

Interactive Effects of Arbuscular Mycorrhizae and Maize (*Zea mays* L.) Straws on Wheat (*Triticum aestivum* L.) Growth and Organic Carbon Storage in a Sandy Loam Soil

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Abstract

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A pot experiment was conducted to study interactive effects of arbuscular mycorrhizae (AMs) and maize (*Zea mays* L.) straws on wheat (*Triticum aestivum* L.) growth and organic carbon (C) storage in a sterilized sandy loam soil. The experiment included four treatments: control, inoculation with AM fungus *Glomus caledonium* (M), amendment with maize straw (S), and amendment with maize straw plus inoculation with *G. caledonium* (S + M). The inoculation of *G. caledonium* significantly ($P < 0.05$) increased wheat root biomass and root-to-straw ratio, but had no significant effects on shoot biomass, grain yield, and soil parameters. The amendment of maize straw significantly ($P < 0.05$) decreased soil pH, wheat root biomass, and root-to-straw ratio, and significantly ($P < 0.05$) increased soil invertase and alkaline phosphatase activities, but had no significant effects on shoot biomass, grain yield, soil organic C content, and urease activity. The combined application of *G. caledonium* and maize straw had no significant effects on root mycorrhizal colonization rate compared to the M treatment, while significantly ($P < 0.05$) increased wheat root biomass and significantly ($P < 0.05$) decreased soil pH compared to the S treatment, and also significantly ($P < 0.05$) increased grain yield, soil organic C content, and urease activity compared to the control. The Two-Way ANOVA also showed interactive effects of *G. caledonium* and maize straw on soil pH ($P < 0.05$) and wheat grain yield ($P < 0.01$), and the redundancy analysis result indicated the potential application of AM fungi in straw-returned fields.

Keywords: *Glomus caledonium*; rhizosphere acidification; root-to-straw ratio; soil enzyme; straw return

Soil organic carbon (C), an important component of terrestrial ecosystems, does not only affect the soil fertility, but also determines many of the environmental soil functions (BALKOVIČ *et al.* 2011). It is a crucial regulator of C fluxes between the biosphere and the atmosphere. Mechanisms influencing soil organic C pool depend mainly on net primary production and the distribution of photosynthate between above-ground and underground structures (ZHU & MILLER 2003). It is commonly known that arbuscular mycorrhizae (AMs) play an important role in facilitating

C translocation from above-ground to underground structures: AM fungi help plants to capture water and nutrients from the soil, and in return, the plant provides the fungus with relatively constant and direct access to carbohydrates (SMITH & READ 2008), which are translocated from their source to root tissue and on to fungal partners. It is also generally accepted that AM fungi receive all their carbohydrate from host plant and that the association could create a sink demand for carbohydrate, which could result in a 4–20% drain of C from the host plant and could indirectly

influence C storage in soils (GRAHAM 2000). With regard to sequestering C, however, soil organic C content should not be significantly affected by AM inoculation alone (HU *et al.* 2010), but should be greatly influenced by various factors.

Although primary production is a major determinant in the sequestration of C in soils, organic amendments are essential for improving soil organic C content (ZHONG *et al.* 2010). It has long been recognized that the increase in soil organic C storage by increasing crop biomass alone is much lower than that by inputting organic materials such as crop residues (CAI & QIN 2006). However, uncertainties still remain about the interactive and/or additive effects of AMs and organic amendments on crop growth and soil organic C storage, and also about their related mechanisms. Although AMs are thought to be unable to decompose organic materials directly due to a lack of saprotrophic capacity (READ & PEREZ-MORENO 2003), they may alter rhizosphere soil directly or indirectly through changes in root exudation patterns or fungal exudates (LINDERMAN 1992), and still be involved in decomposition processes (TU *et al.* 2006). For instance, AMs have been found to proliferate in decomposing organic residues (JOHN *et al.* 1983). In addition, soil enzymatic activities have been suggested to monitor soil microbial activity related to nutrient transformation (DICK 1994). For example, invertase drives C cycling by catalyzing the hydrolysis of sucrose – thus, testing the activity of soil invertase may be useful for evaluating soil capability of decomposing complex organic compounds into subunits that can be assimilated by microbes or plants. Furthermore, urease is closely related to N mineralization potential because it is required to break down urea to liberate the N into a usable form for plants, and phosphatase may play an important role in the P nutrition of plants because it mediates the release of inorganic P from organically bound P. Thus, such enzymatic activities are often measured to provide and/or explain immediate and accurate information about small changes in soils (ALBIACH *et al.* 2000; WANG *et al.* 2006; HU *et al.* 2011).

