



## Mixed grazing and clipping is beneficial to ecosystem recovery but may increase potential N<sub>2</sub>O emissions in a semi-arid grassland



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### ABSTRACT

A more detailed understanding of the soil nitrogen (N) cycling and the associated functional microbial groups of nitrous oxide (N<sub>2</sub>O) production under different management practices is essential for adopting proper practice to achieve sustainability of grassland systems. We investigated soil inorganic N, the potential emissions of N<sub>2</sub>O, and the abundance of nitrifying and denitrifying communities in different grazing management systems, grazing intensities and topographies in a semi-arid grassland of Inner Mongolia, China. Four grazing intensities (0, 3, 6, and 9 sheep ha<sup>-1</sup>) were applied in two management systems (traditional grazing; and mixed grazing with clipping) in flat or sloped (3–4°) blocks. Results showed that soil inorganic N, the gene abundance of *amoA* (ammonia monooxygenase) gene of ammonia-oxidizing archaea (AOA) and bacteria (AOB), and the *narG* (nitrate reductase) gene, as well as the potential rates of N<sub>2</sub>O production from nitrification (N<sub>N2O</sub>) and denitrification (D<sub>N2O</sub>) significantly decreased with the increase of grazing intensity, particularly in sloped plots; however the effect of increasing grazing intensity in decreasing soil inorganic N, gene abundance and potential N<sub>2</sub>O emissions was alleviated in mixed grazing and clipping system in flat plots, which resulted in greater potential N<sub>2</sub>O emissions in mixed grazing and clipping system than in traditional grazing system. Soil moisture was found to be the controlling factor for N<sub>2</sub>O production in traditional grazing system while soil organic matter and nutrients (total N, soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) were most important in determining N<sub>2</sub>O production in mixed system. Our results suggest that after ten years of consistent grazing management, mixed grazing with clipping alleviated the suppressed N cycle under the traditional grazing, and changed the limiting factor for N<sub>2</sub>O production, shifting from soil moisture under traditional grazing to soil organic matter and nutrient status. The research highlights that mixed grazing with clipping can be considered as an effective management practice in alleviating a suppressed N cycle, and consequently the ecosystem recovery of this semi-arid grassland would likely be associated with an increase in N<sub>2</sub>O emissions.

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### 1. Introduction

Soil nitrogen (N) cycling and the terrestrial N budget are crucial for ecosystem functioning and are critical in regulating the

responses of terrestrial ecosystems to climate change (Pastor and Post, 1986; Melillo et al., 2002). Nitrous oxide (N<sub>2</sub>O), a potent greenhouse gas that contributes significantly to global warming, is a product of soil microbial processes such as nitrification and denitrification (IPCC, 2007), which are key processes of soil N cycling that control N availability and loss in terrestrial systems. The formation and consumption of N<sub>2</sub>O are mediated by specific functional microbial groups (Chroňáková et al., 2009; Zhong et al., 2014). Consequently, changes in the abundance of specific functional

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groups can alter N availability or N<sub>2</sub>O loss from the ecosystem. A number of studies have reported that the abundance of soil functional microbial groups are regulated by a wide range of biotic and abiotic factors such as litter inputs, temperature and soil moisture (e.g. Saggari et al., 2004; Luo et al., 2010); however, the effects of management practices on functional abundance appear to be site-specific and may differ in various ecosystems.

Temperate grasslands cover 11% of the earth's terrestrial surface (Sala et al., 2001) and are the major sources of atmospheric N<sub>2</sub>O (Oenema et al., 2007). Grazing, the major land-use type in grassland ecosystem, can significantly influence N<sub>2</sub>O production and microbial abundance and structure (Holland and Detling, 1990). Grazing would impact on the environment in three ways: vegetation removal, manure deposition, and trampling (Saggari et al., 2004; Oenema et al., 2007; Houlbrooke et al., 2008). Grazing modifies the allocation of carbon (C) and N between above- and below-ground through plant biomass removal (Holland and Detling, 1990), accelerates N cycling by recycling nutrients via animal manure (Kohler et al., 2005), and by animal trampling compacting soil, and decreases air permeability and hydraulic conductivity (Lin et al., 2009). Different intensities of grazing can have contrasting effects on soil microbes and overgrazing is believed to adversely impact on functional microbial abundance and N availability (Ingram et al., 2008). Moreover, the effect of grazing on the soil environment is apparently variable under different topographical conditions (Zhong et al., 2016). Soil nutrition and microbial activity on sloped land are generally lower than flat land since soil nutrition on sloped land is more likely to get lost through runoff (Luo et al., 2013). The optimization of grazing intensity and prescribing appropriate management practices under different topographical conditions to achieve long-term sustainability and mitigate N<sub>2</sub>O emissions from grasslands ecosystems remains a challenge.

