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Effects of intercropping and Rhizobial inoculation on the ammonia-oxidizing microorganisms in rhizospheres of maize and faba bean plants

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ABSTRACT

To clarify whether intercropping and the *Rhizobium leguminosarum* inoculation affected the nitrification and subsequent nitrate leaching in soil, quantitative and qualitative analyses of total bacteria (TB), total archaea (TA), ammonia-oxidizing bacteria (AOB) and archaea (AOA) in the rhizospheres of maize and faba bean were performed. The greater abundances of TB than TA and of AOA than AOB were observed in all the samples, with ratios of TB:TA 21.1 and 8.0; and AOA:AOB 24.5 and 2.3, at the anthesis and pod-bearing stages, respectively. In faba bean rhizosphere, the intercropping and/or inoculation of rhizobia decreased the abundances of TB, TA, AOB and AOA at the anthesis stage; and inoculation of rhizobia alone decreased AOA at the pod-bearing stage. In maize rhizosphere, intercropping alone only enhanced the AOA at the pod bearing stage; while the combination of intercropping and rhizobial inoculation decreased the TA at the anthesis stage, and enhanced TB, AOB and AOA at the pod-bearing stage. The diversity of AOB and AOA were pronounced by intercropping and rhizobial inoculation treatments: at the anthesis stage for AOB and at the pod-bearing stage for AOA. However, *Nitrosospira* always remained as the predominant AOB in all the treatments. Conclusively, intercropping and rhizobial inoculation brought various shifts in microbial abundance and community compositions in rhizosphere depending on the plants and growth stages, which may decrease the nitrification in rhizosphere. Furthermore, the combination of intercropping and rhizobial inoculation presented stronger effects in most cases than the separated treatments.

1. Introduction

Intercropping, or the simultaneous cultivation of leguminous plants and cereal plants in the same field, with or without inoculation of the legume by rhizobia, is a world-wide agricultural practice. Its potential to increase the grain yields of plants has been clearly shown elsewhere, and this efficiency has been attributed to the enhanced utilization of space, time, light (Muoneke and Mbah, 2007 Zhang et al., 2008) and water (Jahansooz et al., 2007; Xu et al., 2008), an increased plant nutrient supply resulting from the stimulation of biological nitrogen fixation in legume–rhizobia symbiosis (Inal et al., 2007; Zhang and Li, 2003; Zhang et al., 2004), and the improved mobilization (Khan et al., 1997) and uptake (Li et al., 2003, 2007; Wang et al., 2007) of phosphorus (P). These benefits may result from the modifications of the microbial interactions and activities in the rhizospheres of intercropped plants (Song et al., 2007; Wang et al., 2007).

Furthermore, intercropping has also been shown to reduce nitrate leaching without loss of plant yield (Whitmore and Schröder, 2007). Whitmore and Schröder (2007) discussed possible mechanisms for the decrease in nitrate leaching, and they concluded that the levels of denitrification, or loss of nitrogen, were similar for both intercropping and monocropping systems, but the intercropping increased plants' uptake efficiency of nitrate.

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Huang et al. (2011) and Li et al. (2005) found that the intercropping cereals with legumes could reduce nitrate accumulation in the soil, but the ammonia accumulation was not affected, which might reduce the nitrate leaching. However, these studies did not assess the nitrification potential rate and related microorganisms, which might be the reason for the reduced nitrate leaching or accumulation.

Although some nitrate salts are used as fertilizers, nitrates in the soil mainly come from the nitrification process. The oxidation of ammonia to nitrite and of nitrite to nitrate is performed by prokaryotes. The oxidation of ammonia to nitrite by the ammonia-oxidizing bacteria (AOB) and archaea (AOA) is the rate-limiting step in this process (Francis et al., 2007; Shen et al., 2008). The nitrification potential of soil is positively related to the numbers of AOB and AOA (Sims et al., 2012; Taylor et al., 2012), but the relative abundance and contribution of each group varies according to the soil conditions, the plant species and its physiological status, like pH and contents of ammonia (Alvey et al., 2003; Chen et al., 2008; Prosser and Nicol, 2012; Rooney and Clipson, 2008). In some soils, AOA were the dominant ammonia-oxidizers (Leininger et al., 2006), and they were more abundant in the rhizosphere than in the bulk soil (Chen et al., 2008). However, little is known about the persistent effects of intercropping and rhizobial inoculation on AOB and AOA and their relationship to the nitrogen cycle. Sun et al. (2009) revealed that the relative abundance of *Nitrosomonas* and *Nitrosospira* (AOB) were modified in intercropped alfalfa-Siberian wild rye with rhizobial inoculation, but no AOA were analyzed in that study.

Considering that most of the previous studies were focused on the effects of intercropping or rhizobial inoculation at growth stage, we performed the present study to clarify whether intercropping and rhizobial inoculation and intercropping–inoculation affected the nitrification and subsequent nitrate leaching of soil. Quantitative and qualitative analyses of total bacteria (TB), total archaea (TA), AOA and AOB in the rhizosphere soils of maize and faba bean plants at two different growth stages were performed. The changes in the microbial community structure were monitored by terminal restriction fragment length polymorphism (T-RFLP) analysis, and the relative abundance of TB, TA, AOB and AOA was quantified using real-time quantitative PCR (qPCR).

