Mycorrhiza
https://doi.org/10.1007/s00572-018-0863-7

ORIGINAL ARTICLE

Rapid temporal changes in root colonization by arbuscular mycorrhizal fungi and fine root endophytes, not dark septate endophytes, track plant activity and environment in an alpine ecosystem

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Received: 22 May 2018 / Accepted: 17 August 2018

Abstract
Fungal root endophytes play an important role in plant nutrition, helping plants acquire nutrients in exchange for photosynthates. We sought to characterize the progression of root colonization by arbuscular mycorrhizal fungi (AMF), dark septate endophytes (DSE), and fine root endophytes (FRE) over an alpine growing season, and to understand the role of the host plant and environment in driving colonization levels. We sampled four forbs on a regular schedule from June 26th–September 11th from a moist meadow (3535 m a.s.l) on Niwot Ridge, Rocky Mountain Front Range, CO, USA. We quantified the degree of root colonization by storage structures, exchange structures, and hyphae of all three groups of fungi. AMF and FRE percent colonization fluctuated significantly over time, while DSE did not. All AMF structures changed over time, and the degree of change in vesicles differed by plant species. FRE hyphae, AMF arbuscules and AMF vesicles peaked late in the season as plants produced seeds. AMF hyphae levels started high, decreased, and then increased within 20 days, highlighting the dynamic nature of plant-fungal interactions. Overall, our results show that AMF and FRE, not DSE, root colonization rapidly changes over the course of a growing season and these changes are driven by plant phenology and seasonal changes in the environment.

Keywords Arbuscular mycorrhizal fungi · Dark septate endophytes · Fine root endophytes · Root colonization · Temporal dynamics · Phenology

Introduction

Arbuscular mycorrhizal fungi (AMF), dark septate endophytes (DSE), and fine root endophytes (FRE) are three widespread groups of root endophytes that have been shown to improve the fitness of a wide range of plants. AMF are obligate plant symbionts in the phylum Glomeromycota (Schüßler et al. 2001; Tedersoo et al. 2018) that have been described as associating with 80% of land plants (Smith and Read 2008). In exchange for carbon compounds, AMF colonize plant roots and help plants acquire both nitrogen and phosphorus (van der Heijden et al. 2008; Sikes et al. 2009; Bonfante and Genre 2010) and cope with drought stress (Augé 2001; Wu and Xia 2006) as well as protecting plants from pathogens (Pozo et al. 2002; Sikes et al. 2009). DSE are facultative symbionts in the phylum Ascomycota that have been described in over 600 plant species (Jumpponen and Trappe 1998). DSE have also been shown to improve plant growth, likely by way of nitrogen mineralization and uptake and protection from pathogens (Mullen et al. 1998; Mandyam and Jumpponen 2005; Newsham 2011). However, their function is still open to debate (Mandyam and Jumpponen 2015), and they have not improved the growth of as many hosts as AMF nor under as wide a range of conditions as AMF.

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00572-018-0863-7) contains supplementary material, which is available to authorized users.

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Published online: 23 August 2018

Furthermore, other literature has also shown that DSE can have negative effects on plant growth (Alberton et al. 2010; Mayerhofer et al. 2013). FRE, once called *Glomus tenue* and placed in the Glomeromycota with AMF, are now recognized as the genus *Planticonsortium* in the phylum Mucoromycotina (Orchard et al. 2017a, Walker et al. 2018). The effects of FRE on host plants are much less studied than AMF. FRE form arbuscules and have been shown to increase plant P uptake under low P conditions, but also can be parasitic as soil fertility increases (Orchard et al. 2017b). A key question regarding these three fungal groups' interactions with plants is how the presence and abundance of endophytic fungal structures varies over time, and whether fluctuations are driven by the host plant or the environment.

Interactions between AMF and plants are controlled bidirectionally, i.e., both host plant and fungal partner control their contribution of nutrients/carbon compounds based on the reward they receive in return (Kiers et al. 2011). Logically, the two partners would engage in a cooperative exchange only at a time when both benefit from the mutualism, although there are exceptions to tight reciprocity and switches from mutualism to parasitism have also been observed (Johnson et al. 2010). For example, plants can cut off photosynthate supply to the fungus if nutrients are no longer limiting their growth (Högberg et al. 2003). The percent of plant root colonized by fungi is also expected to be contingent to an extent on environmental conditions, as fungi may not be able to grow at extreme temperatures or in extremely dry or waterlogged soils (Apple et al. 2005; Escudero and Mendoza 2005). Given conditions that enable fungal growth, the amount of fungi may increase or decrease based on the nutritional demands of the plant and how much photosynthate the plant is donating to the fungus, as well as the nutrient demands of the fungus and the quantity of mineral nutrients the fungus is donating to the plant (Kiers et al. 2011). Another layer of complexity to these interactions is that the amount of nutrients available in the soils can also change over time, and this could affect how much photosynthate the plant donates to the fungi (Muthukumar and Udaiyan 2002).

