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ORIGINAL PAPER

Rapid change of AM fungal community in a rain-fed wheat field with short-term plastic film mulching practice

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Abstract Plastic film mulching (PFM) is a widely used agricultural practice in the temperate semi-arid Loess Plateau of China. However, how beneficial soil microbes, arbuscular mycorrhizal (AM) fungi in particular, respond to the PFM practice is not known. Here, a field experiment was performed to study the effects of a 3-month short-term PFM practice on AM fungi in plots planted with spring wheat (*Triticum aestivum* L. cv. Dingxi-2) in the Loess Plateau. AM colonization, spore density, wheat spike weight, and grain phosphorus (P) content were significantly increased in the PFM treatments, and these changes were mainly attributable to changes in soil properties such as available P and soil moisture. Alkaline phosphatase activity was significantly higher in PFM soils, but levels of AM fungal-related glomalin were similar between treatments. A total of nine AM fungal phylotypes were detected in root samples based on AM fungal SSU rDNA analyses, with six and five phylotypes in PFM and no-PFM plots, respectively. Although AM fungal phylotype richness was not statistically different between treatments, the community compositions were different, with four and three specific phylotypes in the PFM and no-PFM plots, respectively. A significant and rapid change in AM fungal, wheat, and soil variables following PFM suggested that the functioning of the AM symbiosis had been changed in the wheat field under PFM. Future studies are needed to investigate whether PFM applied over a longer term has a similar effect on the AM fungal community and their functioning in an agricultural ecosystem.

Keywords Arbuscular mycorrhizal fungi · Mycorrhizal community · Phosphorus · Plastic film mulch · Soil moisture · Wheat

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Introduction

World demand for grain will double by 2050, and an increase in crop yields is critical to the global food supply for future populations (Tilman et al. 2002). In rain-fed agricultural systems, however, such as in the conventional dry-land farming system of the semi-arid Loess Plateau of northwestern China, wheat yields can be as low as 700–800 kg ha^{-1} due to limited water availability and low soil temperature (Li et al. 2004a). To reduce evaporation from soil and increase soil temperature, plastic film mulching (PFM) has recently been widely used, and crop yields have thus been significantly increased in this dry-land farming system (Li et al. 2004a, b; Liu et al. 2009a; Hou et al. 2010). However, the use of PFM also alters soil biological characteristics that may result in loss of soil quality and sustainability (Liu et al. 2001; Li et al. 2004a, b; Yu et al. 2008), emphasizing the need for further studies.

Soil microbes play a key role in biogeochemical cycles (Kennedy and Smith 1995; Mäder et al. 2002; Falkowski et al. 2008). Among these microbes, the arbuscular mycorrhizal (AM) fungi, which form a mutualistic relationship with all cultivated grain crops, are especially significant because of their multiple roles, including the promotion of plant nutrient uptake, especially phosphorus (P) and nitrogen (N), resistance to root pathogens, and tolerance to water or salt stress (He and Nara 2007; Hamel and Plenchette 2007; Hodge and Fitter 2010). Moreover, glomalin, a glycoprotein produced by AM fungi, appears to play a role in the formation of water-stable macroaggregates and hence soil aggregation (Rillig et al. 2002; Wilson et al. 2009). As a result, AM fungi could potentially be used as a bio-fertilizer and indicator of soil sustainability in agricultural ecosystems (Roesti et al. 2006; Bedini et al. 2007; Siddiqui et al. 2008).

On the other hand, the activity and community composition of AM fungi can be significantly changed by agricultural practices including fertilization and land use intensity (e.g., Mäder et al. 2002; Hijri et al. 2006; Oehl et al. 2010). However, no studies have investigated how PFM practice affect the functioning and community composition of AM fungi. We hypothesize that the alteration of soil properties under PFM practice could have a potential impact on the activity and community composition of AM fungi, and the resulting changes could have implications for crop growth and productivity. We therefore conducted a field experiment with or without PFM coverage in wheat fields in the Loess Plateau of northwestern China to address: (1) How does PFM affect AM fungal colonization, spore density, community composition, and soil glomalin contents? (2) Are changes in AM fungal variables related to the changes in soil physical and chemical properties and wheat yield derived from the PFM practice?

