The effects of arbuscular mycorrhizas on soil aggregation depend on the interaction between plant and fungal species

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Summary

• Arbuscular mycorrhizal fungi (AMF) and roots mediate soil stabilization, although the mechanisms and how their interactions affect soil stabilization are not known. We tested the effects of specific plant–fungus combinations on aggregate stabilization, and whether hyphal length and root biomass determine stabilization, predicting that fungi producing more hyphae, and plants with higher root biomasses, would better stabilize soils.

• The percentage of water-stable aggregates ($\%WSA_{1-2\ mm}$), hyphal lengths, and root biomass were measured from a five AMF × nine plant factorial experiment. Arbuscular mycorrhizal fungi with greater extradical mycelium production were represented by the Gigasporaceae and plants of high root biomass by grasses. Other taxa represented lower hyphal lengths and root biomass.

• An interaction between symbionts with respect to $\%WSA_{1-2\ mm}$ was observed. Root biomass and total hyphal lengths were not positively correlated with %WSA. Combinations of grasses with Gigasporaceae fungi had the lowest %WSA.

• Mechanisms underlying aggregation were not elucidated by measuring root biomass and total hyphal lengths alone, suggesting other physiological or architectural mechanisms may be responsible.

Key words: arbuscular mycorrhizal fungi, erosion, plant–fungus interaction, soil aggregation, water stable aggregates.

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Introduction

Arbuscular mycorrhizal fungi (AMF) are functional components of terrestrial ecosystems world-wide. These fungi in the phylum Glomeromycota (Schüssler *et al.*, 2001) form symbiotic relationships with the majority of land plants. Among the benefits AMF confer to their plant hosts are enhanced mineral nutrition (Smith & Read, 1997) and greater root-pathogen resistance (Newsham *et al.*, 1995). In exchange, the obligate biotrophic fungi receive carbon. These effects at the scale of the individual plant can influence processes at the scale of the ecosystem through their ability to aggregate soils (Rillig, 2004b).

Soil aggregation is a complex, hierarchical process mediated by both biotic and abiotic factors (Tisdall & Oades, 1982). Aggregation is essential to maintaining soil porosity, allowing gas exchange and water infiltration, and facilitating biogeochemical cycling (Diaz-Zorita *et al.*, 2002). Soil structure is also crucial to the success of sustainable agriculture and erosion resistance. Over one-third of the world's arable land was damaged by erosion over the last 40 yr (Pimentel *et al.*, 1995), and much of the focus of sustainable agriculture has shifted towards managing for well-aggregated soils.

Hyphae of AMF are considered to be primary soil aggregators for several reasons: the extraradical hyphae of AMF have a significant biomass in most soils (Rillig & Allen., 1999), as obligate biotrophs these fungi do not need to compete with saprobes for soil carbon and AMF hyphae are more resistant to fungivory than saprobic fungi (Klironomos & Kendrick,

1996). Arbuscular mycorrhizal fungi may stabilize soils up to 5 months after their host's death (Tisdall & Oades, 1980). A positive correlation between AMF hyphae and aggregate stabilization in natural systems is described by Miller & Jastrow (1990) and Jastrow *et al.* (1998). Rillig *et al.* (2002) described significant indirect effects of AMF hyphal length on water-stable aggregate (WSA) stabilization via the production of glomalin-related soil protein (GRSP) in a natural grassland system. Arbuscular mycorrhizal fungi showed similar results on the five plant hosts used, but as in the other grassland studies no AMF species involved were described (Rillig *et al.*, 2002).

Little is known about the effects of different AMF taxa on aggregate stabilization. Schreiner *et al.*, 1997) tested the WSA-forming ability of three AMF species on soybean (*Glycine max*). The authors found that *Glomus mosseae* stabilized aggregates in the 2–4 mm size class significantly more than *Glomus etunicatum* and *Gigaspora rosea*, but there were no differences between species in the 1–2 mm or 0.25–1 mm size classes. In natural grasslands, Miller and Jastrow (1992) found a correlation between spore densities of *Gigaspora gigantea* with %WSA, but not densities of *G. etunicatum*.

Plants with dense, fibrous root systems (such as grasses) assist aggregate formation (Oades, 1993; Amézketa, 1999). Similarly, hyphal characteristics may contribute to aggregation ability. An AMF with dense hyphal clusters may hold soil particles together better than diffuse hyphae, but this hypothesis has never been tested. These mechanisms of aggregation may be, like other AMF characters, species dependent and this has given rise to our hypothesis that AMF species exist that are particularly adept at soil aggregation. The idea of an aggregation 'specialist' is attractive to agriculture as well as to applications in ecosystem restoration. If a species of AMF promoted WSA stabilization independent of plant host or soil type, it could be used to inoculate crops or other soils with poor water aggregate stability.

