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Mycorrhizas effects on nutrient interception in two riparian grass species

Hamid Reza Asghari [a,*], Timothy Richard Cavagnaro [b]

[a] Shahrood University of Technology, Faculty of Agriculture, Shahrood, Iran

[b] School of Biological Sciences , Monash University, Australian Centre for Biodiversity, Clayton, Victoria, Australia

Abstract

Effects of arbuscular mycorrhizal (AM) fungi on plant growth and soil nutrient depletion are well known, but their roles as nutrient interceptor in riparian areas are less clear. The effects of AM fungi on growth, soil nutrient depletion and nutrient leaching were investigated in columns with two riparian grass species. Mycorrhizal and non mycorrhizal (NM) plants were grown in a mixture of riparian soil and sand (60% and 40%, w/w respectively) for 8 weeks under glasshouse conditions. Mycorrhizal colonization, AM external hyphae development, plant growth, nutrient uptake and NO_3, NH_4 and available P in soil and leachate were measured. Mycorrhizal fungi highly colonized roots of exotic grass *Phalaris aquatica* and significantly increased plant growth and nutrient uptake. Columns containing of AM *Phalaris aquatica* had higher levels of AM external hyphae, lower levels of NO_3, NH_4 and available P in soil and leachate than NM columns. Although roots of native grass *Austrodanthonia caespitosa* had moderately high levels of AM colonization and AM external hyphae in soil, AM inoculation had no significant effects on plant growth, soil and leachate concentration of NO_3 and NH_4. But AM inoculation decreased available soil P concentration in deeper soil layer and had no effects on dissolved P in leachate. Although both grass species had nearly the same biomass, results showed that leachate collected from *Austrodanthonia caespitosa* columns significantly had lower levels of NO_3, NH_4 and dissolve P than leachate from exotic *Phalaris aquatica* columns. Taken together, these data shows that native plant species intercept higher nutrient than exotic plant species and had no responsiveness to AM fungi related to nutrient leaching, but AM fungi play an important role in interception of nutrient in exotic plant species.

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Introduction

Nitrogen (N) and phosphorus (P) are the two soil nutrients that most often limit plant growth. Consequently, there are applied to agricultural soils to help achieved acceptable crop yields (Jackson et al. 2008). These elements have been used as important fertilizers in crop production and rangelands management, and a major portion of yields are attributable to these fertilizers. Usually a high amount of these nutrients as fertilizers apply to the soil, but because of the soluble fraction of these nutrients can be taken up by plant, the amount of these nutrients taken up by the plant relative to the amount of N and P applied to the soil is low (Vassilev and Vassileva, 2003). In this situation high levels of applied N and P to the soil are potentially available to transfer to water ecosystem via runoff, which may pollute the ecosystem. The growing concern about water pollution and then eutrophication has made it urgent to restrict losses of nutrient from land use activities and developing methods for predicting such losses (Torrent and Delgado, 2000). Mitigation of nitrogen and phosphorus runoff has become a major goal in riparian ecosystems. Riparian zones are the

* Corresponding author.
Shahrood University of Technology, Faculty of Agriculture, 36137 Shahrood, Iran
Tel.: +989122734282
E-mail address: hamidasghari@gmail.com
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interface between land and a stream. Because of the role of riparian areas in soil conservation, biodiversity, and the influence they have on aquatic ecosystems these areas have critically important ecological functions. Riparian zones can act as a filter strip (buffer), which can intercept sediment and nutrient from reaching streams, and thereby help to protect and improve water quality in aquatic ecosystems (Kovar and Claassen, 2009).

In nutrient interception progress in riparian zone the role of vegetation is highly important, which can slow down the runoff, and cause sediment and attached nutrients to be deposited on the land before they can reach the water reservoir. Different plant species has different potential to intercept and minimize the flow of nutrients and sediments into waterways (Chantachon et al., 2004; Zhuang et al., 2005). In a solution-culture experiment Kovar and Claassen (2009) suggest that *Phalaris arundinacea* L. and *Panicum vitgatum* L. would more effectively uptake and deplete dissolved P than would *Bromus inermis* Leyss. Although the amounts of nutrient uptake by plants from soils are directly depending on plant characteristics (particularly root volume), soil microflora could have significant effects on soil nutrient availability and uptake by plants. Among the soil microorganisms, arbuscular mycorrhizal (AM) fungi are ubiquitous and are the most widespread mutualistic symbiosis on earth (Barea and Jeffries, 1995). One of the most important benefits of AM fungi for plants is improved access to limiting soil resources. They are only ones providing a direct link between soil and roots, so they can aid in ecosystem bioremediation such as heavy metals phytoremadiation (Göhre and Paszkowski, 2006; Khade and Adholeya, 2007). The contribution of AM hyphae to nutrient uptake from the soil is widely investigated. In some cases 100% of a special nutrient can uptake via mycorrhizal hyphae (Smith et al., 2003). Contribution of AM fungi on nutrient interception by enhanced nutrient depletion and plant uptake (phytoextraction) and nutrient immobilization within the soil (phytostabilization) has received less attention. The purpose of this study was to investigate the effects of mycorrhizal colonization on the mitigation of N and P leaching in a native and an exotic riparian grass species.

