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Short communication

# High nitrogen deposition decreases the contribution of fungal residues to soil carbon pools in a tropical forest ecosystem

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ABSTRACT

Soil carbon (C) dynamics are closely mediated by microorganisms and microbial residues could constitute a significant pool of soil organic C (SOC). However, little is known about the nitrogen (N) deposition effect on the contribution of microbial residues to SOC balance in tropical forest ecosystems. Here, we assessed microbial residues using amino sugar biomarkers in surface soils of a tropical forest ecosystem under 11-year continuous N addition at different rates (0, 50, 100 and 150 kg N $ha^{-1}$ $yr^{-1}$). Nitrogen addition didn't affect either fungal or bacterial residues in soil apparently, but high-N addition (150 kg N $ha^{-1}$ $yr^{-1}$) significantly decreased the contribution of fungal residues to SOC indicated by both ratios of fungal/bacterial amino sugars and fungal residues/SOC. Consequently, high-N addition whittled down microbial contribution to SOC pools. Those findings may have implications for our predictions of global change impacts on soil C dynamics.



Soil carbon (C) dynamics are heavily influenced by catabolic and anabolic activities of microorganisms (Liang et al., 2011; Schimel and Schaeffer, 2012). Microbial residues represent a significant source of stable C and may play a greater role in long-term C accumulation in soils than traditionally believed (Simpson et al., 2007; Liang et al., 2011). Global change drivers such as nitrogen (N) deposition can exert direct (i.e., altering physiological activity) and indirect (i.e., altering resource supply) influences on soil microbial communities. Consequently, the retention of microbial debris and the regulation in key process in soil C turnover can also be affected by N deposition to some extent (Zak et al., 2011). However, main understanding of N deposition effects on soil microbial-mediated C dynamics is largely based on work in temperate ecosystems, where N is mostly limited (Griepentrog et al., 2014). Comparable data are generally lacking for tropical forest ecosystems, where soil is often N-rich or even N-saturated, thus the systems are featured by rapid N cycling rates and high net primary productivity (Matson et al., 1999; Wright et al., 2011; Brookshire et al., 2012). Meanwhile, most tropical soils are often highly acidic and poorly buffered against acidification (Sollins et al., 1988; Matson et al., 1999). As a result, conclusions based on studies conducted in temperate regions are of little relevance for the tropics under elevated N deposition. To our knowledge, there is limited information regarding the effect of N deposition on soil microbial contribution to soil C pools in tropical forest ecosystems, which store approximately 13% of global soil C contributing greatly to the global C cycle (Lu et al., 2013).

The dynamics of microbial residues and their contribution to SOC can be indicated by soil amino sugars. They are cell wall components of microorganisms (Lauer et al., 2011) and can be stabilized in soil after cell dies. Amino sugars can serve as a time-integrated biomarker to indicate microbial community structure (Glaser et al., 2004). Muramic acid (MurN) originates exclusively from bacteria, whereas glucosamine (GluN) predominantly originates from fungi (Amelung, 2001). The origin of soil galactosamine

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**Table 1**
Soil properties and fungal and bacterial amino sugars parameters under different N treatment.

| | Control | Low-N | Medium-N | High-N | *P* value |
|---|---|---|---|---|---|
| SOC (g kg$^{-1}$) | 37.77a | 47.57a | 47.38a | 47.92a | 0.404 |
| Soil N (g kg$^{-1}$) | 2.54a | 3.21a | 3.28a | 3.31a | 0.136 |
| pH | 3.91a | 3.81a | 3.77a | 3.75b | 0.031 |
| Total amino sugar (mg kg$^{-1}$) | 1972.03a | 1925.26a | 2066.98a | 1634.30a | 0.599 |
| GluN[a] (mg kg$^{-1}$) | 1372.18a | 1365.85a | 1411.90a | 1040.69a | 0.395 |
| GalN[b] (mg kg$^{-1}$) | 480.24a | 450.88a | 526.36a | 462.94a | 0.829 |
| MurN[c] (mg kg$^{-1}$) | 119.60a | 108.53a | 128.69a | 130.67a | 0.644 |
| GluN/GalN | 2.86a | 3.02a | 2.67ab | 2.30b | 0.045 |
| GluN/MurN | 11.61a | 12.47a | 10.87ab | 8.00b | 0.008 |
| Fungal residues/bacterial residues | 2.04a | 2.21a | 1.89ab | 1.31b | 0.008 |

The effects were significant at $P < 0.05$ and different letters indicate significant difference between different treatments.
[a] GluN, glucosamine which predominantly originates from fungi.
[b] GalN, galactosamine which origin is uncertain, but is generally considered to be derived from bacteria.
[c] MurN, muramic acid which originates exclusively from bacteria.

(GalN) is uncertain (Engelking et al., 2007), but is generally considered to be derived from bacteria (Amelung, 2001). Accordingly, ratios of different individual amino sugars can be used to track the relative contributions of fungi and bacteria to SOC pools (Liang et al., 2013).

