Arbuscular mycorrhizal fungi enhance soil carbon sequestration in the coalfields, northwest China

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Carbon storage is affected by photosynthesis ($P_n$) and soil respiration ($R_s$), which have been studied extensively in natural and agricultural systems. However, the effects of $P_n$ and $R_s$ on carbon storage in the presence of arbuscular mycorrhizal fungi (AMF) in coalfields remain unclear. A field experiment was established in 2014 in Shendong coal mining subsidence area. The treatments comprised two inoculation levels (inoculated with or without 100 g AMF inoculums per seedlings) and four plant species [wild cherry (Prunus discadenia Koebne L.), cerasus humilis (Prunus dictyneura Diels L.), shiny leaf Yellow horn (Xanthoceras sorbifolium Bunge L.) and apricot (Armeniaca sibirica L.)]. AMF increased $P_n$ of four species ranging from 15.3% to 33.1% and carbon storage, averaged by 17.2% compared to controls. Soil organic carbon (OC), easily extractable glomalin-relation soil protein (EE-GRSP), and total glomalin-relation soil protein (T-GRSP) were significantly increased by AMF treatment. The effect of AMF on the sensitivity of $R_s$ depended on soil temperature. The results highlighted the exponential models to explain the responses of $R_s$ to soil temperature, and for the first time quantified AMF caused carbon sequestration and $R_s$. Thus, to our knowledge, AMF is beneficial to ecosystems through facilitating carbon conservation in coalfield soils.

Carbon storage depends on the balance between carbon sequestration by plant $P_n$ and carbon release to atmosphere through $R_s$. Therefore, if plant $P_n$ or $R_s$ is altered, carbon balance will be affected. $R_s$, especially seasonal variations, are significantly affected by soil temperature and moisture. However, the impact of soil microbes on $R_s$ or carbon storage remains unclear. So, far quantified contribution of soil microbes, especially AMF has not been reported yet. AMF are obligate plant symbions that associates with the roots of more than 80% vascular land plants, which significantly enhance long-term success of mine site reclamation. Studies showed that AMF are helpful for building up a productive, healthy, and sustainable post-mine land ecosystem with vegetation cover. The positive impact of AMF on reclaimed soil fertility and plant communities succession has been well documented. However, few studies focused on the effects of AMF on $R_s$ in post-mine land. A recent study conducted in Germany grassland communities reported that AMF stimulated $R_s$ on pasture soil, leading to elevated CO$_2$ level and temperature, with most carbon sequestered in belowground parts, making $R_s$ an important component of carbon balance. Factors such as soil moisture, soil temperature, CO$_2$ enrichment, and precipitation changes were all affected $R_s$. However, relevant studies remain scare and the complexity of various interactions that affect $R_s$ (such as soil temperature vs moisture, soil temperature vs microbes, moisture vs microbes interactions) are still poorly understood.

$R_s$ includes integrated CO$_2$ flux of root respiration, mycorrhizal respiration (considered as part of autotrophic respiration), and heterotrophic respiration, which are controlled by carbon supply and temperature. Carbon supply is an important $R_s$ impact factor, which primarily depends on plant productivity and generally responds positively to CO$_2$ enrichment with the increased $P_n$. Numerous studies confirmed that carbon storage depended on plant $P_n$ and $R_s$ via AMF in greenhouse or field experiments. AMF usually form symbiotic associations with trees in diverse forests. The fungi rely on host plant to obtain carbon supply and utilize 5–20% of the net photosynthesize of the symbiotic system. Global forest soil releases 24 Pg Carbon per year into atmosphere via CO$_2$ efflux and generates CO$_2$ from a widely variety of belowground organisms, with AMF as the dominant carbon source. AMF as an important carbon and soil CO$_2$ efflux source.
have been well studied under various temperature and moisture conditions. A pulse-labeling experiment showed that in temperate prairie, about 4–6% photoassimilates were facilitated via AMF. Simultaneously, AMF enhanced 16% soil CO2 efflux when *Lolium perenne* roots were colonized and 8% carbon supply was derived from AMF in a barley field. To date, no studies have been carried out on the contribution of AMF on carbon storage in coal mining soil.