Materials and Methods

Experimental design

Two experiments were carried out to investigate the effects of AM fungi on N and P interception in soil under leaching of repacked columns of a riparian soil growing Phalaris aquatica or Austrodanthonia caespitosa. Each experiment was arranged in a completely randomized design with one plant species, 2 mycorrhizal levels (Mycorrhizal and Non-mycorrhizal), 3 fertilizers treatments (Control, nitrogen and phosphorous) and 4 replications for a total of 24 columns for each plant.

Soil preparation

Soil was collected from riparian vegetation site in the southern Murray-Darling Basin in south-eastern Australia called Faithful Creek. Soil type in the region was grey, yellow and brown sodosols and chromosols (Ladson et al. 1999), formed by alluvium deposits from the Cainozoic period. The soil was air-dried and passed through a 2-mm sieve. Then the soil was mixed with washed sand (40%, w/w). For non-mycorrhizal treatments, the mixed soil and sand autoclaved (121 °C, 1 h, twice at 48 h intervals) to remove indigenous AM fungal propagules. Columns containing autoclaved soil received a 10 ml filtrate (passed through a 11 µm nylon filter) from 5 gram original non-autoclaved soil to reintroduce soil microflora except mycorrhizal fungi (Smith and Smith, 1980).

Column description and growth conditions

The 9 cm diameter × 30 cm long polyvinyl chloride cylinder used in the experiment. The columns were caped (with a hole) at the end and 2 cm of dried and autoclaved washed sand (140 g) was placed with a cotton mesh layer at the bottom. Then the columns were packed with 1.8 kg of autoclaved or non-autoclaved mixed soil and sand (columns were filled to 25 cm) leaving a 5 cm space for irrigation at the top of each column. Seeds of two grass species were soaked for 10 min in a mixture of 1 part sodium hypochlorite (NaOCl 12.5% w/v) and two parts of reverse osmosis (RO) water, and subsequently rinsed three times with RO water. Four seeds of each species were planted in each column and were covered with 1.0 cm of sand. Seeds were watered with RO water daily. After 2 weeks seedlings were thinned to one per column. Columns were irrigated every two days with RO water at 80% of the field capacity of the whole soil core for 8 weeks (leachate did not flow through columns when 80% of field capacity was applied). The plants were grown in a

glasshouse during August to October 2009 (late winter). Long Ashton nutrient solution (Hewitt, 1966) without P was added (20 mL per pot) once per week for 3 weeks, after 4 weeks of plant growth.

Fertilizer application and leachate collection

Each N treated column received 102 mg of ammonium nitrate (NH_4NO_3) which was equal to 200 kg N ha^{-1}. Each P column received 740 mg of potassium dihydrogen phosphate (KH_2PO_4) which was equal to 326 kg P ha^{-1}. Columns receiving no chemical fertilizer were used as control. Fertilizers were dissolved in RO water and add to the columns 7 days before harvesting.

Harvesting

At harvesting time plants were cut from top of the soil. After irrigation of the columns up to 80% of field capacity, 500 mL of RO water added to each column. Leachate was collected at the end of the columns in plastic bottles during 18 h after final irrigation and stored in fridge. A sub sample of the leachate was centrifuged at 14000 rpm for 5 min and then stored to -20 °C for future analysis of supernatant. After 24 h of leachate collection columns were cut into three sections (0–5, 5-10, 10–25 cm) and 2 soil samples were collected from each section for NO_3, NH_4 and P analyses and length of mycorrhizal hyphae measurement. Then the roots were separated from the soil by water pressure.

Mycorrhizal assessment

Sub-samples of plant roots (0.2 g of fresh root) were washed carefully and AM colonization was assessed after clearing roots with 10% KOH (W/V) for 3 days at room temperature and staining with Trypan blue using a modification of the method of Phillips and Hayman (1970), omitting phenol from the reagents. Roots were observed at × 40 magnification and AM fungal colonization was evaluated for roots by a line intersect method (Giovannetti and Mosse 1980). The length of AM external hyphal in the middle soil layer (5-10 cm) was measured using the method of Jakobsen et al. (1992) modified by using 5 mL aliquots for each sample and Trypan blue for staining the hyphae. This staining method does not distinguish between living and dead hyphae. Mycorrhizal growth response (MGR) was calculated using the individual total plant dry weight (DW) of M and the mean dry weight of NM plants as follows:

$$\%\,\mathrm{MGR} = \frac{\mathrm{DW(M)} - \text{mean DW (NM)}}{\text{mean DW (NM)}} \times 100$$

