




SHORT COMMUNICATION

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Sensitivity of soil nitrifying and denitrifying microorganisms to nitrogen deposition on the Qinghai–Tibetan plateau

Minjie Xu^{1,2}, Tingting Li², Wei Liu², Junjun Ding², Lili Gao², Xingguo Han^{1*} and Ximei Zhang^{2*} 

Abstract

Purpose: Nitrogen deposition at rate not more than 50 kg ha⁻¹ year⁻¹ is generally considered to stimulate soil nitrifying and denitrifying microorganisms via increases in soil nitrogen content. However, this phenomenon in alpine ecosystems remains largely untested.

Methods: We conducted an 8-year nitrogen deposition experiment on the Qinghai–Tibetan Plateau, with four nitrogen deposition rates of 10 (atmospheric deposition), 20, 30, and 50 kg ha⁻¹ year⁻¹.

Results: The abundances of two nitrifying genes and four denitrifying genes and the N₂O emission rate initially increased and subsequently decreased as the nitrogen deposition rate increased. The observed decrease in these indices at the rate of 50 kg ha⁻¹ year⁻¹ was caused by the toxicity of excessive NH₄⁺.

Conclusions: Our study demonstrates the vulnerability of alpine microorganisms under global changes.

Keywords: Ammonium toxicity, Denitrification, N₂O, Nitrification, Nitrogen deposition, Qinghai–Tibetan plateau

Over the past few decades, environmental changes caused by anthropogenic activity, such as climate warming and nitrogen (N) deposition, have played a large role in the disturbance of biological diversity and ecosystem functions (Klein et al. 2004; Chen et al. 2013). However, the effects of N deposition on soil microbial communities and their underlying mechanisms within the alpine area are yet to be fully understood. In many tropical and temperate ecosystems, N deposition at rate not more than 50 kg ha⁻¹ year⁻¹ stimulates the nitrification and denitrification of soil microbial communities by elevating the substrate contents of the two processes (Erickson et al. 2001; Baer and Blair 2008). N deposition at rate > 50 kg ha⁻¹ year⁻¹ often continues to stimulate the microbial nitrification process (Lehtovirta-Morley et al. 2011;

Zhang et al. 2012), yet depresses the microbial denitrification process (Gao et al. 2015). However, it is unclear whether the N deposition at rate not more than 50 kg ha⁻¹ year⁻¹ will also stimulate microbial nitrification and denitrification in alpine ecosystems.

In this study, we conducted an 8-year (2007–2015) N addition experiment to simulate N deposition in a meadow ecosystem on the Qinghai–Tibetan Plateau, China (N 37° 37', E 101° 19'). The Qinghai–Tibetan Plateau has served as the roof of the world, the **water tower of Asia**, and the third pole of the Earth for 65 million years (Zheng et al. 1979; Wu and Yin 2002; Sun et al. 2012). The current N deposition rate at the Qinghai–Tibetan Plateau is recorded at approximately 10 kg ha⁻¹ year⁻¹, with an increasing trend predicted for the next few decades (Lu and Tian 2007). The vegetation type is a typical *Kobresia humilis* meadow. The soils developed in the *Kobresia* meadow are Mat–Gryic Cambisol (Fang et al. 2012). Four N addition rates were applied (0, 10, 20, and 40 kg ha⁻¹ year⁻¹), and thus, the actual total N deposition rates (anthropogenically added

* Correspondence: xghan@ibcas.ac.cn; zhangximei@caas.cn

¹State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

²Key Laboratory of Dryland Agriculture, Ministry of Agriculture, Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing 100081, China



rate + naturally deposited rate) were approximately 10, 20, 30, and 50 kg ha⁻¹ year⁻¹, respectively. Two types of nitrogenous compounds, NH₄Cl and KNO₃, were added for each of the latter three rates. Thus, there were seven treatments, with three replicates (in three blocks) for

each treatment. In 2015, the abundances of two nitrifying (AOA-*amoA* and AOB-*amoA*) and four denitrifying genes (*nirS*, *nirK*, *narG*, and *nosZ*) of soil microbial communities were quantified using real-time PCR analysis. The N₂O emission rate was quantified in situ via gas

