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The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species

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Abstract

Soil aggregation and soil structure are fundamental properties of natural and managed ecosystems. However, most of our knowledge on the role of plant species in soil aggregation is derived from work in agroecosystems or with agriculturally important plants. Here we examined the effects of five plant species on soil aggregate water stability. The five species (three grasses, one forb, and a legume) were from the same natural grassland, and were grown in monoculture plots in the field. Our first goal was to test if productivity-related or species-specific factors would prevail in determining soil aggregation. We also tested what the relative importance of the soil protein glomalin (produced by arbuscular mycorrhizal fungi, AMF) in soil aggregation is, compared to other factors, including AMF hyphal and root length and percent plant cover. We found significant differences in soil aggregate water stability (1–2 mm size class) for the five plant species examined, and corresponding differences in plant cover, root weight and length, AMF soil hyphal length, and glomalin concentrations. A structural equation modeling approach (path analysis) was used to distinguish direct from indirect effects of factors on soil aggregation based on covariance structures. Root length, soil glomalin, and percent cover contributed equally strong paths to water-stable aggregation. The direct effect of glomalin was much stronger than the direct effect of AMF hyphae themselves, suggesting that this protein is involved in a very important hypha-mediated mechanism of soil aggregate stabilization, at least for the 1–2-mm size class of aggregates.

Introduction

Soil structure is central to soil and ecosystem functioning as it controls fluxes of water, gases and nutrients. Soils also serve as large repositories for carbon, with carbon storage capacity greatly depending on soil structure. The vast majority of knowledge about soil structure in general, and on the influence of organisms (e.g. plants) on soil aggregation is derived from experiments and observations made in agroecosystems or using agriculturally important plants (Angers and Caron 1998; Haynes and Beare 1997). While many of these observations will also be transferable to natural ecosystems as general principles, it is important to realize that effects on soil structure can perhaps not always be separated from management practices, like tillage and fertilization, associated with agricultural cultivation (Angers and Caron, 1998). Few studies have addressed biological factors involved in soil aggregation in natural ecosystems (e.g., Jastrow et al., 1998; Miller and Jastrow, 1990). The focus of this study was to elucidate contributions of mycorrhizal fungi and other biological factors on soil aggregation using native plants from a grassland ecosystem.

Given constant abiotic factors, fungal hyphae are among the most important, if not the most important agents in soil aggregate stabilization among the soil biota (Degens, 1997), although effects of roots, soil bacteria and fauna are clearly significant as well (Degens,

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1997; Oades, 1984). Among the fungi, arbuscular mycorrhizal fungi (AMF) appear to be the most important mediators of soil aggregation for three reasons. The extraradical hyphae of AMF represent a substantial, often dominant component of soil microbial biomass (e.g. Allen, 1991; Miller et al., 1995; Rillig et al., 1999). By directly tapping into carbon resources of the plant, they are independent of the limiting carbon supply in bulk soil on which saprobic fungi depend (Smith and Read, 1997). Additionally, since grazers prefer saprobic hyphae over AMF hyphae (Klironomos and Kendrick, 1996), AMF hyphae appear to have a longer residence time in soil, allowing for a less transient contribution to soil aggregate stabilization than saprobic hyphae. As a consequence, AMF hyphae were one of the most important components in a path analysis model describing biotic influences on soil aggregation compared to numerous other biological factors (Jastrow et al., 1998).

Recently, a new factor of presumably great importance in soil aggregation was discovered: glomalin (Wright and Upadhyaya, 1996). Glomalin is a glycoprotein, produced by AMF, and its concentration in aggregates (Wright and Upadhyaya, 1998) and soil (Rillig et al., 2001) correlates with the percentage of water-stable aggregates (WSA). We were interested in examining the relative importance of glomalin in comparison with other biological factors in explaining the proportion of WSA in soil. Specifically, we were interested in separating the direct effects of hyphae from those caused by glomalin, and how plant species can alter these effects.

