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著者	SAITO Katsuharu, SUGAWARA Kazuo
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Seasonal Variation of Arbuscular Mycorrhizal Fungal Colonization for Coexisting Plant Species in *Miscanthus*-Type Semi-Natural Grassland

Katsuharu SAITO^{1*} and Kazuo SUGAWARA²

¹Faculty of Agriculture, Shinshu University, Minamiminowa, Nagano 399-4598, Japan

²Graduate School of Agricultural Science, Tohoku University, Naruko-Onsen, Osaki, Miyagi 989-6711, Japan

*e-mail: saitok@shinshu-u.ac.jp

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Abstract

Many *Miscanthus*-type semi-natural grasslands in Japan are located on volcanic ash soil, which is short of available phosphate. Arbuscular mycorrhiza (AM) is a symbiotic association between AM fungi and plant roots, in which fungi are known to play an important role in phosphate uptake. For this study, we investigated seasonal variation in AM colonization of coexisting plant species in a *Miscanthus*-type grassland for two years. We selected three major plant species represented in vertical strata of *Miscanthus*-type grasslands: *Miscanthus sinensis*, a tall grass that was the dominant species in the stand; *Lysimachia clethroides*, a forb found in the middlelayer of the stand; *Potentilla freyniana*, a short forb occupying the lower layer of the stand. Mycorrhizal colonization of *M. sinensis* and *L. clethroides* was low in the early growing season. It subsequently increased concomitantly with increased plant dry weight. The mycorrhizal colonization reached a maximum level when the host plants reached a plateau of growth and entered a reproductive growth phase. During the late growing season, the colonization level decreased. Actually, *P. freyniana* showed a high level of mycorrhizal colonization over its growing season and different patterns of colonization between two growing seasons. These results demonstrate that seasonal variations of mycorrhizal colonization of *M. sinensis* and *L. clethroides* were related to their growth patterns and that *P. freyniana* might be susceptible to environmental fluctuations.

Introduction

Arbuscular mycorrhiza (AM) is a symbiotic asso-

ciation between AM fungi and plant roots. The AM fungi are associated with the vast majority of vascular plants (Maeda, 1954; Harley and Harley, 1987) and promote nutrient uptake of the host plant, especially phosphate acquisition from the soil (Smith and Read, 1997). In return, AM fungi gain photosynthate from the host plant. The AM fungi are an important microbe for nutrient cycling in terrestrial ecosystems (Allen, 1991).

Many native grasslands in Japan are located on volcanic ash soil, which is short of available phosphate. Under such conditions, AM symbiosis is expected to play an important role in phosphate uptake of plants. *Miscanthus*-type grasslands are representative of mesic tallgrass-type semi-natural grasslands in Japan (Numata, 1969). Some grasslands have been used for mowing or grazing. Their productivity and community structure are well characterized. *Miscanthus*-type grasslands form three vertical strata in a community: the upper, middle, and lower layers (Koike, 1969). Plants of the upper layer have a higher elongation rate and relative growth rate (RGR) in the early growth stage and a longer elongation term. The middle-layer plants show a higher elongation rate in the middle growth stage than in the early growth stage. The lower layer plants have a low elongation rate and RGR throughout the growing period and cease elongation in the early growing stage. Plant phenology also differs among layers: the lower the layer species of the community, the earlier the flowering time.

The AM colonizing pattern is closely related to plant growth and phenology (Gay et al., 1982; Brundrett and Kendrick, 1988; DeMars and Boerner, 1995; Johnson-Green et al., 1995; Merryweather and

Fitter, 1995; Allen *et al.*, 1998; Ruotsalainen *et al.*, 2002) as well as environmental factors such as temperature, precipitation, soil moisture and phosphorus content in soil (Rabatin, 1979; DeMars and Boerner, 1995; Johnson-Green *et al.*, 1995; Merryweather and Fitter, 1998; Hartnett and Wilson, 1999). However, these effects on AM colonization are not always consistent, probably because of the complicated interaction among factors (DeMars and Boerner, 1995). In *Miscanthus*-type grasslands, plant species for which characteristics of growth and phenology differ coexist in one place. These plant species might exhibit differential AM colonizing patterns under the same environmental conditions. Knowledge of AM colonizing patterns in response to plant characteristics can contribute to an explanation of the dynamics of AM fungi in plant communities.

