Journal of Animal and Veterinary Advances 11 (24): 4553-4558, 2012
ISSN: 1680-5593

The Impact of Soil Available Phosphorus on the AM Fungi and the Organic Acids Exudation at Different Patches in Northern Steppe of China

Hanshu Zhou, Gaowen Yang and Yingjun Zhang
Institute of Grassland Science, College of Animal Science and Technology,
Agricultural University, West Road 2 Yuan Ming Yuan, 1000193 Beijing, P.R. China

Abstract: The research is conducted at a degraded steppe in Hebei province, China. Researchers took four sample areas which are dominated by fringed sagebrush (*Artemisia frigida*), narrowleaf stellera (*Stellera chamaejasme*), shining speargrass (*Achnatherum splendens*), white swordflag (*Iris lactea*), respectively. Phosphorus (P) was applied at four levels (0, 5, 10 and 15 mg kg^{-1} soil) in each sample area. The AM fungi colonization and the organic acid exudation in the rhizosphere were affected by the concentration of soil available phosphorus. These results suggested that the difference of soil P content is probably the cause of the appearance of four patches.

Key words: Phosphorus, arbuscular mycorrhizal fungi, organic acid, rhizosphere, grassland patches

INTRODUCTION

The appearance of patches is an important characterization of the degradation of Northern China grassland ecosystem. Soil heterogeneity determines plant community structure, combining with soil Phosphorus (P) level impact on species composition and community succession (Hinsinger, 2001; Vance *et al.*, 2003). Although, bound P is quite abundant in many soils, it is largely unavailable for uptake because plants acquire phosphorus as phosphate anions from the soil solution thus P is frequently the most limiting element for plant growth and development (Vance *et al.*, 2003). However, plants have evolved different strategies to cope with restricted P supplies that either increase P-use efficiency or help to extract more P from the soil (Raghothama and Karthikeyan, 2005; Schachtman *et al.*, 1998). These strategies include one acidification of the rhizosphere which can promote the release of nutrients from soil minerals by exudation of organic acid anions (Ryan and Delhaize, 2001) of which the malate and citrate appear to be the primary components released by roots under P deficiency (Jones and Brassington, 1998) and the other initiation of mycorrhizal associations by forming symbiotic associations with the roots of the majority of plant species to enhance the resource acquisition of some host plant species more than others (Caldwell *et al.*, 1985); Meanwhile, many studies have found that AM fungi have strong impacts on plant community structure, species diversity and the competition between different species (Dobson and Crawley, 1994; Moora and Zobel, 1996) and the organic anions can also stimulate microbial activity in the rhizosphere to influence the availability of other minerals and nutrients as well (Ryan and Delhaize, 2001).

The studies on relationship of soil available phosphorus and AM fungi and the organic acids exudation at different degradation patches in North steppe of China have never been done. The overall aim of this study was to examine the interaction between AM fungi, organic acids exudation in the rhizosphere and the soil P level on the degraded steppe and then deduce the possible cause of the appearance of patches in heavy degraded grassland.

MATERIALS AND METHODS

The research was conducted at a degraded steppe in Saibei adiministrative region of ZhangJiakou in Hebei province (41°45'~41°57'N, 115°39'~115°51'E) for 2 years. The average annual temperature is 1.4°C and the average annual total precipitation is about 300 mm with 70% falling during July, August and Sep. Researchers took four sample areas which are dominated by shining speargrass (*Achnatherum splendens*) (sample A), narrowleaf stellera (*Stellera chamaejasme*) (sample B), white swordflag (*Iris lactea*) (sample C), fringed sagebrush (*Artemisia frigida*) (sample D), respectively and a control sample area which is dominated by Chinese leymus (*Leymus chinensis*). Each sample area is 15×15 m. Phosphorus (P) was applied at four levels (0, 5, 10 and

Corresponding Author: Yingjun Zhang, Institute of Grassland Science, College of Animal Science and Technology, Agricultural University, West Road 2 Yuan Ming Yuan, 1000193 Beijing, P.R. China

15 mg P kg^{-1} soil) in each sample area in July with a 2 m space between each treatment. Each treatment had three replicates which area is 4×2 m. The mycorrhizal infection rate and the organic acids content in the rhizosphere were measured monthly till September.