Plant species can influence soil aggregation (Reid and Goss, 1981; Scott, 1998, Tisdall and Oades, 1979) through a number of mechanisms, including: Root structure and distribution, quality of carbon inputs, quantity of carbon inputs, effects on soil microclimate, and influences on microbial communities and their activities (e.g. bacterial extracullular polysaccharide production, mycorrhizae). In prairies, water stable aggregates >0.2 and >2 mm diameter have been associated with the presence of grass species more than with any other vegetation group (Jastrow, 1987). We examined soil aggregation associated with different grassland species to test if species-specific factors or more general, productivity-related factors could be held responsible for observed differences in water stability of aggregates. There is a scarcity of information on the effects of co-occurring plant species from a natural ecosystem on soil aggregation (Angers and Caron, 1998), although examples from agroecosystems are available (e.g. Degens et al., 1994).

Materials and methods

Field experiment

This research took place at the University of California Hopland Research and Extension Center, located in Mendocino County, California (39°00′ N latitude, 123°04′ W longitude). The ecosystem is a California annual grassland in the coastal range of northern California. The area experiences a Mediterranean climate, with wet, cool winters, and hot, dry summers. The soil was a fine-loamy over clayey, mixed, mesic Ultic Haploxeralf (Sutherlin series).

In the summer of 1997, a 30 m × 60 m area was mowed, and litter was removed and subsequently autoclaved to kill the seeds in the litter. In order to minimize the existing seedbank, the area was irrigated (5 cm of water), and the resident seedbank was allowed to establish, as it would in a typical fall germinating rain. After irrigation, the germinated vegetation was killed using glyphosate (Roundup). This process was repeated once more to almost eliminate the pre-existing seedbank. We established 1-m^2 plots of different plant monocultures, with 9 replicates of each species treatment (seven of which were used for this study). These were layed out in a randomized block design. Seeds of each species were planted at a density planned to achieve constant end of season biomass among the species (based on preliminary greenhouse data; V. Eviner, unpublished). Seeds were raked into the top cm of soil, and then the autoclaved litter was placed back into the plots. The seeds were allowed to germinate naturally with the fall germinating rains. Species composition was maintained through weeding. Percentage plant cover and density were representative of surrounding area of grassland (V. Eviner, unpublished).

The plant species used for this experiment were: 3 grass species [*Avena barbata* (slender wild oats n = 6), *Aegilops triuncialis* (barbed goatgrass; n = 6), *Taeniatherum caput-medusae* (medusa head; n = 7)]; 1 forb species, *Amsinckia douglasiana* (fiddleneck; n = 6); and 1 legume, *Trifolium microcephalum* (maiden clover n = 7). In April of 1999. after 2 growing seasons, samples were extracted from experimental species plots with a 2 cm diameter corer to a depth of 15cm. Three cores per plot were taken, spaced approximately equidistantly in the center area of the plot

(to minimize edge effects), and pooled. Soil samples were air-dried and stored in paper bags until analysis.

Water-stable aggregates in the 1–2-mm size class ($WSA_{1-2\text{-}mm}$)

All soils had been stored as air-dried samples >4 months. We concentrated on macro-aggregates of 1–2 mm diameter, since the amounts of these aggregates are sensitive to short term (< 2 yr) management and treatment of soils (Kemper and Rosenau, 1986); also most effects of glomalin have been observed for this size class. Replicate 4 g samples of soil aggregates were moistened by capillary action for 10 min. Water-stability of aggregates was then measured with a wet-sieving method using the apparatus and procedure described in Kemper and Rosenau (1986). Percentage of water-stable aggregates ($WSA_{1-2\text{-}mm}$) is calculated using the mass of aggregated soil remaining after wet sieving and the total mass of aggregates at the beginning. The initial and final weights of aggregates were corrected for the weight of coarse particles (> 0.25 mm).

Percent cover and aboveground biomass

In early June of 1999, a visual percent plant cover estimation was made for each of the plots. This cover estimation is substantially lower than the actual growing season% cover values for *Amsinckia* and *Trifolium*, since these measurements incorporate high gopher activity on these plots that occurred between early April and the time of sampling. At the time of belowground sampling, measurements of aboveground biomass were made by harvesting plant material in a 10.16-cm-diameter ring , and drying, and weighing it.

