Arbuscular Mycorrhizal Colonization Increased Above-Belowground Feedback of Maize In a Degraded Coal Mining Area Soil By Increasing The Photosynthetic Carbon Assimilation And Allocation of Maize

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Abstract

A three-compartment culture system was used to study the mechanism by which the AM fungus *Funneliformis mosseae* influences host plant growth and soil organic carbon (SOC) content in a coal mining area. A $^{13}\text{CO}_2$ pulse tracing technique traced the allocation of maize photosynthetic C in shoots, roots, AM fungus and soil to detect C accumulation and allocation in mycorrhizal (inoculated with *Funneliformis mosseae*) and non-mycorrhizal treatments. AM fungal inoculation significantly increased the ^{13}C concentration and content in both above- and below-ground plant parts. Mycorrhizal inoculation significantly enhanced the anti-aging ability by increasing soluble sugars and catalase activity (CAT) in maize leaves while reducing foliar malondialdehyde content (MDA) and leaf temperature to promote plant growth. AM fungi also increased P uptake to promote maize growth. Soil organic carbon (SOC), glomalin, microbial biomass carbon (MBC) and nitrogen (MBN) contents increased significantly after inoculation. A mutually beneficial system was established involving maize, the AM fungus and the microbiome, and the AM fungus became an important regulator of C flux between the above- and below-ground parts of the system. Inoculation with the AM fungus promoted plant growth, C fixation and allocation belowground to enhance soil quality. The positive above-belowground feedback appeared to be established.

Introduction

In a natural system there is positive above-belowground feedback that is essential for a healthy ecosystem (Mariotte et al. 2018). However, some artificial practices such as coal mining disrupt this and lead to environmental degradation (Rocha-Nicoleite et al. 2017). It is therefore important to devise methods for the ecological restoration of mining areas (De and Mitra 2002; Shrestha and Lal 2006). Inoculation with arbuscular mycorrhizal fungi has been demonstrated to be an effective biotic method to achieve ecological restoration of degraded mining areas (Bi et al. 2018 and 2019; Vahter et al. 2020; Wang 2017). However, how the AM fungal inoculation influences the positive above-belowground feedback of mining areas remains unclear. The soil organic carbon (SOC) pool is an important part of soil fertility, productivity and quality (Schlesinger 1999; Wiesmeier et al. 2019). Changes in SOC are considered an indicator of feedback effects (Kirschbaum 2000; Liu et al. 2019). Carbon is derived mainly from the primary net productivity of higher plants and the allocation of photosynthates belowground (Malhi et al. 2011). A large portion of the C fixed by photosynthesis is distributed directly to plant roots and root-associated soil microorganisms which indirectly affect soil C sequestration (Zhu and Miller 2003; Heinemeyer et al. 2007; Dijkstra and Cheng 2007). Microbially mediated plant-soil feedback affects plant growth and community assembly (van der Heijden et al. 1998; Kardol et al. 2007). Arbuscular mycorrhizal (AM) fungi can form a potentially symbiotic mutual association with the roots of 80% of terrestrial plant species, accelerate the growth of host plants, and promote plant photosynthetic C fixation (Rodrigues and Rodrigues 2014) through enhancing nutrition uptake, water supply and C source-sink relations (Bethlenfalvay et al. 1982 and 1988; Andrade et al. 1998; Black et al. 2000; Baier et al. 2010). In return, AM fungi store a portion of the photosynthetic products for their own growth (Godbold et al. 2006; Kiers et al. 2011). Four to twenty percent of plant photosynthetic products are transferred to the AM fungi to build hyphae that extend into the soil and influence soil C storage (Bago et al. 2000; Godbold et al. 2006). A ^{13}C labelling experiment has found that AMF received 4.3% plant fixed C in 24h (Tomè et al. 2015). Kaiser et al. (2015) found that mycorrhizal fungi directly transported C from plant photosynthetic products to the soil and even to soil microbes (Kaiser et al. 2015). AM fungi therefore plays an important role in C cycling from above- to below-ground.

Previous studies show that AM fungi affect the association between roots and soil by increasing nutrient uptake and the resistance of host plants to a range of environmental stresses and by promoting plant growth and development (Evelin et al. 2009; Hajiboland et al. 2010; Lee et al. 2012; Klein et al. 2016). A number of mechanisms are involved. Mycorrhizal inoculation can increase the chlorophyll content and the rate of photosynthesis and further increase plant C accumulation (Talaat and Shawky 2014). Plants inoculating with AM fungi show decreased H_2O_2 and increased malondialdehyde (MDA) contents compared to non-AM plants and increased soluble proteins and sugars, thereby increasing both resistance to environmental stress and the productivity of the host plants (Abdel Latef et al. 2016). Furthermore, inoculation facilitates the acquisition of mineral nutrients, especially P (Řezáčová et al. 2018). These studies indicate that AM fungal inoculation influences plant physiological traits and promotes C sequestration by host plants (Miller et al. 2002; Zhu and Miller 2003). In return, photosynthate is allocated belowground. An increase in root biomass may increase the space for colonization by AM fungi while AM inoculation decrease the root-to-shoot ratio through ameliorating plant nutrient status (Veresoglou et al. 2012). In addition, the AM fungi produce hyphae that extend into the soil and then exert important effects on the soil environment (Zhu and Miller 2003; Godbold et al. 2006). Hyphae, spores and vesicles of AM fungi are widely present in soils and the extraradical biomass can form up to 32% of the total soil microbial biomass, contributing up to 15% of SOC (Miller and Kling 2000). Some studies suggest an increased or accelerated photosynthetic down-regulation with AMF under greenhouse conditions (Goicoechea et al. 2014). Establishment of mycorrhizal symbiosis often increases allocation of C to the roots and further to the mycorrhizal fungi (Řezáčová et

