Applied Soil Ecology 95 (2015) 90–98

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Composition of soil microbial communities in the rhizosphere of cucumber cultivars with differing nitrogen acquisition efficiency

Xingang Zhou[a,b], Songna Guan[a,c], Fengzhi Wu[a,*]

[a] Department of Horticulture, Northeast Agricultural University, Harbin 150030, PR China
[b] Postdoctoral Station of Biology, Northeast Agricultural University, Harbin 150030, PR China
[c] Agriculture Technology Popularizing Center of Tangyuan, Tangyuan 154700, PR China

ARTICLE INFO

Article history:
Received 26 February 2015
Received in revised form 15 June 2015
Accepted 19 June 2015
Available online 2 July 2015

Keywords:
Cucumis sativus L.
Nitrogen acquisition efficiency
Nitrogen cycling
Rhizosphere microbial community

ABSTRACT

Crop cultivars with high nitrogen acquisition efficiency (NAE) in agriculture can alleviate the problem of excessive use of nitrogen fertilizer. Soil microbial communities play an important role in nutrient cycling. However, the relationship between rhizosphere microbial communities and plant NAE is still not clear. In this experiment, cucumber cultivars with high NAE (Jinyou-2 and Jinyou-31) and low NAE (Jinlv-30 and Meihao) were grown in pots under limiting and not limiting N supply. The composition of rhizosphere microbial communities was analyzed with the most probable number, real-time PCR and PCR-denaturing gradient gel electrophoresis (DGGE). Generally, N supply rate increased the abundance of rhizosphere ammonia-oxidizing bacteria (AOB), and changed the composition of bacterial, fungal and AOB communities. The abundances of rhizosphere ammonifiers were higher in cucumber cultivars with high NAE than in cultivars with low NAE under limiting N supply. The composition of rhizosphere AOB community, rather than the bacterial and fungal communities, was more similar between cultivars with the same NAE than between cultivars with contrasting NAE. When subjected to the same N fertilization rate, no differences in rhizosphere microbial biomass N and C, abundances of bacteria, fungi and AOB were found between cultivars with low and high NAE. Overall, our results suggest that the abundance of rhizosphere ammonifiers and the composition of AOB community may be linked to the NAE of cucumber.

1. Introduction

The phytoavailability of nitrogen (N) is generally considered one of the major limiting elements in both marine and terrestrial ecosystems (Canfield et al., 2010). Fertilization is an essential practice to optimize crop production in modern agriculture. However, low N use efficiency was widely observed and over 50% of the applied N can be lost from agricultural systems (Adesemoye and Kloepper, 2009; Ju et al., 2006; Tilman et al., 2002). High N fertilization rate with decreasing plant N use efficiency have contributed to severe environmental problems, such as eutrophication and global warming (Bingham et al., 2012; de Vries and Bardgett, 2012). Therefore, it is generally accepted that crop N efficiency must be improved to maintain productivity but reduce inputs of N fertilizer (Bingham et al., 2012; Tilman et al., 2002).

Use of crop cultivars with high N efficiency, as determined by the N use efficiency (NUE) and N acquisition efficiency (NAE), is one of the most effective practices to decrease N use in agriculture (Lynch, 2007; Tilman et al., 2002). Some promising advances have recently been achieved in several field crops, such as barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and oilseed rape (*Brassica napus* L.) (Hirel et al., 2007; White et al., 2013). However, the underlying mechanisms of N efficiency differences among crop cultivars are largely unknown.

Soil microorganisms play an essential role in terrestrial ecosystem functioning and services (Mendes et al., 2013; Philippot et al., 2013). Important N biogeochemical processes, including N fixation, ammonification, nitrification and denitrification, are primarily mediated by microorganisms (Canfield et al., 2010; White et al., 2013). In the rhizosphere, plant and soil microorganisms dynamically interact with each other with profound effects on plant nutrient acquisition and growth (Philippot et al., 2013). On one hand, rhizosphere microorganisms can affect plant nutrition either directly, by influencing nutrient availability and uptake, or indirectly through plant growth promotion (Dodd and

* Corresponding author at: Department of Horticulture, Northeast Agricultural University, Mucai 59, Xiangfang, Harbin 150030, PR China. Fax: +86 451 55190443.
E-mail address: fzwuneau@yahoo.com (F. Wu).

http://dx.doi.org/10.1016/j.apsoil.2015.06.014
0929-1393/

Ruiz-Lozano, 2012; Yang et al., 2009). On the other hand, plants can affect the activity, diversity and abundance of soil microorganisms through releasing diverse organic compounds (i.e., rhizodeposition) (Mark et al., 2005; Micallef et al., 2009).

Empirical studies showed that there are cultivar-specific selection of rhizosphere communities (Lundberg et al., 2012; Micallef et al., 2009), and rhizosphere microbial N processes can differ among different cultivars of rice (Briones et al., 2003; Li et al., 2008), barley (Glaser et al., 2010) and potato (*Solanum tuberosum* L.) (Dias et al., 2012). However, our knowledge of the physical and ecological significance of these differences in rhizosphere microbial communities is limited. Plants can modulate the composition and activity of the rhizosphere microbial communities to have beneficial effects, such as enhancing plant health or nutrient acquisition (Chaparro et al., 2014; Zhang et al., 2009). Therefore, it could be speculated that the differences in the composition of rhizosphere microbial communities may play some role in the rhizosphere N processes including plant N uptake.

