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ORIGINAL PAPER

Seasonal and temporal dynamics of arbuscular mycorrhizal and dark septate endophytic fungi in a tallgrass prairie ecosystem are minimally affected by nitrogen enrichment

Keerthi Mandyam · Ari Jumpponen

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Abstract Root colonization by arbuscular mycorrhizae (AM) and dark septate endophytic (DSE) fungi in nitrogen amended and unamended mixed tallgrass prairie communities were analyzed monthly over two growing seasons. Roots were stained with Trypan blue and Sudan IV and fungal structures quantified using the modified magnified intersections method. Root length colonized (RLC) by DSE exceeded AM colonization during early part of the growing season. Fungal colonization varied among the years and was greater in 2003 than in 2002. Seasonal variation among the months within a growing season was observed in 2002 but not in 2003 for both AM and DSE. AM fungi were most abundant during the peak growing season of dominant C_4 vegetation while DSE were most abundant during the early part of the growing season. Hyperparasitism of AM hyphal coils by melanized septate fungi was frequently observed and increased with AM coil frequency. Although nitrogen amendment had altered the plant community composition, it had no impact on the colonization by AM or DSE fungi.

Keywords Arbsucular mycorrhiza (AM) · Dark septate endophytes (DSE) · Nitrogen amendment · Hyperparasitism

Introduction

Arbuscular mycorrhizal (AM) fungi are important mutualists in the grasslands (Hartnett et al. 1993; Hartnett et al. 1994; Hartnett and Wilson 1999). Mycorrhizal status of grassland plants varies from obligately mycotrophic C_4 plants to the facultative C_3 grasses and many nonmycorrhizal plants (Hetrick et al. 1988). A strong relationship exists between phenology of prairie grasses and their mycorrhizal responsiveness, while it is less apparent in forbs that are C_3 plants and range from obligate to facultative mycotrophs (Hetrick et al. 1992).

Another group of root colonizing fungi, the dark septate endophytes (DSE), has been reported from different habitats worldwide. They coexist with the AM fungi and have been isolated from many grasses and forbs (Jumpponen and Trappe 1998). Although little studied, DSE may be as abundant as AM fungi. In a recent study of the sandy grasslands of Hungarian plains, Kovács and Szigetvári (2002) showed that DSE colonised as many plant species as AM fungi. Plant roots in semi-arid grassland were most abundantly colonised by DSE (Barrow and Aaltonen 2001). While the role of AM symbiosis is known to some extent, very little is known about the DSE symbiosis. However, based on the limited reports of DSE associations, it has been suggested that they be considered "mycorrhizal" (Jumpponen 2001). To clearly understand the significance of these endophytes in the functioning of ecosystems, we must understand their relative abundance compared to other root-associated fungi.

Seasonality of AM fungi has been studied in different habitats. While many studies suggest that AM root colonization is seasonally dynamic, some have found no seasonal patterns (Boerner 1986; Brundrett and Kendrick 1988; Ruotsalainen et al. 2002). Temporal variation of AM hyphae, arbuscules, vesicles, spores, extramatrical hyphae, and even of glomalin (Lutgen et al. 2003), an AM protein, have been studied thus far. AM colonization varies within (Hetrick and Bloom 1982; Bentivenga and Hetrick 1992b; Sanders and Fitter 1992; Mullen and Schmidt 1993;

K. Mandyam (✉) · A. Jumpponen
Division of Biology, Kansas State University,
Manhattan, KS 66506, USA
e-mail: kgm9595@ksu.edu

DeMars and Boerner 1995; Kabir et al. 1997; Titus and Lepš 2000; Lugo et al. 2003; Li et al. 2005) and between years (Sanders and Fitter 1992) as well as among plant species (Bentivenga and Hetrick 1992b; Sanders and Fitter 1992; DeMars and Boerner 1995; Titus and Lepš 2000; Ruotsalainen et al. 2002; Lugo et al. 2003; Fuchs and Haselwandter 2004; Li et al. 2005). Regardless of the broad range of seasonal and temporal variation, many studies have reported similar patterns of AM seasonality across habitats. Maximum abundance occurs during summer and colonization declines during winter and early spring (Bentivenga and Hetrick 1992b; DeMars and Boerner 1995; Kabir et al. 1997; Lugo et al. 2003).

While AM seasonality is relatively well studied, seasonal dynamics of DSE fungi are unknown. Ruotsalainen et al. (2002) followed the DSE colonization of four plant species over one growing season in an oroarctic region in Northern Europe. While no overall seasonal variation in DSE colonization was evident, species-specific differences in colonization were seen. Barrow and Aaltonen (2001) followed the abundance of the different morphological structures of DSE in *Atriplex canescens* (Prush) Nutt. during 1 year in an arid grassland. The study suggested that the abundance of various fungal structures was related to precipitation events and plant phenology. Fuchs and Haselwandter (2004) quantified AM and DSE colonization of seven endangered plant species in two wetland sites (fen meadow vs. peat bog). In that study, DSE colonization was comparable to AM colonization in the fen meadow site whereas in the peat bog the AM colonization was considerably greater. Li et al. (2005) compared AM and DSE abundance monthly for a year in five grassland species. In this study, they found that AM were more abundant than DSE and showed seasonal variation in both AM and DSE colonization in some species.

