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Distribution of soil fractions and location of soil bacteria in a vertisol under cultivation and perennial grass

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Abstract

Effects of soil management on soil characteristics were investigated on the rhizosphere (RPP) and the non-rhizosphere (NRPP) soil of a re-grass vertisol under *Digitaria decumbens* and in the soil under continuous cultivation (CC). A low energy technique allowed to separate eight size and density fractions, including macro- and micro-aggregates while preserving soil bacteria. Organic C and N, microbial biomass C and the number of total bacteria (AODC) and of *Azospirillum brasilense* and their distribution were determined in soil fractions isolated from the CC, NRPP and RPP soils. Soil macroaggregates (>2000 μm) were similarly predominant in the NRPP and RPP soils when the dispersible clay size fraction (<2 μm) represented more than 25% of the CC soil mass. The main increase of C content in RPP originated from the macroaggregates (> 2000 μm) and from the root fraction, not from the finer separates. The proportion of organic C as microbial biomass C revealed the low turnover of microbial C in the PP situations, especially in the clay size fraction of the NRPP soil. A common shift of AODC toward the finer separates from planted soils (CC and RPP) revealed the influence of living plants on the distribution of soil bacteria. The relative abundance of *A. brasilense* showed the presence of the active roots of *Digitaria* in the macroaggregates and their contact with the dispersible clay size fraction of the rhizosphere soil.

Introduction

Permanent grasses provide a consistantly active rhizosphere over the year contrasting with annual crops. Consequently, grasslands maintain a higher content of soil organic C (Tiessen and Stewart, 1983), a higher microbial biomass C content (Collins et al., 1992) and higher levels of aggregation and structure stability (Hart et al., 1988; Tiessen and Stewart, 1983) than cultivated soils. From a biological point of view, Hassink et al. (1993) showed that bacteria constituted the largest biomass pool in grassland soils with a maximum in fine textured soils. The rhizosphere of most of the Gramineae is considered to affect soil microorganism distribution and metabolism either directly (i.e. substrate supply through exudates or root decay, chemotaxis) or through the modification of their environment. Some microbial species are typically rhizosphere inhabitants and have been isolated from different root surfaces or from soil attached to active roots. Döbereiner and Pedrosa (1987) observed that the majority of diazotrophic microorganisms are closely associated with roots. Different studies have been conducted on the penetration of bacterial cells into the roots as to localize *Azospirillum* spp. (O'Hara et al., 1983; Patriquin et al., 1983). In natural conditions, *Azospirillum* is not the only microorganism capable to colonize roots. But the use of the fluorescent antibody technique allows to specifically identify *Azospirillum* cells in the rhizosphere of *Digitaria decumbens* (Schank et al., 1979) from which *A. brasilense* was first isolated by Day and Döbereiner (1976).

The presence of active roots may also influence bacterial metabolism. Sparling et al. (1981) found that phenolic-degrading microorganisms are stimulated by the presence of some graminaceous crops. Denitrification is increased in the rhizosphere environment (Smith and Tiedje, 1979), and specific respiration would be reduced by high CO_2 production in the rhizosphere of many grasses (Santruckova and Straskraba, 1991). One of the benefits due to the presence of Gramineae would be a better conservation of organic C in soil due to the presence of more numerous microorganisms with lower C turnover.

Soil components may also influence the distribution and activity of microorganisms. Gray and Williams (1971) suggested that the preferential zones of microbial activity are associated with sites where fresh decomposable organic matter is released in soils, i.e. the root environment and organic fragments from litter fall or crop residues. More researchers have investigated the role of clay material on microbial life in soils. Stotzky and Burns (1982) indicated that direct interaction of clays, i.e. sorption of microbial cells or colonies, may influence the distribution and the activity of microorganisms. Ladd and Foster (1987), Amato and Ladd (1992) observed that soil microbial biomass content is correlated with CEC (i.e. clay content). The abundance of microbes and of microbial biomass C in clay soils may be attributed to the stabilization (slow turn-over) of microbial cells (Amato and Ladd, 1992; Oades, 1988). Specific respiration (CO_2 released per microbial cell) is negatively correlated with clay content in soil (Chaussod et al., 1986). The stabilization of organic matter in clay soils is also attributed to the slower diffusion of O_2 and of organic solutes to microorganisms located in the aggregates (Stotzky and Burns, 1982).

Habitats of soil microorganisms are dependent on soil structure which implies the presence of voids of various shapes and sizes. Hassink et al. (1993) found that bacterial cells were more abundant in clay and loamy soils, where narrow pores predominate, than in coarse textured soils. An other aspect of soil structure concerns the size and the appearance of the individual lumps which form the soil body on or in which microorganisms are not evenly distributed (Russell, 1973). Jocteur Monrozier et al. (1991) observed that the microaggregates (20–2 μm) isolated from a deep clay layer of massive structure were less porous and contained a lower proportion of soil microbial biomass C than when isolated from a crumbly rhizosphere soil with the same clay content.

