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Distribution of a Population of *Rhizobium leguminosarum* bv. trifolii among Different Size Classes of Soil Aggregates†

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A combination of the plant infection-soil dilution technique (most-probable-number [MPN] technique) and immunofluorescence direct count (IFDC) microscopy was used to examine the effects of three winter cover crop treatments on the distribution of a soil population of *Rhizobium leguminosarum* bv. trifolii across different size classes of soil aggregates (<0.25, 0.25 to 0.5, 0.5 to 1.0, 1.0 to 2.0, and 2.0 to 5.0 mm). The aggregates were prepared from a Willamette silt loam soil immediately after harvest of broccoli (September 1995) and before planting and after harvest of sweet corn (June and September 1996, respectively). The summer crops were grown in soil that had been either fallowed or planted with a cover crop of red clover (legume) or triticale (cereal) from September to April. The *Rhizobium* soil population was heterogeneously distributed across the different size classes of soil aggregates, and the distribution was influenced by cover crop treatment and sampling time. On both September samplings, the smallest size class of aggregates (<0.25 mm) recovered from the red clover plots carried between 30 and 70% of the total nodulating *R. leguminosarum* population, as estimated by the MPN procedure, while the same aggregate size class from the June sampling carried only ~6% of the population. In June, IDFC microscopy revealed that the 1.0- to 2.0-mm size class of aggregates from the red clover treatment carried a significantly greater population density of the successful nodule-occupying serotype, AR18, than did the aggregate size classes of <0.5 mm, and 2 to 5 mm. In September, however, the population profile of AR18 had shifted such that the density was significantly greater in the 0.25- to 0.5-mm size class than in aggregates of <0.25 mm and >1.0 mm. The populations of two other *Rhizobium* serotypes (AR6 and AS36) followed the same trends of distribution in the June and September samplings. These data indicate the existence of structural microsites that vary in their suitabilities to support growth and protection of bacteria and that are influenced by the presence and type of plant grown in the soil.

Recently, it has been shown that changes in the microbial community composition of soils can be brought about by either abusive or improved management practices (1, 11, 12, 26, 37). However, the factors that dictate how such changes in composition occur are generally unknown. Because soil structural properties often change when management practices are modified (10), interest has been shown in the phenomenon of soil aggregation and its influence on the distribution of microorganisms and their activities throughout the soil fabric (2, 13–15, 21, 22, 29). We wished to examine the relationship between soil aggregate size and the distribution and dynamics of soil bacteria under a cover crop management system which has the potential to promote change in soil structural properties (21).

In this particular study, we chose to examine the distribution of an individual bacterial species, *Rhizobium leguminosarum* bv. trifolii. Despite being recognized primarily for its ability to form symbiotic associations with species of *Trifolium*, the bacterium is a successful soil saprophyte with the ability to support soil populations ranging from 10^8 cells g^{-1} in rhizosphere to <10 cells g^{-1} in the prolonged absence of the host plant (4). Furthermore, well-established methods for extracting populations of *Rhizobium* from soil and enumerating them with fluorescent antibodies exist (3, 8). Although several studies have examined the impact of soil pore size on the fate of introduced rhizobial strains (16, 23–25), there are no reports describing the distribution of an indigenous population of *Rhizobium* sp. or any other bacterial species across different soil aggregate size classes. The presence of red clover (*Trifolium pratense* L.) in the winter cover crop-summer vegetable crop rotational system at the North Willamette Research and Extension Center, Aurora, Oreg., provided us with an opportunity to compare the dynamics and distribution across aggregates of *R. leguminosarum* bv. trifolii under conditions in which the host legume was intermittently present each year and the presence of nonhost plants and soil cultivation provided additional physical and biological pressures on the soil ecosystem.