Most seasonality studies have focused on representative plant species rather than the whole plant community. Sanders and Fitter (1992) suggest that it is essential to follow the seasonal dynamics of AM fungi in whole plant communities and emphasize that true seasonal patterns can be observed when colonization levels are followed for more than a year. Understanding the fungal colonization at the community level provides valuable information especially in a Long-Term Ecological Research (LTER) site where system-level perspective is essential to understanding grassland dynamics.

Environmental change impacts many ecosystem processes including mycorrhizal symbiosis. An important global change phenomenon is the accelerating anthropogenic N enrichment. Anthropogenic N additions exceed natural N_2 fixation (Vitousek 1994). This N enrichment can affect both the community composition and extent of AM colonization. N enrichment can increase, decrease, or have no effects on AM colonization (Aerts 2002). An increase in the AM colonization has been demonstrated in the tallgrass prairie ecosystem (Eom et al. 1999; Johnson et al. 2003). N addition can also impact the AM community composition with or without affecting the root colonization (Johnson 1993; Hartnett and Wilson 1999; Egerton-Warburton and Allen 2000; Treseder and Allen 2002; Jumpponen et al. 2005). While considerable research has focused on the effects of N on AM fungi, no information is available regarding the effect of N on DSE fungi.

In this study, we followed the root colonization by both AM and DSE fungi in a mixed native tallgrass prairie plant community exposed to N-enrichment. The temporal variation in AM and DSE colonization was assessed monthly for two growing seasons. The primary questions addressed in this study were: (1) How do the AM and DSE colonization levels compare over the growing seasons in a tallgrass prairie community? (2) Do the structures associated with AM and DSE change within and between years and if so, are these changes related to temperature or precipitation?, and (3) Does N amendment influence root colonization by AM and DSE?

Materials and methods

Site description

The Konza Prairie Biological Station (KPBS, 39°05′ N, 96° 35′ W), a LTER site, represents a native tallgrass prairie in the Flint Hills of eastern Kansas, USA. KPBS spans 3,487 ha and remains undisturbed by agriculture. The vegetation is dominated by big blue stem (*Andropogon gerardii* Vitman), Indian grass (*Sorghastrum nutans* (L.) Nash.), little bluestem (*Schizachyrium scoparium* (Michx.) Nash.), and switch grass (*Panicum virgatum* L) (see Towne 2002 for a complete list of vascular plants at KPBS). The soil parent material is chert-bearing limestone with the soil bulk density of 1.0 g/cm^3. Top soils in this area are typically slightly acidic (pH=5.6) becoming more alkaline in depth (>40 cm, pH up to 8.3). Soil organic matter is relatively low (<2% to slightly over 5% in top 20 cm). Soil texture is silt loam with sand (22%) and loam (24%) (Hayden 1998). January mean temperature is −3°C (range −9°C to 3°C) and the July mean is 27°C (range 20°C to 33°C). Annual precipitation averages 835 mm, 75% of which falls in the growing season. Site selected for this research is managed by annual burning. The air temperature and precipitation data for the 2 years were obtained from the on-line database provided by Konza LTER (Fig. 1).

Sampling

Twelve permanent lowland plots (4 m^2) at KPBS were selected for sampling. Six of these plots have received 10 g m^{-2}

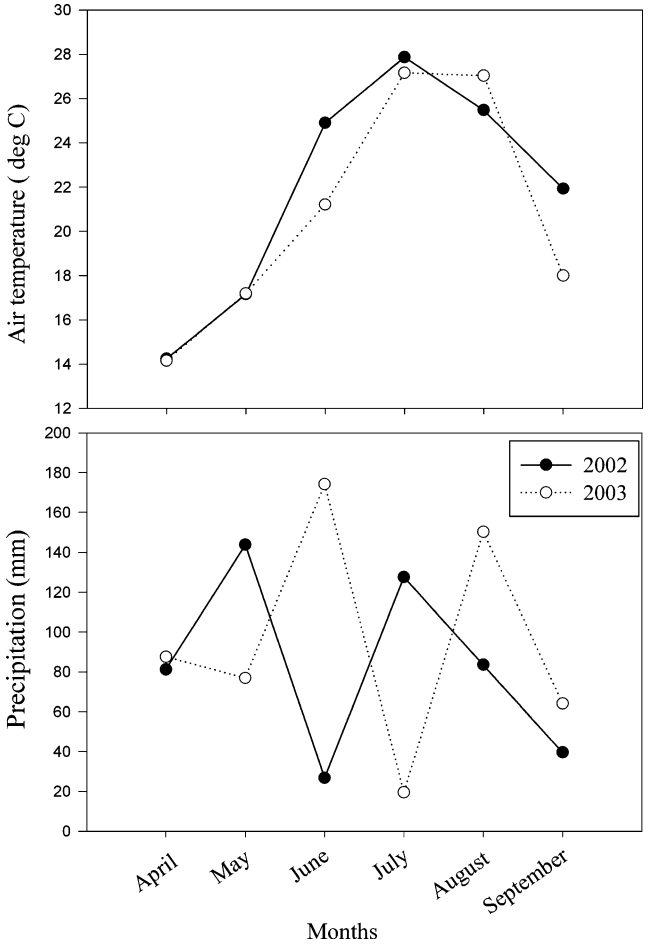

Fig. 1 Monthly precipitation and mean temperature at KPBS for years 2002 and 2003. Mean monthly temperatures of June and September were significantly different in 2002 and 2003 ($P<0.01$)

of NH_4NO_3 mid-summer annually since 1999. Sampling was performed monthly, from April to November in 2002 and from March to September in 2003. Two random soil cores (2 cm in diameter, 20 cm in depth) from each plot were pooled and manually homogenized to reduce with-in plot variation. Roots were immediately washed free of soil and stored in 90% alcohol. They were transferred to the laboratory and stored at 4°C until further processing. The samples were usually processed within 2 days.