In this paper, we studied the location of a typical rhizosphere bacterium, *Azospirillum brasilense*, together with the distribution of the total soil bacteria and of the microbial biomass carbon in a clay soil submitted to intense cultivation or permanent pasture. After seven years of *Digitaria decumbens* pasture, soil structure and aggregate stability have been strongly improved and average organic C content increased from 12 g per kg to more than 50 g per kg of soil. The objective of this work was to assess if the changes in soil physics and organic matter observed when permanent pasture replaced continuous cultivation resulted in concomitant changes in the distribution and abundance of the soil microflora and of the rhizosphere microorganisms.

Material and methods

Field sites and soil sampling

The soil was a Vertisol (black earth) which was sampled from two sites located in the southeast of Martinique (14° 3' N and 62° 34' W, a French island in the West Indies). One site was located in an area of permanent pasture (PP) of *Digitaria decumbens* and the other in a nearby area under continuous cultivation (CC) of seasonal crops. The permanent pasture soil exhibited vertic cracks between soil under *Digitaria* shoots with dense root hair (RPP) and the next bare soil between the tufts with merely no rhizosphere (NRPP), which were separately sampled. The upper 10 cm layer of each soil was carefully cored to avoid structure disturbance and stored unsieved at 4°C. Fifteen replicates of each soil (approximately 100 g) were sampled in the field. Six of them were used for fractionation and the others for soil analyses. The main characteristics of soils sampled from the three situations CC, NRPP and RPP are described in Table 1.

Physical fractionation of soil

Physical fractionation of soils was performed according to Chotte et al. (1993) (Fig.1). Subsamples of moist unsieved soil weight equivalent to 100 g of dry soil were submerged in 250 mL of cold (4°C) distilled water in a 400 mL plastic beaker and kept at 4°C for 36 hours. Before fractionation (Fig. 1), coarse roots and plant debris (>20 mm) were picked up with tweezers, rinsed with distilled water and collected for further analyses (root fraction). The soil suspension plus the root fraction leachates were successively passed

Table 1. Main characteristics of the studied Vertisol soils

Soil characteristics		Continuous cultivation bulk soil CC	Non-rhizosphere permanent pasture soil NRPP	Rhizosphere permanent pasture soil RPP
Root mass (g kg^{-1} soil)		1.2 ± 1.3a	2.17 ± 1.15a	13.24 ± 7.2b
Gravel (> 2000 μm)		0	0	0
C (g kg^{-1})		11.9 ± 0.8c	35.6 ± 3.1d	54 ± 2.6d*
N (g kg^{-1})		1.1 ± 0.17e	3.5 ± 0.4f	4.4 ± 0.2f*
Biomass C (mg C kg^{-1})		316 ± 45g*	1960 ± 196h	2485 ± 673h
Mechanical analysis	(< 2000 μm %)			
	Clay (< 2 μm)	50.6i	53.7i	55.4i
	Fine loam (2–20 μm)	27.9j	31.0j	25.5j
	Coarse loam (20–50 μm)	6.4k	6.5k	9.0k
	Fine sand (50–200 μm)	6.4l	5.9l	7.9l
	Coarse sand (200–2000 μm)	8.7m	2.8m	2.2m

Values are mean ± standard deviation. Values with * are means of two replicates only. Mean values with unlike letters are significantly different ($p < 0.05$) by a Fisher PLSD test.

through 2000 μm, 200 μm and 50 μm mesh size sieves by gentle washing with distilled water to separate fractions >2000 μm, 2000–200 μm size and 200–50 μm size with the minimum of disturbance. The fraction 2000–200 μm was divided by flotation in water into light (d<1 g cm^{-3}) and dense (d>1 g cm^{-3}) fraction noticed FL and FD respectively. The remaining soil fraction (<50 μm size) was collected and fractionated at increasing *g* into three fractions. The fraction of 50–20 μm size was allowed to settle in 1250 mL glass cylinders at 1*g*, when particles and microaggregates of 20–2 μm size were pelleted from the unsettled 1*g* suspension by centrifugation (90 *g*, 3 times) at 10°C. The resulting supernatant containing the <2 μm size fraction was flocculated by addition of $CaCl_2$ (0.5 *M* final) and centrifugated at 2460 *g* to concentrate the <2 μm particles as a slurry. Aliquots of the wet fractions were stored at 4°C if microbial analyses could not be performed immediately. Twenty four hours was the usual time required to achieve the fractionation process of one soil in duplicate.