MATERIALS AND METHODS

Experimental site description. Soil samples were collected from a vegetable crop rotational experiment initiated in 1989 at the North Willamette Research and Extension Center on a Willamette silt loam (Pachic Ultic Argixeroll). The general characteristics of the site have been described in detail elsewhere (7). Field treatments include three winter management treatments in a vegetable crop rotation that alternates two summer crops, sweet corn (*Zea mays* L. cv. Jubilee) and broccoli (*Brassica oleracea* L. botrytis group cv. Gem) grown between May-June and September of alternate years. The three winter treatments are fallow and cover crops of either red clover (*T. pratense* L. cv. Kenland) or Celia triticale (X *Triticosecale wittmack*). The cover crops are relayed into the summer crop during late July and are grown until the following April, whereupon they are tilled into the soil. Hereafter, the three winter management treatments will be referred to as fallow, cereal, and legume, respectively. The experimental design is a randomized complete block, split-plot with four replications of each treatment. Winter cover crops are the three main treatments, and three N rates are subplots. In this study, soil samples were taken only from the subplots receiving 0 kg of N ha^{-1} to avoid the influence of N fertilizer on legume nodulation.

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Soil sampling protocol. In June and September 1994 to 1996, soil samples were collected to a depth of 20 cm from each of the four field replicate plots of each of the three winter management treatments. The soil cores were taken with a 2.5-cm-diameter tube auger, gently broken up and mixed by hand in the field, and transported to the laboratory in Ziploc bags. In June 1994, the size of the nodulating population of *R. leguminosarum* bv. trifolii was examined in whole soil samples from each replicate plot of each of the three treatments. In September 1995 and 1996, the population density of *R. leguminosarum* bv. trifolii was examined in different size classes of aggregates prepared from soil sampled after summer crops of broccoli (1995) and sweet corn (1996) had been harvested. In June 1996, we examined the population density of *R. leguminosarum* bv. trifolii in aggregate size classes prepared from soil sampled immediately after the seed bed had been prepared for planting of the summer crop, about 1 month after winter cover crop incorporation. The moisture content of the soil samples collected throughout this study period ranged between 10 and 20% (wt/wt).

Method of aggregate preparation. Our preliminary studies conducted on aggregate preparation showed that the distribution of soil among different aggregate size classes was not influenced by the soil water content at the time of sieving, provided that the latter was <15% (wt/wt). As a result, the following protocol was developed for preparation of aggregates. Field-moist soil samples were spread onto paper to a depth of approximately 1 cm and allowed to air dry for approximately 7 days in a cold room at 4°C. This treatment effectively lowered the soil water content to ≤10% (wt/wt). We presumed that slow drying in this manner would lessen any negative impact of drying on the *Rhizobium* populations. Subsamples (100 g) of soil were placed in the top of a nest of sieves and sieved for 3 min on a Tyler Ro-Tap shaker (Combustion Engineering Inc., Mentor, Ohio) into the following size classes of aggregates: <0.25, 0.25 to 0.5, 0.5 to 1.0, 1.0 to 2.0, and 2.0 to 5.0 mm. Results of preliminary experiments indicated that sieving for 3 min was sufficient to promote good separation of the different size classes (data not shown). The procedure was repeated on another 100-g portion of soil from the same field treatment. Aggregates and whole soil samples were stored in polyethylene bags at 4°C until the time of the analyses. Most-probable-number (MPN) analyses were conducted on aggregates over a 1- to 2-week period after their preparation.

MPN estimates of *R. leguminosarum* bv. trifolii populations. The population size of *Rhizobium* organisms was determined by the plant infection-soil dilution method, as described elsewhere (9). Briefly, seeds of red clover (*T. pratense* cv. Kenland) were surface sterilized, germinated on water agar, and transferred in pairs to sterile test tubes (20 by 2.5 cm) containing 20 ml of N-free mineral nutrient solution solidified with 15 g of Bacto Agar (Difco) per liter. Portions (5 g) of whole soil (or aggregates) were suspended and shaken vigorously in 47.5 ml of a mineral salts solution (per liter, NaCl [0.1 g], $CaCl_2$ [0.05 g], $MgSO_4$ [0.2 g], K_2HPO_4 [0.34 g], and KH_2PO_4 [0.16 g] [pH 6.5]). Fivefold dilution series were carried out to achieve final dilutions of $1:(1.56 \times 10^5)$, $1:(7.81 \times 10^5)$, and $1:(1.95 \times 10^7)$ of the fallow, cereal, and legume treatments, respectively. One-milliliter portions of each dilution were pipetted into each of four replicate tubes containing the red clover seedlings. The seedlings were grown in a greenhouse, as described elsewhere (9). After 6 weeks of growth, MPN values were calculated with the MPNES program (36). In June 1994, MPN determinations of the population sizes of nodulating *R. leguminosarum* bv. trifolii were carried out on samples of soil obtained from each of the four replicate field plots of each of the three treatments (12 soil samples). In September 1995 MPN estimates were carried out on composite samples of whole soil prepared by combining portions of soil from each of the four replicates of a particular field treatment (three samples per date). In the case of aggregates, portions of a specific aggregate size prepared from each of the four field replicates of a treatment were pooled, and an MPN determination was carried out on each of those composite samples (15 samples per date). In June 1996, MPN determinations of population size were made only on composite samples of each of the aggregate size classes from the legume treatment (five samples) and on composite whole soil samples from the three treatments (three samples).