The seasonal dynamics of AMF and DSE colonization levels have been examined in a variety of ecosystems, particularly for AMF (Fig. 1, Supplementary Table 1). In an exhaustive literature review (see Supplementary Table 1 methodology details), we found 59 studies that examined temporal change in colonization, with 86% focusing on AMF colonization. The majority of these studies (84 and 75% for AMF and DSE, respectively) found that colonization levels do change over the course of a year or growing season (e.g., Abbott and Robson 1991; Mandyam and Jumpponen 2008). We did not perform a literature review on the seasonality of FRE colonization because of confusion over their taxonomy leading to their inclusion with AMF in many studies.

Important factors driving colonization include plant species (e.g., Lingfei et al. 2005), year (i.e., seasonal variation depends on the year; e.g., Mandyam and Jumpponen 2008), site (e.g., Liu et al. 2009), climate (e.g., Likar et al. 2009), and soil nutrients (e.g., Apple et al. 2005, Supplementary Table 1). Host plant phenology often was related to changes in fungal colonization, with increases in colonization tracking plant growth and seed production (Hayman 1970, Roldan-Fajardo et al. 1982; Abbott and Robson 1991; Kabir et al. 1997), although this may not be the case in legumes (Jakobsen and Nielsen 1983; Liu et al. 2009). Some researchers have argued that soil temperature and soil moisture also play a role in colonization levels, as the optimum temperature and moisture

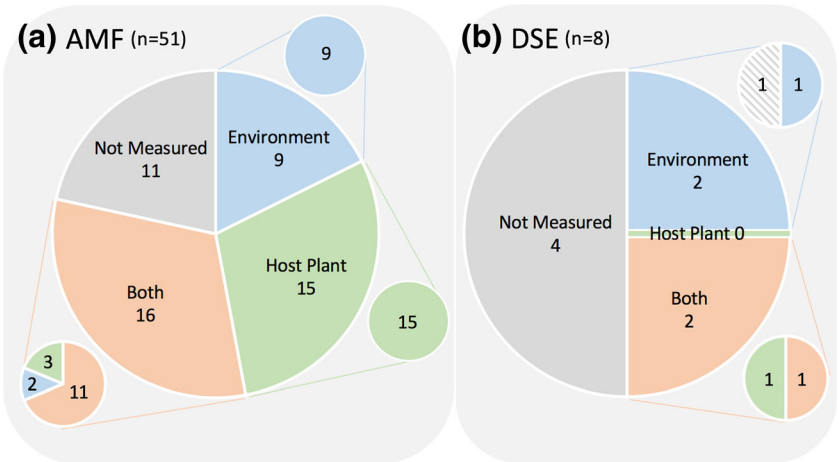

Fig. 1 A summary of 59 studies that have examined the seasonality of AMF and DSE shows that both host plant and environment can play a role in driving the temporal dynamics of root colonization by these two fungal groups. Numbers in the large pies represent the number of studies that measured at least one environmental variable (climate, soil moisture, soil temperature), host plant growth or phenology, both environment and host plant, or neither. Small pies indicate how many of those studies found that the factor affected AMF and DSE colonization levels. A more detailed summary is presented in Supplementary Table 1

conditions for fungal growth varies seasonally (Fig. 1). Unfortunately, many studies did not measure plant phenology and environmental variables together, and very few studies were conducted at multiple sites across multiple years (Supplementary Table 1). One interesting trend across several studies of AMF is that the highest levels of root colonization often occur at the peak or late in the growing season (Mandyam and Jumpponen 2008).

While the temporal dynamics of the plant-fungus symbioses is largely unstudied for alpine ecosystems ($n = 3$), they are ideal systems for studying the symbioses. The growing season only lasts 1–3 months, environmental conditions change rapidly after snowmelt occurs as soils thaw and then dry, and plants must uptake sufficient nutrients quickly to complete their life cycles (Bowman and Seastedt 2001). Fungal symbionts can assist plants in meeting their nutritional demands, but to do so must colonize plant roots and develop the necessary mutualistic structures rapidly enough to track plant phenology and environmental change. Studies of AMF and DSE in the alpine zone have found temporal fluctuations in some but not all plant species (Mullen and Schmidt 1993; Mullen et al. 1998; Ruotsalainen et al. 2002). Mullen and Schmidt (1993) reported that an increased abundance of arbuscules corresponded to increased levels of phosphorus in the host after seed production depleted the plant's phosphorus. Mullen et al. (1998) found that DSE colonization is very high at the onset of the growing season, which may help plants increase nitrogen uptake as nitrogen is flushed from the system as snow melts.

