Plant Soil (2016) 398:195–206
DOI 10.1007/s11104-015-2656-5

REGULAR ARTICLE

Indigenous arbuscular mycorrhizal fungi can alleviate salt stress and promote growth of cotton and maize in saline fields

Shenglin Liu · Xiuli Guo · Gu Feng · Baidengsha Maimaitiaili · Jialin Fan · Xinhua He

Received: 27 January 2015 / Accepted: 26 August 2015 / Published online: 4 September 2015

Abstract
Aims The function of indigenous mycorrhizal fungi in improving crop growth is not well addressed because of methodological limitations. In this study, we determined the effects of the indigenous arbuscular mycorrhizal (AM) fungal community on the growth responses and salt tolerance of cotton and maize.
Methods Through a 2-year field trial with in-growth microcosms constructed by polyvinylchloride (PVC) tube cores and 30-μm nylon mesh that were buried in

Highlights
- We quantified the role of indigenous AM fungi in promoting crop growth in saline field.
- AM fungi improved P uptake, Na^+/K^+ ratio, proline or/and soluble sugar accumulation.
- Indigenous AM fungi alleviate high salinity stress of crop in intensified farming system.
- The in-growth core system was modified using a non-mycorrhizal plant species *Beta vulgaris*.

Responsible Editor: Tatsuhiro Ezawa.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-015-2656-5) contains supplementary material, which is available to authorized users.

S. Liu · X. Guo · G. Feng (✉)
Center for Resources, Environment and Food Security, China Agricultural University, Beijing 100193, China
e-mail: fenggu@cau.edu.cn

S. Liu
e-mail: shengtai1143@163.com

X. Guo
e-mail: maryshz@126.com

S. Liu · X. Guo · G. Feng
College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China

B. Maimaitiaili
Institute of Nuclear Technology and Biotechnology, Xinjiang Academy of Agricultural Science, Urumqi, China
e-mail: baidengsha@126.com

J. Fan
Isotope Institute, Henan Academy of Sciences, Zhengzhou, Henan 450052, People's Republic of China
e-mail: hnskxyfjl@163.com

X. He
Department of Environmental Sciences, University of Sydney, Eveleigh, NSW 2015, Australia
e-mail: xinhua.he@uwa.edu.au

X. He
School of Plant Biology, University of Western Australia, Crawley, WA 6009, Australia

X. Guo
Agricultural College of Shihezi University, Shihezi, China

different saline soils, two core treatments, static (freely allowed AM fungal colonization) and vibrating (patting the top core edge twice every day to break any extraradical hyphae that intends to access into the core to reduce AM fungal colonization), were applied in field conditions.

Results The results showed that vibration did not affect the growth of the control non-mycorrhizal plant, sugar beet, but significantly affected the growth of the mycorrhizal plants, cotton and maize. These data indicated that such core systems could provide a reliable method to quantify the functions of the AM fungal community in situ. Mycorrhizal colonization of cotton and maize significantly declined in the vibrating treatment compared to the static treatment. Phosphorus (P) uptake and biomass production of cotton and maize were significantly higher in the static than the vibrating. The indigenous AM fungal community promoted leaf proline accumulation in cotton and a higher K^+/Na^+ ratio via selective preferential uptake of K^+ over Na^+. These effects and enhanced P uptake derived from AM fungi were related to alleviating salt stress and promoted the growth of cotton and maize in saline soils.

Conclusions Our results demonstrated that indigenous AM fungi play a role in improving crop growth by alleviating the harmful effects of high salinity in intensified cropping systems.

Keywords AM fungi · Indigenous community · In-growth core system · Osmotic regulation · Salinity · *Beta vulgaris* L. · *Gossypium hirsutum* L. · *Zea mays* L.

Introduction

Arbuscular mycorrhizal (AM) fungi are a major component of soil biofertility. They are present in arable soils and improve nutrient (particularly P) acquisition and crop resistance to biotic and abiotic stresses, primarily because of their extensive extra-radical mycelium networks, which extend the roots' absorbing area beyond the depletion zone around the root (Smith and Read 2008) and enhance soil P availability (Feng et al. 2002a). Application of AM fungal inocula to the soil might reduce the dependence of crop performance on P fertilizers or otherwise increase crop production (Jeffries et al. 2003; Lekberg et al. 2008), although such effects in arable lands could be positive (David et al. 2007), neutral (Grant et al. 2005), or even negative (Li et al. 2006, 2005; Stewart et al. 2005). The possible mechanisms causing neutral or negative effects are as follows: (i) the introduced AM fungi are less competitive than their indigenous counterparts (Muok et al. 2009); (ii) the use of fertilizers and pesticides inhibits colonization of AM fungi (Bethlenfalvay et al. 1996; Busse et al. 2004; Garciaromera and Ocampo 1988); (iii) modern crop varieties are less dependent on AM fungi because they are selected under high soil fertility (Zhu and Smith 2001); (iv) the AM fungi colonizing on plant roots could sometime become parasitic due to higher soil fertility or the genetic traits of lower mycorrhizal responsiveness of the crops (Chu et al. 2013; Janos 2007; Nogueira and Cardoso 2006). However, a rich community of AM fungi in both species and population densities in terms of both natural and agricultural ecosystems have been observed with intensive crop management (Oehl et al. 2004) even when available soil P is high (Thomson et al. 1992; Vestberg et al. 2011). It therefore seems reasonable to take advantage of the indigenous AM fungal community to improve crop resistance to those harsh edaphic conditions and promote crop growth.

Salt-affected soils cover more than 7 % of the earth's land surface and represent a major limiting factor in crop production (Jain et al. 1989; Rozema and Flowers 2008; Munns and Gilliham 2015). Progress in understanding the integrated physiological mechanisms of plant tolerance to soil salinity have been made in the last decade. These include morphological changes of some plant organs (roots, leaves, etc.), synthesis and accumulation of compatible solutes, maintenance of ion homeostasis and photosynthesis, regulation of water uptake and distribution to plant tissues and reduction of oxidative damage (Ruiz-Lozano et al. 2012). Developing salt-tolerant crops is a much-desired practical goal with little success to date because few major genetically determined traits of salt tolerance have been identified (Flowers 2004; Munns and Tester 2008; Schubert et al. 2009; Roy et al. 2014). Alternative approaches including the use of beneficial rhizosphere microbes to improve the salt tolerance of crops have been considered (Dodd and Pérez-Alfocea 2012; Chen et al. 2014; Munns and Gilliham 2015). For instance, purified indigenous AM fungal isolates from saline soils showed variable effects on crop growth when inoculated individually (Campagnac and Khasa 2014; Daei et al. 2009; Paluch 2011; Tian et al. 2004), but salt stress was alleviated (Feng et al. 2002b; Miransari et al. 2007; Murugensan et al. 2014). The possible roles of AM fungi in promoting host plant salt tolerance in saline soils might occur due to enhanced