Collection and analysis of isolates from the indigenous soil population of *R. leguminosarum* bv. trifolii. As many nodules as possible were recovered from plants nodulated by the highest soil dilutions of the first MPN estimate (June 1994). One hundred fifty-seven isolates (one per nodule) were obtained from the three treatments (47 to 62 isolates per treatment). In April 1996, between 8 and 10 red clover plants were recovered from each of the four replicate plots of the legume treatment. Approximately 10 nodules were obtained from each of the plants in a replicate plot, combined, and surface sterilized, and isolates were obtained by standard procedures (35). We attempted to obtain an isolate from each of about 40 nodules per replicate. We were successful in getting 120 isolates into culture with this strategy. The isolates were screened by immunofluorescence with fluorescein-labeled immunoglobulin conjugates (FAs), as described elsewhere (20).

Immunofluorescence enumeration of *Rhizobium* serotypes in soil. Bacteria were extracted and enumerated from rhizosphere and nonrhizosphere soil, as described elsewhere (8). Population densities of serotype AR18 were determined in aggregate size classes prepared from the four replicates of each of the three treatments in June and September 1996. Serotypes AS6, AS36, and AR6 were enumerated only in aggregates prepared from the four replicates of the legume treatment.

TABLE 1. Serological identity of *R. leguminosarum* bv. trifolii isolates[a]

Serotype	No. of isolates from cover crop treatment[b]		
	Fallow	Cereal	Legume
AS36	1	0	2
AS6	1	0	0
AR6	0	1	22
AR18	18	9	14
Total	20 (43)	10 (21)	38 (61)

[a] Isolates recovered from nodules formed on plants inoculated with the highest positive dilutions of soil from fallow, cereal, and legume winter cover crop treatments.

[b] Totals of 47, 48, and 62 isolates were recovered from the fallow, cereal, and legume treatments, respectively. Numbers in parentheses are the percentages of isolates from each treatment that were antigenically related to one of the four serotypes.

Enumeration of total soil bacteria. Total soil bacteria were enumerated by epifluorescence microscopy with the DNA-specific stain DAPI (4′,6-diamidino-2-phenylindole), as described elsewhere (5).

Statistical analysis. At each sampling time, the distributions of AR18 and total soil bacteria across aggregate size classes from the three treatments were analyzed by repeated-measures analyses of variance (ANOVA); aggregate size was the repeated term (28). To compare the distribution of different *Rhizobium* serotypes across aggregates from the legume treatment, individual ANOVA were conducted on each serotype by comparing the densities found in the five aggregate size classes. Main effects were separated by Fisher's least significant difference test at P of 0.05.

RESULTS

Identification of antigenically distinguishable serotypes in the soil population. Serological analysis of the *Rhizobium* isolates recovered from nodules on field-grown plants and from terminal dilutions of the MPN revealed that antigenically distinguishable serotypes existed in the Willamette silt loam. Forty-one of 120 isolates from field nodules reacted positively with an immunoglobulin conjugate to serotype AR18. In addition, a substantial percentage of isolates recovered from the MPN terminal soil dilutions of fallow (38%), cereal (19%), and legume (23%) treatments belonged to serotype AR18 (Table 1). Immunofluorescence analysis confirmed the existence of serotypes AS36, AS6, AR6, and AR18 directly in soil samples recovered from the fallow, cereal, and legume treatments (Table 2). Populations of serotypes AS6 and AS36 existed at similar densities in nonrhizosphere soil in all three treatments, whereas the population densities of serotypes AR6 and AR18