Staining and microscopy

Randomly selected roots were cut into 1-cm fragments and cleared by autoclaving (121°C) in 2.5% potassium hydroxide for 5 min followed by several washes with water and neutralization with acetic acid. To observe AM and DSE colonization, half of the cleared roots were soaked in Trypan blue (Phillips and Hayman 1970) and half immersed in Sudan IV (Barrow and Aaltonen 2001) and autoclaved for 4 min followed by washing in several changes of water. The stained roots were allowed to destain in acidic glycerol (50%) overnight.

Stained root fragments were observed by magnified intersections method (McGonigle et al. 1990) at 200× magnification for total percent root length colonized (% RLC) by AM and DSE fungi. In each case, ten randomly selected roots (1 cm) were used for quantification. Percent colonization of AM (overall colonization, hyphae, vesicles, arbuscules, and AM coils) and DSE (overall colonization, melanized septate hyphae, microsclerotia, hyaline septate hyphae, and hyaline vesicles) were recorded. Overall colonization of the endophyte refers to the presence of the endophyte irrespective of the structure.

Statistical analyses

Statistical analysis was performed using SAS (Version 9). The DSE data were not normally distributed and variances were not homogeneous, whereas the AM data were normally distributed and the variances were homogeneous. To correct for these violations of the assumptions for analyses of variance (ANOVA), all data were arcsine square root transformed. Since we were mainly interested in the annual and seasonal changes in the abundance of the AM and DSE endophytes, we used multivariate analysis of variance (MANOVA). Although the roots were sampled randomly from the same plots each month, we opted against repeated measures model to allow elucidation of the seasonal and annual effects. To compare the overall colonization between the two different types of root associated fungi (AM vs. DSE) and to test whether the colonization rates differed, a nested model was used: block, nitrogen, type, year, month (year), nitrogen × type, nitrogen × year, nitrogen × month (year), type × year, type × month (year), nitrogen × type × year, and nitrogen × type × month (year). MANOVAs of AM and DSE colonization were carried out separately for each type of colonization to evaluate the abundance of their structures. In these analyses, the model effects included block, nitrogen, year, month (year), nitrogen × year, and nitrogen × month (year). Pillai's trace was used to calculate the multivariate F ratio as it is statistically powerful and immune to violations of assumptions for ANOVA. Univariate analyses of the individual fungal structures were performed to further elucidate responses when multivariate F ratios were significant. Pair-wise differences, when necessary, were determined by a conservative Bonferroni test. To correct for multiple comparisons using the same data, we chose a conservative level of significance ($\alpha=0.01$). The correlations among the fungal structures were determined by partial correlation coefficient from MANOVA. Spearman's rank correlation was used to study the association between abiotic factors (air temperature and precipitation) and AM

or DSE colonization (structures and overall), association between AM and DSE colonization as well as association between AM coils and proportion of infected coils.

Results

Comparison of overall colonization by AM and DSE

The ANOVA results for the comparison of overall abundance of AM and DSE are presented in Table 1. There were no significant three-way interactions. "N" effect was not significant indicating that N amendment had no effect on fungal abundance. The overall DSE colonization was greater than AM colonization as indicated by a significant "type" effect. Overall colonization was greater in 2003 than in 2002 (significant "year" term). The significant "month (year)" term indicates that the overall fungal colonization varied among the months within the years. Seasonal trends between DSE and AM were dissimilar as indicated by a significant interaction "type × month(year)" (Fig. 2). This effect was seen due to the high early DSE colonization that remained invariable while AM colonization was low initially and peaked in mid-late summer. There was no significant correlation between AM and DSE colonization in either of the 2 years (Spearman rank correlation test: year 2002, $r=-0.6$, $P=0.2080$; year 2003, $r=-0.2571$, $P=0.6228$).

Seasonality in AM colonization

AM colonization varied both annually and seasonally as indicated by MANOVA (Table 2). AM hyphae, and vesicles were more abundant in 2003 than 2002, while abundance of arbuscules did not differ between years (Table 3). In 2002, the seasonal variation in AM hyphae, vesicles, arbuscules, and coils were observed (Table 3, significant "month (year)" term; Figs. 3a–d). In contrast, in 2003, only arbuscules varied seasonally (Fig. 3c). In 2002, the colonization by AM hyphae was similar to the overall AM colonization pattern with the highest colonization in mid-summer and lowest in early spring. The %RLC by vesicles varied significantly and was highest from early spring to early summer and declined thereafter. Seasonal variation in %RLC by arbuscules was observed in both years: arbuscules were most abundant during late summer. Abundance of AM coils was not different among the 2 years, but varied within the growing seasons. The abundance of coils was low in early spring followed by maximum colonization in late spring and decline in late summer. AM vesicles and arbuscules were positively correlated with air temperature during 2003 (Spearman rank correlation test: $r=0.94286$, $P=0.0048$ for vesicles; $r=0.88571$, $P=0.01$ for arbuscules). No significant correlations between average temperature or precipitation and AM colonization (neither AM structures nor overall) were evident in 2002.

