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SEASONAL DYNAMICS IN ARBUSCULAR MYCORRHIZAL FUNGAL COLONIZATION AND SPORE NUMBERS IN THE RHIZOSPHERE OF *DACTYLIS GLOMERATA* L. AND *TRIFOLIUM REPENS* L.

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Abstract

The seasonal dynamics in the colonization of the rhizosphere of orchardgrass (*Dactylis glomerata* L.) and white clover (*Trifolium repens* L.) pastures by arbuscular mycorrhizal (AM) fungi and the production of spores in an artifical Japanese grassland was investigated over 12 months (between December 2001 and December 2002). The results showed that the AM fungal colonization fluctuated seasonally in the rhizosphere of both pastures. The total AM fungal colonization of the two pastures decreased during winter, then increased from March to June as the pastures grew, but slightly decreased again in July and August, and again followed an increase in September. There was significant difference of the colonization by arbuscules and vesicles between the two pastures ($p<0.05$). Besides, the vesicular colonization of orchardgrass was higher than that of white clover, but the opposite trend was observed for arbuscular colonization. Similarly, the numbers of AM fungal spores in the pastures varied throughout the year, decreasing from spring to summer, then slowly increasing in late summer, reaching peak levels in winter. There is significant correlation between the frequency of spores in the rhizosphere soil and both soil temperature and pH.

Introduction

Arbuscular mycorrhizal (AM) fungi are important soil microbes that form symbiotic associations with the root systems of most terrestrial plant species (van der Heijden *et al.*, 1998). These symbiotic associations are mutualistic. AM fungi gain carbohydrates from the host plants, while the plants can increase their uptake of nutrients (especially phosphorus) through the external mycelium (Smith & Read, 1997). Besides, much research has well documented that AM fungi perform pivotal roles such as in fertilizer management, pathogenic resistance and ecological protection in host plants (Li & Feng, 2001), as well as in plant regrowth after cutting (Wu *et al.*, 2011). However, the interactions of fungus-plant partners in the field are related with many factors, including *inter alia* fungus and plant, inherent plant growth rates, soil properties, management practices and climatic variables.

Most investigations concerning AM fungal colonization focused on seasonal dynamics temporal variations in AM hyphae, arbuscules, vesicles, spores, extramatrical hyphae and glomalin, which is a glycoprotein produced by AM fungi (Mandyam & Jumpponen, 2008). For example, seasonal changes occurred in abundance of healthy, moribund and dead *Gigaspora gigantean* spores (Lee & Koske, 1994), as well as spores of *G. mosseae*, *G. fasciculatum* and *G. monosporum* (Nasim *et al.*, 2008). Muthukumar & Udaiyan, (2002) found temporal variation in mycorrhizal colonization and the number of spores in the rhizosphere of *Cyperus iria* L., and *C. rotundus* L., which grew in a semi-arid tropical grassland. Lugo *et al.*, (2003) reported a marked seasonal variation in endomycorrhizal colonization; colonization rates were higher during summer, while declined during winter and early spring. Lutgen *et al.*, (2003) found that significant seasonal changes occurred in AM hyphal colonization and in the concentration of glomalin in rhizosphere soil. Schreiner, (2005) found that arbuscular colonization of grapevine (*Vitis vinifera* L.) roots was strongly seasonal in an investigation of the spatial and temporal development of grapevine roots and their associated mycorrhizal fungi in 1999 and 2000. Recent experiments by Mandyam & Jumpponen, (2008) indicated that AM fungi were most abundant during the peak growing season of the dominant C_4 vegetation in a tallgrass prairie ecosystem. In contrast, some studies did not show such regular seasonal changes in AM fungi colonization (e.g. Boerner, 1986; Brundrett & Kendrick, 1988). For example, Ruotsalainen *et al.*, (2002) did not find that AM fungi colonization fluctuates throughout the growing season in a study of meadow plants.