TABLE 2. Population densities of *Rhizobium* serotypes and total soil bacteria in rhizosphere and nonrhizosphere soil recovered from each cover crop treatment

Serotype	Density[a] with fallow treatment	Cereal			Legume		
		Density[a]		R/NR	Density[a]		R/NR
		R	NR		R	NR	
AS6	0.7	3.8	1.4	2.7	15	1.5[b]	10.0
AS36	5.9	12	6.9	1.7	21	7.5	2.8
AR6	1.6	11	4.3	2.5	770	19[b]	40.5
AR18	5.6	32	6.6[b]	4.8	630	60[b]	10.5
Total soil bacteria	1.4	9.8	1.9[b]	5.2	35	2.4[b]	14.6

[a] Densities of *Rhizobium* serotypes are 10^5 cells g of soil^{-1}; densities of total soil bacteria are 10^8 cells g of soil^{-1}. R, rhizosphere; NR, nonrhizosphere.

[b] Significantly different from the corresponding density of the same serotype or total soil bacteria in rhizosphere ($P < 0.05$).

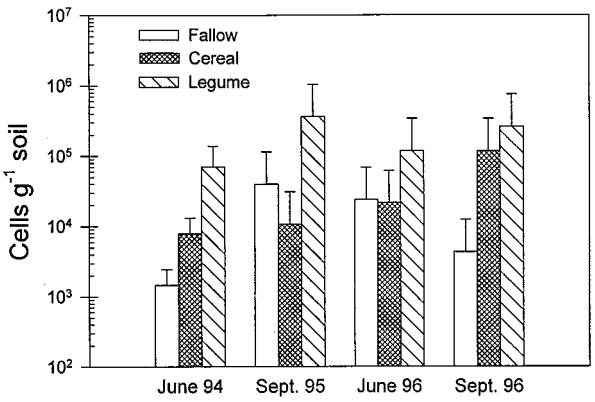

FIG. 1. Population densities of *R. leguminosarum* bv. trifolii in whole soil samples taken from the three winter cover crop treatments, as determined by an MPN procedure. For June 1994, the error bars represent the standard errors of MPN determinations conducted on each of the four field replicates of a treatment. For the other sampling times, MPN determinations were conducted on single composite samples of soil prepared by combining the four field replicates of each treatment. In the latter cases, the error bars represent the upper limits of the confidence intervals ($P < 0.05$).

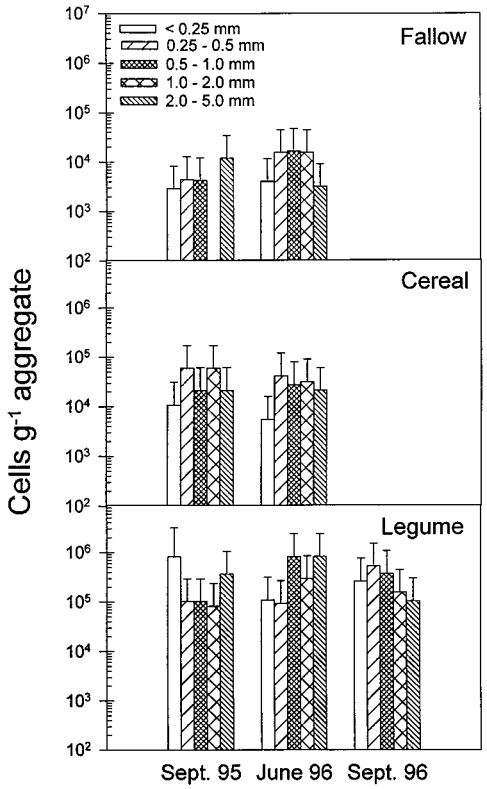

FIG. 2. Population densities of *R. leguminosarum* bv. trifolii in different soil aggregate size classes prepared from the three winter cover crop treatments, as determined by an MPN procedure. Determinations were conducted on a single composite sample of a specific aggregate size class produced by combining portions of that size class which had been prepared from each of the four field replicates of the respective field treatment. Missing data account for the absence of a value for the 1.0- to 2.0-mm size class of fallow treatment (September 1995). Error bars represent the upper limits of the confidence intervals ($P < 0.05$).

were 5 to 10 times greater in nonrhizosphere soil from the red clover treatment than from fallow and cereal soil. Although the rhizosphere/nonrhizosphere ratios for *Rhizobium* serotypes and the total soil bacterial population were always higher in the legume treatment than in the cereal treatment, the ratios in the former varied widely among serotypes, ranging between 2.8 and 41 (Table 2). Statistically significant differences between rhizosphere and nonrhizosphere populations were observed for AR6, AR18, and total bacteria in the legume treatment and for AR18 and total bacteria in the cereal treatment.