Chemical analyses

Phosphorous was extracted from the soil by 0.5 M $NaHCO_3$ (pH 8.5) solution and available P in different sections of each column was measured by Fixen and Grove (1991) method. The amounts of available P in soil and leachate were determined by Cary® 50 UV-Vis spectrophotometer. Ammonium and nitrate were extracted from the soil by 2 M KCl solution. A total of 25 mL of 2 M KCl was added to 10 g of moist soil samples in a 50 mL falcon tube, shaken on an orbital shaker for 30 min at 200 rpm. Then the solution centrifuged at 2000 rpm for 5 min. The supernatant was collected and stored at −20 °C until analysis. Ammonium and nitrate concentration in soil extraction and in leachate were determined colorimetrically by Thermo Scientific Multiskan® Ascen spectrophotometer. Ammonium concentration determined by a method adapted by Forster (1995) and nitrite concentration was determined by a method adapted by Miranda et al. (2001) and Doane and Horwath (2003). Soil gravimetric moisture was determined after drying 10 g of moist soil at 105 °C for 48 h. Harvested shoots and roots were dried at 80 °C for 48 h and weighed. Nutrient concentrations in shoots and roots sub samples were determined by inductively coupled plasma emission spectroscopy (ICP).

Statistical analysis

Data from AM and NM treatments (Control, P treated and N treated) were analyzed using two-way analysis of variance (ANOVA) using Genstat® 11.0 statistical software. Significant differences between treatments were found ($P<0.05$) using the least significant difference (LSD) method. In some cases two-way ANOVA revealed no significant differences between nutrients irrespective of mycorrhizae. The large differences between nutrient treatments may have masked mycorrhizal effects within the various nutrient addition treatments. Therefore individual t-test was performed to compare AM and NM plants within nutrients addition treatments. Plants were analyzed separately and we present as such in the results.

Results and Discussion

Mycorrhizal colonization and external hyphae

No AM fungal colonization was found in non-mycorrhizal roots of both plants (results not shown). Control plants of both species had higher AM colonization than other treatments (Figure 1), also there were no differences in AM colonization between P and N treated plants. The highest level AM colonization in *Phalaris aquatica* was about 87% in roots of control plants, but in roots of *Austrodanthonia caespitosa* the highest level of AM colonization was about 37% where found in control treatment. Development of external hyphae in soil of both plants was significant in AM columns in all treatments compared to NM columns, but no significant differences were found in different nutrient treatments in AM plants (results not shown). The highest levels of length of AM external hyphae in *Phalaris aquatica* and *Austrodanthonia caespitosa* were about 9.9 and 12 meter per gram of soil of control treatment respectively.

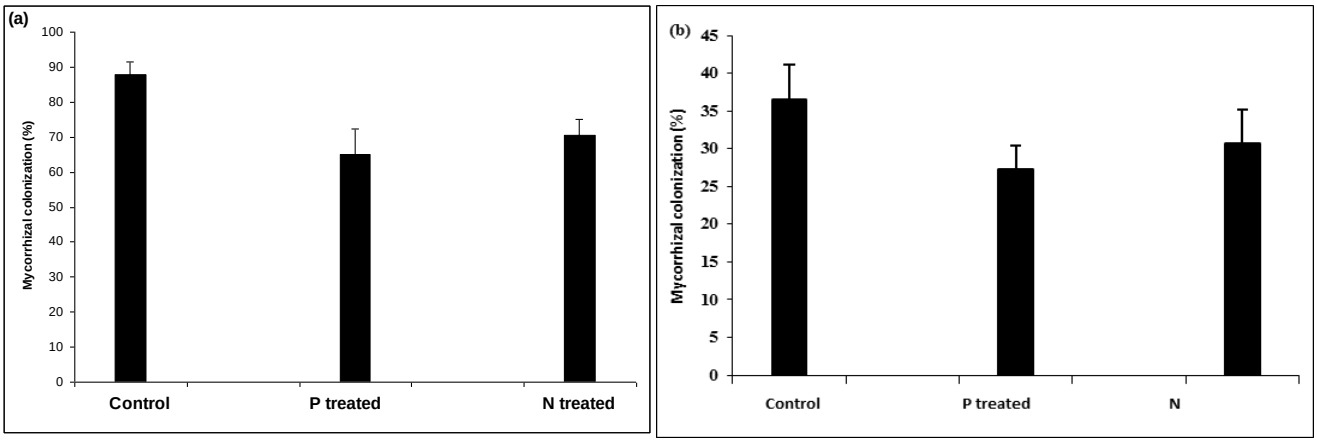

Figure 1. Mean percentage of root length colonized in *Phalaris aquatic* (a) and *Austrodanthonia caespitosa* (b) grown under different nutrient treatments after 8 weeks in glasshouse conditions. Vertical bars represent standard error of the means, n=4.

Plant growth and mycorrhizal responses

In *Phalaris aquatica*, shoot dry weight, root dry weight in AM plants were higher than NM plants in control and N treated plants, no differences were found in AM and NM treated with P (Figure 2a). But, shoot and root dry weight of *Austrodanthonia caespitosa* were not significantly affected by mycorrhizal fungi, N addition and P addition treatments, alone or in combination (Figure 2b). Mycorrhizal growth responses (MGR) in terms of total dry weight at different nutrient levels are shown in Fig 3. *Phalaris aquatica* plants showed a positive response to AM inoculation in all nutrient levels, where the biggest MGR was at control level and the smallest was at P treated plants (Figure 3a). In *Austrodanthonia caespitosa*, although, control treatment showed a positive response to AM inoculation, but P and N treated plants had a negative response (Figure 3b).

