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Soil aggregation status and rhizobacteria in the mycorrhizosphere

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Abstract

Soil aggregation is a dynamic process in which plants and the soil microbiota play a major role. This experiment was conducted to determine whether the effects of mycorrhizae on the stability of water-stable soil aggregates (WSA) and on selected groups of soil microorganisms are interrelated. Soil containers consisting of four compartments were utilized. Two compartments on each side of a solid barrier were separated by a 43 μm screen that permitted the passage of hyphae, but not of roots. The roots of *Sorghum bicolor* plants were split over the center barrier, and the roots on one side were inoculated with an arbuscular-mycorrhizal (AM) fungus. This design produced mycorrhizosphere soils (M) by AM roots or hyphosphere (H) soils by AM hyphae in the two compartments on the one side of the barrier, and rhizosphere soils (R) by nonAM roots or root- and hypha-free bulk soil (S) in the two compartments on the other side. At harvest (10 wk), there were significant differences in WSA between soils in the order: M>R>H>S, and WSA stability was significantly correlated with root or hyphal length. Numbers of colony-forming units of the microflora (total bacteria, actinomycetes, anaerobes, P solubilizers, and nonAM fungi) were in general not correlated with root or hyphal length, but in some cases were significantly correlated with WSA. Bacteria isolated from the water-stable soil-aggregate fraction tended to be more numerous than from the unstable fraction. The difference was significant in the M soil for total bacteria and P solubilizing bacteria. NonAM fungi were more numerous in the unstable fraction of the M soil. The data show that the root and fungal components of mycorrhizae enhance WSA stability individually and additively in concert, and suggest that they affect microorganism numbers indirectly by providing a favorable and protective habitat through the creation of habitable pore space in the WSA.

Introduction

Rhizobacteria can promote or inhibit plant growth according to environmental conditions, host genotype or mycorrhizal status (Germida and Walley, 1996; Nehl et al., 1996), and their effects may range from promoting the development of one plant organ to inhibiting another at the same time (Andrade et al., 1995). Similarly, rhizobacteria can stimulate (Gryndler and Vosátka, 1996) or inhibit (Bethlenfalvay et al., 1997) mycorrhiza formation, while arbuscular-mycorrhizal (AM) fungi, in turn, may increase (Olsson et al., 1996) or decrease (Christensen and Jakobsen, 1993) soil bacterial populations.

The activities of soil organisms depend to a large extent on the stability of the structural matrix in which they occur. Soil water content, drying and wetting cycles, and compaction of the soil fabric by root development are well-known factors that affect the stability of soil aggregates (Emerson and Greenland, 1990; Perfect et al., 1990). Soil structure is one of the most important properties controlling plant growth (De Freitas et al., 1996), and although AM effects on the

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binding of soil to AM hyphae has long been recognized (Graham et al., 1982), most reports describing plant-AM fungus–rhizobacterium relationships do not take into account the stability of the soil matrix where these interactions occur (Schreiner et al., 1997). Soil aggregates may be assessed for their stability by wet-sieving, compaction or dispersion (Burns and Davies, 1986). Wet-sieving (Kemper and Rosenau, 1986) is a practicable method for the measurement of microorganisms in stable and unstable aggregate fractions as it is based on a physical separation of the fractions.

Mycorrhiza effects on soil macroaggregate stability have been described (Tisdall and Oades, 1982), and separate effects of mycorrhizae, AM hyphae, and nonAM roots on the mycorrhizosphere, hyphosphere, rhizosphere, respectively, have been demonstrated and related to aggregation in the (mycorrhiza-free) bulk soil (Thomas et al., 1993). Within the macroaggregates, a number of processes contribute to the formation and stabilization of microaggregates. These include bacterial activity and the deposition of microbial by-products, and are influenced by the accrual of organic matter and pore-size distribution (Miller and Dick, 1995). Microorganisms thus are a key to the dynamic process of aggregate formation and degradation (Sollins et al., 1996; Wright and Upadhaya, 1996), and differences in their distribution by aggregate size (Drazkiewitz, 1994) indicates a positive relationship between bacterial populations and metabolism and aggregate stability.

