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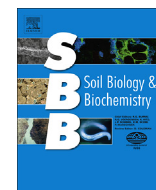

Identifying response groups of soil nitrifiers and denitrifiers to grazing and associated soil environmental drivers in Tibetan alpine meadows

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ABSTRACT

Defining response groups within N-related microbial communities is needed to predict land management effect on soil N dynamics, but information on such response groups and associated environmental drivers is scarce. We investigated the abundance and major populations of ammonia-oxidizing archaea (AOA) and bacteria (AOB), and *nirS*- and *nirK*-harboring denitrifiers under different grazing managements in Tibetan alpine meadow soils. Grazing increased AOB and AOA abundances up to 42 fold and 3.7 fold, respectively, and increased the percentage of AOB within total ammonia oxidizers from 3.1% to 10.8%. The abundance of *nirK*-like denitrifiers increased with grazing intensity, while the abundance of *nirS*-like denitrifiers tended to decrease. However, sub-groups within each of these broad groups of (de)nitrifiers responded differently to grazing. Soil nitrate was the main driver of the abundance of denitrifier sub-groups (*nirK* or *nirS*) positively responding to grazing, while soil moisture and carbon concentration were the main drivers of the abundance of denitrifier sub-groups negatively responding to grazing. AOB and *nirK*-harboring denitrifiers thus generally responded more positively to grazing than AOA and *nirS*-harboring denitrifiers, but significant functional diversity existed within each group. Our approach demonstrates the usefulness of the concept of response groups to better characterize and understand (de)nitrifier response to grazing.

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1. Introduction

Grazing is a common land use in grasslands that influences plant species diversity, drives community successions, and can stimulate belowground nutrient cycling and energy flows (Milchunas and Lauenroth, 1993; Augustine and Frank, 2001; Altesor et al., 2005). In particular, grazing often alters soil nitrogen (N) cycling and influences aboveground primary production which is limited by N in many ecosystems (Vitousek and Howarth, 1991; Bardgett and Wardle, 2003; Leriche et al., 2003). Soil microorganisms are the key drivers of soil N cycling (Falkowski, 1997; Heijden et al., 2008) and play an important role in regulating soil N available to plants and preventing undesirable N loss from ecosystems (Yao et al., 2011). Understanding how grazing influences microbial communities is thus of paramount importance to predict grazing-induced changes in soil N cycling and fertility.

Two key processes, i.e. nitrification and denitrification, are particularly important in this context. Nitrification is believed to be driven by ammonia-oxidizing bacteria, AOB (Prosser, 1989) and ammonia-oxidizing archaea, AOA (Könneke et al., 2005; Leininger et al., 2006). Denitrification is driven by different groups, in particular nitrite-reducers harboring a nitrite reductase encoded by either *nirS* or *nirK* genes (Zumft, 1997). Many studies have shown that AOA and AOB are ubiquitous in many ecosystems such as soils (He et al., 2007). Similarly, *nirK*- and *nirS*-type denitrifiers have frequently been detected in natural environments such as grasslands (Bremer et al., 2009), forests (Szukics et al., 2010) and glacier forelands (Kandeler et al., 2006). Several studies have demonstrated the effects of different grazing managements practices on nitrification and denitrification, in soil: enhanced denitrification and nitrification rates were frequently observed (Le Roux et al., 2003; Patra et al., 2005; Xu et al., 2008; Khalil and Richards, 2011), and grazing-induced changes in the abundance and community structure of nitrifiers and denitrifiers were reported (Patra

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et al., 2006; Liu et al., 2011). However, description of grazing-induced changes in nitrifying and denitrifying communities is not sufficient to improve our understanding of the observed responses and associated mechanisms. For example, previous studies reported that the relative abundance of *nirS*- and *nirK*-type denitrifiers differ between ecosystems and between management practices in a given ecosystem (Kandeler et al., 2006; Hallin et al., 2009; Yoshida et al., 2009; Bárta et al., 2010; Su et al., 2010; Dandie et al., 2011; Banerjee and Siciliano, 2012; Palmer et al., 2012; Palmer and Horn, 2012; Le Roux et al., 2013), but the underlying environmental determinants remain largely obscure. It is likely that defining *nirS*- versus *nirK*-types is not enough to understand and predict the response of nitrite reducers to environmental change. In this context, the concept of functional response groups, i.e. groups of organisms that consistently respond to given disturbance or management regimes and associated environmental drivers (Lavorel and Garnier, 2002), and the associated concept of microbial functional traits (Krause et al., 2014), are promising to promote more predictive microbial ecological studies.

Here, we tested whether response groups of nitrifying and denitrifying micro-organisms and driving soil environmental factors could be defined along a grazing gradient in the Qinghai-Tibetan Plateau alpine meadows. In these grazed ecosystems, overgrazing has contributed to several environmental problems such as land degradation (Wu et al., 2010; Zhang et al., 2011), while accelerated soil N mineralization, enhanced denitrification and nitrification, and increased emission of N_2O by soils have been reported (Gao et al., 2008; Lin et al., 2009; Hu et al., 2010; Chen et al., 2011; Liu et al., 2011; Zheng et al., 2012). However, little is known about the response of N cycling-related microorganisms to grazing in these grasslands.

