Journal of Microbiology (2015) Vol. 53, No. 7, pp. 454–461
DOI 10.1007/s12275-015-5108-2

Arbuscular mycorrhizal fungal diversity, root colonization, and soil alkaline phosphatase activity in response to maize-wheat rotation and no-tillage in North China

Junli Hu[1,3], Anna Yang[2], Anning Zhu[1], Junhua Wang[1,3], Jue Dai[1,3], Ming Hung Wong[3,4,5], and Xiangui Lin[1,3]*

[1]*State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, East Beijing Road 71, Nanjing 210008, P. R. China*
[2]*Provincial Key Laboratory of Biotic Environment and Ecological Security in Anhui, College of Life Sciences, Anhui Normal University, East Beijing Road 1, Wuhu 241000, P. R. China*
[3]*Joint Open Laboratory of Soil and the Environment, Hong Kong Baptist University and Institute of Soil Science, Chinese Academy of Sciences, East Beijing Road 71, Nanjing 210008, P. R. China*
[4]*Croucher Institute for Environmental Sciences, Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, P. R. China*
[5]*Consortium on Health, Environment, Education and Research, Department of Science and Environmental Studies, The Hong Kong Institute of Education, Tai Po, Hong Kong SAR, P. R. China*

(Received Mar 2, 2015 / Revised Jun 2, 2015 / Accepted Jun 9, 2015)

Monitoring the effects of no-tillage (NT) in comparison with conventional tillage (CT) on soil microbes could improve our understanding of soil biochemical processes and thus help us to develop sound management strategies. The objective of this study was to compare the species composition and ecological function of soil arbuscular mycorrhizal (AM) fungi during the growth and rotation of crops under NT and CT. From late June 2009 to early June 2010, 32 topsoil (0–15 cm) samples from four individual plots per treatment (CT and NT) were collected at both the jointing and maturation stages of maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) from a long-term experimental field that was established in an Aquic Inceptisol in North China in June 2006. The AM fungal spores were isolated and identified and then used to calculate species diversity indices, including the Shannon-Wiener index (H'), Evenness (E), and Simpson's index (D). The root mycorrhizal colonization and soil alkaline phosphatase activity were also determined. A total of 34 species of AM fungi within nine genera were recorded. Compared with NT, CT negatively affected the soil AM fungal community at the maize sowing stage, leading to decreases in the average diversity indices (from 2.12, 0.79, and 0.82 to 1.79, 0.72, and 0.74 for H', E, and D, respectively), root mycorrhizal colonization (from 28% to 20%), soil alkaline phosphatase activity (from 0.24 to 0.19 mg/g/24 h) and available phosphorus concentration (from 17.4 to 10.5 mg/kg) at the maize jointing stage. However, reductions in diversity indices of H', E, and D were restored to 2.20, 0.81, and 0.84, respectively, at the maize maturation stage. CT should affect the community again at the wheat sowing stage; however, a similar restoration in the species diversity of AM fungi was completed before the wheat jointing stage, and the highest Jaccard index (0.800) for similarity in the species composition of soil AM fungi between CT and NT was recorded at the wheat maturation stage. Our results also demonstrated that NT resulted in the positive protection of the community structure of AM fungi and played an important role in maintaining their functionality especially for maize seedlings.

Keywords: functionality, jointing stage, maturation stage, restoration, species richness, spore density

Introduction

The North China Plain is often referred to as "the bread basket of China". The sustainable use of agricultural soil in this region may affect China's food security. However, phosphorus (P) is one of the major essential nutrients that limit plant growth owing to its low availability in soils, and the soils in this area are extremely P-deficient. Although applying P fertilizer is one of the most effective ways to increase crop yields, long-term fertilizer applications increase the risk of P accumulation in soils. Therefore, improving P availability is an alternative for managing low P soils and for enhancing the efficiency of P fertilizers (Zhu *et al.*, 2003). Arbuscular mycorrhizal (AM) fungi are ubiquitous mutualists that are found in both natural and agricultural ecosystems, and they provide a direct link between soil and roots. They are also renowned for their ability to increase plant nutrient acquisition, notably P acquisition (Smith and Read, 2008). It has been widely reported that mycorrhizal colonization improves the growth and/or P-acquisition of host plants. The enhancement of soil phosphatase activity is one of the major mechanisms involved in the "mycorrhization effect" on plant nutrition, because mycorrhizal roots may release more exudates containing enzymes owing to the larger root system and/or improved nutrition. AM fungi also have beneficial influences on agro-ecosystem functions (Cameron, 2010), such as increases in plant resistance to soil pathogens and foliar-feeding insects as well as tolerance to drought, salinity and pollutants. A higher spore density and diversity of AM fungi are suggested to be advantageous for improving

*For correspondence. E-mail: xglin@issas.ac.cn; Tel.: +86-25-86881589; Fax: +86-25-86881000

root colonization and subsequent crop growth. However, soil AM fungi are sensitive to changes in land-use patterns and management regimes (Martinez and Johnson, 2010; Oehl *et al.*, 2010). Thus, the species composition and root colonization of AM fungi are often measured to provide immediate and accurate information about small changes in soils (Gosling *et al.*, 2006; Hu *et al.*, 2009; Sun *et al.*, 2013).