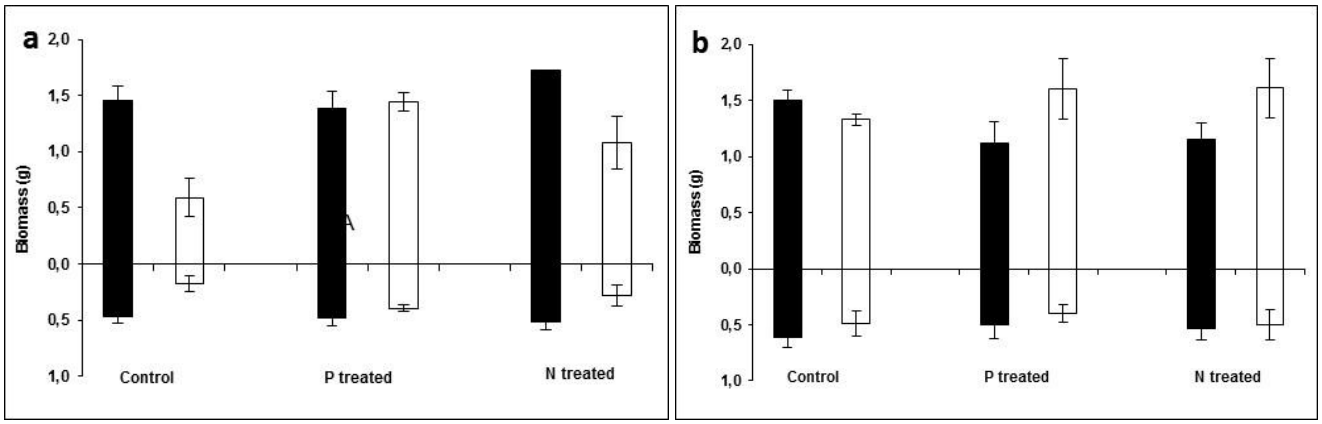

Figure 2. Mean shoot (above *X*-axis) and root (below *X*-axis) dry weights of mycorrhizal (AM) (solid bar) and non mycorrhizal (NM) (open bar) *Phalaris aquatica* (a) and *Austrodanthonia caespitosa* (b) after 8 weeks growing under glasshouse conditions. Vertical bars represent standard error of the means, n=4.

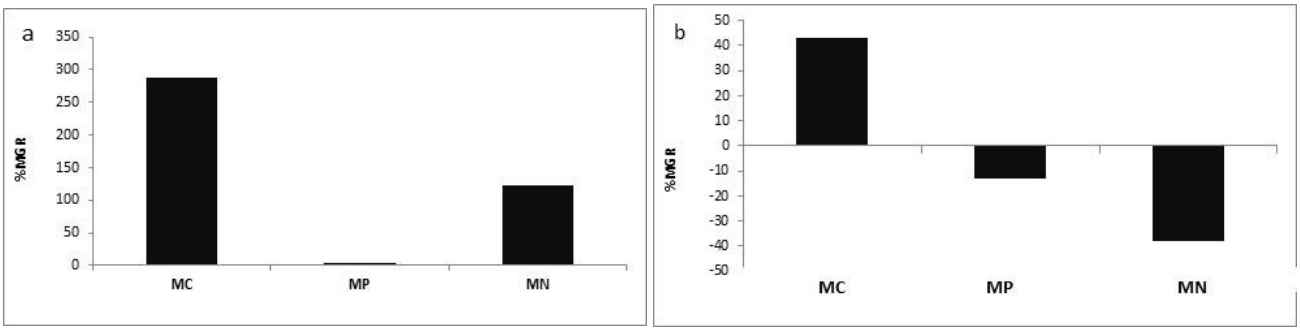

Figure 3. Mycorrhizal growth response (MGR) in *Phalaris aquatica* (a) and *Austrodanthonia caespitosa* (b) in different nutrient levels after 8 weeks growing under glasshouse conditions

Soil NO_3, NH_4 and P concentrations

Nitrate

In columns of both plants, increased soil depth decreased soil NO_3 concentration and the highest NO_3 concentration were found in surface layer of NM columns treated with N. The highest level of nitrate in soil containing *Phalaris aquatica* plants was about 2.14 mg/kg soil, but it was about 1.1 mg/kg soil in *Austrodanthonia caespitosa* (Table 1 and 2).

Ammonium

Concentration of NH_4 was decreased by soil depth in soil of both plants. Non mycorrhizal in N treated columns had the highest NH_4 concentration in soil containing *Phalaris aquatica* plants (about 39.8 mg/kg soil), but the highest level of NH_4 was found in soil of *Austrodanthonia caespitosa* in mycorrhizal N treated l columns (about 19.2 mg/kg soil) (Table 1 and 2).

