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Seasonality of arbuscular mycorrhizal hyphae and glomalin in a western Montana grassland

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Abstract

In order to more fully understand the basic biology of arbuscular mycorrhizal fungi (AMF), and their role in natural ecosystems, it is necessary to document seasonal changes of various aspects of the life history of these fungi. Due to their unique position at the root-soil interface, AMF have been described as 'keystone mutualists' in ecosystems. Despite the importance of AMF in ecosystems, few studies exist that examine the seasonality of external hyphae and their exuded products (e.g. glomalin), the AMF variables directly related to ecosystem function through their contributions to soil aggregation. This study examined seasonal dynamics of several soil variables, with a specific interest in the seasonality of external hyphae and glomalin, a glycoprotein produced by AMF, which is correlated with soil aggregate stability. Here we measured glomalin concentrations and external AMF and non-AMF hyphal length, as well as soil moisture, percent fungal root colonization (AMF and non-AMF), and root length in soil in an intermountain grassland in western Montana over one growing season (13 time points). Of the glomalin pools and hyphal lengths measured, significant seasonal changes occurred for total glomalin (TG; 24.5% change), immunoreactive easily extractable glomalin (IREEG; 53.8% change), and AM hyphal length (107% change). Prior studies on glomalin in natural systems have not considered seasonal effects. The small seasonal change in glomalin pools lends further support to the hypothesis that glomalin is relatively stable in soils, and suggests that one-time sampling may be sufficient to satisfactorily capture this response variable. However, the generality of this observation has yet to be tested in a wider range of ecosystems.

Introduction

AMF are obligately biotrophic fungi that are closely associated with both host plants and the soil environment, function as an extension of the root system into the soil, and have numerous effects on plant physiology and plant communities (e.g. Allen, 1991; Smith and Read, 1997; van der Heijden et al., 1998). AMF play an integral role in the translocation of carbon to soil, having direct access to root carbon (Smith and Read, 1997). Due to their unique position at the root-soil interface, AMF have been described as 'keystone mutualists' in ecosystems (O'Neill et al., 1991).

In order to more fully comprehend the role of AMF in natural ecosystems, as well as their basic biology, it is important to document seasonal changes of various aspects of the life history of these fungi. While numerous studies have examined seasonality of AMF spore production and root colonization (e.g., Anderson et al., 1984; Gay et al., 1982; Johnson et al., 1991; Mullen and Schmidt, 1993; Sanders and Fitter, 1992), studies examining the seasonality of AMF extraradical hyphae and their exuded products are sparse. Although fungal spore production and root colonization are important for elucidating fungal life histories (Hart and Reader, 2002), neither of these characteristics directly

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relate to an ecosystem process or state variable. Conversely, extraradical hyphae and their products, such as glomalin, can be directly related to ecosystem processes, e.g. by virtue of their contributions to soil aggregate stability (Jastrow and Miller, 1997; Wright and Upadhyahya, 1998). More specifically, AMF contribute to soil aggregation through the hyphal entanglement process, assisting in soil aggregate formation (Jastrow and Miller, 1997). AMF produce extracellular polymeric compounds on hyphal surfaces, which can adhere to inorganic materials, helping to stabilize soil aggregates (Jastrow and Miller, 1997). As an example, extraradical hyphae of all AMF genera produce glomalin, a glycoprotein that is highly correlated with the percentage of water-stable aggregates (WSA) in soil (Wright and Upadhyahya, 1998; Rillig et al., 2001). Glomalin is not produced by other groups of soil fungi so far tested (Wright et al., 1996).

Few studies have examined the seasonality of extraradical hyphae in the field, and sampling intensity was generally low. Kabir et al. (1997) examined the seasonal changes of extraradical and intraradical arbuscular mycorrhizal hyphae affected by tillage and fertilization in an agricultural soil over a growing season (n=4). Abundance of AM hyphae fluctuated significantly within a growing season, with lowest hyphal densities found in the spring. Seasonal variation in mycorrhizal root colonization followed corn plant development, increasing up to silking and decreasing thereafter. Miller et al. (1995) examined external hyphal production and its relation to gross root morphology (specific root length: SRL) over a season in two temperate grassland communities (n=4). SRL was strongly associated with external hyphal lengths, where root systems with low SRL had greater lengths of external hyphae. Staddon et al. (2003) found changes in extraradical hyphal length (over a growing season from April to November; n=4) in a pasture, ranging almost across an order of magnitude as a function of treatment and sampling time. From these studies, it is clear that important seasonal patterns in extraradical hyphal length may exist, but the database is too small to draw firm conclusions. Seasonal dynamics of AMF hyphal products, such as glomalin, are unknown.

The field study described herein examines the seasonality of AMF extraradical hyphae and glomalin in a grassland invaded by spotted knapweed. We also relate these values to intraradical colonization. Total root length and the root length of two root diameter size classes were also measured to examine the relationship of hyphae and their products to plant root morphology.

Table 1. Physical and chemical properties of soils* at the North Hills field site, Missoula, MT

	Percent
Organic matter	5.72
Sand	63.50
Silt	21.50
Clay	14.50
Kjeldahl N	0.32
	mg/g
Nitrate-N	10.80
Olsen P	12.25
K	358.2
Fe	39.1
CEC (meq/100 g)	13.50

* determined by South Dakota State University Soil Testing Laboratory.

Materials and methods

Site description

This research site was located in a grassland with an initial invasion of spotted knapweed approximately 1 km north of Missoula, Montana, in the North Hills area. The plant community of this area is an Idaho fescue/bluebunch wheatgrass community type (*Festuca idahoensis*/*Agropyron spicatum*) (Mueggler and Stuart, 1980). The soil at this site is a sandy loam with pH 6.6 (Table 1).