Although there is increasingly interest in AM resources, including their classification and ecological effects, few studies focuse on the seasonal dynamics of AM colonization and the production of spores in artificial grasslands. The main objective of this study was to investigate the seasonality in AM fungal colonization and spore numbers under natural field conditions. We investigated the interactions between AM fungi and two common pasture plant species, orchardgrass (*Dactylis glomerata* L.) and white clover (*Trifolium repens* L.), which were the dominant constructive species in an artificial grassland in Japan. We aimed to determine: 1) how seasonal dynamics influence AM fungi colonization and the number of spores in the rhizospheres of the orchardgrass and white clover plants; and 2) if there are differences in the seasonality of AM fungi colonization or numbers of spores between the plant species.

Materials and Methods

Site description: The study site is located in an artificial grassland that was established in the 1970s in the Field Science Centre of Tohoku University, Miyagi Prefecture, Japan (latitude, 38°44´N; longitude, 140°15´E). The dominant species at this site was orchardgrass (*Dactylis glomerata* L.), while white clover (*Trifolium repens* L.) and sweet vernal grass (*Anthoxanthum odoratum* L.) were also relatively abundant. This artificial grassland covers an area of approximately 0.33 ha. The soil is a highly acidic allophone andosol with high humus content and a high capacity to adsorb phosphoric acid. The soil pH ranged from 4.0 to 5.5. Annual rainfall in this area amounts to approximately 1, 800 mm. The average annual temperature at this site is approximately 11.1°C, with the highest and lowest temperatures being 34.4°C and -4.3°C, respectively.

Sampling methods: Each root and rhizosphere soil sample from the orchardgrass and white clover was collected at a depth of 0-10 cm in the meadow, within a 10 cm ×10 cm quadrate. Four replicates were collected, each containing a single plant tiller tuft. During the period of our investigation, the herbage was cut three times (on 14th May, 23rd July and 3rd October, 2002) and fertilized four times with 200 kg ha^{-1} of N, 100 kg ha^{-1} of P_2O_5 and of 200 kg ha^{-1} K_2O per year. Both pastures were sampled 12 times from December 2001 to December 2002 at 4- to 6-week intervals. The sampling dates were December 23, 2001, and February 1, March 10, April 11, May 6, June 6, July 4, August 5, September 5, October 2, November 5 and December 4 in 2002.

AM colonization and soil analysis: The samples were carefully separated into soil and plant components. Part of each root sample was carefully rinsed with tap water and cut into 1 cm-long fragments. Then the root fragments were heated at 100°C for 10 min in 10% KOH and rinsed with tap water. These samples were soaked in 2% HCl for 3-5 min and rinsed again. Finally, the samples were stained for 5 min at 100°C with trypan blue (Phillips & Hayman, 1970). Percentage colonization of the roots by AM fungi was determined according to the magnified line intersection method described by Giovannetti & Mosse, (1980).

The soil temperature at a depth of 0-10 cm in each quadrat was determined using a soil thermometer (TR-71/72) on each sampling occasion, and 10 g of fresh soil from each sample (separated from roots as described above) was wet-sieved to collect spores using the method described by Gerdemann & Nicolson, (1963). In addition, the pH of the soil samples was measured using a glass electrode after making a 1:2.5 soil: water paste from 10 g of dried soil and 25 ml of distilled water.

Statistical analysis: Statistical analyses were performed using the SPSS software package version 12.0 (SPSS Inc., USA). Means of soil pH and temperature, as well as means and standard errors of AM colonization and numbers of spores, were calculated by descriptive statistics from four replicate samples in each month. One-way analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) at the 5% confidence level, was used to estimate any differences in AM colonization (including total colonization, arbuscular colonization and vesicular colonization) and spore numbers between the two tested pasture species in each month. The same test was used to determine the difference in AM colonization and spore numbers among 12 months. In addition, Pearson's product-moment correlation coefficients were conducted to examine the relationships between mycorrhizal colonization as well as numbers of spores and soil factors (pH and temperature). To test whether the explanatory variables had mutual effects on AM colonization and the numbers of spores, the effects of 'species (orchardgrass versus white clover)', 'month (year)' and 'species ×month (year)' were also analyzed using Univariate ANOVAs.