Associations between functional groups of rhizobacteria and AM fungi (Andrade et al., 1997; Meyer and Linderman, 1986; Secilia and Bagyaraj, 1987) may be influenced by preferences of these associated organisms for soil micro-environments provided by different states of soil aggregation, as has been shown for nonAM microfungi (Drazkiewitz, 1996). The purpose of this experiment was to study relationships between AM fungi, groups of rhizobacteria and soil aggregation to determine whether (1) the mycorrhiza and its root and fungal components change soil micro-organism numbers and composition, and (2) mycorrhiza-mediated changes in the soil microflora affect soil aggregation.

Materials and methods

The experimental unit and design

Plants were grown in containers made of 6-mm thick black acrylic material. The containers consisted of four compartments (9 × 15 × 20 cm each). There were two compartments on either side of a solid barrier. Two adjacent compartments were separated by screened (43 μm, Tetko Inc., Lancaster, New York) openings (12 × 17 cm). The screens were clamped between compartment walls, and the four compartments were secured tightly to each other by screws.

Plant roots were split and trained to grow into the soils of the two (inside) compartments on either side of the central solid barrier. The soil on one side was inoculated with an AM fungus; the other was not (nonAM). The screens were placed to prevent penetration by roots into the outside compartments but to permit the growth of hyphae and an exchange of the soil microflora and fauna and of the soil solution between compartments. By use of this arrangement, we obtained bulk soil (S, no roots, no AM hyphae) and rhizosphere soil (R, nonAM roots) on the nonAM side, and mycorrhizosphere soil (M, AM roots, AM hyphae) and hyphosphere soil (H, AM hyphae only) on the AM side of the central barrier.

There were nine experimental units, each containing soils of the four treatments (S, R, M and H). The soils of each treatment (compartment) were cut in two across the length of the compartment upon harvest, and each half was sampled separately for all response variables. The means of these two samples were used to evaluate the data (n = 9) by analysis of variance, correlation analysis, or Student's t-test (for two-way comparisons only). We presented actual probability values (p) instead of arbitrary probability levels ($p \leq 0.05$ or $p \leq 0.01$) where applicable, to permit the reader to interpret significance (Nelson, 1989). We may interpret differences to be biologically significant up to $p = 0.1$.

Soil and biological materials

Each compartment was filled with 1.5 L (≈1 kg) of a steam-pasteurized (75 °C, 3 h) mix of coarse sand and sandy-loam soil (1:1, v:v). The soil (pH 6.5, from the bank of the Willamette River near Corvallis, OR) contained after pasteurization (mg kg^{-1}): NH_4–N, 1.9; NO_3–N 24.1; $NaHCO_3$-extractable P, 1.0; total P, 0.5; K, 176; Ca, 88; Mg, 3.5; S, 0.8 and nonlimiting micronutrients (Central Analytical Laboratory, Dept. Crop and Soil Sciences, Oregon State University, Corvallis, OR).

The soil of the M compartment was inoculated with spores of *Glomus mosseae* (Nicol. & Gerd.) Gerd

and Trappe. The inoculum (INVAM[1] isolate # CA110) was cultured on sorghum (*Sorghum bicolor* L.), wet-sieved, and washed. Each M compartment received 150 spores placed 5 cm below the roots. Sorghum seeds were surface-sterilized (ethanol, 70%, v:v, 2 min) and germinated in vermiculite. The seedlings were selected for uniformity when the roots were approximately 10-cm long and were transferred to the growth compartments, where an equal number of roots were trained to separate over the central solid barrier and grow into the soils of the R and M treatments.

Growth conditions

Plants were grown during the fall of 1996 in a greenhouse at Corvallis, OR, and harvested 10 wk after transplanting to the four-chamber containers. The soils of the inside (R and M) compartments were watered daily with tap water and those of the outside compartments (S and H) once a week to keep all compartments at similar moisture contents. Soil surfaces were covered with Styrofoam to minimize evaporation, and the containers were covered with reflective aluminum foil as protection from direct sunlight. Soils of the outside compartments were not watered for 7 d before harvest to permit them to approximate moisture conditions of the inner, root-containing soils, where water loss was more rapid due to transpiration. Similar moisture conditions are of consequence, as soil water content at sampling can be a major source of variation for the structural stability of soils (Bresson and Moran, 1995). Sunlight was supplemented by 1000 W phosphor-coated metal halide lamps, providing 16 h of photosynthetically active radiation (450 μmole m^{-2} sec^{-1}) at soil surface level. Temperatures were kept within 18 and 21 °C by automatic controls.