Distribution of *R. leguminosarum* bv. trifolii across aggregate size classes determined by the MPN procedure. MPN analysis of whole soil showed that the population density of *Rhizobium* organisms in soil of the legume treatment was greater than in soil from either the fallow or cereal treatments (Fig. 1). The magnitude of this difference varied from year to year and ranged between 2- and 50-fold. Aggregates recovered from the legume treatment carried *Rhizobium* population densities that ranged from 19 to 284 and from 5 to 77 times greater than the populations from the corresponding aggregate size classes of the fallow and cereal treatments, respectively (Fig. 2). In September 1995, however, there were two exceptions in the cereal treatment. Size classes of 0.25 to 0.5 mm and 1.0 to 2.0 mm contained *Rhizobium* densities (6.0×10^4 g of soil^{-1}) similar to their counterpart size classes in the legume treatment (8.2×10^4 to 10.2×10^4 g of soil^{-1}).

Despite the large confidence intervals typically associated with MPN population estimates (35, 36), there were indications that population densities differed among aggregate size classes within each treatment. For example, in September 1995 and June 1996, the microaggregate size class (<0.25 mm) from both the fallow and cereal treatments was among the size classes with the lowest *Rhizobium* populations. In contrast, microaggregates recovered from the legume treatment in September 1995 and 1996 contained *Rhizobium* population densities similar to, and sometimes greater than, those found in some of the larger aggregate size classes. By contrast, in June, the lowest densities in the legume treatment were found in the size classes of <0.25 and 0.25 to 0.5 mm. These differences in population distribution can also be illustrated in terms of the percent contribution that each aggregate size class makes to the whole soil (data not shown). For example, in September 1995, even though a similar percentage of soil was found in the <0.25-mm aggregate size class of the three cover crop treatments (33.3%), the proportion of the nodulating *Rhizobium* population found in this aggregate size class differed greatly among treatments (18.7, 12.1, and 69.5% for fallow, cereal, and legume, respectively). In contrast, in June 1996, the proportion of nodulating *Rhizobium* populations found in the <0.25-mm size class was similar in all three treatments (4.5 to 8.0%).

Distribution of *Rhizobium* serotypes across aggregate size classes, as determined by immunofluorescence. In June and September 1996, the population density of AR18 varied significantly as a function of aggregate size (Fig. 3 and Table 3). Furthermore, the interaction between aggregate size and treatment was significant, indicating that AR18 distribution among aggregate size classes differed among the treatments (Table 3). For example, in June, a significantly greater density of AR18 was found in the aggregate size class of 0.5 to 1.0 mm of the fallow treatment than in the 0.25- to 0.5-mm and 2.0- to 5.0-mm size classes of the same treatment (Fig. 3). Similarly, the 1.0- to 2.0-mm size class of the legume treatment contained a significantly greater density of AR18 than the size classes of <0.25, 0.25 to 0.5, and 2.0 to 5.0 mm. However, in September, AR18 densities in all of the size classes of >0.5 mm of the fallow treatment were greater than those found in the <0.5-mm size classes. In contrast, in the legume treatment, one of the smallest size classes (0.25 to 0.5 mm) contained a