Materials and methods

Study site and experimental design

This study was carried out in a traditional agricultural field near Zhonglianchuan village (36°02′N, 104°25′E; 2,370 m above sea level) in Yuzhong County, Gansu, China. The climate is temperate semi-arid, with a mean annual temperature of 6.5°C (19.0°C maximum in July and −8.0°C minimum in January), mean annual precipitation of 320 mm (most between July and September), and mean annual free water evaporation of ~1,300 mm (Liu et al. 2009a). The soil is classified as Heima (Calcic Kastanozems, FAO Taxonomy) with a 23% field water holding capacity. Potato, wheat, and maize are the main crops in this region, and their productivity is mainly dependent on rainfall.

On 22 March 2008, two treatments with or without plastic film mulching (PFM or no-PFM) were performed in a 40-m^2 field. Before treatment, 0.6 kg urea (c. 7 gm^{-2} N) and 1.8 kg tricalcium phosphate (c. 9 gm^{-2} P) were carefully hand broadcasted, and the top soils (~20 cm) were adequately mixed using a rotary tillage machine to ensure that the original soil conditions were identical in all experimental plots. Ten 2×1 m plots, with five replicates per treatment, were completely randomly arranged as two columns by five rows with 0.5-m buffer strips between plots. For the PFM treatment, plastic film was mulched on the soil surface, and the film edges were buried with soils. Six hundred spring wheat seeds (*Triticum aestivum* L. cv. Dingxi-2) were sown in 120 (15×8) holes in each plot with a hole-seeding machine. No irrigation or other fertilization was applied during the wheat growth season, during which 78 mm of rain fell.

Sampling procedure

Soil samples were collected on 10 June 2008 and mature wheat spikes on 16 July 2008. Five soil cores (3.8 cm in diameter, 25 cm in height) were randomly taken in each plot and mixed as two subsamples, and then stored in sealed bags in an icebox. Fine roots from one subsample were carefully washed with tap water and then cut into 1-cm lengths for AM colonization and molecular analyses. After the root separation, soils were air-dried, sieved (1 mm), and stored at 4°C for glomalin and alkaline phosphatase analyses. The other subsample was used for soil properties analysis and AM fungal spore isolation. Mean spike weight and grain weight of each plot were estimated based on 100 randomly picked air-dried spikes and 1,000 air-dried grains, respectively. Grain samples were dried for 48 h at 60°C, milled, and the total P content determined following wet-ashing of aliquots of tissue (150 mg) and a colorimetric assay of digest P (Chen et al. 1956).

Soil properties and phosphatase analyses

Soil moisture content was measured gravimetrically, and the soil pH was measured in 1 M KCl (1:5 *w*/*v*). Soil organic carbon and total nitrogen concentrations were analyzed using the CHNS-analyser system (Elementar Analysensysteme GmbH, Hanau, Germany) with the burning method at 450° C and 1,250°C, respectively. Soil available P was extracted using the Olsen method and measured with the molybdate-blue colorimetrical method (Olsen et al. 1954). Measurement of soil alkaline phosphatase activity was followed by Guan (1986).

Analysis of AM colonization, spore density, and glomalin

Determinations of percentage root AM colonization and spore density were followed by McGonigle et al. (1990)

and Brundrett et al. (1994), respectively. Glomalin was extracted from 1-g air-dried soil sample using the easily extracted glomalin protocol and determined by the Bradford dye-binding assay with bovine serum albumin as the standard (Wright and Upadhyaya 1998).

