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Nitrogen fertilizer enhances growth and nutrient uptake of *Medicago sativa* inoculated with *Glomus tortuosum* grown in Cd-contaminated acidic soil

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HIGHLIGHTS

- AMF inoculations increased shoot biomass and decreased shoot Cd concentration.
- N fertilizer only elevated plant performance of alfalfa with Gt inoculation.
- Gt inoculation in combination with N is a more suitable agronomic practice.

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ABSTRACT

This study aimed to explore whether nitrogen availability could influence mycorrhizal function and their associations with host plants in Cd-contaminated acidic soils or not. A greenhouse pot experiment was conducted to assess the effects of mycorrhizal inoculation (non-mycorrhizal inoculation (NM), *Glomus aggregatum* (Ga), *G. tortuosum* (Gt) and *G. versiforme* (Gv)) and inorganic N amendment on the growth, nutrient and Cd uptake of *Medicago sativa* grown in Cd-contaminated acidic soils (10 mg Cd kg^{-1} soil). AMF inoculations significantly increased the shoot and total biomass and decreased the shoot Cd concentration in comparison to plants uninoculated. N addition increased markedly concentration and content of N and decreased those of P in plants at all inoculation treatments. Shoot K, Na and Mg concentration in plants inoculated with Ga and Gv were decreased by N addition, whereas shoot K, Na, Ca and Mg concentration in plants inoculated with Gt were not negatively affected. It was observed that N addition only increased mycorrhizal colonization, shoot biomass, shoot K, Ca and Mg content of plants inoculated with Gt. Irrespective of N addition, plants with Gt inoculation got the maximum shoot and root P concentration and content, as well as P/Cd concentration molar ratio among all inoculation treatment. Neither AMF nor N fertilizer contributed to the decrease of soil exchangeable Cd and increase of soil pH. These results suggested that N fertilizer only elevated plant performance of alfalfa with Gt inoculation grown in acidic soil, by diluting Cd concentration and alleviating of nutrient deficiency, especially P.

1. Introduction

Due to various industrial and agricultural practices, such as mining, metal smelting and application of sewage sludge, heavy metal contamination in soil has attracted considerable public attention worldwide (Tang et al., 2015). Cadmium (Cd) enters the food chain through plant uptake from polluted soils (Aghababaei et al., 2014). Cd is a potentially toxic metal, which could inhibit plant growth with high toxicity at low levels (Yang et al., 2009).

Arbuscular mycorrhizal fungi (AMF) can form a symbiotic association with approximately 80% of terrestrial plant species. They play an important role in the soil/plant system, influencing soil fertility and plant nutrition (Smith and Read, 2008). The extra-radical hyphae extend the surface area and the explorable soil volume for nutrient uptake and enhance the diffusion-limited transport (Lehmann and Rillig, 2015). AMF are considered to not only increase the nutrient uptake of their host plants, but also elevate their tolerance to heavy metal (Aggangan et al., 1998). AMF

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could promote the precipitation or chelation of elements in the rhizosphere and reduce heavy metal uptake, therefore, the morphological, physiological and molecular changes in host plants are improved (Kaldorf et al., 1999; Andrade et al., 2010). On the other hand, some plants could respond negatively to AMF inoculation in contaminated soils (Liao et al., 2003; Liu et al., 2014). Different combinations of host plant and AMF may exhibit different morphology, nutritional status, symbiotic efficiency and gene expression pattern (Feddermann et al., 2010). Subsequently, inoculation with suitable AMF has been proposed to overcome unfavourable conditions (e.g. low nutrient content, high salinity, heavy metal pollution) for plant establishment (Kohler et al., 2015).

Nitrogen is an essential macronutrient and is involved in many metabolic processes including the biosynthesis of amino acids and proteins in plants. Nitrogen fertilizers could alter Cd availability in soils and its accumulation in plants (Valdez-Gonzalez et al., 2014). Results regarding the interaction effect of N supply and metal accumulation in plants are contradictory. Numerous studies showed that inorganic N fertilizer augmented heavy metal concentration in plants (Carrasco-Gil et al., 2012; Bauddh and Singh, 2015; Liu et al., 2016; Moreno-Jimenez et al., 2016; Yang et al., 2016). On the contrary, the nutrition status could influence greatly the capability of plants to accumulate toxic heavy metals, which had been studied in a number of crops to minimize the metal concentration (Sarwar et al., 2010; Singh et al., 2010). Meanwhile, fertilizer can increase or decrease mycorrhizal function and the magnitude of the effect varies among different systems (Treseder, 2004). Such inconsistencies among studies could depend on soil fertility, climate, disturbance regime, and host community, etc (Johnson et al., 2003). If AMF and host plants have the potential to develop mycorrhizal associations in heavy fertilized soils, the manipulation of most beneficial AMF species inoculation or fertilizer practices would be required in agrosystems. However, little is known about the influence of the AMF inoculation in combination with N fertilizer on the growth, nutrient and Cd uptake by plants grown in Cd-contaminated acidic soils.