Phosphorous

Soil available P concentration decreased by the soil depth, and increased by addition of P in columns of both plants. The highest level of available P concentration in soil containing *Phalaris aquatica* and *Austrodanthonia caespitosa* plants was found in non-mycorrhizal P treated plants about 107 and 115 mg/kg soil respectively (Table 1 and 2).

Leachate volume and nutrient concentration

No significant differences of volumes of leachate were found between the treatments by the end of leachate collection in both plants (results not shown). Mycorrhizal treatment significantly reduced the concentration of NO_3, NH_4 and dissolved P in leachate of *Phalaris aquatica* (Figure 4). The highest concentration of NO_3 in leachate was found in N treated NM columns, where NH_4 concentration was highest in NM control columns. Addition of P increased concentration of dissolved P in leachate of columns, but leachate from AM columns had significantly lower level of dissolved P than NM columns.

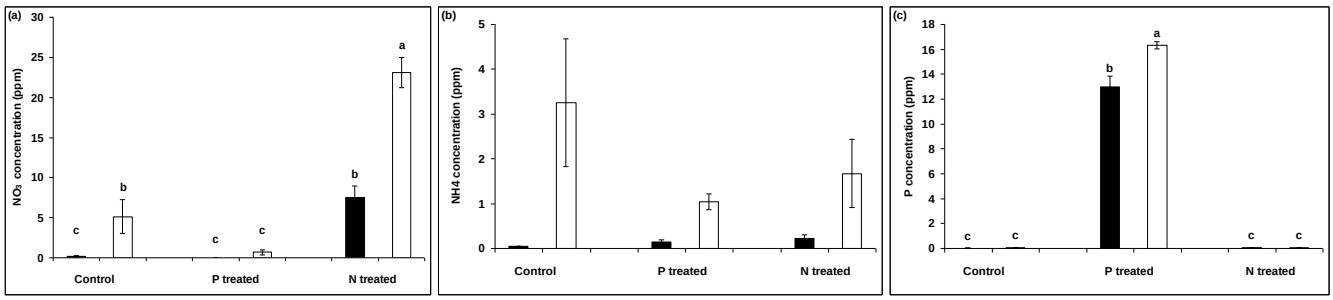

Figure 4. Concentration of NO_3 (a), NH_4 (b) and dissolved P (c) in leachate, collected from columns of mycorrhizal (AM) and non mycorrhizal (NM) *Phalaris aquatica*, grown under different nutrient treatments after 8 weeks in glasshouse conditions. Solid bars and open bars show mycorrhizal (AM) and non mycorrhizal (NM) treatments respectively. Vertical bars represent standard error of the means, n=4. Means followed by the same letter are not significantly different at the $P \leq 0.05$ level. N.B. the concentration NH4+ in the leachate differed significantly between mycorrhizal and non-mycorrizal treatments, irrespective of nutrient addition treatments, see text for details.

Table 1. Extracted nutrient concentrations of soil samples from different depths of columns containing mycorrhizal (AM) and non mycorrhizal (NM) *Phalaris aquatica*, after 8 weeks growing under glasshouse conditions. Means ± standard error (n = 4). Note: valid statistical comparisons cannot be made between soil depths.

Treatments		KCL-extractable NO_3^- - N (mg kg^{-1} soil)			KCL-extractable NH_4^+ - N (mg kg^{-1} soil)			Bicarbonate-extractable PO_4^{3-} - P (mg kg^{-1} soil)		
	Soil depth (cm)	0-5	5-10	10-22	0-5	5-10	10-22	0-5	5-10	10-22
Mycorrhizal (AM)	Control	0.13±0.03 c	0.09±0.02	0.08±0.01 c	1.51±0.26 d	0.81±0.05	0.71±0.04	1.67±0.47	0.70±0.42	0.37±0.30 c
	P treated	0.27±0.04 c	0.13±0.01	0.07±0.02 c	3.16±0.31 cd	0.76±0.03	0.76±0.12	98.6±7.56	23.06±5.39	12.39±4.12 b
	N treated	0.54±0.15 b	0.71±0.22	0.19±0.11 c	11.85±1.91 c	2.00±0.51	1.09±0.09	1.23±0.56	0.82±0.42	0.21±0.05 c
Non-mycorrhizal (NM)	Control	0.70±0.25 b	0.56±0.11	0.47±0.16 b	20.94±1.54 b	17.58±1.74	16.20±2.61	5.01±0.34	2.48±0.44	2.07±0.29 c
	P treated	0.17±0.03 c	0.24±0.08	0.10±0.03 c	8.50±1..20 cd	15.01±2.20	13.86±1.12	106.99±12.23	34.64±2.65	29.20±3.96 a
	N treated	2.14±0.21 a	2.08±0.06	1.33±0.22 a	39.80±3.57 a	19.56±1.65	13.48±2.91	5.08±0.66	1.36±0.41	1.51±0.4 c
Mycorrhizae		***	**	***	***	***	***	ns	*	**
Nutrient		***	***	***	***	ns	ns	***	***	***
Mycorrhizae × Nutrient		***	ns	**	***	ns	ns	ns	ns	**

*, **, *** significant at P ≤ 0.05, 0.01, 0.001 respectively.
ns: not significant at P ≤ 0.05.