Soil AM fungal hyphae, non-mycorrhizal fungal hyphae, and roots

Hyphae were extracted from a 4 g soil subsample by an aqueous extraction and membrane filter technique modified after Jakobsen et al. (1992), as described in Rillig et al. (1999). Soil samples were mixed and suspended in 100 mL of deionized water, to which 12 mL of a sodiumhexametaphosphate solution (35 g L^{-1}) was added. The soil suspensions were shaken for 30 s (end-over-end), left on the bench for 30 min, and then decanted quantitatively through a 38 μm sieve to retain hyphae, roots and organic matter. The material on the sieve was sprayed gently with deionized water to remove clay particles, and then transferred into a 250 mL Erlenmeyer flask with 200 mL of deionized water. The flask was shaken vigorously by hand for 5 s, left on the bench for 1 min, and then a 2 mL aliquot was taken and pipetted onto 25 mm Millipore filters. The material on the filter was stained with 0.05% Trypan Blue in lactoglycerol and transferred to microscope slides. Hyphal length was measured with a grid-line intersect method at 200 × magnification, distinguishing hyphae into mycorrhizal and non-mycorrhizal hyphae according to Rillig et al. (1999).

Roots were extracted from 10-g soil samples by floatation and wet-sieving. Soils were suspended in 1 L of water in a beaker and stirred vigorously; the floating roots were decanted onto a 0.50 mm sieve, rinsed, and picked with forceps. This process was repeated until no further roots were retained on the sieve. Root weight was measured after drying over night at 105 °C. Root lengths were measured using the WinRhizo V 3.10B root image analysis system (Régent Instruments Inc, Québec, Canada).

Glomalin

Glomalin extractions from whole-soil subsamples were carried out as described by Wright and Updahyaya (1998). Easily-extractable glomalin (EEG) was extracted with 20 mm citrate, pH 7.0 at 121 °C for 30 min. Total glomalin (TG) was extracted with 50 mm citrate, pH 8.0 at 121 °C in rounds of 60 min each. For the sequential extractions, the supernatant was removed by centrifugation at 5000 × *g* for 20 min. Extraction of a sample continued until the supernatant showed none of the red-brown color typical of glomalin. Extracts from each replicate were pooled and then analyzed. After extraction cycles were completed, samples were centrifuged at 10000 × *g* to remove soil particles, and protein in the supernatant was determined by ELISA using the monoclonal antibody MAb 32B11 (Wright and Upadhyaya, 1998). Immunoreactive fractions of glomalin are designated by the prefix IR, hence we obtained the two glomalin fractions IREEG and IRTG. We also measured glomalin using a Bradford assay (Wright and Upadhyaya 1998), yielding the two fractions called TG and EEG (without the IR prefix). Concentrations of glomalin were extrapolated to mg/g for all four measured fractions (EEG, TG, IREEG, IRTG) by correcting for the dry weight of coarse fragments (> 0.25 mm) included in the extraction of soil.

Statistical methods

Means were compared using analysis of variance, with the factor plant species used as a fixed effect. We also tested for a block effect. Residuals were examined for normality (Shapiro-Wilks W test), and homogeneity of variances was tested (Levene's test).

Path analysis (structural equation modeling) has been used previously to test causal relationships among interacting biological factors on aggregate stability (Jastrow et al., 1998). We used the AMOS 4.01 software package (SmallWaters Corporation, Chicago, IL., USA) to design the model, and calculate path coefficients, squared multiple correlations, and model fit. To test for collinearity among independent variables we used the ridge regression procedure of the NCSS 2000 software package (NCSS Statistical Software, Kaysville, UT, USA).

Results

Effects of plant species

The block effect was never significant and was, therefore, dropped from the analysis. The proportion of $WSA_{1-2\text{-}mm}$ differed significantly (F = 4.54, P = 0.006) in the soils underneath the five plant species (Figure 1), ranging from 72 to 85% stable aggregates in this size class. Percent plant cover (F = 10.3; $P < 0.0001$) and aboveground plant biomass (F = 13.7; $P < 0.0001$) both were significantly different between plant species (Figure 2a and b). The latter result indicates that the goal to achieve similar plant biomass was not achieved in the field. Root weight (F = 9.00; $P < 0.0001$) and length (F = 9.39, $P < 0.0001$) were both significantly different as well for the five species, the pattern closely following that for water-stable aggregates (Figure 3). AMF soil hyphal length (F = 14.89; $P < 0.0001$) and non-mycorrhizal fungal hyphal length (F = 6.91; P = 0.0006) differed in the soils under the five plant species as well, but NM hyphal length exhibited quite a different pattern than AMF (Figure 4). AMF hyphal length was also always higher than NM fungal hyphal length. We only found a significant difference among the five plant species for the immunoreactive easily-extractable glomalin fraction (IREEG; F = 7.94; P = 0.0002)), not for the total immunoreactive glomalin pool (IRTG; F = 1.55; P = 0.21) (Figure 5). This pattern also held true for the protein fractions measured using the Bradford assay (EEG and TG) (Figure 6).