To reduce costs and environmental changes through reductions in both soil erosion and the use of diesel fuel, modern agriculture has greatly reduced the use of tillage, which is a common practice in conventional agriculture. No-tillage (NT), which is a category of conservation tillage that causes the least soil disturbance and consequently has beneficial impacts on crop productivity, seems to be superior to tillage for increasing carbon (C) deposits in the soil (Alvear *et al.*, 2005), and for conserving soil and water (Blanco-Canqui and Lal, 2008). However, NT may also affect soil microbial characteristics, including the species composition (Jansa *et al.*, 2003) and root colonization (Mirás-Avalos *et al.*, 2011) of AM fungi. A filament network of AM fungi that was left intact from the previous season can readily serve as AM inoculum and colonize the roots of post-harvest seedlings (Castillo *et al.*, 2006). This inoculum promotes nutrient uptake by crops through enhanced root mycorrhizal colonization and related enzyme activities, such as phosphatase activity, in the soils. However, long-term NT farming may also result in problems such as the surface hardening of soil and more limited O_2 supplies for soil organisms (Álvaro-Fuentes *et al.*, 2008), which may be unfavorable for the distribution of AM fungi propagules. For example, Curaqueo *et al.* (2011) observed degradation in the soil AM fungal community after long-term NT management in central Chile. This variance could in turn influence the growth of cultivated crops. However, uncertainties still remain about NT influences on the diversity and functionality of soil AM fungi in many other regions around the world. Consequently, more field experiments that address different soil types and crop species are urgently needed to improve our knowledge of changes in AM fungi under various tillage regimes, and investigating their seasonal dynamics may help to ensure the feasibility of NT farming in arable soils.

There are concerns in China about the degradation of soil quality from the replacement of conventional tillage (CT) with conservation tillage, and thus a number of long-term field experiments have been set up in agricultural regions to monitor changes in soil fertility and quality (He *et al.*, 2011). The soil quality reportedly tended to increase in response to 6-year NT farming in a Haplic Cambisol in the North China Plain, and the crop yields were not significantly affected by different tillage regimes (Qin *et al.*, 2010). Therefore, there is an urgent need to investigate the influences of continuous NT on the community composition and ecological function of soil AM fungi. It was hypothesized that CT could lead to decreases in soil AM fungal diversity and functionality relative to NT, and the subsequent restoration might start at the sowing stage and end at different growing stages with differential crop species. In this study, one long-term field experiment, which was set up in a sandy loam soil in the North China Plain, was used to monitor changes in the AM fungal community. The aims of this study were to compare the species composition and root colonization of AM fungi at both the jointing and maturation stages of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) during a rotation cycle that took place within one year, and to evaluate the effects of NT and CT on AM fungal propagules and related enzyme activities. This work could contribute to the development of management schemes for soil AM fungi based on specific functions.

Materials and Methods

Description of the experimental site and soil sampling

A long-term experiment for testing the NT regime was conducted in a well-drained field in which wheat and maize are cultivated during the winter and summer, respectively. This site is part of the State Experimental Station for Agro-Ecology in Fengqiu county (35°00′N, 114°24′E), Henan province, China. This area has a temperate monsoon climate with a mean annual temperature of 13.9°C and a mean annual precipitation of 615 mm. The lowest and highest mean monthly values are -1.0°C in January and 27.2°C in July, respectively, and two-thirds of the precipitation falls from June-September. The soil has a sandy loam texture (containing 90 and 218 g/kg of clay and silt, respectively) in the plow layer and loam in the subsoil, and it was derived from alluvial sediments of the Yellow River and classified as an Aquic Inceptisol (IUSS, 2006). Maize was directly sown in early/mid June and harvested in late September. Wheat was then directly sown in early/middle October and harvested in early June of the next year. Fertilizers were applied in the form of urea, super phosphate, and potassium (K) sulfate at medium levels, for 300, 60, and 150 kg of nitrogen (N), P, and K ha^{-1} per year, respectively. In June 2006, two tillage treatments (CT and NT) were randomly arranged with four replicates (each plot was 14 × 6.5 m) in a common field with a long cultivation history under maize-wheat rotation. The soil contained ca. 5.6, 0.6, 0.8, and 20 g/kg of organic C, total N, P, and K, and a pH of 8.5 at the beginning of the experiment. The field was surrounded with a 1.5 m-wide buffer zone, and the space between adjacent plots was 20 cm. The CT plots were plowed with a moldboard to a depth of 20 cm. The soil was later disked twice with a disk harrow before sowing the seeds. The NT plots were managed similarly to the CT plots, except for the tillage, which continued to be NT with a NT planter for seed sowing. At harvest time, the above-ground biomass was removed along with the grain except for the crop stubble. Each plot was treated with the same tillage regime every year starting during the maize season in 2006. On 27 June and 20 September 2009, and 31 March and 8 June 2010, i.e., at the jointing and maturation stages of wheat and maize, soil samples were collected from 16 points at a depth of 0–15 cm from each plot and then mixed (at approx. 200 g each) and homogenized with a 2-mm mesh sieve to remove above-ground plant materials and stones. Soil samples from the maturation stages were collected just before maize and wheat harvest. Tiny maize or wheat roots that were left in the soil samples were collected for mycorrhizal colonization assessment. Soil samples were divided into two subsamples, with one that was stored at 4°C for the enzyme activity determi-

nation, and the other, which was air-dried for about two weeks and then stored at 4°C for chemical analysis and spore extraction.

Root mycorrhizal colonization and soil chemical/enzymatic properties analysis

The fresh roots that were collected from soil samples were cleared with 10% potassium hydroxide and stained with acid fuchsin (Phillips and Hayman, 1970). The root mycorrhizal colonization was then assessed with a light microscope by grid-line intersect method (Giovannetti and Mosse, 1980). The soil total P was digested with hydrofluoric and perchloric acids (Jackson, 1958), and the soil available P was extracted with sodium bicarbonate (Olsen *et al.*, 1954), and both were

Table 1. Spore density (per 20 g air-dried soil), species composition, and relative abundance (%) of soil AM fungi in conventional tillage (CT) and no tillage (NT) treatments at jointing and maturation stages of maize and wheat