Alfalfa (*Medicago sativa* L.), is widely cultivated for its high yield, nutritional quality and high protein content (Ashrafi et al., 2014). Alfalfa is a worldwide forage legume that is considered to improve soil quality resulting from nitrogen fixation (Campanelli et al., 2013). *M. sativa* could be an efficient tool for the rehabilitation of Cd contaminated soils by phytoextraction (Ghnaya et al., 2015). The objectives of this study were to: (1) examine the effects of inoculation with AMF on the on growth, nutrient status and Cd uptake of *M. sativa*, (2) determine whether nitrogen shift the plant-mycorrhizal interactions in Cd-contaminated soil or not, (3) explore soil Cd availability in response to AMF inoculation and N fertilizer.

2. Methods

2.1. Experimental design

The experiment included four mycorrhizal inoculation treatments and two N fertilizer treatments with four replicates. These eight treatments were no mycorrhizal inoculation (NM), no mycorrhizal inoculation with N fertilizer (NM + N), *Glomus aggregatum* inoculation (Ga), *G. aggregatum* inoculation with N fertilizer (Ga + N), *G. tortuosum* inoculation (Gt), *G. tortuosum* inoculation with N fertilizer (Gt + N), *G. versiforme* inoculation (Gv) and *G. versiforme* inoculation with N fertilizer (Gv + N). The AMF were purchased from Beijing Academy of Agriculture and Forestry, China. The AMF inocula species were propagated on maize in autoclaved substrate (sand/soil, 1:2) for two successive propagation cycles, each three months long. The soil used in the experiment was collected from farmland located in Xinghua City, Jiangsu Province, China (119°46′23″E, 33°02′33″N). The substrate used for experiment was mixed with river sand (sand/soil, 1:2). The soil had the following properties: pH 4.38, total N 1.55 g kg^{-1}, total P 0.327 g kg^{-1}, total K 5.28 g kg^{-1}, total organic carbon 12.22 g kg^{-1}, available N 71.2 mg kg^{-1}. The substrate was milled and homogenized by sieving through a 2 mm mesh sieve, sterilized by autoclaving at 120 °C for 2 h. Cd was added in the form of aliquots of aqueous solutions $CdCl_2$, at a rate of 10 mg Cd kg^{-1}, and thoroughly mixed in each treatments. And then the soils were allowed to stabilize for 45 days. The plants were cultured in plastic pots containing 560 g soil sterilized soil was used for the control treatments. Mycorrhizal treatments received 50 g of fresh inoculum while non-mycorrhizal plants received 50 g of sterilized inoculum. The inoculum in each pot contains approximately 1600 spores for each AMF species. To establish and reactivate soil microorganisms, 50 ml suspension was added to each pot to inoculate the autoclaved soil with fresh microorganisms. 100 g fresh soil was suspended in 300 ml deionized water and filtered through a 25 μm for eliminating AMF spores.

Alfalfa seeds were surface-sterilized with 0.5% NaClO solution, and then sown into pots. During the growth period, plants were watered with distilled water to maintain 60% water holding capacity. Basal nutrients applied at the following amount expressed in micrograms per pot: KH_2PO_4 1088, $MgSO_4 \cdot 7H_2O$ 7088, Fe(II)-EDTA 8.816, H_2BO_3 22.88, $ZnSO_4 \cdot 7H_2O$ 1.76, $MnCl_2 \cdot 4H_2O$ 14.48, $CuSO_4 \cdot 5H_2O$ 0.408, $Na_2MoO_4 \cdot 2H_2O$ 0.96. Nitrogen fertilization was divided between two applications, delivered at 60 and 100 days after sowing. The aqueous solutions of NH_4NO_3 (0.2 g N kg^{-1}) were added to the soils at each fertilizer application.

2.2. Measurements

The plants were harvest after growing for 130 days. The roots were washed with distilled water. Thirty pieces of 1-cm long fine root samples were randomly collected from plants at each pot. Roots were cleared in 10% KOH at 90 °C for 30 min in a hot-water bath and stained with 0.05% trypan blue. And then mycorrhizal colonization was determined by the grid intersection method. The shoots and rest roots were dried at 70 °C for 72 h to constant weight. The dried plant materials were wet-digested in a mixture of HNO_3 and $HClO_4$ (2:1, v/v). Soil exchangeable Cd concentrations were extracted with 0.5 M $MgCl_2$. The plant tissue P, K, Na, Ca, Mg and Cd concentrations, as well as soil exchangeable Cd concentrations, were determined using ICP-OES (PerkinElmer Optima 8000). The N concentrations in plants were determined with Kjeldahl method. Soil mineral N (NO_3^-, and NH_4^+) was extracted with 1 mol L^{-1} K_2SO_4, and then the concentrations were determined using an ultraviolet spectrophotometer (PerkinElmer Lambda 25). Soil pH was measured with a pH meter using a soil: water ratio of 1: 2.5. The translocation factor (TF) indicated the ability of plants to translocate cadmium from the roots to the shoots (Valdez-Gonzalez et al., 2014). TF was calculated as: TF = Cd concentration in shoots/Cd concentration in roots.

2.3. Statistical analysis

Except mycorrhizal colonization, all results were tested by one-way ANOVA or two-way ANOVA analysis of variance. The LSD multiple tests was used for post-hoc comparison at a significant level of $P < 0.05$. Non-parametric analysis (Kruskal-Wallis rank test) was used for the effect of treatments on the mycorrhizal colonization. All statistical tests were performed on SPSS 13.0 package.