Molecular analysis

Thirty root fragments (c. 1 cm in length) were randomly taken from each root sample and the total DNA was extracted using a Plant DNA Extraction Kit following the manufacturer's recommendation (Tiangen Biotech, Beijing, China). The extracted DNA was diluted with ddH_2O (1:10) and subjected to a nested PCR. The first PCR was performed with the universal fungal primers GeoA2 and Geo11 to amplify a c. 1.8-kb fragment of the 18S rDNA (Schwarzott and Schüßler 2001). PCRs were carried out in a final volume of 25 μl with 2 μl of template (c. 20 ng) and 1 μl of each primer (10 μM) using the *Pfu* PCR mastermix system (Tiangen, Biotech, Beijing, China) with the following cycles: 94°C for 2 min, 30×(94°C for 30 s, 59°C for 1 min, and 72°C for 2.5 min) and 72°C for 10 min. The first amplification products were diluted with ddH_2O (1:100), and 2 μl of this dilution was used as a template for the second PCR amplification, with a universal eukaryotic primer NS31 (Simon et al. 1992) and an AM fungal specific primer AML2 (Lee et al. 2008). The second PCRs were the same as the first PCR but with the following cycles: 94°C for 2 min, 30×(94°C for 30 s, 58°C for 1 min, and 72°C for 80 s) and 72°C for 10 min. All PCRs were run on a GeneAmp® PCR system 2700 (Applied Biosystem, San Francisco, CA, USA), and the PCR products were examined on a 1.5% (*w*/*v*) agarose gel with ethidium bromide staining to confirm the product integrity.

The second PCR products were purified using the Gel and PCR Clean-Up System (Promega, Madison, WI, USA), and the expected DNA fragments (c. 560 bp) were obtained. Purified DNA derived from each root sample was used for the construction of a total of ten clone libraries. After an A-tailing procedure, the DNA fragments were ligated into pGEM-T vector (Promega, Madison, WI, USA) and cloned into *Escherichia coli* DH5α according to the manufacturer's recommended protocol. For each clone library, 50 randomly selected white clones were re-amplified using the primer set of NS31/AML2 with the same PCR condition described above. All positive PCR products were then screened using the restriction fragment length polymorphism (RFLP) with the restriction enzymes HinfI and Hin1II (MBI, Lithuania). Representatives of each RFLP type were sequenced by the Major Biotech Company (Shanghai, China) using the vector primer T7. All DNA sequences were edited and compared with public databases using the basic local alignment search tool (BLAST) (Altschul et al. 1997) and then used for the neighbor-joining phylogenetic analysis with the Kimura 2-parameter model (Tamura et al. 2007).

Statistical analysis

Significant differences between the PFM and no-PFM treatments were tested using the independent samples *t* test at $P \leq 0.05$. The correlations among soil, wheat, and AM fungal variables were tested using Pearson's correlation coefficient. To assess which soil factor could best explain the variation of AM fungal and wheat variables, a series of stepwise regression analyses were performed in which the AM fungal and wheat variables served as dependent variables and soil properties (soil moisture, pH, total N, organic C, available P, and alkaline phosphatase) as independent variables. The AM fungal community composition was calculated on the clone numbers of each phylotype in a root sample, and then ordered using the non-metric multidimensional scaling (NMDS) based on Bray–Curtis community dissimilarities between samples. To explore the correlations between soil factors and AM fungal community composition, soil factors were fitted as vectors onto the NMDS plot using the function "envfit" from the Vegan library of R package. All statistical analyses were performed using the SPSS 13.0 (SPSS Inc., Chicago, IL, USA) or R version 2.12.1 (R Development Core Team 2010), and all data used in the correlation and regression analyses were ln-transformed, except for AM colonization that was arcsine square-root transformed.

Results

Effects of PFM on soil properties and wheat yields

Soil moisture, pH, available P, and alkaline phosphatase activity were significantly higher whereas the soil total N and organic C were significantly lower under the PFM than under the no-PFM treatment (Table 1). Moreover, the alkaline phosphatase activity showed a significantly positive correlation with soil available P ($r=0.89$, $P=0.001$).