In this study, we sought to quantify the relative importance of abiotic versus biotic drivers in fungal colonization by characterizing the temporal dynamics of plant-fungus mutualisms in an alpine system. Importantly, to disentangle the effect of plant host phenophase and potential environmental drivers of colonization, we chose four forb species that grow in close proximity to each other but whose phenologies differ. We hypothesized that all fungal structures vary across time and species, and the effect of time differs across plant species due to differences in plant phenology. Additionally, based on previous work, we predicted AMF levels to increase throughout the season to aid in phosphorus uptake while DSE levels would start high to help with nitrogen uptake and then decline (Fig. 2, Mullen and Schmidt 1993; Mullen et al. 1998).

Materials and methods

Study system and sampling

Our study took place in an alpine tundra moist meadow plant community in the Saddle at the Niwot Ridge Long Term Ecological Research site, CO, USA (Lat = 40.056177; Lon = −105.589355; 3535 m above sea level). This site is located in the Front Range of the Rocky Mountains. Detailed descriptions of temperature and precipitation trends are described elsewhere (McGuire et al. 2012; Kittel et al. 2015). Moist meadow communities are characterized by high soil moisture and high primary production relative to other plant communities, which favors the growth of the community's two dominant species, the forb *Geum rossii* and the bunchgrass *Deschampsia cespitosa*. Our moist meadow study site is typically covered by snow from October to mid to late June. The short growing season combined with low temperatures limits microbial activity and, subsequently, plant growth via nutrient availability (Bowman and Seastedt 2001). Nutrient addition experiments have demonstrated that both nitrogen and phosphorus co-limit primary production in moist meadow communities and play a role in determining the plant assemblage (Gasarch and Seastedt 2016). The majority of the plant species in alpine tundra moist meadow communities are perennial and many use nutrients and sugars stored from the previous year to begin growing at the beginning of the summer.

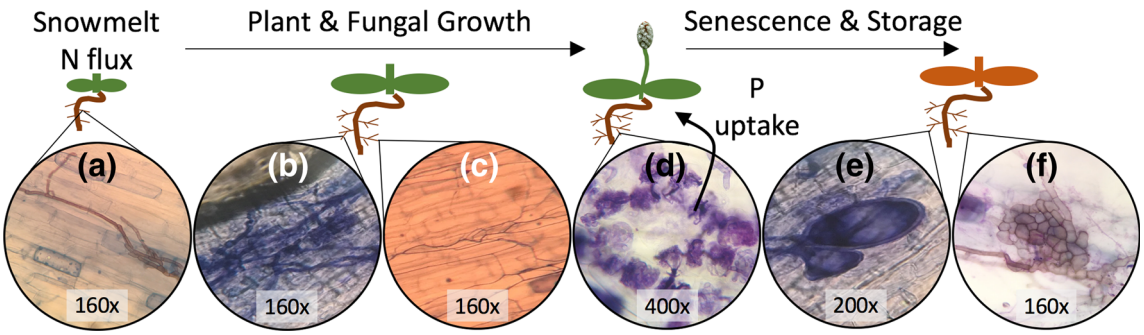

Fig. 2 We predict that AMF, DSE, and FRE fungal structures (A–F) change over time as a function of plant phenology and environmental changes. AMF and FRE hyphae and arbuscules are predicted to increase then decrease, DSE hyphae are predicted start high and then decrease, and storage structures (vesicles and microsclerotia) are predicted to increase late in the growing season. **a** = DSE hyphae (growth structure), **b** = AMF hyphae (growth structure), **c** = FRE hyphae (growth structure), **d** = AMF arbuscule (nutrient exchange structure), **e** = AMF vesicle (storage structure), **f** = DSE microsclerotia (storage structure). The number at the bottom of each image is the magnification at which the photo was taken. Photos by CPB and CMM

We quantified mycorrhizal colonization in the roots of four moist meadow perennial forb species: *Polygonum bistortoides*, *Gentiana algida*, *Artemisia scopulorum*, and *Geum rossii*. We selected these species to encompass different plant phenologies (Fig. 3f). *Geum*, *Artemesia*, and *Polygonum* flower at approximately the same time in the middle of the growing season, while *Gentiana* flowers several weeks later, towards the end of the growing season (Fig. 3f, Smith et al. 2012). Despite flowering at the same time, *Geum* and *Polygonum* differ in the timing of their nutrient uptake, with *Geum* experiencing peak tissue nitrogen concentrations in June, and *Polygonum* peaking in tissue nitrogen in August (Jaeger et al. 1999). The root structure of the four species also differs markedly. *Geum* and *Polygonum* have coarse, subligneous tap roots from which fine lateral roots radiate while *Gentiana* and *Artemesia* have fibrous root systems. *Geum* grows in clonal patches and individuals are connected via rhizomes. *Gentiana* also grows in clumps, but individuals within a clump do not share roots and are not clonal.