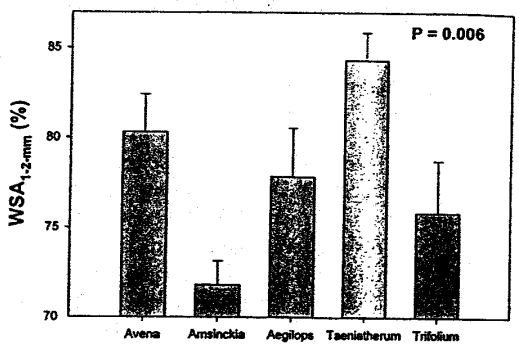

Figure 1. Effect of plant species on the proportion of water stable aggregates ($WSA_{1-2\text{-}mm}$) in the 1–2 mm size class. Plant species are (number of replicates): *Avena barbata* (n = 6), *Amsinckia douglasiana* (n = 6), *Aegilops triuncialis* (n = 6), *Taeniatherum caput-medusae* (n = 7), *Trifolium microcephalum* (n = 7). Error bars are standard errors of the mean (P-value from ANOVA).

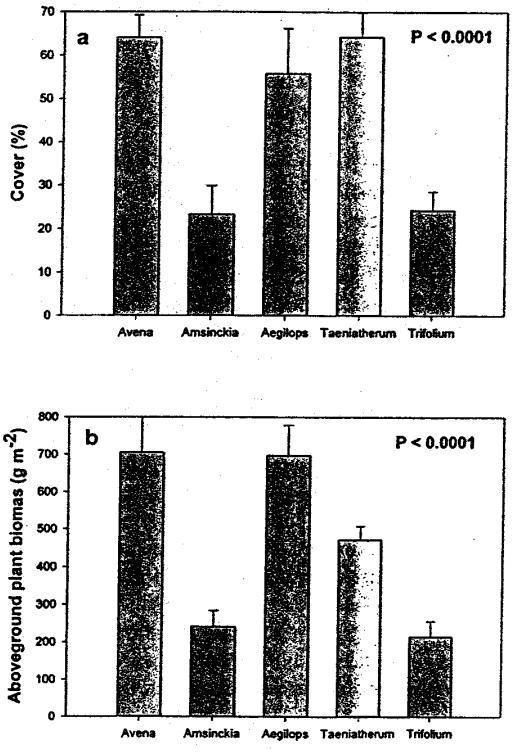

Figure 2. Effect of plant species on percent plant cover (a) and aboveground plant biomass (b). Error bars are standard errors of the mean (P-values from ANOVA). For plant species names and number of replicates see Figure 1.

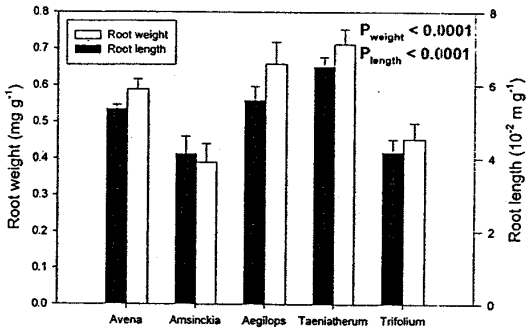

Figure 3. Effect of plant species on root weight (mg g^{-1} soil) and length (cm g^{-1} soil). Error bars are standard errors of the mean (*P*-values from ANOVA). For plant species names and number of replicates see Figure 1.

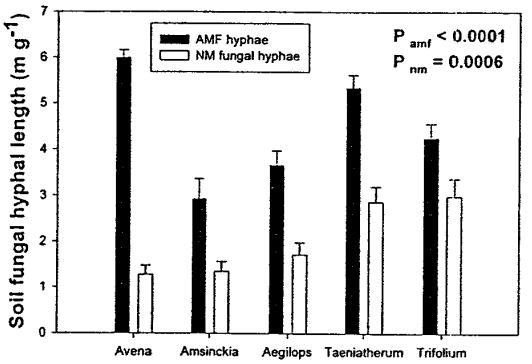

Figure 4. Effect of plant species on arbuscular mycorrhizal fungal (AMF; black bars) and non-mycorrhizal fungal (white bars) hyphal lengths (m g^{-1} soil). Error bars are standard errors of the mean (P-values from ANOVA). For plant species names and number of replicates see Figure 1.

