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Substrate-driven microbial response: A novel mechanism contributes significantly to temperature sensitivity of N_2O emissions in upland arable soil

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ABSTRACT

The mechanism by which temperature sensitivity (TS) of soil N_2O emissions is increased by agricultural management with application of nitrogen fertilizer (AMN) is unclear. We hypothesized that a higher TS of N_2O emission induced by AMN is the result of the faster growth of specific microorganisms in response to faster nitrogen (N) mineralization at higher temperatures. To test this hypothesis, we used reciprocal transplants to separate the contributions of abiotic and microbial components to the TS of N_2O emissions in an arable soil receiving organic and inorganic fertilizers and its neighboring natural grassland soil treated with two levels of N. N_2O sources were separated with acetylene, and the abundances of N_2O-producing microbes were assessed by quantifying the copy numbers of the associated functional genes. Compared with natural soil, only changes in abiotic properties increased the Q_{10} (the factor by which the rate increases with a 10 °C rise in temperature) by 105.7%, while changes in both abiotic and the microbiome increased the Q_{10} by 225.2%. Higher TS of N_2O emission in the arable soil induced by a microbiome shift was associated with faster N mineralization, increased proportion of nitrification-N_2O emission, and faster growth of ammonia-oxidizing bacteria at higher temperatures. Addition of ammonium nitrate further enhanced the TS of N_2O emissions, the proportion of nitrification-N_2O emission, and the increased extent of the growth of ammonia-oxidizing bacteria in the soil with AMN compared to the natural grassland soil. Substrate-driven growth of ammonia-oxidizing bacteria with higher substrate preference contributes significantly to the higher TS of N_2O emission caused by AMN.

1. Introduction

Nitrous oxide (N_2O) is the third most important greenhouse gas (IPCC, 2007) and is a major cause of stratospheric ozone depletion (Ravishankara et al., 2009). About 60% of anthropogenic N_2O is emitted from agricultural soils, mostly derived from applications of synthetic fertilizer N and animal manure (Smith, 2017; Zhou et al., 2017). Besides by N sources, the emission of N_2O is controlled by many other factors, including contents of soil water and carbon and temperature (Weier et al., 1993; Conrad, 1996; Avrahami et al., 2003; Baggs and Philippot, 2010; Blagodatskaya et al., 2014; Smith, 2017). Of these, temperature is particularly significant because it influences current N_2O emissions and also impacts future N_2O emissions via positive or negative feedback (Smith, 1997). We analyzed the results in the review by Smith (1997) and subsequent studies and found that N fertilization increases the N_2O-emission rate and also its temperature sensitivity (TS). The Q_{10} levels in soils with AMN are higher than in soils without N fertilizer in both disperse studies and studies with paired N and no N treatments (Fig. 1, Table S1). However, the mechanisms underlying the AMN-enhanced TS of soil N_2O emission are not well known.

The variation in TS of soil N_2O emission has been attributed mostly to anaerobic-zone development (AZD) in aggregated soils (Smith, 1997, 2017). This explanation suggests that a temperature increase leads to an

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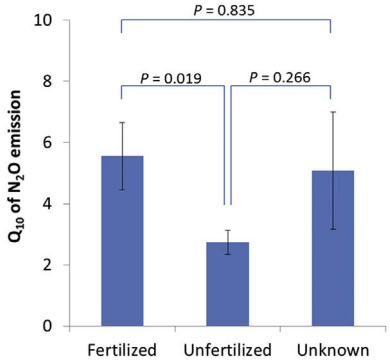

Fig. 1. Q_{10} of N_2O emission reported in the literature. The data are divided into fertilized, unfertilized, and unknown according to application of nitrogen fertilizer. P values were obtained from t-tests.

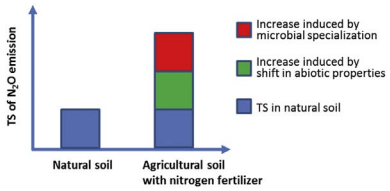

Fig. 2. Hypothesized controls on temperature sensitivity (TS) of N_2O emission in agricultural soil amended with nitrogen fertilizer. Agricultural management using nitrogen fertilizer increased the TS of soil N_2O emission by: 1) increasing the supply of substrates including mineral nitrogen and dissolved carbon; 2) increasing the growth of N_2O-generating microbes in response to higher nitrogen mineralization at high temperature.

increase in both the size of the zone where denitrification N_2O occurs (because increased respiration depletes the oxygen supply) and the rate of denitrification per unit of anaerobic volume. Consequently, the overall change in the rate of N_2O production in a soil mass is due to the comprehensive effects of these two factors. This theory could be easily modeled, but is hard to empirically test. In addition, it only accounts for N_2O emitted from denitrification and thus seems unsuitable for interpreting the effect of AMN on the TS of N_2O emission in upland soils where N_2O can be generated via other pathways (Baggs and Philippot, 2010; Hu et al., 2015).