nutrient uptake (Navarro et al. 2014), maintenance of osmotic balance by ion homeostasis (Estrada et al. 2013) and osmolytes (Evelin et al. 2009), and alleviation of salt-induced oxidative stress (Latef et al. 2011). Accumulation of lower-molecular-weight organic compounds, such as proline (Sharifi et al. 2007) and soluble sugar (Al-Garni 2006) is usually correlated with salt tolerance of plants, and can be induced by AM fungi colonization (Feng et al. 2002b). However, diverse AM fungi colonize plant roots in the field, and different AM fungal species could have competitive or facilitative effects on plant nutrient uptake and growth. The AM fungal status of the indigenous community in the roots and their functions on salinity tolerance of crops in saline fields are obviously different from these in pot experiments in greenhouses. At present, limited information is available from real saline field conditions to demonstrate the direct effects of the indigenous AM fungal community on crop nutrient uptake and growth due to the inability to establish a true non-mycorrhizal control to quantify mycorrhizal responsiveness.

To test the role of the indigenous AM fungal community in nutrient uptake and seedling growth in the field based on the difference in diameter of AM fungal mycelia and root hairs, ~37 μm fine mesh has been used to allow access to AM mycelia, but not the roots. For instance, Schweiger et al. (2001), Schweiger and Jakobsen (1999) designed a nylon mesh-enclosed soil cylinder as the hyphae compartment and used fungicides to create a non-mycorrhizal control. However, the fungicide sometimes not only inhibited the colonization of AM fungi, but also other soil-borne pathogen fungi (Newsham et al. 1994). The sensitivities of different AM fungal species to fungicides were also variable (Chiocchio et al. 2000; Schreiner and Bethlenfalvay 1997). Johnson et al. (2001) set up a novel in-growth core system that enabled a functional test of the natural AM fungal mycelium network in the field. This method can create normal colonization or relatively lower colonization (as control) by utilizing either a static or rotating nylon mesh-enclosed soil cylinder in which the test plant grows. This method has been successfully used for small biomass plants growing in different ecological systems, e.g., grassland and arid grassland system (Babikova et al. 2013; Nottingham et al. 2013; Zhang et al. 2012). However, information as to whether this in-growth microcosm would be suitable to quantify AM fungal function in crops with a larger canopy is limited. A possible defect of the in-growth system is that rotating the soil cylinder in which the test plant grows may disrupt soil moisture flow, thereby limiting plant growth.

Here, we present results from a 2-year field trial based on in-growth microcosms constructed with 5-cm-diameter polyvinylchloride (PVC) tube cores covered by 30-μm nylon mesh. In two experiments on three common crops that grow widely in saline soil in northwest China, we tested whether a modified core system could affect the growth of crops inside the core and quantified the effect of AM fungi on salinity tolerance of cotton and maize in saline agricultural conditions. We hypothesized that indigenous AM fungi would enhance the salt tolerance of AM cotton and maize by improving their P uptake and ionic balance.

Materials and methods

Study area

The study site locates in the Cotton Experimental Station (44°17′57″N, 86°22′6″E, 400 m above sea level) of the Xinjiang Academy of Agricultural Sciences in Manas County, Xinjiang, northwestern China, where cotton has been widely grown in sodium-sulphuric saline soils since 2005. The annual mean temperature is 7.2 °C, and the annual mean rainfall is 276 mm in this region. Based on the total soluble salt concentration (1:5 soil :water), the saline strength of a soil is classified as none (<3 g kg^{-1} DW soil), slightly (3–6 g kg^{-1} DW soil), moderately (6–10 g kg^{-1} DW soil), highly (10–20 g kg^{-1} DW soil) and extremely high saline (>20 g kg^{-1} DW soil) in this region (Abuduwaili et al. 2012). We used slightly, moderately, highly and extremely high saline soils in our studies. The chemical characteristics of the soils are shown in Table 1.

Design of cores

The design of in-growth system was based on Johnson et al. (2001) (Fig. S1). Polyvinylchloride (PVC) cores were constructed using PVC water pipe (5-cm diameter × 25-cm height). Two symmetrical rectangular open windows (4-cm width × 12-cm height) were cut at 5-cm depth towards the core bottom. The area of the window consisted of approximately 50 % of surface area of the cylinder. PVC glue was spread homogeneously on the window margin to secure the 30-μm nylon mesh covering each window to allow hyphae but not plant root

Table 1 Chemical characteristics of soil before seeding in experiment 1 and 2

Experiment	Soil salinity level	Total soluble salt (g kg^{-1} DW soil)	Electric conductivity (ms cm^{-1})	pH	SOC (g kg^{-1} DW soil)	Available P (mg kg^{-1} DW soil)	Inorganic N (mg kg^{-1} DW soil)
Experiment 1	Slight	5.69	1.02	7.98	4.36	7.22	30.11
	High	11.87	2.01	8.02	4.12	6.89	28.02
	Extreme	24.36	4.01	7.99	3.02	5.81	20.11
Experiment 2	Moderate	8.33	1.44	8.12	5.19	4.11	39.81
	High	12.56	2.15	8.06	5.67	3.95	34.80

penetration. The base of each core was sealed with a rubber plug secured with PVC glue to prevent the entrance of mycelia or roots.

The soils with different saline strengths were collected independently from different sites from the Cotton Experimental Station, sieved through 2 mm, and then sterilized with 10 k Gy ^{60}Co γ-rays (Institute of Nuclear Technology and Biotechnology, Xinjiang Academy of Agricultural Science, Urumqi) after air dry. Each PVC core was filled with 200 g of 2 mm sieved soils (each of a distinctive saline strength) to within ~2 cm of the core surface. Uniform cotton, maize and sugar beet seeds from a local farm were sterilized with 10 % hydrogen peroxide for 10 min and rinsed 10 times with deionized water. Seeds were then soaked with warm water (28 °C) for 12 h and germinated on moist filter paper at 28 °C in the dark for 48 h in an incubator. After 3 days, four germinated seeds were sown in each core, but only one seedling remained at day seven.

