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High temperatures inhibited the growth of soil bacteria and archaea but not that of fungi and altered nitrous oxide production mechanisms from different nitrogen sources in an acidic soil

Xiaoya Xu [a], Xiaorui Liu [a], Yong Li [a, *], Yu Ran [a], Yapeng Liu [a], Qichun Zhang [a], Zheng Li [b], Yan He [a], Jianming Xu [a], Hongjie Di [a]

[a] Institute of Soil and Water Resources and Environmental Science, College of Environmental and Resource Sciences, Zhejiang Provincial Key Laboratory of Agricultural Resources and Environment, Zhejiang University, Hangzhou, 310058, China
[b] Changzhou Industrial Technology Research Institute of Zhejiang University, Changzhou, China

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ABSTRACT

More frequent extreme heat conditions, forecasted in the context of climate change, are likely to significantly affect microbial communities that are important for nitrogen (N) cycling and N_2O emissions. Here we report an incubation study on the effect of a range of temperatures (20, 30 and 40 °C) on the growth of bacteria (ammonia oxidizing bacteria, AOB), archaea (ammonia oxidizing archaea, AOA), fungi (18SrRNA) and key denitrifying communities (*nirK*-, *nirS*- and *nosZ*-denitrifiers), and effects on N_2O emissions, following the application of urea and an organic manure in an acidic soil. Results showed that the growth of ammonia oxidizers and denitrifiers were strongly inhibited by the higher temperatures, particularly at 40 °C. AOB grew well with the application of urea and manure but AOA grew in the Control at 20 °C. Bacterial denitrifiers only grew mostly at 20 °C and did not grow at the higher temperatures. In contrast, fungi communities, including fungal *nirK*-type communities, grew under all three temperatures in the manure treatment, but not in the urea treatment, showing fungal tolerance to high temperatures and an interaction between nitrogen (N) source and fungal growth. N_2O emissions increased in the urea treatments and decreased in the manure treatments with increasing temperature, possibly pointing to different mechanisms of N_2O production in the urea and manure treated soils. These results suggest that different microbial communities will respond differently to extreme heat conditions and the type of N source applied will also have an interactive effect with temperature on the microbial growth and N_2O emissions.

1. Introduction

Nitrous oxide (N_2O) is a powerful greenhouse gas and the most significant ozone-depleting substance (Ravishankara et al., 2009). Agriculture is by far the largest source of anthropogenic N_2O emissions, mainly from nitrogen (N) fertilizers and animal manure (Galloway et al., 2004). N_2O emissions will increase further in the future as greater amounts of N fertilizers and animal manures are used to grow more food to feed the growing world population.

Nitrous oxide is produced in the soil mainly from two microbial processes: nitrification and denitrification. In the nitrification process, ammonium (or ammonia) is first oxidized to nitrite (NO_2^-) which is further oxidized to nitrate (NO_3^-). N_2O is produced as a by-product of the nitrification process (Wrage et al., 2001; Di et al., 2014; Di and Cameron, 2016). The first and rate-limiting step of the nitrification process, ammonia oxidation is carried out by ammonia oxidizing bacteria (AOB) or archaea (AOA), which carry the *amoA* gene encoding the ammonia monooxygenase. The relative importance of the two ammonia oxidizing communities may vary, depending on soil conditions such as N status and pH (He et al., 2007; Nicol et al., 2008; Di et al., 2009). It was hypothesized that AOA may substantially contribute to N_2O production via the ammonia oxidation pathway in strongly acidic soils due to their high affinity for ammonia substrates (Hu et al., 2015). N_2O is also produced from denitrification where NO_3^- is reduced to NO_2^-, nitric oxide (NO), N_2O, and N_2 under anaerobic conditions (Firestone and

* Corresponding author.
E-mail address: yongl@zju.edu.cn (Y. Li).

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Davidson, 1989; Mosier et al., 1998). These reduction processes are carried out mainly by a wide range of heterotrophs which use organic carbon as an electron donor. A number of functional genes that encode the enzymes involved in the denitrification processes have been identified, including *narG*, *nirS/nirK*, and *nosZ* (Wrage et al., 2001). In addition, recent studies showed that fungi also played an important role in N_2O emissions through fungal denitrification under certain soil conditions, e.g. in strongly acidic soils (Wei et al., 2014). The development of specific primers targeting fungal *nirK* makes it possible to study the role of fungi in N_2O emissions (Long et al., 2015; Wei et al., 2015).

The growth of the bacterial, archaeal, and fungal communities and their contribution to N_2O emissions are likely to vary, depending on soil and environmental conditions and the type of N fertilizer or animal manure applied. In addition, as global warming worsens over time, the world is likely to experience extreme climatic conditions such as extreme heat. These extreme climatic conditions are likely to affect the growth of the different microbial communities and N_2O emissions. Different microbial communities may differ in their ability to adapt and grow under the extreme conditions (Tourna et al., 2008; Szukics et al., 2010; Cui et al., 2016). For example, some archaea communities are extremophiles and therefore may be better adapted to extreme heat than AOB (Schleper and Nicol, 2010; Hatzenpichler, 2012). The *nirS*-communities were also reported to be less sensitive to high temperatures than other denitrifier communities (Cui et al., 2016). However, our understanding on how extreme temperatures may impact on the growth of the different microbial communities and related N_2O emissions, following the application of different N sources in acidic soils, is very limited.

