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Seasonality of arbuscular mycorrhizal symbiosis and dark septate endophytes in a grassland site in southwest China

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Abstract

Arbuscular mycorrhizal and dark septate endophytic fungal colonization in a grassland in Kunming, southwest China, was investigated monthly over one year. All plant roots surveyed were co-colonized by arbuscular mycorrhizal and dark septate endophytic fungi in this grassland. Both arbuscular mycorrhizal and dark septate endophytic fungal colonization fluctuated significantly throughout the year, and their seasonal patterns were different in each plant species. The relationships between environmental (climatic and edaphic) factors and fungal colonization were also studied. Correlation analysis demonstrated that arbuscular mycorrhizal colonization was significantly correlative with environmental factors (rainfall, sunlight hours, soil P, etc.), but dark septate endophytic fungal colonization was only correlative with relative humidity and sunlight hours.

Keywords: Seasonality; Arbuscular mycorrhizal fungi; Dark septate endophytic fungi; Environmental factors

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are important components of rhizosphere microbial communities in natural ecosystems, forming symbiotic association with the majority of land plant roots [1]. AMF benefit from this association by obtaining carbon compounds [2,3], which are necessary for their growth. In return, AMF have diverse, beneficial impacts on plants and soils [4,5]. They can promote host plant growth by mitigating nutrient-deficient and water stresses [6–9], improve soil stabilization [10–12] and increase plants resistance to herbivores and soil-borne pathogens [13–15]. Their extraradical mycelia can translocate nutrients from one host to another through a common mycelial network [16,17]. As different plants have different responses to varied AMF, mycorrhizal fungal diversity may potentially determine plant biodiversity, ecosystem variability and productivity [18].

Previous studies reported that some host plants of AMF were co-colonized by dark septate endophytic fungi (DSE) [19–22]. To understand the functions of AMF and DSE in natural ecosystems, as well as their basic biology, it is essential to document the seasonal variation of these fungi and diverse factors affecting them. Root colonization and fungal spore production are important for elucidating fungal life history [23]. Although some studies have examined the seasonality of AMF colonization in roots [24–27], most of them failed to observe consecutive seasonal patterns of AMF due to the fact that samples were usually collected only four times per year. Over the past several years there has been growing attention to DSE, but there were few studies concerning their seasonality. The objectives

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of the present study were to examine whether plants were co-colonized by AMF and DSE in the surveyed grassland, to investigate seasonal variation of AMF and DSE colonization, and to assess the correlation between fungal colonization and environmental (climatic and edaphic) factors.

2. Materials and methods

2.1. Site description

The study site was located in the campus of Yunnan University, Kunming (25°1′N, 102°4′E, 1891 m a.s.l.), southwest China. This area has low-latitude monsoon climate with sunlight of 2250 h, frost-free of 230 days, mean annual temperature of 15.1 °C and mean annual precipitation of about 1075 mm. Most rainfall occurs from May to October (data are from Meteorological Station of Kunming for 20 years statistics). It is not bitterly cold in winter and not extremely hot in summer. The monthly changes of mean temperature, rainfall, relative humidity and sunlight hours were illustrated in Fig. 1. Due to the climate of this area, we could continuously collect samples throughout the year. *Cynodon dactylon*, *Cyperus rotundus*, *Digitaria cruciata*, *Digitaria ischaemum*, *Paspalum distichum*, *Plantago asiatica*, *Poa annua*, *Taraxacum mongolicum* and *Trifolium repens* comprised the plant community in the studied site, and some of these plants occurred sporadically or infrequently in the year (Table 1). *C. dactylon*, *P. asiatica* and *T. repens* were the dominant species in the community according to their coverage and occurrence frequencies throughout the year.

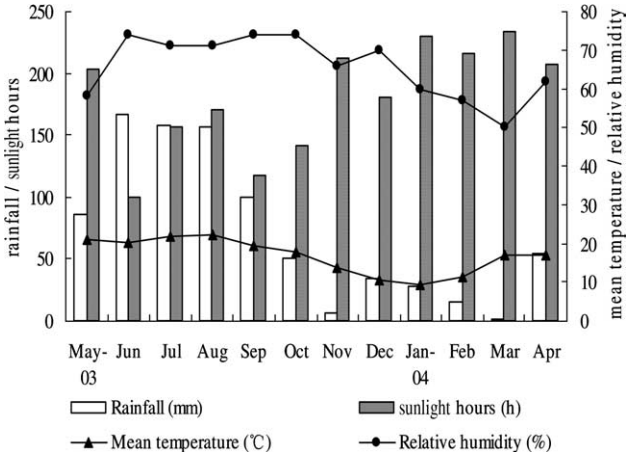

Fig. 1. Monthly values for climatic factors during the study period.