The mechanical analysis was performed in parallel on air dried subsamples of each soil. After dry sieving at 2 mm, organic matter was destroyed by oxidation with boiling hydrogen peroxide. The mineral residue was dispersed in water by addition of NH_4Cl (1 *M* final) and shaking for 1 hour. The resulting dispersion was sieved to collect the coarse sand (2000 to 200 μm) and the fine sand (200 to 50 μm), which were oven dried and weighed. The distribution of the <50 μm size particles was obtained by a laser diffraction analyser (Mastersizer/E, Malvern).

C, N and microbial biomass C

Organic C and N were determined by catharometry of CO_2 and N_2 after combustion (900°C under O_2 flow) of 50 mg of crushed air dried soils or of 50–10 mg of crushed oven dried fractions in a CNS analyzer (Na 1500 Carlo Erba, Milano, Italy).

Microbial biomass C of unfractionated moist soils (CC, RPP, NRPP) (5 replicates) or of aliquots of moist soil fractions (in duplicate) was determined by a fumigation – extraction method (Amato and Ladd, 1988), with determination of ninhydrin-N reactive compounds extracted from soils with 2 *M* KCl after a 10 day-fumigation period. Biomass C = Ninhydrin-N × 21 (μg g^{-1} of dry soil or soil fraction).

Direct enumeration of bacteria

Direct enumeration of soil bacteria was performed on soil suspensions. Five grams of unfractionated soil or particle size fractions were homogenized in 50 mL ster-

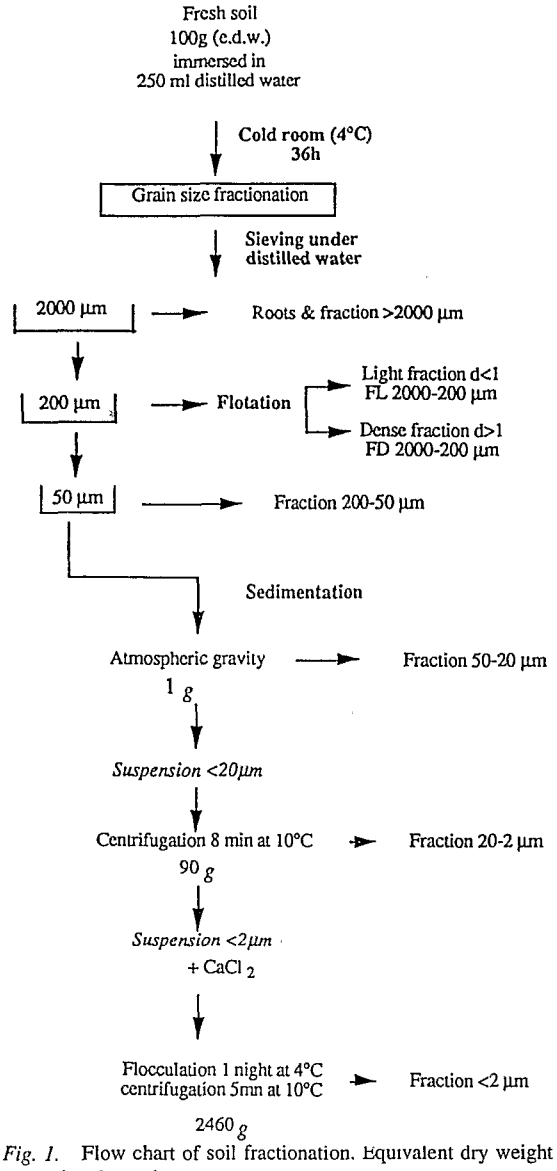

Fig. 1. Flow chart of soil fractionation. Equivalent dry weight is abbreviated as e.d.w.

ile distilled water in a Waring blender (Eberbac Corp) for 2 minutes and bacterial cells were stained with Acridine Orange (A.O., 0.1% in 0.2 µm filtered sterile distilled water) according to Ramsay and Bawden (1983).

The immuno-fluorescence technique (IF) described by Schmidt (1974) was used for the direct enumeration of the population of *Azospirillum brasilense*. In our study, we used a serum which has been shown to be specific to *Azospirillum brasilense* species (sp245) (Gamard, 1991). The stained cells (AODC or IF) were

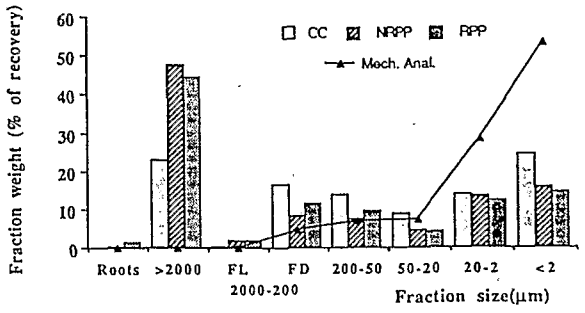

Fig. 2. Distribution of the size fractions isolated from the Vertisol soils in the three situations: cultivated soil (CC), non-rhizosphere pasture soil (NRPP) and rhizosphere pasture soil (RPP). Mech. Anal.: average grain-size distribution resulting from the mechanical analysis of the texture of the Vertisol under CC, NRPP and RPP situations.

enumerated using the epifluorescence microscope. At least 20 microscope fields were counted, when 5 or more cells were present per field. All the results presented here were expressed per g of dry soil or dry fraction.