An interesting observation in this study was the occurrence of parasitized AM coils (Fig. 4). Aseptate AM coils were colonized by septate, melanized hyphae and their occurrence and seasonal variation were followed. The proportion of infected coils increased with overall abundance of coils (Spearman rank correlation: $r=0.1897$, $P=0.038$).

Table 1 ANOVA results for the overall AM and DSE colonization

Model effect	*F*	*P*
Block	1.0	0.3182
N	3.85	0.051
Type	245.19	<0.0001
Year	41.77	<0.0001
Month (Year)	4.3	<0.0001
N × Type	0.48	0.4902
N × Year	1.15	0.284
N × Month (Year)	0.81	0.6344
Type × Year	1.22	0.2708
Type × Month (Year)	6.52	<0.0001
N × Type × Year	0.68	0.4105
N × Type × Month (Year)	0.81	0.6029

Significance was set at a conservative level ($P \leq 0.01$)

Seasonality in DSE colonization

DSE abundance varied seasonally and annually as indicated by MANOVA (Table 4). Univariate analyses were performed for significant effects in multivariate analysis (Table 5). All DSE structures except hyaline hyphae and vesicles were more abundant in 2003 than 2002. However, seasonal variation was seen only in 2002. In 2002, DSE hyphae showed low colonization in the spring followed by an increase in the colonization during the summer (Fig. 5a). Microsclerotia and hyaline vesicles were most abundant in early spring but their occurrence decreased during the summer (Figs. 5b–d). Hyaline hyphae showed greatest colonization in early spring and declined steeply thereafter. Correlation analysis indicated no significant effect of temperature or precipitation on DSE colonization (neither structures nor overall) in 2002 or 2003.

Effects of N enrichment

N amendment altered the plant community composition. Unamended plots were dominated by *A. gerardii*, *S. nutans* and *S. scoparius*, whereas in N-amended plots *A. gerardii* decreased and *P. virgatum* increased in abundance (Loretta Johnson, personal communication).

N amendment did not exert significant effect on overall abundance of AM and DSE (Table 1). The separate analysis

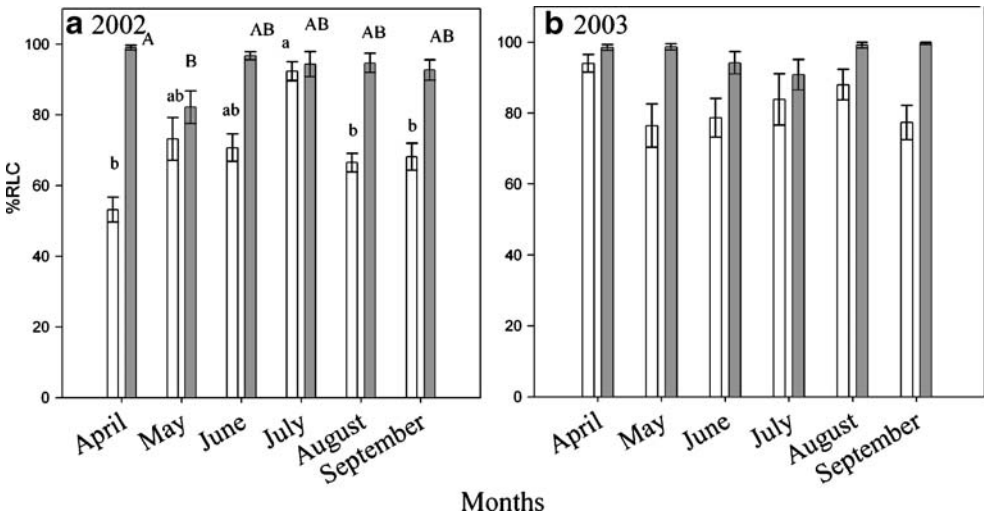

Fig. 2 Seasonal variation of AM and DSE colonization: **a** year 2002, and **b** year 2003. *Letters in lower-case* indicate statistically significant mean monthly variation in AM colonization (Bonferroni test $P<0.01$) and *letters in upper-case* signify statistically significant difference in mean monthly variation in DSE colonization in year 2002. No significant monthly variation was observed in 2003 for AM or DSE fungi. Colonization data combined from N-treated and untreated plots. *Open bars* AM; *gray bars* DSE. Standard error is indicated

of AM colonization showed a significant N effect ($P=0.0005$). Univariate analyses on the structures showed that AM coils responded to N amendment. To correct for the effects of pseudoreplication in the analyses of fungal colonization responses to N amendment, we redid the MANOVA with N as a nested variable, N (month). These analyses indicated that N did not have a true effect on AM colonization (Pillai's trace=0.1298, $F=0.79$, $df=20, 472$ and $P=0.724$), but the AM responses to N were driven by larger than true replication. Multivariate analysis on DSE abundance showed that N was not a significant driver.

Discussion

Multivariate analyses rather than repeated measures were used as our main goal was to improve understanding of annual and seasonal changes in AM and DSE abundance. Agreeably, this approach may have resulted in pseudoreplication as suggested by our results on AM responses to N-amendment. However, to avoid Type I error, we confirmed that N effects on AM colonization likely were driven by exaggerated replication.