We sampled five individuals of each species at seven times throughout the growing season, from June 26, 2017 (2 days after snowmelt) to September 11, 2017 (79 days after snowmelt). All 140 individuals sampled were taken from a 5 × 24 m rectangular area. Individuals of approximately the same size and in the same phenophase were selected and dug up with a soil plug 7–10 cm wide and 10–14 cm deep that contained their roots. Plants were transported to the lab, and placed in a 4 °C refrigerator within 3 h. We defined five phenophase categories—leaves, budding, flowering, fruiting, and mature seeds—and recorded the phenophase of each sampled individual. On each sampling day, we also measured volumetric soil moisture with a hydrosense II probe (Campbell Scientific, Logan, UT) and soil temperature with a HI 9063 probe (Hanna Instruments, Woonsocket, RI) at seven locations throughout the sampling rectangle.

Within 4 days of collection, roots were rinsed and new, fine roots were selected for analysis. We only sampled fine roots that could be traced back to the target host plant. The roots of each species differed in character, but we strove to collect the lightest-colored and finest roots, as we suspected these were the newest growth and had not suberized. For *Geum,* we collected light brown lateral roots that branched from the plant's tap roots. For *Artemesia* and *Gentiana*, we collected very pale yellow and often white lateral roots branching off the primary roots as well as very fine primary roots. For *Polygonum*, we collected pale lateral roots that branched off the tap root. The selected fine roots were placed in formaldehyde acetic acid alcohol (FAA) solution and stored at 4 °C for a maximum of 1 month until staining and microscopy. In addition to collecting roots, aboveground biomass was clipped, dried at 60 °C for 48 h, and weighed on a Secura 225d-1S balance (Sartorius, Arvada, CO).

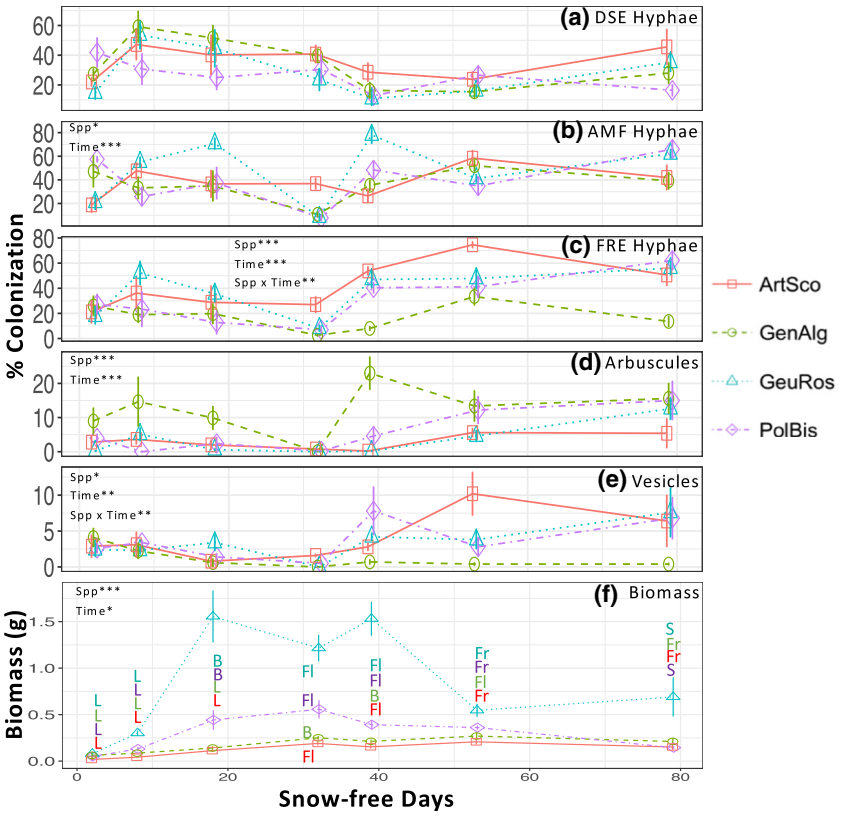

Fig. 3 Mean (± SE) percent colonization of five abundant fungal structures (**a**–**e**) and plant aboveground biomass (**f**) in the four plant species over time. Statistics are from a repeated measures mixed-effects ANOVA with observer as a random effect (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). Also depicted in panel **f** is the phenological stage of the plant (L = leaves, B = budding, Fl = flowering, Fr = fruiting, S = mature seeds). Note that letters are the same as in Fig. 2 except that panel **f** here is biomass (not microsclerotia). Note also that the scale of the *y*-axis changes among panels