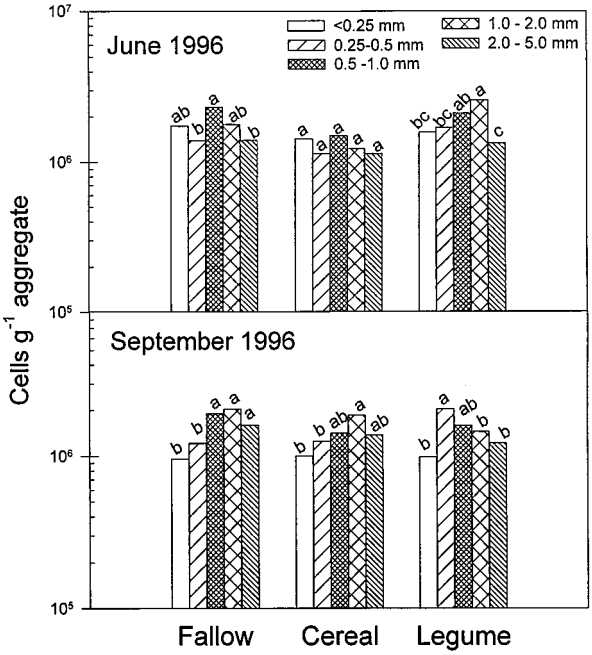

FIG. 3. Comparison of the distribution of *R. leguminosarum* bv. trifolii serotype AR18 across different aggregate size classes prepared from the three winter cover crop treatments in June and September 1996. Within each cover crop treatment and time, bars (mean values) not headed by the same letter (a, b, or c) are significantly different at P of <0.05.

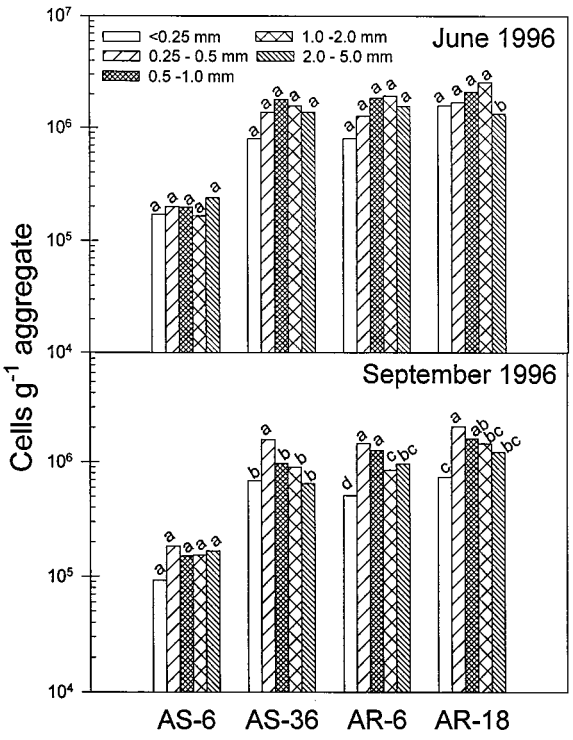

FIG. 4. Comparison of the population densities of four *R. leguminosarum* bv. trifolii serotypes (AS6, AS36, AR6, and AR18) in different aggregate size classes prepared from the legume winter cover crop treatment in June and September 1996. For each serotype and time, bars (mean values) not headed by the same letter (a, b, or c) are significantly different at P of <0.05.

significantly higher density of AR18 than was found in the size classes of <0.25, 1.0 to 2.0, and 2 to 5 mm. In June 1996, although there was a trend for the lowest densities of serotypes AS6, AS36, and AR18 to be found in the <0.25-mm size class of the legume treatment, the population densities did not differ significantly across the different aggregate size classes (Fig. 4). Nevertheless, in September a shift had occurred in population distribution, with the highest density of AS36 being found in the size class of 0.25 to 0.5 mm and the highest densities of AR6 in the 0.25- to 0.5-mm and 0.5- to 1.0-mm size classes.

Distribution of total soil bacteria across aggregate size classes. The densities of total soil bacteria in aggregates from the fallow, cereal, and legume treatments were determined in June and September 1996 (Fig. 5). At both sampling times, the total soil bacterial population varied significantly as a function of aggregate size and the distribution across aggregate size classes was influenced by the treatment (Table 3). Two distinct patterns of distribution were observed across aggregate sizes.

TABLE 3. Summary of repeated-measures ANOVA of the effects of winter cover crop and aggregate size class on the population densities of serotype AR18 and total soil bacteria

Source of variation	Probability level ($P > F$)[a]			
	June 1996		September 1996	
	AR18	TSB	AR18	TSB
Treatment	0.0028	0.0097	0.2631	0.0672
Aggregate size	0.0095	0.0001	0.0001	0.0001
Treatment × Aggregate size	0.0163	0.0053	0.0007	0.0010

[a] See Materials and Methods for a description of statistical procedures. TSB, total soil bacteria.