Field experiment and sampling

Soil samples (n=5) were collected along a 5-m transect. A soil sample consisted of 3–4 soil cores (2 cm diameter) taken within a 15 × 15 cm area and pooled together. Samples were taken beginning on May 13, 2001, and then approximately bi-weekly until the last sampling date, November 30, 2001, for a total of 13 time points. Samples were repeatedly taken within the same 15 × 15 cm area and 5-m transect through time. Gravimetric soil moisture was determined on a subsample of soil (5 g) from each sample at each time point. All soil samples were dried overnight at 70 °C. Soil samples were sealed in polyethylene bags and stored at –20 °C until analysis.

Monthly precipitation and temperature data were obtained from the Missoula International Airport weather station, approximately 11 km southwest of

the study site (Western Regional Climate Center and National Climatic Data Center).

Extraradical hyphal and glomalin measurements

Extraradical hyphae were extracted from soil samples (4 g) using an aqueous extraction and filtration method (Rillig et al., 1999). Arbuscular mycorrhizal (AM) hyphae were distinguished from non-mycorrhizal hyphae at 200× magnification using similar criteria to Miller et al. (1995). Hyphal length was determined using the line intersect method as described in Jakobsen et al. (1992) and Tennant (1975).

Two detection methods are used to quantify glomalin: the Bradford protein assay, yielding the easily extractable glomalin (EEG) and the total glomalin (TG) fractions, and an ELISA assay (employing the monoclonal antibody developed against crushed spores of *Glomus intraradices*; Wright and Upadhyahya, 1998), yielding the immunoreactive easily extractable glomalin (IREEG) and immunoreactive total glomalin (IRTG) fractions. These glomalin fractions are operationally defined based on their extractability/solubility and detection methods (much like other soil fractions, such as humic acids). While the ELISA assay is a very specific detection method for glomalin, the more general Bradford protein assay is also utilized. This protein assay may capture glomalin protein that has undergone small (perhaps microbially-mediated) changes, possibly resulting in the destruction or concealment of the epitope for the monoclonal antibody. Because of well-documented and strong correlations with soil aggregate stability (e.g., Wright and Upadhyahya, 1998), these glomalin fractions continue to be quantified. Although the Bradford protein assay is generally utilized to measure total protein, it has been shown by SDS–PAGE that glomalin crude extract from soil (as measured in the Bradford assay) has similar banding patterns to glomalin extracts from single-species AMF sand cultures (Rillig et al., 2001).

Glomalin extractions from soil (1 g) were carried out as described by Wright and Upadhyahya (1998). The EEG fraction was extracted with 20 m*M* sodium citrate, pH 7.0 at 121 °C for 30 min. Following the EEG extraction, the TG fraction was extracted with 50 mM sodium citrate, pH 8.0 at 121 °C for 60 min cycles until the supernatant showed none of the red-brown color typical of glomalin. Both fractions of glomalin were analyzed using the Bradford Protein Assay (Bio-Rad, Melville, NY). The glomalin fractions were further analyzed using an enzyme-linked immunosorbent assay (ELISA) using the monoclonal antibody MAb32B11. Thus, after all glomalin analyses were completed, four fractions of glomalin values were obtained: EEG, TG, IREEG, and IRTG.

Root extraction and quantification

Roots were removed from the soil samples by a hand flotation and sieving method modified from Cook et al. (1988) and Miller et al. (1995). A 10 g subsample from each soil sample was soaked in 100 mL of tap water and 20 mL sodium hexametaphosphate (35 g L^{-1}) for 30 min. The soil suspension was then added to 880 mL tap water (total volume: 1000 mL), manually agitated to suspend roots, and poured through 212 μm and 0.5 mm sieves to retain roots. This process was repeated five times with all soil samples to maximize retrieval of roots. Separated roots were washed with tap water several times to remove any attached soil. No attempt was made to distinguish between current and previous year's growth. Obvious organic material and other debris were removed. Extracted roots were dried in a drying oven at 70 °C overnight and stored at room temperature until analysis. Total root length and root lengths of two root diameter size classes, fine roots (>0.25 mm diameter) and very fine roots (<0.25 mm diameter), were measured using the Win-Rhizo V. 5.0A root image analysis system (Régent Instruments Inc, Québec, Canada). Total root length colonized by AM hyphae was calculated by multiplying root length by percent AM hyphal colonization for each sample. The efficiency of the root extraction procedure was determined by re-extracting soils (n=3). Any visible roots were removed from the dried soil and measured using the Win-Rhizo root image analysis system. Extraction efficiency was determined to be 98%.

Percent AM root colonization

Roots were cleared in 10% KOH for 1 h at 80 °C, acidified with 1% HCl for 15 min, and then stained with trypan blue in lactoglycerol (0.05%). Roots were left in clear lactoglycerol overnight, and then 1 cm pieces were placed on microscope slides for analysis. Percent colonization was measured by the gridline intersect method as described by Rillig et al. (1999).