Staining and microscopy

Staining and microscopy were performed following established protocols (Koske and Gemma 1989; McGonigle et al. 1990; Schmidt et al. 2008). Roots were rinsed three times with DI water to remove FAA and then cleared with 10% KOH for 1 h in a 90 °C water bath. Roots were rinsed with water to remove KOH and then soaked in 1% HCl at room temperature for 20 min. Roots were then soaked overnight in acidic glycerol with 0.05% trypan blue. In the morning, roots were destained with acidic glycerol and stored in acidic glycerol at 4 °C until microscopy was performed within 1 week. Several fine root segments and their branches, totaling 10–20 cm of root, were placed horizontally across slides, covered with a cover slip, and viewed at × 200 magnification under a microscope with a crosshair on the ocular. Passes were made up and down the slide at random intervals and the presence of AMF or DSE structures at each of 100 intersections with the crosshair was recorded. We counted the following structures: AMF hyphae (> 2 μm), AMF arbuscules, AMF vesicles, FRE hyphae (< 2 μm, with typical "fan-shaped" arrangements) DSE hyphae, and DSE microsclerotia (Fig. 2). Hyphae are long, branching, filamentous structures, arbuscules are highly branched nutrient exchange sites between the plant and fungus, and vesicles and microsclerotia are storage structures. Percent colonization for each fungal structure is the number of times it was present at each of the 100 intersections. For plants from which we were only able to collect < 5 cm of root (four instances during the study), we only made 50 vertical passes and multiplied the number of "hits" for each fungal structure by 2.

Statistical analyses

Structures that occurred in enough roots (AMF hyphae, FRE hyphae, DSE hyphae, arbuscules, vesicles) were analyzed with a repeated measures ANOVA model that tested for the effect of time (snow-free days), plant species, and the time by species interaction. The observer was included as a random effect to account for any observer bias (three people conducted the microscopy). Plant aboveground biomass was also analyzed with a repeated measures ANOVA. We used the 'nlme' *R* package for these models (Pinheiro et al. 2017). To test for relationships between root colonization and plant biomass, plant phenology, soil moisture, and soil temperature, we ran a repeated measures ANOVA model within each species with all four of these factors in a single model as fixed effects and observer as a random effect. We also conducted variance partitioning of fungal colonization by these four factors using the varpart function in the R package 'vegan' (Oksanen et al. 2018). Lastly, we examined correlations between the three fungal groups within each plant species using Pearson correlations. All analyses were performed using the software R, version 3.4.0 (R Core Team 2017).

Data accessibility Data will be accessible via the Niwot Ridge Long-Term Ecological Research website (niwot.colorado.edu) upon acceptance.

Results

All four plant species peaked in biomass around 1 month into the growing season (Fig. 3f). The plants differed in the timing of leafout, budding, flowering, fruiting, and producing mature seeds (Fig. 3f). *Geum* and *Polygonum* followed the same phenological trajectory, while *Artemesia* and *Gentiana* lagged behind, with *Gentiana* having the latest timing of events. Soil moisture declined steadily from almost 50% volumetric water content to less than 15% (Fig. 4). Soil temperature increased from ~ 5 to ~12 °C in the middle of the summer, and then declined to ~7 °C by September (Fig. 4).

All four plant species were highly colonized by AMF, DSE, and FRE throughout the study period (mean total AMF = 40%, mean total DSE = 32%, mean FRE = 32%). AMF hyphae varied significantly over time and across species, starting high early in the season, decreasing in the middle of the season, and then increasing late in the season (Fig. 3b). FRE hyphae varied significantly over time and across species, and there was also a significant interaction between time and species; the magnitude of change over time was less in *Gentiana*. The percent colonization by FRE hyphae started low and then increased drastically in August. Colonization by FRE hyphae then declined in September for *Artemesia* and *Geum*, but was still relatively high compared to earlier