Effects of N fertilization on fungal colonization

Nitrogen is the most limiting nutrient in a tallgrass prairie and its availability can vary with fire, grazing, topography, soil texture, and depth and precipitation (Blair et al. 1998). Typical N additions occur through atmospheric deposition, N fixation by free-living microbes and through symbiotic association. This accrued N is lost mainly through three processes: hydrologic fluxes (insignificant), denitrification (significantly greater in unburned than in burned sites), and volatilization by fire (greater in annually burned than infrequently burned sites; Blair et al. 1998 and references therein). Leaching losses of N are generally small and the N fertilization (10 g m^{-2} $year^{-1}$ of NH_4NO_3) applied in our field experiment is nearly double that is lost via the above processes. It should be noted that all these estimates about N cycling come from non-amended, ungrazed prairie that were subjected to different fire frequencies (Blair et al.

Table 2 MANOVA results for response variables from AM colonization: hyphae, vesicles, arbuscules, and coils

Model effect	Pillai's trace	*F*	*df* (numerator, denominator)	*P*
Block	0.0606	1.85	4, 115	0.1235
N	0.1587	5.42	4, 115	0.0005[a]
Year	0.4304	21.73	4, 115	<0.0001
Month (Year)	1. 449	6.7	40, 472	<0.0001
N × Year	0.0202	0.59	4, 115	0.6683
N × Month (Year)	0.2093	0.65	40, 472	0.9520

Pillai's trace was used as the multivariate criterion
[a] This significance disappeared when pseudoreplication was accounted for by using N (month) in the analysis. Pillai's trace=0.1298, $F=0.79$, $df=20, 472$ and $P=0.724$

Table 3 *F*-values for univariate ANOVAs for the response variables for AM colonization

Model effect	Hyphae	Vesicles	Arbuscules	Coils
Block	2.0 ns	0.27 ns	1.31 ns	4.76*
N	0.0 ns	2.8 ns	2.54 ns	7.61**
Year	69.6***	52.89***	0.39 ns	2.82 ns
Month (Year)	8.11***	4.49***	6.48***	10.03***
N × Year	0.02 ns	0.19 ns	0.02 ns	2.4 ns
N × Month (Year)	0.38 ns	0.69 ns	0.79 ns	0.6 ns

ns nonsignificant $P>0.05$
*$P<0.05$
**$P<0.01$
***$P<0.0001$

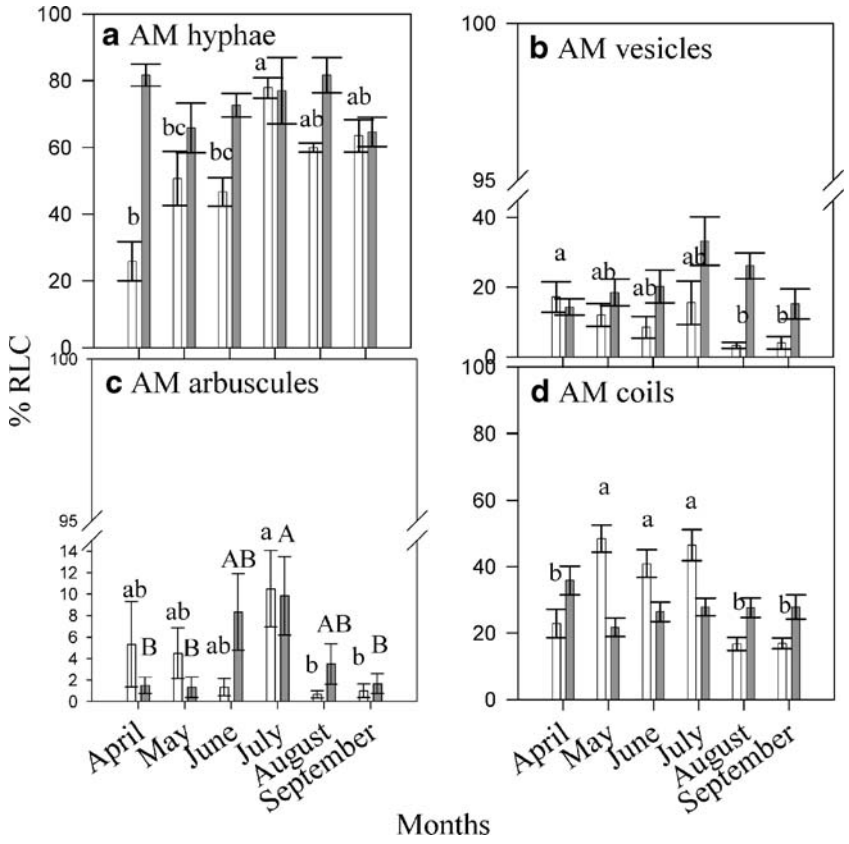

Fig. 3 Seasonal variation of the different AM structures for years 2002 and 2003: **a** hyphae, **b** vesicles, **c** arbuscules, and **d** coils. *Letters in lower-case* signify statistically significant mean monthly variation in 2002 (Bonferroni test $P<0.01$) and *letters in upper-case* signify statistically significant difference in mean monthly variation in 2003. In 2003, seasonal variation was seen only in %RLC by arbuscules. Colonization data combined from N-treated and untreated plots. *Open bars* year 2002; *gray bars* year 2003. Standard error is indicated

1998). Plants respond positively to N additions by accumulating N in roots and rhizomes resulting in increased productivity in annually burned tallgrass prairie (Knapp et al. 1998).

In this study, although N addition was relatively large, it did not affect overall colonization. Similarly, N amendment had no effects on the DSE and AM colonization when analyzed separately suggesting that N is not an important driver of fungal host colonization at Konza prairie. Although N was significant for AM colonization, this effect became non-significant when the possible pseudoreplication was accounted for.