Enzymatically, the TS of a biochemical reaction rate is largely determined by the chemical structure of the substrate and the configuration of the enyzme catalyzing the substrate's degradation (Davidson and Janssens, 2006). The enzymatic theories have been applied as paradigms to interpret the TS of soil respiration with oxygen as electron acceptors but rarely the TS of N_2O emissions (Davidson and Janssens, 2006; Conant et al., 2011). Compared with soil organic carbon, the chemical structures of substrates for N_2O production are relatively simple (Baggs and Philippot, 2010; Hu et al., 2015). The activation energy will be stable for specific N_2O-producing pathways but varies among different pathways. Thus, the TS of N_2O emission would be decided by the relative proportions of the different N_2O-generating pathways. In addition, N_2O emissions are always stimulated by application of N fertilizer (Cui et al., 2013; Shcherbak et al., 2014), showing that the substrate for N_2O production is unsaturated. The abundance of N_2O-producing microbes is often positively correlated with N_2O emission (Morales et al., 2010; Cui et al., 2013), showing that N_2O-generating enzymes can also be rate limiting for N_2O production in soil. Thus, a stronger increase in N mineralization and number of N_2O-generating microbes at higher temperature would lead to a stronger increase in N availability, more functioning microbes and consequently higher TS of N_2O emission. Also, the affinity of the substrate and N_2O-generating-enzymes differs among different microbial taxa (Prosser and Nicol, 2012), which would catalyze the N_2O-generating processes at different rates at certain N levels. However, it is unknown if AMN shapes the soil microbiome to have above-mentioned functioning potentials, leading to higher TS of N_2O emission.

In this study, we hypothesized that a higher TS of N_2O emission induced by AMN results from microbial specialization with faster growth of specific functioning microbial taxa in response to faster N mineralization at higher temperatures (Fig. 2). To test this hypothesis, we used a reciporocal transplant approach to separate the contributions of substrate and microbiome to TS of N_2O emission in an arable soil treated with organic and inorganic fertilizers and a nearby natural grassland soil treated with two levels of N. In addition, we used acetylene, a nitrification inhibitor, to differentiate N_2O sources and estimate microbial reponses by quantifying functional genes associated with N_2O production with real-time quantitative PCR (qPCR).

2. Materials and methods

2.1. Literature review

We obtained Q_{10} estimates of soil N_2O emission before 1997 from the literature cited in Smith (1997). The Q_{10} values of soil N_2O emission after 1997 were retrieved from the Web of Science (www.isiknowledge.com). The search parameters were set as "TOPIC: (soil) AND TOPIC: (temperature) AND TOPIC: (nitrous oxide) AND TOPIC: (Q10)". A total of 90 Q_{10} values were obtained. The data were divided into fertilized or unfertilized according to whether they received N fertilizer or not. Data were designated as "unknown" if there was no fertilizer information. T-tests were used to examine the significance of differences between the data groups.

2.2. Soil collection and incubation

Agricultural farmland soil (AGR) and a natural grassland soil (NAT) were collected for the incubation experiment. The soil samples were from Qiyang County (26 °45′12″N, 111 °52′32″E), Hunan province, southern China. This site has an average annual temperature of 18 °C and average annual precipitation of 1250 mm. The soils, developed from Quaternary red clay, are classified as ferralic cambisols (FAO). The AGR and NAT were located within 20 m and were the same type of grasslands about 40 years ago. AGR soil was collected from the conventional fertilization treatment in the long-term fertilization experiment of the Chinese Academy of Agricultural Sciences, which has been previously described (Zhang et al., 2009). Since 1990, the N application rate of the treatment has been 300 kg N ha^{-1}, with 30% N from urea and 70% from pig manure. The application rates of P_2O_5 and K_2O fertilizers have each been 120 kg ha^{-1} $year^{-1}$ in the form of calcium superphosphate and potassium chloride. The cropping system was a wheat-maize rotation. NAT was collected from the grassland near the field trial of the long-term fertilization experiment. No crops had been planted in the NAT soil and the main vegetation was cogon grass (*Imperata cylindrica*). The soil samples were collected at several randomly chosen locations (5 cm diameter × 20 cm depth) and pooled to form

Table 1
Properties of the agricultural soil (AGR) and neighboring grassland soil (NAT) used in the present study.

	pH	organic C (g kg^{-1})	total N (g kg^{-1})	available P (mg kg^{-1})	available K (mg kg^{-1})	NO_3^- (mg kg^{-1})	NH_4^+ (mg kg^{-1})
NAT	4.23	12.20	2.10	2.87	56.93	13.69	9.44
AGR	5.7	8.58	1.07	10.8	122	106.24	29.67