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The response patterns of community traits of N_2O emission-related functional guilds to temperature across different arable soils under inorganic fertilization

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ABSTRACT

Temperature is an important factor governing the community traits of N_2O-emission related functional guilds (mainly autotrophic ammonia oxidizers and heterotrophic denitrifiers) and their activities. However, there have been few attempts to explore the broad response patterns of these guilds to temperature changes across arable soils. For this, a temperature-controlled (15, 25 and 35 °C) microcosm experiment was conducted using three arable soils (Fujian, Gansu, and Jiangsu) in China under two different fertilizations (no fertilization control (CK) and inorganic fertilization (NPK)). In conjunction with the measurement of N_2O emission, the community structure and abundance of ammonia oxidizing archaea (AOA) and bacteria (AOB), as well as *nirS*- and *nirK*-denitrifiers were assessed using T-RFLP and quantitative PCR, respectively. The analysis of community traits indicated a consistent response pattern of AOAs to temperature in terms of guild abundance, and a consistent effect of inorganic fertilization on the abundance of AOBs, but soil-dependent response patterns to fertilization and temperature were found for *nirS*- and *nirK*-denitrifiers in terms of abundance and community structure. The correlation analysis suggested that AOAs possibly assumed a role in N_2O emission in all the tested soils, and *nirS*-denitrifiers probably participated in N_2O emission in both the Fujian and Gansu soil, while a considerable amount of N_2O emission in the Jiangsu soil might have been derived from heterotrophic nitrification.

1. Introduction

Temperature is a major factor governing microbial metabolism in general (Paul and Clark, 1989) and the emission of greenhouse gases in particular, such as those of CO_2, CH_4 and N_2O (Smith, 1997). Among these, N_2O is of particular interest due to both its net greenhouse effect per unit mass approximating to 300 times larger than that of CO_2 on a 100-year time span (Lashof and Ahuja, 1990), and its ability to deplete the ozone layer (Ravishankara et al., 2009). Meanwhile, ~50% of the global anthropogenic N_2O emission is derived from agricultural soil, thus the N_2O emission in agricultural soils is of significant environmental consequences (Mosier et al., 1998). As shown in previous experiments, temperature can impact N_2O emission in soils both directly and indirectly (Smith, 1997), and the sensitivity of N_2O emission to temperature varies among different soils, with both increases and declines in N_2O emission in response to temperature increases being reported (e.g. Focht and Verstraete, 1977; Maag and Vinther, 1996; Smith, 1997; Godde and Conrad, 1999; Dobbie and Smith, 2001; Avrahami and Bohannan, 2009). Most research suggests that N_2O emission is positively correlated with soil temperature.

The effect of temperature on N_2O emission has a profound microbial basis (Smith, 1997; Braker and Conrad, 2011; Butterbach-

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Bahl et al., 2013). Convincing evidence has shown that both autotrophic nitrification and heterotrophic denitrification account for the majority of N_2O emission from soils (Firestone and Davidson, 1989). Both are temperature-sensitive processes (Focht and Verstraete, 1977; Smith, 1997; Godde and Conrad, 1999; Avrahami and Bohannan, 2009; Braker et al., 2010; Cui et al., 2016), and performed by diverse microorganisms (Butterbach-Bahl et al., 2013; Hu et al., 2015). The N_2O emission via ammonia oxidization is carried out by a few phylogenetically closely related taxa of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Shaw et al., 2008; Jung et al., 2014; Stieglmeier et al., 2014; Hu et al., 2015; Hink et al., 2016). AOBs can function as denitrifiers (nitrifier denitrification) (Shaw et al., 2008), while the corresponding AOAs' denitrification ability is still a subject of debate (Jung et al., 2014; Stieglmeier et al., 2014; Hu et al., 2015). In contrast, canonical denitrification, i.e. the ability for respiratory nitrate reduction, is a polyphyletic trait which has been found in a broad range on phylogenetically unrelated microorganisms, including bacteria, archaea, and fungi, which can be divided into two exclusive genotypic groups based on species differences in their active sites of nitrite reductase, namely *nirK*-denitrifiers and *nirS*-denitrifiers (Zumft, 1997).