2. Materials and methods

2.1. Experimental design

In this study, the treatments were (1) monoculture of maize (sample M), (2) monoculture of faba beans (sample F), (3) monoculture of faba beans inoculated with rhizobia (sample RF), (4) maize–faba bean intercropping (samples IM for maize and IF for faba beans), (5) maize–faba bean intercropping–inoculation of rhizobia on faba beans (samples IRM for maize and IRF for faba bean). Fifteen pots of 30 cm × 30 cm × 40 cm for each treatment were included. Each pot was filled with 30 kg of air-dried and sieved (2 mm sieve) sandy loam soil collected from 0 to 20 cm depth at Shangzhuang Experimental Station of China Agricultural University, Beijing, China. The physical and chemical characteristics of the soil were as follows: pH 8.03; organic matter, 0.92 g kg^{-1}; available nitrogen, 22.42 mg kg^{-1}; available phosphorous, 6.94 mg kg^{-1}; available potassium, 59.88 mg kg^{-1}. The contents are extremely low for organic matter and available N; and moderately rich for available P and K according to the fertility grading criteria for Chinese soil (http://www.top17.net/news_info/1826.html). According to Li et al. (2007), basal nutrients in solution were added to soil at the following rates (kg ha^{-1}): K_2SO_4, 200; $MgSO_4{\cdot}7H_2O$, 100; $MnSO_4{\cdot}H_2O$, 10; $ZnSO_4{\cdot}7H_2O$, 10; $CuSO_4{\cdot}5H_2O$, 10; H_3BO_3, 1.4; $Na_2MoO_4{\cdot}2H_2O$, 10; $FeSO_4{\cdot}7H_2O$, 10; $CoSO_4{\cdot}7H_2O$, 1.2. These data were converted from the doses in micrograms per kilograms soil, considering 2×10^6 kg soil ha^{-1} in the depth of 0–15 cm. No nitrogen fertilizer was supplied in order to avoid its effects on the nodulation of faba beans. The phosphorous fertilizer was not used since available P could strengthen the interaction between the intercropped maize and faba bean plants by improving the P uptake of maize (Li et al., 2007).

Plants were grown under sunlight in the greenhouse with natural relative humidity and temperature controlled between 25 °C and 18 °C in the day/night cycle (12 h/12 h), during March–August 2008. After pre-germination, two seeds of faba bean (cultivar Lincan 5) and two of maize (cultivar Zhongnong 4), which were obtained from Beijing Cau Grand Technology Development Co., Ltd. and Professor Long Li (College of Resources and Environmental Sciences, China Agricultural University, Beijing), were sown in each pot for intercropping, and four seeds of maize or faba beans were sown in each pot for monoculture assays, with a 20 cm distance from each other. Some of the faba bean seeds were inoculated in the corresponding treatments, as described in the subsequent part, and the pot was watered with the nutrient solution (see above) immediately after sowing. Similar to the field production, the faba beans were sown at the end of March, and the maize was sown in mid-April. After the seeds sprouted (about one week after sowing), one maize and one faba bean were maintained in intercropping, and two individuals of maize or faba bean were maintained in monoculture experiments by eliminating the excess ones. All pots were irrigated to field capacity twice a week, by adding deionized water until the excess water was drained from the bottom of the pot.

2.2. Preparation of rhizobial inoculant

The strain used for inoculation was *Rhizobium leguminosarum* bv. viciae CCBAU 1253, an effective symbiotic bacterium for faba bean (Xiao et al., 2004), was gifted by W. F. Chen (*Rhizobium* Research Center, China Agricultural University, Beijing). To prepare the inoculant, liquid yeast mannitol medium (Vincent, 1970) was inoculated with a fresh culture of CCBAU 1253 at a ratio of 1% (v/v) and then incubated at 28 °C for 72 h with shaking (150 rpm). The cell numbers of the culture were estimated microscopically with a Neubaur Chamber and was justified with fresh medium at a concentration of 5×10^6 cells mL^{-1}. An aliquot of 2 mL of the justified culture was applied to each pre-germinated faba bean seed in the pot.

2.3. Sampling of the rhizosphere soils

The maize and faba bean rhizosphere soils were sampled on May 25 and August 27 of 2008, corresponding to the anthesis and pod-bearing stages of faba beans (the jointing and bell stages for maize), respectively. For each treatment, 12 plants for each species were excavated carefully, which were divided into three groups (containing four plants in each) as three replicates. After shaking off the loosely adhering soils, the tightly adhering soil (rhizosphere soil) was collected from the four plants to give a pooled rhizosphere sample, which was immediately stored at 4 °C. The rhizosphere soil was passed through a 2 mm sieve and stored at −20 °C until it was analyzed.

2.4. T-RFLP analysis for DNA extraction from rhizosphere soil

The metagenomic DNA was extracted from 0.5 g of each soil sample using the Fast DNA spin sample kit (for soil; Bio 101, Lajolla, CA), following the manufacturer's protocol. The quality of the extracted DNA was determined using a Qubit fluorometer (Invitrogen, USA). Bacteria and archaea 16S rRNA and *amoA* (ammonia