This study investigated seasonal variations in AM colonization of several coexisting plant species in a *Miscanthus*-type grassland during two years. We selected three major plant species representing a range of structural characteristics: (i) *Miscanthus sinensis* Anderss. (Gramineae), a tall grass that was the dominant species in the stand; (ii) *Lysimachia clethroides* Duby (Primulaceae), a forb found in the middle layer of the stand; (iii) *Potentilla freyniana* Bornm. (Rosaceae), a short forb occupying the lower layer of the stand.

Materials and Methods

Study area

The experiment was conducted in a *Miscanthus*-type grazing land at an experimental Farm of Tohoku University, Miyagi, Japan, in 1999 and 2000. Soil was nonallophanic andisol (pH 4.7) with available phosphorus concentrations of 33.6 $\mu\text{g g}^{-1}$ (Bray II method). The 1-ha *Miscanthus*-type grassland was grazed by 10 head of cattle on 16–23 July and 30 September – 4 October in 1999, and 4–11 July and 22–25 September in 2000.

Sampling procedure

2 m \times 2 m plots were situated randomly on the *Miscanthus*-type grassland on every sampling date. Numbers of plots were, respectively, eight and six in 1999 and 2000. One plant was harvested from each plot for *M. sinensis*, *L. clethroides*, and *P. freyniana*. Sampling dates were on 23 May, 23 June, 5 August, 1 September, 6 October, and 2 November in 1999,

and 16 May, 16 June, 17 July, 18 August, 15 September, and 16 October in 2000. Plants were removed with a 10 cm \times 10 cm \times 10 cm soil core. Plant parts were separated from soil and subdivided into roots, rhizomes, stems, leaves, and heads. Roots were used for measurement of the mycorrhizal colonization. Plant parts, except for roots, were dried at 70°C for 48 hr and then weighed. Total phosphorus contents in aboveground parts were determined using the ascorbic acid – molybdate blue method (Watanabe and Olsen, 1965) after digestion with concentrated sulfuric acid and hydrogen peroxide at 200°C for 120 min.

Arbuscular mycorrhizal colonization

Fresh lateral roots were cleared with 10% KOH and stained with trypan blue [a phenol-free modification of the method of Phillips and Hayman (Phillips and Hayman, 1970)]. The percentage of root length colonized (%RLC) by all AM fungal materials was determined using a magnified intersection method at 150 \times (McGonigle *et al.*, 1990).

Results

Meteorology

Mean temperature and precipitation during the growing season in 1999 was higher than that of the prior ten years (+1.0°C and +180 mm; Figs. 1A and 1B). In the early growing season of 2000, the mean temperature was lower than that of the prior ten years (-1.0°C; Fig. 1A). It was hotter and drier in the summer of 2000 than in the prior ten years (+1.4°C and -216 mm, June–August; Figs. 1A and 1B).

Dry matter production

Dry weight of *M. sinensis* increased toward August and reached a stationary growth stage (Fig. 2A). Their panicles began to emerge from late July. Subsequently, *M. sinensis* occupied the upper layer of the stand. Most dry matter was allocated to its stem to reach top of the stand (data not shown). Patterns of dry matter production were similar between 1999 and 2000. In 2000, the total dry weight decreased during August–September, probably because of the hot and dry summer. *L. clethroides* rapidly grew toward July–August and blossomed in July (Fig. 2B). Although the growth pattern of *L. clethroides* was similar between the two growing seasons, the total dry weight decreased during August–September in 2000, probably because of the hot and dry summer, as shown for