The mean spike weight and grain P content, but not the mean grain weight, were significantly higher under the PFM than under the no-PFM treatment (Table 1). The increase in wheat spike weight was correlated with soil moisture, whereas grain P content was most strongly correlated with soil available P (Table 2).

AM colonization, spore density, and glomalin content

Significantly higher AM colonization and spore density were observed under the PFM than under the no-PFM treatment (Table 1). In contrast, the soil glomalin contents

Table 1 Differences of soil, AM fungal, and wheat variables between the PFM and no-PFM treatments

	Soil variables						AM fungal variables				Wheat variables		
	Moisture (%)	pH	Total N (mg kg^{-1})	Organic C (g kg^{-1})	Available P (mg kg^{-1})	Alkaline phosphatase (μg Phenol g^{-1} h^{-1})	AM colonization (%)	Phylotype richness	Spore density (spores g^{-1} soil DW)	Glomalin (μg BSA g^{-1} soil DW)	Grain weight (mg)	Spike weight (g)	Grain P (mg/kg)
PFM	5.4±0.1	7.6±0.02	496.9±3.8	11.8±0.05	11.33±0.04	57.4±0.7	57.4±2.9	5±0.7	4.4±0.3	0.48±0.02	46.9±0.3	3.5±0.06	62±0.9
no-PFM	4.0±0.1	7.4±0.01	525.8±4	12.3±0.07	9.23±0.04	52.8±0.4	39.3±3.1	4.6±0.5	3.0±0.3	0.47±0.03	45.9±0.3	2.9±0.06	54.2±0.6
P value	<0.001	<0.001	0.001	0.001	<0.001	0.001	0.003	0.347	0.003	0.790	0.052	<0.001	<0.001

Data are means ± SE, $n=5$

Table 2 Soil factors that best explain the variation in AM fungal and wheat variables

	AM colonization	Spore density	Spike weight	Grain P content
Predictors	Soil available P	Soil total N	Soil moisture	Soil available P
r	0.82	−0.91	0.94	0.94
P value	0.004	<0.001	<0.001	<0.001

AM fungal phylotype richness, glomalin content, and wheat grain weight were not presented since they could not be explained by any soil factor

were similar between these two treatments (Table 1). Stepwise regression analyses showed that the soil available P and total N were respectively the best predictors for AM colonization and spore density (Table 2). However, the glomalin content could not be explained by any soil variables (Table 2) and was not significantly correlated with any wheat variables (Table 3). In addition, both AM colonization and spore density showed significant positive correlations with the spike weight, grain P content, and soil alkaline phosphatase activity (Table 3).

Molecular identification and the AM fungal community

PCR products at c. 1.8 kb generated from the first PCR and c. 560 bp from the second PCR were successfully amplified from all samples. A total of 500 white clones (50 clones per sample) were screened, and 497 out of these clones containing insert of correct size were submitted to RFLP typing. One representative clone per RFLP type was sequenced, comprising a total of 29 sequences. The remaining clones were classified by RFLP typing. Among the 29 sequences, the BLAST results showed that 23 sequences had high homology (≥95% identity) to members of Glomeromycota, four sequences to Ascomycota, one

Table 3 Correlations between AM fungal variables with wheat variables and the soil alkaline phosphatase

	Spike weight	Grain weight	Grain P content	Alkaline phosphatase
AM colonization	+0.608*	+0.303	+0.733*	+0.611*
Spore density	+0.796**	+0.639*	+0.799**	+0.667*
Glomalin content	+0.127	+0.116	+0.113	+0.170
Phylotype richness	+0.479	+0.258	+0.181	+0.367