Collection of root exudates: Root system were held by the stem base and gently lifted out of the soil. The roots were carefully shaken to remove extra soil and clods trapped between roots were shaken out. The soil which still adhered to the roots was defined as rhizosphere soil. The root system was transferred to a beaker and rhizosphere soil was washed off the roots by adding 0.2 mM $CaCl_2$ and shaking the beaker gently. Organic acids of malate and citrate were measured by using HPLC according to the methods of Woulterwood (Wouterlood *et al*., 2004).

Soil analyses: Soil samples were taken at 0-10 cm depth across a site (3 replicates). Soil available phosphorus was determined by Olsen Method.

Plant measurements: The mycorrhizal infection rate of plant were measured according to the methods of McGonigle *et al*., 1990).

Vegetation survey: The compositions of plant across the plots were recorded at the beginning and end of experiment from 2008 to 2009.

Statistics: All statistical analysis was performed using SPSS11.5 for Windows. Data were compared using a one-way ANOVA and significantly different values had a probability of $p<0.05$.

RESULTS

Soil available phosphorus content: The original soil available phosphorus content of the control area and the *Achnatherum splendens* sample area are significantly lower than the others while the highest 6.57 mg kg^{-1} was in *Stellera chamaejasme* sample area ($p\leq 0.05$, Table 1). After fertilized P in each sample area, the soil P content increased according to the treatment (Fig. 1). The highest content of soil P appeared in the *Stellera chamaejasme* patch in August but it decreased in September. The lowest soil P content was in *Achnatherum splendens* patch during the 2 months.

Table 1: Soil available phosphorus content in each sample area with no addition of phosphorus

Species	Soil available phosphorus content (mg kg^{-1})
Leymus chinensis	3.03^a
Achnatherum splendens	3.88^a
Artemisia frigida	5.61^b
Iris lactea	6.02^b
Stellera chamaejasme	6.57^b

Values followed by different letters are significantly different ($p\leq 0.05$, n = 3)

AMF colonization: Patterns of infection intensity of the mycorrhizal colonization are shown in Table 2. There were significant differences among the four treatments for *Achnatherum splendens* and *Artemisia frigida*. The mycorrhizal infection rate decreased for *Achnatherum splendens* and *Stellera chamaejasme* as the soil available phosphorus increased. However, for the *Iris lacteal* species and *Artemisia frigida* species, the infection rate fluctuated. For the *Iris lacteal* species, the mycorrhizal infection rate reduced from 14-7% as the soil available phosphorus content increased from 6.02-9.31 mg kg^{-1} and it rose from 7-15% as the soil available phosphorus content increased from 9.31-11.88 mg kg^{-1}. For the *Artemisia frigida*, the mycorrhizal infection rate rose from 8-26% while the soil available phosphorus content increased from 5.62-9.84 mg kg^{-1} and it declined from 26-18% while the soil available phosphorus content increased from 9.84-14.98 mg kg^{-1}.

Organic acids in the rhizosphere: The concentration of total organic acids in the rhizosphere of the 30 mm apical parts of lateral roots first increased then dropped dramatically as the p level rose (Fig. 2). For the

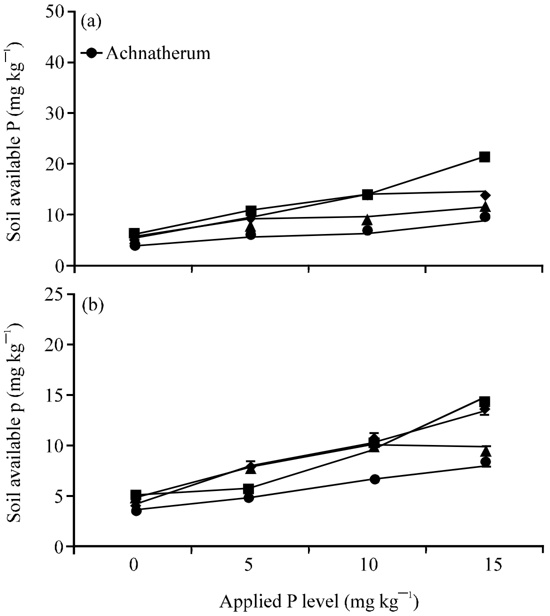

Fig. 1: Soil available phosphorus concentration after addition of phosphorus. a) August; b) Sep. Values represent means±SE (n = 3)