2.2. Root sampling and treatments

For the seasonality analysis, root samples were collected monthly from May 2003 to April 2004. We collected all plant species in a current month if the aboveground part of plants were present (Table 1). Four samples for each species were collected at each time and assured that roots were connected to the sampled plants. Young roots (with root tips) were fixed in 1/2 FAA (formalin + glacial acetic acid + 70% ethanol in 1:1:18 ratio by volume, diluted twice), and stored at 4 °C.

Roots were taken out from 1/2 FAA, washed several times in tap water and cleared in 10% (w/v) KOH by heating to approximately 90 °C in a water bath for 2–3 h. The time of heating depended on the structure of the roots and their pigmentation. The cooled root samples were washed and stained with 0.5% acid fuchsin according to Berch and Kendruck's method [28]. Fungal colonization was quantified using the magnified intersection method [29] under a compound-light microscope (OLYMPUS-BX51) at magnification 200×. One hundred and fifty intersections were observed for each sample. The presence of arbuscular mycorrhizal hyphae, vesicles, arbuscules and dark septate endophytic fungi was recorded.

2.3. Analysis of soil characters

Soils collected from the rhizospheres of the sampled plants each month were ground and mixed completely. Soil total nitrogen (N), phosphate (P), potassium (K),

Table 1
Plant species sampled in different months

Plants species	Living forms	2003								2004			
		May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Cynodon dactylon	Perennial	√	√	√	√	√	√	√	√	√	√	√	√
Cyperus rotundus	Perennial			√		√		√					
Digitaria cruciata	Annual			√	√	√	√	√	√	√			
Digitaria ischaemum	Annual					√	√						
Paspalum distichum	Perennial			√	√	√	√						√
Plantago asiatica	Perennial	√	√	√	√	√	√	√	√	√	√	√	√
Poa annua	Annual			√	√	√	√	√	√	√	√	√	√
Taraxacum mongolicum	Perennial		√		√								
Trifolium repens	Perennial	√	√	√	√	√	√	√	√	√	√	√	√

organic matter and available P were analyzed monthly using the methods described by Tan [30]: total N was determined by Kjeldahl method; total P and total K were digested by $HNO_3 + HClO_4$ and were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES); organic matter was calculated from the percent organic carbon determined by the $K_2Cr_2O_7$ wet combustion method; available P was extracted with $NH_4HCO_3 + DTPA$ (dethylenetriaminepentaacetatic acid) and was measured by ICP-AES; soil pH was determined by potentiometric method.

2.4. Statistical analysis

All statistical analyses were performed with SPSS software package (version 12.0). Fungal colonization percentages were arcsine transformed to match analysis of variance (ANOVA). The normality of the data and the homogeneity of the variances were tested before ANOVA. Repeated measures ANOVA were performed to determine the effects of time (within-subjects variables) and plant species (between-subjects factors) on fungal colonization. If Mauchly's sphericity tests indicated heterogeneity of covariance, we used the adjusted Huynh–Feldt values. Pearson's correlation coefficients were employed to determine the relationships between fungal colonization parameters and environmental factors, as well as the correlations between fungal colonization parameters.

3. Results

3.1. Soil characteristics

Monthly changes of physical and chemical properties of soils are presented in Fig. 2. The ranges of total N, total P and total K were from 0.28% to 0.40%, 0.18% to 0.24% and 0.29% to 0.48%, respectively. Available P ranged from 19.9 to 38.7 mg/kg, organic matter from 5.42% to 7.94%, and pH varied from 7.14 to 7.92.

3.2. Seasonality of arbuscular mycorrhizal colonization

All plants surveyed were colonized heavily by AMF and formed typical arbuscular mycorrhizal (AM) structures. Intra- and intercellular hyphae, hyphal coils, vesicles, arbuscules and occasional intraradical spores were observed in the root tissues. These AM structures, which characterized AMF colonization, were stained light red to red and were abundant in the roots and sometimes occurred in clusters.