In agricultural ecosystems, both maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) are the world's most important cereal crops in terms of either cultivated area or produced amount, and the demands for maize and wheat production will continue to increase in the coming decades due to the population growth and cropland losses. For example, the succession of summer maize and winter wheat is very popular in the North China Plain, and the sustainable utilization of

agricultural soil in this highly grain-producing area may affect China's food security (GONG *et al.* 2009). The management of straw in the field as crop residue for returning the organic matter as well as nutrients removed to the soils is significant in agricultural production (TUYEN & TAN 2001). In the North China Plain, maize is sown in June and harvested in late September, and then wheat is sown in October and harvested in early June of the next year. As a result, maize straws are sometimes returned to the field before the wheat season. It was hypothesized that AMs could play an important role in increasing soil organic C storage and enhance wheat growth in the case of maize straws amendment via altering soil enzymatic activities. Therefore, the objectives of this study were to investigate the interactive effects of AMs and maize straws on wheat growth and organic C storage in a sandy loam soil collected from the North China Plain, and analyze the possible mechanisms that influence these parameters.

MATERIAL AND METHODS

Mycorrhizal inoculum. *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerdemann 90036 was isolated from a fluvo-aquic soil in Hennan Province, China (LIAO *et al.* 2003), and deposited at the Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China. The AM inocula were propagated on Sudan grass (*Sorghum sudanese* (Piper) Stapf.) grown in an autoclaved (121°C for 1 h on 3 successive days) substrate for two successive propagation cycles (2 months each). The inocula were a mixture of rhizospheric soil containing spores, hyphae, and mycorrhizal root fragments, and were air-dried and sieved (2 mm). At the same time, the non-mycorrhizal inoculum was also prepared with the same sterilized substratum on which Sudan grass was cultivated under the same conditions.

Soil preparation. A subsurface soil sample was collected from an arable agricultural land at Fengqiu County (35°00'N, 114°24'E), Henan Province, China. The air-dried soil sample was ground with a wooden pestle, homogenized by sieving through a 5 mm sieve, autoclaved (121°C for 1 h on 3 successive days), and stored for the pot experiment. The soil was derived from alluvial sediments of the Yellow River and classified as Aquic Inceptisol with the following properties: soil pH 8.6 (soil-to-water ratio = 1:2.5 (m/m)), 3.66 g/kg organic C, 0.35 g/kg total N, 0.55 g/kg total P, and 22 g/kg total K.

Pot experiment. Four treatments were established in the pot experiment: control, inoculation with

G. caledonium (M), amendment with maize straw (S), amendment with maize straw and inoculation with *G. caledonium* (S+M). Each square polyvinyl chloride pot (30 × 30 × 30 cm) contained 7.5 kg of soil, which was mixed with 375 g of mycorrhizal/non-mycorrhizal inoculum, and each straw amendment treatment was also mixed with 37.5 g of dried and neutral maize straw chip (< 1 cm). The amendment amount of maize straw was thus 10% of mycorrhizal inoculum, and was equal to 50% residue returning on crop fields in North China (WANG *et al.* 2011). Fifty wheat seeds were sown into each box. Plants were grown in a sunlit glasshouse with 30/22°C day/night temperature, 40–60% relative humidity, and 60–70% water-holding capacity. Pots were randomly arranged with four replicates per treatment. After 220 days of growing, all wheat plants were harvested, and soil samples were collected.

Mycorrhizal colonization and plant biomass analysis. Wheat plants were divided into grains, straws, and roots, and weighed after oven drying at 70°C for 48 h. All roots were thoroughly rinsed with tap water before drying, and weighed subsamples of fresh roots were used for mycorrhizal colonization assessment by the grid-line intersect method (GIOVANNETTI & MOSSE 1980) after clearing with 10% (m/m) KOH and staining with acid fuchsin (PHILLIPS & HAYMAN 1970).