Data analysis

All response variables were analyzed first using repeated measures multivariate analysis of variance by

Table 2. Monthly mean temperature and precipitation data for Missoula, MT for 2001 and long-term (LT) averages

Month	Temperature (°C)		Precipitation (cm)	
	2001	LT average	2001	LT average
January	–5.5	–5.2	1.78	3.15
February	–5.1	–1.6	1.70	2.01
March	2.9	2.1	2.03	2.46
April	6.0	6.8	2.92	2.44
May	12.6	11.0	1.02	4.52
June	14.6	15.6	9.93	4.52
July	19.2	19.3	3.99	2.31
August	21.2	18.8	0.23	3.05
September	16.3	13.2	0.79	2.84
October	6.8	6.8	5.38	1.88
November	2.2	0.2	1.12	2.06
December	–3.4	–4.8	2.84	2.95

GLM procedures of SPSS statistical software (SPSS Inc., version 11.0.1, 2001) since these variables cannot reasonably be assumed to be independent (Scheiner and Gurevitch, 1993). The effect of time on each response variable was then determined using univariate repeated measures analysis of variance in the procedure of SPSS (SPSS Inc., version 11.0.1, 2001). All response variables were tested with the adjusted Huynh and Feldt (1976) *F*-test ($P< 0.05$). Pearson product-moment correlations (r) on the means (n=13) were determined in the procedure of JMP (version 3.1.6.2, 1996). The coefficient of variation (cov) was calculated by dividing the standard deviation of means by the grand mean for each response variable. The percent change for response variables was calculated as ((mean X_{max} – mean X_{min}) / mean X_{min})*100.

Results

Repeated measures multivariate analysis of the response variables measured revealed a significant difference (RM-MANOVA: $F_{180,118}$: 2.413, $P<0.001$), justifying further analysis of individual response variables.

Monthly mean temperature and precipitation data for Missoula, MT in the year 2001 and long-term averages are presented in Table 2. In 2001, Montana experienced severe drought conditions (Montana Drought Monitoring, 2001). The mean percent soil moisture for the study site at each sampling time is presented in Figure 1. Variation in the mean percent soil moisture through time was significant (Table 3).

Table 3. *F*- and *P*- values from univariate repeated measures analysis of variance for glomalin, arbuscular mycorrhizal (AM), and root parameters. *P*-values <0.05 are bolded. All response variables were tested with the adjusted Huynh and Feldt *F*-test

Variable	*F*	*P*
Percent soil moisture	109.2	**<0.001**
Easily extractable glomalin (EEG)	0.942	0.515
Total glomalin (TG)	2.405	**0.016**
Immunoreactive easily extractable glomalin (IREEG)	2.097	**0.047**
Immunoreactive total glomalin (IRTG)	0.494	0.259
AM hyphal length	2.416	**0.033**
Non-AM hyphal length	1.697	0.097
Percent AM hyphal colonization	4.678	**0.001**
Percent AM vesicle colonization	1.459	0.227
Percent AM arbuscule colonization	3.414	**0.009**
Percent non-AM hyphal colonization	3.361	**0.003**
Total root length	1.398	0.200
Fine root length (>0.25 mm diameter)	3.037	**0.003**
Very fine root length (<0.25 mm diameter)	1.299	0.250
AM colonized root length	2.576	**0.028**

The concentration of TG fluctuated significantly through time (Table 3), with a 24.5% change between the lowest and highest average concentrations. The low coefficient of variation indicates that individual samples did not vary greatly from the overall mean. There was an increase in TG concentration from late May to June (Julian day 145 – 162), followed by a general downward trend until early November (Julian day 307; Figure 2a). In November, TG concentrations increased until the end of the sampling period (Figure 2a). Conversely, there were no significant differences in the EEG fraction (Table 3).

Variation in the IREEG fraction, but not the IRTG fraction, was significant through time (Table 3), and also had a low coefficient of variation (Figure 2b). There was a 53.8% change between the lowest and highest average concentrations of IREEG. An initial decrease in the concentration of IREEG occurred from May to June (Julian day 133 – 162), with IREEG concentrations generally rising thereafter until October (Julian day 286). After October, the concentration of IREEG again decreased (Figure 2b). The IRTG pattern through time was similar to that of the IREEG fraction (Figure 2b). However, due to large variation at some

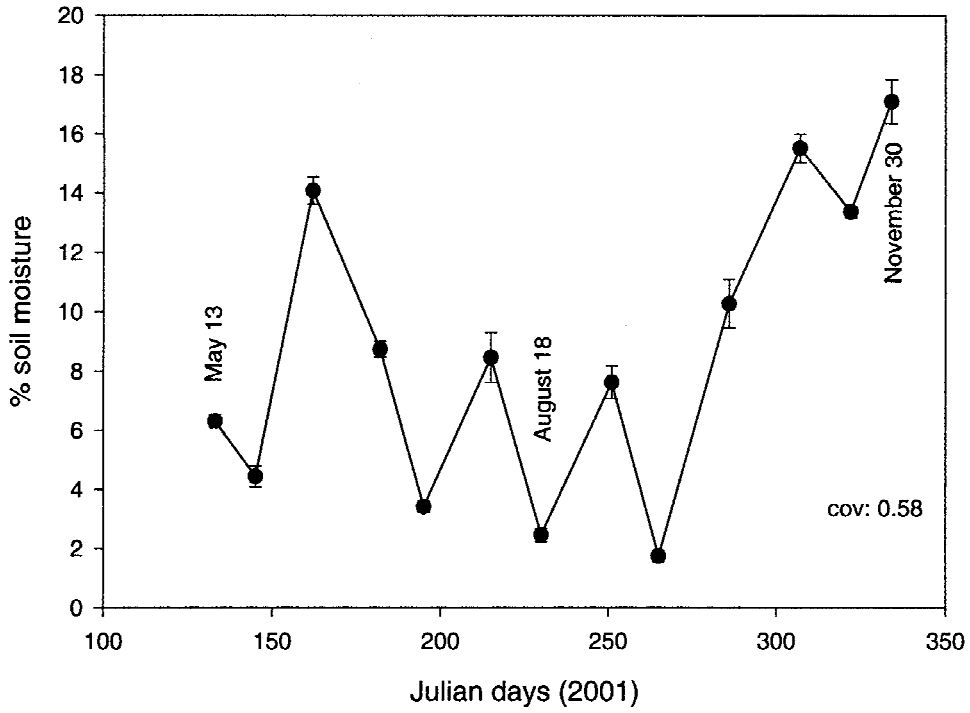

Figure 1. Average percent soil moisture through time (Julian days). Standard errors of the mean (n=5) and coefficient of variation (cov) are shown. Univariate repeated measures ANOVA P-value is presented in Table 3. cov=variability of the means (n=13).

sampling times, there was not enough power to observe a significant variance of the IRTG concentrations through time.