The data are Pearson's correlation coefficients

*$P \leq 0.05$

**$P \leq 0.01$

sequence to Metazoa, and another to wheat. A total of 409 clones (82.3%) were identified as AM fungal sequences; the remaining non-AM fungal sequences were not analyzed. A total of nine sequence groups (≥98% identity), which were revealed by the phylogenetic analysis from the 23 Glomeromycota sequences and the closest reference sequences, were referred to as phylotypes (Fig. 1). Among these nine phylotypes, seven belonged to the *Glomus* group A (GA), one to the *Glomus* group B (GB), and another to the *Archaeosporales* (Ar) (Fig. 1). Among the eight *Glomerales* phylotypes, three (GA-1, GA-2, and GB-1) showed ≥98% similarity to sequences of *Glomus intraradices/fasciculatum*, *Glomus mosseae*, and *Glomus claroideum*, respectively; five phylotypes were highly related (≥98% identity) to root-derived, but taxonomically unknown sequences. The phylotype Ar-1 showed a 95% identity to the sequences of *Ambispora fennica* and was thus designated into the *Archaeosporales*.

AM fungal phylotype richness was not significantly different under the PFM and the no-PFM treatments (Table 1), and was not significantly correlated with any soil (Table 2) or wheat variables (Table 3). On the other