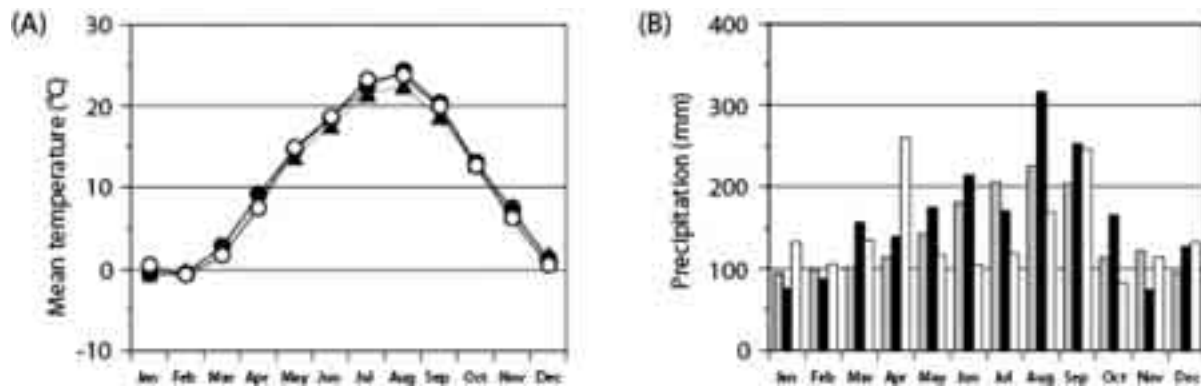


Fig. 1. (A) Monthly mean temperatures at the Kawatabi Meteorological Station near the study site in 1999 (black circle), 2000 (white circle), and 1991–2000 (black triangle) and (B) monthly precipitation in 1999 (black column), 2000 (white column), and 1991–2000 (gray column).

M. sinensis. The growth patterns of *P. freyniana* differed between the two growing seasons (Fig. 2C). In 1999, the dry weight of *P. freyniana* increased rapidly during May–June; then it was maintained as almost constant. In 2000, the dry weight was very low in the early growing season (May–July), possibly because of the low amount of precipitation during the season and low temperature in April. The dry weight increased slightly after August. Flowering of *P. freyniana* was observed in May of each year. Actually, *P. freyniana* allocated a large part of its dry matter to rhizome development (data not shown).

AM colonization

The AM colonization of *M. sinensis* showed a similar pattern between two growing seasons (Fig. 3A). The AM colonization was low in the early growing season, but it rapidly increased concomitantly with increasing plant dry weight. The colonization level reached a maximum of 60–70% in August, coincident with the flowering time of *M. sinensis*. In the late growing season, the colonization level decreased markedly. In *L. clethroides*, the AM colonization increased concomitantly with increasing plant dry weight to August (Fig. 3B). The colonization level reached a maximum of around 70% in August. During the late growing season, the colonization level decreased in both years, but the decrease was drastic in 1999 and gradual in 2000. Only in 1999 was the high level of AM colonization observed in November when the aboveground part of *L. clethroides* died. In contrast to *M. sinensis* and *L. clethroides*, the AM colonization of *P. freyniana* was high overall during its growing season (Fig. 3C). The patterns of AM col-

onization differed between the two growing seasons. The AM colonization level in 1999 was maintained at an almost constant of 60–80%, but the colonization in 2000 initially tended to be lower than that in 1999.

Phosphorus content

Phosphorus concentration of shoots was higher in May than in other months for the three plant species, especially *L. clethroides* (Fig. 4). No differences of seasonal variation were found between the two growing seasons, except in May. The phosphorus concentration of *M. sinensis* was 0.11% in May; it gradually decreased thereafter to 0.03% (Fig. 4A). Both *L. clethroides* and *P. freyniana* showed high levels of phosphorus concentration in May, although the levels differed between years (Figs. 4B and 4C). After June, the concentrations in each plant type remained between 0.04–0.09%. The total phosphorus content aboveground was correlated with plant dry weight (Figs. 4D, 4E and 4F).

