

Anthropogenic inputs of reactive N to terrestrial ecosystems have increased considerably over the past several decades (Gruber and Galloway, 2008; Galloway et al., 2008). In agricultural systems, these inputs are mainly in the form of mineral fertilizers, while in unmanaged ecosystems atmospheric deposition is the primary pathway for N input. Nitrogen inputs have been largely responsible for the substantial crop yield increases realized since the 1950s (Fageria and Baligar, 2005; Ladha et al., 2011). In natural ecosystems, increased N inputs can also have strong effects on not only plant productivity but also plant community composition (Suding et al., 2005; Bobbink et al., 2010).

Belowground soil communities are also sensitive to inputs of nutrients to the soil (Allison and Martiny, 2008). However, while plant growth in terrestrial ecosystems is generally N limited, soil microorganisms are more likely to be carbon (C) limited and less frequently N limited (Wardle, 1992). The types of responses of soil microbes therefore differ from responses of plant communities. Soil microorganisms provide numerous ecosystem services such as decomposition of organic material, nutrient cycling, and biotransformation of organic pollutants (Schimel, 1995; Thiele-Bruhn et al., 2012). Furthermore, the release of CO<sub>2</sub>, a product of decomposition, closely links microbial activity to the global C cycle as the ratio between decomposition and C input from plants determines whether soils are a sink or source of C (Gougoulas et al., 2014). Consequently, factors affecting soil microbial biomass and activity may have local and global impacts.

In a previous study, focusing on long-term trials with annual crops, we found that mineral fertilizer application led to a 15.1% increase in the SMB compared to unfertilized control treatments. Mineral fertilization also increased soil organic carbon (C<sub>org</sub>) content and our results suggested that C<sub>org</sub> is a major factor contributing to the overall increase in SMB with mineral fertilization (Geisseler and Scow, 2014). These results were surprising, as several recent meta-analyses based on data predominantly from unmanaged ecosystems found that increasing N inputs suppress soil microorganisms (Treseder, 2008; Liu and Greaver, 2010; Lu et al., 2011). Our results differed from previous meta-analyses most likely because our focus was on annual cropping systems but perhaps also because we only included long-term trials in the analysis. With the present study we are examining these two factors.

World food production is dominated by grass species (Poaceae or Gramineae) and this was evident from the long-term data included in our previous meta-analysis. Roughly half of the trials involved continuous cultivation of corn or cereals or a rotation with only these crops. While grass species also dominate natural and managed grassland, grasslands differ considerably from systems with annual crops because they experience less soil disturbance by tillage, have continuity of plant cover and higher plant species richness. Overall, grassland soils are less disturbed and provide a temporally less variable habitat for soil microorganisms while the spatial variability may be higher.

The objectives of this study were to compare the long- and short-term effects of N input on microbial biomass, activity and community composition in permanent grassland and annual cropping systems. This study builds on results of our previous

analysis where we focused on long-term N impacts on cropping systems (Geisseler and Scow, 2014). In the current study, we included shorter-term studies and grasslands to test the hypotheses that (i) the response of the microbial biomass to N addition differs between annual cropping systems and permanent grassland, that (ii) the effects of N addition become more pronounced over time, and that (iii) the differences can be explained by shifts in the microbial community composition. These hypotheses were tested with a meta-analysis of a global dataset. Based on the results, we formulated a set of additional hypotheses to investigate potential factors responsible for the observed trends and differences.

## 2. Material and methods

We used a meta-analysis approach to analyze results from a number of peer-reviewed studies to quantify the effect of N fertilizer additions on soil microorganisms. We searched the online database Web of Science for papers using the title words 'input', 'deposition', or 'fertilizer' and the keywords 'soil', 'nitrogen', 'microbial' and 'biomass'. More than 1000 articles met these criteria and were screened for data on microbial biomass. In addition; articles cited in review papers or meta-analyses that analyzed data from N fertilization trials were included in our search. The following criteria were applied to select appropriate studies: (i) the data were from field trials with annual crops (except lowland rice cropping systems in paddy soils) or permanent grassland and (ii) the study reported microbial biomass both from an unfertilized control and a treatment with mineral N addition. Although urea is chemically an organic molecule; its behavior in soil is much more like that of a mineral fertilizer and we consider it as such in our analysis. Trials in which fertilization treatments were discontinued more than a year before soil sampling were not included in the analysis. The literature search was concluded in May 2015.

Meta-analysis requires that datasets are independent. To meet this requirement, we only included values from the topsoil when data from several soil layers were reported. When studies measured microbial biomass repeatedly over time, only one sampling date was used for the meta-analysis. The sampling date at which other soil properties, such as pH or C<sub>org</sub> were also determined, was preferred. We also preferred pre-plant or post-harvest sampling dates over in-season dates to minimize the effects of recently added fertilizers. If several sampling dates met these criteria, random numbers were assigned to each date and the date with the highest number was used for the meta-analysis. The same criteria were used when several studies reported data from the same trial.

Trials that included different crop rotations or plant communities, each with an unfertilized control, were considered independent and entered as separate datasets, as were results from different trials at the same location. Some trials included treatments with different N application rates or different N fertilizers, with treatments sharing a control. In this case, the treatments were considered dependent and the average response and a composite standard deviation (Borenstein et al., 2009) were calculated and used for the meta-analysis. However, for the comparison of the

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