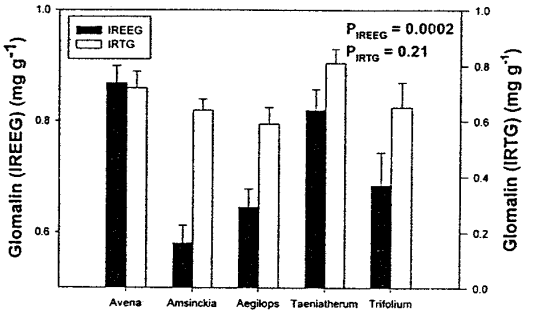

Figure 5. Effects of plant species on soil concentrations (mg g^{-1} soil) of glomalin. IREEG (black bars) is the easily extractable glomalin fraction: IRTG (white bars) is the total glomalin fraction (IR indicates that glomalin was measured by *i*mmuno-*r*eactivity). Error bars are standard errors of the mean (*P*-values from ANOVA). For plant species names and number of replicates see Figure 1.

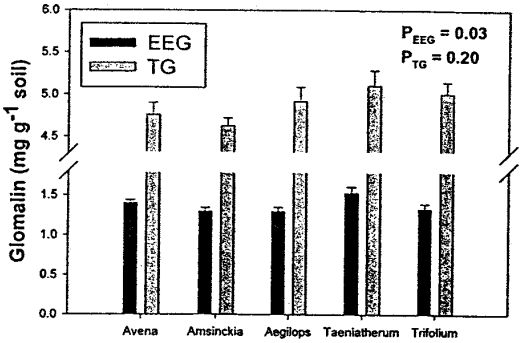

Figure 6. Effects of plant species on soil concentrations (mg g^{-1} soil) of glomalin. EEG (black bars) is the easily extractable glomalin fraction; TG (white bars) is the total glomalin fraction (these fractions were measured using the Bradford assay). Error bars are standard errors of the mean (*P*-values from ANOVA). For plant species names and number of replicates see Figure 1.

Construction of path model

Root weight and root length were highly correlated, as there was no change in specific root length among the plant species (data not shown; $F = 1.97$; $P = 0.13$). Previous studies have found a better model fit with root length (Jastrow et al., 1998), and this was also the case here; we therefore used root length in the model. Root length was causally linked with hyphal length and directly with percent $WSA_{1-2\text{-}mm}$. Percent plant cover was linked with root length and $WSA_{1-2\text{-}mm}$. The latter was included since protection of the soil surface by plant material could lessen the impact of rainwater, which could cause aggregate disintegration (e.g. Angers and Caron, 1998). We constructed a direct and an indirect path (via glomalin) from hyphal length to $WSA_{1-2\text{-}mm}$. We did not include length of non-AMF fungal hyphae in the model (Figure 4). Initially, we also included labile carbon pools in the model (carbon respired after 48 or 120 hours of incubation; data not shown), but this did not improve model fit or the multiple correlation coefficient for $WSA_{1-2\text{-}mm}$; we therefore dropped these variables from the model.

Path analysis

Almost 50% of the variability in water-stable aggregates in the 1–2 mm size class was explained by the variables hypothesized to have an effect on aggregation, as included in the path diagram (Figure 7). Product-moment correlations between the variables included in the path model are shown in Table 1.

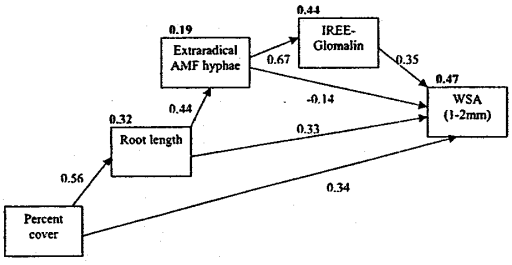

Figure 7. Path model depicting the hypothesized causal relationship of dependent and independent variables. Numbers on arrows are standardized path coefficients. Numbers in bold are estimates of the proportion of total variance explained (squared multiple correlations) for each dependent variable (i.e. all except root length). Each arrow signifies a hypothesized direct causal relationship in the direction of the arrow. Indirect causal effects occur if one variable is linked to another via other, intermediate variables. The model fit was significant ($\chi^2 = 6.61$; df = 3; $P > 0.05$).