Respiratory quotient (Q10) is defined as the temperature sensitivity of Rs which is derived from substrate availability, which is the respiration variation ratio when soil temperature rises 10°C. Q10 is largely affected by a series of environmental factors, such as soil physicochemical properties and soil moisture, etc. However, few studies have quantified the effects of AMF on apparent Q10 from different tree species. Thus, knowledge about the function of AMF on Rs, Pn, and carbon storage is insufficient.

Notably, CO2 concentration indirectly influences Rs and carbon allocation from host species to fungus, suggesting that root participates in carbon fixation. Most colonized plants enhanced productivity or nutritional status of the plant symbioses system when exposed to higher CO2 concentration. In addition, mycorrhizal hyphae assisted carbon re-distribution from aboveground parts to roots via utilizing the photosynthate and provides multiple adherent agents such as extracellular polymer, and amino acid, etc than non-colonized plants. Briefly, AMF enhance soil carbon proportion in higher CO2 environment. However, lacking a better understanding of these responses in coal mining soil limited to prediction soil carbon storage when AMF existed in current scenarios.

Thus, the experiment planted wild cherry, *cerasus humilis*, shiny leaf Yellowhorn and apricot in coal mining soil and half seedlings were inoculated with AMF inoculums. Based on the experimental results, two hypotheses was proposed that: (i) plant species preferentially facilitate their photosynthetic ability and increase Rs rates, leading to different carbon storage amount; and (ii) soil temperature rather than soil moisture is sensitive to Rs due to the extreme drought. The future goal is to investigate the effect of AMF on carbon sequestration in coal mining fields.

**Results**

**Pn and Rs temporal dynamics.** *Pn* was significantly greater with AMF inoculation in all the four species from July to September (Fig. 1). Specifically, AMF treatment increased *Pn* in wild cherry, *cerasus humilis*, shiny leaf Yellowhorn, and apricot by 21.2%, 15.0%, 53.1%, and 7.4%, respectively. Meanwhile, *Pn* in all four tree species increased from July to August and then strikingly decreased from August to September (Fig. 1).

In all tree species, Rs was significantly decreased from July to September, with slight decrease from July to August and reduced sudden reduction of 21.3–26.9% in September (Fig. 2).

**Cumulative carbon measures.** Annual cumulative carbon in AMF treated wild cherry, *cerasus humilis*, shiny leaf Yellowhorn, and apricot were about 1706, 1784, 2049 and 2065 g C.m−2.yr−1 (Fig. 3a–d). Compared to the corresponding controls, the annual cumulative carbon levels in the four tree species were increased by 28.3%, 44.6%, 31.8% and 33.5%, respectively (Fig. 3a–d). In growing season, AMF increased the carbon storage by 29.4%, 45.7%, 32.4% and 34.4% (*P* < 0.0001) (Fig. 3a–d). AMF also significantly increased the annual cumulative Rs carbon in the four tree species by 32.4%, 36.6%, 25.2% and 29.9%, when compared to the corresponding controls (*P* < 0.0001) (Fig. 4a–d).

**Soil temperature sensitivity to Rs.** Rs was calculated using exponential function (Eq. 1), temperature dynamic showed over 70% Rs variation in AMF treated groups and the correlation of Rs showed only 40% variation in control groups (Table 1). Apparent Q10 and the coefficient *b* were decreased significantly in control...
Effects of AMF inoculation on carbon-linked parameters. In control of wild cherry, cerasus humilis, shiny leaf yellowh orn, and apricot, the production of OC, EE-GRSP and T-GRSP almost did not change with apricot and wild cherry ($P < 0.05$) (Table 1; Fig. 5a,d). However, AMF decreased previous two parameters in cerasus humilis and shiny leaf Yellowh orn (Fig. 5b,c). Apparent $Q_{10}$ responded differently to temperature (the precipitation in 2014 was higher than 10 year’s average precipitation) in August from that in July or September (Fig. 6a–d).
In contrast, the production of soil OC, EE-GRSP and T-GRSP in AMF treated of four species increased significantly over time (Table 2). After three months of treatment, the increment of OC, EE-GRSP and T-GRSP in AMF treated groups were all significant (27–37%, 34–45% and 19–22%, respectively (Table 2). After five months of AMF treatments, compared to the control, the levels of OC, EE-GRSP and T-GRSP in AMF treated groups were remarkable enhanced by 52–61%, 55–70% and 36–44%, respectively (Table 2). Collectively, AMF had a positively impacted on soil carbon-linked parameters over time in all of the four tree species (Table 2).

