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Contributions of ammonia-oxidising bacteria and archaea to nitrification under long-term application of green manure in alkaline paddy soil

Songjuan Gao[a], Gpuopeng Zhou[b], Yulin Liao[c], Yanhong Lu[c], Jun Nie[c,*], Weidong Cao[a,b,*]

[a] College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China

[b] Key Laboratory of Plant Nutrition and Fertilizer, Ministry of Agriculture / Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China

[c] Soil and Fertilizer Institute of Hunan Province, Hunan Academy of Agricultural Sciences, Changsha. 410125, China

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ABSTRACT

Rice–rice–green manure rotation is a cropping system that effectively maintains high yields and improves soil fertility. Nitrification, a critical process in N cycling, is affected by different fertilisation regimes, but the roles of ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA) in nitrification and their shift in abundance and diversity in response to long-term green manure application in alkaline paddy soil is poorly understood. In the present work, nitrification potential (NP), the contributions of AOA and AOB to nitrification, abundances, and communities of AOA and AOB were studied to mechanistically understand the effects of green manuring and fertilisation regimes on nitrification and ammonia oxidisers. Results showed that the combination of green manure and reduced chemical fertiliser increased grain yields. Soil NP increased with increasing amount of chemical fertiliser. The combination of green manure and different rates of chemical fertiliser promoted the soil recovered nitrification potential (RNP) and the contribution of AOB to nitrification (RNP_{AOB}). Differences in AOA and AOB communities revealed that an increase in the abundance of *Nitrosopumilus* in AOA and a decrease in the abundance of *Nirosomonas* in AOB were observed in green manure treatment compared with those of chemical fertiliser treatments. Partial least squares path modelling showed that chemical fertilisation exerts direct effects on NP whereas green manuring markedly influenced RNP. For the relative contributions of AOA (RNP_{AOA}) and AOB, RNP_{AOB} were correlated with chemical fertilisation, whereas green manuring directly affected RNP_{AOA}. In conclusion, green manuring changed the nitrification process by affecting RNP, whereas chemical fertiliser directly influenced NP. Changes in AOA composition and their impact on nitrification might reflect a mechanism worthy of further research.

1. Introduction

Application of green manure is an effective method to improve soil fertility and significantly increase soil C and N stocks (Drinkwater et al., 1998; Tejada et al., 2008; Zhang et al., 2019a). The utilisation of green manure can reduce chemical fertiliser usage in farmland (Macguidwin et al., 2012; Xie et al., 2016); thus, confirming that the strategy is a feasible approach for fertiliser reduction and sustainable agricultural development. Given the intensive double-rice cropping system in south China, large amounts of chemical fertiliser are required to maintain crop yields. The utilisation of leguminous green manure provides an alternative approach that can bridge the gap between sustainable development and high yield (Zhu et al., 2014). Milk vetch (*Astragalus sinicus* L.) is widely used in south China as winter green manure. Many studies have reported that application of milk vetch in paddy soil can provide potential N sources for rice production and improve soil quality (Zhu et al., 2014; Xie et al., 2016; Zhang et al., 2017b; Yang et al., 2019), and can substitute 20%-40% of N fertiliser for the production of next rice season in south China (Xie et al., 2016; Yang et al., 2019).

The main forms of N uptake by rice roots are respectively ammonia in flooded paddy soil and nitrate in upland soil, and the mixed supply of the two N forms improves N uptake and grain yields of rice (Qian et al., 2004). Nitrification is the conversion of ammonium via nitrite to nitrate, and is critical in microbe-regulated N cycling in cropland (Beeckman et al., 2018). The process of nitrification involves three cohorts of microorganisms, that is, ammonia oxidisers, nitrite oxidisers, and complete ammonia oxidisers) (Stein and Klotz, 2016). Ammonia oxidisation, the first and rate-limiting step in nitrification, is catalysed by ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA) (Kowalchuk and Stephen, 2001). The abundances and

* Corresponding authors at: College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China (W. Cao).
E-mail addresses: niejun197@163.com (J. Nie), caoweidongnj@njau.edu.cn (W. Cao).

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