al. 2017). As feedback, the roots and AM fungi exude sugars and organic acids to the soil and thus to soil microorganisms, which may be activated to metabolize accumulated soil mineral nutrients for the growth of the host plants (Zhang et al. 2016). When hyphae die, a portion of their C is rapidly decomposed by other microorganisms and converted to CO₂ which may enter the atmosphere or some recalcitrant organic compounds such as chitin and glomalin that can remain in the soil for years to decades (Gleixner et al. 2002; Wilson et al. 2009; Smith and Read 2010; Treseder 2013). In addition, the contribution of AM fungi to the C cycle also depends on both the ERH and exudates from AM fungal hyphae such as glomalin which can contribute to soil C aggregation and soil quality enhancement (Wright and Upadhyaya 1996; Tisdall et al. 1997; Zhu and Miller 2003; Zhang et al. 2016). However, several research showed that plant C allocated to hyphae was high enough to cause the depression of host plants in some experiments especially in nutrient-limited areas or the establishment stages of roots and hyphae (Jakobsen 1999; Gavito et al. 2019).

It is important to promote vegetation restoration and soil organic matter accumulation for a mining area ecological restoration, understand the mechanisms by which AM fungi promote soil C storage is helpful. However, most studies have been conducted under field conditions and factors such as soil heterogeneity and climatic variability have had a substantial impact on the results. Recent efforts have been made to quantify how AM associations may affect the overall C balance and C fluxes in different types of photosynthetic metabolism using stable ¹³C isotopes, but have ignored how much C the AM fungi receive because of the paucity of available methods to quantify this directly (Lendenmann et al. 2011; Slavíková et al. 2017; Řezáčová et al. 2017 and 2018). In previous studies, different results were got under different nutritional conditions, host plants and growth stages. Quantification of the contribution of AM fungi to the exchange of photosynthetic products between above- and below-ground pools have been based mainly on δ¹³C and C% value, methods which cannot give a direct indication of the allocation of primary products. Here, a greenhouse experiment was conducted and ¹³CO₂ pulse labeling was used to quantify the effects of AM inoculation on the allocation of photosynthetic products above- and below-ground and the effects of AM fungal inoculation on C storage in plants and soil. The effects of hyphae on C allocation were studied using three-compartment microcosms to collect AM fungal biomass and then calculate the amount of ¹³C in the biomass. The work aimed to unveil the mechanisms by which AM fungi promote C sinks in plants and soil under controlled conditions, and to provide a theoretical basis for the role of AM fungi in C storage in the soil and in photosynthate allocation above- and below-ground in a nutrient-poor soil from a coal mining area in northwest China.

Materials And Methods

Soil, plants and AM fungus

Sandy soil collected from Daliuta mining area, Yulin, Shanxi Province, China was sieved (2 mm), sterilized at 121°C and 103 kPa for 2 h and air-dried for three days before use. Selected soil physicochemical properties were: available phosphorus (AP, NaHCO₃ extracted), 2.97 mg kg⁻¹; available potassium (AK), 12.9 mg kg⁻¹; organic matter content, 1.25 g kg⁻¹; pH, 7.39; conductivity, 834 μS cm⁻¹; and water holding capacity, 18.6%. The low AP, AK and conductivity indicate a nutrient-limited environment for plants. Maize (*Zea mays* L., cultivar Nuoyu 2) was selected as the host plant and seeds were provided by the Seeds Company of the Chinese Academy of Agricultural Sciences. They were surface sterilized by immersion in 10% (v/v) H₂O₂ for 10 min, rinsed several times with distilled water and germinated at 25°C for 48 h in the dark. The AM fungal inoculum, *Funneliformis mosseae*, was provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China and propagated at the State Key Laboratory for Coal Resources and Safe Mining, China University of Mining and Technology (Beijing). The inoculum consisted of spores (2000 spores per 100 g inoculum), external hyphae and mycorrhizal root fragments.

Experimental design and management

The experiment was conducted using 55 × 28 × 26 cm three-compartment microcosms (Fig. 1a). On the left was the root compartment (RC), in the middle the buffer zone (BC) and on the right the hyphal compartment (HC). We used a 1-mm nylon mesh to allow hyphae and fine roots (but not coarse roots) into the BC in order to avoid the roots extending to the HC and ensuring enough hyphae in the HC for collection. The BC and HC were separated by 30-μm pore nylon mesh to allow hyphae in and exclude all roots. The soil in the RC and BC was sterilized sandy soil sieved to 2 mm and the medium in the HC was 0.8-1.2 mm glass beads immersed in 5% HCl for 24 h and washed with distilled water several times before use to collect clean hyphae. There were 25 kg, 5 kg and 22 kg soil, respectively, in the RC, BC and HC of each pot. Two treatments were established, mycorrhizal (inoculated with *F. mosseae*) and non-mycorrhizal controls (CK). One hundred of inoculum was applied to the root compartment of the mycorrhizal treatment by layering and the non-

mycorrhizal treatment received 100 g sterilized inoculum. the effects of AM fungi on maize growth, stress resistance and C allocation were determined compared with the control. There were three replicates of each treatment and a total of six microcosms were used.