**Discussion**

The present study firstly examined photosynthetic ability, Rs and carbon storage in respond to AMF inoculation in the coalfields. Under AMF treatment, carbon storage was derived from belowground parts of the tree species. Meanwhile, the temporal variability strongly responded to Rs and annual cumulative carbon with exponential models.

**Pn and Rs responses to AMF inoculation.** AMF inoculation incurred a series changes in Pn, Rs and carbon storage via close symbiosis with host plants, leading to enhanced carbon production and improved nutrition status against the detrimental effects\(^{14,23-27}\). Meanwhile, AMF accelerated the organic matter (OM) decomposition rates\(^{28}\) and provided some raw materials, which elevated CO\(_2\) concentration in micro circumstance\(^{29,30}\) and facilitated soil carbon turnover in the agro-or grassland via CO\(_2\) stimulation effects\(^{31}\). In the moist forest, abundant and diverse AMF (e.g. tropical forests) species created a transient carbon sink via N transferring to reduce carbon lost. This implied that AMF promotes carbon production and storage via increasing Pn, which ameliorated the

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**Figure 4.** Soil carbon releases via Rs in growing and non-growing seasons. (a–d) Showed that wild cherry, Cerasus humilis, shiny leaf Yellowhorn, and apricot, respectively. Bar represented means ± SE. –M and +M denoted without and with AMF inoculation, respectively. Different lowercase letters indicated that –M and +M were significantly different at 5% level by LSD.

**Table 1. The exponential model of Q\(_{10}\) value.** Exponential relationship between the Rs and soil temperature (T °C; July to September) and apparent Q\(_{10}\) values.
negative effect caused by unfavorable environment. Recently, strong evidences showed that the colonized plants have higher Pn and ribulose-1,5-bisphosphate carboxylase/oxygenase activity than non-colonized plants, which compensated greater photosynthate. Carbon was produced directly from Pn, and AMF perhaps supported the higher metabolism of plants and greater productivity of carbon in the harsh environment. Furthermore, soil temperature decreasing inhibited substrate movement, lowing microbal activity, which decreased plant Pn and belowground carbon allocation. Temperature also reduced Pn and the belowground coupling, which potentially affected the substrate availability and carbon cycling. The results demonstrated that AMF can increase Pn of the plant, thus increasing carbon allocation.

Carbon partitioning responses to AMF inoculation. Due to its ability to enhance carbon allocation, AMF has been used in agricultural field and grasslands, but information about carbon storage is omitted in mining soil. Carbon accumulation tended to be higher with AMF inoculation in this study in the growing season. AMF sequestered greater carbon via enhanced Pn and photosynthate production, which was transferred from aboveground to roots or microbes. AMF counteracted carbon loss of respiration, due to increase productivity and nutrient acquisition, especially carbon sequestration. In the non-growing seasons, AMF perferentially allocated photosynthate from branchs to belowground parts, increasing the EE-GRSP and T-GRSP of carbon stocks. Estimately, AMF utilized a large proportion (about 20%) of photosynthates, leading to more carbon retention via symbiosis. Consequently, AMF inoculation resulted in remarkable greater carbon sequestration.

Nonlinear response to Rs. AMF affected carbon storage and partition, which has been discussed in the previous study. However, no data have shown whether interactions between species and temperature affect AMF facilitated carbon sequestration. As literatures indicated, carbon productivity of the plants was greater with AMF, due to higher availability of nutrients from the soil environments. The beneficial effect of AMF in carbon sequestration was considered a result of improved availability of nutrients and altered carbon allocation caused by AMF. The exponential models successfully illustrated the changes of Rs in response to soil temperature variation and captured monthly dynamics of all tree species used in the experiment.

