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Composition and carbon utilization of soil microbial communities subjected to long-term nitrogen fertilization in a temperate grassland in northern China

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ABSTRACT

Carbon (C) and nitrogen (N) cycling in soil during microbial decomposition is well studied, yet the mechanism underlying the response of microbial C utilization to the presence of N still remains an open question. This study was designed to determine the effect of long-term N fertilization on grassland microbial communities, and to explore if the alteration of labile C utilization of microbial communities was affected by N. A 35-day multi-factorial incubation experiment with three N fertilization rates 0, 4, or 16 g N m^{-2} yr^{-1} (applied as urea) and one C substrate application, 0.4 mg ^{13}C glucose g^{-1} soil was conducted using a temperate grassland soil. Soil respiration, inorganic N, soil total C (TC) and total N (TN), and ^{13}C-phospholipid fatty acids were measured. High N fertilization rate (16 g N m^{-2} yr^{-1}) increased soil inorganic nitrogen (ION) significantly and resulted in a significant drop of soil pH, which decreased from a neutral (~pH 7) to pH 5.8. Long-term N fertilization caused an increased ^{13}C utilization of gram-positive bacteria and actinomycetes, but reduced ^{13}C utilization of gram-negative bacteria and fungi. Low and high N levels had inconsistent impacts on the temporal patterns of ^{13}C distribution in saprophytic fungi and ratios of incorporated ^{13}C in cyclopropyl to its precursor during the course of the decomposition. Decomposition theories such as 'nutrient stoichiometry' and 'N mining' were both supported in this study, as N mining was least prominent in soil with high N fertilization rates, while optimal nutrient ratio existed when labile C was added in soil under low N level. N fertilization in the temperate grassland might regulate the shift in labile C and SOM between microbial C utilization. To further understand the coupling of soil C and N, future work should focus on the beginning of the decomposition process, and increase the sampling frequency.

1. Introduction

Soil microorganisms modify C decomposition, and thus are critical in controlling the quantity and quality of carbon (C) stored in terrestrial soils. Therefore, understanding how microbial communities will react to nutrient additions will improve predictions of terrestrial C dynamics (Leff et al., 2015). Nitrogen (N) is one of the limiting factors for ecosystem productivity, and increasing atmospheric N has a profound impact on ecosystems worldwide (Galloway et al., 2008). Inconsistent results were obtained for soil respiration (Eberwein et al., 2015; Li et al., 2015), exoenzyme production (Cenini et al., 2015; Wang et al., 2015), dissolved organic C (Evans et al., 2008; Kopáček et al., 2013; Wei et al., 2013), and microbial C use efficiency (Riggs and Hobbie, 2016; Spohn et al., 2016) on the effects of N fertilization on soil C decomposition. Soil C sequestration is considered to be enhanced by soil N input on a global scale due to stimulated plant biomass input (Reay et al., 2008), but soil C accumulation following N addition was not observed in some grassland studies (Creamer et al., 2014; Fornara et al., 2016; Li et al., 2015). Due to the heterogeneity of soil properties, microbial structure, and microbial functioning (Hautier et al., 2014), the response of soil decomposition to N fertilization is still a subject of debate in cellulose-dominated grassland soils.

Labile C input from litter and root exudates can be utilized almost instantly by soil microbial communities, therefore it has a short residence time and the product (e.g. CO_2 and microbial biomass) is easy to measure (Creamer et al., 2014). Despite its small pool size, fluxes of labile C are large due to its continuous inflows and outflows (de Vries and Caruso, 2016). Labile C such as glucose can activate microbial

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Table 1
Baseline soil properties on day 0.

N levels (g N m^{-2} yr^{-1})	Soil pH	Total carbon (%)	Total nitrogen (%)	Inorganic nitrogen (mg kg^{-1})	Soil respiration (umol CO_2 g^{-1} s^{-1})	Phospholipid fatty acids (nmol g^{-1})
0	7.26[a] (0.58)	2.12[a] (0.12)	0.21[a] (0.01)	7.45[a] (0.73)	4.35[a] (0.26)	13.17[a] (1.00)
4	7.08[a] (0.80)	1.91[a] (0.23)	0.19[a] (0.03)	18.03[a] (5.54)	2.70[b] (0.29)	14.24[a] (1.72)
16	5.78[b] (0.10)	1.96[a] (0.10)	0.20[a] (0.01)	44.02[b] (8.66)	0.98[c] (0.03)	10.08[a] (1.60)

Standard error is in parentheses (n = 4). Means followed by the different lower case letters in a column are significantly different at $P < 0.05$.

metabolism and trigger the decomposition of soil C. This will lead to a substantial increase of C loss from the soil, especially on a short timescale.

The classic assumptions about r-selected and K-selected microbial communities are being challenged by emerging evidence. Saprophytic fungi were found to be quick to utilize labile C (Lemanski and Scheu, 2014), and no association occurred between soil organic matter (SOM) and the fungal-to-bacterial dominance (Rousk and Frey, 2015). The latest theory suggests that soil C sequestration can be thought of as a continuum as opposed to highly separate sinks (de Vries and Caruso, 2016; Lehmann and Kleber, 2015). Since the role of labile C as a major C input into the soil is important for studying soil C cycling (de Vries and Caruso, 2016), the interactions among microbial communities and labile C should be addressed.