External arbuscular mycorrhizal (AM) hyphal length varied significantly through time (Table 3), with a large increase from late September to early October (Julian day 265 – 286), followed by a decrease in hyphal length through November (Julian day 286 – 334, Figure 3). The decrease in AM hyphal length at the end of the sampling period was similar to the decrease observed in the IREEG fraction. The percent change between the highest and lowest AM hyphal lengths measured was 107%. Non-AM hyphal length did not vary significantly through time (Table 3).

Percent AM hyphal colonization decreased in November (Julian days 307 – 334), similar to the decrease in external AM hyphal length (Figures 3 and 4; Table 3). Percent vesicle colonization did not vary significantly, while percent arbuscule colonization varied significantly through time (Table 3), with great fluctuation from May to the beginning of August (Julian days 133 – 215), followed by a considerable decrease (Julian day 230; Figure 4) until the end of the sampling period, with colonization leveling out at 2% (Figure 4).

Fine root length (>0.25 mm diameter) changed significantly through time, while very fine root length (<0.25 mm diameter) and total root length did not (Table 3). A decrease in fine root length occurred mid to late May (Julian day 133 – 145), followed by a general increase in fine root length until late September (Julian day 265, Figure 5). Fine root length then decreased from September to the end of November (Julian day 265 – Julian day 334, Figure 5). This decrease was similar to the decrease observed in AM and glomalin parameters (Table 3, Figures 2b, 3, and 4).

AM colonized root length remained constant until July, when AM colonized root length increased and then decreased by August (Julian day 182 – 215; Figure 6). Thereafter, there was an increase until the end of September (Julian day 251), followed by a decrease through the end of the sampling period (Figure 6). Variation in root length colonized by AM hyphae through time was significant (Table 3).

Pearson product-moment correlations (r) for glomalin fractions were strongest between IREEG and root lengths. IREEG was positively correlated with total root length (r=0.82, P<0.01), fine root length (r=0.79, P<0.01), and very fine root length (r=0.80, P<0.01)

Table 4. Correlation matrix of glomalin, arbuscular mycorrhizal (AM), and root variables. Pearson product-moment correlations (r) on the means (n=13) are shown, with those significantly different from zero bolded ($P<0.05$). (EEG-easily extractable glomalin; TG-total glomalin; IREEG-immunoreactive easily extractable glomalin; IRTG-immunoreactive total glomalin)

Variable	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.
1.% soil moisture	1.00														
2. EEG	0.33	1.00													
3. TG	–0.04	–0.32	1.00												
4. IREEG	–0.10	**0.60**	**-0.59**	1.00											
5. IRTG	–0.29	0.49	–0.22	**0.62**	1.00										
6. AM hyphal length	0.37	0.23	**–0.67**	0.45	0.30	1.00									
7. Non-AM hyphal length	0.27	0.25	–0.18	**0.53**	**0.53**	**0.54**	1.00								
8.% AM colonization	**–0.72**	**–0.55**	0.03	–0.25	–0.02	–0.05	–0.18	1.00							
9.% vesicle colonization	–0.19	0.20	0.18	–0.14	0.09	–0.36	–0.08	–0.01	1.00						
10.% arbuscule colonization	–0.20	–0.15	0.48	–0.53	0.20	**–0.61**	–0.07	0.08	0.30	1.00					
11.% non-AM colonization	–0.53	**–0.60**	0.03	–0.30	–0.29	–0.05	–0.29	**0.78**	0.12	0.11	1.00				
12. Total root length	–0.18	0.26	**–0.58**	**0.82**	0.42	**0.60**	0.18	0.08	–0.47	**–0.68**	0.03	1.00			
13. Fine root length (>0.25 mm diameter)	0.09	0.51	**–0.57**	**0.79**	0.45	**0.67**	0.36	–0.17	–0.07	**–0.73**	–0.21	**0.81**	1.00		
14. Very fine root length (<0.25 mm diameter)	–0.21	0.24	**–0.57**	**0.80**	0.39	**0.57**	0.12	0.12	–0.49	**–0.64**	0.09	**0.99**	**0.75**	1.00	
15. AM colonized root length	**–0.67**	–0.22	–0.28	0.36	0.20	0.23	–0.29	**0.70**	–0.23	–0.37	**0.61**	**0.71**	0.42	**0.74**	1.00