Hysteresis between Rs and soil temperature exists in various ecosystems and vegetations. In general, the decoupling of Rs in response to soil temperature is attributed to confound effect, such as precipitation or
physiological drought caused hysteresis, which leads to decreased AMF activity and subsequently lower CO₂ production. Over all, the Rₛ-temperature relationship was affected by Pn, litterfall, and soil microbe activities. However, the detailed mechanisms remain unclear. In this study, due to the existence of hysteresis, Rₛ in control groups was higher than AMF inoculation groups under a given temperature, which was in agreement with the previous hypothesis. Most likely, AMF plants accumulated more fresh litter in a given temperature than non-colonized plants. Q₁₀ was often used to represent the temperature measurement depth in greenhouse or field experimental models. In this work, apparent Q₁₀ increased in AMF treated groups, indicating that AMF

Figure 6. Apparent Q₁₀ values represents the sensitivity of soil respiration and soil temperature when rises 10°C. Monthly apparent Q₁₀ values variation of four tree species without or with AMF inoculation from July to September. Values represented means ± SE. (a–d) Indicated wild cherry, cerasus humilis, shiny leaf Yellowh orn, and apricot of tree species, respectively.

Table 2. Different soil carbon-linked fractions variations. Note: values are means of three replicates. Values followed by the small lowercase letters are not significantly different among different inoculation durations (month) of one tree species with or without AMF (+M or −M) at the 5% level by LSD (vertical comparisons); values followed by the same capital letters are not significantly different between −M and +M at the same durations within the same tree species at the 5% level by LSD (horizontal comparisons) in the identical durations (months). −M, +M represented without and with added mycorrhizal fungi inoculums at 100 g per tree species. Effect of added AMF inoculums on soil OC, EE-GRSP, T-GRSP in four tree species with three inoculation durations.
increases the sensitivity of $R_\text{s}$ to soil temperature. Apparent $Q_{10}$ value was lower in summer than in cooler season, which might be because of higher substrate availability and severe soil moisture limitation in summer. In consistent with the previous publication\textsuperscript{14}, the experiment data showed that $R_\text{s}$ strongly depends on soil temperature in the study.

Previous studies have used exponential model but ignored the function of AMF\textsuperscript{64}. The results suggested that AMF plays a key role in $R_\text{s}$ and carbon stocks. AMF and soil temperature in combination modulate apparent $Q_{10}$ in coalfields.

**Conclusions**

This study provided new insights into the impacts of AMF on $R_\text{s}$ and carbon storage in coalfields. AMF enhances carbon sequestration and the sensitivity of $R_\text{s}$ to temperature. Higher AMF infection rate significantly enhances $R_\text{s}$ and $P_\text{n}$, the positive AMF function is through promoting plant growth, especially increasing leaf area, chlorophyll content, and the $Q_{10}$ value. These results clearly demonstrated that AMF infection increased organic matter and glomalin which may be associated with the enhancement of carbon storage in soil. The model of $R_\text{s}$ to soil temperature should be reassessed to take into account of the interaction between the soil volumetric moisture and mycorrhizal fungus. Thus, further study will focus on long-term AMF effect on carbon stock in a tree species specific manner.

**Materials and Methods**

**Study site and experimental set-up.** This experiment was conducted at the ecological reclaimed region, which located in Dalita town (39°18′N, 110°4′E), Shenmu County, Yulin City, Shaanxi Province, Northwest China at 1,200 m height above sea level. This site was a typical junction area of Shansi, Shaanxi and Inner Mongolia of three Provinces and the south margin of Mu us desert in the loess plateau transition zone. About 70% precipitation falls from June to September, the 10-year (2005–2014) average total precipitation and the potential evaporation were about 150 mm and 2000 mm according to Shenmu Meteorological Station near the experiment site. The experimental location had a typical characteristic of continental climate, with annual average temperature about 8°C. The cumulative temperature above 0 °C and 10 °C were 3,550 °C and 3,210 °C, respectively; the frost-free period is 150 days and total solar radiation is 6,000 MJ m\textsuperscript{-2} year\textsuperscript{-1}. The local soil was classified as Aeolian sandy (FAO/UNESCO, 1988) which contained 75% sand, 22% silt, and 3% clay. The topsoil physico-chemical properties were: soil OM 4.5 g kg\textsuperscript{-1}, total N 0.21 g kg\textsuperscript{-1}, Olsen P 5.3 mg kg\textsuperscript{-1}, exchangeable K 37.8 mg kg\textsuperscript{-1} and pH value (1:2.5 soil: distilled water) 7.9.

