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Long-term nitrogen & phosphorus additions reduce soil microbial respiration but increase its temperature sensitivity in a Tibetan alpine meadow

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ABSTRACT

Nutrient availability may exert major controls over soil microbial respiration, especially in carbon (C)-rich, nitrogen (N)-limited ecosystems in high elevation regions, but how soil organic matter (SOM) decomposition and its temperature sensitivity respond to long-term N & P additions in alpine ecosystems remains unclear. We examined the impact of long-term (15 yr) N & P additions on soil microbial respiration and its temperature sensitivity (Q_{10}), and assessed the relative importance of nutrient-induced alterations in substrate quality and the microbial community composition in explaining the variation in soil respiration and temperature sensitivity. We found that N & P additions significantly reduced microbial respiration rates and cumulative C efflux, but increased the Q_{10} (15/5 °C). Also, N & P additions reduced the biomass of the whole microbial community, gram negative bacteria and fungi, but increased the aromaticity and aliphaticity of soil organic C substrate. Across the treatments, averaged Q_{10} was positively correlated with the complexity of SOM as characterized by ^{13}C-NMR, supporting the prediction based on kinetic theory that SOM with recalcitrant molecular structure is with high temperature sensitivity. Together, our results showed that changes in both substrate quality and soil microbial community induced by long-term nutrient inputs may alter the response of soil microbial respiration to elevated temperature. Because the positive effects of increasing temperature sensitivity for use of lower quality substrates on C emission may be offset by lower absolute rates at any one temperature, long-term N & P additions increase the uncertainty in predicting the net soil C losses in the scenario of warming on Tibetan Plateau.

1. Introduction

Soil respiration (Rs) is the second largest carbon (C) flux between the atmosphere and terrestrial ecosystems behind plant photosynthesis (Raich and Schlesinger, 1992; Bond-Lamberty and Thomson, 2010) and is comprised of auto- and heterotrophic respiration (Luo and Zhou, 2010; Schindlbacher et al., 2009). Because soils contain the largest active C pool on the Earth, a small change in soil respiration may have a large impact on the net C fluxes (Davidson and Janssens, 2006; Shao et al., 2013). Several environmental factors such as temperature and nutrient availability exert major controls over Rs, especially in C-rich, N-limited ecosystems in high elevation or latitude regions such as alpine meadow or tundra (Song et al., 2010; Zhu et al., 2011; Fang et al., 2005).

Alpine ecosystems on Tibetan Plateau are characterized by the cold climate, and thereby store a large amount of soil organic C (averaged at ca. 3.1 kg C m^{-2} from the top 30 cm depth) (Yang et al., 2008) largely due to relatively low rate of SOM decomposition. While potentially acting as a sink of CO_2 and playing a pivotal role in C sequestration, Tibetan Plateau has experienced warmer climate since 1930s (Liu and Chen, 2000). The temperature dependence of

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SOM decomposition is of considerable ecophysiological importance, especially in the context of possible climate-change feedback effects (Kirschbaum, 2006). However, accurate quantitative predictions of C fluxes in Tibetan alpine meadows are still lacking because of limited information about the temperature sensitivity of decomposition.

Soil microbial respiration and its sensitivity to temperature are affected by multiple factors, including substrate quality (Davidson and Janssens, 2006; Hartley and Ineson, 2008; Wagai et al., 2013), the microbial community composition and nutrient availability (Zhang et al., 2005; Jin et al., 2010; Stone et al., 2012; Coucheney et al., 2013; Li et al., 2015; Lin et al., 2015). The enzyme-kinetic hypothesis predicts that SOM with recalcitrant molecular structure is characterized with lower decomposition rates and higher activation energies (Davidson and Janssens, 2006). Therefore, recalcitrant SOM has a higher "intrinsic" temperature sensitivity (commonly expressed as Q_{10}, proportional increase in CO_2 released by soil heterotrophic microbes for a 10 °C increase in temperature) than simple substrates do (Bosatta and Ågren, 1999; Davidson and Janssens, 2006). This hypothesis was broadly supported by both experimental and modeling studies (Fierer et al., 2005; Davidson and Janssens, 2006; Hartley and Ineson, 2008; Craine et al., 2007; Wagai et al., 2013), although in the opposite has also been found (Fang et al., 2005; Rey and Jarvis, 2006). Given there is a relative increase in the fraction of molecules with sufficient energy to react, the soils of alpine ecosystems are predicted to have larger Q_{10} value and higher risk of carbon losses in response to climate warming (von Lützw and Kögel-Knaber, 2009; Zheng et al., 2009; Wagai et al., 2013).