Statistical analyses

Stastistical analyses of the data were performed by One factor or Two factor ANOVA using Microsoft StatView® (Microsoft Corporation). A Fisher PLSD test was used for comparisons between soils and between soil fractions for organic C contents, biomass C contents, total soil bacteria numbers (AODC) and IF counts.

Results

Weight distribution of the particle size fractions

Weight recoveries after soil fractionation amounted to 100% for each situation. The results were expressed as percent of the sum of the fractions. The fractions >2000 µm were predominant in all of the three situations, and accounted for 23% of the cultivated soil (CC), 48% of the non-rhizosphere pasture soil (NRPP) and 45% of the rhizosphere pasture soil (RPP) (Fig. 2). Particles > 2000 µm were not found by mechanical analysis (Table 1).

The weight of the root fraction ranged from 0.02% in CC, 0.15% NRPP to 1.61% in RPP. The collected material was mainly coarse, woody, hard pieces of roots and less shoots or aerial parts of the plants. The

proportion of the FL 2000–200 μm fraction, which also contained plant residues, ranges from 0.25% in the CC soil to about 1.80% in the pasture soil samples (RPP and NRPP). Thus organic residues from plants (roots + FL 2000–200 μm fraction) accounted for less than 0.3% in the CC soil and more than 3.0% in the RPP soil.

The proportion of the FD 2000–200 μm fraction decreased from 16% in CC to 11% in the RPP and 8% in NRPP. The 20–2 μm fraction was found approximately in the same proportion in the three situations (i.e., 13.8% in CC soil, 13.6% in NRPP and 12.5% in RPP) and lower than the proportion of the nominated size particles obtained by the mechanical analysis (i.e. 27.9%, 31.0%, and 25.5% in CC, NRPP, and RPP soil respectively). The clay size fraction (<2 μm) amounted to 24.6% of sample weight in the CC soil, 15.8% in the NRPP soil and 14.5% in the RPP soil. These quantities amounted to 48.6%, 29.4% and 26.2% respectively of the clay content obtained by the mechanical analysis.

Organic C, N and microbial biomass C

The amount of organic C increased from 11.9 mg g^{-1} in the cultivated soil (CC) to 35.6 mg g^{-1} in the non-rhizosphere pasture soil (NRPP) and 54 mg g^{-1} in the rhizosphere pasture soil (RPP) (Table 1). The quantity of N amounted to 1.1 mg g^{-1} in CC, 3.5 mg g^{-1} in NRPP, and 4.4 mg g^{-1} in NRPP. This difference also occurred in the case of microbial biomass C. The total biomass C in unfractionated soils ranged from 316 μg C g^{-1} in CC to 1960 μg C g^{-1} in NRPP and 2485 μg C g^{-1} in RPP. Considering the C, N and biomass C contents of unfractionated soils, the permanent pasture soils (NRPP and RPP) were found to be significantly different (Fisher PLSD test, $p < 0.05$) from the soil under continuous cultivation (CC) but not different from each other.

Organic C and N recovery after fractionation was equal to 99% and 99%, 104% and 111%, 104% and 105% in CC, NRPP and RPP respectively. Microbial biomass C recovery after fractionation amounted to 114% of the unfractionated CC soil content, 74% in NRPP and 105% in RPP. The distribution of C, N and microbial biomass C were expressed as percent of the recovered amounts.

In the cultivated soil (CC), organic C and N were predominantly found in the finest particles (<2 μm) which contained 26% and 30% of the total soil organic C and N (Fig. 3) as well as a high proportion (35%)