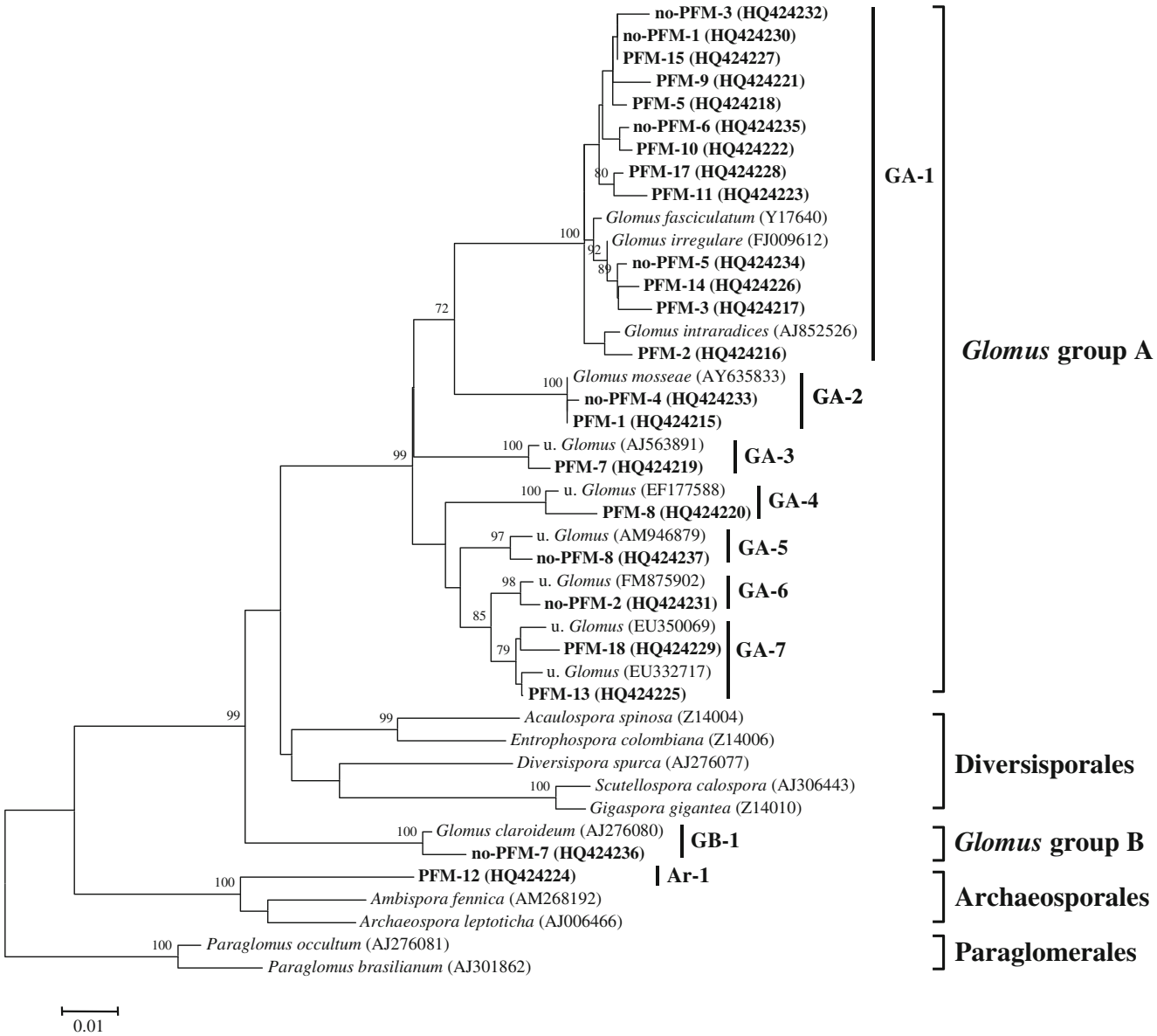


Fig. 1 Neighbor-joining phylogenetic tree of new AM fungal sequences from this study and referenced sequences from the GenBank. The names of new sequences are in *bold*, of which the *letters* of PFM and no-PFM indicate the sequence obtained from wheat roots of PFM and no-PFM treatments, respectively. *Bootstrap values* (1,000 replicates) >70% are shown. *Sequence groups* (*GA-1*, etc.) identify distinct clusters of sequences with similarity ≥98%. All sequences have been submitted in the GenBank database under the accession numbers HQ424215–HQ424237

hand, the AM fungal species compositions were distinctive between the PFM and the no-PFM treatments. Two fungal phylotypes (GA-1 and GA-2) occurred in wheat roots under both treatments, whereas four phylotypes were only detected in the PFM treatment and three only in the no-PFM treatment (Fig. 2). The GA-1 phylotype was dominant in all root samples, representing 79% and 57% of the AM fungal population under the PFM and no-PFM treatments, respectively (Fig. 2). The next most abundant phylotype (GB-1) was only detected under the no-PFM treatment, where it comprised 24% of the population (Fig. 2).

The NMDS ordination showed that scatterplots of AM fungal community composition were obviously separated by the treatment (Fig. 3a). Six fitted vectors onto the NMDS plot showed that all soil factors were significantly correlated with the ordination, of which the soil available P (r^2=0.90, P=0.006) was the most important factor that related to AM fungal community composition (Fig. 3b).

Discussion

In the present study, soil water and available P were significantly higher, but soil organic C and total N were significantly lower after a 3-month short-term PFM treatment. The rapid decrease of soil C and N pools, which may be due to the enhanced degradation of organic matter by soil microbes and the increased nutrient demand of crops under the PFM treatment (Li et al. 2004a), may result in the observed loss of soil quality and sustainability after a long-term PFM treatment (Liu et al. 2001).

Our results revealed that the AM colonization and sporulation were significantly increased under the PFM treatment. The best predictors for AM colonization and spore density were soil available P and total N, respectively, suggesting that the increase in AM fungal fitness by the PFM practice could well be explained by the changes of soil properties. Nonetheless, our data did not distinguish between a direct response of AM fungi to the change in soil chemical properties and an indirect response due to the increased changes in temperature and moisture in the PFM soils. The low temperature and water content in soils of the study region are the most limiting factors for crop growth (Zhou et al. 2009), and they may also restrain the activities of AM fungi and other soil microbes. Temperature increase often results in the improvement of both AM colonization and mycorrhiza-mediated P uptake (Heinemeyer and Fitter 2004; Kytöviita and Ruotsalainen 2007). Although soil temperature was not measured in this study, Liu et al. (2009a) and Zhou et al. (2009) did find that soil temperature and moisture were significantly higher in soils under the PFM than under the no-PFM in a nearby site. Thus, it is possible that the performance of AM fungi could be improved under the PFM practice. However, further studies are needed to address if an increase in soil temperature or moisture under the PMF affects the AM symbiosis and its functioning in this semi-arid region.