Using these PVC cores, two field experiments were performed to determine the effects of indigenous AM fungi on crop growth and salt resistance in two consecutive years. The first investigated the effects on one crop (cotton) in three saline soils but with a 3-week sowing lag time within the same season in the slightly saline (see below) soil due to low germination (experiment 1). The second evaluated the effects on three crops (sugar beet, cotton and maize) in two saline (see below) soils (experiment 2).

Experimental design and samples analyses

Experiment 1

Soils with three saline strengths (slightly saline, 5.69 g kg^{-1} DW soil; highly saline, 11.87 g kg^{-1} DW soil and extremely high saline, 24.36 g kg^{-1} DW soil) and one crop of cotton (*Gossypium hirsutum* L., Xinluzao 18) were used. A total of 96 PVC cores (32 cores in each saline soil) were constructed. For each saline soil, a total of 32 PVC cores were installed in two rows beside a drip pipe in the cotton field with either static (16 PVC cores) or vibrating (16 PVC cores) core treatments (Fig. S1). The distance was 30 cm between rows and 20 cm between cores in the same row. The static treatment freely allowed AM fungal colonization while the vibrating treatment (gently patting the top core edge ten times along the circumference with a pliers every dawn and sunset) reduced AM fungal colonization. Germinated cotton seeds were sown on June 30th, 2010 in all three saline soils. Seedlings were irrigated with 20 ml of water twice a day and harvested 8 weeks after sowing.

Analyses of growth responses and K^+, Na^+, Cl^- and P concentration

Eight PVC cores from the static or vibrating treatments were randomly selected to determine cotton height, area of functional leaf (the last 4th leaf from the top of plant), root length and tissue biomass. Leaf area and root length were measured using a MRS-9600TFU2 (MICROTEK) Scanner with WinRhizo software (Regent Instrument Inc., Quebec, Canada). Shoot and root biomass were oven dried at 70 °C for 48 h until a consistent weight was reached.

A total of 0.25 g DW leaf samples from functional leaf was digested with 5 ml of sulphuric acid for 12 h and diluted to 50 ml with deionized water. The P concentration was then analysed by atomic absorption spectrophotometry (Johnson and Ulrich 1959) at 450 nm with a VIS-723 Spectrophotometer (Third Shanghai Analytical Instrument Factory, Shanghai, China). Two

grams of functional leaves was digested in 70 % $HClO_4$ and concentrated HNO_3 (1:2 *v*/*v*) for K^+ and Na^+ determination using flame photometry (Precision Scientific Instrument Co., Ltd., Shanghai, China). Cl^- concentrations in the functional leaves were measured after extraction with water at 100 °C and determined by titration with $AgNO_3$.

Analyses of mycorrhizal colonization and plant physiological variables

Another eight PVC cores from the static or vibrating treatments were used to determine the SPAD values (an indicator of chlorophyll content) of the functional leaves, mycorrhizal colonization, proline concentration and soluble sugar concentrations of the roots. The SPAD values were measured by a SPAD-502 plus Chlorophyll Meter (Top Instrument Co., Ltd., Hangzhou, Zhejiang, China).

Approximately 1-cm fresh root segments (30 in each replicate) were cleared with 10 % (*w*/*v*) KOH at 90 °C in a water bath for 30 min and stained with 0.5 % (*w*/*v*) Trypan blue. Mycorrhizal colonization (%) was measured using the gridline-intersection method (Giovannetti and Mosse 1980).

The soluble sugar concentration in fresh roots was tested by the anthrone colorimetry method at 620 nm with a VIS-723 Spectrophotometer (Third Shanghai Analytical Instrument Factory, Shanghai, China). Proline was extracted from 0.3 g of fresh leaves and measured at 520 nm after the ninhydrin reaction according to Bates et al. (1973).

Experiment 2

Soils at two saline strengths (moderately saline, 8.33 g kg^{-1} DW soil and highly saline, 12.56 g kg^{-1} DW soil) and three crops, i.e. sugar beet (*Beta vulgaris* L., Xintian 14), cotton (*Gossypium hirsutum* L., Xinluzao 18) and maize (*Zea mays* L., Zhengda 128), were used. A total of 120 PVC cores (60 cores for each saline soil and 20 cores for each plant species) were constructed, and the core-installation procedures were the same as in experiment 1 (Fig. S1). Four germinated sugar beet, cotton or maize seeds were sown on May 30th, 2011, and one seedling was maintained in each core. Plants were irrigated with 20 ml of water twice each day and harvested 8 weeks after sowing.

Analyses of samples

All cores were harvest together, and the fresh weight of the shoot and root was measured. Measurement of mycorrhizal colonization was the same as in the experiment 1 but with ~200 fresh root segments in each replicate. The remainder of the shoots and roots were oven dried at 70 °C for 48 h. Shoot P concentrations were determined as described in the experiment 1.

Statistical analyses

Data were subjected to either one-way or two-way analysis of variance (ANOVA) using SPSS software version 16.0. Percentage data, e.g., mycorrhizal colonization rate, were arcsine transformed prior to the statistical analysis. Significant differences between static and vibrating treatments in the same soil or between saline soils in the same static and vibrating treatment were compared by the least significant difference (LSD) test at $P \leq 0.05$.

Results

Growth response of sugar beet to vibrating treatment

There were no significant differences in the biomass of leaves (Fig. S2A, $P = 0.08$) or roots (Fig. S2B, $P = 0.32$) of sugar beet between the static and vibrating treatments in both the moderately and the highly saline soils in experiment 2. These results indicated that the nylon mesh on the PVC cores did not limit water and nutrient flow from the soil to the roots, and thus, the in-growth system was able to evaluate the role of AM fungi on crop growth in the field.

The sugar beet leaf (Fig. S2A) biomass was significantly lower in the highly saline soil than in the moderately saline soil ($P \leq 0.05$), regardless of static or vibrating treatment, suggesting that high salinity suppressed plant growth, although the sugar beet is a salt-tolerant crop.

Mycorrhizal colonization and growth responses of cotton and maize to the indigenous AM fungal community

Mycorrhizal colonization of cotton or maize of static treatments were not affected by salinity strengths from

slight to moderate and high (Fig. 1). This may be attributed to the slight and high saline soils of experiment 1 were similar to moderate and high saline soils of experiment 2 (Table 1), respectively.