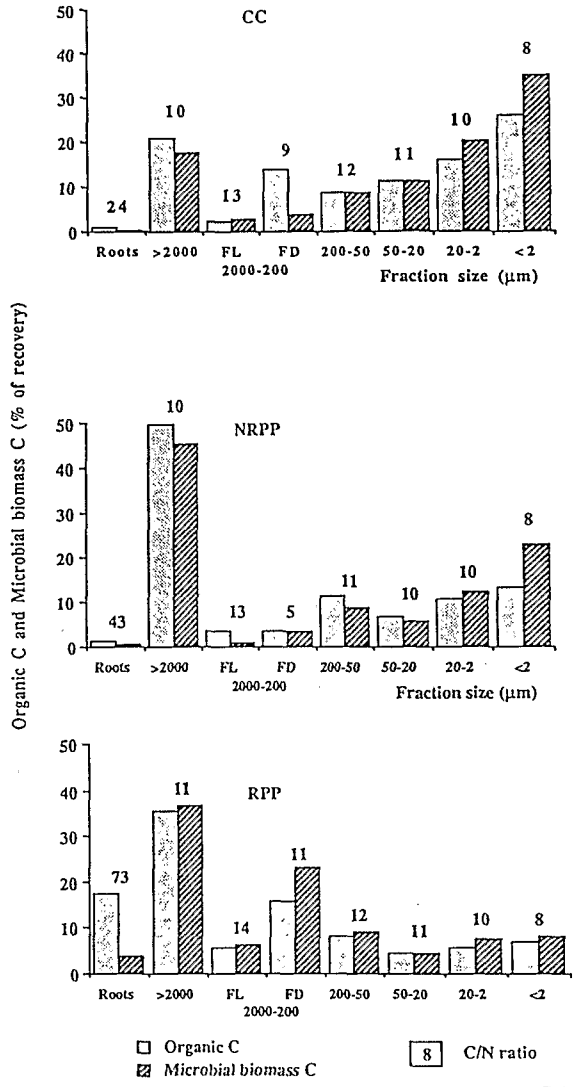

Fig. 3. Distribution of organic C and microbial biomass C in the fractions of the cultivated soil (CC), non-rhizosphere pasture soil (NRPP) and rhizosphere pasture soil (RPP). C/N ratios of the fractions are at the top of the bars.

of the microbial biomass C. By contrast, organic C, N and microbial biomass C were concentrated in the macroaggregates (> 2000 μm) of the non-rhizosphere soil (NRPP) (50% of the organic C, 49% of N and 39% of the biomass C) and to a lesser extent in the macroaggregates of RPP (35% of the organic C, 41% of the unfractionated soil N and 37% of the biomass C). Except for RPP, the root fraction plus the FL 2000–200 μm fraction represented a small proportion of soil C, N and microbial biomass C due to the low mass of organic debris.

Table 2. Organic C and microbial biomass C contents in fractions of the Vertisol soils under continuous cultivation (CC), and under permanent pasture of *Digitaria decumbens* in non-rhizosphere (NRPP) and rhizosphere (RPP) situation

Soil type	CC			NRPP			RPP		
	C	Biomass C		C	Biomass C		C	Biomass C	
Fractions	(mg g^{-1} fraction)	($\mu g\ g^{-1}$ fraction)	(% fraction C)	(mg g^{-1} fraction)	($\mu g\ g^{-1}$ fraction)	(% fraction C)	(mg g^{-1} fraction)	($\mu g\ g^{-1}$ fraction)	(% fraction C)
Roots	385 ± 49	3223*	0.8	364 ± 48	6504 ± 309	1.8	453 ± 8	7724*	1.7
> 2000 μm	10.8 ± 1.1	295 ± 20	2.7	37.4 ± 2.1	1668 ± 260	3.1	48.5 ± 6.4	2421 ± 368	5.0
FL 2000–200 μm	112 ± 26	4544*	4.1	139.7 ± 7.6	1865 ± 67	1.3	149.0 ± 6.0	7844 ± 140	5.3
FD 2000–200 μm	10.0 ± 0.3	88 ± 38	0.9	8.6 ± 0.6	423 ± 65	4.9	50.5 ± 2.8	3534 ± 180	7.1
200–50 μm	7.4 ± 0.1	240 ± 58	3.2	39.2 ± 4.2	1507 ± 24	3.8	49.5 ± 2.3	2725 ± 51	5.5
50–20 μm	15.2 ± 0.9	490 ± 2	3.2	43.0 ± 2.2	1748 ± 44	4.1	56.3 ± 5.8	2684 ± 150	4.8
20–2 μm	13.7 ± 0.8	559 ± 4	4.1	27.0 ± 1.0	1503 ± 141	5.6	28.4 ± 7.4	1816 ± 290	6.4
< 2 μm	12.6 ± 1.7	547 ± 38	4.3	30.0 ± 0.9	2520 ± 158	8.4	35.7 ± 3.0	2024 ± 267	5.7

Values are mean ± standard deviation. Values with * are data with one replicate only. Biomass C % fraction C: proportion (in %) of fraction C in biomass C.