Sampling date/stage	27 Jun (maize jointing)		20 Sep (maize maturation)		31 Mar (wheat jointing)		8 Jun (wheat maturation)	
Treatment	CT	NT	CT	NT	CT	NT	CT	NT
Spore density	411 ± 137 a	505 ± 178 a	356 ± 258 a	421 ± 156 a	434 ± 93 a	594 ± 165 a	563 ± 65 a	566 ± 87 a
Acaulospora (11 species)								
A. bireticulata	1.7 ± 2.3 a	1.8 ± 2.6 a	3.2 ± 2.3 a	1.6 ± 2.3 a	1.5 ± 1.1 a	4.6 ± 2.9 a	2.8 ± 3.2 a	2.3 ± 1.8 a
A. cavernata	0.0 ± 0.0 b	0.7 ± 1.4 ab	2.0 ± 2.4 a	0.0 ± 0.0 b	0.0 ± 0.0 b	0.9 ± 1.0 ab	1.2 ± 1.6 ab	0.6 ± 0.7 ab
A. denticulata	47.3 ± 9.4 a	38.4 ± 8.7 ab	35.2 ± 3.4 b	36.1 ± 3.8 ab	37.1 ± 5.3 ab	32.7 ± 6.9 b	29.4 ± 10.1 b	27.7 ± 6.0 b
A. elegans	0.5 ± 0.9 b	0.8 ± 0.9 ab	1.8 ± 2.3 ab	1.6 ± 1.9 ab	3.0 ± 2.5 ab	1.7 ± 3.4 ab	2.0 ± 1.6 ab	5.4 ± 5.7 a
A. foveata	0.0 ± 0.0 a	1.0 ± 2.1 a	0.0 ± 0.0 a	0.7 ± 1.4 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.8 ± 1.7 a	0.6 ± 1.2 a
A. lacunosa	4.3 ± 2.0 a	0.0 ± 0.0 c	2.2 ± 0.9 b	2.5 ± 1.4 b	5.0 ± 1.0 ab	1.2 ± 0.9 bc	0.8 ± 0.9 bc	2.0 ± 1.5 b
A. laevis	0.0 ± 0.0 a	0.0 ± 0.0 a	1.0 ± 1.2 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
A. mellea	0.5 ± 1.0 bc	1.0 ± 2.1 bc	0.0 ± 0.0 c	0.3 ± 0.6 bc	0.8 ± 1.5 bc	0.0 ± 0.0 c	4.9 ± 2.4 a	2.7 ± 2.6 ab
A. rehmii	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.3 ± 0.7 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
A. spinosa	1.7 ± 2.7 a	2.9 ± 1.2 a	2.6 ± 1.9 a	2.0 ± 2.5 a	1.4 ± 1.9 a	1.0 ± 2.0 a	3.9 ± 4.5 a	1.0 ± 1.2 a
A. tuberculata	3.8 ± 2.9 ab	2.0 ± 2.2 ab	6.8 ± 1.8 a	6.0 ±2.6 ab	4.5 ±3.8 ab	2.6 ± 4.2 ab	1.5 ± 2.1 b	4.8 ± 3.8 ab
Claroideoglomus (2 species)								
C. claroideum	1.0 ± 2.0 a	2.7 ± 2.8 a	3.7 ± 2.2 a	1.2 ± 2.4 a	3.5 ± 2.1 a	2.2 ± 1.9 a	1.2 ± 1.7 a	2.7 ± 1.5 a
C. etunicatum	4.9 ± 4.2 ab	5.2 ± 1.8 ab	0.0 ± 0.0 b	4.7 ± 5.4 ab	8.2 ± 1.8 ab	6.9 ± 5.2 ab	2.7 ± 3.1 ab	4.6 ± 2.4 ab
Diversispora (1 species)								
D. spurca	0.0 ± 0.0 a	0.0 ± 0.0 a	0.3 ± 0.6 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.5 ± 1.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Entrophospora (1 species)								
E. infrequens	0.0 ± 0.0 a	0.7 ± 1.4 a	0.0 ± 0.0 a	0.3 ± 0.6 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.3 ± 0.6 a
Funneliformis (5 species)								
F. caledonium	1.0 ± 1.1 b	1.2 ± 1.6 b	1.7 ± 1.5 b	1.8 ± 2.1 b	4.6 ± 1.3 a	2.5 ± 2.1 ab	1.2 ± 1.5 b	0.3 ± 0.6 b
F. constrictum	0.4 ± 0.8 c	4.4 ± 1.4 a	4.1 ± 1.1 ab	1.7 ± 2.4 bc	0.0 ± 0.0 c	1.9 ± 1.3 bc	2.3 ± 2.5 abc	0.9 ± 1.2 c
F. geosporum	0.0 ± 0.0 b	0.8 ± 0.9 a	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.3 ± 0.6 ab
F. mossea	3.7 ± 3.4 bc	1.3 ± 1.5 c	10.4 ± 5.5 a	6.0 ± 2.0 abc	2.5 ± 2.0 bc	5.3 ± 3.0 bc	4.8 ± 5.2 bc	7.1 ± 2.3 ab
F. verruculosum	15.2 ± 4.6 a	13.5 ± 7.5 ab	11.2 ± 6.3 abc	16.1 ± 9.2 a	2.9 ± 2.1 c	4.8 ± 3.3 bc	5.3 ± 4.4 bc	2.8 ± 3.5 c
Glomus (5 species)								
G. globiferum	0.0 ± 0.0 a	0.8 ± 1.7 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.4 ± 0.7 a	0.0 ± 0.0 a	0.0 ± 0.0 a
G. monosporum	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	1.1 ± 1.3 a	0.0 ± 0.0 b	0.7 ± 0.9 ab
G. pansihalos	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.9 ± 1.7 a
G. viscosum	0.0 ± 0.0 a	0.0 ± 0.0 a	1.9 ± 2.9 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.4 ± 0.8 a	1.5 ± 3.0 a
Glomus sp. I	0.0 ± 0.0 b	0.8 ± 0.9 ab	0.0 ± 0.0 b	0.4 ± 0.9 b	0.0 ± 0.0 b	1.0 ± 2.0 b	2.7 ± 2.6 a	0.7 ± 0.9 ab
Racocetra (2 species)								
Ra. persica	5.1 ± 3.5 bc	7.4 ± 2.6 abc	2.6 ± 2.5 c	5.4 ± 1.9 bc	5.5 ± 4.1 bc	11.2 ± 5.7 a	12.2 ± 3.1 a	10.5 ± 1.5 ab
Ra. verrucosa	0.7 ± 1.3 bc	4.8 ± 3.5 ab	0.0 ± 0.0 c	3.5 ± 1.7 abc	7.0 ± 3.6 a	5.6 ± 2.7 a	5.4 ± 3.8 a	3.3 ± 3.2 abc
Rhizophagus (2 species)								
Rh. clarus	0.0 ± 0.0 a	0.7 ± 1.4 a	1.1 ± 1.6 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	1.0 ± 1.2 a
Rh. intraradices	0.9 ± 1.9 a	1.0 ± 1.9 a	3.4 ± 4.0 a	1.8 ± 3.5 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.9 ± 1.9 a	0.3 ± 0.6 a
Scutellospora (5 species)								
S. heterogama	0.0 ± 0.0 a	0.0 ± 0.0 a	0.3 ± 0.6 a	0.3 ± 0.6 a	0.8 ± 1.5 a	1.2 ± 1.7 a	0.4 ± 0.9 a	0.3 ± 0.6 a
S. nigra	1.1 ± 1.5 a	0.6 ± 1.3 a	0.6 ± 1.2 a	0.3 ± 0.6 a	0.4 ± 0.8 a	0.0 ± 0.0 a	1.1 ± 2.2 a	0.3 ± 0.6 a
S. reticulata	4.2 ± 2.7 bcd	1.4 ± 2.0 d	1.5 ± 1.9 d	2.5 ± 2.0 cd	3.4 ± 2.1 bcd	6.7 ± 4.4 abc	8.5 ± 4.0 a	7.4 ± 0.8 ab
Scutellospora sp. I	1.2 ± 0.8 a	1.7 ± 2.1 a	0.5 ± 0.6 a	1.7 ± 2.6 a	2.1 ± 3.1 a	1.1 ± 0.8 a	2.3 ± 2.8 a	1.3 ± 1.0 a
Scutellospora sp. II	0.8 ± 1.6 b	2.5 ± 3.3 ab	1.7 ± 2.2 b	1.6 ± 1.9 b	5.6 ± 1.3 a	2.8 ± 3.0 ab	2.3 ± 1.9 ab	5.6 ± 2.9 a