Harvest and assays

The soils were removed in one cohesive clump from their compartments at harvest, were cut in two and the halves were sampled separately. Soils were crumbled, shaken from the roots where applicable, and immediately sieved through stacked 1- and 2-mm sieves. The 1 – 2 mm soil fraction was used for subsequent assays. After sieving, subsamples were immediately weighed for a gravimetric determination of soil moisture content.

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Roots were collected from the soil and subsamples were taken. Total and subsample root fresh weights were determined. The subsamples were washed, cleared in KOH solution (5% KOH, w:v, 30 min, 90 °C), and stained with trypan blue in lacto-glycerol (0.05%, 10 min, 90 °C). Total and AM root length was estimated for the subsamples by the grid-line intersect method (Giovanetti and Mosse, 1980). Total and AM root length for the entire sample was then calculated from total and subsample fresh weights. Roots were weighed after drying (2 d at 70 °C). Two samples from each treatment soil were used for a determination of AM-fungal hyphal length by the grid-line intersect method (Sylvia, 1992), as modified by Andrade et al. (1997).

Soil aggregation was determined on samples of moist soil (8 g, 1–2 mm). The samples were vapor-wetted (20 min) on sieves (3.5-cm dia., 1 mm screen) in a humidifier and then agitated in distilled water for 10 min in a sieving apparatus (stroke length 1.3 cm, 35 cycles min^{-1}) described by Kemper and Rosenau (1986). Materials that passed through the sieves were considered to be the water-unstable (WU) fraction. Materials that remained on the sieves consisted of sand grains and aggregations of small soil particles. All materials were collected in aluminum cans, oven-dried (110 °C), and weighed. After drying, the materials that remained on the sieves were separated into sand (>1 mm) and soil (<1 mm) by rubbing through 1 mm sieves. The sand was discarded, and the soil was considered to be the water-stable (WS) fraction. Water-stable soil aggregation (WSA) was calculated as a percentage of the stable and unstable materials [WSA = 100 × WS/(WU+WS)] contained in the 1–2 mm soil fraction.

Soil organism determinations were made from whole soil and from the separated WS and WU soil fractions. Samples (7 g) were taken from the whole soil (1–2 mm size fraction). The entire WS or WU soil fraction was used to sample for soil organisms before drying these soils for the weight determination. The soils were suspended in 100 mL of phosphate-buffered saline solution (Zuberer, 1994) and sonicated (4 min). Samples (1 mL) were taken from a constantly stirred suspension for dilutions. Serial dilutions (1:10) were made and aliquots (0.1 mL) were spread on duplicate plates. Plates were incubated at 27 °C and counted after 7 d. Sonicated and stirred suspensions of the WS and WU aggregate fractions were also sampled for plating, as described above, immediately after the wet-sieving process. Counts of colony forming units (cfu)