Table 2: Effect of the root system mycorrhizal infection rate in different supplied P level (%)

		Date			
		August		September	
Species	Treatment (mg kg^{-1})	Soil P content (mg kg^{-1}) (0-10 cm)	Mycorrhizal infection rate	Soil P content (mg kg^{-1}) (0-10 cm)	Mycorrhizal infection rate
Achnatherum splendens	0	3.87	21.54^{a}	3.57	17.44^{a}
	5	5.76	18.82^{b}	5.11	14.37^{b}
	10	6.49	16.36^{c}	6.59	13.63^{c}
	15	8.82	11.03^{d}	8.04	9.87^{d}
Stellera chamaejasme	0	6.58	6.33^{a}	5.30	5.16^{a}
	5	11.39	5.29^{b}	5.84	3.55^{b}
	10	14.17	4.77^{bc}	9.36	3.03^{c}
	15	21.65	4.56^{c}	14.53	2.86^{c}
Iris lactea	0	6.02	14.52^{a}	4.96	9.80^{a}
	5	9.31	7.66^{b}	8.07	5.15^{b}
	10	10.03	15.15^{c}	9.63	12.49^{c}
	15	11.88	15.41^{c}	10.03	12.60^{c}
Artemisia frigida	0	5.62	8.32^{a}	4.03	7.51^{a}
	5	9.84	26.89^{b}	8.14	15.27^{b}
	10	14.06	23.38^{c}	10.20	18.05^{c}
	15	14.98	18.26^{d}	13.41	10.05^{d}

Multiple comparison is limited to the same grass name. Means with different letters in the same column are significantly different at the 0.05 level