Data are mean values with standard deviation ($n = 4$). Data within a row followed by different letters indicate a significant difference by the Least Significant Difference multiple range test ($P < 0.05$).

determined by molybdenum blue method. The soil alkaline phosphatase activity was determined by incubating at 37°C with borate buffer (pH 9) according to Tabatabai (1982), and the results were presented in units of mg *p*-nitrophenol produced g^{-1} soil 24 h^{-1}. All results were expressed on the basis of the oven-dried soil weight by correcting the water content in the soil (105°C, 24 h).

Recovery and counting of AM fungal spores

The AM fungal spores were extracted from 20 g of soil via wet-sieving method. Spores were collected in 70, 100, 150, and 900 μm sieves, filtered onto a filter paper and placed in a Petri dish for examination under a binocular stereomicroscope. The intact healthy spores were sorted into groups and counted. The spores were mounted in polyvinyl lactic acid and polyvinyl lactic acid mixed 1:1 (v/v) with Meltzer's reagent for species identification under a light microscope (Olympus CX31). The identification was based on morphological descriptions provided by the International Collection of (Vesicular) AM Fungi (http://invam.caf.wvu.edu), newly published species descriptions, and recent advances in *Glomeromycota* taxonomy (Schüßler and Walker, 2010; Oehl *et al.*, 2011; Krüger *et al.*, 2012). If the species had not yet been described, it was marked as *Glomus* sp. I, *Scutellospora* sp. II, etc.

AM fungal community structure and diversity analysis

The community structure of the AM fungi was evaluated on the basis of six selected parameters (Yang *et al.*, 2011). The spore density was calculated from direct counts of AM fungal spores under a binocular stereomicroscope and all the isolated spores were counted, including some spores that lacked distinguishable morphological characteristics. The spore specimens that were identified to species were used to analyze the relative abundance and the species richness of AM fungi. Three diversity indices of AM fungi, including the Shannon-Wiener index (H'), Evenness (E), and Simpson's index (D), were evaluated as sensitive indicators of the relative richness of the species, the species evenness of the community, and the dominance of the most common species in the community, respectively. The Jaccard index of similarity was calculated to compare the species compositions of AM fungi under the two treatments at the crop jointing and maturation stages.

Statistical analyses

An analysis of variance analysis (ANOVA) was performed with SPSS software (version 13.0). The significance of the parameters was tested by the Least Significant Difference multiple range test at $P < 0.05$ after the one-way ANOVA.

