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Relationships between soil aggregation and mycorrhizae as influenced by soil biota and nitrogen nutrition

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Abstract The effect of the form of N nutrition on soil stability is an important consideration for the management of sustainable agricultural systems. We grew soybean [*Glycine max* (L.) Merr.] plants in pot cultures in unsterilized soil, and treated them by (1) inoculating them with *Bradyrhizobium japonicum*, fertilizing with (2) nitrate or (3) ammonia, or (4) by providing only minimum N amendment for the controls. The soils were sampled at 3-week intervals to determine changes in water-stable soil aggregates (WSA), soil pH, the development of roots, arbuscular mycorrhizal (AM) soil and root colonization, and selected functional groups of soil bacteria. The soil fauna was assayed at the end of the experiment (9 weeks). WSA was correlated positively with root and AM soil mycelium development, but negatively with total bacterial counts. Soil arthropod (Collembola) numbers were negatively correlated with AM hyphal length. Soils of nodulated and ammonia-fertilized plants had the highest levels of WSA and the lowest pH at week 9. Sparse root development in the soils of the N-deficient, control plants indicated that WSA formation was primarily influenced by AM hyphae. The ratio of bacterial counts in the water-stable versus water-unstable soil fractions increased for the first 6 weeks and then declined, while counts of anaerobic bacteria increased with increasing WSA. The numbers of soil invertebrates (nematodes) and protozoans did not correlate with bacterial counts or AM soil-hyphal lengths. Soil pH did not affect mycorrhiza development, but actinomycete counts declined with decreasing soil pH. AM fungi and roots interacted as the factors that affect soil aggregation, regardless of N nutrition.

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Introduction

Since the pioneering work on interactions of arbuscular-mycorrhizal (AM) fungi with rhizobacteria (Barea et at. 1975; Meyer and Linderman 1986; Secilia and Bagyaraj 1987), soil aggregation (Tisdall and Oades 1979) and the soil biota (Schenck and Kinlock 1974; Warnock et al. 1982), AM fungi have come to be viewed not only as plant symbionts, but as symbionts of both plant and soil (Bethlenfalvay and Linderman 1992; Schreiner and Bethlenfalvay 1995). Such a view does not replace the phytocentric evaluation of mycorrhizae with an agrocentric one, but considers AM fungi in a larger context: as a link between plant and soil in the plant-soil continuum. Within this context, plant responses to AM fungi may be influenced, perhaps decisively, by the soil microflora and fauna with which the soil mycelia of AM fungi associate. Such soil-mediated influences vary under different cultural stresses, such as fertilization or cultivation regimes (Johnson and Pfleger 1992), and although they are being explored and reviewed at an accelerated pace (Fitter and Sanders 1992; Miller and Jastrow 1992; Linderman 1994), the AM-fungal linkage between plants and the soil food web is still poorly understood (Wardle 1995).

We investigated relationships between mycorrhizae and soil factors under different N regimes because of the adverse effects of N_2-fixing legumes on soil aggregation that are sometimes noted in the field (Albers and Wendt 1985) or under controlled conditions (Bethlenfalvay et al. 1997). The purpose of this experiment was to (1) test the hypothesis that soil acidification resulting from ammonia-based nutrition (N_2–fixation or urea fertilization) has an adverse effect on the water stability of soil aggregates, (2) investigate the effects of N nutrition on various groups of the soil biota, and (3)

determine if N effects on the biota affected soil aggregation.

Materials and methods

Experimental design and statistics

The experiment had four treatments with five replications. The experimental units, potted plants, were arranged on a greenhouse bench in a completely random manner. The N-regime treatments consisted of N input through N_2 fixation in nodulated plants, through N fertilization with nitrate-N or ammonia-N, or of minimal N input to the controls. The results were evaluated by analysis of variance (ANOVA) and analysis of covariance (ANCOVA). To determine treatment differences, we used orthogonal contrasts and linear regression to evaluate relationships between response variables. We present actual probability values instead of the traditional, arbitrary significance criteria ($P \leq 0.05$) to permit the reader to interpret significance (Nelson 1989).

Biological materials and soil

Nodulating and non-nodulating isolines of soybean seeds [*Glycine max* (L.) Merr., cultivars Harosoy 63 and L66-2470, respectively, obtained from the USDA-ARS Soybean Germplasm Collection, Urbana, Ill., USA] were germinated, selected for uniformity (root length ≈ 3 cm), planted in 1.5 l plastic pots, and thinned later (1 week) to one plant per pot. Seedlings of the nodulating isoline were inoculated with a suspension (20 ml, 10^8 cells ml^{-1}) of *Bradyrhizobium japonicum,* strain USDA 110 (USDA-ARS Rhizobium Collection, Beltsville, Md.) at planting. The N-fertilized and the control plants were non-nodulating isolines.