Various grazing management practices including decreasing the grazing intensity, rotational grazing, and periodic fencing off from grazing have been proposed to reduce the adverse impact of overgrazing in temperate grasslands (Luo et al., 2010; He et al., 2011; Zhong et al., 2016; Braker et al., 2015). Studies showed that these grazing management regimes had contrasting effects on N cycling processes depending on climate and grassland types. In most managed temperate grasslands under humid climates, N<sub>2</sub>O emissions and the abundance of the associated functional microbial groups increased with an increase in grazing intensity, as a result of greater urine/dung return (Luo et al., 2010; Zhong et al., 2015). However, in semi-arid grasslands grazing was found to decrease N<sub>2</sub>O emissions and caused grassland degradation as a result of suppressed N cycle (Wang et al., 2005; Xu et al., 2008). However, previous studies merely compared the responses of N<sub>2</sub>O emissions to limited grazing treatments, for example, non-grazing, moderate grazing or heavy grazing (Chroňáková et al., 2009; Di et al., 2010; Hu et al., 2010; Zhong et al., 2014, 2015). There is a lack of research on the optimal magnitude of grazing intensity in relation to N<sub>2</sub>O emissions in grasslands. Furthermore, a coherent comparison between traditional grazing and mixed grazing with other management practices is still lacking.

The Inner Mongolia grassland covers an area of about  $8.67 \times 10^7$  ha and is one of the most well-known rangelands in the eastern part of the Eurasian steppe (Coupland, 1993). In recent decades, the rapid increase in livestock populations has induced a series of grassland degradation (Li et al., 2000, 2008) and decreased more than 40% of N<sub>2</sub>O emissions (Xu et al., 2008). Apart from grazing, clipping is another common management practice commonly used by local inhabitants for hay making. The alternation between clipping and grazing in the mixed system would provide several advantages including recovery phases for grazed swards and the wider distribution of excremental nutrients, which

would partially compensate for the nutrient removal by clipping, and would be more resilient to moderate and heavy grazing compared to the traditional system (Schönbach et al., 2011). However, previous studies on grazing management mainly focused on productivity recovery (Jing et al., 2013) or C and N mineralization (Wu et al., 2012), few studies had explicit consideration of N<sub>2</sub>O emissions especially the role that microorganisms played in N<sub>2</sub>O production under different grazing managements (Chroňáková et al., 2009). In this study we assessed the potential emissions of N<sub>2</sub>O and the abundance of nitrifying and denitrifying communities in different grazing management systems (traditional grazing, or mixed grazing with clipping), grazing intensities (0, 3, 6, and 9 sheep ha<sup>-1</sup>) and topographies (flat or sloped (3–4°) blocks) in a typical steppe in Xilin River Basin, Inner Mongolia. We hypothesized that: (1) grazing would decrease the potential N<sub>2</sub>O emissions, soil inorganic N and microbial abundance that were related to N cycling, and increasing grazing intensity would had greater effects particularly in sloped pasture; (2) mixed grazing with clipping would alleviate the effects of increased grazing intensity on soil N cycle but would enhance potential N<sub>2</sub>O emissions.

## 2. Materials and methods

### 2.1. Experimental grassland

This study was conducted at the Inner Mongolia Grassland Research Station (IMGERS, 43° 38' N, 116° 42' E) of the Chinese Academy of Sciences, which was located in the Xilin River Basin of Inner Mongolia, China (Bai et al., 2004). The topography consists of low rolling hills, with an elevation ranging from 1200 m to 1280 m above sea level. The mean annual precipitation is 346.1 mm, with about 60–80% falling as rainfall in the growing season (April to September). The mean annual temperature is 0.3 °C. The soil is classified as dark chestnut (Calcic Chernozem according ISSS Working Group RB, 1998). Soil organic matter content is 2.76%, while soil total N content is 1.73%. Soil pH is 7.52, and the sand, silt and clay content is 80.2%, 17.6%, and 2.22%, respectively (Bai et al., 2010). *Stipa grandis* P.A.Smirn. (perennial bunchgrass) and *Leymus chinensis* (Trin.) Tzvel. (perennial rhizomatous grass) are two dominant species in the study area, which altogether account for 60–80% of total aboveground biomass (AB). The experimental area, with a total area of 128 ha, had been grazed by sheep freely until 2003 when the experiment was established, and in order to make an equal starting point, the whole area was cut to 3–5 cm in stubble height at end of the growing season in 2004. In 2005 a split–split plot in a random complete block design was established. The 128 ha area was first divided topographically into two blocks (sloped and flat blocks, the slope class was about 3–4°), and each block was divided into two paddocks (traditional grazing) and mixed systems (each plot had a shift between grazing and clipping year after year), while each paddock was further divided into 4 plots (0, 3, 6 and 9 sheep ha<sup>-1</sup>).

The layout of experiment in 2005 and 2006 were showed in Wan et al. (2011). A total of 16 experimental units were used in this research and one unit was two ha in size. Grazing started from the beginning of June until end of September and hay making was done once a year at the middle of August. Due to spatial heterogeneity of vegetation, plots with the same stocking rate were exposed to different grazing pressure. In order to keep the same grazing pressure on the sward under target grazing intensity, we switched grazing management from the fixed stocking rates to herbage allowance in 2007. The number of sheep was then adjusted monthly in each grazing plot according to the herbage on offer. There were four grazing intensities including control (0 sheep ha<sup>-1</sup>), low-intensity grazing (3 sheep ha<sup>-1</sup>), moderate-intensity

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