Results

Soil temperature and pH: The pH of the artificial grassland soil was acidic, ranging from 4.96 to 6.05 (Fig. 1), but the mean monthly values remained roughly constant throughout the study. In contrast, mean soil temperatures changed on a monthly basis, ranging between 1.1°C in February to 25.3°C in August 2002.

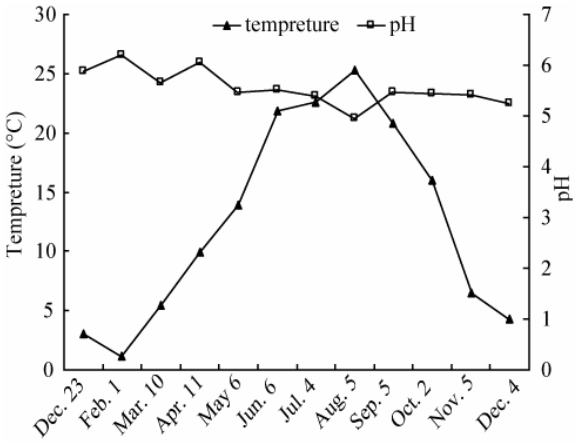

Fig. 1. Monthly mean soil temperature and pH at the four sampling sites over the study period from December 2001 to December 2002.

Seasonal dynamics in AM colonization of roots of the two pasture species: Univariate ANOVAs results showed that the effects of 'month (year)' on the total colonization, arbuscular colonization and vesicular colonization were all significant (p<0.001); this indicated that AM colonization varied seasonally (Table 1). The pasture species had significant effects on arbuscular colonization and vesicular colonization (p<0.05), but not on total colonization (p>0.05). Moreover, the interaction term 'species × month (year)' was significant for total colonization and vesicular colonization (p<0.01), but not for arbuscular colonization (p>0.05).

Table 1. F-values, from univariate ANOVAs, of the effects of species, month and species × month on AM colonization and spore numbers.

	TC	TA	TV	Spore numbers
Species	1.561	5.616*	5.584*	8.309**
Month (year)	3.654***	4.993**	4.517***	18.188***
Species × Month (year)	3.324**	1.012	2.618**	1.279

TC, TA and TV indicate total colonization, total arbuscular colonization and total vesicular colonization, respectively (n=96)
*p<0.05, ** p<0.01, *** p<0.001

Table 2. F-values from one-way ANOVAs, of effects of month on AM colonization and spore numbers.

	TC	TA	TV	Spore numbers
Orchardgrass	3.219**	5.168***	3.235**	11.998***
White clover	4.051**	1.625	4.385***	6.938***

TC, TA and TV indicate total colonization, total arbuscular colonization and total vesicular colonization, respectively (n=48)
** p<0.01, *** p<0.001

The total AM colonization of both orchardgrass and white clover varied significantly (p<0.01) over the 12 months (Table 2). During the survey year, total AM colonization of both pastures initially decreased to minimal value in March (ca. 21.2% for orchardgrass and 23.52% for white clover; Fig. 2a), then increased along with the pasture growth to the first peak in June. Colonization then moderately declined in July and August. During these periods, the changes of total AM colonization of the two species were similar, but there was a rapid divergence thereafter, during the second phase of increasing colonization. The total colonization of orchardgrass continued to increase from August until November, and reached highest (53.87%), but then decreased. In contrast, white clover colonization peaked in September and colonization then decreased. Besides, the total colonization of white clover was significantly higher than that of orchardgrass in February, but significantly lower than that of orchardgrass in November (p<0.05). However, there was no significant difference in the total AM colonization between orchardgrass and white clover over the 12 months (p>0.05).