Table 2. Extracted nutrient concentrations of soil samples from different depths of columns containing mycorrhizal (AM) and non mycorrhizal (NM) *Austrodanthonia caespitosa*, after 8 weeks growing under glasshouse conditions. Means ± standard error (n = 4). Note: valid statistical comparisons cannot be made between soil depths.

Treatments		KCL-extractable NO_3^- - N (mg kg^{-1} soil)			KCL-extractable NH_4^+ - N (mg kg^{-1} soil)			Bicarbonate-extractable PO_4^{3-} - P (mg kg^{-1} soil)		
	Soil depth (cm)	0-5	5-10	10-22	0-5	5-10	10-22	0-5	5-10	10-22
Mycorrhizal (AM)	Control	0.13±0.04	0.08±0.01	0.06±.01	3.55±1.11	1.04±0.06 b	1.03±0.15 ab	2.92±0.61	0.67±0.16 c	0.02±0.00 c
	P treated	0.16±0.02	0.09±0.01	0.05±0.01	3.28±0.60	1.09±0.08 b	0.91±0.07 b	108.30±23.94	17.55±2.11 b	13.74±1.75 b
	N treated	0.6±0.13	0.42±0.14	0.21±0.07	19.20±2.90	4.70±1.60 a	3.67±0.66 a	2.21±0.75	0.78±0.34 c	0.75±0.46 c
Non-mycorrhizal (NM)	Control	0.22±0.11	0.11±0.02	0.11±0.05	6.72±1.41	4.36±1.88 ab	3.24±1.48 ab	2.45±0.67	1.88±0.77 c	1.40±0.55 c
	P treated	0.15±0.05	0.11±0.03	0.05±0.01	8.23±2.85	3.98±1.48 ab	3.60±1.48 ab	115.11±11.90	36.39±7.07 a	27.35±5.07 a
	N treated	1.1±0.40	0.97±0.66	0.45±0.41	13.70±3.99	1.67±0.32 b	1.27±0.07 ab	11.65±5.59	5.54±3.70 c	1.25±0.55 c
Mycorrhizae		ns	ns	ns	ns	ns	ns	ns	**	*
Nutrient		**	ns	ns	***	ns	ns	***	***	***
Mycorrhizae × Nutrient		ns	ns	ns	ns	*	*	ns	*	*

*, **, *** significant at P ≤ 0.05, 0.01, 0.001 respectively.
ns: not significant at P ≤ 0.05.

In *Austrodanthonia caespitosa*, the volumes of collected leachate from AM and NM columns were the same (results not shown). The averages of volume of leachate in AM and NM treated columns were 317.58, and 327.41 respectively. Although leachate from AM columns contained lower concentration of NO_3, the difference was not statistically significant (Figure 5). Addition of N to the columns increased NO_3 leaching. Non mycorrhizal columns treated with N had the highest level of NO_3 in leachate (5.12 ppm). There were no effects of N addition and mycorrhizal fungi on NH_4 concentration in leachate. Mycorrhizal treatment had no significant effects on dissolved P concentration in leachate, but addition of P increased dissolved P concentration in leachate.