In the fallow treatment (June and September) and in the legume treatment (September), the two extremes of aggregate size classes (<0.25 and 2.0 to 5.0 mm) contained the lowest densities of total bacteria. In the cereal treatment (June and September) and in the legume treatment (June) the <0.25-mm size class contained significantly lower bacterial densities than all larger size classes.

DISCUSSION

Heterogeneous distribution of microbial biomass and its activities in soil aggregates of different sizes has been observed previously (13, 22, 29). However, our data are the first of their kind to illustrate that the population of a soil-borne bacterial species can be heterogeneously distributed in soil fabric. Some features of the distribution of *R. leguminosarum* across the different soil aggregate size classes were particularly interesting. While it is logical to expect that the population of nodulating *Rhizobium* organisms would be greater in a legume cover crop soil than in either the cereal or fallow soil, aggregate size classes in which the population densities of nodulating *Rhizobium* were similar in the cereal and legume treatments were identified. These data indicate the existence of microniches in the legume cover crop soil in which the *Rhizobium* population is stimulated to different degrees by the presence of the legume.

Data supporting the idea that microaggregates (<0.25 mm) are a less favorable habitat than macroaggregates for microbial activity exist in the literature (13, 31, 34). Microaggregates are thought to represent the primary nuclei of the aggregation process and to be relatively isolated from current biological

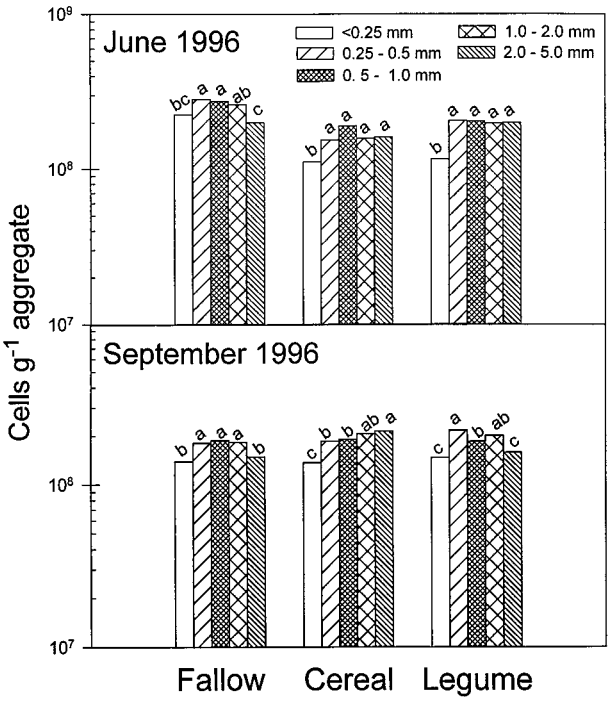

FIG. 5. Comparison of the population densities of total soil bacteria in different aggregate size classes prepared from the three winter cover crop treatments in June and September 1996. Within each cover crop treatment and time, bars (mean values) not headed by the same letter (a, b, or c) are significantly different at P of <0.05.

events occurring in soil, and they contain a low level of organic matter which is complex and somewhat recalcitrant to microbial attack (34). Nonetheless, other reports have shown that microaggregates can be as biologically active as macroaggregates (2, 17, 18, 29). Although some of our microscopic data support the idea that aggregates of <0.25 mm are less favorable habitats for microbial growth in our soil because they contain either the lowest or one of the lowest DAPI (Fig. 5) and immunofluorescence (Fig. 4) counts, some of our *Rhizobium* MPN data contradict that notion (Fig. 2). For example, in September 1995 and 1996, we observed that the <0.25-mm size class of the legume treatment contained densities of nodulating *Rhizobium* organisms that were equal to or greater than those found in larger size aggregate size classes. These data might indicate that the population of nodulating *Rhizobium* in the <0.25-mm size class responds more favorably to growth conditions in this niche than the majority of the soil bacterial population. Further experiments are required, however, to investigate the possibility that the <0.25-mm size class of aggregate simply provides better physical protection over the summer months than larger size classes for nodulating *Rhizobium* organisms. Furthermore, we cannot exclude the possibility that the nodulating populations in the larger size classes of aggregates might have been artificially deflated during the processing of September soil for aggregate preparation.