Hyphal colonization was quite present in all surveyed species and often reached above 60%, occasionally, even beyond 95% in *C. dactylon* and *T. repens*. Hyphal colonization of each species reached peak and trough in different time (Fig. 3). Repeated measures ANOVA showed that hyphal colonization of each species fluctuated significantly through time (Table 2), and that the fluctuations differed from each other in the three dominant plants (*C. dactylon*, *P. asiatica*, and *T. repens*) (Table 3).

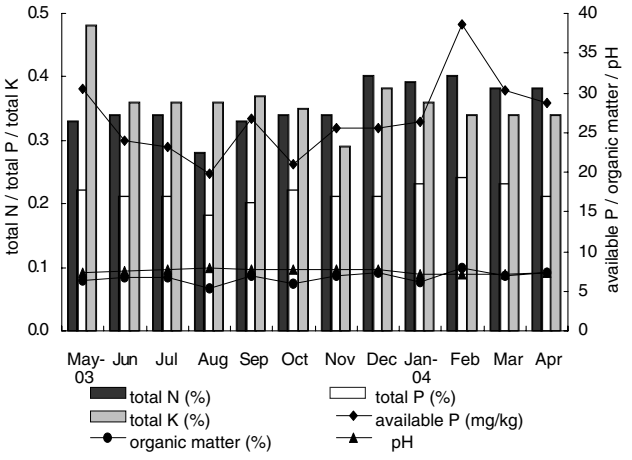

Fig. 2. Patterns of edaphic factors in the surveyed grassland during the study period.

Arbuscules were abundant in all samples. Arbuscular colonization of *T. repens* was always higher than that of other species every month except for July 2003 and reached the highest value in October 2003. Arbuscular colonization reached its maximum and minimum in different months (Fig. 3) for different plants. Repeated measures ANOVA showed that arbuscular colonization of each species highly varied throughout the growing season (Table 2), and that it differed significantly among the three dominant plants (Table 3).

Compared with hyphal and arbuscular colonization, vesicular colonization was often low in all species except for *C. dactylon*. The time with maximum and minimum of vesicular colonization for each species appeared different (Fig. 3). Similar to hyphal and arbuscular colonization, significant seasonality of vesicular colonization in all species was detected (Table 2) and varied with species (Table 3).

3.3. Seasonality of dark septate endophytic colonization

All plant roots were co-colonized by AMF and DSE. The hyphae of DSE fungi could be readily distinguished from AM hyphae by their color with dark red-brown to dark brown, thicker lateral wall, and frequent septa.

DSE colonization in all sampled plants was also lower than AMF hyphal and arbuscular colonization every month. It tended to reach peak and trough in different time for different species (Fig. 3). Repeated measures ANOVA indicated that variation in DSE colonization of each species was significant through time (Table 2)