Two hypotheses seem to be at odds when illustrating the association between microbial decomposition and the processes of soil C and N cycling. The 'nutrient stoichiometry' model says that the fate of C is determined by the demands for nutrients of each organism relative to its resources (Hessen et al., 2014). Contrastingly the 'N mining' theory is based on the concept that microorganisms utilize labile C as an energy source and produce exoenzymes to decompose recalcitrant C when N is limited (Craine et al., 2007). Thus, to reveal the coupling of C and N cycling in soil during microbial decomposition, C utilization by microbial communities should be studied within N enriched soils with a labile C isotope to allow tracing.

Grasslands today cover 26% of the world land area and 70% of the world agricultural area (Conant, 2010), playing a crucial role in ecoservice and functioning. Since the critical N load for the typical grassland in China is around 5 g N m^{-2} yr^{-1} (Liu et al., 2011), the effects of N input at the concentration lower and higher than the N threshold are supposed to vary on grassland soil. To explore the alteration of the grassland soil microbial communities and the dynamics of their labile C utilization under a long-term N addition scenario, an incubation experiment was established using grassland soil. The soil was subjected to 3 N fertilization rates, with and without ^{13}C labeled glucose addition. The hypotheses of the study were: (1) Long-term N fertilization will have a significant impact on the relative abundance of microbial communities and their contributions to glucose utilization; (2) N fertilization will affect the dynamics of glucose utilization by microbial communities during the course of decomposition; and (3) N fertilization will shift the soil C components for microbial C utilization, and thus lead to a change of soil C accumulation.

2. Materials and methods

2.1. Study site

The study site is located in Duolun County, Inner Mongolia, China (42.02°N, 116.17°E; 1341 m above sea level). The mean annual temperature is 2.1 °C, and average monthly temperatures range from a minimum of −17.5 °C in January to a maximum of 18.9 °C in July. The mean annual precipitation is 379.4 mm, most occurs as rainfall from May through September (Wang et al., 2015). The typical soil in this region is a chestnut soil (Chinese Soil Taxonomy Research Group, ISSAS 2001), corresponding to Petrocalcic Aridisol based on the U.S. Soil Taxonomy (Soil Survey Staff, 2014). The area is dominated by perennial grasses, such as *Stipa krylovii, Leymus chinensis, Artemisia frigida, Agropyron cristatum* and *Allium bidentatum*. The long-term N fertilization experimental site was established by the Duolun Restoration Ecology Research Station (part of the Institute of Botany, Chinese Academy of Sciences). Since July 2003, dry urea N fertilizer ($CO(NH_2)_2$, 46.67% N) has been manually spread on the surface of the plots (15m × 10 m each) in mid-July (during the rainy season) of each year.

2.2. Experimental design

The experiment had a multi-factorial design with three N fertilization rates 0, 4 or 16 g N m^{-2} yr^{-1} (applied as urea), and one C substrate (^{13}C labeled glucose). Soil without C substrate addition served as control. Every treatment was replicated four times. In late July 2015, three soil cores (0–20 cm depth) were randomly collected from each plot, then mixed, homogenized and sieved (2 mm). A 50 g dry weight soil sample was transferred into a 150 mL flask and the soil moisture was adjusted to 40% of the water holding capacity (WHC).

Soil samples were incubated in the dark at 20 °C, until soil CO_2 rate was stable (Garcia-Pausas and Paterson, 2011). Soil properties were measured after stabilization, and this data was considered as day 0 (Table 1). On the same day, 1 mg ^{13}C labeled glucose (99.9 atom% ^{13}C, Cambridge Isotope Laboratories, Inc., MA, USA) was dissolved in deionized water and sprayed on each glucose treated soil sample (final concentration of 0.4 mg ^{13}C g^{-1} soil). Controls were treated with the same amount of deionized water. Soil moisture was kept at 70%WHC during the incubation. Three destructive samplings took place on days 7, 21, and 35 after glucose addition.

2.3. Analysis of soil properties

Air-dried soil samples for days 0 and 35 were ground and sieved (0.25 mm), then measured for total nitrogen (TN) and total carbon (TC) by an elemental analyzer (Perkin-Elmer, MA, USA).

Soil respiration was measured for days 0, 7, 21 and 35 on fresh soil. The flask was sealed with a rubber stopper and 2 tubes connected the flask to an automated soil gas flux system (LI-COR, NE, USA). The CO_2 rate was calculated by measuring the linear increase of CO_2 concentration in the flask for 60 s. The flux rate was calculated as μmol CO_2 g^{-1} soil s^{-1} based on LI-COR 8100 Manual (Eberwein et al., 2015).

Soil inorganic nitrogen (ION, the sum of ammonium nitrogen and nitrate nitrogen) was measured using fresh soil samples on days 0, 7, 21 and 35. Soil was extracted with deionized water for 1 h (the soil to extractant ratio was 1:10), and ION in the extractant was measured using an ion chromatograph analyzer (DIONEX, CA, USA).

Phospholipid fatty acids (PLFA) were extracted based on the Bligh and Dyer (1959) procedure and analyzed via gas chromatography (Agilent, CA, USA). Saturated FAMEs were 15:0, 16:0 and 18:0; PLFA biomarkers indicating gram-positive bacteria were i15:0, a15:0, i16:0, i17:0, and a17:0; gram-negative bacteria biomarkers were 16:1w7c, cy17:0, 17:1w8c, 18:1w7c, and 18:1w5c; saprophytic fungal biomarkers were 18:2w6c and 18:1w9c; arbuscular mycorrhizal fungi (AMF) biomarker was 16:1 w5c; and actinomycete biomarkers were 10Me17:0 and 10Me18:0 (Bossio and Scow, 1998; Wei et al., 2013).