3. Results

3.1. Root colonization and plant biomass

Regardless of N addition, plants inoculated with Gt got the highest mycorrhizal colonization, following by Ga and Gv (Fig. 1). No significant mycorrhizal inoculation × N addition interaction was observed in shoot, root and total biomass, and root/shoot ratio (Table 2). N addition only increased mycorrhizal colonization and shoot biomass in plants inoculated with Gt (Fig. 1 and Table 1). Compared with non-inoculation treatments, AMF inoculation significantly increased the shoot and total biomass, irrespective of N amendment. N fertilizer input caused significantly reductions of root biomass and root/shoot ratio at all AMF inoculation treatments. Maximum and minimum root/shoot ratio occurred in the NM and Gt + N treatments, respectively. N addition decreased the total biomass of alfalfa uninoculated and inoculated with Gv, whereas those of plants inoculated with Ga and Gt was not affected by N addition (Table 1).

3.2. Plant nutrient uptake

Shoot N, P, K, Na, Ca and Mg concentration, root N, P, K and Na concentration was significantly affected by N addition (Table 2). N fertilizer significantly increased shoot and root N concentration at all AMF inoculation treatments. Shoot and root N concentrations showed the lowest value in the Gv inoculation treatments in absence of N fertilizer, while those exhibited lowest value in the Gt + N treatments in presence of N fertilizer (Fig. 2A). Regardless of N addition, compared with non-inoculation treatments, shoot and root P concentration was increased by Gt inoculation and shoot K, Na, Ca and Mg concentration was not promoted by AMF inoculation (Fig. 2B, C, E, F). Although shoot K, Na and Mg concentrations were decreased by N addition in plants inoculated with Ga and Gv, that of plants inoculated with Gt was not influenced by N fertilizer. Root Na concentration remained constant among all treatments without N addition, whereas the root Na concentrations were increased by Ga and Gt inoculations in presence of N fertilizer (Fig. 2D). Root K and Mg concentrations were decreased by AMF inoculation in absence of N fertilizer, but when N fertilizer was applied, only Gt inoculation reduced root K and Mg concentration, in comparison to non-mycorrhizal inoculation treatment (Fig. 2C, F).

N, P, K, Na, Ca and Mg concentrations of shoots were significantly affected by mycorrhizal inoculation, while those of root were influenced by N addition (Table 2). The maximum shoot N, K, Ca, Mg and Na content occurred in the Gt + N treatments, while the maximum shoot P content occurred in the Gt treatments (Fig. 3A–F). N fertilizers significantly increased shoot and root N contents and decreased P contents at all AMF inoculation treatments (Fig. 3A and B). Except plants inoculated with Gt, the shoot and root K content was significantly decreased by N fertilizer application (Fig. 3C). Root K, Na, Ca and Mg contents of plants uninoculated and inoculated with Gv were decreased by N fertilizer application.

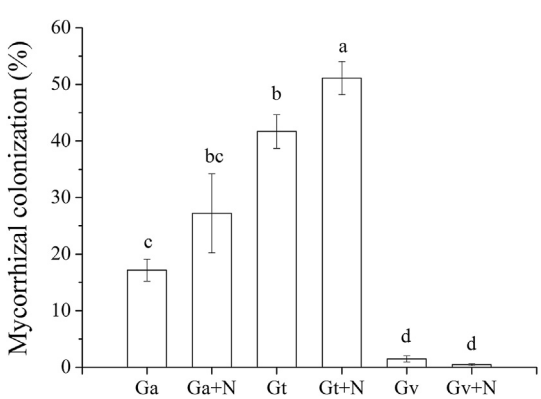

Fig. 1. Effects of AMF inoculation and N fertilizer on mycorrhizal colonization of alfalfa plants. Ga, Gt and Gv represent inoculation with *Glomus aggregatum*, *G. tortuosum* and *G. versiforme*, respectively. Means ± S.E. Different letters represent significant difference according to Kruskal-Wallis rank test.

3.3. Plant Cd uptake

Interactions between mycorrhizal inoculation and N addition had significant effects on Cd concentrations and contents in both shoot and root (Table 2). AMF inoculations significantly decreased shoot Cd concentration in comparison to the non-mycorrhizal inoculation treatments, irrespective of N addition. Maximum and minimum shoot Cd concentration occurred in NM and Gt treatments, respectively. Nitrogen fertilizer application only decreased shoot Cd concentration in plants without AMF inoculation. When plants were inoculated with AMF, it was found that N fertilizer application has no effect on shoot Cd concentration. On the contrary, root Cd concentration was increased by N addition at all inoculation treatments (Fig. 4A). When plants inoculated with Ga and Gv, N fertilizer application did not affect shoot Cd content. It was observed that N amendment increased the shoot Cd content in plants inoculated with Gt but decreased shoot Cd content in plants without AMF inoculation. For root Cd accumulation, N amendment only enhanced plants inoculated with Ga (Fig. 4B).