Seasonal changes in arbuscular colonization (Fig. 2c) and vesicular colonization (Fig. 2b) patterns were similar to those of total colonization for both species. There was significant difference (p<0.001) in arbuscular colonization of orchardgrass among 12 months, but not for white clover (Table 2). However, vesicular colonization of both species significantly differed (p<0.01) among the 12 months. The vesicular colonization of both species was lowest in April (during the first decreasing phase), 1.62% for orchardgrass and 1.78% for white clover. In contrast, while the vesicular colonization of orchardgrass was highest in November (17.52%), during the second increasing phase, it was highest for white clover (10.92%) in May, during the first increasing phase. During December of both 2001 and 2002, vesicular colonization of two species differed significantly (p<0.05). Similarly, the arbuscular colonization of orchardgrass was lowest (2.82%) in April, during the first decreasing phase, and highest (20.32%) in November, during the third increasing phase (Fig. 2c), while for white clover it was highest (17.53%) in June, during the first increasing phase, and lowest (5.95%) in March, during the first decreasing phase.

We also investigated the ratio of vesicular colonization to arbuscular colonization in each of the months (Fig. 2d). Before March 2002, the ratios were higher for white clover than for orchardgrass. In March, the ratios for the two species were the same. Then, from March to December 2002, the ratio was higher for orchardgrass than for white clover, indicating that vesicular colonization was higher in orchardgrass, whereas arbuscular colonization was higher in white clover.

Seasonal dynamics of spore numbers in the rhizosphere of the two pasture species: Spore numbers in the rhizosphere of the two tested species varied seasonally, as shown in Table 1. During the survey year, the spore numbers in the rhizosphere of both pastures were highest in winter, then decreased from spring to summer, and then increased slowly in early autumn (Fig. 3). Significantly seasonal differences in the number of spores were found among the 12 months for both species (p<0.001) (Table 2). The spore numbers were highest in the rhizospheres of orchardgrass and white clover in February and March, respectively, and lowest (in both cases) in July. There were also significant effects of 'species' on spore numbers (p<0.05). Overall, spores were more abundant in the rhizosphere soil of orchardgrass than that of white clover, except in May, August and December 2002. One-way ANOVA result showed that the spore number in the rhizosphere of orchardgrass was significantly higher than that of white clover in February (p<0.05). The 'species × month (year)' interaction was not significant for spore number (p>0.05).

Correlations between soil temperature, soil pH and AM variables: Correlations between the soil characters that were measured and the AM variables are shown in Table 3. In the growing season, the number of spores in the rhizosphere had a significant negative correlation with soil temperature (p<0.01), while it was significantly positively correlated with soil pH (p<0.01). However, there was no significant relationship between soil characters and AM colonization (p>0.05).