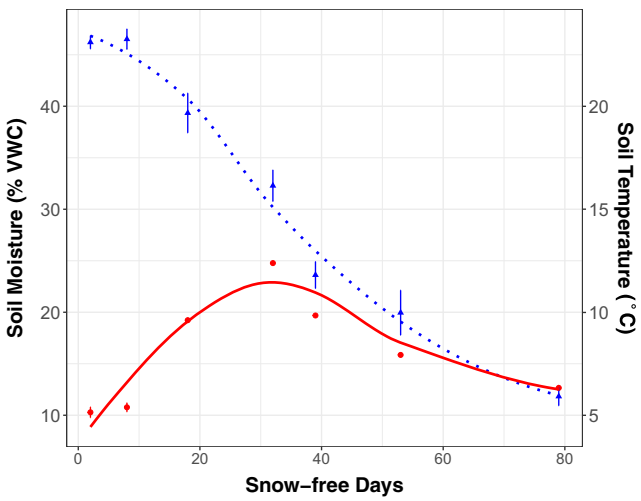

Fig. 4 Mean (± SE) soil moisture (blue, triangles, dotted line) and temperature (red, circles, solid line) at the sampling site over time. Lines were drawn using loess functions

in the season (Fig. 3c). DSE hyphae did not change significantly over time or differ among species, but fluctuated randomly throughout the season, hovering around 30% root length colonized (Fig. 3a). DSE microsclerotia were not abundant enough for analysis ($n = 63$ samples, rarely > 5% colonization). Both arbuscules and vesicles differed significantly across plant species, and were significantly more abundant later in the season when plants were producing seeds (Fig. 3d, e). AMF and FRE hyphae were consistently positively correlated with each other (Fig. 5). There were no significant correlations between AMF and DSE, or DSE and FRE (Fig. 5).

Relationships between colonization and plant biomass, phenophase, soil temperature, and soil moisture differed among fungal structures and plant species (Table 1). Colonization by AMF hyphae varied significantly with biomass, soil temperature and moisture in three plant species, and with phenophase in two species. There was no relationship between number of arbuscules and biomass, but significant relationships with phenophase and moisture in two species, and temperature in one species. There were no relationships between any of the variables and vesicle colonization. FRE hyphae colonization was significantly affected by phenophase and temperature in two species, and biomass and moisture in one species. DSE hyphae colonization was significantly related to each variable in one species (Table 1). Both plant and environmental variables explained variation in AMF, FRE, and DSE colonization, and the importance of plants versus the environment differed based on fungal structure and plant species (Table 2).

Discussion

The high levels of colonization by AMF, FRE, and DSE highlight the potential importance of these fungal groups for alpine plants. We observed significant fluctuations in root colonization by AMF and FRE structures over the growing season consistent with our hypothesis, while DSE did not vary significantly over time, contrary to our hypothesis. While it is difficult to decouple plant and environmental variables, our results show that plant variables such as biomass and phenophase can drive fungal colonization in some plant hosts and for some fungal structures, while for other species and fungal structures, moisture and temperature may play a more important role.

Our data show rapid fluctuations in fungal colonization, and relationships with both plant phenology and biomass, as well as soil moisture and soil temperature. While soil moisture showed steady declines throughout the season, and soil