Pots (1.5 l) were filled with 2 kg soil (Chehalis Series, Mesic Cumulic Ultic Haploxeroll) collected (0–30 cm depth) at the Vegetable Research Farm (Willamette River flood plains near Corvallis, Ore.), Oregon State University, in mid-April. It was stored moist and unsterilized (4 °C, 4 weeks), and was comminuted by sieving (2- and 1-cm sieves), removing earthworms. At potting, it had a pH of 6.3, organic matter content of 5.6%, and moisture content of 19.1% H_2O (field capacity ~31%). The soil was a silty loam and contained 1.1 g kg^{-1} of total P. Other nutrients were (mg kg^{-1}): P ($NaHCO_3$-extractable), 56.4; NH_4^+-N, 2.2; NO_3^--N, 8.6; K, 320; Ca, 15.1; Mg, 4.5; SO_4-S, 2.0; B, 0.4; Cu, 4.0; Fe, 168.0; Mn, 14.4; and Zn, 7.8.

The AM inoculum was the native soil itself and included *Acaulospora trappei* Ames & Linderman, *Entrophospora infrequens* Ames & Schneider, *Glomus aggregatum* Schenck & Smith, *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe, and at least three other unidentified *Glomus* isolates.

Growth conditions

Plants were grown in a greenhouse at Corvallis (summer 1996, mid-day mean light intensities ≈ 800 $\mu E \cdot m^{-2}\ s^{-1}$ of photosynthetically active radiation) and harvested at 9 weeks. Temperatures varied between 18 °C and 32 °C. Plants were watered with tap water for 2 weeks. From then on, the nodulated (R-plants) and the control plants (C-plants) received a nutrient solution low in N (mM): NH_4NO_3, 1; K_2SO_4, 4; $MgSO_4$, 0.5; $CaCl_2$, 3. This solution was chosen to encourage nodulation in the R-plants and to sustain the C-plants in this N-deficient soil. The nitrate-fertilized plants (NO_3^--plants) and the ammonium-fertilized plants (NH_4^+-plants) received nutrient solutions with N concentrations (mM) at one-half strength Hoagland's solution (NO_3^--plants: $Ca(NO_3)_2$, 3; KNO_3, 2; K_2SO_4, 1; $MgSO_4$, 0.5; NH_4^+-plants: urea-$[CO(NH_2)_2]$, 4; K_2SO_4, 1; $MgSO_4$, 0.5; $CaCl_2$, 3. Since the R-plants outgrew the NO_3^-- and NH_4^+-plants by the 6th week, the nutrient solutions of the latter were adjusted to double their N concentrations.

From 3 weeks on, plants were watered daily with the same amount of nutrient solution, and twice daily during a hot spell. The small C-plants received less solution to equalize soil moisture status among all treatments. The pots were weighed during the 2 days preceding each sampling date so that watering could be adjusted to produce similar soil moisture contents and friable soil samples. Similar soil moisture content was of consequence, as its status can be a major source of variation in aggregate stability (Perfect et al. 1990; Schreiner et al. 1997).

Sampling

Soil samples were taken from each pot at 3-week intervals by non-destructively lifting the entire, cohesive root-soil complex out of the pot, using nylon meshes placed in the pots for this purpose. A wedge of soil (~80 g) was cut from each replicate, weighed, and placed in plastic zip-lock bags to maintain soil moisture content. The root-soil complex was then returned to its pot and the hole was filled with autoclaved soil.

The soil samples were gently crumbled and rapidly sieved through stacked 3.5 mm and 1 mm sieves. Crumbs larger than 3.5 mm and smaller than 1 mm were discarded after retrieval of roots from the entire sample. This 1- to 3.5-mm size fraction was placed into plastic bags immediately upon sieving and collecting of roots to minimize water loss, and was then successively divided into subsamples for the determination of soil moisture content, soil pH, water-stable soil aggregates (WSA), soil bacterial number, and soil hyphal length. Bacteria were also quantified from the separated water-stable and water-unstable aggregate fractions, providing two additional measures of the bacterial presence. Bacterial samples were processed for plate counts immediately upon availability of the soil suspensions. Subsamples for hyphal-length determination were frozen at harvest for subsequent measurement. The soil fauna and shoot dry weight was measured at the final sampling at week 9.