We added 100 mg N (as NH_4NO_3), 30 mg P (as KH_2PO_4), and 150 mg K (as K_2SO_4) per kg to the RC to meet the basic nutrient demand of maize at the seedling stage, watered the soil to maximum water holding capacity and then preconditioned the soil for 24 h before transplanting. Maize seeds were sown in plastic seedling pots and then transplanted to the RC at the three-leaf stage on April 5th, 2017. There were two maize plants in each RC. An EM50 data logger (ICT International, Armidale, NSW, Australia) was used daily to maintain the soil moisture content within 75 to 80% of the water holding capacity. Deionized water (100 ml) was added to the HC every day plus 100 ml 1/10 Hoagland's nutrient solution every three days to ensure hyphal growth. Maize growth was maintained by adding 50 mg N (as NH_4NO_3), 15 mg P (as KH_2PO_4), and 75 mg K (as K_2SO_4) to the RC soil 25 days after transplanting to alleviate plant nutrient deficiency.

$^{13}\text{CO}_2$ pulse labeling chamber and procedure

Previous studies indicate that ^{13}C from photosynthesis may be transferred to each plant part, AM fungi and soil and even to soil microbes within one day (Kuzyakov and Cheng 2004; Kaiser et al. 2015). $^{13}\text{CO}_2$ stable isotope pulse labeling for three days starting at the tasseling stage 63 days after transplanting was used to trace the distribution of maize photosynthetic C among plant parts, AM fungus and soil. The labeling chamber was $110 \times 110 \times 75$ cm in size (Fig. 1b). The mycorrhizal treatments and non-mycorrhizal controls were randomly placed in two labeling chambers with good light transmission and repositioned randomly each day to minimize variation due to the labeling process. LI-6400 handheld probes (LI-COR Biosciences, Lincoln, NE; to probe the real time concentration of CO_2), air fans, silica gel and thermometers (to determine the real-time temperature) were placed in the airtight chambers before labeling as shown in Fig. 1b. The $^{12}\text{CO}_2$ in the labeling chamber was consumed by placing maize pots in the glasshouse chambers at 08:00 in the morning. When the CO_2 concentration in the labeling chambers was ≈ 50 ppm at 09:00 the $^{13}\text{CO}_2$ (atom $> 99.99\%$) gas was injected at 30-min intervals into the chambers to maintain a constant $^{13}\text{CO}_2$ concentration of 300-450 ppm. At 15:00 $^{13}\text{CO}_2$ gas was injected for the last time and the concentration of $^{13}\text{CO}_2$ was ≤ 450 ppm. The labeling chambers were opened at 17:00 when labeling ended to ensure maize respiration during the night. The experiment was conducted in Beijing at China University of Mining and Technology (40°N , 116°E). The weather was sunny and the temperatures on the three ^{13}C -tracing days were, respectively, 40.7, 37.5, and 37.3 $^\circ\text{C}$.

Sampling and measurement

The photosynthetic rates of the third and fourth fully expanded leaves (from the top) were measured at 10:00 one day before labeling to test the effects of the AM fungus on plant photosynthetic ability. An LI-6400 handheld probe (Licor Biosciences, Lincoln, NE) was used and the built-in red and blue light source, with a red light photosynthetic effective radiation flux density of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ was used. The leaf chlorophyll content was measured with a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan; Ling et al. 2011). Leaf starch and soluble sugar contents (fresh samples) were determined by anthrone colorimetry (Leach and Braun 2016) to determine plant stress resistance. Soluble protein was determined by the Coomassie brilliant blue G-250 staining method (Murphey et al. 1989) and malondialdehyde (MDA) content by the thiobarbituric acid (TBA) method (Schmedes and Hølmer 1989). Catalase (CAT) activity was determined by the potassium permanganate titration method (Chen et al. 2015). The plant and soil samples were collected after three days of labeling. The plants were divided into six parts to determine the allocation of carbon among plant parts, namely young leaves (leaves not fully expanded), intermediate leaves, senescent leaves (leaves with yellow edges and signs of senescence), dry leaves (completely withered leaves), stems, and roots. Leaf areas and shoot and root biomass were determined. All plant parts were oven-dried to constant weight and ground to 0.15 mm. Shoot P and K concentrations were determined by digestion with $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$ and analysis using ICP-AES (Thomas et al. 1967). Rhizosphere soil was sampled by the quartering method and then passed through a 2-mm sieve. The soil was divided into two portions to determine the carbon allocation to the soil and the effects of the AM fungus on soil properties. One portion was stored at 4°C to determine microbial biomass and the remainder was air-dried outdoors. Wet sieving was used to collect hyphae. The glass beads in the hyphal compartment were transferred to buckets and washed five times with distilled water. Glass rods were used to mixed the glass beads and water. The supernatant was immediately passed through a 30- μm pore sieve to collect the hyphae. The AM fungal hyphae were dried at 80°C , ground to 0.15 mm and homogenized before analysis.

The carbon allocation, $\delta^{13}\text{C}$ value and C% content of each plant part and soil and hyphal samples were fully quantified using a Deltaplus XP mass spectrometer (Thermo Fisher, Waltham, MA) and an elemental analyzer at the stable isotope laboratory of the Chinese Academy of Agricultural Sciences. The accuracies of measurement of C content and C isotopic composition were 0.1% and 0.2‰, respectively (Deniro and Epstein 1978).