Decomposition of correlations into direct, indirect and total effects and their statistical significance (from bootstrap analyses given by the Amos software) are shown in Table 2 for all variables in the model. Root length, percent plant cover, and IREE-Glomalin had about equally strong direct paths to $WSA_{1-2\text{-}mm}$, with 0.33, 0.34 and 0.35, respectively (all $P < 0.1$; see Table 2 for details). The direct path from extraradical hyphal length was weak (and not statistically significant). There was a strong path from hyphal length to IREE-Glomalin. Root length contributed a strong path to hyphal length (0.67). The total effect of root length on $WSA_{1-2\text{-}mm}$ is composed of the direct effect, and the sum of all the indirect effects (each obtained by multiplying the path coefficients along the direction of a causation path). The total effect of root length was 0.37. The total effect of percent plant cover on $WSA_{1-2\text{-}mm}$ was 0.55. While the direct effect of hyphal length on $WSA_{1-2\text{-}mm}$ was −0.14, the total effect, including the indirect effect via glomalin, was +0.10. The indirect path of hyphae via glomalin (0.23) was hence greater than the direct effect of hyphae on $WSA_{1-2\text{-}mm}$.

Using maximum likelihood estimation, we obtained a χ^2 of 6.61 for this model (df = 3; P = 0.09). The goodness-of-fit (χ^2) test tests the null hypothesis that the covariance matrix implied by the model (expected) reproduces the observed covariance matrix. Failure to reject that null hypothesis ($P > 0.05$) therefore indicates that the model was a good fit. We also calculated a c.f.i. (comparative fit index, ranging from 0 to 1) of 0.93, and a Tucker-Lewis Index (an index that appears least affected by sample size, ranging mostly from 0 to 1) of 0.79, further supporting that an acceptable fit of the model to the data was achieved. The model-implied and observed correlation coefficients were, as a consequence of model fit, well correlated ($r^2 = 0.75$). The independent model (observed variables are assumed to be uncorrelated with each other) fit was non-significant ($\chi^2 = 66.4$; df =10; $P < 0.0001$). We examined normality of the data by Mahalanobis distances (d^2) of individual data points from the multivariate centroid of the data set, in addition to examining kurtosis and skewness. Based on these tests, we excluded three observations from the final analysis (this lead to a reduction of n for three plant species from 7 to 6). Multicollinearity in the data set was not a problem, as examined by calculating variance inflation factors (all < 10) and condition numbers (based on the eigenvalues of the correlation matrix; all < 100).

Discussion

Our results show that plant species from the same grassland can affect soil aggregate water stability, perhaps primarily via their different biomass and percent cover. As has been found previously, grass species in our study were associated with higher aggregation than other plant types (Jastrow, 1987, Tisdall and Oades, 1979), while differences among grass species were not statistically significant (Scott, 1998). These differences among plant species provided us with an opportunity to examine which factors were responsible for bringing about these aggregation changes. Particularly, we focused on plant species differences in productivity, *versus* potential species-specific mechanisms.

Species-specific versus productivity-related mechanisms

All of the examined plant species were host plants for AMF (Rillig, unpublished). While AM fungi are mostly believed to be non-host specific, there is a preferential association of mycobionts with certain plant hosts (Bever et al., 1996). AMF can differ in a variety of physiological and ecological traits, for example in hyphal production (Giovanetti and Gianinazzi-Pearson, 1994), production of glomalin per hyphal length (Wright et al., 1996), and promotion of aggregate stability (Schreiner and Bethlenfalvay,

Table 1. Pearson's product-moment correlations between variables included in the path model (Figure 7)

	Cover (%)	Root length	AMF hyphal length	IREEG	WSA (%)
Cover (%)	1.00				
Root length	0.56	1.00			
AMF hyphal length	0.42	0.44	1.00		
IREEG	0.48	0.52	0.66	1.00	
WSA (%)	0.60	0.61	0.36	0.57	1.00

1995). Therefore it is conceivable that the different host plants, being colonized by different subsets of the AMF community, could give rise to species-specific changes in aggregate stability. However, species-specific mechanisms determining aggregation did not appear to be important in our study. Although these species differed in C inputs (data not shown), our path analysis indicates that these did not contribute to the aggregation patterns. The five plant species did also not differ significantly in our estimate of gross root architecture, i.e. specific root length; however, they have been shown to be associated with very different root growth rates and root distributions (Peters, 1994).