Arbuscular mycorrhizal fungal effects can run the gamut from mutualist to parasite depending on its host plant. Klironomos (2003) illustrated that the effects of a single AMF on many hosts ranged from greatly stimulating shoot biomass to drastically reducing it. We propose that these types of interactions may also manifest in differential amounts of soil aggregation. If the effects of an AMF species vary widely from host to host, then the search for an applicable aggregation 'specialist' may be complicated. If a certain AMF interacts with specific hosts to strongly promote aggregation, AMF–host combinations could be customized to yield the highest aggregate formation.

Overall, little is known about the effects of species of AMF on soil aggregation in natural ecosystems or agroecosystems. We know of no study in which the ability to promote stable soil aggregation has been compared among several hosts and fungal species combinations. This is the focus of the present study. Importantly, many studies have used biological material that was not derived from the same soil and ecosystem. The existence of intraspecific variation in AMF with respect to their ecosystem origin casts doubts on the degree of ecological realism of such studies (Klironomos, 2003). This experiment takes advantage of co-occurring inhabitants of a long-term mycorrhizal research site to study possible effects of plant–fungi interactions on WSA formation. To help explain the mechanism of aggregate stabilization we measured root biomass and hyphal lengths. As these variables are thought to be major determinants of stabilization, correlation between these and aggregate percentage could predict which fungi–plant combinations are most suited to form WSAs.

We tested the following hypotheses:

1 Fungi of the family Gigasporaceae will be better soil aggregators independent of their plant host compared with non-Gigasporaceae members, as this family produces greater hyphal lengths and denser hyphal clusters (Hart & Reader, 2002).

2 Plants in the Poaceae (grasses) will be better soil aggregators independent of their AMF partner, as grasses have more fibrous root biomass to ensnare soil particles (Amézketa, 1999).

3 There will be three levels of aggregation based on the above assumptions. The combinations of grasses with Gigasporaceae fungi will yield the most WSAs, followed by the combinations of Gigasporaceae fungi and non-grasses and grasses and non-Gigasporaceae fungi, finally the lowest percentage of WSAs will be found in pots with the combination of non-grasses and non-Gigasporaceae fungi.

Materials and Methods

Experimental design and materials

The plant and AMF species used in this experiment are listed in Fig. 1. All organisms were collected from the Long-Term Mycorrhiza Research Site (LTMRS) at the University of Guelph, Ontario, Canada (43°32′30″ N, 80°13′00″ W). Plant seeds were collected from May to September 2000 and stored under dry conditions at 4°C before the experiment. The five AMF species were isolated from the soil by first allowing them to sporulate in trap cultures containing *Allium porrum* L. cv. Giant Musselburgh, and then using single AMF spores to start single species cultures (Brundrett *et al.*, 1996). For a period of approx. 4 yr before setting up the experiments described below; all the AMF were grown in dual-pot culture with the host *A. porrum* under similar glasshouse conditions. During that time, AMF were subcultured at 3-month intervals to keep the cultures clean and viable.

This experiment was performed using a (five AMF × nine plants) factorial design. The AMF factor consisted of one of five AMF species (Table 1). The plant factor consisted of one of nine plant species (Table 1). Each treatment combination consisted of 10 replicated units, making a total of 450 experimental units. Each unit was positioned in a completely randomized design on benches in a glasshouse. The experiment ran from May 2001 to April 2002 under ambient light