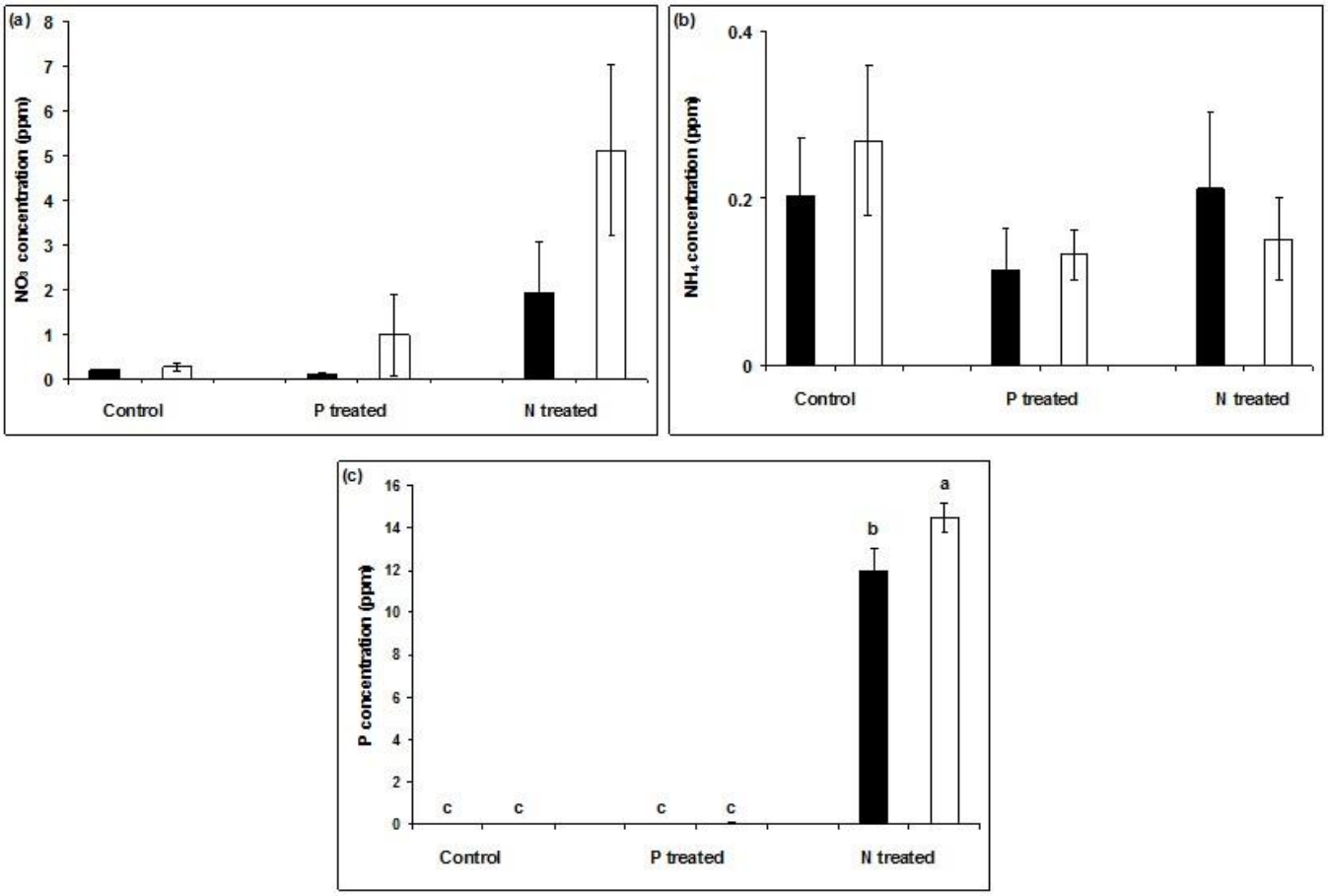

Figure 5. Concentration of NO_3 (a), NH_4 (b) and dissolved P (c) in leachate, collected from columns of mycorrhizal (AM) and non mycorrhizal (NM) *Austrodanthonia caespitosa*, grown under different nutrient treatments after 8 weeks in glasshouse conditions. Solid bars and open bars show mycorrhizal (AM) and non mycorrhizal (NM) treatments respectively. Vertical bars represent standard error of the means, n=4. Means followed by the same letter are not significantly different at the $P \leq 0.05$ level

The results of this experiment showed that, volume of leachate collected from columns of both grasses were nearly the same, but native grass *Austrodanthonia caespitosa* significantly had lower levels of NO_3, NH_4 and dissolve P in the leachate than exotic grass *Phalaris aquatica*. The differences were more obvious in NO_3 and NH_4. *Austrodanthonia caespitosa* reduced NO_3 leaching in control and N treated columns 12 and 4 times (respectively) more than *Phalaris aquatica*. The same trend was found for NH_4. Beside plant size, there are some other important factors that effects of nutrient leaching in soil profile, such as species and genotype (Baird, 1997), root morphological characteristics (Sullivan et al., 2000), root density and architecture (Dunbabin et al., 2003), chelate-induced nutrient leaching, root exudation (Quartacci et al., 2009), soil microbial activities and nutrient availability (Bardgett and Wardle, 2003; Zogg et al., 2000) and so on. Nearly, the biomass of *Phalaris aquatica* and *Austrodanthonia caespitosa* and nitrogen content of two species was the same after 8 weeks growing in glasshouse conditions (results not shown). Although the root shoot ratio in *Austrodanthonia caespitosa* was higher than *Phalaris aquatica* (0.37 and 0.31 respectively), it seems that some other factors than root shoot ratio are contributing to nutrient leaching in this experiment. Results of a glasshouse experiment showed that a big differences in nitrogen leaching between six different grass

species, which species adapted to nutrient-rich sites (*Lolium perenne*) lose more nitrogen than species from less fertile soils (*Festuca ovina* and *Molinia caerulea*) (Vázquez de Aldana et al., 1996). Fast growing and competitative species compared to slow growing and native species had higher rate of N cycling, higher rate of N mineralization and finally higher rate of N leaching from the soil (Waelker et al., 2001). The results of this study showed that, native *Austrodanthonia caespitosa* grass species is more nutrient (N) conserving and have higher retention of nutrient in soil than exotic *Phalaris aquatica*.