Soil parameter analysis. Soil samples were air-dried and homogenized by sieving through a 2 mm mesh sieve to remove visible maize straw. Soil pH was determined with a glass electrode using a soil-to-water ratio of 1:2.5 (m/m). Soil organic C and total N were determined by dichromate oxidation (MEBIUS 1960) and Kjeldahl digestion (BREMNER 1965), respectively. Soil total P was digested by HF-HClO₄ (JACKSON 1958) and determined by molybdenum-blue spectrophotometry. Soil invertase activity was analyzed using the constant temperature incubation method as described by SRINIVASULU and RANGASWAMY (2006), and soil extracts were passed through Whatman No.1 filter paper and glucose in the filtrate was assayed (NELSON 1944). Soil urease and alkaline phosphatase activities were determined according to the methods of TABATABAI (1982) by incubation at 37°C with citrate buffer (pH 6.7) and borate buffer (pH 9), and were given in units of mg NH₄⁺-N and *p*-nitrophenol produced per g soil per 24 h, respectively. All these results were expressed on an oven-dried soil weight basis by correcting for water content in the soil (105°C, 24 h).

Statistical analysis. The means and standard deviations of the four replicates were computed. Analysis

of Variance was carried out using both One-Way and Two-Way ANOVA procedure with SPSS software (Version 13.0, 2001). The comparison of mean effects was based on least significant difference (LSD) test ($P < 0.05$). Redundancy analysis (RDA), a multivariate direct gradient analysis method, was performed using Canoco software (Version 4.5, 2002) to elucidate the relationships between plant parameters, soil properties, and experimental treatments.

RESULTS

Mycorrhizal colonization, vegetative biomass, root-to-straw ratio, and grain yield. Mycorrhization in wheat roots was shown in two *G. caledonium*-inoculated treatments (Figure 1a), and the amendment of maize straw had no significant effects on the colonization rate. There were no significant differences in wheat straw biomass among the 4 treatments (Figure 1b), while both the root biomass and the root-to-straw biomass ratio were significantly ($P < 0.05$) affected by either inoculation of *G. caledonium* or amendment of maize straw, and differed in the following order: S < S + M and control < M (Figures 1b and 1c). Compared to the control, wheat grain yield was significantly ($P < 0.05$) elevated only with the combined application of *G. caledonium* and maize straw (Figure 1d).

Soil pH, basic nutrient contents, and key enzyme activities. Compared to the control, the inoculation of *G. caledonium* had no significant effects on soil pH, organic C content, and the activities of invertase, urease, and alkaline phosphatase (Table 1), while the amendment of maize straw significantly ($P < 0.05$) decreased soil pH, and significantly ($P < 0.05$) increased soil invertase and alkaline phosphatase activities, but had no significant effects on soil organic C content and urease activity as well. The combined application of *G. caledonium* and maize straw significantly ($P < 0.05$) increased soil organic C content and urease activity compared to the control, and also significantly ($P < 0.05$) decreased soil pH compared to the S treatment, but did not affect soil invertase and alkaline phosphatase activities in relation to the S treatment. In addition, the inoculation of *G. caledonium* and/or the amendment of maize straw had no significant effects on soil total N and total P contents.

Two-Way ANOVA of maize straw amendment and AM fungal inoculation. The Two-Way ANOVA results of maize straw amendment and AM fungal inoculation are shown in Table 2. On the one hand, maize straw amendment systematically affected wheat root

