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Effects of warming and grazing on dissolved organic nitrogen in a Tibetan alpine meadow ecosystem

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A B S T R A C T

The production and fate of inorganic N as regulated by environmental change are relatively well understood, but we know comparatively little about how these factors influence dissolved organic nitrogen (DON). We measured total N, DON and dissolved inorganic nitrogen (DIN) concentrations in the soil solution and plant N uptake in a factorial warming × grazing experiment in a Tibetan alpine meadow. Results showed that warming significantly decreased DON concentration by up to 36%. The effect of warming on DON concentration in the soil solution varied with sampling date and soil depth. However, our results showed that moderate grazing set off the effect of warming on DON concentration in the soil solution. Grazing increased soil DON, opposite to the effect of warming. Previous studies have found warming to increase a range of factors which contribute to the supply of DON in soil. Our results show that the observed decrease of DON under warming was correlated with plant N uptake, suggesting that warming stimulated processes making DON available to plants. Our study highlights the complex interaction of land management regime and climate warming in the regulation of DON cycling in N-limiting environments.

1. Introduction

Although the availability of inorganic nitrogen (N) is one of the primary limitations for plant growth in terrestrial ecosystems (Vitousek and Howarth, 1991), the conversion of insoluble organic N to DON can also be a major constraint of the supply of N to plants (Farrell et al., 2011; Jan et al., 2009). The production and fate of inorganic N as modulated by temperature and land use are relatively well understood (Bai et al., 2013; Ueda et al., 2013), but relatively few studies have been carried out on how these factors affect DON, especially in alpine ecosystems.

DON concentrations in soil are the result from the net balance between input and removal processes which mainly arise from plant and microbial turnover and root exudation, and there is indeed a correlation between soil DON concentrations and plant uptake of DON from the soil solution (Jones et al., 2004, 2005; Xu et al., 2006). DON, however, also provides a vital source of C and N for soil microorganisms and is an important precursor leading to the production of NH_4^+ and NO_3^- in soil (Bai et al., 2013; Bardgett et al., 2003). DON consists of a large spectrum of organic N compounds, including high molecular weight compounds which are prone to precipitation and low molecular weight compounds which are highly bioavailable (e.g. amino acids, peptides). Plant roots and soil microorganisms do not only take up inorganic N, but also can directly take up and assimilate low molecular weight DON (Jones et al., 2004; Nordin et al., 2004; Weigelt et al., 2005; Jiang et al., 2015). Previous studies show positive influences of warming on DON as warming accelerates litter decomposition (Kalbitz et al., 2004), whereas negative effects are found in forests due to higher mineralization rates (Ueda et al., 2013). Shan et al. (2014) found that a component of the DON pool (e.g. amino acids) increased with altitude, and plants preferentially utilized DON over inorganic N in a cold-temperate forest ecosystem using elevation as a proxy of climate (Averill and Finzi, 2011). However, concomitant changes of temperature with moisture and vegetation composition along

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the elevational gradient make the interpretation of the results difficult. So far, few studies have included direct in situ measurements of DON dynamics under climate warming.

Grazing can decrease soil DON through the stimulation of soil net N mineralization (Frank et al., 2000). However, it has been shown that grazing may have opposite effects for low productive ecosystems (Harrison and Bardgett, 2004). In alpine meadows, previous results have shown that grazing may stimulate the production of dissolved organic C (DOC) (Luo et al., 2009) as it increased above-and below-ground plant biomass production (Wang et al., 2012) and increased rates of litter decomposition (Luo et al., 2010), while grazing did not change soil net N mineralization rates (Wang et al., 2012). Thus, we infer that concentrations of DON will increase under grazing in alpine meadows.

Tibetan alpine meadows are particularly sensitive to global climate change; the average surface temperature in Tibet is expected to increase 2 °C more than the global average by 2050 (Thompson et al., 2000). Grazing is the main land use type for meadow ecosystems and grazing pressure is expected to substantially increase in the near future due to the rise in human population within the region (Chen et al., 2013). Here, a field experiment was performed to test the effects of warming and grazing on soil DON dynamics in Tibetan alpine meadows. First, we tested the hypothesis that warming decreases soil DON. This could result from a correlation of soil DON with greater demand of N for plant and soil microbial production or microbial N turning (Xu et al., 2006; Hu et al., 2010; Rui et al., 2011). Second, we tested the hypothesis that grazing will increase soil DON as we previously found that grazing increased rates of litter decay (Luo et al., 2010) and soil dissolved organic carbon in soil solution (Luo et al., 2009), while grazing did not significantly change soil N net mineralization rates (Wang et al., 2012). In addition, we explicitly tested the interactive effects of warming and grazing on soil DON in the Tibetan alpine meadow.