Two-way ANOVA showed that significant main effects were found in N/Cd and P/Cd concentration molar ratio in shoot and root (Table 2). The shoot N/Cd concentration molar ratios showed no significant difference among the plants with AMF inoculation in presence of N amendment. Root N/Cd concentration molar ratio got the highest value in the Gt + N treatments. When no N fertilizer was applied, shoot and root N/Cd concentration molar ratios of Gt treatments were significantly higher than other inoculation treatments. Regardless of N fertilizer, shoot and root P/Cd concentration molar ratio of Gt treatments was significantly higher than other inoculation treatments. TF did not show significant differences among the inoculation treatments without N fertilizer input. TF did not affected by N addition in plants inoculated with Gt but decreased in other inoculation treatments (Table 1).

3.4. Soil pH, inorganic N and exchangeable Cd concentration

Neither AMF nor N addition affected soil exchangeable Cd concentration (Fig. 5C). Fig. 5D showed that pH in soil significantly decreased in Gv + N treatments in comparison to NM treatments. Mycorrhizal inoculation × N addition interaction only had significant effect on NO_3^--N (Table 2). Compared with NM + N treatments, the soil NH_4^+-N and NO_3^--N concentration in the Ga + N and Gt + N treatments significantly decreased. For the non-N amendment treatments, NH_4^+-N concentration of Ga and Gt treatments was significantly lower than that of NM treatments, whereas the NO_3^--N concentration remained constant throughout all inoculation treatments (Fig. 5C and D).

4. Discussion

4.1. Effects of AMF inoculation

Similar to previous studies, the increased biomass accompanying with decreased Cd concentration after AMF inoculation was observed (Fig. 4A, Table 1) (Hu et al., 2013; Liu et al., 2014, 2015).

Table 1
Effects of AMF inoculation and N fertilizer on plant biomass, root/shoot ratio, N/Cd concentration molar ratio, P/Cd concentration ratio and translocation factor after alfalfa harvest. NM, Ga, Gt and Gv represent the non-mycorrhizal treatment, inoculation with *Glomus aggregatum*, *G. tortuosum* and *G. versiforme*, respectively. Means ± S.E. Different letters represent significant difference according to LSD multiple tests.

Treatments	Shoot biomass (g pot^{-1})	Root biomass (g pot^{-1})	Total biomass (g pot^{-1})	Root/Shoot ratio	Shoot N/Cd concentration molar ratio	Shoot P/Cd concentration molar ratio	Root N/Cd concentration molar ratio	Root P/Cd concentration molar ratio	Translocation factor (TF)
NM	0.54 ± 0.02d	0.68 ± 0.03bc	1.23 ± 0.03d	1.26 ± 0.07a	3702.48 ± 134.34e	101.61 ± 4.87c	498.08 ± 38.05e	17.72 ± 1.92d	0.18 ± 0.02a
NM + N	0.5 ± 0.07d	0.41 ± 0.02d	0.96 ± 0.08e	0.75 ± 0.07ef	9635.24 ± 273.50bc	76.69 ± 6.58d	927.18 ± 19.27c	6.15 ± 0.43f	0.09 ± 0.00c
Ga	0.79 ± 0.02bc	0.81 ± 0.07ab	1.60 ± 0.07ab	1.03 ± 0.09bc	5943.43 ± 396.30d	165.88 ± 5.29b	694.28 ± 48.40d	30.62 ± 1.97b	0.17 ± 0.02a
Ga + N	0.78 ± 0.07c	0.68 ± 0.04bc	1.46 ± 0.08bc	0.88 ± 0.10cde	12,240.39 ± 814.21a	98.38 ± 5.64c	1022.86 ± 67.44c	7.61 ± 0.81f	0.08 ± 0.01c
Gt	0.92 ± 0.05b	0.89 ± 0.07a	1.81 ± 0.11a	0.97 ± 0.04bcd	8113.71 ± 441.83c	289.18 ± 25.87a	1132.26 ± 97.80bc	63.91 ± 5.98a	0.19 ± 0.01a
Gt + N	1.09 ± 0.06a	0.68 ± 0.04bc	1.78 ± 0.10a	0.62 ± 0.02f	11,090.82 ± 536.06ab	143.06 ± 13.05b	1645.16 ± 159.96a	18.63 ± 1.02cd	0.15 ± 0.02ab
Gv	0.77 ± 0.03c	0.86 ± 0.05a	1.63 ± 0.04ab	1.12 ± 0.11ab	4887.58 ± 530.52de	154.68 ± 12.01b	678.10 ± 63.30d	23.85 ± 2.21c	0.18 ± 0.03a
Gv + N	0.75 ± 0.01c	0.60 ± 0.03c	1.35 ± 0.03cd	0.80 ± 0.47def	11,086.70 ± 717.51ab	82.91 ± 4.68cd	1285.29 ± 11.80b	9.92 ± 0.31e	0.12 ± 0.01bc

Table 2
Significant levels of mycorrhizal inoculation, N addition and their interactions on variables on a two-way ANOVA analysis.