Results

Spore density, species composition, and relative abundance of AM fungi

There was no significant difference in the spore densities of AM fungi between NT and CT at all sampling times (Table 1). A total of 34 species of AM fungi within nine genera were recorded, including three previously undescribed species. Based on the relative abundances, the most abundant genus was *Acaulospora* (44.4–59.8%), followed by *Funneliformis* (10.0–27.4%), *Racocetra* (2.6–16.8%), *Scutellospora* (4.6–14.9%), and *Claroideoglomus* (3.7–11.7%); *Rhizophagus* (0–

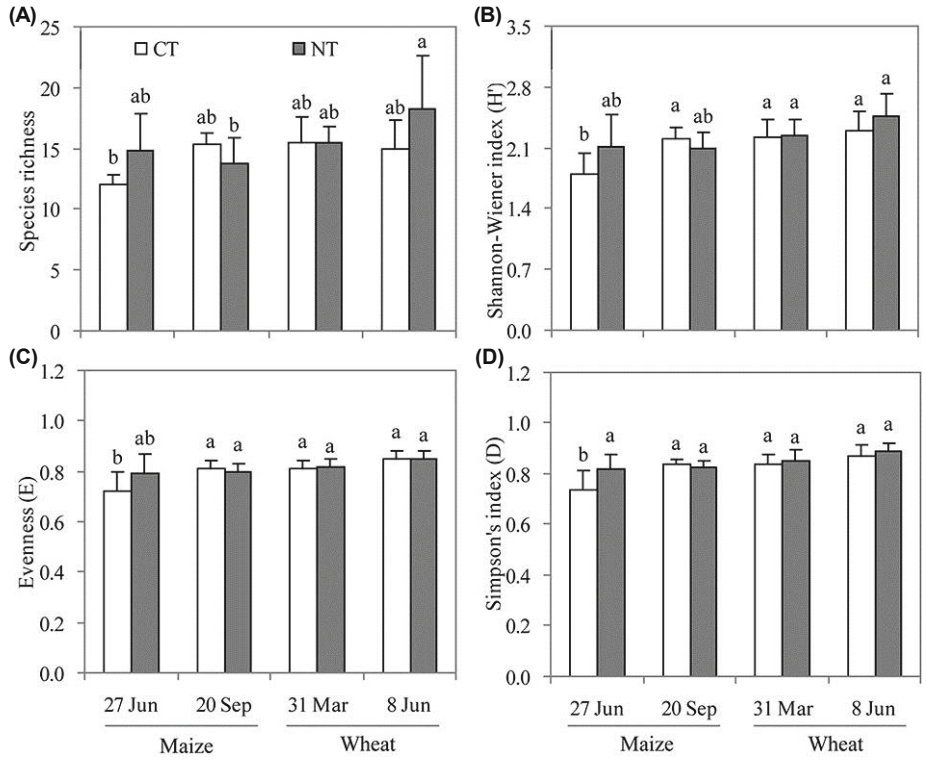

Fig. 1. Soil AM fungal species richness (A) and community diversity indices, including (B) the Shannon-Wiener index (H'), (C) Evenness (E), and (D) Simpson's index (D), under no tillage (NT) and conventional tillage (CT) treatments at the jointing and maturation stages of maize and wheat. Maize and wheat were sown in June and October 2009, respectively. Soil samples from the maturation stages were collected just before the maize and wheat harvest. *Vertical T bars* indicate standard deviations. *Bars* not topped by the same letter indicate a significant difference in values according to the Least Significant Difference multiple range test ($P < 0.05$).

4.5%), *Glomus* (0–3.9%), *Entrophospora* (0–0.7%), and *Diversispora* (0–0.5%) were rarely found. Only 15 AM fungal species were recorded simultaneously from the two treatments during all four sampling seasons, and the most abundant one was *A. denticulata* (27.7–47.3%), followed by *F. verruculosum* (2.8–16.1%), *Ra. persica* (2.6–12.2%), *F. mosseae* (1.3–10.4%), *S. reticulata* (1.4–8.5%), and *A. tuberculata* (1.5–6.8%). A number of AM fungal species was only recorded in the NT treatment, such as *E. infrequens* in three stages, *F. geosporum*, *G. globiferum*, and *G. monosporum* in two stages, and *G. pansihalos* in one stage. However, *A. laevis* and *A. rehmii* were only recorded in the CT treatment in one stage. The relative abundances of spores for many other species were relatively consistent across the two treatments.

Species richness, diversity, and similarity of AM fungi

There were no significant differences in the species richness, Shannon-Wiener index (H'), *Evenness* (E), and Simpson's index (D) of the soil AM fungal community between NT and CT for all sampling seasons (Fig. 1), except for a significantly higher ($P < 0.05$) D in the NT treatment compared with the CT treatment in the jointing stage for maize. With repect to the total number of AM fungal species that were extracted from all four replicate plots for each treatment at each sampling time, it was higher in the NT treatment (26, 24, 23, and 30, respectively) than in the CT treatment (20, 23, 20, and 24, respectively). The highest Jaccard index of similarity in species composition was recorded at the maturation stage of wheat (0.800), followed by the jointing stages of maize (0.704) and wheat (0.654), and the lowest one was observed at the maturation stage of maize (0.621). With regards to the seasonal dynamics, there were significant increases ($P < 0.05$) in the diversity indices (H', E, and D) of AM fungi from the jointing to maturation stages of maize in the CT treatment as well as a significantly higher ($P < 0.05$) species richness at the maturation stage of wheat compared with that at the maturation stage of maize in the NT treatment.

Root mycorrhizal colonization, soil alkaline phosphatase activity, and soil P concentrations

There were no significant differences in the root mycorrhizal colonizations between NT and CT at each of the sampling times (Fig. 2). In both the NT and CT treatments, the highest root mycorrhizal colonization was recorded at the maize jointing stage, when the root colonization under NT was significantly higher ($P < 0.05$) than those under CT for the other three sampling seasons. The NT significantly increased ($P < 0.05$) the soil alkaline phosphatase activity at the jointing stages of both maize and wheat, but it had no significant effects at the maturation stages. The highest soil alkaline phosphatase activity was recorded in the NT treatment at the jointing stage of wheat, and the lowest one was recorded in the CT treatment at the jointing stage of maize. NT had no significant effects on soil P concentrations, except for a significantly ($P < 0.05$) higher available P concentration at the jointing stage of maize, which was 0.66 times greater than that of the CT treatment.