The objective of this study was to elucidate the differences in rhizosphere microbial communities between plant cultivars with high and low NAE, and evaluate whether rhizosphere microbial community composition can be linked to plant NAE. We selected cucumber (*Cucumis sativus* L.) as the model plant species, which is commonly cultivated in the greenhouse and requires high rate of N supply. The pot experiment was performed with limiting and non-limiting N supply under greenhouse conditions. Our previous greenhouse studies showed that cucumber cultivars, Jinyou-2 and Jinyou-31, produced more aboveground dry biomass (77.3 and 84.5 g plant^{-1}, respectively) and assimilated more N (2.03 and 2.08 g N plant^{-1}, respectively) than the other two cultivars, Jinlv-30 and Meihao (biomass, 25.4 and 24.6 g plant^{-1}; assimilated N, 0.43 and 0.47 g N plant^{-1}, respectively), under limiting N supply; while the differences in plant biomass and N acquisition were much smaller under non-limiting N supply (Jiang et al., 2012). These indicated that Jinyou-2 and Jinyou-31 can acquire N from the soil more efficiently than Jinlv-30 and Meihao under limiting N supply. Since, there are inherent differences in rhizosphere soil microbial communities among plant cultivars (Lundberg et al., 2012; Micallef et al., 2009), we hypothesized that community structures of rhizosphere microorganisms involved N cycling, rather than total bacterial and fungal communities, are related to cucumber NAE. Specially, we also hypothesized that cucumber cultivars with high NAE under limiting N supply are characterized with higher number of rhizosphere ammonifiers, which can facilitate cucumber with NH_4^+ by mineralizing organic N, but lower number of ammonia-oxidizing bacteria (AOB), which can compete N with cucumber.

2. Materials and methods

2.1. Plant material and soil basic properties

Four cucumber cultivars differing in NAE were used in this experiment (Guan et al., 2013). Cultivar Jinyou-2 and Jinyou-31 with high NAE were provided by Tianjin kernel cucumber research institute; Jinlv-30 and Meihao with low NAE were provided by Tianjin Lvfeng Horticultural High-tech Company and Jinzhou Youerte High-tech Agricultural Company, respectively.

Sandy loam soil was collected from the upper soil layer (0–20 cm) of a greenhouse in the experimental station of Northeast Agricultural University, Harbin, China (45°41′N, 126°37′E). The soil contained organic matter, 21.7 g kg^{-1}; inorganic N, 56 mg kg^{-1}; available P, 38 mg kg^{-1}; available K, 141 mg kg^{-1}; EC (1:2.5, w/v), 0.59 mS cm^{-1}; and pH (1:2.5, w/v), 7.4. After sampling, soils were thoroughly mixed and sieved (4 mm) prior to use.

2.2. Greenhouse experiment

The pot experiment was performed with two N fertilization rates, limiting (0 g kg^{-1}) and non-limiting (0.72 g kg^{-1}) N supply in the form of urea. All treatments received the same amount of P and K fertilizers at the rate of 0.36 g kg^{-1} and 1.44 g kg^{-1}, respectively, as monopotassium phosphate and potassium sulfate. One thirds of the N fertilizer and all P and K fertilizers were applied as basal fertilizer; and the other two thirds of the N fertilizer were applied as top dressing on 30 and 50 d after cucumber transplanting, respectively.

Cucumber seeds were sterilized with 2.5% NaClO and germinated in perlite in the dark at 28 °C. After emergence, seedlings with two cotyledons were planted in plastic pots (diameter 6 cm and height 8 cm) containing 100 g soil, and grown in a greenhouse (32 °C day/22 °C night, relative humidity of 60–80%, 16 h light/8 h dark). Four weeks later, seedlings with three leaves were transplanted into the plastic pots (diameter 25 cm and height 30 cm) containing 12.5 kg soil. There was one cucumber plant per pot. Cucumber plants were watered with tap water every day to keep the soil water content at about 60% of its water-holding capacity, and grown in the greenhouse as described above. Weeds were manually removed. The experiment was done in triplicate and there were 10 pots for each cultivar at each N fertilization rate.

2.3. Rhizosphere soil sampling

Rhizosphere soil samples were collected at 20, 40 and 60 transplanting (DAT), respectively, as described before (Zhou and Wu, 2012). Samples from three plants in each treatment were mixed to make a composite sample. Part of these fresh sampled soils was used to measure rhizosphere soil microbial biomass and culturable microbial population; the other part was stored at −70 °C for DNA extraction.

2.4. Rhizosphere soil microbial biomass estimation

Rhizosphere soil microbial biomass C (MBC) and N (MBN) were determined by the chloroform fumigation extraction method (Vance et al., 1987). MBC and MBN were calculated from the differences between total extractable C and N in the fumigated and unfumigated samples using efficiency factors (Kec and Ken) of 0.38 and 0.45, respectively (Brookes et al., 1985; Vance et al., 1987).

2.5. Culturable microbial populations

The number of soil bacteria, fungi and ammonifiers were estimated by the most probable number (MPN) method with selective medium (Rowe et al., 1977). Agar plates with beef extract-peptone medium and Martin's Rose Bengal agar medium were used for growing bacteria and fungi, respectively. Soil ammonifiers were evaluated in Winogradsky's saline solution with L-asparagine as the only N and C source (Kidd et al., 2008). CFU were counted and the population per gram of soil (CFU g^{-1}) was calculated.

2.6. DNA extraction and real-time PCR

Total soil DNA was extracted with the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA) according to the manufacturer's instructions.

Rhizosphere soil AOB community size was estimated by real-time PCR assays in an IQ5 real-time PCR system (Bio-Rad Lab, LA, USA). The gene encoding ammonia monooxygenase catalytic subunit A (*amoA*) was amplified using the primer set of *amoA*1F/*amoA*2R (Rotthauwe et al., 1997) according to the methods described by Glaser et al. (2010). All amplifications were