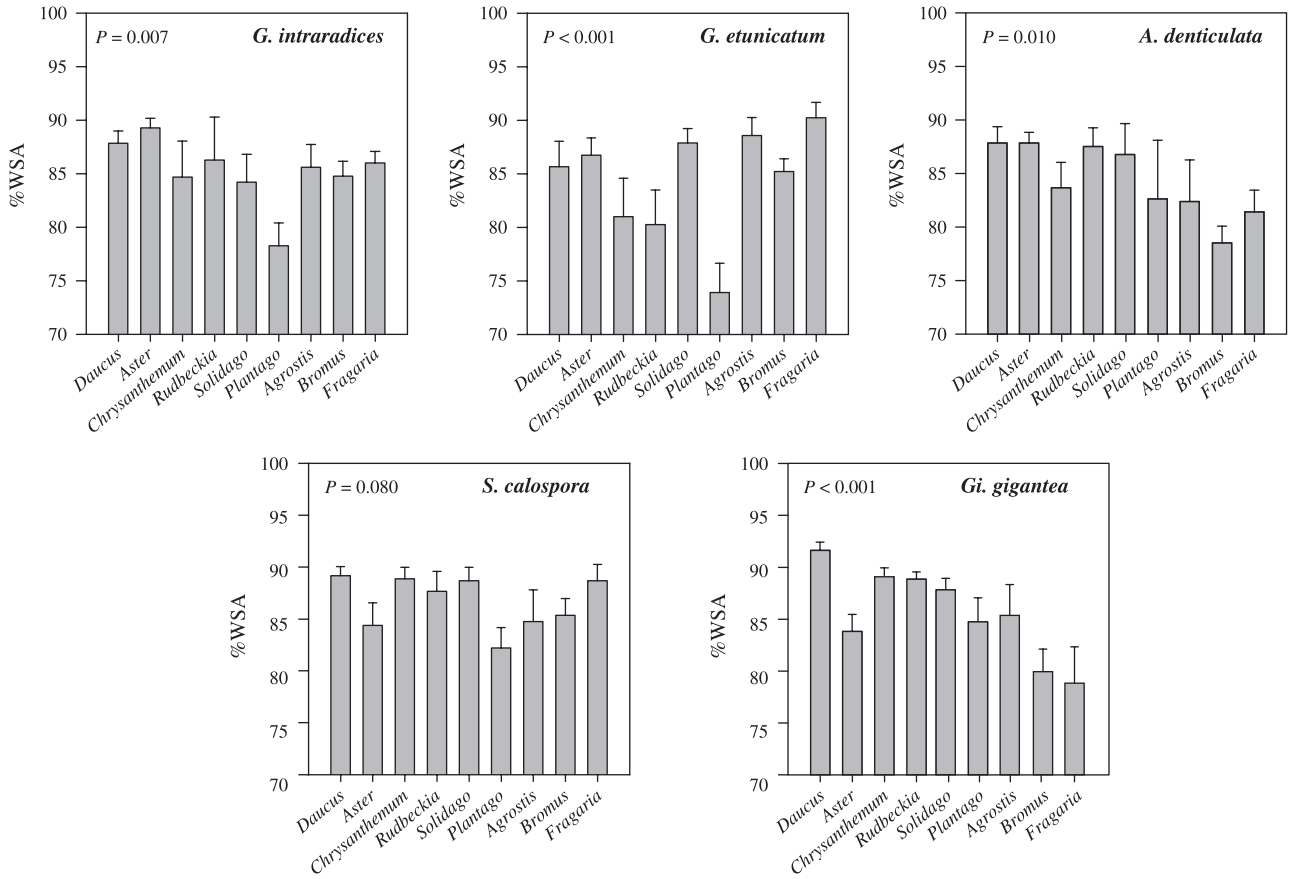

Fig. 1 Mean percentage (+ SE) of 1–2 mm water-stable aggregates (WSA) containing one of five arbuscular mycorrhizal fungal (AMF) isolates (*Acaulospora denticulata*, *Gigaspora gigantea*, *Glomus etunicatum*, *Glomus intraradices*, *Scutellospora calospora*) in combination with nine plant species. *P*-values are from Kruskal–Wallis tests. Standard errors shown are not computed on the log-transformed scale used in analysis of these data.

Table 1 Species and families of arbuscular mycorrhizal (AM) fungi and plants used in this study

Plant family	Species
Apiaceae	*Daucus carota*
Asteraceae	*Aster novae-angliae*
	Chrysanthemum leucanthemum
	Rudbeckia hirta
	Solidago canadensis
Plantaginaceae	*Plantago lanceolata*
Poaceae	*Agrostis gigantea*
	Bromus inermis
Rosaceae	*Fragaria virginiana*
Fungus family	
Acaulosporaceae	*Acaulospora denticulata*
Gigasporaceae	*Gigaspora gigantea*
	Scutellospora calospora
Glomaceae	*Glomus etunicatum*
	Glomus intraradices

conditions, 22.1°C/16.7°C average day/night temperatures, and 48.5%/74.2% average day/night relative humidity.

Each experimental unit consisted of a single pot (15 cm deep, 60 cm long) containing sterile field soil, AMF inoculum and an individual plant. The sandy-loam soil was collected from the LTMRS (total nitrogen (N) = 80.5 mmol kg^{-1}; total phosphorus (P) = 6.9 mmol kg^{-1}; percentage organic matter = 5.7). Soil was autoclaved and then added to individual pots. At a depth of 2 cm below the surface of the soil, we added a band of AMF inoculum with a mass of approx. 1 g. This inoculum was composed of sheared *A. porrum* roots (precolonized by one of the AMF isolates) and approx. 100 spores. To correct for possible differences in microbial communities, each experimental unit received a 50-ml filtered washing comprised of microbial extract from every AMF isolate used (Koide & Li, 1989). Plant seeds were germinated in a growth chamber at 20°C on moist filter paper. Individual seedlings were then transferred to the pots. We initially added two seedlings, but after 1 wk we removed one plant. The remaining plant in each pot was left to grow for a period of 1 yr. In some experimental units, the second plant died during the course of

the experiment, so these units were removed from the final analysis. Removal of these dead plants from this analysis is valid because the treatments (plant × fungus combination) themselves did not cause the plants to die. All plants were watered every 2 d or as needed with deionized water. They were also fertilized once per week with a modified Long-Ashton Nutrient solution (half-strength P; Hewitt, 1966).