Our results on AM responses to N enrichment are in contrast with previous studies at KPBS. Johnson et al. (2003) reported an increase in overall internal colonization, intraradical hyphae, and spores in N enriched plots while vesicles decreased, and arbuscules and coils did not vary. Similarly, Eom et al. (1999) reported an increase in AM hyphae and extramatrical hyphae after N addition, whereas Bentivenga and Hetrick (1992a) reported a decrease in total

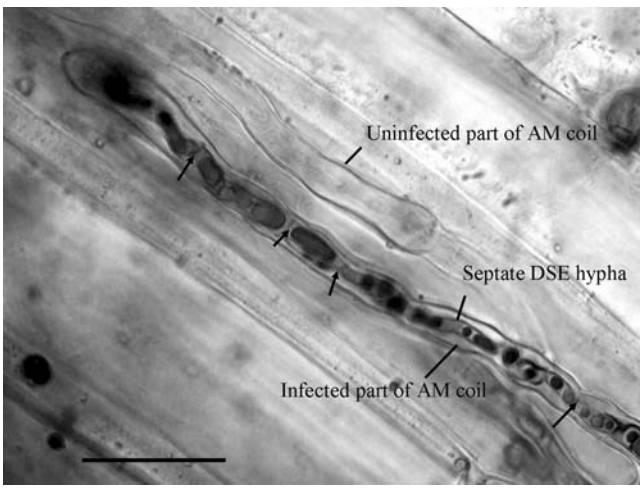

Fig. 4 Hyperparasitism of AM coil by a septate fungus. *Arrows* point to septa of the parasitizing fungal hyphae (1,000×). Bar=10 μm

Table 4 MANOVA results for response variables from DSE colonization: hyphae, microsclerotia, hyaline hyphae, and hyaline vesicles

Model effect	Pillai's trace	*F*	*df* (numerator, denominator)	*P*
Block	0.0249	0.74	4, 116	0.5654
N	0.0281	0.84	4, 116	0.5037
Year	0.5928	42.22	4, 116	<0.0001
Month (Year)	1.1939	5.06	40, 476	<0.0001
N × Year	0.02	0.59	4, 116	0.6684
N × Month (Year)	0.293	0.94	40, 476	0.5752

Pillai's trace was used as the multivariate criterion

Table 5 *F*-values for univariate ANOVAs for the response variables for DSE colonization

Model effect	Overall	Hyphae	Microsclerotia	Hyaline hyphae	Hyaline vesicles
Block	0.48 ns	0.11 ns	1.11 ns	1.20 ns	0.39 ns
N	0.97 ns	1.24 ns	0.43 ns	0.62 ns	0.59 ns
Year	16.36***	123.86***	3.88 ns	29.3***	1.93 ns
Month (Year)	3.11***	6.5***	6.16***	16.01***	3.24**
N × Year	0.03 ns	0.0 ns	1.3 ns	0.14 ns	0.44 ns
N × Month (Year)	1.61 ns	1.19 ns	1.33 ns	0.43 ns	0.6 ns

ns nonsignificant $P>0.05$
*$P<0.05$
**$P<0.01$
***$P<0.0001$

root colonization. These observed differences in responses to N amendment may be due to the variable sampling periods in the different studies. Johnson et al. (2003) sampled for 3 years. In the first year, they sampled three times corresponding to three seasons (early, mid, and late growing season) and, in the next 2 years, sampling was conducted in the mid and late growing season. While they observed an overall increase in intraradical AM colonization in response to N fertilization, this response was not consistent across seasons and years. Bentivenga and Hetrick (1992a) sampled every 2 months (Sep 1989 to Sep 1991) for 3 years, including fall and winter and found that N fertilization significantly reduced AM colonization. Our results support the large variability in AM responses to N additions.

Overall colonization

We show in this study that DSE colonization may equal or exceed AM colonization. So far, three studies (Ruotsalainen et al. 2002; Fuchs and Haselwandter 2004; Li et al. 2005) have compared DSE and AM colonization. While the first two studies did not detect any seasonal patterns, Li et al. (2005) found seasonality in DSE colonization. In addition to demonstrating the great DSE abundance at KPBS, we present data showing that both AM and DSE vary both annually and seasonally. Unlike Li et al. (2005) who found positive correlation between DSE and AM, we observed no (positive or negative) correlation. Similarly, neither temperature nor precipitation was correlated with overall AM or DSE.

AM colonization

AM hyphae, vesicles, arbuscules, and coils varied across the growing season in 2002, while no such variation occurred in 2003 except for arbuscules. Colonization by AM hyphae was low in early spring and peaked in July. Maximum colonization by arbuscules in both years oc-