Variables	Mycorrhizal inoculation	N addition	Mycorrhizal inoculation × N addition
Shoot biomass	***	ns	ns
Root biomass	ns	***	ns
Total biomass	**	*	ns
Root/Shoot ratio	ns	***	ns
Shoot N/Cd concentration molar ratio	*	***	*
Shoot P/Cd concentration molar ratio	***	***	ns
Root N/Cd concentration molar ratio	***	***	ns
Root P/Cd concentration molar ratio	***	***	*
Translocation factor	*	***	ns
Shoot N concentration	ns	***	***
Shoot P concentration	***	***	ns
Shoot K concentration	ns	***	**
Shoot Na concentration	***	*	ns
Shoot Ca concentration	ns	***	**
Shoot Mg concentration	ns	**	**
Shoot Cd concentration	**	ns	*
Root N concentration	**	***	***
Root P concentration	***	***	**
Root K concentration	ns	***	ns
Root Na concentration	**	***	ns
Root Ca concentration	**	ns	ns
Root Mg concentration	**	ns	ns
Root Cd concentration	***	***	*
Shoot N content	***	***	ns
Shoot P content	***	***	ns
Shoot K content	***	*	***
Shoot Na content	***	ns	ns
Shoot Ca content	**	***	***
Shoot Mg content	***	ns	***
Shoot Cd content	ns	ns	**
Root N content	ns	***	ns
Root P content	***	***	*
Root K content	ns	***	ns
Root Na content	**	***	ns
Root Ca content	**	**	*
Root Mg content	**	**	ns
Root Cd content	***	***	**
NH_4^+-N concentration	**	***	ns
NO_3^--N concentration	*	***	***
Exchangeable Cd concentration	ns	ns	ns
pH	*	**	ns

ns: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

This elevated plant biomass may result in "growth dilute effect" in plants, thus minimizing the heavy metal phytotoxicity (Chen et al., 2007). Although absorption of macronutrients such as N, P, K and Mg concentrations were increased by AMF inoculation in shoots (Sarkar et al., 2015; Zhao et al., 2015), it was observed that only P concentration in shoots was increased by AMF inoculation in comparison to non-mycorrhizal treatments in this study (Fig. 2). AMF could elevate nutrient uptake of their host plant, in particular P-uptake efficiency (Smith and Read, 2008). It was reported that host plants inoculated with different AMF species exhibited different tolerances in heavy metal polluted soils (Wu et al., 2009). This symbiotic efficiency mainly depended on heavy metal concentrations, soil physicochemical characteristics and species both of host plant and AMF (Liu et al., 2015). This variation in both plant and fungal capabilities termed as "functional diversity" (Burleigh et al., 2002). We found that plants with Gt inoculation got the highest mycorrhizal colonization, shoot, root and total biomass, shoot and root P concentration and content, P/Cd concentration