The role of AM fungi in plant growth and nutrient development in many plant species has been well documented. Mycorrhizal colonization is usually coincide with AM external hyphal development in soil which leads to increase of N and P acquisition, transport and delivery to plant (Asghari et al., 2005; Johansen et al., 1993b; Leigh et al., 2009; Li et al., 2009; Tanaka and Yano, 2005). In our experiment roots of *Phalaris aquatica* were more colonized by AM fungi than *Austrodanthonia caespitosa*, which is in agreement with previous work (Tran and Cavagnaro, 2010). *Phalaris aquatica* showed a positive growth response to AM colonization, but *Austrodanthonia caespitosa* had a negative response. Although the length of AM hyphae in soil of mycorrhizal columns of both species was nearly the same, AM treatment in *Phalaris aquatica* columns showed a significant effect on scavenging and depletion of soil NO_3, NH_4 and available P in all soil depths, where AM fungi in *Austrodanthonia caespitosa* columns just decreased available P in deeper sections of soil and had no significant effects on soil NO_3 and NH_4. There was no AM colonization in soil of NM columns, so the external hyphae in these columns are likely to be from dead or saprophytic fungi (Asghari et al., 2005). Plants can vary widely in their response to an association with a mycorrhizal fungus. Our results indicate that exotic grass *Phalaris aquatica* is highly responsive to AM fungi, but native grass *Austrodanthonia caespitosa* had no response to AM fungi related to plant growth and nutrient interception. This results is in contrast with other studies which demonstrated that, native plant species had a greater responsiveness to inoculation with mycorrhizal fungi than exotic plant species and mycorrhizal dependency reduced during plant invasions (Pringle et al., 2009; Seifert et al., 2009). Many researchers indicated that native plant species are more responsive to AM fungi than exotic or invasive plant species (De Deyn et al., 2003; Hart et al., 2001; Klironomos, 2003). In another survey on responses of different plant species to AM fungi, mycorrhizal inoculation positively increased growth of the late-successional native species and negatively affected growth of the early-successional native and invasive species (Rowe et al., 2007). Beside plant species there are different factors that can effects on mycorrhizal responsiveness in plant species and no single mechanism can explain AM responsiveness. Plant responsiveness to AM fungi is not depend to plant species or fungus species individually, and it come from the interaction of independent plant and fungus genomes (Frey and Schuepp, 1993; Janos, 2007).

Soil and leachate of AM columns in *Phalaris aquatica* had significantly lower available P compared with NM columns (Figure 3 and Table 2). Soil available P depletion, transport and delivery to plant roots via AM external hyphae have been reported previously (Deressa and Schenk, 2008; Smith et al., 2003; 2004). The importance of AM pathway is more obvious at lower level of soil P, which in some cases hyphae accounted for nearly the whole P uptake (Deressa and Schenk, 2008; Smith et al., 2003).

Contribution of AM fungi to N depletion in soil and reduction of N in leachate has received less attention. The only related work is the effect of AM plants in reducing leaching of nitrate (Haines and Best 1976), but in this case the roles of AM fungi themselves could not be evaluated because AM and NM plants were of markedly different size. In *Phalaris aquatica* AM fungi had a significant contribution to reduction of NO_3 and NH_4 in soil and leachate, which reduction in leachate was from 3 (N treated columns) to 27 (control columns) times for NO_3 and from 7 (P and N treated columns) to 75 (control columns) times for NH_4. Soil N depletion (NO_3 and NH_4) and transport from growth medium toward the host plant by AM external hyphae was reported previously (Johansen et al., 1993a; Tanaka and Yano, 2005). The reduction of NH_4 in soils of *Phalaris aquatica* via AM treatment was higher than NO_3 (particularly in top layers), considering higher capability of AM fungi to deplete NH_4 than NO_3. This result is in agreement with Tanaka and Yano (2005).

Nearly, a large amount of AM colonization in roots and high level of hyphal length were found in AM treated *Austrodanthonia caespitosa* columns, but mycorrhizal treatment had no significant effects of NO_3, NH_4 and P reduction in leachate in these columns (as found in *Phalaris aquatica*). Presence of AM colonization and external hyphae dos not necessarily show the beneficial effects of AM fungi, on the other hand absence of AM benefits in AM inoculated plants dos not musk the role of mycorrhizal fungi in plant and fungi symbiosis. Although biomass of cucumber plants were not affected by AM fungi, and the hyphal N transport was not appear to effect plant growth, a large amount of N uptake in plants was taken up and transported via AM

external hyphae. In our experiment, at least some parts of N in *Austrodanthonia caespitosa* may were transported via AM external hyphae.

Although some researchers indicated that some parts of increased N uptake of AM plants have been contributed to translocation via AM external hyphae (Frey and Schuepp, 1993; Hawkins et al., 2000; Johansen et al., 1993a; Subramanian and Charest, 1999), Tanaka and Yano (2005) suggested that, some parts of N acquire in external hyphae and dos not necessarily transferred or delivered to the host plants. It means that AM external hyphae can accumulate or immobilized N without transferring to plant which may leads to conserving the N from leaching process. Little is known about the mechanisms of N uptake by AM hyphae and further investigation is required.

Conclusion

In conclusion, our results suggested that beside the soil which have a very high capacity to accumulate and immobilized the nutrients, plant genotype and AM fungi can intercept a large amount of nitrate, ammonium and phosphorus. Native plants which are adapted to riparian ecosystems are more efficiently immobilized nutrient than exotic plants. Mycorrhizal fungi association was not important to assist native species to decrease nutrient leaching, but this association was highly effective in exotic species to slow down the deleterious effects of nutrient leaching. These results may be of practical importance, as they highlight the potential of using proper plant species and associated mycorrhizal fungi in riparian zones.

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