Soil moisture content and pH

Soil moisture was determined gravimetrically. Soil pH (5 g air-dry soil in 5 ml distilled water) was determined according to McLean (1982) on soil of the 1- to 3.5-mm size fraction. The rate of change in aggregate stability as a function of soil drying was measured in soils of potted plants that were not part of the experimental set. These data were used to ascertain if there was a significant correlation between soil moisture and aggregate stability within the range of our experimental soil moisture contents.

Soil aggregation

Samples of moist soil (7 g, 1–3.5 mm) were vapor-wetted (20 min) on sieves (5-cm diameter, 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 were essentially free of sand particles larger than 1 mm and were considered to be the water-stable (WS) fraction requiring no correction for sand content. The WU and WS materials were collected in aluminum cans, oven-dried (110 °C) and weighed. WSA was calculated as a percentage of the stable to unstable materials [$WSA = 100 \times WS/(WU + WS)$] contained in the 1- to 3.5-mm soil fraction.

Soil bacteria

Soil samples (7 g) from the 1- to 3.5-mm size fraction were suspended in 100 ml of phosphate-buffered saline solution (Zuberer 1994) and sonicated (4 min, best level of disaggregation without a

decrease in cell viability). Samples (1 ml) were taken from the constantly stirred suspension for spreading on agar plates. 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 days. Stirred and sonicated suspensions of the WS and WU aggregate fractions were also sampled for plating as described above immediately after the wet-sieving process.

A non-specific medium (one-tenth strength tripticase soy agar, Kirchner et al. 1993) was used to count colony-forming units (cfu) of "total" bacteria. Actinomycetes were enumerated on starch-casein agar (Wollum 1982) and anaerobic bacteria by the method of Kaspar and Tiedje (1994). Actinomycetes were singled out as a group because their filamentous structures are thought to be important in soil-aggregate stabilization (Harris et al. 1966). Anaerobic bacteria were of interest because low oxygen concentrations within soil aggregates (Tiedje et al. 1984) stimulate these organisms, which were therefore expected to proliferate in the WSA fraction of our soil.

Soil hyphae and roots

Hyphal length was measured by the filtration-gridline method (Sylvia 1992) as modified below. Subsamples (7 g, fresh weight) were thawed, suspended in 200 ml of a mixture of glycerol, HCl, and water (12:1:7, v:v:v), and blended (medium speed, 20 s) in a Virtis blender. After permitting the heavier particles to settle (1 min), the suspension was sieved (34 μm) to eliminate small particles, and the hyphae were transferred from the sieve to a beaker by rinsing with water. The suspension was made up to 200 ml and stirred while a 10-ml aliquot was taken. A gridded membrane filter (GN6, 0.45 μm pore size, 47-mm diameter, gridline interval 3 mm, Gelman Scientific, Ann Arbor, Mich.) was placed on a filter holder attached to a vacuum apparatus. The membrane was wetted before placing the 10-ml aliquot of the hyphal suspension on it. After filtering off the water, the hyphae were stained with 5 ml of a staining solution (0.05 trypan blue in a mixture of lactic acid:glycerol:water, 1:2:1, v:v:v, 10 min), resuspended in water to ensure a homogeneous distribution on the membrane, and then filtered again.

A drop of the staining solution was placed on the inside the cover of a small Petri dish. After removing the membrane from the filter holder, it was placed against this cover with the hyphae between the membrane and the cover. Air bubbles were removed, and the cover was placed on the Petri dish containing 1 ml of water to avoid desiccating the membrane. Hyphal length was calculated by the grid-line intersect method (Giovanetti and Mosse 1980).

The root fragments contained in the soil samples were collected at each sampling time and weighed. Total and AM root lengths were estimated by a grid-line intersect method (Giovanetti and Mosse 1980) and calculated per gram dry soil at weeks 3 and 6 and as m/pot from the entire root mass at week 9.

Soil fauna

Soil fauna numbers were determined at the final harvest after mixing soil that remained in each pot. Protozoa were enumerated on soil extract agar (Darbyshire et al. 1974) by the Soil Microbial Biomass Service, Oregon State University, Corvallis, Ore. Nematodes were extracted for 72 h from 50 g of moist soil by the Baermann-funnel method (Ingham 1994), and subsequently counted and categorized to broad functional groups based on stomal and esophageal morphology (Freckman and Baldwin 1990). Soil arthropods were enumerated after Berlese-funnel extraction (Moldenke 1994) from the remaining soil.