Discussion

The major objective of this study was to compare the species composition and ecological function of soil AM fungi dur-

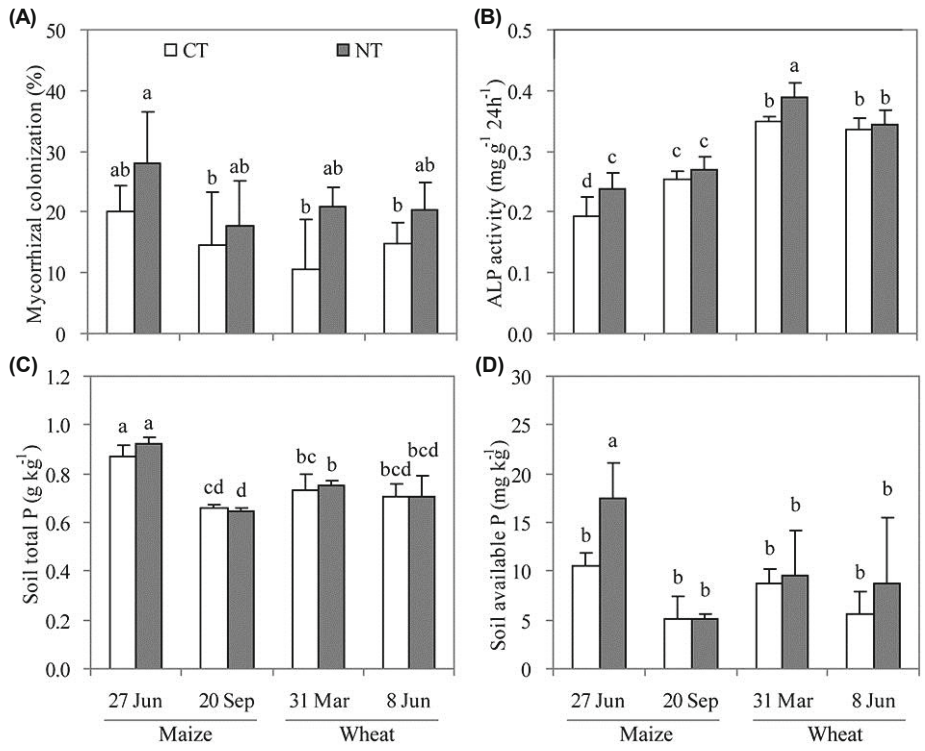

Fig. 2. Root mycorrhizal colonization (A), soil alkaline phosphatase (ALP) activity (B), total P concentration (C) and available P concentration (D) under no tillage (NT) and conventional tillage (CT) treatments at the jointing and maturation stages of maize and wheat. Maize and wheat were sown in June and October 2009, respectively. Soil samples from the maturation stages were collected just before the maize and wheat harvest. *Vertical T bars* indicate standard deviations. *Bars* not topped by the same letter indicate a significant difference in values according to the Least Significant Difference multiple range test ($P < 0.05$).

ing the growth and rotation of maize and wheat under NT and CT regimes in North China. The dominance of AM fungal genera is related to their sporogenous characteristics. The *Acaulospora* species produce the smallest sized spores in large numbers within a short time, and these spores are distributed easily (Hepper, 1984). Similar to the total number of 35 AM fungal species reported from the same arable land (Wang *et al.*, 2011), a total of 34 was recorded in this study, and the largest number of species was found for *Acaulospora*, which was also the most abundant genus because of one dominant species, namely *A. denticulata*, which produced the most spores, at 27.7–47.3% (Table 1). However, different tillage regimes also caused differential effects on the species diversity of AM fungi. CT was hypothesized to decrease the diversity and functionality of AM fungi. For example, at the jointing stage of maize, there was a remarkable increase in one diversity index, that is, the dominance of the most common species (*D*), for AM fungal spores under NT (Fig. 1D). The causal mechanisms are not fully understood, but they appear to be caused by the decrease in destruction by tillage in the soil physico-chemical environment (Tabaglio *et al.*, 2009). It has been demonstrated that long-term periods under the NT regime may result in compacting processes that are derived from the use of planting machinery (Botta *et al.*, 2009). However, these processes may also contribute to the longevity of microbes in the soils (Yang *et al.*, 2012). Consequently, at the jointing stage of maize in the present study, seven AM fungal species, namely, *A. foveata*, *A. scrobiculata*, *Entrophospora infrequens*, *Funneliformis geosporum*, *Glomus globiferum*, *Glomus* sp. I, and *Rhizophagus clarum* were recorded in association with NT treatment alone, with only *A. lacunosa* being recorded in association with the CT treatment alone.

The responses of host plants to tillage regimes can be helpful for understanding the changes in the ecological parameters of the AM fungal community, such as the spore density. In a similar sandy loam soil in western Iran, NT resulted in higher soil bulk density and lower root length density compared with CT (Mosaddeghi *et al.*, 2009). Moreover, in a typical Vertisol in southern Spain, lower values were also found for the root length density and root biomass in the topmost 10 cm of soil under NT than under CT (Muñoz-Romero *et al.*, 2010). Therefore, it can be deduced that there were possible decreases in the root biomass or length density under NT in this experimental field, which might provide less carbohydrates for feeding AM fungi. However, as mentioned earlier, the reductions in physical disturbances to the system under NT might also contribute to the longevity of soil AM fungi. Because of the two contrary effects of NT, i.e., the decreasing carbohydrates from post-harvest crops and the increasing longevity of existing AM fungi, tillage had no significant effect on the spore densities of AM fungi at all sampling times in this study, even at the jointing stage of either wheat or maize (Table 1). Compared with the plots that were maintained under CT, there were also no significant changes in the spore densities of AM fungi in response to both 6-year and 10-year NT in a thermic Entic Haploxeroll under wheat-maize rotation in central Chile (Curaqueo *et al.*, 2011). By contrast, more AM fungi spores were formed under NT than under CT from the second to fourth year after the beginning of an experiment in a typic Chilean Ultisol under wheat-oat (*Avena sativa L.*) rotation in southern Chile (Castillo *et al.*, 2006). Because the result derived from this study was only based on a rotation cycle that took place within one year, a longitudinal study should be conducted in the future.