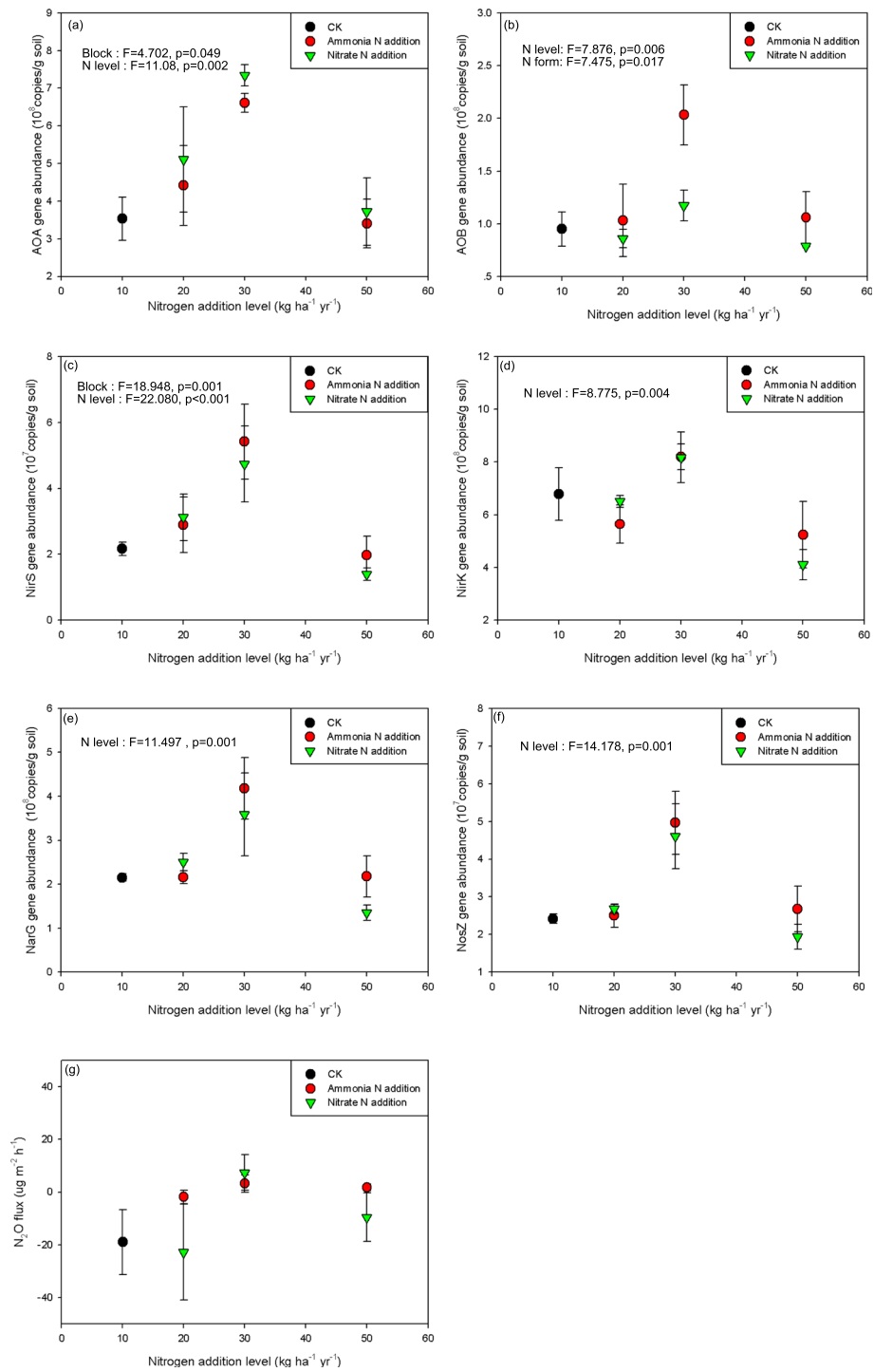


Fig. 1 Relationship between community size of **a, b** nitrifying and **c-f** denitrifying microorganisms, **g** N₂O emission rate, and nitrogen deposition rate. Error bars represent one standard error

chromatography (Wang and Wang 2003). In addition, plant community biomass and soil physicochemical indices (soil water content, pH, dissolved organic carbon content, NO₃⁻-N content, and NH₄⁺-N content) were also measured (see Method S1).

