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Warmer and drier conditions alter the nitrifier and denitrifier communities and reduce N_2O emissions in fertilized vegetable soils

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ABSTRACT

Nitrous oxide (N_2O) is a potent greenhouse gas and is mainly produced from agricultural soils especially vegetable soils with large N fertilizer input. How future projected climate change may impact on the N_2O emissions and the related key (de)nitrifier communities in such ecosystem is poorly understood. The aim of this field study was to determine the interactive effects of a simulated warmer and drier climate on (de) nitrifier communities and N_2O emissions in a vegetable soil. A warmer (+3.3 °C) and drier climate (−14.4% soil moisture content) was created with greenhouses with or without urea N fertilizer application. The variation of microbial population abundance and community structure of Ammonia-oxidizing archaea (AOA), bacteria (AOB) and denitrifiers (*nirK/S*, *nosZ*) were determined using Real time-PCR and sequencing. The results showed a strong interactive effect of simulated climate change with N fertilizer applications, whereby the impacts of warmer and drier conditions on the microbial communities and N_2O emissions were more evident when N fertilizer was applied. The simulated warmer and drier conditions in the greenhouses significantly decreased N_2O emissions largely due to the drier soil conditions. The abundance and community structure of AOB showed more rapid responses than AOA under the simulated climate conditions when N fertilizer was applied. Changes of AOB community structure were significantly correlated with soil moisture content and NH_4^+-N concentration. The simulated climate change did not affect the *nirS* gene abundance, but significantly increased *nirK* gene abundance, and significantly decreased *nosZ* gene abundance with urea application. N_2O emissions were positively correlated with the bacterial *amoA* abundance and with the ratio of *nirK*/*nosZ* gene abundance. Therefore, bacterial *amoA*, *nirK*- and *nosZ*-type denitrifiers are the dominant microbial communities which were affected by the simulated climate conditions and are thus critically important for N cycling in vegetable soils under a changing climate.

1. Introduction

Nitrous oxide (N_2O) is the third most important greenhouse gas, after carbon dioxide (CO_2) and methane (CH_4), with a global warming potential of about 298 times that of carbon dioxide (CO_2) (IPCC, 2007). It is also the most significant ozone-depleting substance (Ravishankara et al., 2009). Two-thirds of anthropogenic N_2O emissions originate from agriculture soils, especially following the application of nitrogen (N) fertilizers or animal manures (Mosier et al., 1998). To meet the rising global food demands, the amount of N fertilizers used for agricultural production is expected to increase even further (Galloway et al., 2008), potentially leading to rising N_2O emissions. The rising concentrations of greenhouse gases, such as CO_2, CH_4 and N_2O, have already resulted in regional and global climatic changes. It is projected that global surface temperature may rise between 2 and 4 °C by the end of the 21st century and extreme weather patterns such as heat waves accompanied by severe droughts are likely to become more common (IPCC, 2007, 2014). These new weather conditions are likely to create new challenges for agricultural

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production, including nutrient and fertilizer management to feed the growing world population.

Many studies have sought to elaborate on the effect of climate change drivers such as elevated CO_2 (eCO_2), warming and drought on N_2O emissions. On the one hand, these studies were conducted on grassland, heathland, dryland, steppe, and alpine meadow ecosystems (Hu et al., 2010, 2015, 2016; Brown et al., 2012; Cantarel et al., 2011, 2012; Carter et al., 2011; Larsen et al., 2011; Hartmann et al., 2013), and revealed that the N_2O fluxes in response to simulated climate change varied with local climate conditions, land use types and agricultural management. Among these factors, agricultural management, such as grazing perturbations and fertilizer application might alter the extent of climatic change effect on N_2O fluxes (Hu et al., 2010; Hartmann and Niklaus, 2012; Hartmann et al., 2013; Tian et al., 2015). These studies suggested that positive effects of warming on N_2O emissions were because warming induced N mineralization, thus increasing N availability in the ecosystem (Cantarel et al., 2011, 2012). However, few studies have been reported on the effect of climate change on N_2O emissions from intensive agroecosystems, such as vegetable soils, where large N inputs occur annually (He et al., 2009).

On the other hand, the interactive effects of climate drivers on N_2O emissions were much more complex than single-factor effects. Results from multiple climate change studies indicated that the interactive effects tend to cause smaller changes in N_2O efflux than from single-factor manipulations as the effect of individual treatments may negate each other if they act in opposite directions (e.g. summer drought and warming) (Larsen et al., 2011; Brown et al., 2012). Therefore, single-factor studies might overestimate the effect of climate change on N_2O emissions. Previous studies demonstrated that changed temperature and soil moisture content were two main direct climatic factors that affect N_2O emissions, while the effect of eCO_2 was often indirect through changing soil moisture content and/or nitrogen turnover (Billings et al., 2002; Cantarel et al., 2011, 2012; Larsen et al., 2011; Liu et al., 2015). Thus, a study on the interactive effect of warming and drought might be a more effective and realistic approach to understanding the impact of climate change on N_2O emissions in vegetable soils.

