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Green manuring inhibits nitrification in a typical paddy soil by changing the contributions of ammonia-oxidizing archaea and bacteria

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ABSTRACT

Rice–rice–green manure rotations in south China are characterized by high efficiency and good environmental performance, and the application of green manure plays an important role in N management. Nitrification is a key process in N cycling and is highly correlated with the N utilization of crops and with leaching losses. As a potential N loss pathway, the nitrification process and nitrifiers as affected by green manuring are of critical importance. A pot experiment covering green manure-double rice rotation was conducted to evaluate the effects of green manure and N fertilizer on soil nitrification and to achieve a mechanistic understanding of underlying processes in an alkaline paddy soil. Soil nitrification potential (NP) and the recovered nitrification potential (RNP) were measured. Relative contributions of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in soil nitrification were studied using specific bacterial inhibitors. In the alkaline paddy soil, soil NP and nitrate concentrations were significantly decreased by green manuring but increased with increasing N fertilization. Soil nitrification was dominated by AOB and that the relative contributions of AOB to RNP ranged from 65% to 79% at different sampling stages despite the larger abundance of AOA *amoA* gene numbers. RNP and the contribution of AOB to RNP were significantly increased by N fertilizer, whereas the contribution of AOA was decreased by green manuring. We concluded that the application of inorganic N enhanced NP while green manures reduced it, which is consistent with the variation of observed soil nitrate concentrations. These results indicate that the utilization of winter green manure is an effective practice to improve N management in paddy rice.

1. Introduction

Green manuring offers a potentially valuable approach to supporting green agriculture. Utilization of green manures can contribute to high and stable yields (Zhou et al., 2016a), improve soil fertility (Tejada et al., 2008), and benefit soil carbon and nitrogen cycles (León Castro and Whalen, 2019; Yao et al., 2019; Zhang et al., 2019a). Green manuring is also an effective way to reduce chemical N application (Zhu et al., 2014). After being incorporated, green manures can release available N and reduce the dependence on mineral fertilizers for subsequent plants. A previous study reported that the N released from green manure provides approximately 17% of the crop N requirement during the growing season (Gardner and Drinkwater, 2009). In south China, milk vetch is the most traditional green manure in rice cropping system. Many studies have proved that the application of milk vetch in rice cropping systems can substitute for 20%–40% of mineral fertilizer nitrogen and maintain high rice yields (Xie et al., 2016; Xie et al., 2018; Yang et al., 2019a; Zhou et al., 2016a; Zhu et al., 2014). The use of leguminous crops in rice cropping system is currently of interest because of their ability to provide an N input by biological N fixation. The legume green manure milk vetch may have more influence than other green manure varieties on soil N cycling processes, but the mechanisms underlying the effects need further investigation.

Nitrification, a key process in N cycling controlling the transformation between ammonium and the more mobile nitrate, which is regulated by nitrifiers. The conversion of ammonium to nitrite is the first and rate-limiting step in nitrification (Stein and Klotz, 2016). Although complete nitrification by *Nitrospira* bacteria has been reported (Daims et al., 2015), most studies still focus on ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Carey et al., 2016). In agricultural soils, the abundances and communities of AOA and AOB respond differently because of varied soil properties and

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