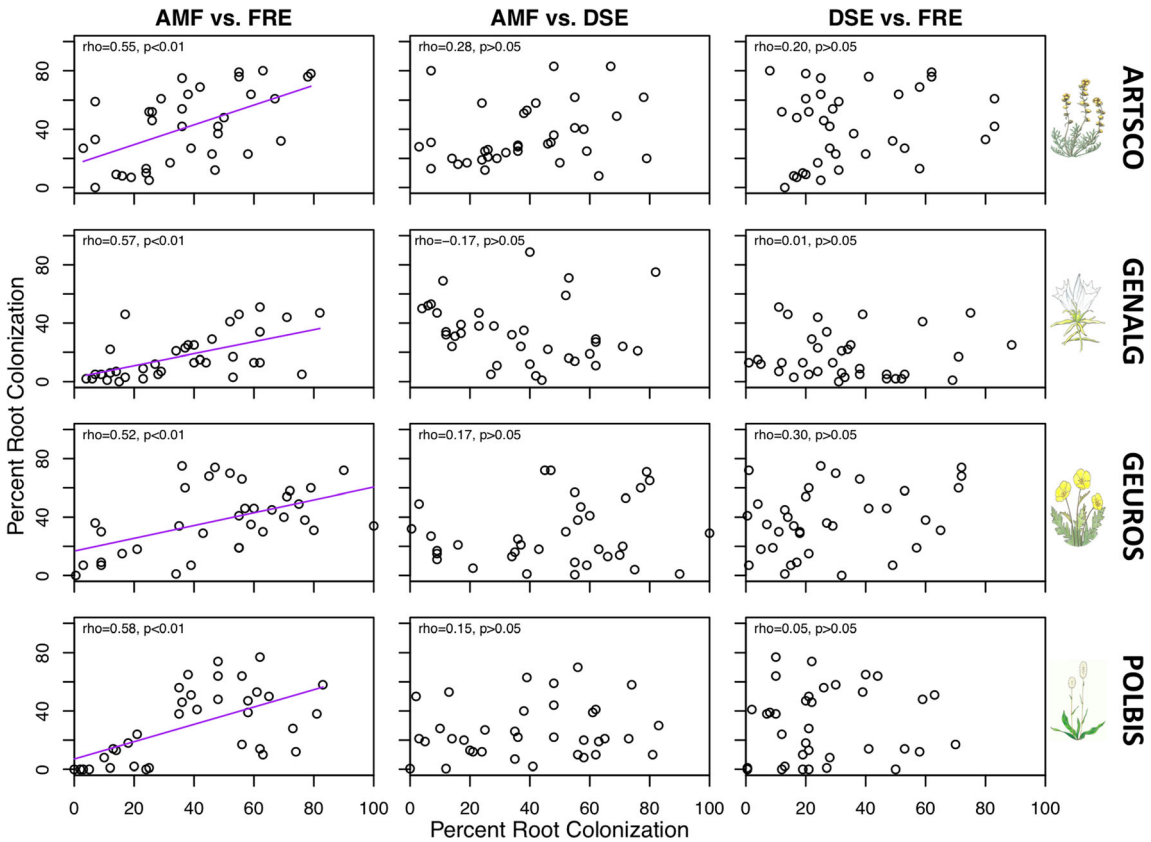

Fig. 5 Pearson correlations between AMF (*x*-axis) and FRE (*y*-axis), AMF and DSE, and DSE and FRE hyphae in the four plant species. Regression lines are shown for significant correlations. Botanical illustrations by Jane G. Smith

Table 1 Results of repeated measures ANOVA analyses within each plant species (ARTSCO = *Artemesia scopulorum*, GENALG = *Gentiana algida*, GEUROS = *Geum rossii*, POLBIS = *Polygonum bistortoides*) with the percent root length colonized by each fungal structure as a response variable and biomass (*B*), phenophase (*P*), soil temperature (*T*), and soil moisture (*M*) as fixed effects and microscopy observer as a random effect. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

	ARTSCO				GENALG				GEUROS				POLBIS			
Structure	*B*	*P*	*T*	*M*	*B*	*P*	*T*	*M*	*B*	*P*	*T*	*M*	*B*	*P*	*T*	*M*
AMF Hyphae	*		**	**	**				*	***	***	***		***	***	**
AMF Arbuscule				*		**	*	***		**						
AMF Vesicle																
FRE Hyphae	*		*			***				**	***	***				
DSE Hyphae										***	***	***	*			

temperature showed a bell-shaped curve, fungal colonization levels may not necessarily follow these trends exactly due to complex interactions between both fungal growth and root growth. Abrupt changes in colonization could have been caused by delays in fungus colonizing new root growth; other authors have noted that mycorrhizae may not be able to spread as fast as roots can grow (Douds and Chaney 1982; Dodd and Jeffries 1986; Abbott and Robson 1991; Miller 2000). Our data also show that in just a few days, fungi can spread into the new roots, or colonize the roots from propagules in the soil, leading to increased colonization after a lag.

While our analyses show some effect of the environment on fungal colonization, plant phenology clearly was an important factor, as has been found in several other studies (Lugo et al. 2003; Bohrer et al. 2004; Liu et al. 2009). Phenophase was either a significant predictor variable or explained the most variation of at least one fungal structure in all four plant species. Our species sampling scheme enabled us, to some extent, to tease apart the effect of environmental variables and host plant phenology, because, while the plants experienced the same environmental conditions, the timing of phenological events differed. This is especially evident for FRE hyphae and arbuscules, where there was a significant interaction between plant species and time. For example, on August 16th, the three species already producing seed had higher levels of FRE hyphae than the one species still in flower, despite growing in the same environmental conditions. Colonization levels may also reflect the timing of nutrient acquisition. Peaks in the fungal colonization of *Geum* occurred earlier than those in *Polygonum*, and previous work has shown that peak tissue nitrogen levels occur early in the season in *Geum* and late in the season in *Polygonum* (Jaeger et al. 1999).

Amidst the temporal fluctuations in fungal levels, a trend that emerged for all of the AMF fungal structures across all of the plant species was that the highest colonization typically occurred in August as plants were producing seeds. Importantly, arbuscules, the exchange site between plants and AMF, were the most abundant at this time, suggesting that plants were exchanging photosynthates for phosphorus at a higher rate than at any other point in the growing season, probably to meet the phosphorus demand of producing seeds (Saif 1977; Roldan-Fajardo et al. 1982; Dodd and Jeffries 1986; Tian et al. 2011). At this point in the growing season, plants have many established leaves and likely have an abundance of photosynthates to exchange with AMF. Alternatively, at this point in the growing season, plants may allocate less carbon to tissue regeneration and more to the belowground compartment, enabling increased fungal colonization.