Results

Relationships between soil moisture and WSA

The stability of soil aggregates increased slightly as the soil lost moisture due to evapotranspiration (data not shown). Water loss was gradual in the 14–20% moisture-content range of our experiment. It was not correlated ($r = 0.455$, $P = 0.257$) with WSA and therefore unlikely to significantly influence treatment effects on WSA. Nevertheless, we recalculated WSA values using soil moisture as a covariant, which decreased treatment-related differences between the recalculated WSA values (Fig. 1A). Treatment effects on WSA were significant at all sampling times for both original and recalculated WSA (3 weeks: $P = 0.063$, 0.076; 6 weeks: $P = 0.001$, 0.002; 9 weeks: $P = < 0.001$, < 0.001, respectively). Levels of WSA were higher at all sampling times in the soils of R- and NH_4^+-plants than in those of the NO_3^-- or C-plants (Fig. 1A).

WSA, pH and mycorrhiza relationships

The lag phase of mycorrhiza development up to week 3 (Fig. 2A, C) was associated with a decline in WSA (Fig. 1A). Proliferation of AM soil hyphae (3–6 weeks) was accompanied by a commensurate increase in WSA. The rate of change of both variables declined thereafter. Thus, the pattern of WSA formation was more closely related to the development of AM soil hyphae (Fig. 2A) than to AM root colonization (Fig. 2C) or total root growth (Fig. 2D), since roots of the 3 N treatments grew most rapidly from 6 to 9 weeks. The relationship of WSA with the AM-mycelium was particularly pronounced in the soil of the C-plants, where strong hypha development following the lag phase was accompanied

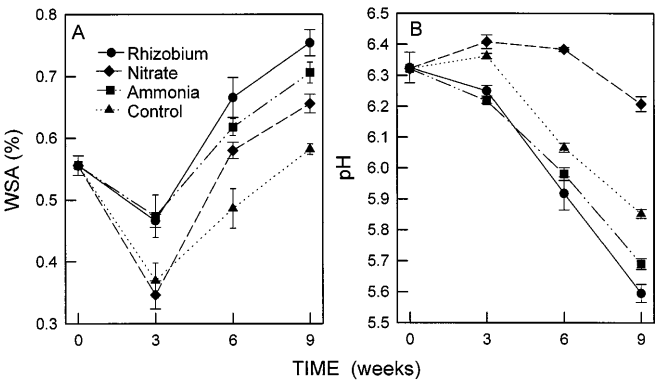

Fig. 1 Changes in water-stable soil aggregation (*WSA*) and pH in a silt-loam soil with time, as affected by plant growth. One soybean plant grew in a rooting volume of 1.5 l, and was either nodulated (Rhizobium), fertilized (nitrate or ammonia) or received only a minimal N supplement (control). Data points represent the means (±SE) of five replications

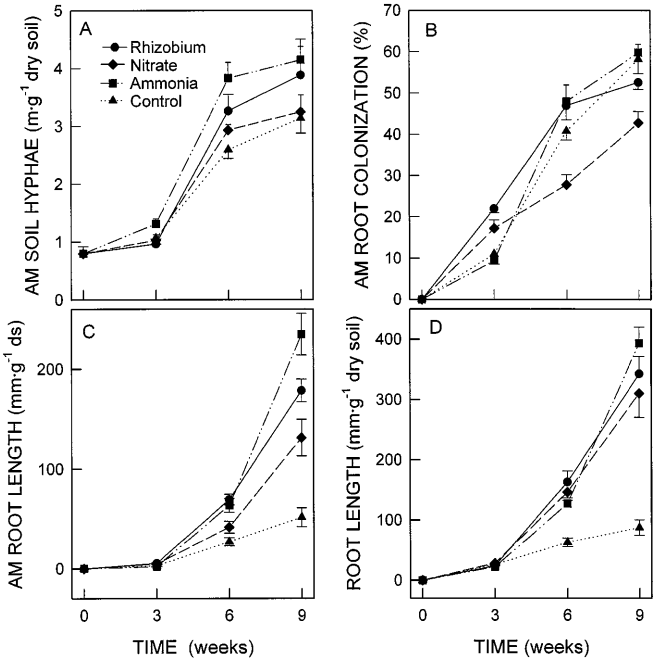

Fig. 2A–D Arbuscular mycorrhiza (*AM*) development of soybean plants in 1.5-l pots in unsterilized silt-loam soil. Soil ("extraradical") mycelium (**A**), root ("intraradical") mycelium (**B**), AM-fungus-colonized root length (**C**), and total root length was measured at 3-week intervals. Plants were either nodulated (Rhizobium), fertilized (nitrate or ammonia) or received only a minimal N supplement (control). Data points represent the means (±SE) of five replications