Root segments were stained with 0.05% Trypan blue and examined under a microscope (Nikon, Tokyo, Japan) to determine the percentage of root length colonized (Phillips and Hayman 1970). The mycorrhizal colonization in every compartment was calculated as the number of mycorrhizal root segments/total number of root segments examined $\times$ 100%, which may be described as the percentage of root length colonized or the colonization frequency. The hyphal length density was determined by the method of Jakobsen et al. (1992). SOC content was determined by standard dichromate oxidation (Bremner and Jenkinson 1960). Briefly, the easily extractable soil glomalin (EEG) was extracted with alkaline citrate (20 mM, pH 7) by autoclaving for 30 min (121°C) followed by centrifuging at 4000 rpm for 5 min and collecting the supernatant. The supernatant was stained with Coomassie brilliant blue G-250 and detected by UV spectrophotometry. Total glomalin (TG) was extracted with alkaline citrate (50 mM, pH 8) by autoclaving for 90 min (121°C) and centrifuging at 4000 rpm for 5 min. The cycle of extraction and centrifugation was repeated four times until the supernatant was almost transparent. The supernatants extracted each time were combined and estimated as TG (Wright and Upadhyaya 1996). AP and AK, respectively, were extracted with 0.5 M NaHCO_3 and 1 M ammonium acetate (Zebec et al. 2017) and then determined by ICP-AES. Soil microbial biomass C (MBC) and soil microbial biomass N (MBN) were determined by chloroform fumigation and extraction with 0.5 M K_2SO_4 solution. MBC was determined using a total C analyzer and MBN using an automatic Kjeldahl nitrogen analyzer (Nguyen et al. 2016).

Data calculations

Total area of maize leaves (only green leaves) was calculated by the following equations.

S_i = leaf length $\times$ maximum leaf width $\times$ 0.75 (1a)

$$S = \sum_i^1 S_i(1b)$$

where S_i is area of a single leaf i and S is the total leaf area.

^{13}C distribution rate in each plant part:

$$\delta^{13}\text{C} = \frac{R_{\text{samples}} - R_{\text{PDB}}}{R_{\text{PDB}}} \times 1000(2)$$

R , ^{13}C concentration/ ^{12}C concentration; PDB, Peedee belemnite in the Cretaceous Peedee formation, South Carolina, $R_{\text{PDB}} = (11237.2 \pm 90) \times 10^{-6}$.

$$C\% = \frac{13 \times ^{13}\text{C} + 12 \times ^{12}\text{C}}{M} \times 100(3)$$

M , dry weight of samples; ^{13}C , the ^{13}C content in samples; ^{12}C , the ^{12}C content in samples

$$c(^{13}\text{C}) = \frac{13 \times \frac{C\%}{100}}{\frac{12 \times 1000}{(\delta^{13}\text{C} + 1000) \times R_{\text{PDB}}}}(4)$$

$c(^{13}\text{C})$, indicates the ^{13}C concentration in each plant and soil part

$$m(^{13}\text{C}) = \frac{m \times \frac{C\%}{100}}{13 + \frac{12 \times 1000}{(\delta^{13}\text{C} + 1000) \times R_{\text{PDB}}}} \times 13(5)$$

$m(^{13}\text{C})$, ^{13}C content; indicates the ^{13}C weight in each plant and soil part

The mycorrhizal contribution of each plant biomass and shoot P and K nutrition (MC%):

$$MC\% = \frac{\text{index}(\text{inoculated}) - \text{index}(\text{CK})}{\text{index}(\text{inoculated})} \times 100\% \quad (6)$$

Data analysis

All results in the Tables and Figures are mean value $\pm$ standard error. The mean values of all data were compared to determine the difference between the inoculated treatment and uninoculated control. Student's t-test was used to determine the effects of inoculation at $P < 0.05$ using the IBM SPSS 20.0 software package (IBM, Armonk, NY). The root mycorrhizal colonization data were normalized by arcsine-transformation before statistical analysis.

Differences between mycorrhizal treatment and non-mycorrhizal control of mycorrhizal colonization, hyphal length density, shoot and root biomass, P and K concentration, SPAD value, total leaf area, MDA, CAT, soluble protein, soluble sugar, and starch were compared by Student's t-test at $P < 0.05$. The differences in photosynthetic indexes and soil physical and chemical properties between mycorrhizal and non-mycorrhizal treatments were compared by Student's t-test. Differences in $\delta^{13}\text{C}_{\text{C}\%}$, ^{13}C concentration and ^{13}C content in plants and soil samples between mycorrhizal and non-mycorrhizal treatments were also examined using Student's t-test.

Results

Mycorrhizal colonization, hyphal length density and plant biomass and P and K concentrations

As shown in Table 1 the roots in the inoculated treatment were colonized by *F. mosseae* in both the root and buffer compartments in which the root mycorrhizal colonization rate reached 77 and 81%, respectively. The hyphal length density was 1.56 m g^{-1} soil in the root compartment with no colonization or mycelium found in the non-mycorrhizal control. Both mycorrhizal colonization and hyphal length density indicate that the AM fungus colonized the maize roots and the hyphae extended into the soil. Shoot, root and total biomass values were significantly higher in the mycorrhizal treatment than in the non-mycorrhizal control. The mycorrhizal contributions to shoot, root and total biomass were 13.6, 25.4 and 17.3%, respectively. In addition, AM fungal inoculation significantly increased shoot P concentrations and contributed 45.4%, which was 3.5 mg g^{-1} in mycorrhizal treatment but only 1.9 mg g^{-1} in non-mycorrhizal treatment. There was no influence observed on K concentrations (Table 1).