There are also seasonal variations in the species composition of AM fungi (Castillo *et al.*, 2006), such as a trend towards lower relative abundances of *A. denticulata* and *F. verruculosum* from the jointing stage of maize to the maturation stage of wheat as observed in this study (Table 1). It should be noted that there were significant increases in the diversity indices (H', *E*, and *D*) of AM fungi from the jointing to the maturation stages of maize with the CT treatment (Fig. 1). In considering the species composition (Table 1), there were nine increased or decreased species and four significantly changed relative abundances in the specific species from the jointing to the maturation stages. During the same period, there were only six altered species and a significant decrease in the relative abundance of one species under the NT treatment. Thus, the seasonal variations in the AM fungal community under NT were more moderate than the ones found under CT. Based on a comparison between diversity indices that were performed on 27 June 2009 and 8 June 2010, it can be deduced that there was a great influence from CT on the AM fungal community at the sowing stage of maize in early June 2009. Conversely, the AM fungal filament network that was left intact from the previous wheat season under NT could colonize the roots of maize seedlings (Castillo *et al.*, 2006), which could lead to enhanced root colonization, as well as increased soil alkaline phosphatase activity and available P concentrations (Fig. 2) at the jointing stage of maize. In this study, the increase in the soil alkaline phosphatase activity and a trend towards higher root mycorrhizal colonization at the jointing stage indicated that NT could play an important role in the improvement in the ecological functions of soil AM fungal community, especially for crop seedlings.

There should be similar findings in the soil AM fungal community by CT at the sowing stage of wheat in October 2009. However, the period from the sowing to the jointing stages is approximately 2–3 weeks for maize, but it is more than five months for wheat. There might be a significant restoration in the community structure of AM fungi from the sowing to the jointing stages of wheat. Although there was also enhanced root colonization and soil alkaline phosphatase activity at the wheat jointing stage under NT relative to CT (Fig. 2B), there was no significant increase in the soil available P concentration (Fig. 2D). In other words, the benefits of P nutrition for wheat seedlings from NT through the protection of AM propagules may be more remarkable within a shorter time after the sowing stage. Based on the jointing stage, the NT employed here is more valuable during the maize season than the wheat one. This observation has not been reported elsewhere. However, further work is necessary to determine whether the sustainability and productivity in this system will not depend on the high diversity and abundance of AM fungi or on the abundance of particular species.

In general, the aim of this study was to compare the diver-

sity and functionality of soil AM fungi during the growth and rotation of crops under NT and CT in the North China Plain. The CT employed at the sowing stage affected the species richness, diversity indices (including the H', E, and D), root colonization, and ecological function of AM fungi negatively relative to the NT treatment, and it took several months to restore the reductions during the crop growing season. The NT employed during the maize season was more beneficial than that of the wheat season for maintaining the diversity and functionality of AM fungi, suggesting the prior popularity of NT in maize season. However, these findings were obtained within the fourth year of a long-term experiment, and further in-depth work is still necessary to assess the diversity and functionality of AM fungi under NT and CT in this region.

Acknowledgements

This work was supported by the Strategic Priority Research Program Grant (XDB15030202) of Chinese Academy of Sciences, the National Basic Research Program (2011CB100505) of China, and the Talents Project (Y412010009) of State Key Laboratory of Soil and Sustainable Agriculture. We wish to acknowledge Qi'ao Jiang, Linyun Zhou, Jinfang Wang, and Jian Liu, of the Fengqiu Agro-Ecological Experimental Station, Institute of Soil Science, Chinese Academy of Sciences, for their field management and support with soil sampling, as well as the two anonymous reviewers, for their comments and suggestions on this manuscript.

References

Álvaro-Fuentes, J., Lopez, M.V., Cantero-Martinez, C., and Arrue, J.L. 2008. Tillage effects on soil organic carbon fractions in Mediterranean dry land agroecosystems. *Soil Sci. Soc. Am. J.* **72**, 541–547.

Alvear, M., Rosas, A., Rouanet, J.L., and Borie, F. 2005. Effects of three soil tillage systems on some biological activities in an Ultisol from southern Chile. *Soil Till. Res.* **82**, 195–202.

Blanco-Canqui, H. and Lal, R. 2008. No-tillage and soil-profile carbon sequestration: an on-farm assessment. *Soil Sci. Soc. Am. J.* **72**, 693–701.

Botta, G.F., Becerra, A.T., and Tourn, F.B. 2009. Effect of the number of tractor passes on soil rut depth and compaction in two tillage regimes. *Soil Till. Res.* **103**, 381–386.

Camron, D.D. 2010. Arbuscular mycorrhizal fungi as (agro)ecosystem engineers. *Plant Soil* **333**, 1–5.

Castillo, C.G., Rubio, R., Rouanet, J.L., and Borie, F. 2006. Early effects of tillage and crop rotation on arbuscular mycorrhizal fungal propagules in an Ultisol. *Biol. Fertil. Soils* **42**, 83–92.

Curaqueo, G., Barea, J.M., Acevedo, E., Rubio, R., Cornejo, P., and Borie, F. 2011. Effects of different tillage system on arbuscular mycorrhizal fungal propagules and physical properties in a Mediterranean agroecosystem in central Chile. *Soil Till. Res.* **113**, 11–18.

Giovannetti, M. and Mosse, B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* **84**, 489–500.