by poor root development. However, association of poor root growth with low WSA levels in C-plant soil showed that roots also have an effect on WSA. The characteristic sigmoid pattern of root colonization with time (Bethlenfalvay et al. 1982) was not as pronounced (Fig. 2B) as was that of the soil hyphae. It was essentially linear under all N regimes, indicating that the root ("intraradical") mycelium continued to develop when the rapid growth phase of the soil ("extraradical") mycelium had already ceased. Mycorrhiza formation was lower in NO_3^--plant roots than in the roots of the other treatments from week 3 on (Fig. 2B).

Mycorrhiza and AM-fungus development (Fig. 2A, C) and WSA (Fig. 1A) were lowest and pH (Fig. 1B) highest in the soils of the NO_3^-- and C-plants at final harvest. At week 9, WSA was positively correlated with hyphal length over the entire data set ($r=0.565$, $P=0.009$, $n=20$), even though differences in hyphal lengths between treatment means were not significant (Table 1). WSA was negatively correlated with soil pH ($r=0.432$, $P=0.057$, $n=20$). AM root length differed significantly among treatments (Table 1) and its correlation with WSA over the entire data set ($r=0.654$, $P=0.002$, $n=20$) was significant, as was that of total root length ($r=0.652$, $P=0.002$, $n=20$).

Table 1 Mycorrhizal plant and fungus traits of soybean grown under different N regimes. Plants were inoculated with *Bradyrhizobium japonicum* (Rhizobium), fertilized (nitrate or ammonia) or received minimum N (control) and were harvested 9 weeks after planting. Numbers are means of five replicates and denote values per plant (rooting volume 1.5 l)

N regime	Traits			
	AM hypha (km l soil $^{-1}$)	AM root (m)	Root (m)	Shoot (g)
Rhizobium	4.4	301	578	19.9
Nitrate	3.7	221	524	18.0
Ammonia	4.7	396	663	17.3
Control	3.5	87	148	4.9
ANOVA (probability values)				
	0.199	<0.001	<0.001	<0.001
Orthogonal contrasts (probability values)				
R vs NO_3^-	0.258	0.049	0.444	0.007
R vs NH_4^+	0.636	0.022	0.232	0.001
R vs C	0.186	<0.001	<0.001	<0.001
NO_3^- vs NH_4^+	0.118	<0.001	0.060	0.270
NO_3^- vs C	0.837	0.002	<0.001	<0.001
NH_4^+ vs C	0.081	<0.001	<0.001	<0.001

Plant-mycorrhiza relations

Poor plant growth due to N deficiency in the C-plants did not significantly decrease the development of their AM soil mycelia in comparison with plants of the three N-sufficient treatments (Table 1). Similarly, significant differences in shoot weight or total root length between the N-sufficient plants were not reflected in significant differences in the lengths of their soil hyphae. Thus, soil-hypha development was essentially independent of host-plant growth. This was not true for the development of the root mycelium: colonization of NO_3^--plant roots was significantly ($P<0.02$) lower than that of the roots of the other treatments, even though shoot mass of the NO_3^--plants was greater than that of the NH_4^+- and C-plants. At the same time, colonization of the NH_4^+- and C-plant roots was the same ($P=0.728$) in spite of the difference in their shoot masses and total root lengths. These data suggest that the development of the root mycelium was influenced by the N source and concomitant differences in N metabolism, while hyphal length was limited by the rooting volume (pot size). The N-deficient C-plants had much greater AM hyphal lengths relative to their size than plants of the other treatments, perhaps in response to the need of producing an effective nutrient-uptake organ.

Relationships between soil bacteria, soil pH and WSA

The first 3 weeks of plant growth were accompanied by a significant increase in bacterial numbers isolated from the WSA soil fraction (Fig. 3A). This phase was characterized by a change from storage at 4 °C to summer greenhouse temperatures, regular watering (with a di-