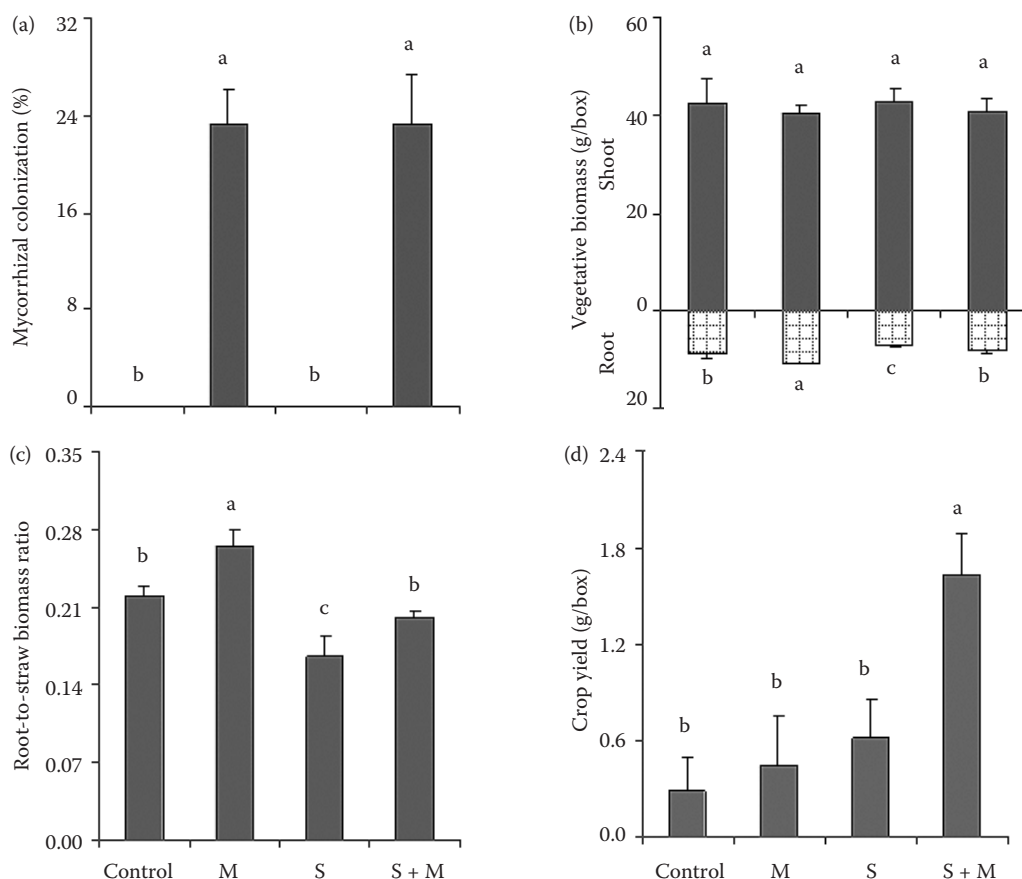


Figure 1. Mycorrhizal colonization (a), vegetative biomass (b), root-to-straw ratio (c), and crop yield (d) of wheat plant under different treatments; control – without inoculation/amendment; M – inoculation with AM fungi; S – amendment with maize straw; S + M – amendment with maize straw plus inoculation with AM fungi; vertical T bars indicate standard deviations; bars not topped by the same letter indicate a significant difference in values ($P < 0.05$)

biomass ($P < 0.001$), root-to-straw ratio ($P < 0.001$), grain yield ($P < 0.01$), soil pH ($P < 0.001$), organic C content ($P < 0.05$), invertase activity ($P < 0.001$), urease activity ($P < 0.01$), and alkaline phosphatase activity ($P < 0.001$). On the other hand, AM fungal inoculation systematically affected wheat root biomass ($P < 0.001$), root-to-straw ratio ($P < 0.001$),

grain yield ($P < 0.001$), and soil pH ($P < 0.01$). Furthermore, interactive effects of these two factors were also observed in wheat grain yield ($P < 0.01$), as well as in soil pH ($P < 0.05$).

Redundancy analysis (RDA) of treatments, and soil and plant parameters. In the RDA ordination plot (see Figure 2), projecting an object (treatment)

Table 1. Soil pH, basic nutrient contents, and key enzyme activities

Treatment	pH (H ₂ O)	Organic C	Total N	Total P	Invertase	Urease	Alkaline phosphatase
		(g/kg)			(mg/(g 24h))		
Control	8.57 (0.03) ^A	4.29 (0.35) ^B	0.38 (0.06) ^A	0.56 (0.02) ^A	4.23 (0.23) ^B	0.99 (0.07) ^B	0.13 (0.01) ^B
M	8.55 (0.02) ^A	4.57 (0.33) ^{AB}	0.36 (0.02) ^A	0.58 (0.02) ^A	3.85 (0.23) ^B	1.01 (0.15) ^B	0.14 (0.02) ^B
S	8.47 (0.05) ^B	4.79 (0.25) ^{AB}	0.38 (0.04) ^A	0.56 (0.03) ^A	5.33 (0.07) ^A	1.12 (0.06) ^{AB}	0.17 (0.01) ^A
S + M	8.35 (0.05) ^C	5.07 (0.61) ^A	0.42 (0.03) ^A	0.61 (0.04) ^A	5.42 (0.26) ^A	1.22 (0.07) ^A	0.19 (0.01) ^A

Control – without inoculation/amendment; M – inoculation with AM fungi; S – amendment with maize straw; S + M, amendment with maize straw plus inoculation with AM fungi; standard deviations are given in parentheses; values within the same column not followed by the same letter differ significantly ($P < 0.05$)