The abundances of the two nitrifying and four denitrifying genes initially increased and subsequently decreased as the N deposition rate increased, with maximum values associated with 30 kg ha⁻¹ year⁻¹ (Fig. 1a–f). The N₂O emission rate exhibited a similar trend as that of the gene abundances, especially for the treatments of KNO₃ addition (Fig. 1g). Specifically, an increase in the N deposition rate from 10 to 30 kg ha⁻¹ year⁻¹ resulted in a rise in the gene abundances and N₂O emission rate due to elevated soil N content. This is consistent with tropical and temperate ecosystems (Xie et al. 2018; Zhang et al. 2018). However, further increases in the N deposition rate (from

30 to 50 kg ha⁻¹ year⁻¹) led to a decline in the index values, differing from results in the literature (Frey et al. 2004; Tian et al. 2014; Zeng et al. 2016).

This unexpected decline in the indices was not attributed to aboveground plant biomass, soil water content, and dissolved organic carbon content, as these variables responded non-significantly to the N deposition rate, as revealed by a two-way analysis of variance (Fig. 2a, c–d). Furthermore, as the N deposition rate increased from 30 to 50 kg ha⁻¹ year⁻¹, no consistent changes were observed for soil pH under the addition of NH₄Cl and KNO₃ (Fig. 2b). Specifically, soil pH levels decreased with the addition of NH₄Cl, yet remained constant with the addition of KNO₃. Thus, soil pH was not an influencing factor for the observed decline in the seven indices. Similarly, soil NO₃⁻-N content remained unchanged under the addition of both NH₄Cl and KNO₃ (Fig. 2e).