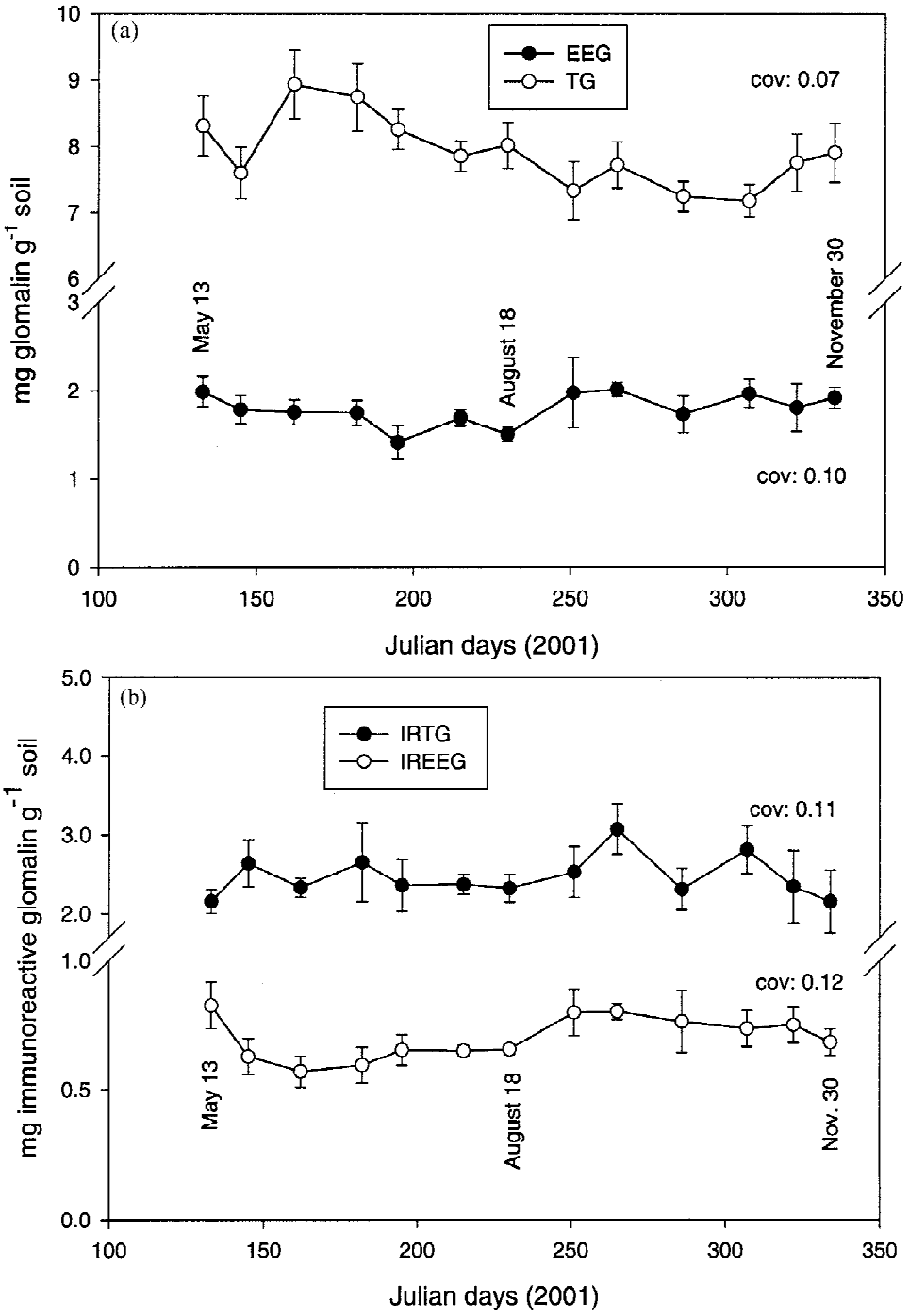

Figure 2. (a) Mean concentrations of glomalin fractions through time (Julian days). Note scale and axis break. Standard errors of the mean (n=5) and coefficient of variation (cov) are shown. Univariate repeated measures ANOVA P-values are presented in Table 3. cov=variability of the means (n=13). (b) Mean concentrations of immunoreactive glomalin fractions through time (Julian days). Note scale and axis break. Standard errors of the mean (n=5) and coefficient of variation (cov) are shown. Univariate repeated measures ANOVA P-values are presented in Table 3. cov=variability of the means (n=13).

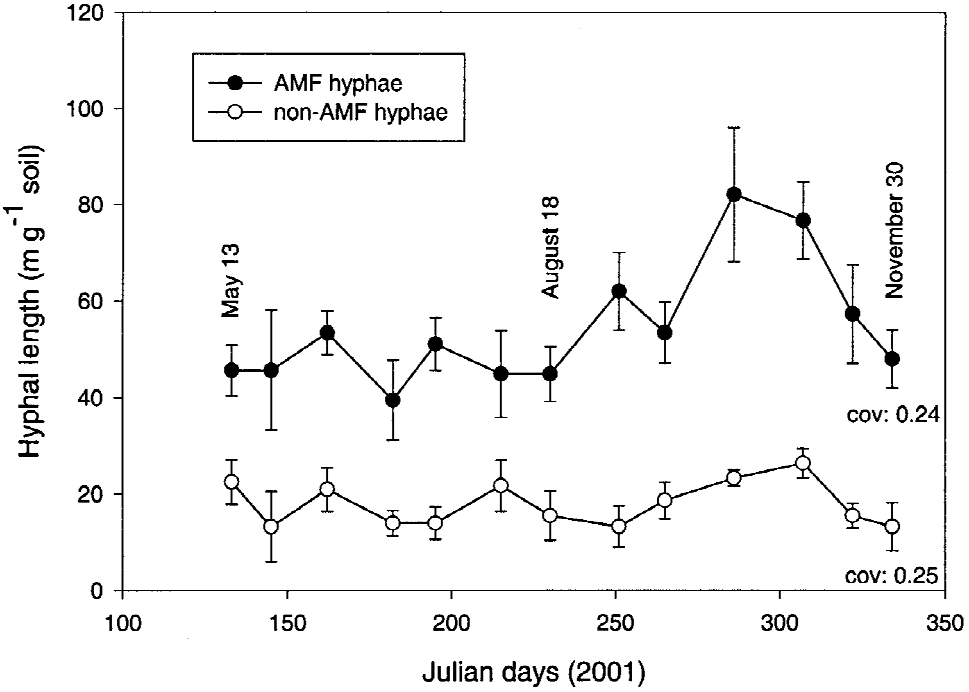

Figure 3. Mean external hyphal length of arbuscular and non-arbuscular mycorrhizae through time (Julian days). Standard errors of the mean (n=5) and coefficient of variation are shown. Univariate repeated measures ANOVA P-values are presented in Table 3. cov=variability of the means (n=13).