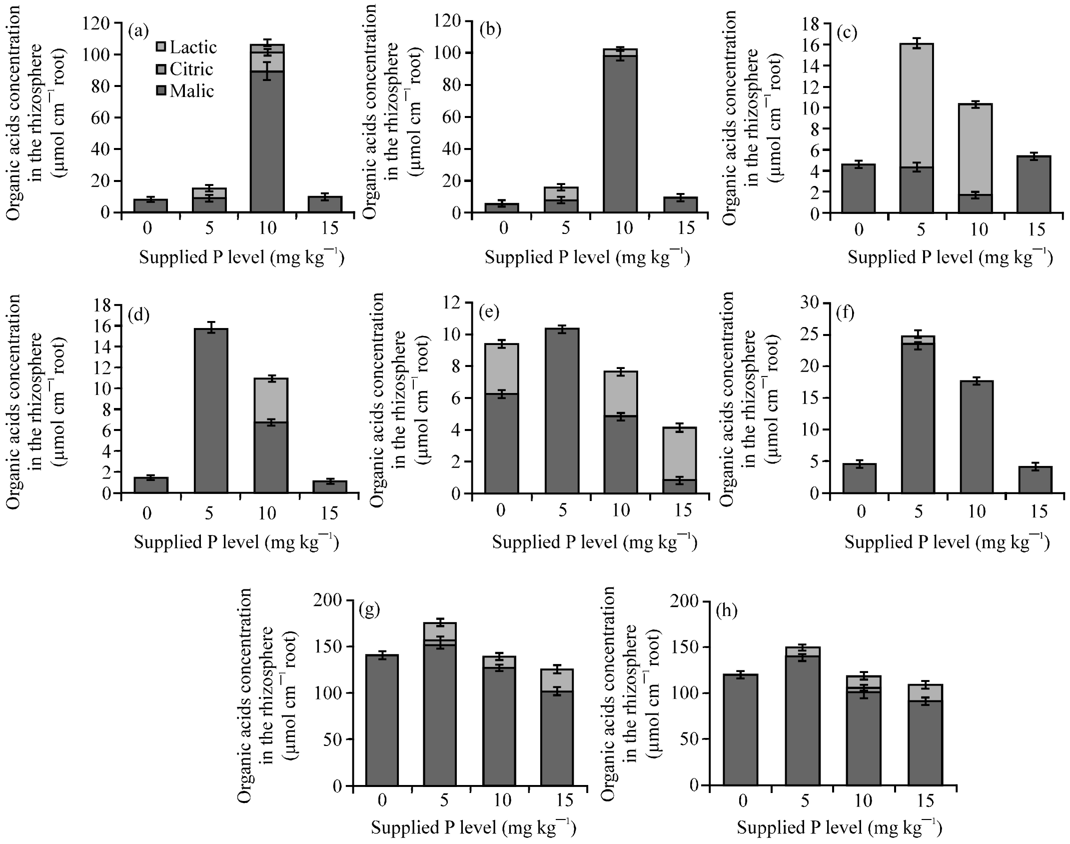

Fig. 2: Concentration of total organic acids in the rhizosphere. Y-axis represents the organic acids concentration in the rhizosphere (μmol cm^{-1} root). X-axis represents the four supplied P level in each sample area. Error bars show SE (n = 3). a) *Stellera chamaejasme* Aug.; b) *Stellera chamaejasme* Sep.; c) *Iris lactea* Aug.; d) *Iris lactea* Sep.; e) *Achnatherum splendens* Aug.; f) *Achnatherum splendens* Sep.; g) *Artemisia frigida* Aug.; h) *Artemisia frigida* Sep.

Achnatherum splendens, *Iris lactea* and *Artemisia frigida* sample area, the organic acids content reach the highest point at the 5 mg kg^{-1} P treatment then decreased as the phosphorus content increased during the 2 months. However, *Stellera chamaejasme* responded differently to the P levels than the other three species. The highest content of total organic acids of it appeared at the 10 mg kg^{-1} P treatment (106.17 μmoL cm^{-1} root in August, 103.77 μmoL cm^{-1} root in September) (Fig. 2). There was an overall trend of reduced organic acids concentrations around roots of every species as the addition P increase. The organic acids content in *Artemisa frigid* was significant higher than any other sample (127.5-176.8 μmoL cm^{-1} root in August, 111.0-151.5 μmoL cm^{-1} root in September). Malic acid appeared to be the primary components released by roots in each sample. Fumaric acid was only detected in small quantities in the rhizosphere of *Stellera chamaejasme*. The carboxylate composition of *Iris lactea* changed gradually with time. In August, the total carboxylate concentration comprised primarily lactic acid at the 5 and 10 mg kg^{-1} whereas in September the amount of lactic acid had decreased and malic acid had become more important.

DISCUSSION

The mycorrhizal infection rate was changed significantly after added phosphorus in the soil. The AMF colonization in the root system is possibly influenced by soil available phosphorus content. It has been discussed that when amount of phosphorus in the soil is abundant, fungal symbionts probably be suppressed and the mycorrhizal infection rate decreased (Schwab *et al*., 1983; Koide and Li, 1990). For the *Artemisia frigid*, the mycorrhizal infection rate rose at the first two P level. It implies that add a small amount of phosphorus can stimulate the growth of the AM fungi. Similar to the results, it is reported that the application of low doses of P as rock phosphate stimulated mycorrhization and enhanced the soil quality parameters (Alguacil *et al*., 2010). And the mycorrhizal infection rate fell at the last two P level, probably because the phosphorus is enough for *Artemisia frigid* to use directly and the fungal symbionts was suppressed. Recent studies indicated that at high nutrient levels, fungi will receive little carbon from plants and will be C-limited (Treseder and Allen, 2002). At lower nutrient levels if N or P concentrations are sufficient for fungal growth, mycorrhizal fungi will proliferate. At the lowest nutrient levels both fungi and plants should be nutrient limited and fungal biomass will be low regardless of C allocation to the fungi by plants (Treseder and Allen, 2002).