We hypothesized that increased grazing intensity would induce strong changes in key soil properties as moisture, temperature, pH or C and N availability, and that different response groups driven by some of these key environmental variables could be identified within nitrifying and denitrifying communities. We also assumed that broad differences in the ecophysiology of AOA versus AOB (French et al., 2012; Prosser and Nicol, 2012) and *nirS*- versus *nirK*-like denitrifiers (Yan et al., 2003; Banerjee and Siciliano, 2012) would imply that these broad groups could be viewed to some extent as different response groups. However, we also anticipated that a substantial functional diversity could exist within each group, which would require a finer look at changes in the diversity of those organisms to identify relevant response groups. We thus determined both the abundance and composition – major populations – of each community (AOA, AOB, and *nirK*- and *nirS*-type denitrifiers) in Qinghai-Tibetan alpine meadows submitted to different grazing managements, along with soil characteristics and aboveground plant biomass. Our objective was to identify major response groups of nitrifiers and denitrifiers to grazing, and the soil environmental variables likely driving each group identified.

2. Materials and methods

2.1. Study site and soil sampling

The study habitat is situated in the eastern part of the Qinghai-Tibetan Plateau within the Maqu experimental site of Alpine Meadow and Wetland Ecosystem Research Station of Lanzhou University (33°39′N, 101°53′E; 3650 m a.s.l.) in Gansu Province, PR China. The climate is cold Humid-Alpine, and mean annual temperature is 1.2 °C, ranging from −10 °C in January to 11.7 °C in July. The annual precipitation is about 672 mm, mainly falling during the short, cool summer (July and August). Cloud-free solar periods represent about 2580 h and there are less than 100 frost days in a year. The soil type of the study areas is mainly Mattic Cryic Cambisols (alpine meadow soil, Cambisols in FAO/UNESCO taxonomy) and grazers are mainly Tibetan sheep and yaks.

The grazing systems were established in 2005 as follows: no grazing (NG), corresponding to a fenced meadow with no livestock, where dominant plant species are *Blysmus sinocompressus* and *Carex meyeriana*; seasonal grazing (SG), corresponding to complete exclusion of livestock by a fence during the plant growing season from April to October, grazing being allowed only during the hay-stage in winter and early spring, and dominant plant species being *Elymus nutans*, *Kobresia capilifolia*, *Nardostachy chinensis*, *Potentilla anserine* and *Sanguisorba filiformis*; continuous grazing (CG), corresponding to non-fenced plots with grazing throughout the year, dominant plant species being *Agrostis hugoniana*, *Carex kansuensis*, *Ligularia virgaurea*, *P. anserine*, *Rumex patientia* and *Saussurea hieracioides*. A 1.6-to-1 proportion of sheep-to-yaks was used for grazing.

Nine soil cores (3.8 cm diameter, 15 cm depth) were randomly taken from each plot (50 m × 50 m) in July 2011 and mixed to obtain one composite sample. A total of 9 composite soil samples (three treatments × three replicates for each treatment) were collected and brought to the laboratory in an ice box within 24 h.

2.2. Soil characteristics and aboveground plant biomass measurements

Soil moisture was measured gravimetrically, and soil pH was measured in 1 M KCL (1:5, w/v). Soil total N and C concentrations were analyzed using a CHNS-analyser (Elementar Analysensysteme GmbH, Hanau, Germany). NO_3^-–N and NH_4^+–N concentrations were analyzed by a FIAstar 5000 Analyzer (FOSS, Hillerød, Denmark). Soil total phosphorus was analyzed by $HClO_4$–H_2SO_4 digestion and measured using the molybdate-blue colorimetric method (Bender and Wood, 2000). Soil available phosphorus (P) was extracted using a Mehlich-3 extractant (Mehlich, 1984) and measured using the molybdate-blue colorimetric method. Above-ground vegetation biomass, an index of grazing intensity, was measured on two 0.25-m^2 quadrats in each sampling site in July 2011. The fresh vegetation and hay were clipped, dried (80 °C for 48 h) and weighed.

2.3. DNA extraction and microbial abundance measurements

Soil DNA was extracted from 0.25 g of mixed soil using a PowerSoil DNA Isolation Kit (MO BIO laboratories, Inc, USA) according to the manufacturer's protocol. Extracted DNA was stored at −80 °C until use.

Copy numbers of the *amoA* gene of AOA and AOB, and *nirK* and *nirS* gene were measured in triplicate. Primer sets Arch-*amoAF*/Arch-*amoAR* (Francis et al., 2005), *amoA-1F*/*amoA-2R* (Rotthauwe et al., 1997), *nirK1F*/*nirK5R* (Braker et al., 1998) and *cd3aF*/*R3cu* (Throback et al., 2004) were used for the amplification of archaeal *amoA* gene, bacterial *amoA* gene, and *nirK* and *nirS* gene fragments, respectively. Real-time PCR was performed on an iCycler iQ 5 thermocycler (Bio-Rad, USA). The 25-μl reaction volume contained 2 μl of DNA template, 1 μl of each primer (0.2 μM) and 12.5 μl SYBR Premix Ex Taq™ II (Takara, Japan). Q-PCR programs were as follows: 94 °C for 3 min, followed by 40 cycles of 94 °C for 30 s, 60 °C for 1 min (for AOA) or 50 s (for AOB) or 30 s (for *nirK* and *nirS*), and extension at 72 °C for 1 min (for AOA) or 50 s (for AOB) or 45 s (for *nirK* and *nirS*). Plasmids carrying the targeted gene (*amoA*, *nirK* and *nirS*) were constructed by cloning the targeted gene fragments into plasmid pGEM-T Easy Vector (Promega, USA). Plasmid DNA was extracted using AxyPrep™ Plasmid Miniprep Kits (Axygen, USA) and the concentrations were determined on a NanoDrop ND-2000

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