Discussion

Results of this study demonstrated that seasonal variations of mycorrhizal colonization of *M. sinensis* and *L. clethroides* are related to their growth patterns. The mycorrhizal colonization was low during the early growing season. It subsequently increased as the plant dry weight increased. The mycorrhizal colonization reached a maximum level when host plants reached a plateau of growth and entered a reproductive growth phase. Phosphorus requirements of host plants might be high at the initial growth stages when the rate of phosphorus content increase was high in aboveground. Increased mycorrhizal colonization

might reflect the high phosphorus requirements of plants in the early growing season. In the late growing season, the colonization level decreased, which implies a low dependence of the host plants on mycorrhizas. During its growing season, *P. freyniana*

showed a high level of mycorrhizal colonization. However, *P. freyniana* is apparently not dependent on mycorrhizae for its phosphorus nutrition because the increased rate of phosphorus content in *P. freyniana* shoots was slight. In *P. freyniana*, AM fungi might be

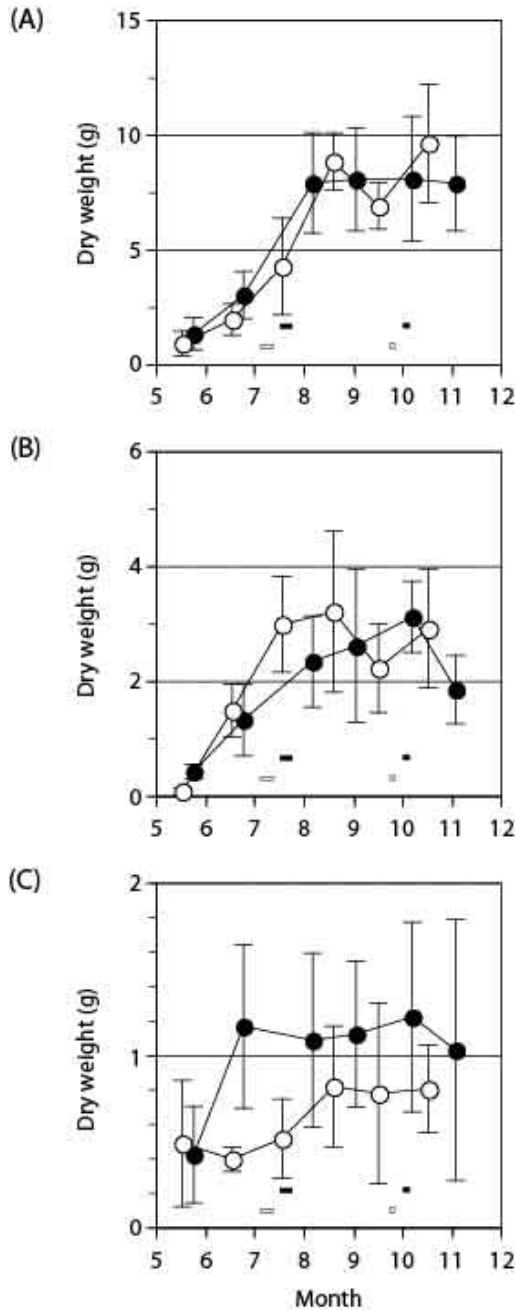


Fig.2. Seasonal variation in total dry matter production (sum of rhizome, stem, leaf, and heads) of (A) *Miscanthus sinensis*, (B) *Lysimachia clethroides* and (C) *Potentilla freyniana* in 1999 (black circle) and 2000 (white circle). Values are means \pm SD ($n = 8$, in 1999; $n = 6$, in 2000). Horizontal bars represent grazing periods in 1999 (black bar) and 2000 (white bar).