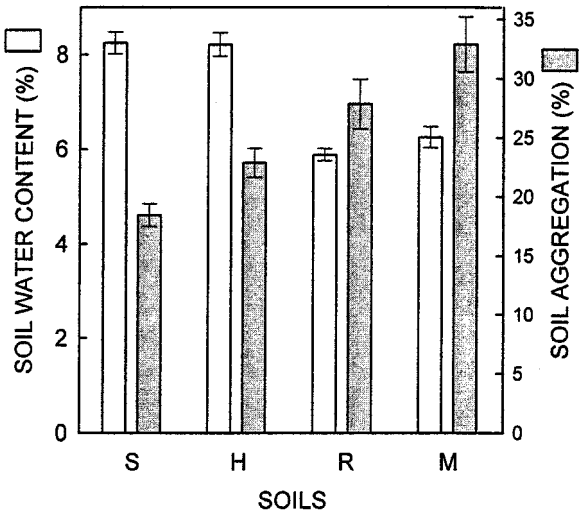

Figure 1. Soil water content and aggregate stability in arbuscular-mycorrhizal (AM) or nonAM soils. Split-root sorghum plants were grown in four-part containers. The soils in the individual compartments were permeated by (1) AM roots and AM hyphae (M), (2) AM hyphae only (H), (3) nonAM roots (R), or (4) were free of roots and AM hyphae as bulk soil (S). Values are the means of 18 samples ± SE.

were calculated per gram of dry soil by using the soil moisture data for whole soil or the oven-dried weights of the WS and WU fractions.

A nonselective medium (one-tenth strength trypticase soy agar, Kirchner et al., 1993) was used to count cfu of 'total' bacteria. Selective media were used to determine the numbers of actinomycetes (starch-casein agar, Wollum, 1982), heterotrophic anaerobic bacteria (Molongoski and Klug, 1976), phosphate solubilizers ($CaCO_3$ medium, Sylvester-Bradley et al., 1982), and (nonAM) soil fungi (Rose Bengal Agar/Streptomycin medium, Martin, 1950). Anaerobic bacteria were of interest as anaerobiosis within soil aggregates (Sierra et al., 1995) encourages their proliferation. Counts from whole soil only were made for the anaerobes, which were incubated for 10 d in BBL Gas Pak systems (Becton Dickinson Microbiology Systems, Cockeyesville, MD). Fungi and actinomycetes were chosen because their hyphal and filamentous structures have been shown to be particularly important in soil-aggregate stabilization (see Harris et al., 1966). Phosphate-solubilizing bacteria were of interest because they facilitate P uptake by AM fungi (Azcón-Aguilar and Barea, 1992) and because their activity in acidifying the soil (Illmer et al., 1995) may destabilize WSA.

Results

Soil moisture, soil aggregation and mycorrhiza

The moisture contents of the two root-free (S and H) soils were equal, as were those of the two root-containing (R and M) soils, but there was a small (≈2%) difference between the moisture contents of the two sets of soils (Figure 1). The difference was due to transpiration, resulting in lower soil water contents in the two root-containing soils. Although soil aggregate stability at the time of harvest tends to increase with decreasing soil water (Perfect et al., 1990), it is conjectural whether such a small change in the water content of this sand-amended soil could significantly affect WSA. A comparison of S vs. H soils showed an increase in WSA in the H soil by 5% (Figure 1). WSA in the M soil was greater than in the R soil by a similar amount.

Root length in R vs. M soils (Figure 2A, 2B), and AM hyphal length in H vs. M soils (Figure 2C, 2D) were significantly correlated with WSA. Increases in WSA of the H, R and M soils relative to the S soil were 24, 51, and 79%, respectively. Root and hypha effects were additive, with the slight difference between their sum (75%) and the M-soil value (79%) attributable to greater root length in M than in R soil (Table 1). The additive nature of hypha and root contributions to WSA was supported by the (statistical) equality of hyphal length in the H and M soils, indicating AM hyphal effects on WSA in these two soils were the same, and that soil moisture effects were too small to effectively interfere with mycorrhiza effects.

Soil microflora and soil aggregation

The relationship of WSA with the microflora in the soil was evaluated after separation into the stable, aggregated (1–2 mm) fraction or in the unstable, disaggregated fraction (<1 mm). The relationships were positive in all cases. For counts based on the entire soil sample, significant correlations were found between WSA and 'total' and anaerobic bacteria, and nonAM fungi (Figure 3), showing an increase in microorganism numbers with increasing WSA. Correlations of WSA with P-solubilizing bacteria and actinomycetes in the entire soil sample were not significant ($p > 0.1$) and are not shown.

