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Nitrous oxide emission and denitrifier communities in drip-irrigated calcareous soil as affected by chemical and organic fertilizers

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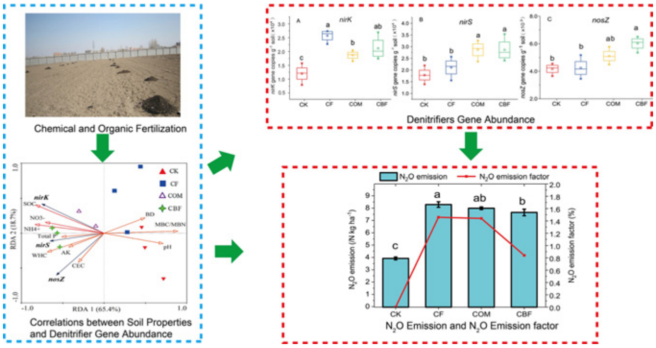
HIGHLIGHTS

- *NirS* and *nosZ* communities were affected by organic but not chemical fertilizer.
- SOC, NO_3^-, NH_4^- and pH were main drivers for the alteration of denitrifier community abundance.
- The significant factors influencing soil N_2O emissions were the *nirS* community, SOC and nitrate
- N_2O emission significantly decreased when biofertilizer was applied with chemical fertilizer.

GRAPHICAL ABSTRACT



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ABSTRACT

The effects of consecutive application of chemical fertilizer with or without organic fertilizer on soil N_2O emissions and denitrifying community structure in a drip-irrigated field were determined. The four fertilizer treatments were (i) unfertilized, (ii) chemical fertilizer, (iii) 60% chemical fertilizer plus cattle manure, and (iv) 60% chemical fertilizer plus biofertilizer. The treatments with organic amendments (i.e. cattle manure and biofertilizer) reduced cumulative N_2O emissions by 4.9–9.9%, reduced the N_2O emission factor by 1.3–42%, and increased denitrifying enzyme activities by 14.3–56.2%. The *nirK* gene copy numbers were greatest in soil which received only chemical fertilizer. In contrast, *nirS*- and *nosZ*-copy numbers were greatest in soil amended with chemical fertilizer plus biofertilizer. Chemical fertilizer application with or without organic fertilizer significantly changed the community structure of *nirK*-type denitrifiers relative to the unfertilized soil. In comparison, the *nirS*- and *nosZ*-type denitrifier genotypes varied in treatments receiving organic fertilizer but not chemical fertilizer alone. The changes in the denitrifier communities were closely associated with soil organic carbon (SOC), NO_3^-, NH_4^+, water holding capacity, and soil pH. Modeling indicated that N_2O emissions in this soil were primarily associated with the abundance of *nirS* type denitrifying bacteria, SOC, and NO_3^-. Overall, our findings indicate that (i) the organic fertilizers increased denitrifying enzyme activity, increased denitrifying-bacteria gene copy numbers, but reduced N_2O emissions, and (ii) *nirS*- and *nosZ*-type denitrifiers were more sensitive than *nirK*-type denitrifiers to the organic fertilizers.

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1. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas and ozone-depleting chemical. It accounts for approximately 6.24% of global radiative forcing and contributes significantly to global warming potential (Davidson, 2009). Atmospheric N_2O concentrations have increased by 0.26% annually over the past several decades, reaching a concentration of 324 ppb in 2014 (IPCC, 2014). Agricultural practices have significantly contributed to the rise in atmospheric N_2O. About 58% of anthropogenic N_2O emissions originate from agriculture, and this amount is expected to rise by 35–60% by 2030 due to increases in N fertilizer use and animal manure production (Smith et al., 2007).

Denitrification is the primary biogeochemical pathway through which N_2O is formed. This is a modular biological process in which nitrate (NO_3^-) is sequentially reduced to nitrite (NO_2^-), nitric oxide (NO), N_2O, and finally dinitrogen (N_2) gas. The process is catalyzed by four key biological transformations: nitrate reductase (*NAR*), nitrite reductase (*NIR*), nitric oxide reductase (*NOR*), and nitrous oxide reductase (*NOS*) (Zumft, 1997). The genes encoding *NIR* and *NOS* (i.e., *nirK*/*nirS* and *nosZ*, respectively) are frequently used as functional markers to analyze the denitrifier communities (Cui et al., 2016; Yang et al., 2017; Azziz et al., 2017). The *nosZ* gene encodes for *NOS* which catalyzes the reduction of N_2O to N_2. However, analysis of the genomes of denitrifying bacterial indicates that, in approximately ⅔ of species, nitrous oxide reductase ability is absent (Jones et al., 2008). As such, N_2O maybe formed, but complete reduction to N_2 cannot occur. Thus, the ratio of *nir*- to *nosZ*-genes has been closely coupled with the N_2O/N_2 emission ratio (Philippot et al., 2011). Harter et al. (2013) observed that emissions of N_2O were inversely related to *nosZ* gene expression. However, Henderson et al. (2010) reported that N_2O emission did not significantly associate with either denitrifier gene abundance or the *m*RNA levels of *nirS* and *nosZ*. Although numerous studies have been carried out, there is still contradictory information related to linkages between N_2O emission and the abundance, diversity, and structure of wider denitrifier community.