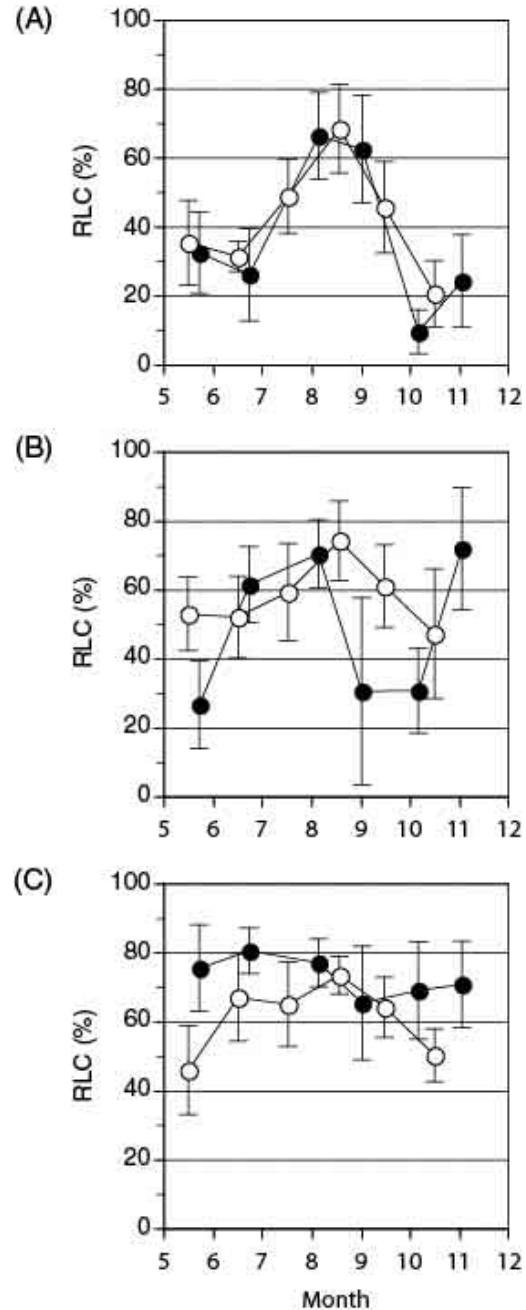


Fig.3. Seasonal variation in mycorrhizal colonization of (A) *Miscanthus sinensis*, (B) *Lysimachia clethroides*, and (C) *Potentilla freyniana* in 1999 (black circle) and 2000 (white circle), measured as a percentage root length colonized (%RLC) by all arbuscular mycorrhizal fungal materials. Values are means \pm SD ($n = 8$ in 1999; $n = 6$ in 2000).

colonizing parasitically, or have other functions for plants such as micro-nutrient uptake, pathogen tolerance, and drought tolerance.

Mycorrhizal colonization of *M. sinensis* and *L. clethroides* showed little annual fluctuation. Particularly, *M. sinensis* showed almost identical seasonal variation in mycorrhizal colonization in 1999 and 2000,

with almost equal dry weight, although the monthly precipitation in 2000 was lower than that of the prior 10 years. In contrast, seasonal variations in mycorrhizal colonization and the dry weight of *P. freyniana* differed between the two growing seasons. Actually, *P. freyniana*, occupying a lower layer of the *Miscanthus*-type grassland, might be susceptible to environmental