Table 1

Mycorrhizal colonization, hyphal length density, maize biomass and mycorrhizal contribution. Values are mean values $\pm$ standard errors. Different lowercase letters represent significant differences between Myc (inoculated treatment) and Ctrl (uninoculated control) at $P < 0.05$. RC, root compartment; BC, buffer compartment; and MC%, mycorrhizal contribution.

Treatment	Mycorrhizal colonization (%)		Hyphal length density (m g^{-1})	Shoot biomass (g plant^{-1})	Root biomass (g plant^{-1})	Total biomass (g plant^{-1})	Shoot P concentration (mg g^{-1})	Shoot K concentration (mg g^{-1})
	RC	BC						
Myc (<i>F. mosseae</i>)	76.67 $\pm$ 3.33	81.11 $\pm$ 3.85	1.56 $\pm$ 0.05	20.5 $\pm$ 0.77 ^a	9.46 $\pm$ 0.28 ^a	30.0 $\pm$ 0.85 ^a	3.50 $\pm$ 0.32 ^a	80.7 $\pm$ 9.43
Control	None	None	None	17.7 $\pm$ 0.48 ^a	7.06 $\pm$ 0.32 ^b	24.8 $\pm$ 0.45 ^b	1.91 $\pm$ 0.34 ^b	73.3 $\pm$ 8.34
MC%	None	None	None	13.6	25.4	17.3	45.4	9.15

Physiological characteristics and photosynthetic indices of maize

Inoculation with *F. mosseae* increased plant resistance to environmental stress and the photosynthetic efficiency. As shown in Table 2, inoculation significantly increased CAT activity and the soluble protein, soluble sugar and starch contents of leaves ($P < 0.05$), by $2 \text{ U g}^{-1} \text{ FW min}^{-1}$, 0.02 mg g^{-1} , 0.5% and 0.11% , respectively. MDA, which can indicate plant senescence, also decreased significantly in

the mycorrhizal treatment. In addition, inoculation significantly promoted plant photosynthetic ability. Furthermore, the Pn significantly increased from 31.0 to 33.5 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ because of AM fungal inoculation (Table 3). Inoculation also increased plant resistance to high temperatures by increasing the Tr and decreasing leaf temperature (Table 3). Inoculation decreased the Ci owing to decreased respiration and increased photosynthesis. Thus, inoculation promoted both resistance of maize to stress and photosynthetic C fixation.

Table 2

Physiological characteristics of maize in the mycorrhizal and non-mycorrhizal treatments. Values are the mean values $\pm$ standard errors. Different lowercase letters represent significant differences between Myc (inoculated treatment) and Ctrl (uninoculated control) at $P < 0.05$. SPAD, leaf chlorophyll content; MDA, malondialdehyde content; CAT, catalase activity. Pn: net photosynthetic rate; Gs: stomatal conductance; Ci: intercellular CO_2 concentration; Tr: transpiration rate; T: leaf temperature.

Treatment	SPAD	Total leaf area (m^2)	MDA ($\mu\text{mol g}^{-1}$)	CAT ($\mu\text{g g}^{-1} \text{ FW min}^{-1}$)	soluble protein (mg g^{-1})	soluble sugar (%)	Starch (%)
Mycorrhizal	38.2 $\pm$ 0.56 ^a	0.15 $\pm$ 0.01 ^a	6.16 $\pm$ 1.31 ^b	4.93 $\pm$ 0.64 ^a	0.22 $\pm$ 0.01 ^a	1.02 $\pm$ 0.09 ^a	0.58 $\pm$ 0.02 ^a
Control	33.7 $\pm$ 1.00 ^b	0.13 $\pm$ 0.00 ^b	15.7 $\pm$ 0.10 ^a	2.95 $\pm$ 0.21 ^b	0.20 $\pm$ 0.00 ^b	0.70 $\pm$ 0.00 ^b	0.47 $\pm$ 0.02 ^b

Table 3

Photosynthetic indices of maize. Values are mean values $\pm$ standard errors. Different lowercase letters represent significant differences between Myc (inoculated treatment) and Ctrl (uninoculated control) at $P < 0.05$. Pn, net photosynthetic rate; Gs, stomatal conductance; Ci, intercellular CO_2 concentration; Tr, transpiration rate; T, leaf temperature.

Treatment	Pn ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Gs ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	Ci ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Tr ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	T ($^{\circ}\text{C}$)
Mycorrhizal	33.5 $\pm$ 0.58 ^a	0.042 $\pm$ 0.00 ^a	84.8 $\pm$ 9.07 ^a	4.39 $\pm$ 0.11 ^a	32.7 $\pm$ 0.18 ^b
Control	31.0 $\pm$ 1.44 ^b	0.046 $\pm$ 0.00 ^a	91.2 $\pm$ 1.89 ^a	4.14 $\pm$ 0.10 ^b	33.27 $\pm$ 0.20 ^a