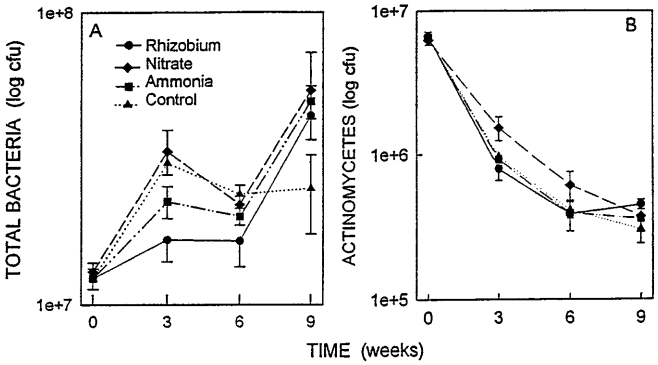

Fig. 3A, B Change in bacterial numbers with time in the mycorrhizosphere as affected by a developing plant-soil system. "Total bacteria" (**A**) denotes the colony-forming units (cfu) isolated from the soil and counted on a nonselective medium after 7 days of incubation. Actinomycete (**B**) counts were made on a selective medium. Plants were either nodulated (Rhizobium), fertilized (nitrate or ammonia) or received only a minimal N supplement (control). Data points represent the means (±SE) of five replications

lute N solution for the first 2 weeks), by relatively stable soil pH (Fig. 1B), by the lag phase of mycorrhiza development, and slow root growth (Fig. 2D). It was therefore a time of stable and benign conditions for aggregate-colonizing bacteria, with adequate levels of well-distributed soil organic matter and limited competition for nutrients by the mycorrhizae, all of which may have contributed to enhance bacterial growth. Bacterial proliferation was associated with a transient, but significant, decline in WSA (Fig. 1A). During the following 3-week period, total bacterial numbers in NO_3^-- and C-plant soils declined and stabilized in the soils of the R- and NH_4^+-plants, while AM hyphal length and root growth accelerated, WSA increased, and pH declined (except in NO_3^--plant soil). The final growth phase showed a renewed increase in bacterial numbers, a steep increase in root growth, declining hyphal growth rate, and continuing stabilization of soil aggregates. Actinomycete numbers generally declined during the entire 9-week period (Fig. 3B) along with pH (Fig. 1B) and tended to have higher in the NO_3^--plant soils that had the highest pH.

At final harvest, there were significant, treatment-connected differences in the numbers of total and anaerobic bacteria and of actinomycetes (Table 2) isolated from the whole soil (before separation into WSA and WUA fractions). Bacteria (total) were most numerous in the soils of plants fertilized with combined N (NO_3^-- and NH_4^+-plants). Actinomycetes, on the other hand, developed best in the soils of the R- and C-plants, i.e. in the absence of large amounts of combined N. Anaerobic bacterial counts, like those of total bacteria, were greatest in the combined-N treatments. Thus, anaerobiosis was not correlated with aggregate stability, as we had expected, for highest (R-plant-soil) and lowest (C-plant-soil) levels of WSA (Fig. 1A) were associated with the same low count of anaerobes.

Table 2 Bacteria in mycorrhizosphere soil of soybean grown under different N regimes. Plants were inoculated with *B. japonicum* (*Rhizobium*), fertilized (nitrate or ammonia) or received minimum N (control) and were harvested 9 weeks after planting. Bacterial counts designated as "total" relate to the establishment of colony-forming units (cfu) on a non-selective medium. Numbers are means of five replicates ±SE

N regime	Rhizobacteria		
	Total bacteria (cfu $\times 10^7$)	Actino-mycete (cfu $\times 10^5$)	Anaer-obes (cfu $\times 10^5$)
Rhizobium	5.4±0.7	3.8±0.9	1.5±0.5
Nitrate	9.2±1.1	2.1±0.2	4.0±2.0
Ammonia	7.3±1.4	2.6±0.2	5.9±1.7
Control	4.5±0.3	4.3±0.8	1.4±0.2
ANOVA (probability values)	0.009	0.045	0.047
Orthogonal contrasts (probability values)			
R vs NO_3^-	0.009	0.062	0.160
R vs NH_4^+	0.148	0.153	0.018
R vs C	0.456	0.447	0.980
NO_3^- vs NH_4^+	0.165	0.618	0.259
NO_3^- vs C	0.002	0.013	0.153
NH_4^+ vs C	0.036	0.037	0.017

The effects of microbial biomass on the processes of soil aggregate formation and N mineralization differ with aggregate size (Gupta and Germida 1988). Our unstable soil fraction (<1 mm) included the aggregate size class that had been defined as microaggregates (<0.25 mm, Tisdall et al. 1997), while the stable fraction (>1 mm) consisted of macroaggregates. Shifts in bacterial populations in our soil fractions relative to each other and with time were therefore an indicator of bacterial influence on WSA. We used the ratio of bacterial counts in the WSA versus WUA (WSA/WUA) fractions as an indicator of bacterial biomass relations with WSA: high ratios reflected increases in the number of bacteria in aggregated soil. For total bacteria, the ratios increased up to the 6-week harvest and then declined (Table 3), suggesting a limit in the capability of the aggregates to sustain bacterial growth. For actinomycetes, the ratio declined during the entire 9-week duration of the experiment, perhaps as a result of increasingly acidic and anaerobic conditions in the aggregates. Anaerobic bacteria, to the contrary, increased in the WSA/WUA fraction during the final 3 weeks in three of the four treatments.