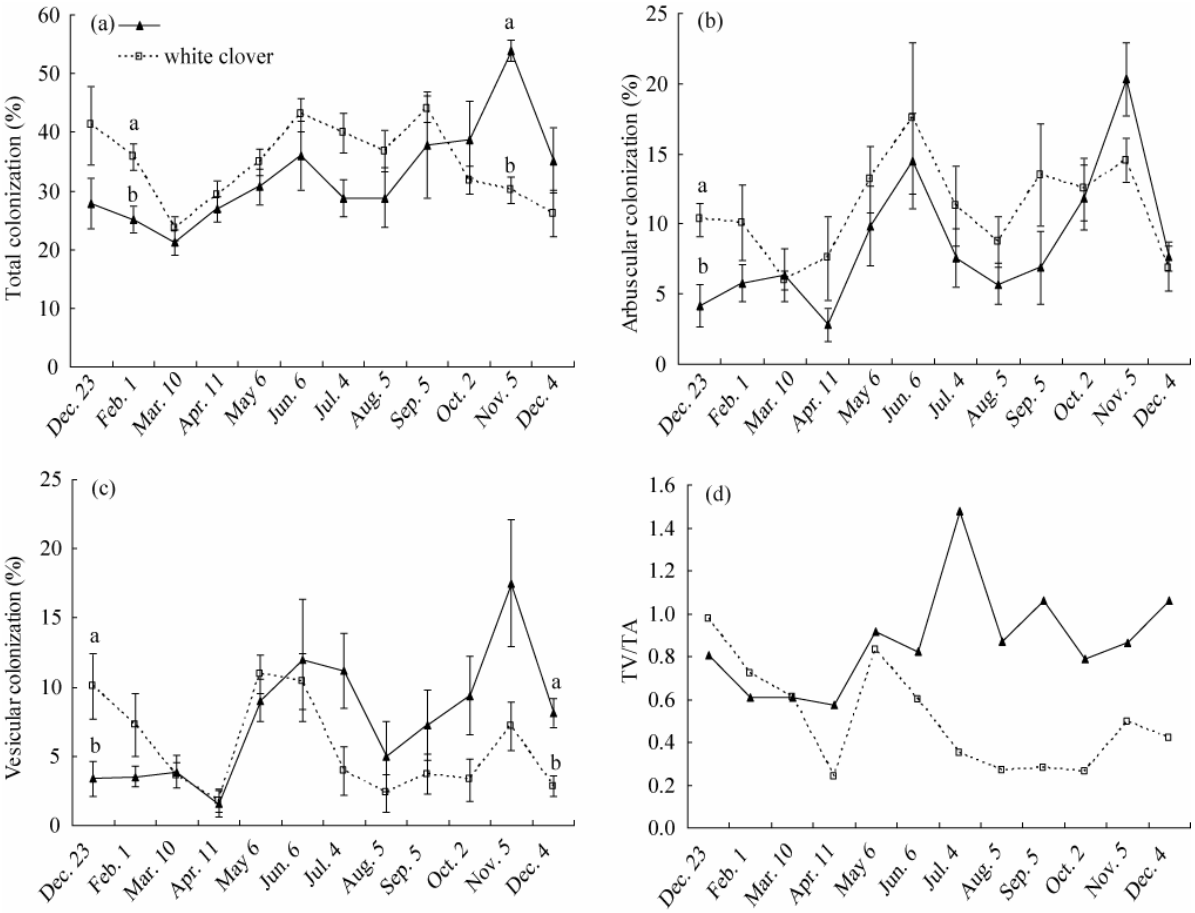

Fig. 2. Seasonal changes in total colonization (a), arbuscular colonization (b), vesicular colonization (c) and TV/TA (d) in orchardgrass and white clover at each sampling date from December 2001 to December 2002. Error bars represent the standard errors of data at the four sampling sites. Different letters indicate significant differences at the 5% probability level between the two pasture species. TV/TA: total vesicular colonization / total arbuscular colonization.

Table 3. Correlations between soil parameters and AM colonization and spore numbers.

	TC	TA	TV	Spore numbers
Soil temperature	0.287	0.222	0.074	-0.644**
Soil pH	-0.096	-0.230	0.028	0.644**

TC, TA and TV indicate total colonization, total arbuscular colonization and total vesicular colonization, respectively (n=24)
** $p<0.01$

Discussion

Liu *et al.*, (2009) found that AM fungal colonization of roots was significantly correlated with the sampling month ($p<0.05$). We also observed conspicuous seasonal fluctuations in AM fungal colonization and the number of spores in the rhizosphere of orchardgrass and white clover. From 2001 to 2002, total AM colonization of the two pasture species decreased during the winter and increased again when plant grew in spring. Colonization rates then declined slowly during the summer. In autumn, the total AM colonization of white clover continued to decline, while in orchardgrass it started to increase. Maximum and minimum total AM colonization levels for the two species were detected in early spring and autumn, separately. Our results were different from several studies which showed AM abundance reached the peak during summer and decline during winter and early spring (see Kabir *et al.*, 1997; Lugo *et al.*, 2003). They also differ from reports by Escudero & Mendoza, (2005) that the colonization of *Lotus glaber* roots was highest in summer or spring and lowest in winter or autumn. These contrasting results are probably due to differences in host plants and habitat types, and possibly other factors that reportedly affect the seasonality of AM fungal colonization. Therefore, further research is needed to determine the effects of these factors.