Mycorrhizal colonization of cotton and maize were significantly higher in the static than in the vibrating treatment, regardless of soil saline strength (Fig. 1), and generally significantly decreased in the high or extremely high salinity treatments in experiment 1 ($P \leq 0.05$, Fig. 1a), but not in experiment 2 (Fig. 1b and c). Interactions of mycorrhizal state and salinity strength on mycorrhizal colonization were observed in experiment 1, but were not in experiment 2, indicating that the response of mycorrhizal colonization of indigenous AM fungi in cotton or maize roots were not always affected by soil saline strength.

Increased colonization of AM fungi significantly enhanced the growth of cotton and maize. For example, in experiment 1, the cotton shoot (Fig. 2a) and root (Fig. 2d) biomass in the slightly saline soil were 73 % and 65 % higher in the static treatment than in the vibrating treatment, respectively. Similar trends in biomass of both cotton and maize were observed in other saline soils in both experiments (Fig. 2a-F), suggesting the indigenous AM fungal community improved the growth of both crops in all soil saline strengths. No interactions of mycorrhizal state and saline strength on cotton and maize were observed in both experiments, suggesting that the soil salinity did not affect the mycorrhizal responsiveness of cotton and maize.

In experiment 1, chlorophyll contents (indicated by SPAD values) were significantly higher in the static treatment than in the vibrating treatment in the slightly and highly saline soils ($P \leq 0.05$), but not in the extremely highly saline soil ($P > 0.05$) (Table S1). Cotton leaf area (Fig. S3A) and root length (Fig. S3B) were significantly higher in the static treatment than in the vibrating treatment, regardless of soil salinity ($P \leq 0.05$). The response of these three indexes to indigenous AM fungi colonization was compatible with that of cotton biomass (Fig. 2a and b).

Phosphorus, sodium, chloride and potassium uptake

Shoot P concentrations of both cotton and maize were significantly greater in the static than in the vibrating treatment in experiment 1 (Fig. 2g) and 2 (Fig. 2h and i). In experiment 1, the cotton leaf Na^+ concentrations (Fig. 3b) were significantly decreased, whereas both the leaf K^+ concentration (Fig. 3a) and the K^+/Na^+ ratio (Fig. 3d) significantly increased ($P \leq 0.05$) in the static compared to the vibrating treatment, regardless of the soil saline strength. Cotton leaf Cl^- concentrations (Fig. 3c) were significantly lower in the static than in the vibrating treatment for both the slightly and highly saline soil ($P \leq 0.05$) but not for the extremely highly saline soil. Interactions of mycorrhizal state and saline strength on cotton leaf Na^+ and Cl^- were observed but not on shoot P concentration, leaf K^+ and the K^+/Na^+ ratio in both experiments, indicating that the effects of indigenous AM fungi on Na^+ and Cl^- uptake were related to soil saline strength.

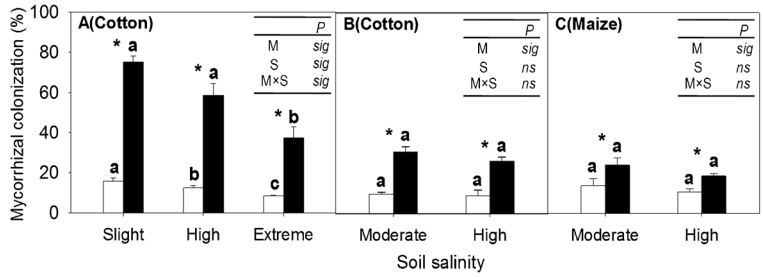

Fig. 1 Effects of vibrating (*open column*) and static (*closed column*) core treatments on mycorrhizal colonization of cotton (*Gossypium hirsutum* L., Xinluzao 18) root in the experiment 1 (**a**) and 2 (**b**) and of maize (*Zea mays* L., Zhengda 128) root in the experiment 2 (**c**) at different soil saline strengths. Data are means ± SE ($n = 8$ in the experiment 1 and 10 in the experiment 2, respectively). Different letters (*a*, *b*) above bars denote significant differences between saline soils for the same vibrating or static treatment, while *asterisks* above bars denote significant differences between vibrating and static treatments for the same saline soil ($P \leq 0.05$). *P* values in the panelled table are results of two way ANOVAs ($P \leq 0.05$) of Mycorrhiza (M) and Salinity (S). *sig* in the table denote significant differences between vibrating and static treatments and/or Salinity (S), while *ns* denote no significant difference. The soil saline strengths in Fig. 1a are slight vs. moderate vs. high = 5.69 vs. 11.87 vs. 24.36 g kg^{-1} DW soil, while those in Fig. 1b and c are moderate vs. high =8.33 vs. 12.56 g kg^{-1} DW soil