Counts of 'total' bacteria and of P solubilizers tended to be higher in the aggregated soil fraction than in the disaggregated one, but they were significantly higher only in the aggregated fractions of the M

Table 1. Mycorrhiza and root traits in soils separated by screens into arbuscular-mycorrhizal (AM) and nonAM compartments

Soil	Response variables			
	Root dry weight (g)	Root length (m kg dry soil^{-1})	AM hypha (m g dry soil^{-1})	Colonization (% ±SE)
Bulk soil	0.01	not available	–	–
Rhizosphere	1.85	64.5	–	–
Mycorrhizosphere	2.57	80.8	18.1	49.6 ± 2.6
Hyphosphere	0	0	20.0	–
t-test (*p*-value)	0.022*	0.181	0.410	–

*Comparison between rhizo- and mycorrhizosphere soils only.

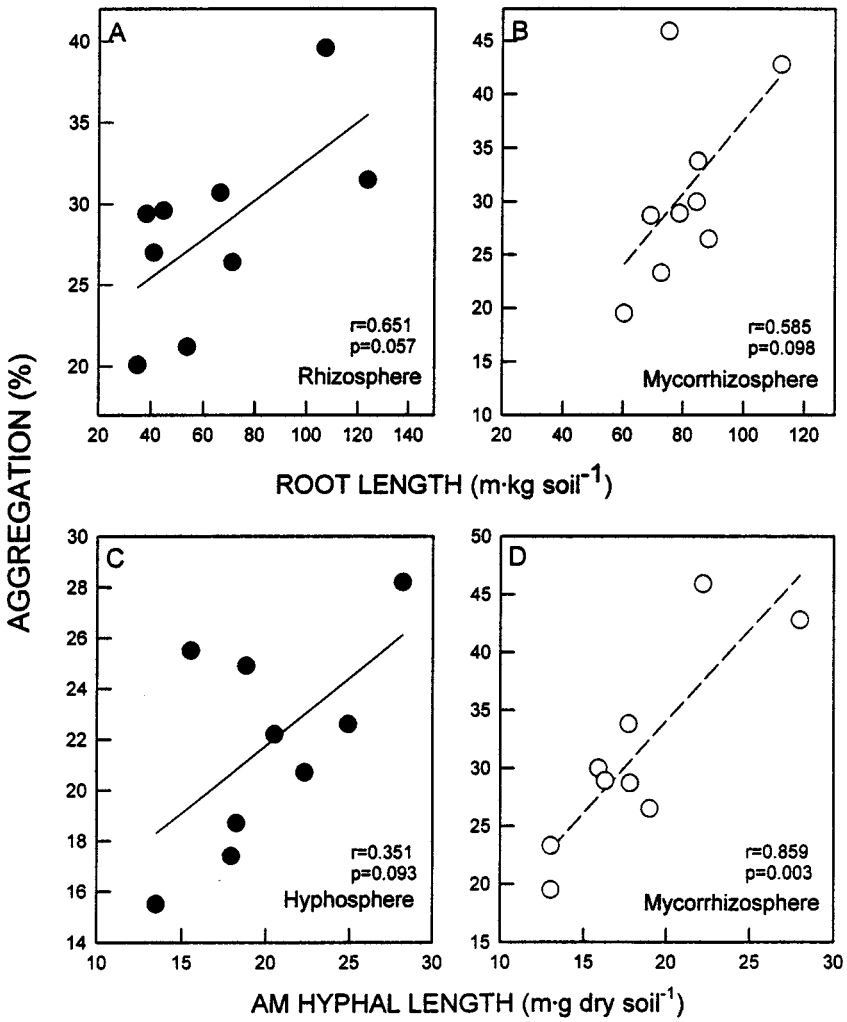

Figure 2. Relationships between arbuscular mycorrhiza (AM) development and soil aggregation. Split-root sorghum plants were grown in multi-compartment containers. Soils in the compartments were classified as rhizosphere soil (nonAM roots only), hyphosphere soil (AM hyphae only) and mycorrhizosphere soil (AM roots and AM hyphae). Each datum point represents an individual measurement.