Feedback responses of the microorganisms related to N_2O emissions caused by warming and drought were different between different ecosystems and regions (Singh et al., 2010), and their inherent habitus resulted in different sensitivity to changed climate conditions. Nitrifiers (AOA and AOB) are the key drivers of nitrification, which are of vital importance in vegetable soils as it can affect the form of nitrogen that can be used for vegetable growth and nitrogen losses (e.g. NO_3^--N leaching and N_2O emission). It was reported that both AOA and AOB were potentially influenced by changed temperature and soil moisture content, although their responses were highly variable in different studies due to different soil parameters (Avrahami and Bohannan, 2009; Gleeson et al., 2010; Chen et al., 2013). Denitrification is a multistep process with each step being mediated by different groups of microorganisms encoded by different functional genes. This process plays an important part in N losses, including N_2O emissions in vegetable soils (Xiong et al., 2006; He et al., 2009; Pang et al., 2009). As denitrifying gene abundances could act as indicators of nitrous oxide emissions from soils (Morales et al., 2010), detailed understanding on denitrifier abundances in response to simulated climate change should be helpful for developing future-proof N_2O mitigation options.

Vegetable soils occupy about 11% of cropland in China, and account for about 20% of the national cropland N_2O emissions (He et al., 2009), far more than that from grassland or forest ecosystems (Brown et al., 2012). However, knowledge on how the projected climate change may impact on N_2O emissions and (de)nitrifiers in vegetable soils, and the feedbacks of these microorganisms through community structural adaption remained scarce. Therefore, corresponding studies on vegetable soils under the projected climate change scenario are needed to bridge this knowledge gap.

We thus conducted an experiment focusing on the interactive effect of warming and drought along with N fertilization on (de) nitrifier communities and N_2O emissions in a vegetable soil. The objectives of this study were to determine possible impacts of simulated climate change (warmer temperatures and drought) in combination with N fertilizer (urea) use on (1) ammonia oxidizing and denitrifying communities; (2) N_2O emissions; and (3) relationships between N_2O emissions and the microbial communities and soil conditions. We hypothesized that the simulated conditions of climate change together with N fertilizer use would alter the ammonia oxidizing and denitrifier communities which in turn would affect N_2O emissions. Greenhouses were built to enable the simulation of warmer (+3.3 °C) and summer drought (−14.4%) to test this hypothesis.

2. Materials & methods

2.1. Site description

A field experiment was carried out in an Experimental Station located in Zhejiang University (30°18′N, 120°05′E), Hangzhou, China. This area has a subtropical monsoon climate with an annual average temperature of 17.8 °C and annual rainfall of 1454 mm. Before the establishment of experiment, the area was used for rotational production of vegetables such as cucumber (*Cucumis sativus* Linn.), cabbages (*Brassica oleracea* var. capitata) and tomatoes (*Lycopersicon esculentum* Mill.). The soil was alfisol (yellow-brown soil) with a sandy loam texture (clay 10.18%, silt 4.07% and sand 85.75%). The soil properties were pH (H_2O) 7.7; organic C 9.98 g kg^{-1}; total N 1.23 g kg^{-1}; Olsen P 12.43 mg kg^{-1}; CEC 10.80 $cmol_c$ kg^{-1}; and base saturation 30%.

2.2. Design of field study

A total 16 plots, each 2 m^2 in size (1 m wide × 2 m long), were established. Greenhouses were established over half of these plots (8 plots) to simulate climate change (higher temperature and drier soil conditions) and half were left uncovered to be exposed to the local natural rainfall and temperature conditions. Two Urea-N rate treatments (0 and 400 kg N ha^{-1}), each with four replicates, were applied to both the greenhouse and non-greenhouse plots. The plots were arranged in the field in a randomized design. The trial was started in August 2013, when the temperature and rainfall in Hangzhou region were high and was completed by November 2013. No crops were grown either inside or outside the greenhouses and therefore there was no difference in terms of vegetation cover between inside and outside of the greenhouses.

The greenhouses were in the form of rectangular transparent glass boxes (1 m wide × 2 m long × 1 m high) large enough to cover the 2 m^2 plots and with enough space inside to allow soil and N_2O sampling (Fig. 1a). These glasshouses would essentially provide a “greenhouse effect” for the plots inside, resulting in higher temperature and drier soil moisture conditions. Two computer-controlled fans were installed on two opposite sides of each glasshouse to control air-flow in and out of the glasshouses to control the temperature inside 3–5 °C above the ambient temperature. Air temperature and soil moisture content inside and outside the greenhouses were recorded by a data logger (MP-406 logger, ZHOMETI). The soil moisture was measured by a soil moisture probe (FDR MP-406T, ZHOMETI). The other plots that were not enclosed by greenhouses received the natural sunshine,