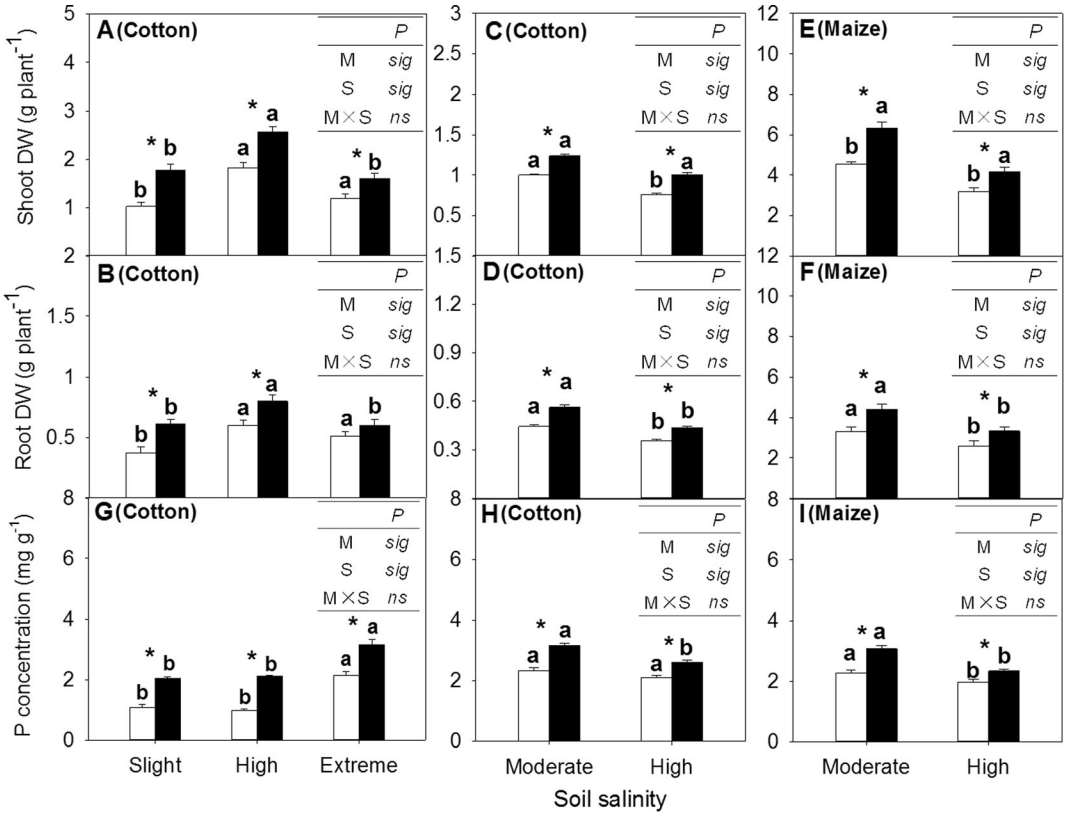

Fig. 2 Effects of vibrating (*open column*) and static (*closed column*) core treatments on dry shoot biomass or dry root biomass or P concentration of cotton (*Gossypium hirsutum* L., Xinluzao 18) in the experiment 1 (**a**, **d**,**g**) and 2 (**b**, **e**,**h**) or maize (*Zea mays* L., Zhengda 128) in the experiment 2 (**c**, **f**,**i**) under different soil saline strengths. Data are means ± SE ($n = 8$ in the experiment 1 and 10 in the experiment 2, respectively). Different letters (*a*, *b*) above bars denote significant differences between saline soils for the same vibrating or static treatment, while *asterisk* above bars denote significant differences between vibrating and static treatments for the same saline soil ($P \leq 0.05$). *P* values in the panelled table are results of two way ANOVAs ($P \leq 0.05$) of Mycorrhiza (M) and Salinity (S). *sig* in the table denote significant differences between vibrating and static treatments and/or Salinity (S), while *ns* denote no significant difference. The soil saline strengths in Fig. 2a, d and g are slight vs. high vs. extreme = 5.69 vs. 11.87 vs. 24.36 g kg^{-1} DW soil, while those in Fig. 2c, d, e, f, h and i are moderate vs. high = 8.33 vs. 12.56 g kg^{-1} DW soil

Response of sugar and proline accumulations to indigenous AM fungi

Significant higher soluble sugar concentration of cotton root was observed in the static than in the vibrating treatments in the extreme highly saline soil (Fig. 3e). Proline concentrations in leaf were significantly higher in the static than in the vibrating treatment in experiment 1 regardless of soil saline strength (Fig. 3f). Interaction between mycorrhizal state and saline strength affected cotton proline concentrations, but not soluble sugar concentrations in experiment 1. These results indicated that the indigenous AM fungi promoted proline synthesis in leaf and soluble sugar accumulation in roots of cotton, and the effects on proline accumulation were related to soil saline strength.

Discussion

Enhancement of salt tolerance of cotton and maize plants by the indigenous AM fungal community in the field

Significantly higher biomass (Fig. 2), leaf area (Fig. S3A), root length (Fig. S3B), plant height and leaf chlorophyll (Table S1) in the static than in the vibrating treatment in the saline soils showed that indigenous AM

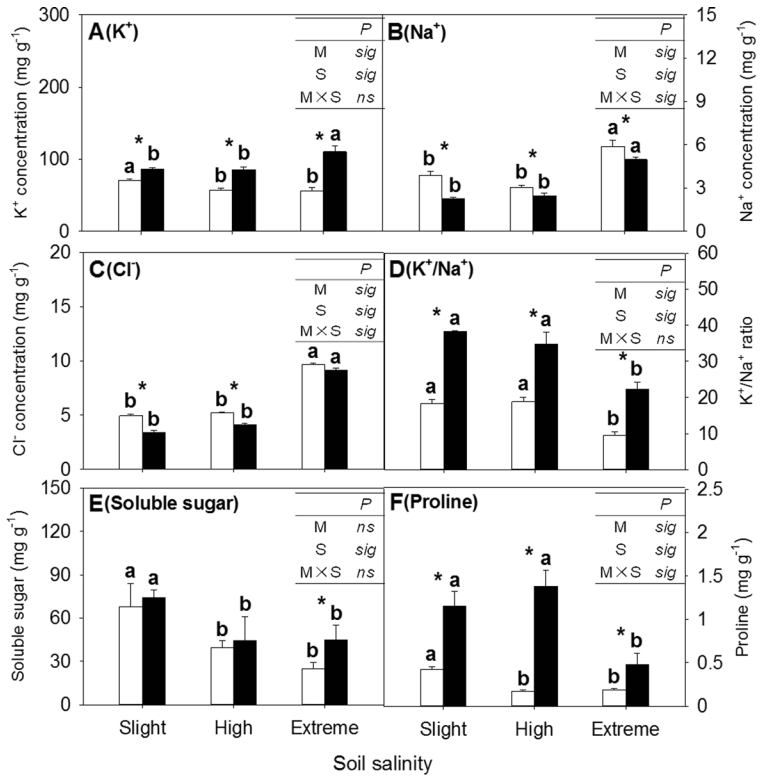

Fig. 3 Effects of vibrating (*open column*) and static (*closed column*) core treatments on cotton (*Gossypium hirsutum* L., Xinluzao 18) K^+ (**a**), Na^+ (**b**), Cl^- (**c**), soluble sugar (**e**) and proline (**f**) concentration and the K^+/Na^+ ratio (**d**) of the last 4th leaf from the top of plant at different soil saline strengths in experiment 1. Data are means ± SE (n = 8). Different letters (*a*, *b*) above bars denote significant differences between saline soils for the same vibrating or static treatment, while *asterisks* above bars denote significant differences between vibrating and static treatments for the same saline soil ($P \leq 0.05$). *P* values in the panelled table are results of two way ANOVAs ($P \leq 0.05$) of Mycorrhiza (M) and Salinity (S). *sig* in the table denote significant differences between vibrating and static treatments and/or Salinity (S), while *ns* denote no significant difference. The soil salinity strengths are slight vs. high vs. extreme = 5.69 vs. 11.87 vs. 24.36 g kg^{-1} DW soil

fungi could alleviate the deleterious effects of salt stress. These field-based results directly demonstrated the positive role of the indigenous AM fungal community in maintaining the growth of cotton and/or maize. It is essential to note that the mycorrhizal colonization and growth responsiveness of crops to AM fungi were still positive with increasing soil salinity, indicating that although soil salt suppresses mycorrhizal colonization, the indigenous AM fungi still existed in the soils and had beneficial effects on crop growth or physiological processes even in extremely high salinity (EC = 4.01 mS cm^{-1}). However, the mycorrhizal colonization did not show correlation with the growth responsiveness of crops to AM fungi in the saline field.