(Table 4). Pearson product-moment correlations (r) for all variables measured are presented in Table 4.

Discussion

The sampling regime for this study was quite intense (bi-weekly from May through November 2001; 13 time points) compared to other field studies on extraradical AM hyphal dynamics (all four sampling time points: Kabir et al., 1997; Miller et al., 1995; Staddon et al., 2003). Hence our study should have had sufficient resolution to detect even relatively short-term fluctuations in glomalin pools, hyphal lengths and other response variables.

Seasonality of glomalin

The main objective of this study was to test whether, in the context of other AMF variables, seasonal changes in glomalin fractions occur over a growing season, as part of a research effort to elucidate the natural history of this compound. We have previously modeled turnover time of glomalin to be in the range of 6–42 years in a tropical forest (Rillig et al., 2001), much longer than the turnover time expected for AM hyphae (Friese and Allen, 1991; Rillig et al., 2001). In a lab incubation comparing glomalin and AMF extraradical hyphae decomposition, glomalin concentrations only decreased by 25% over 150 days, while AMF extraradical hyphal length declined 60% (Steinberg and Rillig, 2003). Rillig et al. (2003) found that ca. 50% of glomalin remained after a 413 day laboratory soil incubation, indicating that some glomalin may be in the slow or recalcitrant soil C fraction. Given the relative persistence of this compound, one could perhaps expect seasonal changes in pool size to be small. Here, we could nevertheless detect significant seasonal changes in glomalin, but in only two of the four fractions (TG and IREEG). Given that these changes were relatively small, results are in agreement with our previous work (Rillig et al., 2003), suggesting that a portion of glomalin is labile while a sizeable proportion of this pool would not be expected to show any measurable seasonal change owing to its slow turnover.

The patterns of change in the TG and IREEG concentrations through time were generally dissimilar. At this time, it is unclear how or why these operationally defined glomalin pools differ from each other in