The alternative hypothesis is that the identity of the species does not matter, but rather their relative productivity. This hypothesis seems to be supported by our data. For example, root length was a good predictor of hyphal length, which, in turn, was a good predictor of glomalin concentration. In a species-specific model, we would have expected to see deviations from a pattern where responses scale linearly with root biomass or length. For instance, a plant species associated with an AMF community with higher average hyphal production or glomalin production should have given rise to significantly lower path coefficients for the root length/ hyphal length – glomalin paths. There was also a relatively strong path from percent cover to $WSA_{1-2\text{-}mm}$, and cover had the strongest total effect on $WSA_{1-2\text{-}mm}$. This is to be expected if the model were essentially productivity-driven.

The path model

Our path model differs somewhat in structure from other models with a similar goal. Whereas our data came from a comparison of different plant species, data for the Jastrow et al. (1998) model was derived from the study of a chronosequence of prairie restoration. For example, we have chosen to include percent plant cover of plots as a predictor variable (to test for productivity-related effects). Jastrow et al. (1998) obtained comparable multiple correlations for aggregate stability to our study in some aggregates size classes they studied (e.g. $r^2 = 0.39$ or 0.69 for the 0.212–1.00 mm and 1.00–2.00 mm size classes, respectively). However, they achieved a higher r^2 in their consideration of macroaggregates as a whole ($r^2 = 0.88$). Their model also included soil carbon pools: soil organic carbon, microbial biomass carbon, and hot water soluble CHO carbon. The paths from these carbon pools were generally weak (the highest being 0.14 from microbial biomass carbon). Including a labile carbon pool in our path model (carbon respired after 48 or 120 hrs of incubation) did not increase model fit in our path model or the multiple correlation coefficient for aggregate stability.

The role of glomalin

We used the path modeling approach to attempt to separate, based on covariance structures, the effects of glomalin from that of the hyphae of AMF themselves (Figure 7). Glomalin, once extracted (i.e. solubilized) from soil, is clearly no longer in its native state. Hence, it is problematic to extract glomalin and simply add it back into soil to study its effect on aggregation separately from that of hyphae. Furthermore, it has been proposed that one of the modes of action of glomalin could be to lead to the formation of a 'sticky' string-bag of hyphae (Jastrow and Miller, 1997) that would stabilize aggregates. It would hence be experimentally difficult to separate the effects of hyphae from those of glomalin associated with their surfaces. Our path model suggests that the indirect effects of hyphae via the production of glomalin were stronger than the direct effect of hyphae, which, according to our model, were rather weak (and as direct effects, not significant).

Table 2. Decomposition of correlations into (standardized) direct, indirect and total effects, and their statistical significance (*P*-value; Monte Carlo analysis). Total effects are the sum of indirect and direct effects (due to rounding to three significant digits, indirect and direct effects do not exactly add up in this table). IREEG only has a direct effect. Direct effects are equal to the path coefficients shown in Figure 7

	Effects on WSA		
	Direct	Indirect	Total
Cover	0.336 (0.049)	0.213 (0.044)	0.548 (0.003)
Root length	0.335 (0.062)	0.042 (0.590)	0.372 (0.044)
Hyphal length	−0.139 (0.512)	0.234 (0.089)	0.096 (0.592)
IREEG	0.352 (0.089)	–	0.352 (0.089)

This does certainly not contradict earlier studies that have attributed a strong effect to hyphae (e.g. Jastrow et al., 1998; Miller and Jastrow 1990; Tisdall and Oades 1982), since the total effect of hyphae would include the glomalin effect. This result does, however, highlight the need for further research into the functioning of this abundant soil protein. We have only examined these relationships for one aggregate size ($WSA_{1-2\text{-}mm}$). It should be the subject of further study to test if the importance (and possibly the function) of glomalin differs at other positions in the aggregate hierarchy (Tisdall and Oades, 1982).

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