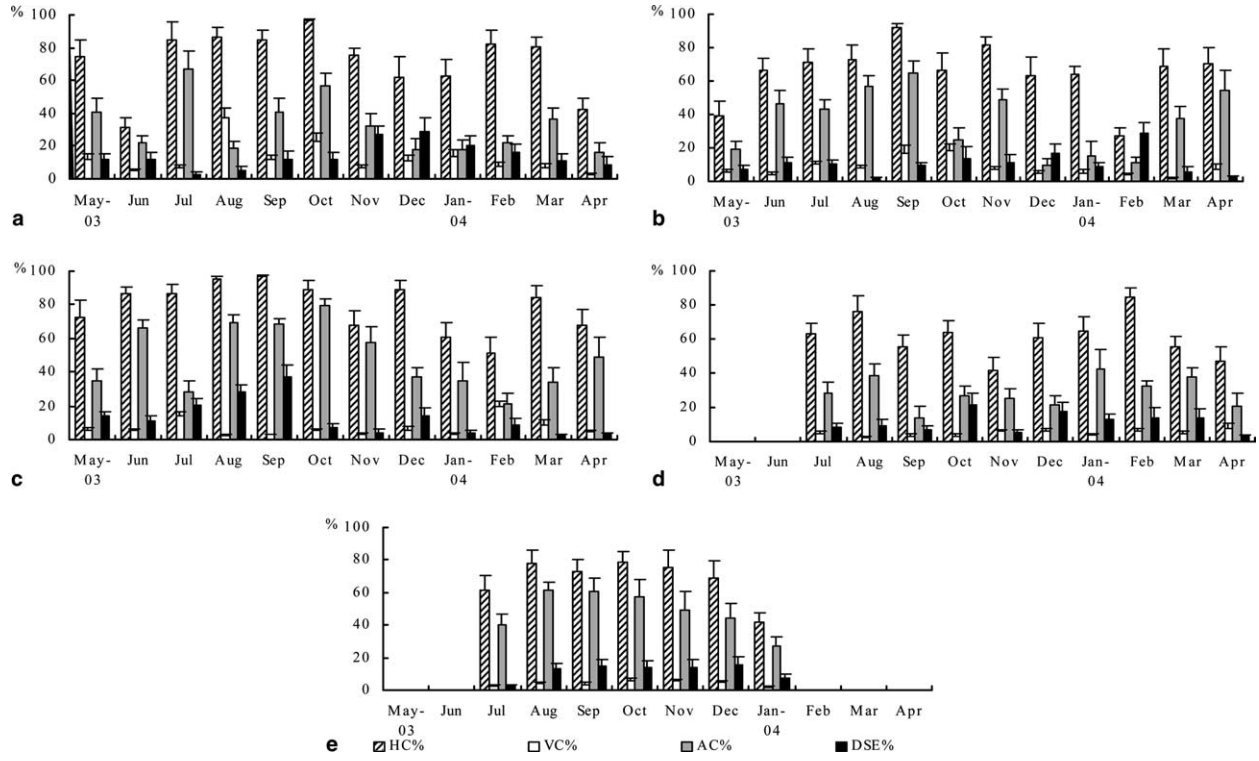

Fig. 3. Seasonal variation of fungal colonization percentage (mean ± SE) in (a) *Cynodon dactylon*, (b) *Plantago asiatica*, (c) *Trifolium repens*, (d) *Poa annua* and (e) *Digitaria cruciata*. HC, hyphal colonization; VC, vesicular colonization; AC, arbuscular colonization; DSE, dark septate endophytic fungal colonization.

Table 2
F-values from repeated measures ANOVA for arbuscular mycorrhizal and dark septate endophytic fungal colonization in each plant species

Plant species	Hyphal colonization	Vesicular colonization	Arbuscular colonization	Dark septate endophytic fungal colonization
Cynodon dactylon	20.721***	37.815***	24.202***	11.419***
Cyperus rotundus	11.976*	15.856*	6.294*	8.028*
Digitaria cruciata	8.560*	9.797***	9.909*	16.009*
Paspalum distichum	23.850***	7.027**	35.533***	40.391**
Plantago asiatica	19.909***	20.436***	42.058***	15.808**
Poa annua	12.025***	7.603***	6.693*	7.773*
Trifolium repens	33.386***	32.497***	49.831***	40.500***

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.
*** Correlation is significant at the 0.001 level.

Table 3
F-values from repeated measures ANOVA for arbuscular mycorrhizal and dark septate endophytic fungal colonization in the three dominant plant species

	Hyphal colonization	Vesicular colonization	Arbuscular colonization	Dark septate endophytic fungal colonization
Within-subjects effects				
Time	36.008***	25.105***	56.823***	18.296***
Time × species	16.439***	33.017***	26.576***	20.221***
Between-subjects effects				
Species	17.231**	40.196***	15.212**	6.103*

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.
*** Correlation is significant at the 0.001 level.

Table 4
Pearson's correlation coefficients between fungal colonization and different parameters

Variable	Hyphal colonization	Vesicular colonization	Arbuscular colonization	Dark septate endophytic fungal colonization
Hyphal colonization	1	0.203***	0.735***	0.159*
Vesicular colonization	0.203***	1	−0.100	−0.043
Arbuscular colonization	0.735***	−0.100	1	0.029
Dark septate endophytic fungal colonization	0.159*	−0.043	0.029	1
Temperature	0.208***	0.064	0.322***	−0.086
Rainfall	0.153**	0.036	0.253***	−0.002
Relative humidity	0.249***	0.084	0.336***	0.204***
Sunlight hours	−0.253***	−0.069	−0.372***	−0.215***
Total N	−0.283***	−0.091	−0.361***	0.010
Total P	−0.203***	−0.018	−0.231***	−0.036
Total K	0.032	0.026	−0.004	0.083
Available P	−0.255***	−0.040	−0.335***	0.035
Organic matter	−0.237***	−0.068	−0.290***	0.121
pH	0.258***	0.076	0.251***	0.138

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.
*** Correlation is significant at the 0.001 level.

and its seasonal differences were significant among the three dominant species, similar to AMF colonization (Table 3).