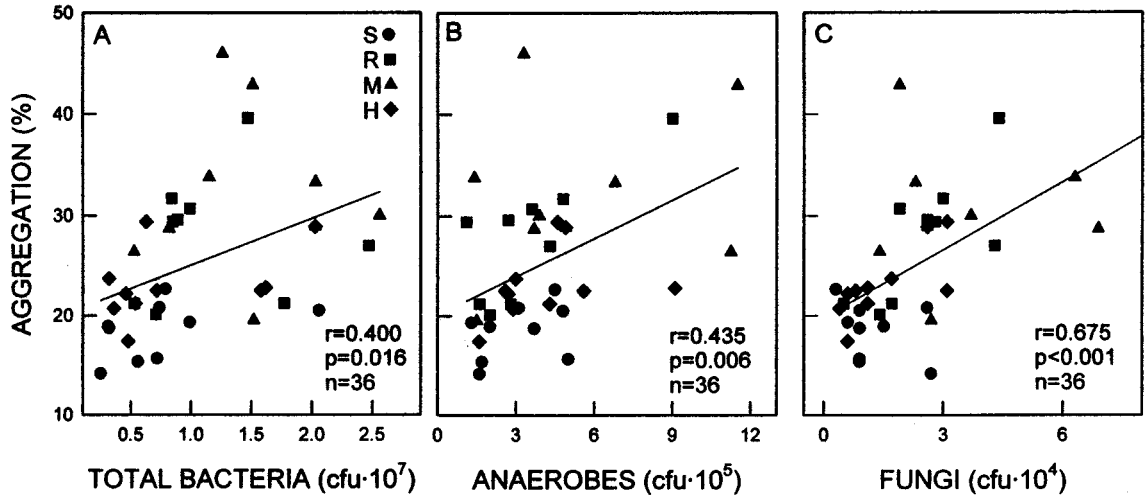

Figure 3. Relationships between soil microorganisms and aggregation. Split-root sorghum plants were grown in multi-compartment containers. The soils in the individual compartments were permeated by (1) AM roots and AM hyphae (M), (2) AM hyphae only (H), (3) nonAM roots (R), or (4) were free of roots and AM hyphae as bulk soil (S). Datum points represent the number of colony-forming units of the groups of organisms assayed. Organisms found in the different compartments are annotated by different symbols.

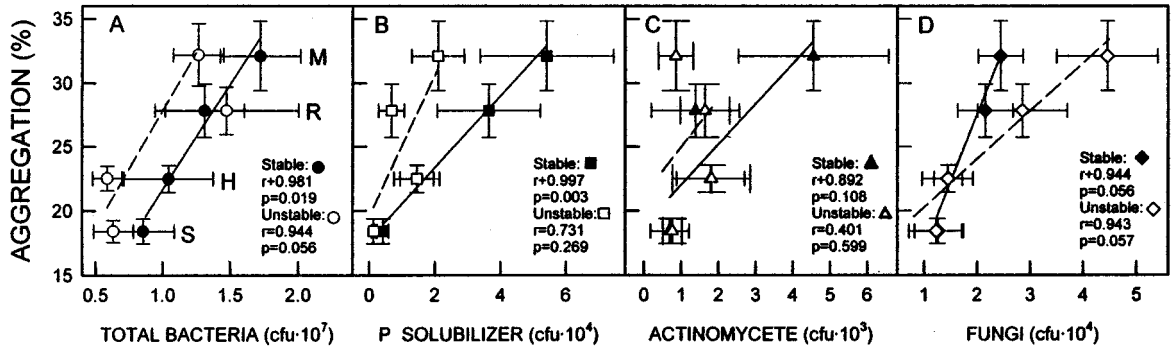

Figure 4. Relationships between soil microorganisms and aggregation in water-stable or unstable soil fractions. Values (means ± SE of 18 samples) represent data from mycorrhizosphere (M), rhizosphere (R), hyphosphere (H), and bulk soils (S).

soils (Figure 4A, 4B). The correlation between 'total' bacteria and WSA was significant for both stable and unstable fractions, while for P solubilizers the correlation was significant only for the stable fraction. There was no significant correlation between WSA and actinomycetes in either soil fraction (Figure 4C). For nonAM fungi, the correlations with WSA were significant for both soil fractions, but unlike for the bacteria, cfu were significantly higher in the unstable, rather than in the stable fraction of the M soil (Figure 4D).