Changes in temperature can impact on community traits, such as community structure and abundance, and also N_2O-emission related to functional guilds, while different response patterns have been reported in different soils (Avrahami et al., 2003; Horz et al., 2004; Tourna et al., 2008; Avrahami and Bohannan, 2009; Braker et al., 2010; Szukics et al., 2010; Jung et al., 2011; Cui et al., 2016; Hu et al., 2016). For instance, variation in temperature has been shown to change the community structures of both AOBs and AOAs in grassland (Avrahami and Conrad, 2005; Avrahami and Bohannan, 2009; Hu et al., 2016), meadow (Avrahami et al., 2003), forest (Szukics et al., 2010), and agricultural soils (Tourna et al., 2008), resulting in the enrichment of particular phylogenetic clades under different temperature regimes (Avrahami et al., 2003; Avrahami and Conrad, 2005; Tourna et al., 2008). Relatively stable community structures of both AOBs and AOAs have also been reported following temperature changes in these arable (Tourna et al., 2008) and forest soils (Szukics et al., 2010). Increases, declines or no change in the abundances of either AOBs or AOAs in response to the elevation of temperature have also been found in forest soil (Szukics et al., 2010), dryland forest soils (Hu et al., 2016), grassland (Horz et al., 2004), and arable soils (Cui et al., 2016), respectively. Similarly, inconsistent response patterns of the denitrifier community to temperature changes have been observed across forest, agricultural, and Anarctic soils (Braker et al., 2010; Szukics et al., 2010; Jung et al., 2011; Cui et al., 2016). Surprisingly, however, there are few cross-arable-soil studies comparing the response patterns of the community traits of these N_2O-emission related microorganisms to temperature, despite the fact that well-structured cross-site research becomes increasingly relevant if soil ecologists want to explore and identify the broad patterns of soil microbiota and their associated processes (Fierer et al., 2009b).

Although some studies failed to establish the link between community dynamics of these functional guilds and N_2O emission (e.g. Dandie et al., 2008; Attard et al., 2011), presumably due to the functional redundancy of different taxa (Wertz et al., 2007), in general, the community traits of these functional guilds underpin their ecosystem processes including N_2O emission (Avrahami and Bohannan, 2009; Morales et al., 2010; Braker and Conrad, 2011; Philippot et al., 2013). It could therefore be expected that the shift of communities of these functional guilds in response to temperature is coupled with variations in N_2O emission. However, few studies have attempted to assess the association of the shifts in community traits of these functional guilds with N_2O emission in the context of temperature change (Szukics et al., 2010; Cui et al., 2016). Also, if the inconsistent response patterns of these guilds to temperature across different soils held true even under well-defined conditions, it implies that different functional guilds are involved in N_2O emission in different soils, but few efforts have been made to test this hypothesis.

Therefore, to explore the response patterns of the functional guilds related to N_2O-emission across different soils and test the previous hypothesis, a microcosm experiment was conducted in this study using three different types of arable soils differing markedly in geography, climate, hydrology, physico-chemical properties, and fertilization history. The majority of anthropogenic N_2O evolved from arable soils is driven by the excess input of inorganic nitrogen fertilizers and animal manures (Shcherbak et al., 2014). It has been widely demonstrated that application of inorganic fertilizer significantly alters the community composition of microorganisms involved in N_2O emission (He et al., 2007; Shen et al., 2008; Hallin et al., 2009; Chen et al., 2010, 2011; Yin et al., 2015; Cui et al., 2016). Therefore, it is interesting to explore whether the application of inorganic fertilizers interact with temperature to change the response patterns of these functional guilds. Hence, the soils either receiving inorganic fertilizer (NPK) or control (CK) were further selected in each site of this study.

Methodologically, exploring and identifying the broad response patterns of these functional guilds to temperature across different soils can be difficult, as the spatial and temporal heterogeneity of soil may have very different unquantified impacts on soil microorganisms (e.g. variation in physical structure, crop residue and rhizosphere deposits, fertilizer residues and the effects of preceding temperature etc.) (Fierer et al., 2009b). Accordingly, a standardized procedure was suggested to smooth out these "noises" (Fierer et al., 2009b). For this, in this study, a one-month-long temperature- and moisture-controlled pre-incubation treatment was adopted to eliminate the lingering effect of the carbon source from the crop and the nitrogen source from fertilizers, as well as the effect of preceding temperature. The soils were also sieved to reduce the impact of soil physical structure.

The polyphyletic distribution of N_2O-producing microorganisms renders those detection methods unsuitable which are based on assessment of genes such as 16S rRNA. Instead, the molecular biomarkers targeting the functional genes are demonstrated to be a valid approach within this context. Thus, the *amoA* gene of ammonia oxidizers, coding for the alpha subunit of ammonia monooxygenase (Avrahami et al., 2003), and *nirK* and *nirS* gene of denitrifies were used as molecular probes in this study (Avrahami and Bohannan, 2009; Hallin et al., 2009; Yin et al., 2015; Cui et al., 2016). Specifically, three questions were addressed; i) how would community traits of the selected functional guilds respond to different temperatures across these three arable soils; ii) were there any consistent response patterns of community traits of these functional guilds to temperature change across soils with a certain history of no and high N fertilization? iii) were there any significant links between the shifts of their community traits and the N_2O emission response to temperature?

2. Material and methods

2.1. Field sites and soil sampling

Soils were sampled from no fertilizer or control (CK) and conventional inorganic fertilization (NPK) treatments in three different fertilization experimental sites in China: Jiangle Experimental Station in Fujian province (Fujian), Xiangquan Experimental Station in Gansu province (Gansu), and Jintan Experimental Station in Jiangsu province (Jiangsu). The sites differed substantially in soil