Table 2. Two-Way ANOVA results of maize straw amendment and AM fungal inoculation

	Maize straws (S)	AM fungi (M)	S × M
Wheat root biomass	62.680***	25.503***	0.861
Wheat straw biomass	0.016	1.380	0.000
Root-to-straw ratio	84.768***	38.147***	0.477
Wheat grain yield	35.259**	20.829***	11.201**
Soil pH	44.896***	10.426**	5.619*
Soil organic C	6.076*	1.827	0.000
Soil total N	0.174	2.014	2.014
Soil total P	4.856	0.920	0.356
Soil invertase	120.451***	1.407	3.695
Soil urease	11.437**	1.187	0.652
Soil alkaline phosphatase	30.533***	1.374	0.252

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

at right angle on a soil or plant variable approximates the value of the object along that variable; the angles between soil and plant variables reflect their correlations, and the relationship between the centroid of a qualitative explanatory variable and a response variable is found by projecting the centroid at right angle on the variable. Compared to the control, the M treatment mostly accelerated root biomass and root-to-straw ratio of wheat, and the former significantly correlated to the latter ($r = 0.999$, $P < 0.01$), while the S+M treatment influenced

soil pH, enzyme activities, organic C content, and crop yield more than the S treatment, which negatively affected wheat root biomass. Both crop yield and soil organic C content negatively correlated to soil pH ($r = -0.971$ and -0.955 , $P < 0.05$). Soil urease activity significantly correlated to soil alkaline phosphatase activity ($r = 0.993$, $P < 0.01$), and both of them significantly correlated to soil organic C content ($r = -0.962$ and 0.977 , $P < 0.05$), and thus negatively correlated to soil pH ($r = -0.993$, $P < 0.01$ and $r = -0.976$, $P < 0.05$).

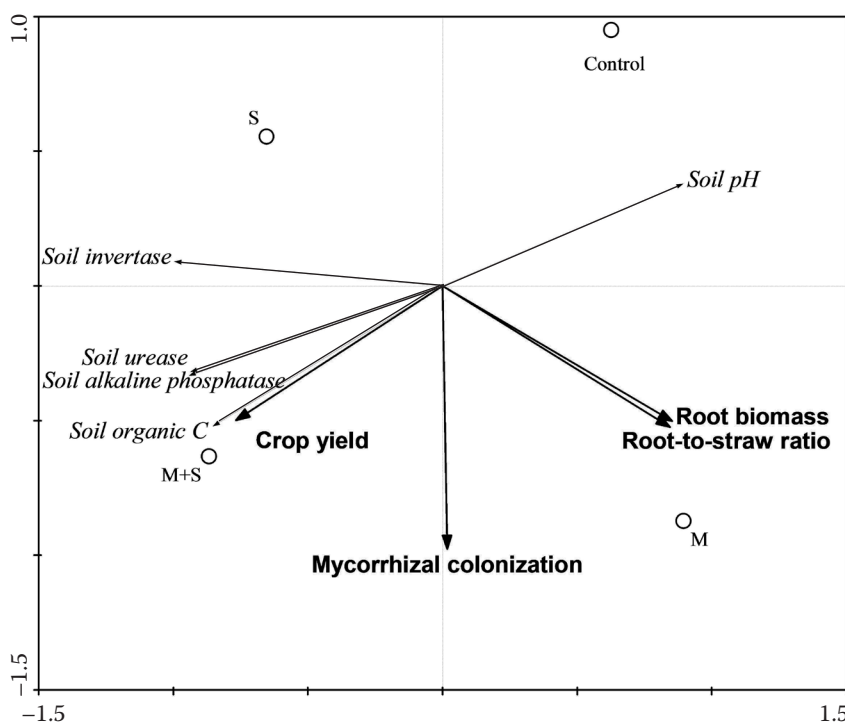


Figure 2. Redundancy analysis (RDA) of soil properties with plant variables under different treatments; control – without inoculation/amendment; M – inoculation with AM fungi; S – amendment with maize straw; S + M – amendment with maize straw plus inoculation with AM fungi