Plant and fungal measurements

At the end of the experiment, plant shoots and roots were harvested. Plant material was then dried at 60°C for 48 h and then weighed to determine biomass. Only root biomass is reported here. Before drying, a subsample of roots was taken from each pot and stored in 50% ethanol. This subsample of roots was then cleared in 10% potassium hydroxide, and stained with Chlorazol Black E (Brundrett *et al.*, 1984) to confirm the presence of AMF structures. In all experimental units, plants were colonized by AMF (data not presented). Extraradical hyphal lengths were estimated by extracting hyphae from two 5-g portions of soil (Miller *et al.*, 1995) and measuring lengths by a gridline-intersect method. Hyphal length (m g^{-1} dry soil) was calculated as in Newman (1966). The hyphae of nonmycorrhizal fungi were distinguished from those of arbuscular mycorrhizal fungi by careful observation of characters normally missing in the latter (melanization, clamp connections or regularly septate hyphae).

Percentage of water-stable aggregate (%$WSA_{1–2\ mm}$) measurement

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 & Rosenau, 1986). Replicate samples of soil aggregates were moistened by capillary action for 10 min. The 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) is calculated using the mass of aggregated soil remaining after wet sieving (5 min) 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) included in the soil samples.

Statistical analysis

All analyses were conducted with SPSS version 10.0 (SPSS, Chicago, IL, USA) or Number Cruncher Statistical Software (NCSS, Kaysville, UT, USA). Univariate analyses of variance (ANOVA) were used to examine the effect of the two factors plant species and fungus type on soil aggregates (WSA), root biomass and hyphal length. Three *Bromus inermis* treatments had unusually high root biomass and were pot bound. Because of these potentially confounding pot-size effects, models were run with and without the three *Bromus inermis* plant treatments grown with the fungi *G. intraradices*, *G. etunicatum* and *Acaulospora denticulata* (BI3). In this way, a total of six ANOVAs were performed. Tukey–Kramer HSD or Kruskal–Wallis *z*-test was used for *post hoc* comparisons, where applicable.

For each of the three responses, the three sets of contrasts outlined earlier on the plant–fungus treatment combinations were also estimated. These contrasts were chosen to represent the following comparisons:

1 Grasses vs non-grass plant types
2 Gigasporaceae vs non-Gigasporaceae fungi types
3 An ordering of treatments as: (grass–Gigasporaceae) > (non-grass–Gigasporaceae, grass–non-Gigasporaceae) > (non-grass–non-Gigasporaceae)

This third contrast was examined in two parts, by first comparing the (grass–Gigasporaceae) treatments with the middle treatments and then the middle treatments with (non-grass–non-Gigasporaceae).

Univariate ANOVAs of WSA, root biomass and hyphal lengths on the factors plant species and fungus type both with and without the *B. inermis* (BI3) treatments were performed. To correct the variance heterogeneity in WSA percentages and root biomass, the log-transformed variables log(100 − WSA) and log(root biomass), respectively, were used in all analyses, and a square-root transformation was used for hyphal lengths. Root biomass and hyphal length were initially considered as covariates in the WSA ANOVA but added nothing significant to the model ($P = 0.819$ and $P = 0.768$, respectively).

Results

Soil aggregate water stability (%$WSA_{1–2\ mm}$)

Factor effects and contrast effects are summarized in the first two columns of Tables 2 and 3. There are significant interactions between plant and fungus species in their effects on WSA ($P < 0.001$) and between plants ($P < 0.001$) but no significant differences among fungus species ($P = 0.253$) whether the interaction is included in the model or not (Table 2). There are significant differences in soil aggregation between grasses and non-grasses ($P = 0.018$) where the median percentage of water unstable aggregates is 1.12 times higher for grasses than non-grasses (Tables 3, $P = 0.18$). The primary contribution to this difference occurs within the fungal type *A. denticulata* (type 3) where the median percentage of water-unstable aggregates is 1.38 times higher for grasses than non-grasses. There are mild differences in soil aggregation between the Gigasporaceae and non-Gigasporaceae (Tables 3, $P = 0.039$) where the median percentage of water unstable aggregates is 1.09 times higher for Gigasporaceae than non-Gigasporaceae. The contrasts identifying the proposed ordering of treatments indicate that the

Table 2 Main-effect *P*-values

Main effects	Models WSA (all data)	WSA (no BI3)	Root biomass (all data)	Root biomass (no BI3)	Hyphal length (all data)	Hyphal length (no BI3)
Plant type	< 0.001	< 0.001	< 0.001	< 0.001	0.011	0.004
Fungus type	0.253	0.257	< 0.001	< 0.001	< 0.001	< 0.001
Plant × fungus	< 0.001	< 0.001	0.001	0.002	< 0.001	< 0.001

WSA, water-stable aggregates. BI3, *Bromus inermis* treatment.