2. Materials and methods

2.1. Experimental site

The study was performed at the Haibei Alpine Meadow Ecosystem Research Station (AMERS) in Qinghai province (37°37′N, 101°12′E) which belongs to the Northwest Institute of Plateau Biology of the CAS. The mean elevation of our study site is 3200 m. The mean annual temperature is −1.7 °C and growing season rainfall from May to September was 449 and 398 mm in 2006 and 2007, respectively. Dominant plant species are *Kobresia humilis* and *Festuca ovina* (Wang et al., 2012). More than 95% of belowground plant biomass was in the upper 20 cm and fine root biomass was higher at 20 cm depth than at 10 cm depth (Wu et al., 2011a,b).

2.2. Controlled warming-grazing experiment

A two-factorial (warming × grazing) experiment with four replicates was set up in May 2006, including control (no warming no grazing: NWNG), grazing (no warming with grazing: NWG), warming (warming with no grazing: WNG), and warming with grazing (WG) treatments. In total, 16 circular plots of 3 m diameter were used in a randomized block design. An infrared heating system was installed at the warming plots (Wang et al., 2012). The temperature during the growing season in the warmed plots was increased by 1.2 °C and 1.7 °C at daytime and nighttime, respectively.

Grazing was performed by enclosing one adult sheep in the grazing plots on 17 August 2006 for 2 h, while two grazing times were performed in the grazing plots using two adult sheep for 1 h on 12 July and 3rd August in 2007, respectively. When the plant height was decreased to approximately half of the initial height before grazing, sheep were removed from the grazing plots (Luo et al., 2009; Wang et al., 2012). This grazing intensity is equivalent to a moderate stocking rate in alpine meadow (Luo et al., 2009; Wang et al., 2012).

Soil temperature was measured automatically with type-K thermocouples (Campbell Scientific, Logan, Utah, U.S.A.) at 10 and 20 cm soil depths every minute and soil moisture was manually measured at depths of 10, 20, 30 and 40 cm though a tube in the ground down to 40 cm depth using a frequency domain reflectometer (FDR) (Diviner-2000, Sen-tek Pty., Ltd., Australia) at 8:00, 14:00, and 20:00 every day from May to September in 2006 and 2007 (Luo et al., 2009).

2.3. Litter, plant biomass, plant n concentration, litter traits, and plant N uptake

Litter and plant biomass were collected from two 10 × 10 cm quadrats in each of the plots in August 2006 and August 2007 (Luo et al., 2009; Wang et al., 2012). Plant dry weight was determined after drying at 80 °C for two days. Sub-samples of roots and shoots were finely ground to determine concentrations of total N on an autoanalyzer (Kjektec System1026 Distilling Unit, Sweden). The litter quality (cellulose content, hemicellulose content, lignin content and lignin-N content) was measured by a forage fiber analyzer (ANKOM 200, Macedon, NewYork, USA) and all nutrient concentrations were calculated on the basis of organic matter (Luo et al., 2009; Jiang et al., 2011). Above-and below-ground plant N uptake was calculated via multiplying biomass by the plant tissue N concentration (Finzi et al., 2007).

2.4. Soil water solution sampling and analysis

Soil water solution was collected on 10 and 24 July, and 16 August in 2006 and on 27 May, 10 and 24 July and 24 August in 2007 (Luo et al., 2009). Within 24 h after rainfall, soil water solution was collected at 10, 20, 30 and 40 cm soil depth in all plots. Soil water solution samples were immediately stored at 4 °C for further analysis. Soil total dissolved N (TDN), dissolved organic C (DOC) and DON concentrations were measured on a TOC/TN analyzer (Shimadzu 5000, Japan). NH_4^+ concentrations were determined according to the method described by Mulvaney (1996). NO_3^- concnetrations were measured according to the method described by Miranda et al. (2001). DON was determined as the difference between the TDN and dissolved inorganic N (DIN; $NH_4^+ + NO_3^-$).

2.5. Statistical analysis

Treatment effects on soil TDN, DON concentration, and NH_4^+ and NO_3^- concentration were tested using repeated-measures analyses of variance (ANOVA), with warming, grazing and their interaction as the main factors (between-subject factors) and with sample date and soil depth as within-subject factors. Multi-comparisons of LSD were performed for all measured variables within each sampling date and each soil depth using a one-way ANOVA. Because all plots were free from grazing until 17 August 2006, the data before that date were analyzed separately. K-S tests was used to check the assumption of normality. Using Levene's tests, the assumption of homogeneity of variances was checked. Data were transformed prior to analysis if the assumptions were not met. All statistical analyses were conducted using SPSS, version 15.0 (SPSS Inc., Chicago, IL, USA).

Simple correlation analyses were conducted to test the relationships between DON concentration across 0–40 cm soil