Soil fauna

Populations of protozoans were highly variable (Table 4). Flagellates and amoebae, which tend to be located in the rhizosphere (Darbyshire and Greaves 1967) were most numerous in the soils of the NH_4^+-plants which also had the highest root lengths. Protozoa numbers did not correlate significantly with bacteria, AM hyphae or WSA ($P>0.1$). The correlaton of flagellates with total

Table 3 Relative abundance of soil bacteria in water-stable (*WSA*) and water-unstable (*WUA*) soil aggregates. Soil was planted with soybean grown under different N regimes and sampled at 3-week intervals. Plants were inoculated with *B. japonicum* (*Rhizobium*), fertilized (nitrate or ammonia) or received minimum N (control). Numbers are means of five replicates and represent the ratio of cfu of bacteria isolated from stable or unstable soil aggregates

Time (week)	Nitrogen nutrition			
	Rhizobium	Nitrate	Ammonia	Control
	——— cfu ratio (WSA/WUA) ———			
Total bacteria				
0	0.35	0.35	0.35	0.35
3	0.43	0.71	0.47	0.65
6	0.69	0.87	0.74	1.04
9	0.47	0.46	0.77	0.85
Actinomycetes				
0	0.73	0.73	0.73	0.73
3	0.73	0.83	0.85	0.86
6	0.54	0.77	0.58	0.74
9	0.41	0.47	0.42	0.44
Anaerobes[a]				
6	0.48	0.56	0.38	1.14
9	0.58	0.62	0.83	1.03

[a] 0 and 3-week data not available

Table 4 Estimated protozoa numbers in soybean mycorrhizosphere soil under different N regimes. Plants were harvested after 9 weeks of growth. Controls were not fertilized or nodulated. Numbers are the means of five replicates

N regime	Protozoans ($10^3 \cdot$g dry soil^{-1})		
	Flagellates	Amoebae	Ciliates
Rhizobium	4.2	2.0	0.22
Nitrate	5.4	4.0	0.55
Ammonia	16.7	7.3	0.13
Control	4.2	4.1	0.28
ANOVA (probability values)	0.131	0.029	0.045
Orthogonal contrasts (probability values)			
R vs NO_3^-	0.840	0.217	0.109
R vs NH_4^+	0.071	0.004	0.512
R vs C	0.831	0.207	0.623
NO_3^- vs NH_4^+	0.048	0.053	0.005
NO_3^- vs C	0.991	0.977	0.052
NH_4^+ vs C	0.047	0.056	0.259

($r=0.491$, $P=0.028$) and AM root length ($r=0.647$, $P=0.002$) was significant, while that of the amoebae was not (total root length, $r=0.287$; $P=0.255$; AM root length, $r=0.327$, $P=0.159$). Ciliates, that are generally excluded from aerated soils with low water contents, were less numerous, and tended to favor the soils of the NO_3^-- and C-plants with the shortest root lengths. Nematode populations (data not shown) were less variable than those of protozoans but did not differ significantly ($P>0.3$) among treatments for any of the functional groups measured (data not shown). Variation in Collembola populations (data not shown) were large and ranged from zero to several hundred per pot. There was no difference in Collembola numbers among treatments ($P>0.6$) but their correlation with AM hyphal length was negative and highly significant ($r=0.641$, $P=0.004$, $n=18$). The variation is ascribed to the presence or absence of egg masses in the soils of some of the replicates.

Discussion

The pattern of WSA development reflected responses to bacteria, hyphae and roots in three discernible phases. The decline in WSA (0–3 weeks) was associated with a significant, though transient, increase in bacterial numbers when mycorrhiza development was in its lag phase. Large WSA consist of small ones (Tisdall et al. 1997), and these are bound together by a labile, temporary, and easily mineralizable organic fraction (Elliott 1986). Cohesion of the small aggregates could have been weakened by the metabolic actvity of the proliferating bacterial populations (Jastrow 1996) during this first phase. In the second phase (3–6 weeks), the rapidly developing hyphal network was related to an equally rapid stabilization of WSA, probably aided by the entanglement of microaggregates (Tisdall et al. 1997) and the production of the soil-particle-binding glycoprotein, glomalin (Wright and Upadhyaya 1998). In the final phase, compaction of the soil fabric by greater root development (Emerson and Greenland 1990), especially under the space constraint imposed by the pots, may have contributed to continued WSA formation or stabilization.