Accumulation of low-molecular-weight compounds, such as proline and soluble sugar, in plant tissues are correlated with higher salt tolerance. Therefore, they are often used to indicate the osmoregulation state of plants. In accordance with Rabie and Almadini (2005) our results that higher proline accumulation occurred in leaves in the static treatment comparing to the vibrating treatment at all saline strengths (Fig. 3f) suggested that the salt tolerance of mycorrhizal plants was related to their higher osmoregulation capacity through more proline synthesis. Feng et al. (2002b) found that maize plants in a low P plus AM fungus treatment had similar P concentrations to those in the high P minus AM fungus treatment, while the mycorrhizal maize had higher dry weight, soluble sugars than non-mycorrhizal plants. The mechanism has been attributed to the AM fungi requirement for carbohydrates that then induces higher soluble sugar accumulation in host root tissues. In the present study, we only observed differences in root sugar concentrations between the static and vibrating treatment at extreme soil salinity (Fig. 3e). This was most likely because the plants in both the static

and the vibrating treatments were colonized by AM fungi, the demand for sugar by AM fungi diminished the differences in root sugar concentrations.

Soil salinity decreases phosphorus availability (Grattan and Grieve 1998) and inhibits Pi uptake by roots and Pi transport in plants (Martinez et al. 1996; Martinez and Läuchli 1994). The presence of indigenous AM fungi improved P uptake of both cotton and maize (Fig. 2) and shoot P concentrations showed a linear positive correlation with shoot dry weights (Fig. S4), suggesting the indigenous AM fungi promoted the growth of maize and cotton by improving P uptake.

The AM fungal community also regulated the K^+/Na^+ ratio (Fig. 3d). The increased K^+/Na^+ ratio is beneficial for plant salt tolerance (Giri et al. 2007). Recent studies also suggested that AM fungi were able to maintain a higher K^+/Na^+ ratio in spores and hyphae to raise the ionic equivalent and avoid Na^+ uptake (Hammer et al. 2011). Our study provided evidence that the common mycorrhizal network plays a role in the regulation of ion balance in plant leaves under natural saline soil conditions.

Utilization of in-growth systems for quantifying the interactions between the indigenous AM fungal community and field crops

Studies of the role of AM fungi in saline soils have mostly been conducted in the laboratory or greenhouse with sterilized soils and a single host plant and/or fungal species. Such experiments are not relevant to natural conditions, and the beneficial effects observed in these experiments may differ from those of an AM fungal network in the field (Jansa et al. 2008) because the AM fungal communities are composed of diverse fungal species (Bharadwaj et al. 2012; Wang et al. 2015). Johnson et al. (2001) created an in-growth system that enabled the functionality of mycorrhizal hyphal networks to be evaluated under conditions closely mimicking those occurring in nature. An essential prerequisite of this approach is that the nylon mesh screen on the PVC core and the disturbance of the PVC core should not have any influence on water flow from the soil to plant roots. The plant growth in the core should not be suppressed by any potential nutrient deficiency due to the mesh barrier. At present, these essential criteria have not been proven for crops with higher and faster transpiration and/or soil water use because of their larger leaf and root systems compared with those of herbaceous plants, e.g. *Trifolium repens*. In the present study, we used a non-mycorrhizal plant, the sugar beet, as a reference. Our results showed that the vibrating treatment did not affect the plant growth of the sugar beet (Fig. S2) but did significantly affect the growth of the mycorrhizal plants, cotton and maize (Fig. 2). Such results suggested that the in-growth system did not limit water flow, and can therefore be used to evaluate the function of the indigenous AM fungal community. However, several factors should be considered in the experimental design when using crop plants. The first is the size of the PVC core and the duration of the experiment. A small-diameter PVC core or longer growth period may result in damage to the mesh windows due to crop root growth and soil fauna activities. The second is the disturbance of the cores. The original method proposed by Johnson et al. (2001) involved rotation or static treatment. In our experience, rotation was not easily performed in the field. Patting the top edge of the PVC core with pliers could cause an up-down vibration and damage the fungal hyphae growing into the core. Use of a non-mycorrhizal plant, such as the sugar beet, is necessary to dismiss all doubts regarding whether a gap is produced between the nylon mesh and soil that could alter the soil water and/or nutrient flow. The third is that the frequency of patting in the vibration treatment should be optimized. In our study, although mycorrhizal colonization of cotton and maize substantially declined after vibrating the cores twice each day compared to the static treatment, 8 % to 15 % root colonization was still observed for both crop plants. These results indicated that it is difficult to establish a true non-mycorrhizal control by inhibiting colonization of the mycorrhizal network in the field. However, this vibrating frequency did establish a low mycorrhizal colonization control, and the extraradical mycelia were limited within the PVC core because vibrating broke the extraradical mycelia that intended to grow out of the core. Therefore, this approach is suitable to explore the on-site interactions between a crop and the indigenous AM fungal community in arable land.

Conclusion

Strategies to improve crop salinity tolerance to enhance productivity in saline soil have focused on the development of salt-tolerant crops by modifying the genetic

traits of salt tolerance (Flowers 2004; Munns and Tester 2008; Schubert et al. 2009; Roy et al. 2014), however, few studies have included plant-microbe associations in these strategies (Ashraf et al. 2008; Ashraf and Foolad 2013; Munns et al. 2006, 2012). Recent studies suggested that modern crop breeding have resulted in large differences in mycorrhizal responsiveness among genotypes (Chu et al. 2013; Janos 2007; Lehmann et al. 2012). Our present results suggest that the indigenous AM fungal community, as a whole, was directly involved in salt tolerance in the field during the seedling stage of cotton and maize. Therefore, an alternative strategy using soil beneficial microbes to enhance crop salt tolerance has received attention recently (Dodd and Pérez-Alfocea 2012; Ruiz-Lozano et al. 2012). Indigenous AM fungi are widespread in arable lands even in high-input farming systems and in saline soils (Tian et al. 2004, 2006; Wang et al. 2015). Our current study may present a new approach for genetic breeders to improve crop salt tolerance by taking advantage of the indigenous AM fungi through screening crop genotypes that are effective mycorrhizal responsiveness under field conditions.