The significant interaction that was measured between the population size of serotype AR18 and the aggregate size and treatment (Fig. 3 and Table 3) presumably reflects the different nutrient sources and soil conditions imposed upon rhizobia during the spring months in the presence or absence of a plant and by the presence of either a host or a nonhost species. It has been recognized for many years that the plant rhizosphere is a zone where microbial activity is greater than in bulk soil and that legume rhizospheres are particularly active (4, 20). Further work is needed to determine to what extent the different distributions of the *Rhizobium* populations in the legume and cereal treatments can be linked to plant-associated rhizosphere processes versus differences attributed to soil disturbance and cover crop incorporation and/or the early stages of cover crop residue decomposition. The changes that occurred between June and September in the distribution of all *Rhizobium* serotypes in the legume treatment presumably reflect the changes occurring in soil conditions over the summer months. During this period, the factors influencing microbial activity are complex, promoted in all treatments by growth of the summer crop and the availability of irrigation water and differentiated among treatments by the presence or absence of decomposing cover crop residues. Further experiments are planned to compare the growth rates and turnover of *Rhizobium* organisms in the different aggregate size classes of the three treatments at the different sampling times.

Since AR18 isolates were recovered from the nodules of red clover plants in the terminal soil dilutions of all three field treatments in the 1994 MPN study, we can assume that the MPN values obtained for the size of the nodulating population of *R. leguminosarum* bv. trifolii also reflect (to an approximation) the density of the nodulating population of serotype AR18 in the soil. A comparison of the immunofluorescence direct count (IFDC) microscopy and MPN values of the AR18 population from the legume treatment show that MPN estimates range between 5 and 65% and between 10 and 30% of IFDC estimates from June and September soil samples, respectively. These values agree reasonably well with each other, if some latitude is given for the large confidence intervals normally associated with the MPN procedure and for the fact that a preliminary study with an immunofluorescence cell elongation assay (6) indicated that ~40% of the immunofluorescence-detectable cells of AR18 were viable (data not shown). However, in the case of the cereal and fallow soil samples, the discrepancies between the MPN and IFDC estimates were greater, with MPN estimates of AR18 representing only 5 to 0.5% of the cereal and fallow IFDC counts, respectively. A variety of possibilities might account for the discrepancy between IFDC and MPN estimates in these treatments. First, unrelated bacteria carrying surface antigens similar to rhizobia might be mistakenly enumerated by the FAs, and their population sizes might vary between treatments. For example, Bohlool and Schmidt (3) reported a cross-reaction by an FA raised to *Bradyrhizobium japonicum* against a soil actinomycete. Second, dead *Rhizobium* cells might be more numerous in the absence of a host plant and persist in microsites that are inaccessible to predators (25, 27). Indeed, the immunofluorescence cell elongation assay indicated that a significantly lower ($P = 0.05$) percentage (~20%) of the immunofluorescence-detectable cells of AR18 were viable in the fallow and cereal treatments than in the legume treatment (data not shown). These observations add credence to the idea that protected pore space exists in soil in which nonviable or dormant bacteria can persist (16, 23, 24). Third, IFDC might enumerate a population of nonsymbiotic rhizobia that are antigenically related to the nodulating serotypes. The existence of nonsymbiotic forms of *Rhizobium* species in soil has been shown on several occasions over the past few years (19, 30, 32, 33), and nonsymbiotic cells have been reported to be 40 times more numerous than the symbiotic forms (30). Nonsymbiotic cells may represent a larger percentage of the population in the absence of a host plant. Further studies are necessary to distinguish between these possibilities and to determine why the percent

viability of the AR18 serotype differs among treatments and if the percentage of viable cells varies across the aggregate sizes among the treatments.

Soils are complex environments in which it is generally recognized that changes in crop systems invariably cause changes in soil physical properties that influence microbial activity. It is not clear, however, to what extent physical properties control the distribution of bacteria and influence their growth, activities, and turnover. We hope that this study will stimulate the interest of other microbiologists to gain an understanding of how soil management might influence the activities of soil microorganisms by modifying soil structural properties.

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