Fertilization strategy (i.e., chemical fertilizer alone or in combination with manure) affects not only soil physical and chemical conditions, but also soil microbial communities involved in N cycling (Bandyopadhyay et al., 2010; Nacke et al., 2011; Cui et al., 2016; Yin et al., 2014, 2015). Studies have shown that NPK fertilization increases denitrifier abundance (Chen et al., 2012; Tang et al., 2010), or alternatively that NPK fertilization decreases denitrifier abundance (Yin et al., 2014) or has little effect on the abundance of denitrifying microbes (Szukics et al., 2009). As a source of energy and nutrients, organic fertilizers usually promote microbial growth (Chen et al., 2012). Some researchers have reported that organic fertilizers significantly altered the composition of denitrifier communities (Chen et al., 2012; Hai et al., 2009). However, published data on the response of denitrifiers abundance and composition to organic fertilization has not always been consistent. For example, Chen et al. (2010) reported that *nirK*-denitrifiers were more responsive than *nirS*-denitrifiers to organic fertilization or environmental disturbance. In contrast, Tang et al. (2010) and Yin et al. (2015) observed that *nirS*-denitrifiers were more susceptive on organic fertilizer. It has been reported that organic fertilizers (i.e., manure and straw) increased the abundance of both *nirK* and *nirS* in tropical (Hai et al., 2009), subtropical (Cui et al., 2016), and temperate agroecosystems (Yin et al., 2015). Molecular-based studies have indicated that organic and inorganic fertilizers have different effects on the denitrifying community, and this is linked to the changes in soil physicochemical properties (e.g., NO_3^-, NH_4^+, SOC, and pH) (Avrahami et al., 2002; Dandie et al., 2011; Palmer et al., 2012; Harter et al., 2013). However, it is still not clear which environmental factors have the greatest effect on denitrification genes and N_2O emissions from soil. This is surprising given the importance of N_2O as a GHG causing chemical and the role of agriculture as a global source of N_2O.

The Xinjiang Uygur autonomous region in northwest of China is a zone of intensive agriculture. The soil in Xinjiang is predominantly calcareous desert soil with low organic carbon (C) content and high pH (7.5–8.5). Due to the scarcity of fresh water, drip irrigation has been extensively used for crop production in this region. In addition, the combined use of chemical and organic fertilizers is recommended as a means of improving nutrient use efficiency and reducing environmental risks due to overuse of chemical fertilizers.

Several studies have explored the effects of chemical fertilizer type and rate on N_2O emissions in drip-irrigated systems (Sánchez-Martín et al., 2010; Kennedy et al., 2013). Although it is important to understand the mechanisms by which different fertilization strategies affect the linkages among soil N_2O emissions, denitrifier community abundance, and soil physiochemical properties, few studies that have examined these associations. We hypothesize that combined chemical and organic fertilizer application could promote N_2O emissions via increasing the abundance and diversity of the denitrifying community, and these effects would be associated with changes in community composition. Furthermore, we sought to determine the underlying links between changes in edaphic parameters, due to the different fertilization methods, and the denitrifying community.

This study focused on a drip-irrigated cotton production system on a calcareous soil. The assessments were made following four years of applying either chemical or chemical fertilizer plus organic fertilizer. In particular, we aimed to (i) compare the effects of chemical fertilizer and organic fertilizer mix chemical fertilizer on N_2O fluxes; (ii) determine how organic plus chemical fertilizer affects the abundance, community structure, and diversity of soil denitrifiers; and (iii) ascertain the relationship among N_2O production, denitrifier gene community abundance, and soil physicochemical properties.

2. Materials and methods

2.1. Experimental site and soil description

The field plot experiment was carried out from 2011 to 2014 at the Shihezi University Agricultural Experiment Station in the Xinjiang Uyghur autonomous region of China (44°23′ N, 85°41′ E). The experimental zone is classified as being temperate, arid, and with a continental climate. The mean annual temperature at the site is 7.8 °C. The annual precipitation is 225 mm, annual evaporation 1340 mm, with no legible annual variation. The soil is a calcareous desert soil (*Calcaric Fluvisol*). The basic soil properties, before the beginning of the experiment, were: pH, 8.21; organic C, 13.5 g kg^{-1}; total N, 0.95 g kg^{-1}; total P, 0.78 g kg^{-1}; alkali-hydrolysable N, 88.6 mg kg^{-1}; available P, 23.4 mg kg^{-1}; and available K, 136 mg kg^{-1}.

2.2. Experimental design

The study used a randomized complete block design with three replicates of four treatments. The four treatments were: (1) CK, unfertilized control; (2) CF, chemical fertilizer consisting of 300 kg N ha^{-1}, 90 kg P_2O_5 ha^{-1}, and 60 kg K_2O ha^{-1}; (3) COM, 60% CF plus 6000 kg ha^{-1} cattle manure; and (4) CBF, 60% CF plus 6000 kg ha^{-1} bio-fertilizer. The cattle manure contained 24.8% organic matter and 1.7% total N. The bio-fertilizer (Jiangsu Xintiandi Amino Acid Fertilizers Ltd., Jiangsu, China) consisted of poultry manure enriched with beneficial microorganisms and various small molecular peptides. The bio-fertilizer (pH 7.6) had 29.7% organic matter and 4.4% total N. It contained approximately 0.5×10^9 CFU g^{-1} of the beneficial bacteria *Bacillus subtilis* HJ5 and *B. subtilis* DF14. These bacteria have been registered in the China General Microbiological Culture Collection Center with the accession numbers CGMCC No. 3301 and CGMCC No. 330, respectively. The bio-fertilizer and cattle manure were spread evenly on the surface of each plot (4.5 m wide × 20 m long) in early April each year, and then completely mixed into the 0 to 20 cm depth with a rotary