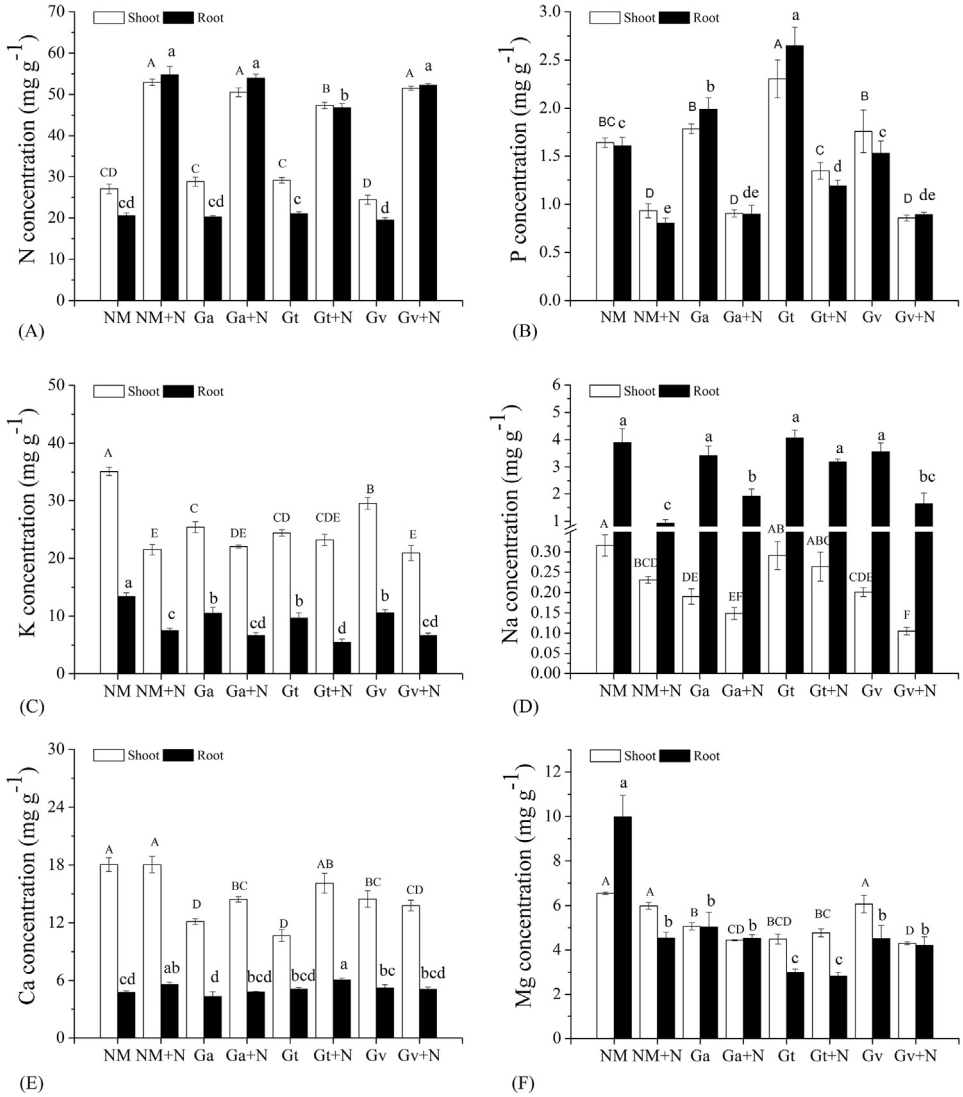

Fig. 2. Effects of AMF inoculation and N fertilizer on nutrient concentrations of alfalfa plants. NM, Ga, Gt and Gv represent the nonmycorrhizal treatment, inoculation with *Glomus aggregatum*, *G. tortuosum* and *G. versiforme*, respectively. Means ± S.E. Different upper letters represent significant differences in shoots, while lower letters represent significant differences in roots according to LSD multiple tests.

molar ratio among all inoculation treatments (Figs. 2 and 3, Table 1). These results indicated that Gt inoculation could increase plant performance through increasing nutrient uptake and decreasing Cd concentration, rather than Ga and Gv inoculation.

4.2. Effects of nitrogen addition

N fertilizers are used to enhance plant biomass and the phytoextraction efficiency of contaminants from soils (Giansoldati et al., 2012). It is known that nitrogen fertilization alter soil solution equilibrium, and root morphological parameters, which might increase heavy metal availability and potentially augment heavy metal uptake (Alloway, 1995). The addition of nitrogen fertilizer significantly elevated the concentration of Hg in alfalfa roots at the seedling stage (Carrasco-Gil et al., 2012). On the other hand, increased N supply would decrease heavy metal concentration due to a dilution effect (Sarwar et al., 2010). It is suggested that N deficiency initiates transcriptional changes that lead to the accumulation of carbohydrates in the shoots and increase the translocation of sucrose to the roots (Hermans et al., 2006). Our results were in agreement with previous study, which reported root/shoot ratio decreased when nitrogen fertilizers were applied (Liu et al., 2016). Therefore, we suggested that the decreased root Cd concentration in plants without nitrogen fertilizer in each inoculation treatments might be due to the increases of root biomasses that could dilute Cd. Nevertheless, Cd concentration in shoots exhibited the opposite trend in response to N input, decreasing by N supply in plants without AMF inoculation and remaining constant in plants with AMF inoculation (Fig. 4). Previous studies using different forms of N to study Cd accumulation in rice suggested an antagonistic effect between Cd and NH_4^+ and a synergistic effect between Cd and NO_3^- (Hassan et al., 2005, 2008; Jalloh et al., 2009). A hydroponic culture experiment showed that a significantly lower Cd uptake was associated with a higher inorganic nitrogen supply in potato (Jonsson and Asp, 2013). The reduction in Cd uptake caused by fertilization might result from the competitive interaction between ions sorption sites at root level (Valdez-Gonzalez et al., 2014). At the end of this experiment, the NH_4^+ in treatment without inoculation was significantly higher than other inoculation treatments (Fig. 5). Moreover, it was found that N addition had no