All experiments were carried out in three replicates. The sub-plots of four tree species, wild cherry (Prunus padiscadenia Koebne L.), cerasus humilis (Prunus dictyneura Diels L.), shiny leaf yellow horn (Xanthoceras sorbofolium Bunge L.), and apricot (Armeniaca sibirica L.) were used in the experiments. Each species was divided into two groups: AMF group and control group. AMF group received the inoculation of AMF at 100 g inoculums/seedling, while the control group did not contain any AMF inoculums.

The four seedlings were obtained from the forest bureau of Shenmu County, Yulin City, Shaanxi Province and Northwest China. Each seedling was transplanted into the plot at temperatures ranging from about 10–15 °C in March 25, 2014 to April 5, 2014. The AMF colonization rate of wild cherry, cerasus humilis, shiny leaf yellow horn, and apricot were 4.8%, 6.3%, 5.4%, and 7.2% before transplanting and 78.1%, 80.2%, 76.4%, and 81.7% at the end of monitoring stage. Two months after transplantation, over 90% of the seedlings were colonized with mycorrhizal fungus.

Each plot had an area of 20 × 12 m\textsuperscript{2}, which consisted of 6 rows, with 10 seedlings per row at 2 m row spacing. 100 g AMF was inoculated in root of AMF plants at seedling transplantation. Funneliformis mosseae BGCXJ01 inoculums (Supplied by Beijing Academy of Agriculture and Forestry Sciences) were propagated on Trifolium repens (clover) for 12 weeks. 100 g inoculums included spores (10–20 spores/g), colonized root fragments (40 root fragments per gram inoculums, 85% root colonization), and external mycelium\textsuperscript{65}.

**$R_\text{s}$ determination.** $R_\text{s}$ was measured from July to September in individual plot with three replicates. Each measurement event was made in the middle of individual month (about 15\textsuperscript{th}) using an Automated Soil Gas Flux System (Li-8100A, Li-cor Inc., Lincoln, Nebraska, USA) coupled with the small PVC chamber (20 cm × 10 cm). The instrument was permanently installed between two rows with identical distance. In each plot, measurements of $R_\text{s}$ were taken inside three replicate PVC chambers with 20 cm in diameter and 10 cm in height, inserted to a depth of 2–3 cm in soil, capturing respirations. The CO\textsubscript{2} efflux from this collar mainly derived from soil organic matter decomposition, and root and microbial respiration. Meanwhile, soil temperature and volumetric moisture at the top of 5 cm was measured with the corresponding probes. Specifically, $R_\text{s}$ was always measured between 8:00 and 12:00 in the morning in the sunny day without wind\textsuperscript{66}.

The cumulative $R_\text{s}$ values were calculated by the method of Bremer et al.\textsuperscript{57}. Additionally, diel respiration (every 2 hours over 24-hour cycles) was periodically measured in all plots and individual collars. All of the measurements were made in four seasons from 2014 to 2015 due to time constraints. The diel measurements were used to calculate the annual growing season (July to September) cumulative $R_\text{s}$. Briefly, respiration measured during the daytime was assumed to be the daily maximum soil CO\textsubscript{2} efflux. Diel measurements were used to calculate the daily minimum efflux as a percentage of maximum efflux. The daily maximum and minimum efflux were used to calculate the average daily efflux. The cumulative flux as the product of average daily flux and the number of days was estimated between each measurement.

**$P_\text{n}$ determination.** Plants assimilated CO\textsubscript{2} via $P_\text{n}$. To quantify photosynthetic performance of plant species, the measurement was with two AMF inoculation levels using a portable open system infrared gas analyzer for
net CO\(_2\) assimilation rate of leaves in individual treatment (Li-6400, Li-cor Inc., Lincoln, Nebraska, USA). The experiment was performed from July to September in 2014, including three candidate trees within individual plot. Each measurement event was carried out at 08:00 to 18:00 in sunny day every 2 h, with three fully expanded healthy sun-exposed leaves of each species. Leaves were carefully positioned in the leaf chamber when they were exposed to the 1000 \(\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}\) saturating quantum flux level. Parameters including the instantaneous \(P_n\), transpiration rate (\(T_t\)), stomatal conductance (\(G_s\)), intercellular CO\(_2\) concentration (\(C_i\)), photosynthetically active radiation (PAR), atmosphere CO\(_2\) concentration (\(C_a\)), atmosphere temperature (\(T_a\)), leaf temperature (\(T_{\text{leaf}}\)), and relative air humidity (RH) were tested.