It is difficult to understand if various influential factors are determinants of the seasonal patterns of AM fungal colonization in the present study, or if the seasonality of AM fungal colonization is an intrinsic property of the fungi. However, numerous studies showed that AM fungal colonization of host-plant roots is influenced by many factors. Firstly, AM fungal colonization rates vary among plant species, for example *Alchemilla glomerulans*, *Carex*

vaginata, *Ranunculus acris* ssp. *pumilus* and *Trollius europaeus* (Ruotsalainen *et al.*, 2002), and five Poaceae plants (Lugo *et al.*, 2003). Our results showed that total colonization did not differ between the two examined pasture species, but 'species' and 'month' had significant mutual-effects (p<0.01). Secondly, several studies have shown that climatic and edaphic factors affect AM fungal colonization (see Muthukumar & Udaiyan, 2002). For instance, Mohammad *et al.*, (1998) found that the AM colonization of winter wheat (*Triticum aestivum* L.) did not change significantly over several months after sowing in autumn, but increased in the following spring as the temperature rose. From December 2001 to February 2002, the total colonization of the two pasture species was high (>25.18%), although the soil temperature was still below 5°C. However, there was no significant correlations (p>0.05) between AM colonization and soil temperature or soil pH. In the future, contents of soil nutrients, such as nitrogen (N) and phosphorus (P), should be considered, since N and P enrichment has been found to increase, decrease, or have no effect on AM colonization in different cases (Aerts, 2002; Muthukumar & Udaiyan, 2002). We summarized some factors above that may be responsible for seasonal changes in AM fungal colonization we detected, but it is hard to figure out the exact causes of the changes in terms of our data. We believe that our investigation of the seasonality of AM fungal colonization is helpful to improve understanding of the interactive effects of AM fungi and plants. In addition, this information may help to optimize pasture management practices, such as the timing of fertilization applications, mowing and grazing.

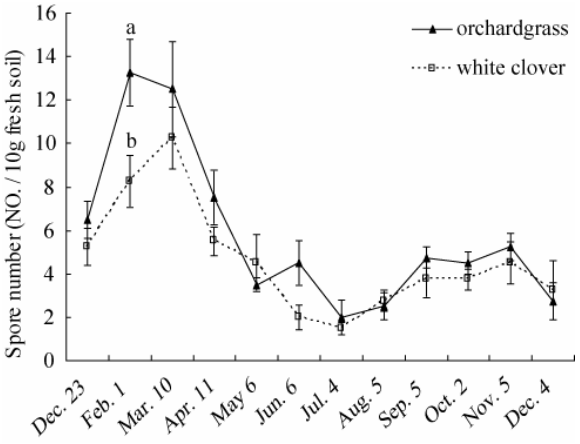

Fig. 3. Seasonal changes in spore numbers in the rhizosphere of orchardgrass and white clover at each sampling date from December 2001 to December 2002. Error bars represent the standard errors of data at the four sampling sites. Different letters indicated significant differences at the 5% probability level between the two pastures.

Both arbuscular colonization and vesicular colonization changed seasonally and differed between orchardgrass and white clover; and vesicular colonization was more frequent in orchardgrass than in white clover, while arbuscular colonization was more abundant in white clover than in orchardgrass. These were supported by other studies. For example, our previous study showed that vesicular colonization of *Rhynchrelyrum repens* (Wild.) C.E. Hubb. was more frequent (4.3-33.3%) than arbuscular colonization (2.9-5.0%) in six quarry sites (Chen *et al.*, 2008). Furthermore, it was reported that vesicular colonization was more frequent (albeit with strong seasonal variations) than arbuscular colonization in the rhizospheres of sedges and plants in a tallgrass prairie ecosystem, respectively (Muthukumar & Udaiyan, 2002; Mandyam & Jumpponen, 2008). The arbuscule and vesicle of AMF are thought to have different functions. Arbuscules are sites where materials are exchanged between AM fungi and plants, while vesicles are used for nutrients storage and propagation (Liu & Chen, 2007). However, there was no other previous research on the differences between arbuscule and vesicle colonization in different plant species in the same ecosystem. Furthermore, other functions of arbuscules and vesicles, particularly with respect to plant growth, remain unknown. Further experiments are needed to investigate whether the structure and function of vesicular and arbuscular formations differ between plants, which coincides with the idea proposed by Burni & Hussain, (2011) in the researches focused on diversity in arbuscular mycorrhizal morphology.