Organic C contents (Table 2) of the root fraction were quite high: 385 mg g^{-1} in CC, 364 mg C g^{-1} in NRPP, and 453 mg g^{-1} in RPP. This evidenced the absence of mineral grains in the root fractions. In contrast, the FL 2000–200 μm fraction with lower organic C concentration (112 mg g^{-1}in CC, 139.7 mg g^{-1} in NRPP, and 149.0 mg g^{-1} in RPP) would contain more than 70% of mineral components. The organic fractions of the cultivated soil were significantly different (Fisher PLSD test, $p < 0.05$) from the same fractions of the rhizosphere pasture soil, while the two pasture soils only differed from each other by the organic content of the root fraction.

Except for these fractions, maximum organic C contents were recorded for the 50–20 μm fraction of each soil ranging from 15.2 mg g^{-1} in CC to 43.0 mg g^{-1} in NRPP and to 56.3 mg g^{-1} in RPP. Lower organic C contents were found in the coarser aggregates of the CC soil. The largest difference between NRPP and RPP organic C content was found in the FD 2000–200 μm fraction which contained 8.6 mg C g^{-1} in NRPP and 50.5 mg C g^{-1} in RPP. The depletion of organic matter also affected the finest particles (20–2 μm and <2 μm) of the NRPP soil.

Generally C/N ratio (Fig. 3) decreased from the root fraction (24/1 in CC, 43/1 in NRPP and 73/1 in RPP) to the FL 2000–200 μm fraction (13–14/1) and from the coarser to the finer sized fractions (8/1), with the noticeable exception of the FD 2000–200 μm fraction of CC (9/1) and NRPP (5/1) soils. The highest concentrations of microbial biomass C (Table 2) were found in the organic fractions (i.e. in the root fraction and in the FL 2000–200 μm fraction). Statistically, the biomass C contents of the root fractions of the three soils were not different (3223 $\mu g\ g^{-1}$ in the CC, 6504 $\mu g\ g^{-1}$ in the NRPP and 7724 $\mu g\ g^{-1}$ in the RPP root fractions) while the biomass C contents of the light 2000–200 μm fractions were significantly different (Fisher PLSD test, $p < 0.05$). The microbial biomass C represented a low proportion of the organic C in the root fraction of the three soils (0.8% of the organic C in the CC soil root fraction, 1.8% in the NRPP and 1.7% in the RPP soil) and in the light fraction of the non-rhizosphere pasture soil (1.3% of the organic C in the NRPP FL 2000–200 μm fraction). Contrastingly, microbial biomass C represented 4.1% and 5.3% of the organic C in the FL 2000–200 μm fraction of the cultivated soil and of the rhizosphere pasture soil.

Among the organo-mineral fractions, the highest biomass C concentration was found in the 20–2 μm (559 $\mu g\ g^{-1}$) and in the <2 μm (547 $\mu g\ g^{-1}$) fractions of the CC soil, in the 50–20 μm (1748 $\mu g\ g^{-1}$) and in the <2 μm (2520 $\mu g\ g^{-1}$) fractions of the NRPP soil and in the FD 2000–200 μm fraction (3534 μg

g^{-1}) of the RPP soil. The biomass C contents of the organo-mineral fractions of the CC soil were significantly different (Fisher PLSD test, $p < 0.05$) from the data obtained from the same fractions of the rhizosphere pasture soil. Besides, the biomass C contents of the medium size fractions (i.e., FD 2000–200 μm, 200–50 μm and 50–20 μm fractions) of the rhizosphere pasture soil were significantly different from the same fractions of the non-rhizosphere soil. Significant differences were not shown between the coarse aggregates (>2000 μm) and between the finest fractions (20–2 μm and <2 μm size fractions) of the two pasture soils. The proportion of the organic C present in biomass (Table 2) in the organo-mineral fractions ranged from 0.9% (FD 2000–200 μm fraction) to 4.3% (<2 μm fraction) in the cultivated soil, from 3.8% (200–50 μm fraction) to 8.4 (<2 μm fraction) in the non-rhizosphere pasture soil and from 4.8% (50–20 μm fraction) to 7.1% (FD 2000–200 μm fraction) in the rhizosphere soil.

Direct enumeration of soil bacteria (AODC)

The number of bacterial cells of the unfractionated soil samples were found to be significantly different (Fisher PLSD test, $p < 0.05$) and increased from the continuously cultivated soil to the non-rhizosphere pasture soil and to the rhizosphere pasture soil (3.0×10^8, 2.0×10^9 and 4.1×10^9 cells per g of soil) (Table 3). The rates of recovered AODC after the fractionation process amounted to 8.1×10^8 in CC, 1.9×10^9 in NRPP and 4.5×10^9 in RPP. The soil bacteria AODC budget was established on the basis of recovered AODC.