Diurnal \(P_n\) amount was the area which surrounded by the curves of net photosynthetic rate > 0 and the time transverse in the diurnal curves variations of species \(P_n\). According to the principle, the diurnal net photosynthetic amount was calculated as follows:

\[
P = \sum_{i=1}^{n} \left( \frac{p_{i+1} + p_i}{2} \times (t_{i+1} - t_i) \times 3600 \div 1000 \right)
\]

where \(P\) was the diurnal total net assimilation amount (mmol.m\(^{-2}\).d\(^{-1}\)), \(p_i\) and \(p_{i+1}\) (\(\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}\)) was the instantaneous photosynthetic rate of the beginning and next measuring point, respectively; \(t_i\) and \(t_{i+1}\) was the time duration of the beginning and next measuring point; \(n\) was the numbers of determination times; one hour equaled to 3600 seconds; 1 mmol equaled to 1000 \(\mu\text{mol}\).

Generally, the 20% diurnal assimilation photosynthates production was consumed by dark respiration, and converted the assimilation into the CO\(_2\) diurnal sequestration as follows:

\[
W_{\text{CO}_2} = P \times (1 - 0.2) \times \frac{44}{1000}
\]

where 44 was CO\(_2\) mole mass; \(W_{\text{CO}_2}\) was CO\(_2\) sequestration mass in the per unit leaf area (g.m\(^{-2}\).d\(^{-1}\)).

According to the \(P_n\) reaction equation CO\(_2\) + 4H\(_2\)O → CH\(_2\)O + 3H\(_2\)O + O\(_2\), which the formula can be calculate the daily CO\(_2\) absorption in the per unit land area of per plant as follow:

\[
Q_{\text{CO}_2} = Y \times W_{\text{CO}_2}
\]

\(Q_{\text{CO}_2}\) was the daily CO\(_2\) sequestration in the per unit land area of per plant species (g.m\(^{-2}\).d\(^{-1}\)).

The average CO\(_2\) uptake per day of individual plants as follow:

\[
Q_{\text{CO}_2} = Y \times P_{\text{average}} \times (1 - 0.2) \times \frac{44}{1000}
\]

\(P_{\text{average}} = (P_{\text{Jul}} + P_{\text{Aug}} + P_{\text{Sep}})/3\)

where \(Q_{\text{CO}_2}\) was the daily average CO\(_2\) sequestration of each plant (g.d\(^{-1}\)); \(P_{\text{average}}\) was the daily assimilation in the per unit land area (mmol.m\(^{-2}\).d\(^{-1}\)) ; \(Y\) was the total leaf area of each plant (m\(^2\)).

**Soil OC, EE-GRSP and T-GRSP fractions.** Soil samples were collected from 0–20 cm of the profile using an auger (35 mm diameter) in each corresponding monitoring stage in 2014. Three cores were collected from individual plot and combined to give one composite sample per plot of each tree species. The composite soil samples were air-dried and sieved through a 2 mm mesh and placed in plastic bags for chemical analysis.

A simple method for routine determination of soil OC by a modified Mebius procedure was described. It involved a digestion of the soil sample with an acidified dichromate (K\(_2\)Cr\(_2\)O\(_7\)-H\(_2\)SO\(_4\)) solution for 30 minutes in a Pyrex digestion tube in (a) 40 tube block digester preheated to 170 °C and (b) estimation of the un-reacted dichromate by titration of the cooled digest with an acidified solution of ferrous ammonium sulfate using the N-phenylanthranilic acid as an indicator.

0.25 g composite sample was extracted with 2 ml of extractant. EE-GRSP was extracted with 20 mM citrate solution; pH 7.0 at 121 °C for 30 min. T-GRSP was extracted with 50 mM citrate solution, pH 8.0 at 121 °C. 90 min was required for one soil sample and six additional sequential extractions. For the sequential extractions, the supernatant was removed by centrifugation at 10,000 × g for 5 min, 2 ml of 50 mM citrate, pH 8.0 was added to the residue, and samples were autoclaved for 60 min. Extraction of a sample continued until the supernatant showed no red-brown color typical of glomalin. Extracts from each replicate were pooled and then analyzed.