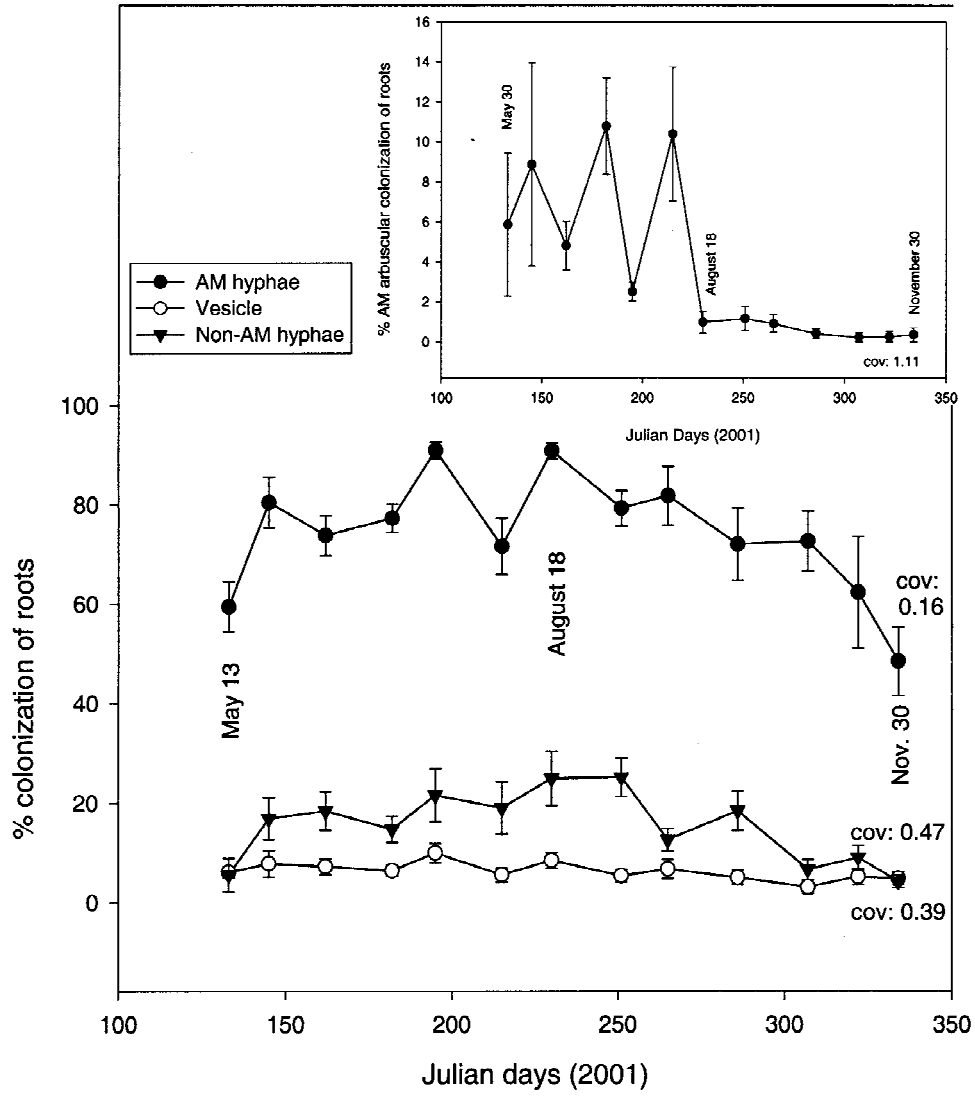

Figure 4. Average percent colonization of arbuscular mycorrhizal (AM) hyphae, vesicles, and non-AM hyphae through time (Julian days). Standard errors of the mean (n=5) and coefficient of variation (cov) are shown. Univariate repeated measures ANOVA P-values are presented in Table 3. cov=variability of the means (n=13) Inset: Mean arbuscular colonization through time (Julian days). Standard errors of the mean (n=5) and coefficient of variation (cov) are shown. Univariate repeated measures ANOVA P-value is presented in Table 3. cov=variability of the means (n=13).

terms of biochemistry, function in soil, and age, or how the production of glomalin is controlled (Rillig et al., 2001, 2003). It is also unclear if the glomalin pools, as currently defined, are a reflection of differential turnover or biochemistry. Initially it was assumed that IREEG consists of glomalin most recently deposited into soil (owing to the ease of its extraction). We recently showed that IREEG concentrations increased in soil with hyphal decomposition, and in the absence of new hyphal glomalin production (Steinberg and Rillig, 2003). The increase in IREEG could be reflective of glomalin liberated from decomposing hyphal walls; this nevertheless cautions against the use of extractability as a predictor of relative age. However, these pools continue to be measured since they are of significance in soil aggregation (Wright and Upadhyaya, 1998).

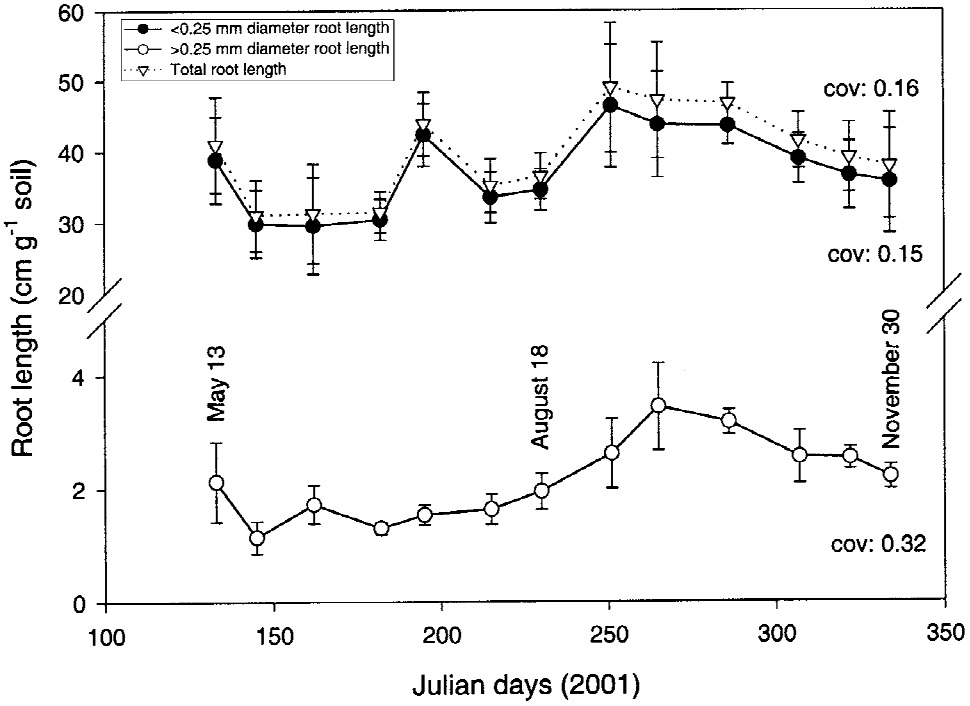

Figure 5. Mean total root length, fine root length (>0.25 Mm diameter), and very fine root length (<0.25 Mm diameter) through time (Julian days). Note scale and axis break. Standard errors of the mean (n=5) and coefficient of variation (cov) are shown. Univariate repeated measures ANOVA P-values are presented in Table 3. cov=variability of the means (n=13).