Increases in total bacterial numbers in the stable versus the unstable soil fractions up to 6 weeks may be explained by an accelerated release of C and mineral growth substrates stored in the "protected" aggregated state (Miller and Jastrow 1994). Larger aggregates have higher mineralization rates (Gupta and Germida 1988), resulting in intense microbial activity within them (Elliott and Coleman 1988). A temporary increase in the numbers of aerobic organisms in the WSA following disturbance (soil sieving and potting) relative to those in small aggregates and dispersed soil (<1 mm, our WUA fraction), where nutrients were depleted by leaching (daily watering), was therefore likely. The decline in aerobic and the increase in anaerobic organisms from 6 to 9 weeks indicates a shift to anaerobic conditions (Tiedje et al. 1984) in the large aggregates: anaerobic incubation has been shown to be more effective in producing WSA than aerobic incubation (Harris et al. 1963).

Although actinomycetes are among the most effective organisms in promoting WSA (McCalla 1946), they probably did not contribute to WSA development as they declined over the entire experimental period. Their decline may have been due to soil acidification,

to which they are sensitive (Waksman 1959). Aggregates influenced by microbial polysaccharides are more stable in acid than in neutral or alkaline soils (Martin et al. 1955), an observation that was confirmed by the relationship between decreasing soil pH and increasing WSA in our experiment. Acidification also affects AM fungi which are inhibited by low soil pH (Wang et al. 1993); the decline in the rate of AM soil-hypha development and declining soil pH during the final phase of our experiment may therefore have been related.

Soil acidification (Marschner 1995) and root development are probably the variables that were most directly affected by N-treatment in our experiment. Although differences between AM soil mycelia suggested some dependence on N-related acidity effects (R- and NH_4^+-plant mycelia and NO_3^-- and C-plant mycelia were grouped and in reverse order to that of pH), treatment differences were not significant by week 9. Although AM fungi may show distinct pH preferences as individual isolates (Abbott and Robson 1991), the lack of a distinct AM pH response here is ascribed to the use of a mixed population of native AM fungi in this experiment: as some members of the community were inhibited by increasing soil acidification, others may have been stimulated. Thus, the distinct soil-hypha effect on WSA was pH-independent. On the other hand, the negative correlation between pH and WSA indicated direct relationship between pH and WSA. While low soil pH may decrease the water stability of aggregates by interfering with the bridging of clays and organic materials by polyvalent cations (Oades 1984), this effect was not observed here, for low pH tended to be associated with high stability. The literature on pH effects on WSA is ambivalent. However, in general, soil pH is thought to affect WSA positively through its effects on microbial activity (Hamblin 1991). An inhibition of bacterial proliferation slows down the degradation of organic stabilizing agents (Martin and Aldrich 1955).

The pattern of microbial populations was not correlated ($P>0.1$) with soil pH, with potential grazers (protozoans, nematodes), or with the development of the source of exudates, the roots and soil hyphae. Nevertheless, there were N-treatment-related significant differences in total bacterial counts that showed a negative relationship with WSA in the treatments of the three N-sufficient plants: highest count of bacteria and lowest WSA in the soil of the NO_3^--plants, and lowest bacterial counts and highest WSA in the soils of the R-plants. The extremely N-deficient and stunted C-plants did not fit into this pattern, as the significantly smaller rhizosphere-soil volumes associated with their lower root densities made comparisons with the other treatments tenuous. Likewise, the coincidence of low actinomycete and high anaerobe numbers in the soils of the NH_4^+- and NO_3^--plants may have been related to the availability of fertilizer N, rather than to more complex interactions with the other biota or soil reactions.

The only significant relationship between the biotic components of our experiment was the significant negative correlation between AM hyphal length and Collembola numbers. Negative effects of grazing by arthropods on AM hyphal length (Larsen and Jakobsen 1996; Warnock et al. 1982) and concomitantly on plant growth (Harris and Boerner 1990) have been reported, while an apparent stimulation of AM-hyphal growth by grazing (Finlay 1985; Klironomos and Kendrick 1995) can be rationalized by the removal of competing non-AM or senescent AM mycelia by the grazers associated with a release of nutrients.

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