The largest proportion of bacteria in the CC soil fractions peaked at 35% of the total AODC in the <2 μm fraction (Fig. 4). Most of the remaining AODC were distributed among three other fractions, namely the fraction 20–2 μm (21%), the fraction >2000 μm (16%), and the FD 2000–200 μm fraction (12%). In NRPP, the 20–2 μm fraction and the >2000 μm fraction respectively contained 42% and 25% of the recovered AODC. In RPP soil, 33% of the recovered AODC were found in the <2 μm and 27% in the 20–2 μm size fractions.

The higher densities of bacteria were enumerated (Table 3) in the root fraction of the three soils (7.6×10^{10}, 2.8×10^{10} and 2.5×10^{10} cells per g of the root fraction in CC, NRPP and RPP soil samples) followed by the light fraction of the pasture soils (1.3×10^{10} in RPP and 1.5×10^{10} in NRPP). Significant differences (Fisher PLSD test, $p < 0.05$) were observed in the distribution of bacterial numbers in the root fraction

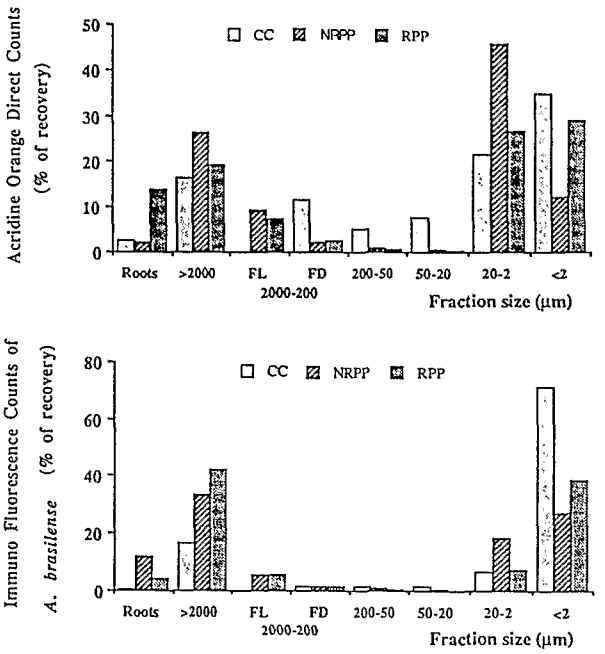

Fig. 4. Distribution of total bacteria enumerated by AODC (above) and of *A. brasilense* enumerated by IF (below) in the fractions of the cultivated soil (CC), non-rhizosphere pasture soil (NRPP) and rhizosphere pasture soil (RPP).

of the three situations. Coarse aggregates also showed significant differences of the total bacterial numbers.

Substantial concentrations of bacteria also were found in the 20–2 μm size fraction of each soil: 1.15×10^9, 5.0×10^9 and 9.4×10^9 cells per g of the fraction in CC, NRPP and RPP respectively. Total soil bacteria numbers were not significantly different (Fisher PLSD test, $p < 0.05$) for most of the fractions of the cultivated soil (except the root fraction). In the pasture soils, the organic fractions (root- and FL 2000–200 μm fractions) exhibited significant differences (Fisher PLSD test, $p < 0.05$) of the numbers of bacteria, while no significant differences were found within the medium size fractions of both soils. The bacterial densities of the two finest fractions (20–2μm and <2μm fractions) were similar in the rhizosphere soil and significantly different (Fisher PLSD test, $p < 0.05$) in the non-rhizosphere soil.

Direct enumeration of Azospirillum brasilense *(IF)*

The numbers of *Azospirillum brasilense* cells (Table 3) were found to be significantly different (Fisher PLSD test, $p < 0.05$) and increased from the CC (2.5×10^5 cells g^{-1}) to the NRPP (3.5×10^6 cells g^{-1}) and to

Table 3. Total bacteria numbers (AODC) and *A. brasilense* numbers (IF) in soils and soil fractions in the cultivated (CC), non-rhizosphere (NRPP) and rhizosphere (RPP) situation