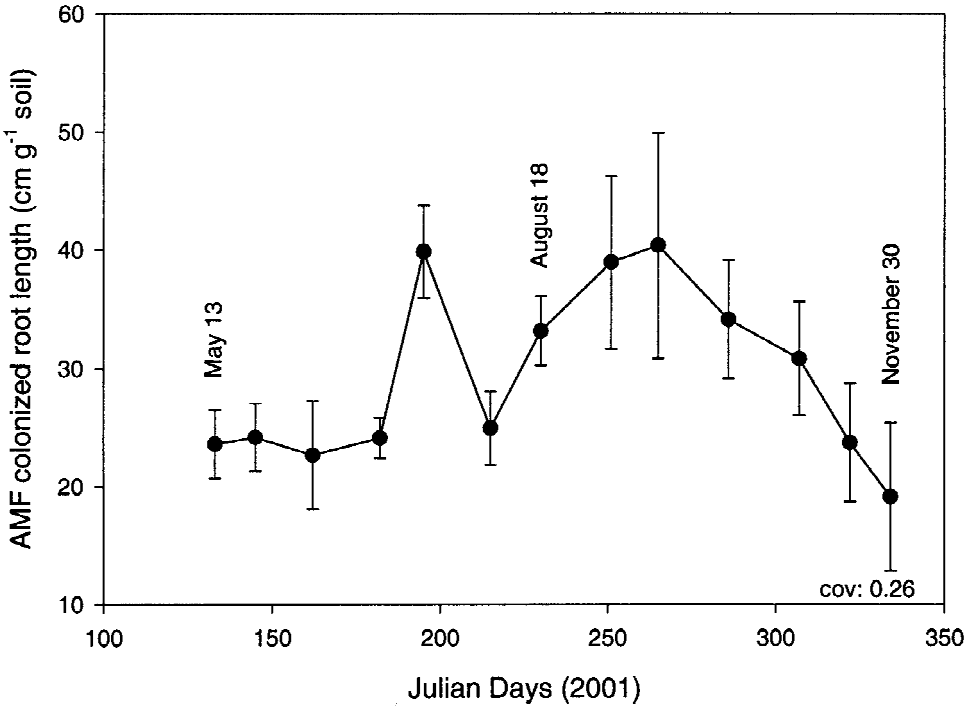

Figure 6. Mean arbuscular mycorrhizal fungal (AMF) root length through time (Julian days). Standard errors of the mean (n=5) and coefficient of variation (cov) are shown. Univariate repeated measures ANOVA P-value is presented in Table 3. cov=variability of the means (n=13).

Seasonality of other AMF and root variables

Several fungal variables fluctuated significantly through time in the soil studied. External AM hyphal length changed significantly over the growing season, lending further support to observations by other researchers in different ecosystems (Kabir et al., 1997; Miller et al., 1995; Staddon et al., 2003). Hyphal length values measured in this study were fairly high, with the highest hyphal length measured to be 82 m g^{-1} soil in October, and an average hyphal length throughout the season of 50 m g^{-1} soil. External hyphal lengths from this study were consistent with hyphal lengths measured in a different intermountain grassland in western Montana (Lutgen and Rillig, unpublished), where hyphal lengths reached 45 m g^{-1} soil. Miller et al. (1995) reported hyphal lengths up to 111 m cm^{-3} in their prairie ecosystem, indicating that our values are within the range of lengths previously reported.

Percent AM and non-AM hyphal colonization in roots decreased in November, similar to the decrease in AM extraradical hyphal length. However, the decrease in fungal root colonization began earlier in the sampling period, during August. A sharp decline in percent AM arbuscule colonization also occurred at this time. It is likely that plants were under severe drought stress, and the carbon allocation from plant to fungus may have decreased as a consequence. Since arbuscules are the structures involved in carbon/ nutrient exchange with plants (Smith and Read, 1997), the drought response in plants could be reflected in the decrease in percent arbuscule colonization. By contrast, others have found increased mycorrhizal colonization under drought in the field (e.g., Augé, 2001; Staddon et al., 2003). This leaves us to hypothesize that perhaps the severity of drought was the overriding factor here.

Root length variables measured did not exhibit much significant change through time, with only the fine root length (>0.25 mm diameter) variable changing through time. The majority of root length measured was found in the very fine root length (<0.25 mm diameter) variable. Fine root length dynamics somewhat follow the dynamics of AM fungal variables over the growing season, and AM hyphal length is positively correlated with all root length variables. AMF are obligate biotrophs, and perhaps less hyphal development is possible when there are fewer roots to colonize.

Correlations between glomalin and other variables

It is very important to keep in mind that correlations were calculated across different time points; hence response variables with different turnover (or even phase-shifted dynamics) would appear non-correlated in this analysis.

There was a general lack of correlation between glomalin fractions and fungal variables, which can seem counterintuitive considering glomalin is produced by AM hyphae. However, we suggest that this lack of correlation is explained by differential turnover of glomalin and fungal structures (e.g. extraradical hyphae; Friese and Allen, 1991; Rillig et al. 2001; Steinberg and Rillig, 2003).

Correlations between glomalin fractions and root variables were only observed between the IREEG fraction and all root length variables, where an increase in root length correlated to an increase in IREEG. As previously mentioned, it is unclear how these operationally defined glomalin pools differ from each other, hence we cannot explain this result based on our current knowledge. It is interesting to speculate that roots themselves contain glomalin (in intraradical fungal structures), and that this is reflected in soil extractions. We did not exclude root fragments from the soils from which glomalin was extracted. Alternatively, if IREEG partly represents glomalin recently liberated from spore walls or soil surfaces (Steinberg and Rillig, 2003), the rhizosphere priming effect of roots on microbial activity (and hence microbial-mediated release of glomalin) could be responsible for this observation.

Conclusions

This is one of the first reports on seasonal behavior of glomalin, and we draw three main conclusions: (1) Although glomalin overall has a slow turnover time in soil, it appears that a proportion of glomalin can fluctuate through a growing season, and we could detect these changes here. Based on the relative size of the recalcitrant and more labile glomalin pool, seasonal changes could be relatively small (as we observed here), or larger. Further studies of glomalin seasonality in other ecosystems could not only test if the pattern we saw holds generally, but could also test the idea that the proportion of labile to recalcitrant glomalin can vary among ecosystems (and give clues as to why that may occur). (2) The small seasonal changes

of glomalin have implications for designing sampling protocols. It would appear that for our site one or two time points per growing season would be sufficient to capture glomalin pools. However, we advise caution in extrapolating this conclusion to other ecosystems (see above). (3) The overall lack of correlation between AM fungal variables and glomalin is important, as it points out that glomalin fractions, as currently defined, may not be useful as indicators of AMF hyphal length and fungal activity in general. The current fractions were not devised with this purpose in mind; however it may be possible to obtain fractions of glomalin that could be useful as an indicator for AM extraradical hyphae.

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