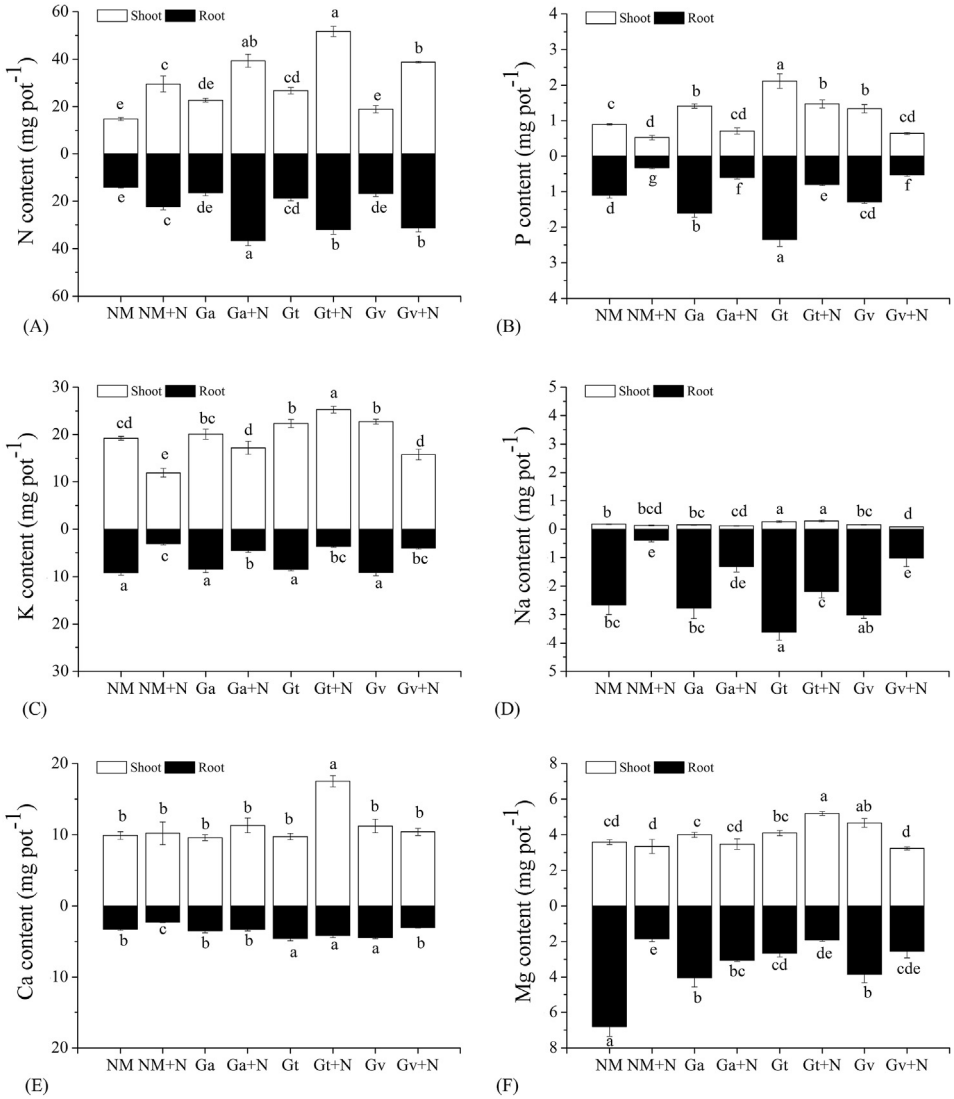

Fig. 3. Effects of AMF inoculation and N fertilizer on nutrient contents of alfalfa plants. NM, Ga, Gt and Gv represent the nonmycorrhizal treatment, inoculation with *Glomus aggregatum*, *G. tortuosum* and *G. versiforme*, respectively. Means ± S.E. Different letters represent significant difference according to LSD multiple tests.

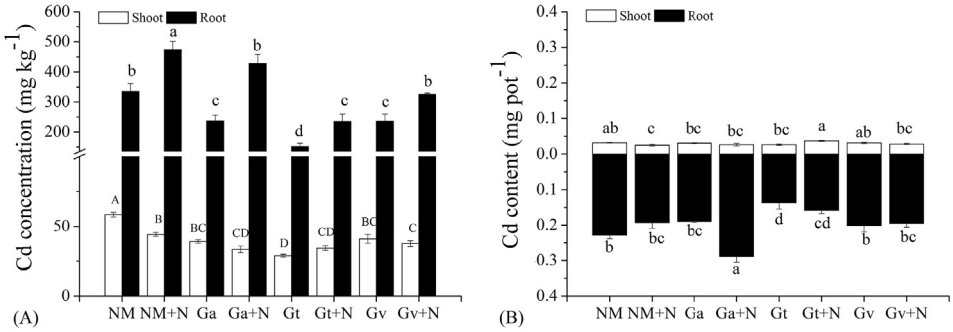

Fig. 4. Effects of AMF inoculation and N fertilizer on cadmium concentrations of alfalfa plants. NM, Ga, Gt and Gv represent the nonmycorrhizal treatment, inoculation with *Glomus aggregatum*, *G. tortuosum* and *G. versiforme*, respectively. Means ± S.E. For Cd concentration, different upper letters represent significant differences in shoot, while lower letters represent significant differences in roots according to LSD multiple tests.

positive effect on the shoot biomass of plant uninoculated (Table 1). Thus, we predicted that the antagonistic effect caused by relatively higher soil NH_4^+ concentration in NM + N treatments might be responsible for the reduction of shoot Cd uptake.

4.3. Effect of N addition on AMF

Species and genera of mycorrhizal fungi appear to vary in responses to nutrient additions in field studies (Treseder and Allen,