Table 3 Contrast effects summary

Contrast	Models Summary	WSA (all data)	WSA (no BI3)	Root biomass (all data)	Root biomass (no BI3)	Hyphal length (all data)	Hyphal length (no BI3)
Grass vs non-grass	Estimate[1]	0.115	0.007	0.258	−0.180	0.019	−0.066
	SE[1]	0.048	0.071	0.040	0.059	0.066	0.097
	P-value	0.018	0.926	< 0.001	0.003	0.775	0.498
Gigasporaceae vs non-Gigasporaceae	Estimate	0.083	0.092	0.234	0.210	−0.688	0.651
	SE	0.040	0.043	0.033	0.036	0.055	0.059
	P-value	0.039	0.034	< 0.001	< 0.001	< 0.001	< 0.001
GG > (GN, NG)	*P*-value[2]	0.967	0.755	0.212	0.979	0.0003	0.269
(GN, NG) > NN	*P*-value[2]	0.091	0.015	0.958	> 0.999	< 0.001	< 0.001

WSA, water-stable aggregates. BI3, *Bromus inermis* treatment. GG, Grass, Gigasporaceae; GN, Grass, non-Gigasporaceae; NG, non-Grass, Gigasporaceae; NN, non-Grass, non-Gigasporaceae.
[1]Estimates and standard errors (SE) are on a log scale for 100-WSA and root biomass and a square-root scale for hyphal length.
[2]*P*-values for the two contrasts testing the ordering of treatments are one-sided.

ordering (grass–Gigasporaceae) > (non-grass-Gigasporaceae, grass–non-Gigasporaceae) > (non-grass–non-Gigasporaceae) is not present in WSAs ($P = 0.967$ and $P = 0.091$, respectively). As indicated in Table 3, mean WSA is highest for the middle group and lowest for the grass–Gigasporaceae treatments.

All fungal species except *Scutellospora calospora* had significant differences in $\%WSA_{1-2\ mm}$ across the plant hosts (Fig. 1). *Glomus etunicatum* had the lowest mean $\%WSA_{1-2\ mm}$ when grown with *Plantago*, *Daucus*, *Chrysanthemum* and *Rudbeckia* but had the highest with *Fragaria*. By contrast, *Gi. gigantea* had the highest mean $\%WSA_{1-2\ mm}$ when associated with *Plantago*, *Daucus*, *Chrysanthemum* and *Rudbeckia*, and had the lowest with *Fragaria*. Both species of *Glomus* had lowest mean $\%WSA_{1-2\ mm}$ with *Plantago*, and the Gigasporaceae species had the highest mean $\%WSA_{1-2\ mm}$ with *Daucus* (Fig. 1). The *Plantago*, *Bromus*, *Daucus*, *Fragaria* and *Rudbeckia* species had significant differences in $\%WSA_{1-2\ mm}$, depending on the AMF associate; the others did not (data not presented).

With the three *B. inermis* treatments (B13) removed, the plants factor and plant–fungus interaction remain highly significant with respect to $\%WSA_{1-2\ mm}$ ($P < 0.001$) with no differences among fungus types ($P = 0.257$), as with the full data (Table 2). However, removal of these treatments resulted in no differences in WSA between grasses and non-grasses (Table 3, $P = 0.926$). This lack of significance stems from the relatively small WSA values for the *Bromus inermis* treatments. Significant differences between Gigasporaceae and non-Gigasporaceae treatments remain with the median percentage of water unstable aggregates 1.10 times higher for Gigasporaceae ($P = 0.034$). Finally, based on the *P*-values in Table 3, there is no evidence of the proposed ordering, with the smallest $\%WSA_{1-2\ mm}$ mean values again residing in the grass–Gigasporaceae treatments (Table 4).

Root biomass

The variability in root biomasses across treatments is presented in Fig. 2. Factor effects and contrast effects are summarized in the middle two columns of Tables 2 and 3. There are significant interactions between plant and fungus species in their effects on root biomass (Table 2, $P = 0.001$), between plants ($P < 0.001$), and among fungus species ($P < 0.001$). There are significant differences in root biomass between grasses and non-grasses where the median root biomass is 1.29 times higher for grasses than non-grasses (Tables 3, $P < 0.001$). There are significant differences in root biomass between fungi of types Gigasporaceae and non-Gigasporaceae ($P < 0.001$) where the median root biomass is 1.26 times lower for Gigasporaceae than non-Gigasporaceae ($P < 0.001$). The main contributors to this difference were *Plantago lanceolata* and *B. inermis*.