DISCUSSION

The objectives of this study were to investigate the interactive effects of AMs and maize straws on wheat growth and soil organic C storage, and to find out their possible mechanisms. Compared to the control, wheat grain yield was elevated only with the combined application of AMs and maize straw (Figure 1d), but the amendment of maize straw had no significant effects on mycorrhizal colonization rate (Figure 1a). Mechanisms causing increased grain yield upon AM fungal inoculation in straw-amended soils are not fully understood, but may be due in part to the rhizosphere acidification (Table 1) and enhanced nutrient acquisition (HU *et al.* 2010). Plant roots alter rhizosphere soil pH by production or consumption of H⁺ or by exudation of organic acids, and thereby induce changes in nutrient availability (LI *et al.* 1991). Such an effect is possibly enhanced by AM fungal inoculation (BAGO *et al.* 1996; HU *et al.* 2010). The Two-Way ANOVA also indicated the interactive effects of AM fungi and maize straw on soil pH (Table 2). However, soil pH does not only play an important role in nutrient availability in soils and uptake by plants, but also influences plant growth and grain yield accordingly. Therefore, the results indicated the potential application of AM fungi in facilitating crop growth in straw-returned fields. It is noteworthy that although rational combination of AM fungi with crop straw can be effective to some extent, the applying strategy still needs an in-depth study.

The inoculation of *G. caledonium* increased the root biomass and the root-to-straw biomass ratio of wheat, regardless of the amendment of maize straw (Figure 1b). Therefore, the trend towards higher soil organic C content upon AM fungal inoculation should be due to the increased distribution of photosynthate in underground structures, since mycorrhizal roots could release more root exudates than non-mycorrhizal roots because of the larger root system and/or improved nutrition (HU *et al.* 2010). While the trend towards higher soil organic C content upon maize straw amendment should be due to the direct input of C sources, which favour plant growth and improve soil organic C content as well (ALBIACH *et al.* 2000). It is the size and activity of soil microbe/enzyme that regulates C accumulation via mineralization and immobilization of plant and microbially derived residues in the soil (ZHU & MILLER 2003). For example, the activities of soil invertase, urease, and alkaline phosphatase were all greatly elevated

upon maize straw amendment (Tables 1 and 2), similarly to the enhancements reported from long-term field experiments upon organic amendment (HU *et al.* 2010, 2011). Nevertheless, some organic acids are reported as toxic to crop development (notably root) at high concentrations (ARMSTRONG & ARMSTRONG 2001), while the production can be enhanced by incorporation of readily decomposable organic matter (DE DATTA 1981), such as maize straw in this study. It appears obvious that wheat root growth was declined with maize straw amendment (Figures 1b and 2), which might be explained by the potential production of organic acids from applied straws. However, although no interactions were observed between maize straw amendment and AM fungal inoculation in soil organic C storage (Table 2), the combined application of AM fungi and maize straws still revealed the strongest influences on soil organic C content (Table 1), which might be explained by the multitude of factors that may act in different but additive ways, such as direct C sources and crop root exudates.

In addition, although the effects of AM fungal inoculation and maize straw amendment on total soil organic C pool and wheat growth have been well evaluated in this study, less is known about the responses of soil organic C fractions to such treatments. Since soil organic C fractions with variable physical and biochemical properties are characterized by differential stabilities and turnover rates (BALDOCK *et al.* 1997), it is needed to examine the effects of agricultural management on soil organic C fractions to assess whether the sequestered C can be stored in the long-term (HUANG *et al.* 2010). In fact, physical fractionation techniques have been employed to separate soil organic C fractions that stabilize C at a long time scale and thus have important implications for soil C sequestration and the mitigation of climate change (JOHN *et al.* 2005). Previous studies have demonstrated that micro-aggregate-associated C played a substantial role in soil organic C stabilization (BALESDENT *et al.* 2000; DENEFF *et al.* 2007), and the stabilization of particulate organic matter within microaggregates was one of the major mechanisms for soil organic C protection (ZOTARELLI *et al.* 2007). In the future study, we thereby need to take into account that only a part of the mentioned C increase (Table 1) can be stabilized. The increase tendency may be related to the increase of the light fraction of organic C compounds, especially root exudates, which may only exhibit a seasonal fluctuation.

CONCLUSION

Both AM fungal inoculation and maize straw amendment tended to increase soil organic C content, and seemed to be due to the increased distribution of photosynthate in underground structures and the direct input of C sources, respectively. The inoculation of AM fungus greatly increased wheat grain yield and soil organic C content in maize straw-amended soils, and seemed to be due to enhanced rhizosphere acidification and nutrient acquisition. Our results suggested the potential of AM fungi in facilitating crop growth and C storage in straw-returned fields, and future study is needed to detect the part of the mentioned C increase that is stabilized.

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