Gosling, P., Hodge, A., Goodlass, G., and Bending, G.D. 2006. Arbuscular mycorrhizal fungi and organic farming. *Agr. Ecosyst. Environ.* **113**, 17–35.

He, J., Li, H., Rasaily, R.G., Wang, Q., Cai, G., Su, Y., Qiao, X., and Liu, L. 2011. Soil properties and crop yields after 11 years of no tillage farming in wheat–maize cropping system in North China Plain. *Soil Till. Res.* **113**, 48–54.

Hepper, C.M. 1984. Isolation and culture of VA mycorrhizal (VAM) fungi, pp. 95–112. *In* Powell, C.L. and Bagyaraj, D.J. (eds.), VA Mycorrhizae. CRC Press, Florida, USA.

Hu, J., Lin, X., Wang, J., Chu, H., Yin, R., and Zhang, J. 2009. Population size and specific potential of P-mineralizing and -solubilizing bacteria under long-term P-deficiency fertilization in a sandy loam soil. *Pedobiologia* **53**, 49–58.

IUSS. 2006. World Reference Base for Soil Resources 2006. World Soil Resources Report no. 103. FAO, Rome.

Jackson, M.L. 1958. Soil chemical analysis, pp. 111–133. Prentice-Hall Inc., Englewood Cliffs, NJ, USA.

Jansa, J., Mozafar, A., Kuhn, G., Anken, T., Ruh, R., Sanders, I.R., and Frossard, E. 2003. Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecol. Appl.* **13**, 1164–1176.

Krüger, M., Krüger, C., Walker, C., Stockinger, H., and Schüßler, A. 2012. Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. *New Phytol.* **193**, 970–984.

Martinez, T.N. and Johnson, N.C. 2010. Agricultural management influences propagule densities and functioning of arbuscular mycorrhizas in low- and high-input agroecosystems in arid environments. *Appl. Soil Ecol.* **46**, 300–306.

Mirás-Avalos, J.M., Antunes, P.M., Koch, A., Khosla, K., Klironomos, J.N., and Dunfield, K.E. 2011. The influence of tillage on the structure of rhizosphere and root-associated arbuscular mycorrhizal fungal communities. *Pedobiologia* **54**, 235–241.

Mosaddeghi, M.R., Mahboubi, A.A., and Safadoust, A. 2009. Short-term effects of tillage and manure on some soil physical properties and maize root growth in a sandy loam soil in western Iran. *Soil Till. Res.* **104**, 173–179.

Muñoz-Romero, V., Benítez-Vega, J., López-Bellido, R.J., Fontán, J.M., and López-Bellido, L. 2010. Effect of tillage system on the root growth of spring wheat. *Plant Soil* **326**, 97–107.

Oehl, F., Laczko, E., Bogenrieder, A., Stahr, K., Bösch, R., van der Heijden, M., and Sieverding, E. 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biol. Biochem.* **42**, 724–738.

Oehl, F., Sieverding, E., Palenzuela, J., Ineichen, K., and Alves da Silva, G. 2011. Advances in *Glomeromycota* taxonomy and classification. *IMA Fungus* **2**, 191–199.

Olsen, S.R., Cole, C.V., Watanabe, F.S., and Dean, L.A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate, Circular 939, p.19. US Department of Agriculture, Washington, D.C., USA.

Phillips, J.M. and Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **55**, 158–161.

Qin, S., He, X., Hu, C., Zhang, Y., and Dong, W. 2010. Responses of soil chemical and microbial indicators to conservational tillage versus traditional tillage in the North China Plain. *Eur. J. Soil Biol.* **46**, 243–247.

Schüßler, A. and Walker, C. 2010. The Glomeromycota: a species list with new families and genera. Libraries at The Royal Botanical Garden Edinburgh, The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University.

Smith, S.E. and Read, D.J. 2008. Mycorrhizal Symbiosis, 3rd edn. Academic Press, London, UK.

Sun, X., Gao, C., and Guo, L.D. 2013. Changes in arbuscular mycorrhizal fungus community along an exotic plant *Eupatorium adenophorum* invasion in a Chinese secondary forest. *J. Microbiol.* **51**, 295–300.

Tabaglio, V., Gavazzi, C., and Menta, C. 2009. Physico-chemical indicators and microarthropod communities as influenced by no-till, conventional tillage and nitrogen fertilisation after four years of continuous maize. *Soil Till. Res.* **105**, 135–142.

Tabatabai, M.A. 1982. Soil enzymes. *In* Page, A.L., Miller, R.H., and Keeney, D.R. (eds.), Methods of Soil Analyses, Part 2, Chemical and Microbiological Properties, 2nd edn., pp. 903–947. American Society of Agronomy, Madison, WI, USA.

Wang, F.Y., Hu, J.L., Lin, X.G., Qin, S.W., and Wang, J.H. 2011. Arbuscular mycorrhizal fungal community structure and diversity in response to long-term fertilization: a field case from China. *World J. Microbiol. Biotechnol.* **27**, 67–74.

Yang, S.K., Kim, M.K., Seo, Y.W., Choi, K.J., Lee, S.T., Kwak, Y.S., and Lee, Y.H. 2012. Soil microbial community analysis of between no-till and tillage in a controlled horticultural field. *World J. Microbiol. Biotechnol.* **28**, 1797–1801.

Yang, A.N., Lu, L., and Zhang, N. 2011. The diversity of arbuscular mycorrhizal fungi in the subtropical forest of Huangshan (Yellow Mountain), East-Central China. *World J. Microb. Biotechnol.* **27**, 2351–2358.

Zhu, Y., Smith, F.A., and Smith, S.E. 2003. Phosphorus efficiencies and responses of barley (*Hordeum vulgare* L.) to arbuscular mycorrhizal fungi grown in highly calcareous soil. *Mycorrhiza* **13**, 93–100.