Acknowledgments This study was supported by the National Science Foundation of China (U1403285, 41461051), the Special Fund for Agro-scientific Research in the Public Interest (201103007) and the Innovative Group Grant of the National Science Foundation of China (31421092).

References

Abuduwaili J, Tang Y, Abulimiti M, Liu DW, Ma L (2012) Spatial distribution of soil moisture, salinity and organic matter in Manas River watershed, Xinjiang, China. J Arid Land 4:441–449

Al-Garni SMS (2006) Increasing NaCl-salt tolerance of a halophatic plant *Phragmites australis* by mycorrhizal symbiosis. Amer-Eurasian J Agri Environ Sci 1:119–126

Ashraf M, Foolad MR (2013) Crop breeding for salt tolerance in the era of molecular markers and marker-assisted selection. Plant Breed 132:10–20

Ashraf M, Athar HR, Harris PJC, Kwon TR (2008) Some prospective strategies for improving crop salt tolerance. Adv Agron 97:45–110

Babikova Z, Gilbert L, Bruce TJ, Birkett M, Caulfield JC, Woodcock C, Pickett JA, Johnson D (2013) Underground signals carried through common mycelial networks warn neighbouring plants of aphid attack. Ecol Lett 16:835–843

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Bethlenfalvay GJ, Mihara KL, Schreiner RP, McDaniel H (1996) Mycorrhizae, biocides, and biocontrol. 1. Herbicide-mycorrhiza interactions in soybean and cocklebur treated with bentazon. Appl Soil Ecol 3:197–204

Bharadwaj DP, Alstrom S, Lundquist PO (2012) Interactions among *Glomus irregulare*, arbuscular mycorrhizal spore-associated bacteria, and plant pathogens under in vitro conditions. Mycorrhiza 22:437–447

Busse MD, Fiddler GO, Ratcliff AW (2004) Ectomycorrhizal formation in herbicide-treated soils of differing clay and organic matter content. Water Air Soil Pollut 152:23–34

Campagnac E, Khasa DP (2014) Relationship between genetic variability in *Rhizophagus irregularis* and tolerance to saline conditions. Mycorrhiza 24:121–129

Chen SL, Hawighorst P, Sun J, Polle A (2014) Salt tolerance in *Populus*: significance of stress signaling networks, mycorrhization, and soil amendments for cellular and whole-plant nutrition. Environ Exp Bot 107:113–124

Chiocchio V, Venedikian N, Martinez AE, Menendez A, Ocampo JA, Godeas A (2000) Effect of the fungicide benomyl on spore germination and hyphal length of the arbuscular mycorrhizal fungus *Glomus mosseae*. Int Microbiol 3:173–175

Chu Q, Wang XX, Yang Y, Chen FJ, Zhang FS, Feng G (2013) Mycorrhizal responsiveness of maize (*Zea mays* L.) genotypes as related to releasing date and available P content in soil. Mycorrhiza 23:497–505

Daei G, Ardekani M, Rejali F, Teimuri S, Miransari M (2009) Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. J Plant Physiol 166:617–625

David Jr DD, Nagahashi G, Reider C, Hepperly PR (2007) Inoculation with arbuscular mycorrhizal fungi increases the yield of potatoes in a high P soil. Biol Agric Hortic 25:67–78

Dodd IC, Pérez-Alfocea F (2012) Microbial amelioration of crop salinity stress. J Exp Bot 63:3415–3428

Estrada B, Aroca R, Azcón-Aguilar C, Barea JM, Ruiz-Lozano JM (2013) Importance of native arbuscular mycorrhizal inoculation in the halophyte *Asteriscus maritimus* for successful establishment and growth under saline conditions. Plant Soil 370:175–185

Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 104:1263–1280

Feng G, Su YB, Li XL, Wang H, Zhang FS, Tang CX, Rengel Z (2002a) Histochemical visualization of phosphatase released by arbuscular mycorrhizal fungi in soil. J Plant Nutr 5:969–980

Feng G, Zhang FS, Li XL, Tian CY, Tang C, Rengel Z (2002b) Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza 12:185–190

Flowers TJ (2004) Improving crop salt tolerance. J Exp Bot 55:307–319

Garciaromera I, Ocampo JA (1988) Effect of the herbicide MCPA on VA mycorrhizal infection and growth of *Pisum sativum*. Z Pflanzenernähr Bodenkd 151:225–228

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vescular arbuscular mycorrhizal infection in root. New Phytol 84:489–500

Giri B, Kapoor R, Mukerji K (2007) Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus*