Discussion

Similar increases in soil aggregate stability in hyphosphere and mycorrhizosphere soils, as compared to soils not containing AM hyphae (Figure 1), indicated that the hyphae contributed to the process of soil-aggregate stabilization independently of contributions by the roots. Hyphae of AM fungi may affect soil aggregation directly by providing the skeletal structure that physically holds soil mineral particles together, by an entanglement of the hyphae serving as a source of organic and inorganic binding agents, and by enmeshing microaggregates into macroaggregates (Miller and Jastrow, 1992). The fungi may act to improve soil structure (Tisdall and Oades, 1982) through the provision of reduced carbon to the extrarhizosphere microflora (Bagyaraj, 1984) of the hyphosphere. Aggregate stability increases with microbial biomass (Lynch, 1981), and AM hyphal growth

outside the rhizosphere extends the flux of organic nutrients from the plant to microorganisms (Jakobsen and Rosendahl, 1990). The hyphosphere is therefore marked by more intense bacterial colonization than the bulk soil devoid of AM hyphae (Foster et al., 1983) as indicated by the positive relationship between hyphal length and aggregate stability (Figure 2).

Our findings showed that even within the short time frame of this experiment, the direct, positive effects of the mycorrhiza on WSA stability were measurable, that roots and hyphae contributed to aggregate stability individually, and that their effects were additive when acting in concert. An influence of mycorrhizae on microbial contributions to WSA could not be interpreted because mycorrhiza development (root and hyphal lengths) was not correlated with microbial numbers. Since the changes in microbial numbers could not be ascribed to direct mediation by the mycorrhiza, our hypothesis that mycorrhiza-mediated changes in soil microorganism numbers would affect soil aggregation, could not be evaluated.

Significant, positive correlations of microbial numbers with WSA status, however, suggested (but did not prove) a causal relationship between these two response variables. The three-way relationship between mycorrhiza development, WSA and microbial numbers indicated that while the mycorrhiza did only enhance WSA directly, the more stable (and perhaps also larger and more numerous) aggregates that resulted could have provided a favored habitat for microbial proliferation. Such a mechanism was, in fact, indicated by the increases in microbial numbers with greater aggregate stability (Figure 4), and had been described by others in terms of 'habitable pore space' (Elliott et al., 1980). If the pore space within macroaggregates (i.e. between its component microaggregates) is of appropriate size for bacterial grazers and for fungi (Elliott et al., 1987), while pores within the microaggregates are small enough only for bacteria (Kilbertus, 1980) providing protection from predation, then an increasing preponderance of WSA within a soil may result in increasing numbers of bacteria.

The larger number of nonAM fungal propagules found at the highest level of WSA (in M soil) indicated an association of fungal sporophores with more aerobic conditions outside the WSA (Figure 4A). Greater numbers of bacteria associated with the aggregated vs. disaggregated soil fractions, on the other hand, showed that WSA created a favored environment for bacteria (see also Elliott et al., 1980). Organisms with interdependent requirements may develop their full potential to function to the benefit of the entire plant-soil system only in such protected microsites. As an example of such interdependence, we cite the relationship of diazotrophic and P-solubilizing bacteria with AM fungi, where each type of organism is the source of an essential product (N, P and C) that enhances both plant growth and soil stability.

Conclusions

The salient aspects of our findings were the lack of a direct mycorrhiza effect on total soil microbe numbers and the positive response of the soil microflora to a mycorrhiza-mediated improvement in soil aggregation. Increases in bacterium numbers with greater aggregate stability was therefore interpreted as an indirect mycorrhiza effect on soil-microbe populations, mediated through the creation of habitable pore space for bacteria. Greater numbers of soil microorganisms in aggregated than in disaggregated soil suggest that favorable growth conditions for soil microbes may enhance the survival of bacteria introduced for specific purposes, such as N_2 fixation, pathogen control, plant growth promotion or soil stabilization. A management of plant-soil systems to guarantee the dominance of beneficial organisms and of benign conditions presupposes an ever-increasing understanding of the relationships between their biotic and abiotic components.

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