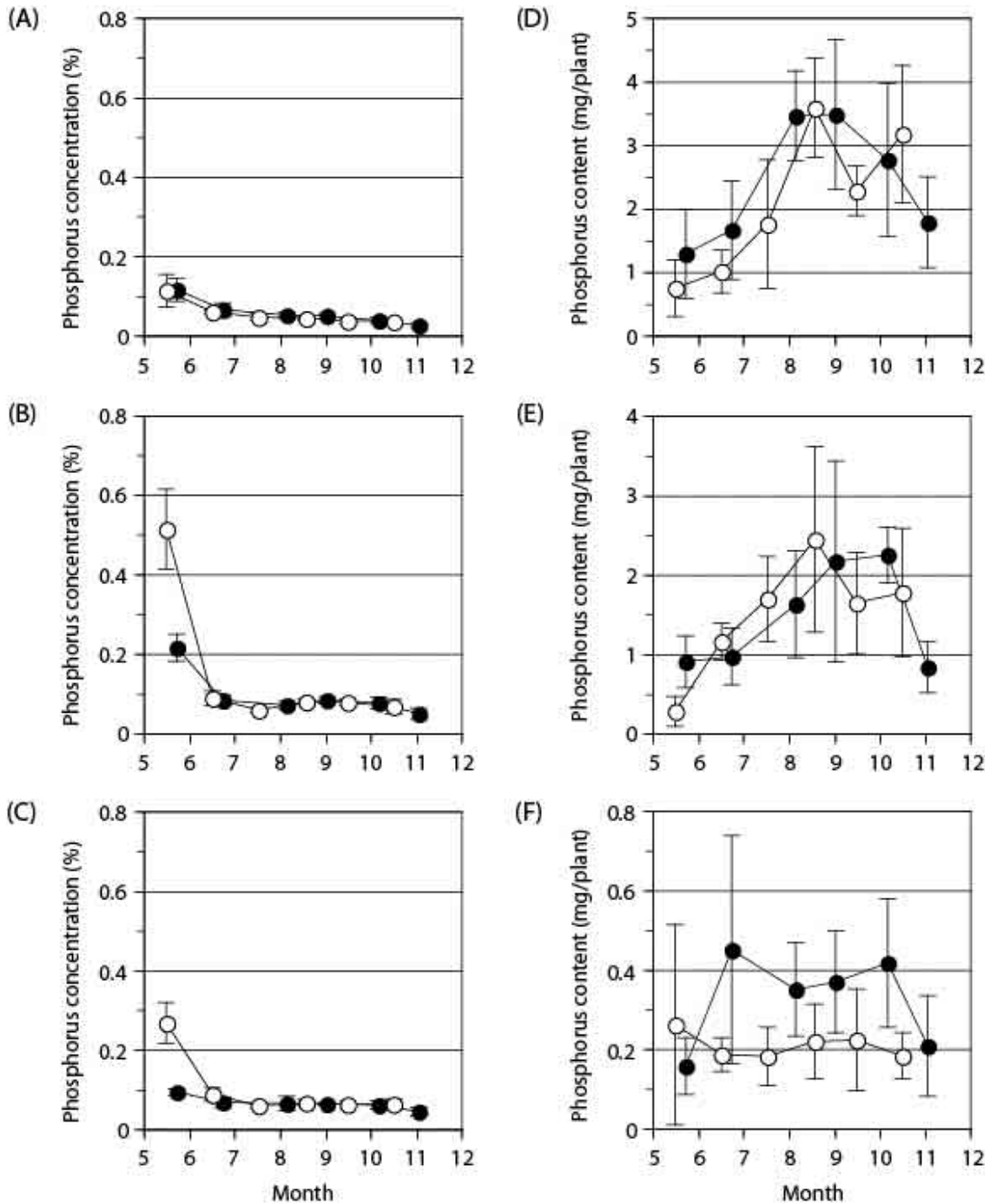


Fig.4. Total phosphorus concentration (A–C) and phosphorus content (D–E) aboveground of (A, D) *Miscanthus sinensis*, (B, E) *Lysimachia clethroides*, and (C, F) *Potentilla freyniana* in 1999 (black circle) and 2000 (white circle). Values are means \pm SD ($n = 8$ in 1999; $n = 6$ in 2000).

fluctuations such as temperature and the amount of precipitation. It is necessary to monitor the annual fluctuation of mycorrhizal colonization over the long term.

Mycorrhizal colonization in a semi-natural grassland is higher than that in an artificial grassland where fertilizers have been applied (Wu-en *et al.*, 2002). In this study, three plant species also showed a high degree of mycorrhizal colonization, although there was seasonal and annual variation, implying high dependence of wild plants on mycorrhizal associations to survive in semi-natural grasslands. The three plants co-occur in the *Miscanthus*-type grasslands. The AM fungal community in the grassland soil would be common to the three plant species. However, patterns of AM fungal colonization differ among the plant species. The differences in colonization patterns might originate from difference of AM fungal response to physiological properties of host plants. Mycorrhizal colonization of *M. sinensis* has been shown to decrease by defoliation, indicating that reduction of photosynthate allocation to mycorrhizal roots affects mycorrhizal colonization (Saito *et al.*, 2004). The defoliation of *M. sinensis* has also caused a change in the AM fungal community structure in roots: some AM fungal groups have decreased because of defoliation (Saito *et al.*, 2004). Some studies have revealed ecological specificity through preferential infection of plants by certain AM fungi in natural communities, although it is considered that no specificity exists between plants and AM fungi (Sanders *et al.*, 1999). Different patterns of mycorrhizal colonization of the three plants observed in our study might also be attributable to differences in AM fungal species colonizing in roots. Molecular studies are needed to ascertain whether the host plants are colonized with different AM fungal species.

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