3.4. Correlation analysis

Correlation analyses indicated that both hyphal and arbuscular colonization were positively correlated with mean temperature, rainfall, relative humidity and pH, while negatively correlated with sunlight hours, total N, total P, available P and organic matter. Vesicular colonization was only significantly positively correlated with hyphal colonization. DSE colonization was correlated with relative humidity and sunlight hours. Pearson's correlation coefficients for all parameters measured were presented in Table 4.

4. Discussion

This study is a densely sampling one (monthly, 12 times per year) compared with other studies on the seasonality of AMF colonization (four times per year) [24–27]. The results might give more detailed information on seasonality of AMF and DSE colonization, and detected relatively short-term fluctuations of AMF and DSE colonization.

AMF colonization has been reported to peak in spring [7,27,31] or in summer [24]. While Brundrett and Abbott [32] observed seasonal fluctuations of AMF colonization were not substantial. In this study, higher AMF colonization occurred from July to October 2003, as this period was the growth stages for most plants in the grassland. It has been reported that AMF colonization could be coordinated with growth stages of plants during the long stress of a strong regimen of drought and rain [33]. AMF colonization for all plant species fluctuated significantly throughout the year, and their seasonal patterns varied among different plant species, which agreed with the view that AM symbiosis was considered to be probably species-specific [26].

Climatic factors and soil nutrient availability have temporal and spatial dynamics. Bohrer et al. [31] concluded that abiotic factors had minimal influence on AMF colonization variation, so AM seasonal dynamics was in response to plant phenology. While our results indicated that correlations were very significant between environmental factors and AMF colonization except for vesicular colonization, which strongly supported the opinion that climatic factors and edaphic factors could influence the AMF colonization [21,34,35].

The positive correlations between rainfall and hyphal/arbuscular colonization in our study were opposite to the previous finding that mycorrhizal variables and rainfall had a negative correlation [21], but consisted with other study [36]. Soil moisture has been reported to be positively correlated with AMF colonization [37], which might be a strong argument supporting our results, as rainfall was an important element of soil moisture. It is generally considered that levels of light were positively correlated with mycorrhizal colonization [38], and higher light levels can enhance the efficiency of photosynthesis, which can contribute more carbon compounds to AMF growth. In our study, sunlight hours were negatively correlative with hyphal and arbuscular colonization, which implied that the relationship between sunlight hours and AMF did not seem simple and its mechanism still remained unknown.

This investigation also indicated that there were significant correlations among edaphic factors and hyphal/arbuscular colonization. Soil P (total P and available P) was negatively correlated with AMF colonization; AMF could enhance plant uptake of P nutrient and other nutrients, especially in nutrient-deficient environment [6,8,39]. Koide and Li [40] pointed out that extremely low levels of P could inhibit AMF colonization, and increase of P levels could promote AMF colonization. Occasionally, AMF colonization did not correlate with soil P [26]. Bohrer et al. [31] outlined that correlation would not occur between soil P and AMF colonization when the amount of P that suppressed AMF colonization up to the level that P no longer limited plants. Hence, the relationships between soil P and AMF colonization appeared to be relatively complex.

DSE have been reported present in the roots of some plant species colonized by AMF [19–22]. In the present study, it was remarkable that all root samples were commonly co-colonized by DSE and AMF. It was found that DSE colonization has less variation than that of AMF. Barrow and Aaltonen [41] suggested that DSE were better adapted to plants than aseptate fungi under certain conditions. Correlation analyses indicated that there were no significant correlations between DSE colonization and environmental factors except for relative humidity and sunlight hours. At present, whether DSE are mycorrhizal fungi has not been documented yet [42]. The role of DSE in roots remains unclear; they may function as pathogens or saprophytes, as well as mutualistic association similar to mycorrhizas [19,42]. However, there is growing evidence that DSE may play roles similar to those of AMF in enhancing host growth and nutrition uptake [41,43–45]. The relationship that DSE colonization was positively correlated with AMF hyphal colonization and the relatively constant and popular colonization of DSE in different plants of the grassland in our study may suggest that DSE do have some ecological functions in this kind of ecosystem, which need to be further elucidated.

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