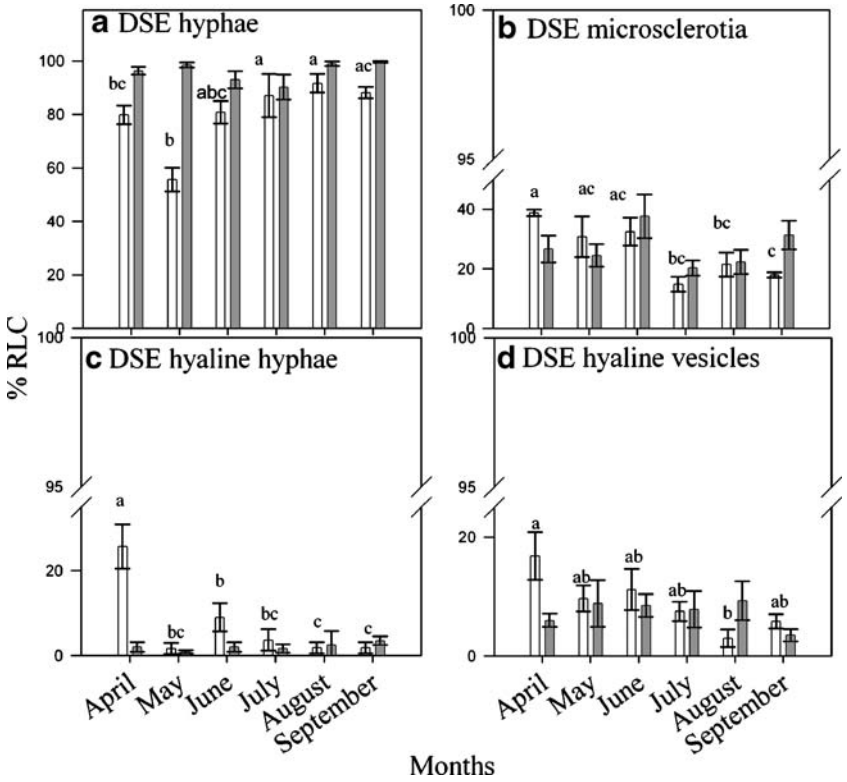

Fig. 5 Seasonal variation of the different DSE structures for years 2002 and 2003; **a** melanized hyphae, **b** microsclerotia, **c** hyaline hyphae, and **d** hyaline vesicles. *Letters in lower-case* signify statistically significant mean monthly variation in 2002 (Bonferroni test $P<0.01$). DSE colonization did not vary in 2003. Colonization data combined from N-treated and untreated plots. *Open bars* year 2002; *gray bars* year 2003. Standard error is indicated

curred in mid-summer. AM vesicles had a stable and relatively high colonization in early spring and lowest colonization in late summer. The *Paris*-type coil abundance peaked in late spring and mid-summer. The seasonal patterns observed at Konza prairie in 2002 are similar to those reported by others. Bentivenga and Hetrick (1992b) followed the colonization of C_3 and C_4 grasses separately at KPBS; our observations largely agree with theirs. Lugo et al. (2003) found maximum abundance of arbuscules during summer in a mountain grassland. The C_3 and C_4 hosts exhibited abundant arbuscules, vesicles and coils in summer. Lugo et al. (2003) observed maximum vesicle abundance in summer, whereas in our study, vesicles were frequent in early and mid-summer but less abundant in late summer. Similar to our results, high colonization during the peak growing season and low colonization during the early or late growing season have been observed in a variety of habitats (Reinhardt and Miller 1990; Mullen and Schmidt 1993; DeMars and Boerner 1995; Kabir et al. 1997; Escudero and Mendoza 2005).

Soil moisture and P availability (Mullen and Schmidt 1993; Titus and Lepš 2000; Ruotsalainen et al. 2002), soil moisture and temperature (DeMars and Boerner 1995; Lugo et al. 2003), plant physiology and metabolism (Bentivenga and Hetrick, 1992b; Kabir et al. 1997; Lugo et al. 2003), growth rate, and turnover of plant roots (Reinhardt and Miller 1990; Titus and Lepš 2000; Lugo et al. 2003) are among the most frequently proposed drivers of AM seasonality. Our research site is typical to annually burned sites and dominated by C_4 grasses and forbs while C_3 hosts constitute a minor component. The warm season grasses have a peak metabolic activity during the warm summer months and senesce in September, while cool-season grasses have peak activity in late spring and again in the fall. In 2002, peak abundance of AM hyphae, arbuscules, and coils in the summer months most likely coincided with the high metabolic rate of C_4 grasses. Since arbuscules are exchange interfaces, their abundance indicates a period of active exchange between AM fungi and their hosts. The significant difference in the AM abundance between 2 years is largely due to the increased colonization in early spring and late summer in 2003. Mean monthly air temperature was higher in 2002 than 2003 (Fig. 1). The lack of correlation with precipitation and the positive correlation between temperature and AM vesicles and arbuscules along with the observed patterns of AM abundance over the 2 years suggest that climatic conditions directly via temperature or indirectly via host physiology may drive AM colonization.

Two morphological types of AM colonization (*Arum*- and *Paris*-type) were observed in this study. Colonization by hyphal coils has been thought to be controlled by host (Smith and Smith 1997). However, it is becoming clear that both host and fungus are responsible for AM morphology (Cavagnaro et al. 2001; Dickson 2004). A review on *Arum*- and *Paris*-type suggests that *Paris*-type is more frequent than *Arum*-type (Smith and Smith 1997). In this study, hyphal coils, characteristic of *Paris*-type, colonized almost 50% of the total root length (Fig. 3d). It is highly likely that the dominant grasses and limited number of forbs present in the plots are mainly colonized by *Paris*-type.