The number of spores in the rhizosphere of the two pasture species varied seasonally. Spore numbers were highest in winter and early spring, then decreased from spring to summer, then increased again slowly in early autumn. Seasonal changes in the abundance of spores have been reported, with the best highest formation rates occurring in June, July and October (Li & Feng, 2001). Liu *et al.*, (2009) also observed significant seasonal variation in spore density, with the highest and the lowest spore densities occurring in October and July, respectively. It appears that autumn may be the best season for spore formation. Although spores that form in the summer and autumn are less likely to sprout immediately than those form in winter, they may accumulate more storage materials. This is consistent with the hypothesis that potential seasonal variation in spore numbers may be the result of due to the formation of new spores in association with root growth (Muthukumar & Udaiyan, 2002). In addition, edaphic factors, such as soil temperature (Dong & Zhao, 2003) and pH (Burni *et al.*, 2011), may play important roles in determining spore abundance. Some studies showed that newly formed spores cannot germinate immediately. The spores of some species need to go pass a dormancy period before germination, while those of other species do not require dormancy. For example, after one month's treatment at 4°C, germination of *Glomus microaggregatum*, *Acaulospora Scrobiculate* and *Scutellospora herergama* spores increased by 35.6%, 39.0% and 28.0%, respectively (Dong & Zhao, 2003). Spore numbers in our study were negatively correlated with soil temperature (p<0.01). Due to the accumulation of snow, the soil temperature remained above 0°C, which contributed to spores living through the winter, but was sufficiently low to release them from dormancy. Thus, when temperature increased in spring, many spores begin to germinate, which may partially explain why we found that AM colonization increased during spring. Although our results showed that spore numbers were positively correlated with soil pH (p<0.01), we could not determine the effects of soil pH on spore density, because soil pH did not change significantly throughout the year.

Conclusions

The results showed that there was seasonal fluctuations of the AM fungal colonization and spore numbers in the rhizosphere of orchardgrass and white clover, and the numbers of spores in the two pasture species was negatively correlated with soil temperature ($p<0.01$). Our results is helpful to improve understanding of the interactions between AM fungi and plants, and may assist with grassland management, including the identification of optimal times for fertilization. Moreover, the vesicular colonization of orchardgrass was higher than that of white clover, while the opposite trend was observed for arbuscular colonization. The study highlights the need for further research to examine differences in structure and function of vesicular and arbuscular colonization.

Acknowledgments

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References

Aerts, R. 2002. The role of various types of mycorrhizal fungi in nutrient cycling and plant competition. *In:* van der Heijden, M. and I. Sanders (Eds.). *Mycorrhizal ecology*. Springer-Verlag, Berlin, pp. 243-261.

Boerner, R.E.J. 1986. Seasonal nutrient dynamics, nutrient resorption, and mycorrhizal infection intensity of two perennial forest herbs. *Am. J. Bot.*, 73(9): 1249-1257.

Brundrett, M.C. and B. Kendrick. 1988. The mycorrhizal status, root anatomy and phenology of plants in a sugar maple forest. *Can. J. Bot.*, 66: 1153-1173.

Burni, T. and F. Hussain. 2011. Diversity in arbuscular mycorrhizal morphology in some medicinal plants of family Lamiaceae. *Pak. J. Bot.*, 43(3): 1789-1792.

Burni, T., F. Hussain and M. Sharief. 2011. Arbuscular mycorrhizal fungi (AMF) associated with the rhizosphere of *Mentha arvensis* L. and *M. longifolia* Huds. *Pak. J. Bot.*, 43(6): 3013-3019.

Chen, Y., J.G. Yuan, Z.Y. Yang, G.R. Xin and L. Fan. 2008. Associations between arbuscular mycorrhizal fungi and *Rhynchrelyrum repens* in abandoned quarrries in southern China. *Plant and Soil*, 304: 257-266.

Dong, C.J. and B. Zhao. 2003. Effect of several factors on germination of AM fungal spores. *Plant Nut. Fer. Sci.* (China), 9(4): 489-494.