Citrate extractants were added to soil samples and disrupted by a brief (3 min) autoclave cycle. When necessary, the extractant was adjusted with HCl solution until the pH stabilized at 7.0 for 20 mM citrate or 8.0 for 50 mM citrate solution. Samples were then subjected to 121 °C for 90 min to extract T-GRSP or 30 min to extract EE-GRSP.

After extraction cycles completed, samples were centrifuged to remove soil particles (10,000 × g for 5 min), and protein in the supernatant was determined by the Bradford dye-binding assay with bovine serum albumin as standard. Concentration of glomalin was extrapolated to mg/g of soil particles by correcting the dry weight of coarse fragments >0.25 mm included in the weight of aggregates and the volume of extractant.

**Data analysis.** To assess the four plant species with and without AMF inoculation treatments to long-term exposure and potentially elucidate the role of AMF in previous response, the relationship of \(R_p\) and \(P_n\), \(R_s\), soil temperature, and carbon storage and AMF inoculation of the key issues have been a focus of this study. The experiment was at two AMF inoculation levels with four plant species. To determine main effects of AMF inoculation on soil temperature and \(R_p\) variables, mixed model restricted maximum likelihood estimation with
repeated measures was used (PROC MIXED; version 8.0; SAS Institute Inc., Cary, NC, USA, 2003). Turkey’s HSD multiple comparison was used to identify the differences between two AMF inoculation levels. An exponential model was used to calculate soil temperature sensitivity on Rs. \( R_s = a e^{bT} \)

where \( R_s \) is soil CO2 efflux (\( \mu \)mol m\(^{-2} \) s\(^{-1} \)), \( T \) is soil temperature (°C) at 0–5 cm depth, \( a \) is the basal \( R_s \), \( b \) is temperature sensitivity of CO2 efflux. The respiratory quotient (\( Q_{10} \)) was calculated as \( Q_{10} = e^{0.1b} \).

Regression coefficients, correlations, figures and curves were obtained by the Sigma-Plot software package (version 11.0, San Jose, California, USA). Analysis was performed to reveal the differences of two AMF inoculation levels with the relevant parameters; differences in means were revealed by LSD (\( P < 0.05 \)) with SAS software package. To determine the effect of AMF on photosynthetic parameters, the data set was divided into two parts; the results were insensitive to variation in species groupings as the sample size was not unduly restricted. Differences in slopes were determined by a dummy variable representing the interaction between independent variable (AMF inoculation level) and plant species in the regression analyses.

Cumulative carbon calculations. To assess the effect of soil volumetric moisture on carbon accumulation, the cumulative carbon (Cumulative-C) was fitted using a combined exponential and quadratic function as follows:

\[ \Delta_{\text{Cumulative-C}} = (ae^{bT}) [2.12(\theta_v - \min \theta_v)(\max \theta_v - \theta_v)^2] \]

where \( \theta_v \) is the volumetric moisture content (the minimum soil volumetric water content of our data set was 3.1% and the maximum was 37.6%, respectively) and \( c \) is the coefficient for soil moisture.

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Acknowledgements
The study was supported by the National Science Foundation of China (51574253) and the Fund for 863 Programs (2013AA102904) of the Ministry of the Science and Technology, PR China and the Bilateral Corporation on the Prospectus of the Open Research Project (SKLCRSM16KFA01) between Kazakhstan and PR China.

Author Contributions
Z.-G.W. initialized the study; Conceived and designed the experiments: Z.-G.W. and Y.-L.B. performed the experiments: Z.-G.W., B.J., Y.Z., W.-W.L., H.L., Z.-G.W. and Y.-L.B. performed the data analysis; Z.-G.W. and Y.-L.B. drafted the manuscript; contributed reagents/materials/analysis tools: Z.-G.W., Y.-L.B., B.J., Y.Z., S.-P., W.-W.L. and H.L.

Additional Information
Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Wang, Z.-G. et al. Arbuscular mycorrhizal fungi enhance soil carbon sequestration in the coalfields, northwest China. Sci. Rep. 6, 34336; doi: 10.1038/srep34336 (2016).

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