Table 4 Grass/Gigasporaceae group means

Treatment groups	Models WSA (all data)	WSA (no BI3)	Root biomass (all data)	Root biomass (no BI3)	Hyphal length (all data)	Hyphal length (no BI3)
Grass–Gigasporaceae	83.81	83.81	6.709	6.709	5.517	2.658
Grass/non-Gigasporaceae	83.94	85.59	10.704	5.068	2.492	2.658
Non-grass–Gigasporaceae	86.67	86.67	4.952	4.952	4.894	4.894
Non-grass–non-Gigasporaceae	84.68	84.68	6.240	6.240	2.534	2.534
Grass	83.89	84.43	9.039	6.131	3.752	4.511
Non-grass	85.48	85.48	5.724	5.724	3.480	3.480
Gigasporaceae	86.05	86.05	5.336	5.336	5.031	5.031
Non-Gigasporaceae	84.53	84.76	7.167	6.132	2.525	2.546

WSA, water-stable aggregates. BI3, *Bromus inermis* treatment.

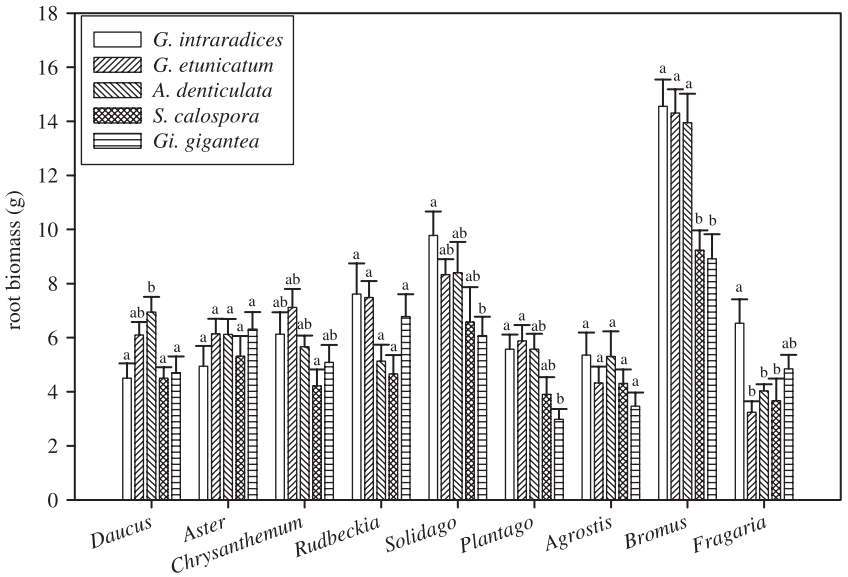

Fig. 2 Mean root biomass (+ SE) of the nine plant hosts associated with the five arbuscular mycorrhizal fungal (AMF) species (*Acaulospora denticulata*, *Gigaspora gigantea*, *Glomus etunicatum*, *Glomus intraradices*, *Scutellospora calospora*). Standard errors shown are not computed on the log-transformed scale used in analysis of these data.

With the three *B. inermis* treatments removed, the plant and fungus factors ($P < 0.001$) as well as the plant by fungus interaction ($P = 0.002$) remain highly significant, as with the full data (Table 2). However, removal of these treatments completely reversed the direction of the difference in root biomass between grasses and non-grasses. With these treatments removed, the median root biomass is now 1.20 times lower for grasses than non-grasses (Table 3, $P = 0.003$). This reversal of effect direction is caused by the very large biomass values for the *B. inermis* treatments. Significant differences between Gigasporaceae and non-Gigasporaceae treatments remain with the median root biomass 1.23 times lower for Gigasporaceae ($P < 0.001$). Finally, based on the *P*-values in Table 3, there is again no evidence of the proposed ordering, with the smallest root biomass values residing in the middle treatment group (Table 4).

Hyphal lengths

Scutellospora calospora and *Gi. gigantea* had the highest mean hyphal lengths on seven of the nine plants tested (Fig. 3). Factor effects and contrast effects are summarized in the middle two columns of Tables 2 and 3. There are significant interactions between plant and fungus species in their effects on root biomass (Table 2, $P = 0.004$), between plants ($P = 0.004$), and among fungus species ($P < 0.001$). There is no difference in hyphal lengths between grasses and non-grasses (Table 3, $P = 0.775$); however, there is a significant difference between fungi of types Gigasporaceae and non-Gigasporaceae ($P < 0.001$) with the mean square root hyphal length (m g^{-1} dry soil) of Gigasporaceae being 0.688 larger than that for non-Gigaspora treatments (Tables 3, $P < 0.001$). This difference in hyphal lengths for the two groups was highly significant within all but the *A. novae-angliae*. The contrasts identifying the proposed ordering of treatments indicate that the ordering (grass–Gigasporaceae) > (non-grass–Gigasporaceae, grass–non-Gigasporaceae) > (non-grass–non-Gigasporaceae) is present in the hyphal lengths ($P < 0.001$). This ordering can be seen through comparison of the mean hyphal lengths in column 5 of Table 2.