fasciculatum may be partly related to elevated K/Na ratios in root and shoot tissues. Microb Ecol 54:753–760
Grant C, Bittman S, Montreal M, Plenchette C, Morel C (2005) Soil and fertilizer phosphorus: effects on plant P supply and mycorrhizal development. Can J Plant Sci 85:3–14
Grattan S, Grieve C (1998) Salinity-mineral nutrient relations in horticultural crops. Sci Hortic 78:127–157
Hammer EC, Nasr H, Pallon J, Olsson PA, Wallander H (2011) Elemental composition of arbuscular mycorrhizal fungi at high salinity. Mycorrhiza 21:117–129
Jain RK, Paruthi IJ, Gupta DC, Mangal JL (1989) Effect of different levels of soil salinity on *Meloidogyne javanica* infecting okra and brinjal. Pak J Nematol 7:115–119
Janos DP (2007) Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. Mycorrhiza 17:75–91
Jansa J, Smith FA, Smith SE (2008) Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? New Phytol 177:779–789
Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 37:1–16
Johnson CM, Ulrich A (1959) Analytical methods for use in plant analysis. Calif Agr Exp Stn Bull 766:25–78
Johnson D, Leake J, Read D (2001) Novel in-growth core system enables functional studies of grassland mycorrhizal mycelial networks. New Phytol 152:555–562
Latef A, Hamed AA, He CX (2011) Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition, antioxidant enzymes activity and fruit yield of tomato grown under salinity stress. Sci Hortic 127:228–233
Lehmann A, Barto EK, Powell JR, Rillig MC (2012) Mycorrhizal responsiveness trends in annual crop plants and their wild relatives—a meta-analysis on studies from 1981 to 2010. Plant Soil 355:231–250
Lekberg Y, Koide RT, Twomlow SJ (2008) Effect of agricultural management practices on arbuscular mycorrhizal fungal abundance in low-input cropping systems of southern Africa: a case study from Zimbabwe. Biol Fertil Soils 44: 917–923
Li HY, Zhu YG, Marschner P, Smith FA, Smith SE (2005) Wheat responses to arbuscular mycorrhizal fungi in a highly calcareous soil differ from those of clover, and change with plant development and P supply. Plant Soil 277:221–232
Li HY, Smith SE, Holloway RE, Zhu YG, Smith FA (2006) Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of positive growth responses. New Phytol 172:536–543
Martinez V, Läuchli A (1994) Salt-induced inhibition of phosphate uptake in plants of cotton (*Gossypium hirsutum* L.). New Phytol 126:609–614
Martinez V, Bernstein N, Läuchli A (1996) Salt-induced inhibition of phosphorus transport in lettuce plants. Physiol Plant 97: 118–122
Miransari M, Bahrami H, Rejali F, Malakouti M, Torabi H (2007) Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on corn (*Zea mays* L.) growth. Soil Biol Biochem 39:2014–2026
Munns R, Gilliham M (2015) Salinity tolerance of crops-what is the cost? New Phytol 206. doi:10.1111/nph.13519
Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681
Munns R, James RA, Läuchli A (2006) Approaches to increasing the salt tolerance of wheat and other cereals. J Exp Bot 57: 1025–1043
Munns R, James RA, Xu B, et al (2012) Wheat grain yield on saline soils is improved by an ancestral Na^+ transporter gene. Nat Biotechnol 30:360–364
Muok BO, Matsumura A, Ishii T, Odee DW (2009) The effect of intercropping *Sclerocarya birrea* (A. Rich.) Hochst., millet and corn in the presence of arbuscular mycorrhizal fungi. Afr J Biotechnol 8:807–812
Murugensan C, Sonia B, Shuijin H, Sang-Hyon O, Tongmin S (2014) A meta-analysis of arbuscular mycorrhizal effects on plants grown under salt stress. Mycorrhiza 171:76–85
Navarro JM, Pérez-Tornero O, Morte A (2014) Alleviation of salt stress in citrus seedlings inoculated with arbuscular mycorrhizal fungi depends on the rootstock salt tolerance. J Plant Physiol 171:76–85
Newsham K, Fitter A, Watkinson A (1994) Root pathogenic and arbuscular mycorrhizal fungi determine fecundity of asymptomatic plants in the field. J Ecol 82:805–814
Nogueira MA, Cardoso EJBN (2006) Plant growth and phosphorus uptake in mycorrhizal rangpur lime seedlings under different levels of phosphorus. Pesq Agrop Brasileira 41:93–99
Nottingham AT, Turner BL, Winter K, Chamberlain PM, Stott A, Tanner EV (2013) Root and arbuscular mycorrhizal mycelial interactions with soil microorganisms in lowland tropical forest. FEMS Microbiol Ecol 4:210–222
Oehl F, Sieverding E, Mader P, Dubois D, Ineichen K, Boller T, Wiemken A (2004) Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. Oecologia 138:574–583
Paluch EC (2011) Can commercial AM FUNGI inoculum improve prairie restoration? Colonization and growth effects of fungi on sand prairie plants and smooth brome in field soils. Master thesis, Universty of Wisconsin-La Crosse
Rabie G, Almadini A (2005) Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. Afr J Biotechnol 4:210–222
Roy SJ, Negrão S, Tester M (2014) Salt resistant crop plants. Curr Opin Biotechnol 26:115–124
Rozema J, Flowers T (2008) Crops for a salinized world. Science 322:1478–1479
Ruiz-Lozano JM, Porcel R, Azcon C, Aroca R (2012) Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. J Exp Bot 63:4033–4044
Schreiner R, Bethlenfalvay G (1997) Mycorrhizae, biocides, and biocontrol 3. Effects of three different fungicides on developmental stages of three AM fungi. Biol Fertil Soils 24:18–26
Schubert S, Neubert A, Schierholt A, Sumer A, Zorb C (2009) Development of salt-resistant maize hybrids: the combination of physiological strategies using conventional breeding methods. Plant Sci 177:196–202
Schweiger P, Jakobsen I (1999) Direct measurement of arbuscular mycorrhizal phosphorus uptake into field-grown winter wheat. Agron J 91:998–1002
Schweiger P, Spliid N, Jakobsen I (2001) Fungicide application and phosphorus uptake by hyphae of arbuscular mycorrhizal

fungi into field-grown peas. Soil Biol Biochem 33:1231–1237

Sharifi M, Ghorbanli M, Ehrahimzadeh H (2007) Improved growth of salinity stressed soybean after inoculation with pre-treated mycorrhizal fungi. J Plant Physiol 164:1144–1151

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Elsevier and Academic Press, New York, London, Burlington, San Diego

Stewart LI, Hamel C, Hogue R, Moutoglis P (2005) Response of strawberry to inoculation with arbuscular mycorrhizal fungi under very high soil phosphorus conditions. Mycorrhiza 15: 612–619

Thomson BD, Robson AD, Abbott LK (1992) The effect of long-term application of phosphorus-fertilizer on populations of vesicular-arbuscular mycorrhizal fungi in pastures. Aust J Agric Res 43:1131–1142

Tian CY, Feng G, Li XL, Zhang FS (2004) Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants. Appl Soil Ecol 26: 143–148

Tian CY, Shi ZY, Chen ZC, Feng G (2006) Arbuscular mycorrhizal associations in the Gurbantunggut Desert. Chin Sci Bull 51:140–146

Vestberg M, Kahiluoto H, Wallius E (2011) Arbuscular mycorrhizal fungal diversity and species dominance in a temperate soil with long-term conventional and low-input cropping systems. Mycorrhiza 21:351–361

Wang XJ, Wang XX, Feng G (2015) Optimized nitrogen fertilizer management achieved higher diversity of arbuscular mycorrhiza fungi and high-yielding maize (*Zea mays* L.). Crop Past Sci. doi:10.1071/CP14160

Zhang T, Shi N, Bai DS, Chen YL, Feng G (2012) Arbuscular mycorrhizal fungi promote the growth of *Ceratocarpus arenarius* (chenopodiaceae) with no enhancement of phosphorus nutrition. PLoS One 7:1–8

Zhu YG, Smith SE (2001) Seed phosphorus (P) content affects growth, and P uptake of wheat plants and theirassociation with arbuscular mycorrhizal (AM) fungi. Plant Soil 231: 105–112