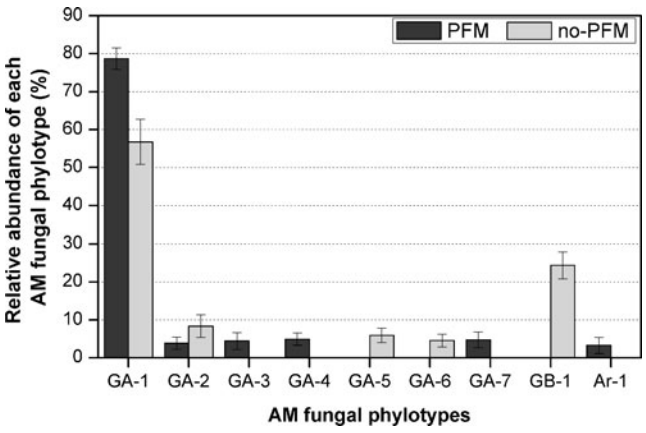


Fig. 2 Relative abundance of each AM fungal phylotype in wheat roots from the PFM and no-PFM treatments. Data were calculated on the proportion of clone numbers of each phylotype in each clone library. Data are means ± SE, n=5

A total of nine AM fungal phylotypes in roots from both PMF and no-PFM treatments indicated a comparatively low diversity of AM fungi in this agricultural system. An asymptotic species accumulation was observed from a rarefied species accumulation curve (data not shown), which indicates that a large proportion of the total AM fungal species had been detected. The number of AM fungal phylotypes in this spring wheat field is similar to that in nearby *Caragana korshinskii* plantations (Liu et al. 2009b) and winter wheat field elsewhere in the Loess Plateau (Wu et al. 2011). Our results strongly support the finding that the AM fungal diversity is generally low in agricultural fields (e.g. Daniell et al. 2001; Öpik et al. 2006; Wu et al. 2011; but see Hijri et al. 2006). Moreover, we found that the *G. intraradices*-related phylotype (GA-1), which has a global distribution (Öpik et al. 2006), showed overdominance (>50% of total abundance), whereas most of the other phylotypes were infrequent under both PFM and no-PFM treatments. This is consistent with previous observations that AM fungal communities are typically dominated by a single taxon in most habitats (Dumbrell et al. 2010a).

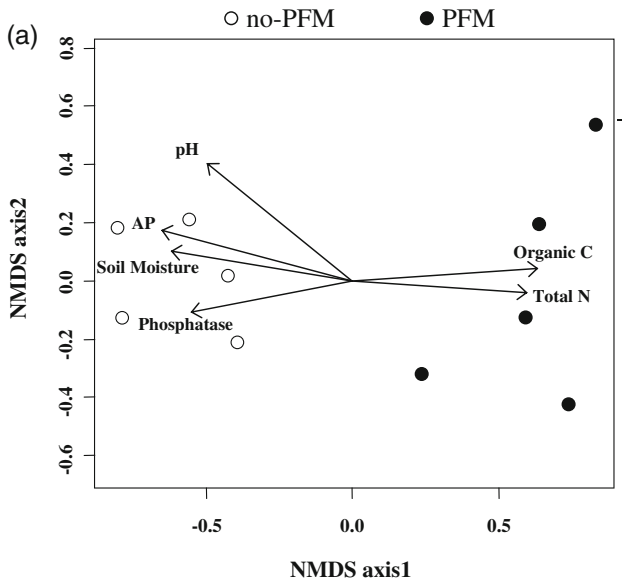
Although the number of phylotypes detected under PFM was similar to that under the no-PFM treatment, the AM fungal species composition was different, with four and three phylotypes occurring uniquely in the PFM and no-PFM treated plots, respectively. This result suggests that the PFM treatment could specifically activate or restrain some AM fungi and result in different community compositions. It seemed that those AM fungi uniquely occurring in PFM or no-PFM treated plots might be attributed to the stochastic