AM fungi have strong impacts on plant community structure, species diversity and the competition between different species (Moora *et al*., 2004; Dobson and Crawley, 1994; Van der Heijden *et al*., 1998; Moora and Zobel, 1996). AM fungi have the capacity to induce community level shifts in species composition by benefiting one plant while inhibiting another co-occurring species (Cameron, 2010; Marler *et al*., 1999; Fitter, 1977; Ayres *et al*., 2006; Hall, 1978). Recent studies observed that when AM fungi is inhibited, the community biomass decrease and the plant proportion change; the biomass of C4 grass fall while the biomass of C3 grass increase (Wilson and Hartnett, 1997). It is also found that in typical steppe, the moderate to heavy grassland degeneration prominently reduced the arbuscular mycorrhizal symbiosis of *L. chinensis* (Wu *et al*., 2009).

The exudation of organic acids from roots is considered to be one of the mechanisms for plants to adapt to P-deficient environment (Jones, 1998; Strom *et al*., 2002) and the amount of it raise under P deficiency. Many studies observed that most plants release more organic acids from their roots under P deficiency in comparison to P sufficient plants (Lipton *et al*., 1987; Imas *et al*., 1997; Schwab *et al*., 1983; Ryan and Delhaize, 2001). The concentration of total organic acids in the rhizosphere of the 30 mm apical parts of lateral roots appeared significant differences among the four p level treatments in each sample area. The amount of the organic acids increased at the 5 mg kg^{-1} P treatment. It implies that add phosphorus appropriately can stimulate the exudation of organic acids by root. There was an overall trend of reduced organic acids concentrations around roots of every species as the addition p increase. In earlier reports, malate and citrate appeared to be the primary components released by roots under P deficiency (Bolan, 1991; Jones, 1998). In this experiment, citrate and malate were present in the major amounts in the exudates, lactic acid was a basic composition as well in *Achnatherum splendens*, *Iris lactea* and *Artemisa frigid*. Succinate constituted the smallest proportion of the organic acid released by plant roots. Similar to the results, Bolan reported that succinate made up a small percentage of the total organic acid in the root exudates (Bolan, 1991). Phosphorus (P) availability is one of the major constraints to plant growth and the exudation of organic acids has the potential to significantly enhance shoot P accumulation and cause a significant enhancement of plant P uptake (Strom *et al*., 2002).

CONCLUSION

According to the recent studies and the result of the research, due to the difference P content in the grassland soil, the infection of AM fungi and the release of organic acid in plant roots responded dissimilarly and the AM fungi infection of *L. chinensis* was decreased probably because of the degeneration. These probably caused the community structure and species diversity change differently in each area. Eventually, four patches dominated by shining speargrass (*Achnatherum splendens*), narrowleaf stellera (*Stellera chamaejasme*), white swordflag (*Iris lactea*), fringed sagebrush (*Artemisia frigida*), respectively appeared in this degraded steppe.

ACKNOWLEDGEMENTS

This research was funded by the National Natural Science Foundation of China (30871822), Projects for agro-industry technology research (200903060) and (CARS-35) and Beijing Key Laboratory of Grassland Science.

REFERENCES

Alguacil, M.D.M., Z. Lozano, M.J. Campoy and A. Roldan, 2010. Phosphorus fertilisation management modifies the biodiversity of AM fungi in a tropical savanna forage system. Soil Biol. Biochem., 42: 1114-1122.

Ayres, R.L., A.C. Gange and D.M. Aplin, 2006. Interactions between arbuscular mycorrhizal fungi and intraspecific competition affect size and size inequality, of *Plantago lanceolata* L. J. Ecol., 94: 285-294.

Bolan, N.S., 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil, 134: 189-207.

Caldwell, M.M., D.M. Eissenstat, J.H. Richards and M.F. Allen, 1985. Competition for phosphorus: Differential uptake from dual-isotope-labeled soil interspaces between shrub and grass. Science, 229: 384-386.

Cameron, D.D., 2010. Arbuscular mycorrhizal fungi as (agro) ecosystem engineers. Plant Soil, 333: 1-5.

Dobson, A. and M. Crawley, 1994. Pathogens and the structure of plant communities. Trends Ecol. Evol., 9: 393-398.

Fitter, A.H., 1977. Influence of mycorrhizal infection on competition for phosphorus and potassium by two grasses. New Phytol., 79: 119-125.