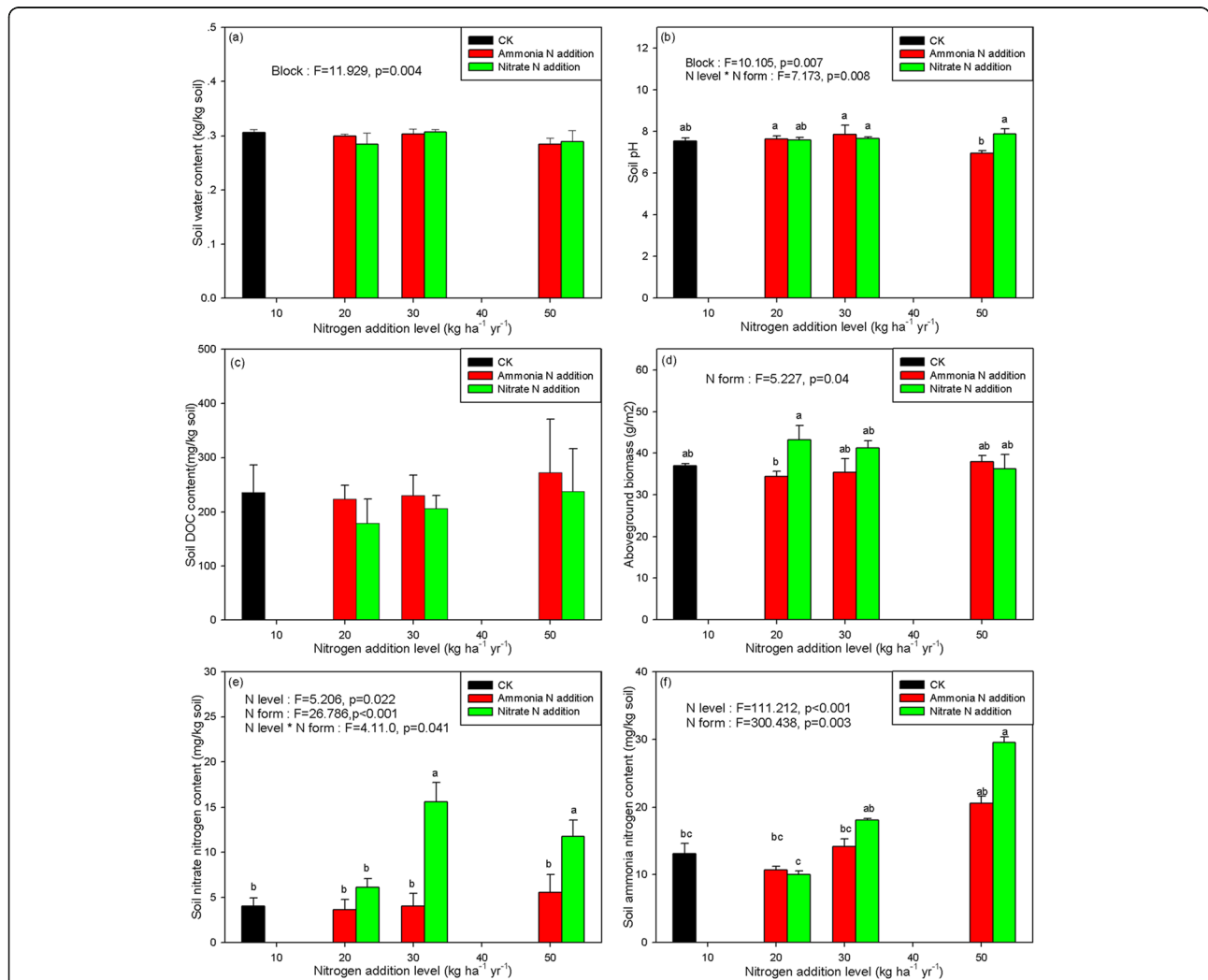


Fig. 2 Effects of N deposition rate on **a** soil water content, **b** soil pH, **c** dissolved organic carbon content, **d** aboveground plant biomass, **e** soil NO₃⁻-N content, and **f** NH₄⁺-N content. Error bars represent one standard error. For clarity, the statistical results with *P* > 0.05 are not shown

In contrast, soil $\text{NH}_4^+\text{-N}$ content increased consistently with the rise in nitrogen deposition under the addition of both NH_4Cl and KNO_3 (Fig. 2f) and is thus identified as a likely cause of the reduced index values.

Excessive soil NH_4^+ concentrations are able to change the ion balance in plants through their uptake of cations and thus are toxic to plants (Britto and Kronzucker 2002; Esteban et al. 2016). Moreover, soil microorganisms are considered to be less resistant to environmental pressures compared to higher plants (Zhang et al. 2015; Zhang et al. 2016). Hence, the excessive NH_4^+ concentration under the nitrogen deposition rate of $50 \text{ kg ha}^{-1} \text{ year}^{-1}$ is likely to be toxic to soil microorganisms, leading to the observed decline in the seven indices (Fig. 1). Note the addition of $\text{NO}_3^-\text{-N}$ (in the form of KNO_3) from 30 to $50 \text{ kg ha}^{-1} \text{ year}^{-1}$ was associated with a slight reduction in soil $\text{NO}_3^-\text{-N}$ content, while $\text{NH}_4^+\text{-N}$ content slightly increased (Fig. 2e, f). This implies that a large amount of $\text{NO}_3^-\text{-N}$ was transformed into $\text{NH}_4^+\text{-N}$, possibly driven by plants and/or microorganisms (Esteban et al. 2016). Both the transformation of $\text{NO}_3^-\text{-N}$ to $\text{NH}_4^+\text{-N}$ and the toxicity of excessive NH_4^+ for microorganisms require further exploration to fully understand the mechanisms.

In summary, N deposition at rate not more than $50 \text{ kg ha}^{-1} \text{ year}^{-1}$ may either stimulate nitrifying and denitrifying microorganisms through increases in soil N content or depress these microorganisms via NH_4^+ toxicity. In some tropical and temperate ecosystems, many microorganisms are resistant to NH_4^+ toxicity, thus exhibiting a net positive response to low deposition rates (Ning et al. 2015; Tian et al. 2018). In contrast, microorganisms found in alpine ecosystems do not present such a resistance to NH_4^+ toxicity, highlighting the vulnerability of microorganisms in such unique ecosystems. Moreover, for other types of ecosystems, N deposition is observed to have a cumulative effect (Dupre et al. 2010). Thus, it is also important to identify whether N deposition rates at rate not more than $30 \text{ kg ha}^{-1} \text{ year}^{-1}$ can cause a net negative effect on microorganisms for longer treatments. A further key point is to determine whether the relationship observed on the Qinghai–Tibetan Plateau between the community size of nitrifying and denitrifying microorganisms and nitrogen deposition at rate not more than $50 \text{ kg ha}^{-1} \text{ year}^{-1}$ also applies to other alpine ecosystems in the world.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13213-020-01619-z>.

Additional file 1: Methods

Acknowledgements

Not applicable.

Authors' contributions

MX designed the research, carried out the experiment, and drafted the manuscript. XZ improved the writing of the manuscript. All authors have read and approved the final manuscript.

Funding

This research was supported by the National Key Research and Development Program (2016YFC0500702) and the China Major Science and Technology Program for Water Pollution Control and Treatment (2017ZX07101003).

Availability of data and materials

All data analyzed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no competing interest.

Received: 1 December 2020 Accepted: 28 December 2020

Published online: 22 January 2021

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