distribution of infrequent phylotypes. However, 24% of the total abundance of the GB-1 phylotype was only observed under the no-PFM treatment, indicating that the effects of PFM on AM fungi might be species specific. The AM fungi are well known to differ in their life history strategy, including germination requirements and responses to environmental variations (Vosátka et al. 1999; Bever et al. 2001). As a result, the significant change of AM fungal population under the PFM treatment may be due to their responses to the changes in soil conditions. Furthermore, we found that the soil factors, especially the available P, were significantly correlated with the AM fungal community composition, consistent with previous reports that soil factors strongly affect the pattern of AM fungal communities (Landis et al. 2004; Dumbrell et al. 2010b). However, as the AM fungal community was synchronously changed with the soil properties under the PFM treatment, it is difficult to determine whether the variation in soil properties controlled the AM fungi or vice versa. It has been suggested that the distribution of AM fungi may be the product of environmental conditions and interspecific competition (Lekberg et al. 2007). Further studies are thus needed to determine if such changes of AM fungi under the PFM treatment is related to interspecific competition.

The main plant benefit from increased AM colonization was an increase in grain P content, which is consistent with the well-known effect of AM symbiosis in the enhancement of plant P uptake (Smith and Read 2008). It has been suggested that higher AM fungal activity can produce more phosphatase and thus transform more P to the host plant needs (Bolan 1991; Joner et al. 2000). As a result, the improved grain P content under PFM treatment may be mainly attributable to an increase in AM fungal activity. In addition, the significant increased dominance of GA-1 phylotype in PFM treated plots indicates that this fungus thrived better with the increase in soil humidity and/or temperature, and may have hence supported a better performance of the spring wheat.

Yields of crops in the temperate semi-arid Loess Plateau of northwestern China, including wheat and maize, can be increased by PFM treatment because of increased soil water due to reduced evaporation (e.g., Li et al. 2004a, b; Gao et al. 2009; Zhou et al. 2009). In this study, mean spike weight was significantly increased under the PFM treatment, an effect best explained by the improvement in soil water status. Although we did not assess the grain number per spike and the total spike numbers in each plot, Li et al. (2004b) showed that both the yield of spring wheat and grain number per spike were significantly increased under PFM treatment in a nearby site. Although mean spike weight was positively correlated with AM colonization and spore density, our data could not demonstrate a causal connection.

In conclusion, this study has shown that a 3-month short-term PFM treatment in the temperate semi-arid Loess Plateau of China could significantly change root colonization, spore density, and community species composition of AM fungi, and that the responses of AM fungi to the PFM were highly related to the variation of soil properties. However, whether the increased AM fungal activity or increased dominance of a particular AM fungus (for example the GA-1 phylotype) under PFM treatment could result in a better performance of the spring wheat needs further research. Furthermore, our results support others highlighting that only one or two AM fungal species dominate in a particular agricultural system, suggesting that these dominant fungi should be firstly



(b)

Vector	r^2	*P*-value
Available P (AP)	0.901	0.006
pH	0.815	0.008
Organic C	0.794	0.009
Soil moisture	0.780	0.010
Total N	0.715	0.021
Phosphatase	0.632	0.034

Fig. 3 Joint plot of NMDS ordination of AM fungal communities under PFM and no-PFM treatments and vectors of soil factors (**a**). Results of correlation analyses (based on 999 permutations) between soil factors and community dissimilarities (**b**)

considered in such agricultural systems. Since the effects of PFM practice on soil characteristics and soil microbial activity are related with the duration of mulch (Li et al. 2004a, b), future investigations are needed to determine whether a long-term PFM practice has a similar effect on the AM fungal community and their functioning. Furthermore, studies of other soil microbial groups, especially those of functional importance such as nitrogen-cycling microbes, are required to accurately evaluate the compatibility of PFM practices with agricultural sustainability (Kennedy and Smith 1995).

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