In this paper, we quantified amino sugars in surface soils of a tropical forest ecosystem under 11-year continuous N addition at different rates. Our goal was to investigate the impact of 11-year N addition on bacterial and fungal residues and their contribution to SOC pools (indicated by the relative proportion of amino sugars in SOC) in a tropical forest ecosystem. It is generally believed that N addition causes a shift from fungal to bacterial dominated microbial communities (Strickland and Rousk, 2010). We hypothesized that N addition would decrease the contribution of fungal residues to SOC pools, while that of bacterial residues would be uncertain due to N-rich and highly acidic features of tropical soil.

We carried out our study at Dinghushan Biosphere Reserve, Guangdong Province, southern China (112°10′E, 23°10′N). The reserve is in tropical moist forest region and it has experienced high rates of atmospheric N deposition (21–38 kg N ha$^{-1}$ yr$^{-1}$, mainly as inorganic N form in bulk precipitation, originated from anthropogenic N) since 1990's (Lu et al., 2014). The soil is classified as Oxisols (Soil Survey Staff, 2003) with a loamy clay texture (Zhang et al., 2008) formed from sandstone approximately 30–70 cm in depth and it was quite homogeneous in soil texture (37.8% sand, 33.0% loam, 29.3% clay) and bulk density (0.98 g cm$^{-3}$) (Wu et al., 1982; Lu et al., 2015). Treatment plots of 10 m × 20 m were established with three replicates in a random design and N addition experiments were initiated in July 2003. There were four N addition treatments: null N (control), 50 kg N ha$^{-1}$ yr$^{-1}$ (low-N), 100 kg N ha$^{-1}$ yr$^{-1}$ (medium-N) and 150 kg N ha$^{-1}$ yr$^{-1}$ (high-N). Ammonium nitrate solution was sprayed monthly onto the plots with equal quantity of solutions and the control plots received equal volumes (20 L) of deionized water. Soils were sampled in July 2014 with a 5-cm-diameter corer to a depth of 10 cm. Each composite soil sample was randomly made from three soil cores in each plot. After large plant residues, root fragments and stones were removed, the soil samples were passed through a 2-mm sieve and then air-dried soil samples were ground before analysis.

Soil organic carbon and soil N were determined by an Elemental Analyzer (vario MACRO cube, Elementar Analysensysteme GmbH, Germany). Soil pH was measured with a glass electrode using a 1: 2.5 soil-water suspension. Soil amino sugars were determined according to Zhang and Amelung (1996). Fungal- and bacterial-derived C as an index for fungal and bacterial residues was calculated according to Engelking et al. (2007), respectively. Total microbial residues were estimated as sum of fungal- and bacterial-derived C. One-way ANOVA with Tukey test was employed to identify the effects of N addition on soil amino sugars at $P < 0.05$ (SPSS Inc.).

Nitrogen addition didn't affect the concentration of SOC and N, the content of any individual amino sugars, and the size of total amino sugar pool in soil significantly (Table 1, $P > 0.05$). However, high-N addition significantly decreased ratios of GluN/MurN, GluN/GalN and fungal residues/bacterial residues (Table 1, $P < 0.05$) by 31.1%, 19.5% and 35.5% compared to control, respectively. The bacterial residues/SOC ratio (Fig. 1) did not change after N addition, while ratios of fungal residues/SOC and total residues/SOC under high-N treatment were significantly decreased by 44.4% and 34.4%, respectively. Unsurprisingly, the pH values were all below 4, but high N addition decreased soil pH further (Table 1, $P < 0.05$).

The microbial contribution to SOC pools is directly related to microbial community dynamics and the balance between formation and degradation of microbial byproducts (Six et al., 2006). Therefore, the lower fungal/bacterial residue ratios could result from both the differences in microbial community composition and different turnover time between fungal and bacterial residues. It is generally believed that fungal products are more chemically recalcitrant and more difficult to mineralize than bacterial products (Nakas and Klein, 1979; Six et al., 2006). Thus, the decrease in fungal/bacterial residue ratios caused by N addition resulted mainly from differences in microbial community composition. Strickland and Rousk (2010) found that N addition causes a shift from fungal to bacterial dominated microbial communities. The shift is likely due to the indirect toxic effect of high N addition on some saprophytic fungi by inhibiting their enzymes (Fog, 1988), and/or outcompeting of less efficient fungi that require less N by highly efficient bacteria that have higher N demands (Ågren et al., 2001). Studies also showed that increased N had a negative effect on arbuscular mycorrhizal fungi (AMF) abundance as AMF become C-limited when N is more available (especially in this tropical region with high ambient N deposition) and plants correspondingly reduce allocation of C to mycorrhizal association (Treseder, 2004;Diepen et al., 2007). Therefore, a decreased trend of fungal contribution to SOC pools induced by high N addition was found in the tropical soil.

On the contrary, 11 years of N addition didn't significantly affect MurN content in our study. Cusack et al. (2011) found that N addition in tropical forests can increase bacterial biomass in the short-term (4–6 years). In our study, however, we could not establish to what extent high N addition initially increased bacterial production due to the nutrient flush, but the soil could become