In this study, we frequently observed hyphal coils parasitized by a septate melanized fungus (Fig. 4). Numerous reports of parasitized AM spores from field and greenhouse studies suggest that hyperparasitism may be a common phenomenon (Gerdemann and Nicholson 1963; Mosse and Bowen 1968; Schenck and Nicholson 1977; Hijri et al. 2002). Daniels and Menge (1980) isolated different fungal parasites (*Humicola fuscoatra* Traaen, *Anguillospora pseudolongissima* Ranzoni and a chytrid, *Phlyctochytrium* sp.) from two different *Glomus* spp. In reciprocal inoculation studies, all tested *Glomus* spp. were susceptible suggesting no specificity among the parasites. Rousseau et al. (1996) studied the steps involved in the infection process of *Glomus intraradices* Schenck & Smith by a common AM parasite *Trichoderma harzianum* Rifai. *Trichoderma* hyphae proliferated abundantly in spores and the emerging hyphae. While the occurrence of parasitized AM spores is fairly well known, we show that *Paris*-type hyphal coils can also be parasitized. Furthermore, the parasitism appears to become more common as the occurrence of coils increases (data not shown). This likely suggests that the parasites thrive when their target organisms are most abundant.

DSE seasonality

DSE colonization was slightly greater in 2003 than in 2002 and showed monthly variation in 2002. Although DSE fungi have been isolated from various C_3 and C_4 grasses and forbs, the nature of this symbiosis is currently unknown. Routinely observed DSE structures are melanized hyphae, microsclerotia, hyaline hyphae, and hyaline vesicles. It is essential to summarize the morphology and function of these structures to better understand their seasonal variation. Melanized hyphae of DSE are septate and are easily recognized without staining. Microsclerotia refer to melanized hyphae packed in the cortical cells (Jumpponen and Trappe 1998 and references therein). According to Yu et al. (2001), the microsclerotia contain storage compounds similar to true sclerotia including glycogen, proteins and polyphosphate, and numerous nuclei suggesting that they may function as vegetative propagules.

Hyaline hyphae are septate and vacuolated. These hyphae stain poorly with Trypan blue and may lack chitin (Barrow and Aaltonen 2001). They can be visualized by

Sudan IV which stains the lipids in the vacuoles. Barrow and Aaltonen (2001) found that hyaline hyphae were present intra- and intercellularly in the cortex and sieve elements of *Atriplex canescens*. While possibly representing a pre-melanization stage of the colonizing DSE hyphae (Yu et al. 2001), the hyaline hyphae may function as an active nutrient-exchange phase between the hosts and DSE fungi (Barrow and Aaltonen 2001). It is essential to note that hyaline hyphae may be seriously underestimated because they are difficult to visualize (Barrow and Aaltonen 2001; Yu et al. 2001).

Hyaline vesicles are usually attached to hyaline hyphae and contain vacuoles with lipids (Barrow and Aaltonen 2001). We believe that hyaline vesicles are the initial stages of microsclerotia. Our microscopic observations included hyaline vesicles with slightly melanized walls (image not included). However, Yu et al. (2001) in their study of *Phialocephala fortinii* Wang & Wilcox colonization of *Asparagus officinalis* L. did not report any formation of hyaline vesicles before microsclerotial development. It is possible that microsclerotia development varies among DSE fungi and some may have prolonged periods of a hyaline state before developing melanin and/or forming microsclerotia.

Our observations on the DSE seasonality in 2002 agree to some extent with Barrow and Aaltonen (2001) and Ruotsalainen et al. (2002). DSE colonization in 2002 was highest during the early part of the growing season and decreased thereafter. The significance of this seasonality remains unclear. Mullen et al. (1998) suggested that N uptake most likely occurs early in the season before new roots and active AM structures are formed. In an alpine tundra ecosystem, they found that *Ranunculus adoneus* Gray was heavily colonized by DSE and proposed that DSE may play a role in N uptake from the early season snow melt. If N is taken up early in the season unlike P whose uptake occurs later in the growing season when AM structures are abundant and if we assume that hyaline hyphae are indeed the active phases involved in nutrient uptake, the maximum abundance of hyaline hyphae in spring would suggest a DSE involvement in N uptake. However, we did not see such a trend in 2003. Although the early season uptake of N by DSE may be a possibility, this function needs to be confirmed experimentally. Since DSE colonization did not respond to air temperature or precipitation, we are unable to pinpoint the reason for the observed seasonality in 1 year and not the other.

This study suggests that DSE fungi are equally or more abundant than AM fungi in a tallgrass prairie ecosystem. This abundance of DSE may be ecologically significant, especially since AM fungi have traditionally been considered to be “keystone mutualists” (O’Neill et al. 1991). If DSE indeed provide some benefit to their host plants, it becomes exceedingly important to know if or how they complement the functions of AM. More research towards understanding the relevance of this symbiosis in ecosystems is necessary.

Conclusions

In this study, we were able to show that DSE colonization was as high as AM colonization, and in some months, even exceeded AM colonization. Our results underline the need for an improved understanding of these root colonizing endophytes. This study also indicates that DSE colonization may change in the course of the growing season and two consecutive years may not show identical seasonal patterns. Accordingly, to obtain a better appreciation for inter- and intra-annual variation in root colonization, cross-site comparisons that span over multiple growing seasons may be necessary. Most AM structures were formed late in the growing season, possibly coinciding with P uptake. It is emphasized that analysis of seasonal dynamics on a plant community level provides valuable information, especially in a LTER site where system-level perspective is essential to understanding grassland dynamics. In addition to community level analysis of variation in fungal root colonization, we have also initiated efforts in understanding the fungal colonization patterns in various groups of plants including cool- and warm-season grasses and forbs. While some roles of AM symbiosis in ecosystem functioning have been identified, no such data are available for DSE. The high DSE abundance underscores the need to elucidate the function of these fungi. Extensive studies are required to assess their roles in ecosystem functioning owing to their high abundance.

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