¹³C assimilation and allocation

AM fungal inoculation significantly influenced the $\delta^{13}\text{C}$, C%, ^{13}C concentration and ^{13}C content of intermediate leaves, roots, soil and hyphae at $P < 0.05$ and significantly increased the C% of the soil (Fig. 2). The ^{13}C concentration and content of maize and soil were significantly higher in the mycorrhizal treatment than in the non-mycorrhizal control, indicating that the AM fungus promoted C fixation in both maize and soil. The ^{13}C concentration of maize followed the sequence: young leaves > stems > intermediate leaves > senescent leaves > roots. The ^{13}C concentration in the hyphae of the AM fungus was about $100 \mu\text{g g}^{-1}$, equivalent to that in the intermediate and senescent leaves of maize and much higher than in the roots (Fig. 2c). The sum of ^{13}C fixation in above- and below-ground parts in the mycorrhizal treatment was 4.00 mg while it was 3.02 mg on the non-mycorrhizal control (Fig. 2d). The MC% to the sum of ^{13}C content in all parts was 25% and was 24% to ^{13}C content only in soil (Fig. 2d). Although the ^{13}C content in maize shoots increased, the percentage of the ^{13}C in maize shoots declined by 5.89%, possibly indicating that inoculation promoted C accumulation and allocation to the belowground parts. Compared to the non-mycorrhizal control, mycorrhizal inoculation reduced the proportion of the ^{13}C distribution to the stems but increased the percentages of the distribution to the roots, soil and hyphae (Fig. 3). The ^{13}C content belowground involved soil, hyphae and roots, accounting for about 17.7% of the total ^{13}C content and much higher than the 11.8% in the non-mycorrhizal control (Fig. 2d). As a result, the AM fungus increased photosynthetic C fixation and soil C content. Inoculation also changed the distribution of photosynthetic products among shoots, roots and soil and tended to promote the allocation of photosynthetic C belowground.

Soil properties

As shown in Table 4 the SOC, EEG and TG values in the inoculated treatment were, respectively, 0.64, 173 and 515 g kg^{-1} and increased by 21.2, 85.9 and 62.4% compared to the non-mycorrhizal control. Inoculation also promoted the biomass of soil microorganisms. The MBC and MBN in the mycorrhizal treatment were 100 and 46.7 mg kg^{-1} and were significantly higher than in the non-mycorrhizal control. Soil AP in the mycorrhizal treatment was significantly lower than in the control but there was no significant difference in soil AK between mycorrhizal and non-mycorrhizal treatments. This may be related to the higher shoot biomass and larger microbial

biomass in the mycorrhizal treatment. Here, mycorrhizal inoculation increased soil C deposition and the biomass of soil microorganisms while reducing soil available P and K contents.

Table 4

Effect of mycorrhizal inoculation on soil parameters. Values are mean values $\pm$ standard errors. Different lowercase letters represent significant differences between Myc (inoculated treatment) and Ctrl (uninoculated control) at $P < 0.05$. AK, available potassium; AP, available phosphorus; SOC, soil organic carbon; TG, total glomalin; EEG, easily extractable glomalin; MBC, soil microbial biomass carbon; MBN, soil microbial biomass nitrogen.

Treatment	AK (mg kg ⁻¹)	AP (mg kg ⁻¹)	SOC (g kg ⁻¹)	TG (mg kg ⁻¹)	EEG (mg kg ⁻¹)	MBC (mg kg ⁻¹)	MBN (mg kg ⁻¹)
Mycorrhizal	148 $\pm$ 18.37 ^a	9.05 $\pm$ 0.87 ^b	0.64 $\pm$ 0.03 ^a	515 $\pm$ 50.05 ^a	137 $\pm$ 32.65 ^a	100 $\pm$ 11.65 ^a	46.7 $\pm$ 3.21 ^a
Control	166 $\pm$ 14.77 ^a	13.3 $\pm$ 0.48 ^a	0.53 $\pm$ 0.04 ^b	317 $\pm$ 52.24 ^b	73.9 $\pm$ 16.69 ^b	62.4 $\pm$ 6.89 ^b	27.2 $\pm$ 3.94 ^b

Discussion

Validation of a novel method for fully quantifying carbon allocation in the plant-AM fungus-soil system

The contribution of AM fungi to C allocation and sequestration belowground has long been a matter of interest and debate because accurate quantification, especially in realistic conditions, is very difficult (Parihar et al. 2020). There are a number of approaches to the estimation of the amount of C allocated to mycorrhiza. Numerous recent efforts have been made to quantify how AM symbiosis would affect the overall C balance and C fluxes in different type of photosynthetic metabolism using the stable ¹³C isotope (Lendenmann et al. 2011; Slavíková et al. 2017; Řezáčová et al. 2017 and 2018). The amounts of plant and soil carbon have been calculated and it has been reported that AM fungi increased assimilation by plants and the carbon allocation to soil. However, how much carbon AM fungi receive is unclear because it is difficult to collect hyphae from soil. In addition, NanoSIMS has demonstrated that AM fungi transferred C to soil and soil microbes (Kaiser et al. 2015). However, it is difficult to calculate the absolute amount of carbon. Here, we used a compartment culture system and mixed glass beads with soil to collect AM fungal hyphae, and combined with hyphal weight, the carbon allocation to the AM fungus was calculated. In addition, separated plant parts were studied in order to understand the carbon allocation in more details. Numerous studies report that aboveground respiration levels in mycorrhizal treatments declined coincident with increased C drain belowground (Řezáčová et al. 2017 and 2018). Here, we focus on the carbon assimilated to organic matter. Although a large percentage of carbon assimilated by photosynthesis is used for respiration, it is related to the carbon in organic matter (Smith and Dukes 2003).