Hall, I.R., 1978. Effects of endomycorrhizas on competitive ability of white clover. N. Z. J. Agric. Res., 21: 509-515.

Hinsinger, P., 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. Plant Soil, 237: 173-195.

Imas, P., B. Bar-Yosef, U. Kafkafi and R. Ganmore-Neumann, 1997. Phosphate induced carboxylate and proton release by tomato roots. Plant Soil, 191: 35-39.

Jones, D.L. and D.S. Brassington, 1998. Sorption of organic acids in acid soils and its implications in the rhizosphere. Eur. J. Soil Sci., 49: 447-455.

Jones, D.L., 1998. Organic acids in the rhizosphere: A critical review. Plant Soil, 205: 25-44.

Koide, R.T. and M.G. Li, 1990. On host regulation of the vesicular-arbuscular mycorrhizal symbiosis. New Phytol., 114: 59-64.

Lipton, D.S., R.W. Blanchar and D.G. Blevins, 1987. Citrate, malate and succinate concentration in exudates from P-sufficient and P-stressed *Medicago sativa* L. seedlings. Plant Physiol., 85: 315-317.

Marler, M.J., C.A. Zabinski and R.M. Callaway, 1999. Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. Ecology, 80: 1180-1186.

McGonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild and J.A. Swan, 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol., 115: 495-501.

Moora, M. and M. Zobel, 1996. Effect of arbuscular mycorrhiza on inter- and intraspecific competition of two grassland species. Oecologia, 108: 79-84.

Moora, M., M. Opik, R. Sen and M. Zobel, 2004. Native arbuscular mycorrhizal fungal communities differentially influence the seedling performance of rare and common Pulsatilla species. Funct. Ecol., 18: 554-562.

Raghothama, K.G. and A.S. Karthikeyan, 2005. Phosphate Acquisition. In: Root Physiology: From Gene to Function, Lambers, H. and T.D. Colmer (Eds.). Springer, Dordrecht, The Netherlands, ISBN-13: 9781402040986, pp: 37-49.

Ryan, P.R. and E. Delhaize, 2001. Function and mechanism of organic anion exudation from plant roots. Annu. Rev. Plant Physiol. Plant Mol. Biol., 52: 527-560.

Schachtman, D.P., R.J. Reid and S.M. Ayling, 1998. Phosphorus uptake by plants from soil to cell. Plant Physiol., 116: 447-453.

Schwab, S.M., J.A. Menge and T.L. Robert, 1983. Quantitative and qualitative effects of phosphorus on extracts and exudates of sudangrass roots in relation to vesicular-arbuscular mycorrhiza formation. Plant Physiol., 73: 761-765.

Strom, L., A.G. Owen, D.L. Godbold and D.L. Jones, 2002. Organic acid mediated P mobilization in the rhizosphere and uptake by maize roots. Soil Biol. Biochem., 34: 703-710.

Treseder, K.K. and M.F. Allen, 2002. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: A model and field test. New Phytol., 155: 507-515.

Van der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis and R. Streitwolf-Engel *et al*., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature, 396: 69-72.

Vance, C.P., C. Uhde-Stone and D.L. Allan, 2003. Phosphorus acquisition and use: Critical adaptations by plants for securing a nonrenewable resource. New Phytol., 157: 423-447.

Wilson, G.W.T. and D.C. Hartnett, 1997. Effects of mycorrhizae on plant growth and dynamics in experimental tall grass prairie microcosms. Am. J. Bot., 84: 478-482.

Wouterlood, M., G.R. Cawthray, T.T. Scanlon, H. Lambers and E.J. Veneklaas, 2004. Carboxylate concentrations in the rhizosphere of lateral roots of chickpea (*Cicer arietinum*) increase during plant development, but are not correlated with phosphorus status of soil or plants. New Phytol., 162: 745-753.

Wu, E., C.X. Li, Y. Zhang, C.M. Wang and J.Y. Yun, 2009. Effect of grassland degradation on arbuscular mycorrhizal symbiosis of *Leymus chinensis* (Trin.) Tzvel. in typical steppe. Acta Agrestia Sinica, 17: 731-734.