With the three *B. inermis* treatments removed, the plants and fungus factors ($P = 0.004$, $P < 0.001$) as well as the

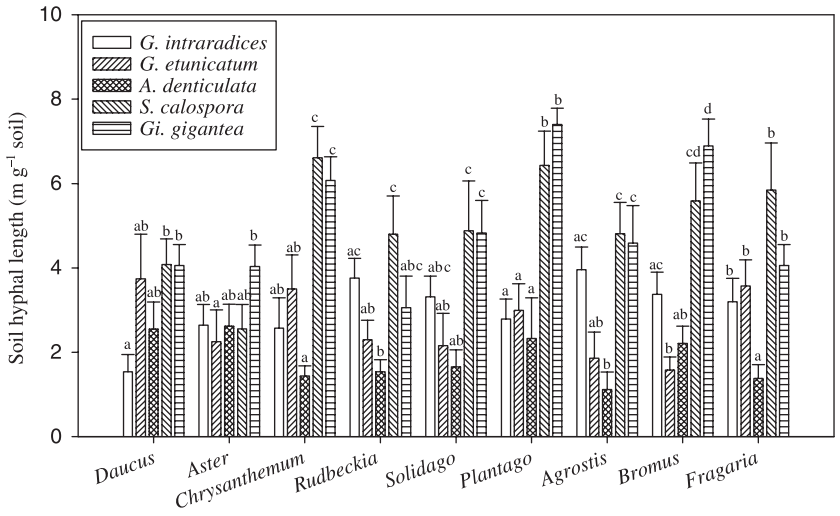

Fig. 3 Mean hyphal length (+ SE) of the five arbuscular mycorrhizal fungal (AMF) species (*Acaulospora denticulata*, *Gigaspora gigantea*, *Glomus etunicatum*, *Glomus intraradices*, *Scutellospora calospora*) associated with the nine plant hosts. Standard errors shown are not computed on the square root-transformed scale used in analysis of these data.

plant–fungus interaction ($P < 0.001$) remain highly significant, as with the full data (Table 2). There is still no difference in hyphal lengths between grasses and non-grasses ($P = 0.498$), and the mean square root hyphal length (m g^{-1} dry soil) remains significantly larger (0.651) for *Gigaspora* treatments than for non-*Gigaspora* treatments (Tables 3, $P < 0.001$). Finally, based on the *P*-values in column 6 of Table 2, although the middle group of treatments (grass–non-Gigasporaceae, non-grass–Gigasporaceae) has a significantly higher square root mean hyphal length than the (non-grass–non-Gigasporaceae) group ($P < 0.001$), the (grass/Gigasporaceae) treatment group is no longer significantly larger in terms of hyphal length than the middle group. This loss of ordering is due to the small hyphal lengths in the grass–non-Gigasporaceae removed treatments (see columns 5 and 6 of Table 3).

Hyphal lengths were negatively correlated with root biomass on *Plantago* ($P < 0.001$), *Bromus* ($P < 0.001$) and *Chrysanthemum* ($P = 0.03$). These lower root biomasses were only present in the Gigasporaceae treatments. *Gigaspora* and *Scutellospora* associated with *Bromus* had a significantly higher mean hyphal length than other fungi ($P < 0.001$); however, the root biomass on *Bromus* with both Gigasporaceae fungi was significantly lower than with the other fungi ($P < 0.001$).

Discussion

Other studies have demonstrated an interaction between AMF and host plant species with respect to host and/or fungal growth and hence net primary productivity (Adjoud *et al.*, 1996; Bever *et al.*, 1996; Eom *et al.*, 2000; Klironomos, 2003). Here, we significantly extend these findings by showing that AMF–host species combinations also differentially control the percentage of water-stable soil aggregates, and thus another major ecosystem state variable (i.e. soil structure). Although the functional significance of changes in %WSA in the range observed here is unknown, this indicates the potential for strong effects under circumstances in which aggregate stability is low (e.g. highly disturbed soil).

Previous studies established that %WSA varied between fungi associated with a single host (Schreiner *et al.*, 1997). However, ours is the first study to use multiple co-occurring plant and fungal species combinations. Klironomos (2003) showed that exotic AMF species have far different effects on their plant host than co-occurring species, and vice versa. Previous pot experiments on aggregation have used soils or fungi that are exotic to the symbionts, adding further complications (Schreiner *et al.*, 1997; Andrade *et al.*, 1998; Bearden & Petersen, 2000).