The AM fungus increased plant growth and C allocation by enhancing the potential of plant stress resistance and nutrient uptake

Numerous previous studies have shown plant growth responses to mycorrhizal inoculation including positive (mutualism), neutral (commensalism) and negative (parasitism) effects (Johnson et al. 1997; Klironomos 2003). AM fungi form potentially symbiotic relationships with host plants based on the exchange of nutrients, especially relatively immobile nutrients such as P, and plant primary products (Kiers et al. 2011). According to the assumption of the bio-market model the effect of AM fungi on the growth of host plants lies in the trade-off between the C cost to and the nutrient acquisition by the host plants (Kiers et al. 2011). Here, the AM fungus formed large amounts of hyphae and increased the biomass, leaf area and P level of maize while decreasing soil available P and K (Tables 1, 2 and 4). These results indicate that the mycorrhizal benefits of nutrient uptake exceeded the C cost to the plant to construct the hyphal network. Especially, the shoot P concentration in mycorrhizal treatment was much higher than it in non-mycorrhizal treatment which was lower than threshold of shoot P concentration from silking stage to mature stage of maize (Jones 1983). Inoculation with the AM fungus also significantly increased leaf chlorophyll content and plant net photosynthetic rate resulting in the accumulation of primary products such as starch, while the intercellular CO₂ concentration decreased in the mycorrhizal treatment and this is consistent with previous studies (Borde et al. 2010; Talaat and Shawky 2014). In addition, soluble sugars and soluble proteins increased as a result of mycorrhiza-mediated enhanced photosynthesis (Table 2). When soluble sugars and soluble proteins increase the resistance to stress of the host plants is enhanced (Latef and Huang 2011; Sheng et al. 2011). Furthermore, the MDA content in the leaves decreased significantly while CAT in the leaves increased in the mycorrhizal treatment (Table 2). Lower H₂O₂ and MDA contents compared to non-mycorrhizal controls reduce oxidative (or other) stresses (Latef and Huang 2011; Talaat and Shawky 2014; Yang et al. 2014). Here, mycorrhizal inoculation reduced the temperature of leaf surfaces and increased the ability of the plants to resist high temperatures that might occur in northern Chinese coal mining areas (Table 3), perhaps by increasing the transpiration rate (Lee et al. 2012). Above all,

AM fungi may increase the potential resistance to environmental stress in coal mining areas and increase their nutrient uptake capacity, as well as contributing to plant growth and the accumulation of photosynthetic products (Xie et al. 1995; Hajiboland et al. 2010).

AM fungi regulate the allocation of photosynthetic products

Symbiotic associations between AM fungi and plants affect the transportation and allocation of photosynthetic products in plants (Lendenmann et al. 2011; Slavíková et al. 2017; Řezáčová et al. 2018). Here, the $^{13}\text{CO}_2$ pulse labeling method was used to label the maize and quantitatively determine the allocation of photosynthetic C in various plant parts and in the soil and the inoculated AM fungus and this is consistent with previous studies. Inoculation with the AM fungus increased the concentration and total amount of ^{13}C in the plants (Fig 2 and 3). This is supported by previous studies showing that AM fungi increase the C fixation capacity of their host plants (Hoeksema et al. 2010). We also observed significantly higher ^{13}C concentrations in the intermediate leaves and roots in the mycorrhizal treatment as well as higher concentrations of ^{13}C in the young and senescent leaves and stems than in non-mycorrhizal controls (Fig 2c). Higher ^{13}C concentrations in plant parts indicate stronger photosynthesis in young leaves than the intermediate and senescent leaves. Here, inoculation with the AM fungus increased the ^{13}C content in the plants and soil and also significantly changed the allocation of ^{13}C between the above- and below-ground parts of the system (Fig 3). Although inoculation increased the concentration of total ^{13}C and each part of the ^{13}C , the above-ground ^{13}C allocation ratio in the inoculated treatment decreased by 5.89%. This indicates that inoculation promoted photosynthesis and altered plant physiology while promoting the transportation of photosynthetic products belowground. This is supported by studies in which associations between AM fungi and host plants have accelerated the transport of photosynthetic products from leaves to roots and through stems. Furthermore, promotion of photosynthesis helps plant photosynthetic C fixation (Jakobsen and Rosendahl 1990; Al-Karaki 2000; Drigo et al. 2010; Olsson et al. 2010). Here, the C% and ^{13}C concentrations in the hyphae were equivalent to those in the leaves and were higher than in the roots (Fig 2b and 2c). This is consistent with a previous study in which ^{14}C was fixed by plants and then transferred to AM fungi within a few minutes (Clemmensen et al. 2013). In fact, AM fungi contain 5 to 20% of the photosynthetic C (Jakobsen and Rosendahl 1990; Johnson et al. 1997). Therefore, AM fungi are regulators of C flux pools from above- to below-ground (Zhu and Miller 2003).

AM fungi enhance C flux to soil microorganisms

Mycorrhizal inoculation significantly increased SOC and glomalin in the soil (Table 4). When a symbiotic relationship has formed a portion of the photosynthetic products is allocated to the AM fungi for extension of hyphae and development of spores in the soil (Gavito et al. 2005; Godbold et al. 2006). Previous studies show that the turnover time is usually 5-6 days. When the AM fungi die, part of their C may be decomposed by other microorganisms and then released to the atmosphere or enter the soil C cycle (Staddon et al. 2003; Treseder 2013). There is also a portion of C produced by AM fungi, for example in their cell walls, in the form of chitin that can remain in the soil for decades. The soil average organic C content in the inoculated treatment increased by 21.3% compared with the uninoculated control (Table 4). Thus, although the AM fungus had a rapid turnover rate in the soil the C stored by the fungus in the soil was measurable, maintaining a stable hyphal network that is important in soil C sequestration (Friesse and Allen 1991). In the mycorrhizal treatment the soil contents of EEG and TG increased significantly (Table 4), indicating that the content of soil glomalin was closely related to the AM fungus (Wright and Upadhyaya 1996). Studies show that AM fungi can produce exudates such as glomalin that are stable in the soil and influence the soil C storage indirectly by stabilizing soil aggregates (Steinberg and Rillig 2003; Rillig and Mummey 2006). This indicates that soil C deposition is not only affected by the C turnover in the mycelium but also by the protection of exudates of AM fungi and hyphal networks (Miller and Jastrow 2000; Zhu and Miller 2003).