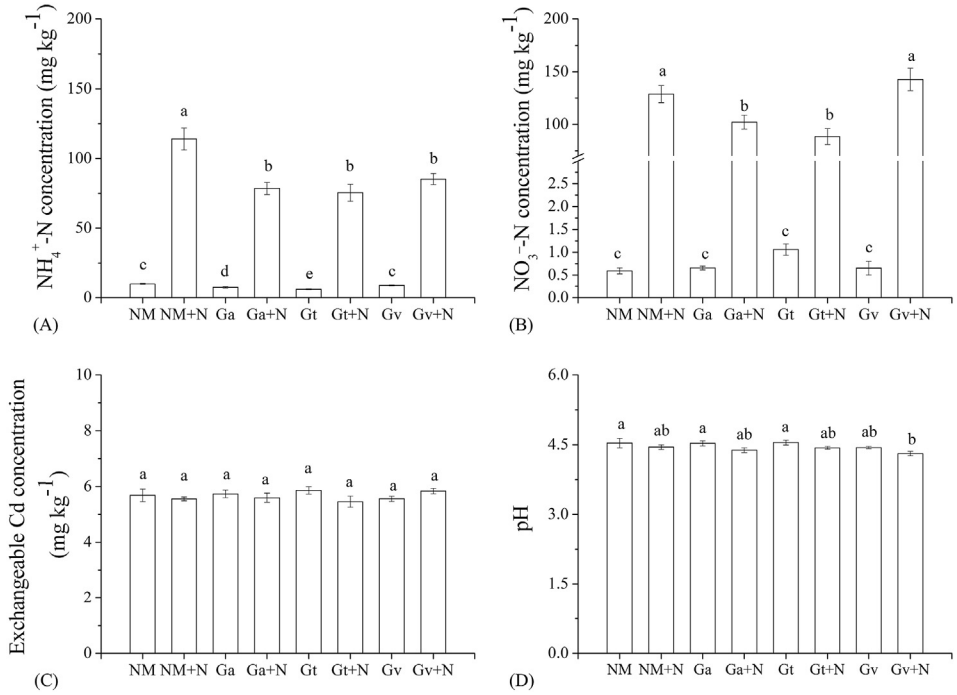

Fig. 5. Effects of AMF inoculation and N fertilizer on inorganic N concentration, soil exchangeable Cd concentration and pH after alfalfa harvest. NM, Ga, Gt and Gv represent the nonmycorrhizal treatment, inoculation with *Glomus aggregatum*, *G. tortuosum* and *G. versiforme*, respectively. Means ± S.E. Different letters represent significant difference according to LSD multiple tests.

2002). It was reported that nitrogen additions decreased relative abundance of *G. gigantea*, *Gigaspora margarita*, *Scutellospora calospora*, and *G. occultum*, and increases that of *G. intraradices* in the Cedar Creek Natural History Area in Minnesota (Johnson, 1993). Mycorrhizal function also differed among different host plants, it was found that root colonization of smooth brome was reduced by N addition but that of big bluestem was not affected (Grman and Robinson, 2013). Treseder and Allen (2002) summarized that N fertilization should increase mycorrhizal growth where mycorrhizal fungi are initially nutrient-limited, decrease mycorrhizal growth where plants are nutrient-limited but fungi are not, and have no effect on fungi where neither organism is nutrient-limited. Hodge and Fitter (2010) suggested that AMF also have high N demand, and this requirement for N could partly explain the limitation of AMF in very infertile soils (Grman and Robinson, 2013). The increase in mycorrhizal colonization, shoot biomass, N, K, Ca, Mg content of plants inoculated with Gt after N addition indicated that increased N availability could increase the potential mycorrhizal function of Gt (Figs. 1–3, Table 1). Although N addition decreased P absorption at all inoculation treatments, Gt inoculation enhanced P absorption of plants, irrespective of N addition. In addition, the soil inorganic N supply remained abundant during the experiment period (Fig. 5). Therefore, we suspected that the elevated shoot biomass induced by N addition might result from the relief of P limitation.

4.4. Soil Cd availability in response to AMF and nitrogen

AMF could enhance the immobilization of heavy metals in the fungi structures (Andrade et al., 2010), and then reducing the transfer of heavy metals from root to shoot of the host plant (Kaldorf et al., 1999). Except "growth dilution effect", the extraradical mycelium of mycorrhizal roots may directly immobilize Cd, and restrict Cd transfer to plants (Nayuki et al., 2014). Additionally, glomalin, a glycoprotein produced by AMF, played an important role in sequestering substantial amounts of heavy metals in soils (Cornejo et al., 2008). Using alfalfa as host plant, AMF species differently affected glomalin-related soil protein concentration and the ability to form extensive and dense mycelial networks (Bedini et al., 2009). Previous studies reported that the soil exchangeable Cd was decreased by the AMF inoculation (Hu et al., 2013; Wang et al., 2016). Meanwhile, the pH increased. On the other hand, other study showed that AMF inoculation could increased Cd bioavailable in 25 and 50 mg Cd kg^{-1} soils and had no effect on that in 100 mg Cd kg^{-1} soils (Liu et al., 2015). Moreover, increased soil pH in the rhizosphere would make the metals more unavailable for plant uptake (Shen et al., 2006), which might be one of the mechanisms that enhanced the phytostabilization and protected the host plant against heavy metal toxicity. Neither AMF inoculation or N supply contributed to the increase of soil pH and decrease of soil exchangeable Cd in this experiment (Fig. 5), indicating the phytostabilization might be not the main reason for the reduced Cd concentration in shoot.

5. Conclusion

N addition increased N concentration and decreased P concentration in plants at all inoculation treatments. Regardless of N fertilizer input, plants with Gt inoculation got the highest mycorrhizal colonization, shoot, root and total biomass, shoot and root P concentration and content, P/Cd concentration molar ratio among all inoculation treatments. In addition, the maximum N, K, Na, Ca, Mg and Cd content occurred in the Gt + N treatments. This promotion might be due to the alleviation of P limitation in plants, irrespective of N addition. Moreover, AMF and nitrogen fertilizer did not contribute to the decrease of soil exchangeable Cd and increase of soil pH. Based on the results, we suggested that the reduction of Cd concentration might be chiefly attributed to biological dilution effect. These results indicated that N fertilizer would only enhance performance of alfalfa inoculated with Gt, which implied that Gt

inoculation in combination with N fertilizer would be a more suitable agronomic practice in Cd-contaminated acidic soil.

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