This glasshouse study contrasts with other field studies showing positive correlation between %WSA and hyphal lengths/root biomass (Jastrow *et al.*, 1998; Rillig *et al.*, 2002). Negative correlations have, however, been observed (Schreiner *et al.*, 1997). A possible explanation for the decrease in $\%WSA_{1-2\ mm}$ with grasses may be a result of our experiment's duration and the extremely high root biomass of *B. inermis*. The plants were grown in pots for a year and some *B. inermis* were pot-bound at harvest time. Such a high density of roots could have inhibited aggregate formation. The differences between the %WSA of grasses and non-grasses disappeared when the *Bromus* treatment combinations yielding extremely high root biomass were removed from the analysis. While root biomass is positively correlated with %WSA in field studies, glasshouse pot experiments must consider the deleterious effects of high root densities on %WSA formation.

In our study, in contrast to our initial hypothesis, the AMF family with greater overall hyphal lengths (Gigasporaceae) produced significantly lower %WSA. While members of the Gigasporaceae generally have more abundant and denser hyphal growth (Hart & Reader, 2002), the species used in our study yielded lower percentages of WSA than members of the Glomaceae and Acaulosporaceae. Although *S. calospora* (Gigasporaceae) hyphal lengths were greater, Jakobsen *et al.*

(1992) states they do not spread as far from the root as A. laevis (Acaulosporaceae), which could explain this difference. Soil aggregate formation may depend more on hyphal spread from the host root than on hyphal length alone. Hyphae that forage farther from the host root could form more %WSAs because a higher proportion of runner hyphae (Friese & Allen, 1991) could 'string' together more soil particles.

Our hypothesis that combinations of grasses and Gigasporaceae fungi would stabilize more aggregates than non-grass and non-Gigasporaceae combinations was not supported since the grass–Gigaspora combination had the lowest mean %WSA. This again suggests that other mechanisms mediated by the symbiont's interaction may dictate WSA stabilization rather than root biomass and total hyphal length. Given that these obvious mechanisms may not function as strongly as first thought in WSA stabilization, we must consider that other aspects of extraradical hyphae and root development could be determined by host interaction and affect $\%WSA_{1-2\ mm}$.

The AMF hyphae, like plant roots, can vary widely in their branching patterns. More highly branched hyphae or roots may be more effective in binding soil particles. Moreover, AMF species can differentially affect root branching (Norman *et al.*, 1995). Future studies should consider measurement of both root and hyphal branching.

Glomalin-related soil protein is strongly positively correlated with $\%WSA_{1-2\ mm}$ (Wright & Upadhyaya, 1998; Wright & Anderson, 2000; Rillig *et al.*, 2001; Rillig, 2004a). Production of GRSP per fungal mycelium biomass may vary as a function of AMF species (Wright *et al.*, 1996), although the AMF species used for that study did not come from the same ecosystem. However, the same pattern appears to hold up for AMF from the same ecosystem (C. Rosier and M. C. Rillig, unpubl. data). Certain hosts might differentially stimulate GRSP production in their AMF symbionts, resulting in increased WSA formation. In this experiment we could not test for this mechanism because background levels of GRSP in the soils used were high and fluxes of GRSP are generally small (M. C. Rillig *et al.* unpubl. obs.). Further, beyond a level of WSA of *c.* 80% (using the WSA measurement technique we used here), the relationship between glomalin concentration and water-stability plateaux (Wright & Upadhyaya, 1998).

We observed negative correlation between hyphal lengths and root biomass in some cases (i.e. *Bromus* with *Scutellospora*). Only gigasporacean fungi caused the decreased root biomass, and it is likely a result of their extraradical growth. Member of the Gigasporaceae generally have greater soil hyphal biomass than the other AMF families (Hart & Reader, 2002), which requires a greater carbon supply from the host.

No evidence of an AMF aggregation 'specialist' was apparent in this study; even so, species less affected by their host, which simultaneously provide overall high WSA (i.e. *S. calospora*) may be better candidates for applications in restoration. These could confer the benefit of higher WSA stabilization to a broad range of hosts in the field. While we do not suggest that field inoculation should only be carried out with one fungal species, our data lend support to the idea of using a cocktail of AMF species, a component of which could be an AMF isolate that is specifically included for promoting soil stabilization.

In situations where a specific host plant is the target, such as in production agroecosystems or in certain restoration and revegetation applications, our data strongly suggest that AMF inoculum could be specifically tailored to maximize aggregate formation. Alternatively, in restoration situations where the host plant is a variable, it is clear that host plant choice can codetermine soil stabilization together with AMF inoculum identity. We conclude that soil aggregation is a function of both the fungi and its host, ranging from a poor interaction to strongly positive, much like other AMF–host exchanges.

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