AM fungi regulate above-belowground feedback

It is reported that soil organisms are important drivers of plant growth and then the effect of plants on soil community may influence the plants that grow later in the soil (Van der Putten et al. 2013; Wilschut et al. 2019). Once a symbiotic relationship is established it can extend the range of uptake by plant roots from a few millimeters to 12 centimeters, increasing the efficiency of soil nutrient utilization (Li et al. 1991). Therefore, in the mycorrhizal treatment the contents of available P in the soil declined significantly (Table 4). The results support the prevalence of a positive plant-soil feedback in the AM system (Bahram et al. 2020). Interaction between the host plants and the AM fungus increased the nutritional status of the host plants and also promoted plant growth and nutrient especially P status and the distribution of photosynthetic products belowground to supply both the roots and the AM fungus (Table 1). The results support the promotion of the accumulation of C in the soil by the AM fungal association by promoting the distribution of

photosynthetic products from the above-ground parts of the host plant to the intra- and extraradical mycelium (Leake et al. 2004). This stimulates the accumulation of microbial biomass. The hyphae release exudates and stabilize the soil structure and can also provide C sources for the soil microbial community and promote the growth of soil microorganisms (Toljander et al. 2007; Jones et al. 2009). Soil microorganisms are the most active part of the soil. They play an important role in soil nutrient acquisition and organic matter mineralization to meet the growth needs of plants and AM fungi (Hodge et al. 2001; Scheublin et al. 2010; Jansa et al. 2013; Wang et al. 2016; Zhang et al. 2018). Therefore, a mutually beneficial system is formed among host plants, AM fungi and soil microorganisms which affects the soil C cycle and enhances soil nutrient conditions. Here, AM inoculation promoted the growth and P nutrition of maize in the nutrient-limited soil and the photosynthetic C allocation to the soil. The enhanced microbial and environmental conditions in the nutrient-poor soil regulated a good feedback mechanism to increase plant growth.

Conclusions

Here, we established a suitable method to quantify the carbon allocation in plants, AM fungus and soil, and additionally in different plant parts. We also determined soil nutrient and SOC change caused by the AM fungus to detect above-belowground feedback regulated by the AM fungal association. Our results indicate that mycorrhizal colonization aided in the promotion of plant growth through increased stress resistance and mineral nutrient uptake. The AM fungus regulated the carbon allocation to functional leaves and increased plant photosynthetic activity. Mycorrhizal colonization also stimulated C allocation belowground to roots, hyphae and microorganisms. Soil available P and K contents decreased significantly because of the mycorrhizal nutrient uptake pathway to host plants. The positive feedback regulated by the AM fungus was established (Fig. 4). Mycorrhizal inoculation may therefore be a useful technique for ecological restoration and land reclamation in mining areas of northwest China, acting through above-belowground interactions and positive feedback. However, the current pot experiment was conducted in greenhouse conditions and further studies are required to verify the results under field conditions.

Declarations

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Not acceptable.

Ethical Approval

Not applicable

Consent to Participate

Not applicable

Consent to Publish

Not applicable

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Authors' contributions

YL Bi, P Christie, Y Cai and X Wang designed the study. Y Cai performed the plant culture and chemical analysis. YL Bi, P Christie and X Wang contributed to statistical interpretation of results and wrote the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1

Schematic diagrams of (a) the three-compartment microcosm and (b) the pulse labeling device. RC, root compartment; BC, buffer compartment; HC, hyphal compartment. One-mm nylon mesh was used to separate the RC and BC and 30 μm nylon mesh was used to separate the BC and HC. 1, rechargeable battery; 2, electric fan; 3, U-shaped silicone plug; 4, injector; 5, semiconductor refrigerator; 6, clip; 7, sink; 8, water; 9, plastic film; 10, thermometer; 11, silica gel; 12, board; 13, three-compartment microcosm; 14, nylon mesh; 15, LI-6400 handheld probe; 16, wire; 17, LI-6400 host; 18, pressure reducing valve; 19, 13CO₂ cylinder; 20, plastic catheter; 21, cock valve; 22, vacuum bag.

Figure 2

(a) $\delta^{13}\text{C}$, (b) C%, (c) ^{13}C concentration and (d) ^{13}C contents in different plant parts and the soil; M, mycorrhizal treatment; CK, non-mycorrhizal control; bars are mean values + standard errors ($n = 3$); different lowercase letters indicate a significant difference between mycorrhizal and non-mycorrhizal treatments at $P < 0.05$.

Figure 3

Distribution of ^{13}C in different plant parts and the soil; bars are mean values + standard errors ($n = 3$); different lowercase letters indicate a significant difference between mycorrhizal and non-mycorrhizal treatments at $P < 0.05$.

Figure 4

Schematic representation of increased above-belowground feedback through enhanced plant carbon assimilation and allocation.