Acidic soils occupy 30% of the earth's ice-free land area (Von and Mutert, 1995). To feed the growing world population, the over-use of fertilizers accelerated world-wide acidification and acidic soils are increasingly used for agricultural production. Acidic soils are considered nutrient-limited with a low diversity of microorganisms and are vulnerable to climate change impacts (He et al., 2012). As denitrifiers in acidic soils often lack the *nosZ* genes which reduce N_2O to N_2, the N_2O emissions were usually higher from acidic soil than from neutral or alkaline soils (Hu et al., 2015). Therefore, studies on potential temperature effect on the microbial communities and N_2O emissions in acidic soils are of particular importance to assess the effect of the ongoing warming climate on the nitrogen cycle and related N_2O emissions in acidic soils.

The objective of this study was to determine the effect of a range of temperatures (20, 30 and 40 °C) on the growth of bacteria, archaea, fungi and key denitrifying communities, and on N_2O emissions, following the application of urea and organic manure in an acidic soil. The 40 °C temperature was used to assess the effect of extreme temperature on the microbial communities and N_2O emissions. We hypothesized that: (1) some microbial communities were better adapted to higher temperatures than others and would thus grow better under the 40 °C heat; (2) the growth of these microbial communities under the different temperatures was also affected by the different N sources applied; and (3) the temperature and N source effect on the microbial communities would also have a subsequent impact on N transformation and N_2O emissions.

2. Materials and methods

2.1. Soil and manure used for the study

A silt loam soil (35% silt, 29% sand and 36% clay) classified as ultisol was used for the study. Surface soil samples (0–10 cm) were collected from a forest in Qu Zhou, Zhejiang province (N 28 °45′; E 118 °20′). The climate in the region is subtropical monsoon climate, with the temperature ranging 2 °C to 33 °C during a year, and a mean annual temperature of 17.3 °C and mean annual rainfall of 1843 mm. The soil was sieved through a 5 mm screen, thoroughly mixed and stored at 4 °C until use. The soil properties were: pH 4.0 (H_2O), 11.2 g kg^{-1} organic C, 2.4 g kg^{-1} total N, 16.0 $cmol_c$ kg^{-1} CEC, 1.4 $cmol_c$ kg^{-1} exchangeable Ca^{2+}, 1.3 $cmol_c$ kg^{-1} exchangeable K^+, 0.3 $cmol_c$ kg^{-1} exchangeable Mg^{2+}, and 10.0% base saturation.

Swine manure was collected from a local piggery farm. The swine manure was composted with sawdust. The properties of the swine manure (based on dry weight) were: pH 7.3 (H_2O), 298.8 g kg^{-1} organic C, 55.1 g kg^{-1} organic matter, 15.5 g kg^{-1} total N, 12.6 g kg^{-1} total P, 110.0 mg kg^{-1} total K, 72.1 mg kg^{-1} NH_4^+-N, 6.3 mg kg^{-1} NO_3^--N.

2.2. Incubation experiment

2.2.1. Treatments

Two parallel sets of incubation experiments were conducted, with one set for soil sampling to determine the growth of microbial communities and mineral N dynamics, and the other for measuring N_2O emissions, similar with that described in Di et al. (2014). The following treatments were applied to the soil: Control; Urea (400 kg N ha^{-1}); Manure (400 kg N ha^{-1}). The N application rate was used to simulate local farming practice where such high N rates are applied in intensive production systems, such as vegetable production systems. Each treatment had four replicates. These treatments were incubated under three different temperatures: 20, 30, and 40 °C.

2.2.2. Experimental setup

For the soil sampling experiment, 1000 g of sieved soil (dry weight basis) were put into plastic incubation containers (1 L volume, 12.0 cm diameter) with two 1 cm diameter holes created on the container lids to allow aeration during incubation. Urea was dissolved in deionized water before being applied and thoroughly mixed with the soil. Manure was sieved through a 3 mm screen and the right amount was then thoroughly mixed with soil.

For N_2O measurement experiment, 500 g of sieved soil (dry weight) were put into 1 L glass jars (10.0 cm diameter) and packed to 1.5 g cm^{-3} bulk density, leaving half of the glass jar empty for N_2O sampling. Urea was dissolved in water and evenly sprayed on to the soil surface. Manure was applied to the soil surface and then thoroughly mixed with soil.

The soil moisture content in all treatments, including the control, manure and urea treatments was adjusted to field capacity (soil moisture content was 24.5%) and was maintained by adjusting the weight of the containers twice per week. All incubation vessels were placed inside incubators in a randomized block design.

2.2.3. Soil sampling and quantification of microbial communities

Soil samples were collected from the incubation containers after 1, 50, 106, 188 days of incubation and used for quantitative PCR analysis (qPCR). Soil genomic DNA was extracted from 0.5 g soil using Fast DNA® SPIN Kit for Soil (Q BIO gene Inc., Carlsbad, CA, USA) according to the manufacturer's instructions with minor modifications: DNA was incubated for 5 min at 55 °C in a heat block before elution and dissolved in 100 μL of TE buffer instead of DES (DNase/Pyrogen-Free Water, which is used to elute DNA from the binding matrix in the Fast DNA® SPIN Kit). DNA concentration was assessed using the Nanodrop® ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

The functional genes of ammonia oxidizers (archaeal and bacterial *amoA* genes) and denitrifiers *(nirK/S, nosZ* gene) were assessed using the primers described in Di et al. (2014). All DNA extractions were diluted 1:10 with nuclease-free DI water to reduce