Microbial decomposition in alpine ecosystems may also be constrained by N availability because many N-enriched enzymes dominate SOM degradation (Schimel and Weintraub, 2003; Allison et al., 2007; Wallenstein et al., 2010). Enhanced microbial decomposition in a warming climate may further stimulate microbial activities through enhancing N availability (Allison et al., 2010; Zhou et al., 2012; Sinsabaugh et al., 2015). More importantly, alpine ecosystems on the Tibetan Plateau is experiencing some unprecedented human perturbations including increasing grazing intensity, degradation, N deposition and fertilization (Harris, 2010; Liu et al., 2015). Increased nutrient (N in particular) inputs can shift plant community composition and change C:N ratios of plant litter and soil properties (e.g., pH, osmotic pressures and metal ions) (Gallo et al., 2004; Fontaine et al., 2007; Liu and Greaver, 2010), further affecting microbial decomposition. The responses of SOM decomposition to increased input of N were reported to vary depending on the type of ecosystems (Lu et al., 2011; Zhou et al., 2014). For example, a meta-analysis has recently showed that N addition significantly increased Rs by 2.0% across all biomes, 7.84% in grassland and 12.4% in croplands but decreased it by 1.44% in forests (Zhou et al., 2014). In Tibetan alpine ecosystems, however, how SOM decomposition and its temperature sensitivity respond to long-term nutrient additions remains unclear.

Taking advantage a long-term N & P additions experiment in an alpine meadow grassland on Tibetan Plateau, we conducted laboratory incubation experiments to assess how long-term nutrient additions affected soil microbial respiration and its temperature sensitivity. Also, we characterized the composition of soil carbon using solid-state ^{13}C nuclear magnetic resonance spectroscopy (^{13}C-NMR) and the microbial community through analyzing the composition of extractable ester-linked phospholipid fatty acids (PLFAs). Our objectives were to 1) quantify the effect of N & P additions on soil C quality, soil respiration and its temperature sensitivity, 2) determine the relationship between altered soil C quality and the temperature sensitivity of soil respiration, and 3) assess the relative importance of substrate C quality and the microbial community composition in affecting soil respiration and its temperature sensitivity.

2. Materials and methods

2.1. The field study site

The long-term field experiment was conducted at the research station of alpine meadow and wetland ecosystems of Lanzhou University, which is located in the eastern tip of the Tibetan Plateau with elevation at approximately 3500 m a.s.l., in Maqu county (35°58′ N, 101°53′ E), Gansu Province, China. Mean annual temperature is 1.2 °C, ranging from −10 °C in January to 11.7 °C in July (Wu et al., 2010). Mean annual precipitation was around 620 mm as recorded in past several decades, and mainly occurred in summer (June–August). The solar radiation is about 2580 h per year (Luo et al., 2006). The soil is typical alpine meadow soil. The plant community is dominated by *Kobresiagr aminifolia, Anemone rivularis, Elymus nutans* and *Carex kansuensis*. The mean aboveground primary productivity is 280–400 g m^{-2} (dry weight), and the species richness is on average 20–35 per 0.25 m^2 (Yang et al., 2011).

2.2. The field experimental design

In 1999, the field experiment was set up at a flat area (450 × 220 m) and was fenced. Grazing was permitted only in winter. Within the fenced area, 16 plots were arranged in a regular 4 × 4 m matrix. Each plot is of 60 m^2 (10 × 6 m), and all plots were separated with at least 1 m interval as buffer zone. In 2000, a randomized design with four fertilization levels and four replicates was established. Slow-release ammonium phosphate pellets $(NH_4)_2HPO_4$ was applied at the rate of 0, 30, 60, 90, and 120 g m^{-2} (0, 6.3, 12.6, 18.9, 25.2 g N m^{-2} and 0, 7.0, 14.0, 21, 28.0 g P m^{-2}) once each year in May.

2.3. Soil sampling and preparations

In the present study, three treatments including the NP 0 (0 g N m^{-2}, and 0 g P m^{-2}), NP 60 (12.6 g N m^{-2} and 14.0 g P m^{-2}) and NP 120 (25.2 g N m^{-2} and 28.0 g P m^{-2}) were selected. In August 2014, four soil cores (2.5 cm diameter × 20 cm depth) were collected from each plot with a soil auger and then bulked to form one composite sample. These soil samples were brought to the laboratory immediately and then were sieved (2 mm mesh) to remove rocks, visible roots and debris. Then a subsample of the soil was air-dried at room temperature (25 °C) and ground with a mill to pass through a 0.25-mm sieve before physicochemical analysis. The other subsample was kept at 4 °C until used for microbial analyses.

2.4. Soil analyses

The total organic carbon (SOC) and total N were analyzed by using Elementar (vario macro) analyzer. Soil pH was measured in a 1:2.5 ratio of soil to deionized water. Soil total P was measured using H_2SO_4 fusion with phosphate detection in neutralized extracts at 880 nm by automated molybdate colorimetry using a Shimadzu ultraviolet spectrophotometer, and available P content was measured using the molybdate blue colorimetric method following the extraction with 0.5 M L^{-1} $NaHCO_3$. Soil NO_3^--N and NH_4^+-N were extracted with 2 M KCl and determined using a flow injection auto analyzer (SEAL-AA3, Germany). Dissolved organic C (DOC) in the extracts was measured by using TOC analyzer (Elementarvario macro). Microbial biomass C (MBC) and N (MBN) were determined using the chloroform extraction methods, as conversion factors K_C (0.38) and K_N (0.45) for MBC and MBN, respectively