Escudero, V. and R. Mendoza. 2005. Seasonal variation of arbuscular mycorrhizal fungi in temperate grasslands along a wide hydrologic gradient. *Mycorrhiza*, 15(4): 291-299.

Gerdemann, J.W. and T.H. Nicolson. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans. Brit. Mycol. Soc.*, 46(2): 235-244.

Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular infection in roots. *New Phytol.*, 84(3): 489-500.

Kabir, Z., I.P. O'Halloran, J.W. Fyles and C. Hamel. 1997. Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization: Hyphal density and mycorrhizal root colonization. *Plant and Soil*, 192(2): 285-293.

Lee, P.J. and R.E. Koske. 1994. *Gigaspora gigantea*: seasonal abundance and ageing of spores in a sand dune. *Mycol., Res.*, 98(4): 453-457.

Li, X.L. and G. Feng. 2001. *Ecological and physiological effects of arbuscular mycorrhiza* (China). Huawen Publishing House, Beijing.

Liu, R.J. and Y.L. Chen. 2007. *Mocorrhizology* (China). Science Press, Beijing.

Liu, Y.J., L. He, L.Z. An, H. Helgason and H.Y. Feng. 2009. Arbuscular mycorrhizal dynamics in a chronosequence of *Caragana korshinskii* plantations. *FEMS Microbiol. Eco.*, 67(1): 81-92.

Lugo, M.A., M.E.G. Maza and M.N. Cabello. 2003. Arbuscular mycorrhizal fungi in a mountain grassland II: Seasonal variation of colonization studied, along with its relation to grazing and metabolic host type. *Mycologia*, 95(3): 407-415.

Lutgen, E.R., D. Muir-Clairmont, J. Graham and M.C. Rillig. 2003. Seasonality of arbuscular mycorrhizal hyphae and glomalin in a western Montana grassland. *Plant and Soil*, 257: 71-83.

Mandyam, K. and A. Jumpponen. 2008. Seasonal and temporal dynamics of arbuscular mycorrhizal and dark septate endophytic fungi in a tallgrass prairie ecosystem are minimally affected by nitrogen enrichment. *Mycorrhiza*, 18(3): 145-155.

Mohammad, M.J., W.L. Pan and A.C. Kennedy. 1998. Seasonal mycorrhizal colonization of winter wheat and its effect on wheat growth under dry land field conditions. *Mycorrhiza*, 8(3): 139-144.

Muthukumar, T. and K. Udaiyan. 2002. Seasonality of vesicular-arbuscular mycorrhizae in sedges in a semi-arid tropical grassland. *Acta Oecol.*, 23(5): 337-347.

Nasim, G., A. Ali, A. Munawar and R. Bajwa. 2008. Seasonal dynamics of AM fungi in sugarcane (*Saccharum officinarum* L. cv. SPF-213) in relation to red rot (*Colletotrichum falcatum*) disease from Punjab, Pakistan. *Pak. J. Bot.*, 40(6): 2587-2600.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.*, 55(1): 158-161.

Ruotsalainen, A.L., H. Väre and M. Vestberg. 2002. Seasonality of root fungal colonization in low-alpine herbs. *Mycorrhiza*, 12(1): 29-36.

Schreiner, R. 2005. Spatial and temporal variation of roots, arbuscular mycorrhizal fungi, and plant and soil nutrients in a mature pinot noir (*Vitis vinifera* L.) vineyard in Oregon, USA. *Plant and Soil*, 276: 219-234.

Smith, S.E. and D.J. Read. 1997. *Mycorrhizal symbiosis*. (2nd Ed) Academic Press, San Diego, New York.

van der Heijden, M.G.A., T. Boller, A. Wiemken and I.R. Sanders. 1998. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology*, 79(6): 2082-2091.

Wu, J., B. Sun, Y.T. Wang, G.R. Xin, S.P. Ye and S.L. Peng. 2011. Arbuscular mycorrhizal fungal colonization improves regrowth of Bermudagrass (*Cynodon dactylon* L.) after cutting. *Pak. J. Bot.*, 43(1): 85-93.

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