Another interesting result from our study is that levels of fungal colonization were also high at the start of the growing season. Other studies from non-alpine systems have found

Table 2 Results of variance partitioning of five fungal structures by plant (biomass and phenophase) and environmental (Env., soil moisture and soil temperature) variables. Numbers are the adjusted R^2 values explained by each variable group, the joint variation explained, and the residual (Res.) variation left unexplained. Bolded numbers highlight the largest contribution of variation explained

Structure	ARTSCO				GENALG				GEUROS				POLBIS			
	Plant	Env.	Joint	Res.	Plant	Env.	Joint	Res.	Plant	Env.	Joint	Res.	Plant	Env.	Joint	Res.
AMF Hyphae	**23.5**	−0.9	−0.1	77.6	2.8	3.8	**8.8**	84.6	**79.5**	74.1	−70.7	17.1	23.5	**40.2**	−11.8	48.0
AMF Arbuscule	**20.0**	5.7	0.8	73.5	11.9	−23.0	**28.5**	82.6	13.1	2.9	**39.3**	44.7	−5.2	1.0	**37.0**	67.2
AMF Vesicle	**28.4**	11.8	4.8	55.0	−3.3	7.8	**26.3**	69.3	−3.1	6.5	**13.8**	82.7	6.7	**19.3**	−8.9	82.9
FRE Hyphae	−4.8	−4.9	**29.7**	80.1	**16.0**	−3.9	13.2	74.7	23.5	**38.9**	−13.9	51.5	−7.1	8.4	**37.2**	61.6
DSE Hyphae	−0.3	−6.1	−0.1	1.06	8.9	7.2	**12.6**	71.3	**36.8**	26.8	−25.7	62.1	**14.6**	2.5	6.0	76.9

very low to no fungal colonization over winter (Hayman 1970; Daniels Hetrick and Bloom 1983), but the roots of the four alpine forbs we studied were highly colonized (25–50% colonization) with AMF, FRE, and DSE fungi just 2 days after snowmelt. Similar results have been found for DSE and AMF in the alpine zone (Mullen and Schmidt 1993; Mullen et al. 1998; Ruotsalainen et al. 2002). While the majority of the fungal structures were hyphae at that time, there were arbuscules present in all four plant species at the first sampling date. This fungal colonization at the onset of the growing season likely started before the snow melted completely, and could help alpine plants jumpstart their growth, which would be particularly beneficial for alpine plants that must cope with a very short growing season. Alpine plants are known to start photosynthesis before snowmelt, and our data suggest that fungi also are active in plants before snowmelt. Future studies should sample plants from under the snowpack at the onset of photosynthesis.

We were surprised to find no significant trends in DSE colonization, which contrasts with Mullen et al. (1998). However, Mullen et al. (1998) only examined roots of one plant species, *Ranunculus adoneus*, while our results were consistent across four plant species. Further work is needed to investigate if DSE are actively helping plants acquire nitrogen (as hypothesized by Mullen et al. 1998), phosphorus (Haselwandter and Read 1982), or if they are performing some other function. The abundance of DSE in the roots (~ 30%) suggests that the plant could be devoting a substantial amount of photosynthate to these fungi, and it may be likely that the DSE are contributing to plant health in some fashion (Mandyam and Jumpponen 2005; Newsham 2011) because all of the plants sampled appeared to be healthy, growing vigorously, and showed no signs of disease or stress.

Our work shows fluctuations of AMF, DSE, and FRE structures over shorter timescales than in most other studies. AMF and FRE structures fluctuated more dramatically and significantly than DSE, and fungal colonization was driven by plant and environmental variables. In summary, fungal colonization in these alpine plants starts high, likely before snowmelt, potentially helping kick-start the short growing season. Colonization levels subsequently fluctuate and then peak, potentially helping plants produce seeds before the first snows come and soils freeze again. Our work highlights the need to study AMF, DSE, and FRE colonization in unison, and to measure both plant phenology and environmental data. Further work should also quantify the relationship between fungal colonization and seed production, seed mass, and seed viability.

Acknowledgements CPB was funded by the Niwot Ridge Long-Term Ecological Research program (National Science Foundation DEB 1637686). CMM was funded by the Research Experience for Undergraduates program at the CU Mountain Research Station (National Science Foundation DBI 1460906). We thank the Niwot Ridge LTER program and CU Mountain Research Station for logistical support. We thank Jane Smith, Sam Sartwell, Joshua Addison, Lauren Zappaterrini, and Alexander Sandberg-Bernard for help in the field and lab. We thank the members of the Suding Lab for helpful feedback on this manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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