Soil type	CC			NRPP			RPP		
Fraction	AODC 10^8 cells g^{-1} fraction	*A. brasilense* 10^6 cells g^{-1} fraction	*A. brasilense* % AODC	AODC 10^8 cells g^{-1} fraction	*A. brasilense* 10^6 cells g^{-1} fraction	*A. brasilense* % AODC	AODC 10^8 cells g^{-1} fraction	*A. brasilense* 10^6 cells g^{-1} fraction	*A. brasilense* % AODC
Roots	765 ± 63	5.7 ± 3.7	0.07	280 ± 14	105 ± 7	0.4	255 ± 7	155 ± 7	0.61
> 2000 μm	5.3 ± 0.7	1.7 ± 0.4	0.32	8.6 ± 0.6	0.3 ± 0.03	0.03	19 ± 0.7	89 ± 6	4.68
FL 2000–200 μm	nd	nd	nd	150 ± 14	26 ± 1.4	0.17	134 ± 7	215 ± 7	1.60
FD 2000–200 μm	5.3 ± 0.5	0.15 ± 0.03	0.03	2.2 ± 0.1	0.53 ± 0.06	0.24	5.6 ± 2.3	7.7 ± 0.8	1.38
200–50 μm	2.87 ± 0.32	0.24 ± 0.02	0.08	1.4 ± 0.01	0.37 ± 0.03	0.26	3.25 ± 0.07	3.0 ± 0.2	0.92
50–20 μm	6.35 ± 0.5	0.34 ± 0.01	0.05	2.15 ± 0.5	0.38 ± 0.04	0.18	3.3 ± 0.1	3.6 ± 0.07	1.09
20–2 μm	11.5 ± 0.7	0.88 ± 0.03	0.08	50 ± 3	6.1 ± 0.1	0.12	94 ± 6	55 ± 6	0.59
< 2 μm	10.5 ± 0.7	1.2 ± 0.3	0.11	11.5 ± 0.7	8.1 ± 0.3	0.7	105 ± 7	305 ± 35	2.90
Unfract. soil	*3.0 ± 0.02*	*0.25 ± 0.03*	*0.08*	*20 ± 2.5*	*3.5 ± 0.2*	*0.18*	*41 ± 0.7*	*28 ± 2*	*0.68*

Dara are means of two replicates ± standard deviation.

the RPP situation (2.8×10^7 cells g^{-1}). The budget of *Azospirillum brasilense* cells showed the same pattern as AODC budget. The results are expressed as percent of total recovery.

The distribution of *A. brasilense* among the soil fractions (Fig. 4) of the three soils evidenced two maxima in the >2000 μm and in the <2 μm size fractions: 17% and 71% of the recovered *A. brasilense* cells in the cultivated soil, 32% and 26% in the non-rhizosphere pasture soil, and 41% and 44% in the rhizosphere soil. In the non-rhizosphere pasture soil, the pattern of *A. brasilense* distribution was slightly different than in the other soils, with substantial percentages of the recovered *A. brasilense* cells found in the root and FL 2000–200 μm fractions (13% and 10% respectively) and in the 20–2 μm fraction (17%).

The density of *A. brasilense* cells (Table 3) was maximum and significantly different (Fisher PLSD test, $p < 0.05$) in the root fraction of the three soils (5.7×10^6 cells g^{-1} in CC, 1.01×10^8 cells g^{-1} in NRPP and 1.55×10^8 cells g^{-1} in RPP). In the FL 2000–200 μm and <2 μm fractions of the rhizosphere soil, high numbers of *A. brasilense* cells (2.15×10^8 cells g^{-1} and 3.0×10^8 cells g^{-1}) also were enumerated.

A. brasilense represented 0.08% of the AODC counts in the cultivated soil, 0.18% in the non rhizosphere pasture soil and 0.68% in the rhizosphere soil (Table 3). The highest proportions of *A. brasilense* cells in the NRPP soil were found in the <2 μm size fraction (0.7%) and then in the root fraction (0.4%). By contrast, in the rhizosphere soil, the rhizospheric bacteria contributed for 4.68% to the bacterial cells in the >2000 μm fraction, 2.90% in the <2 μm size fractions, then for 1.60% and 1.38% in the FL 2000–200 μm and FD 2000–200 μm fractions respectively and for only 0.61% of the bacteria attached to the root fraction.

Discussion

Distribution of soil fractions

The energy used to disperse soil organization is crucial to obtain representative substructures, i.e. macro and microaggregates or particles (Chotte et al., 1992; Christensen, 1992; Elliott, 1986). Ultrasonic energy was not used for dispersion since it was demonstrated to have germicidal effects (Scherba et al., 1991). The low energy method used for the disruption of the Vertisol confirmed the high stability of the >2000 μm macroaggregates in permanent pasture situations. The abundance of the macroaggregates did not distinguish

the active rhizosphere soil (RPP), where 45% of the soil mass was found in the >2000 μm fraction, from the non-rhizosphere soil (NRPP) where 48% of the soil mass was in those macroaggregates. By contrast in the Vertisol under seasonal crops (CC) only 23% of the soil was found as macroaggregates and the fractionation procedure gave more colloidal material (24.6% of the soil mass) because of a weaker cohesion of particles. Considering the rhizosphere characteristics, the most prominent difference between the pasture soils was the abundance of the root fraction which represented 1.61% of the soil mass under rhizosphere control (RPP) and 0.15% in the non-rhizosphere situation (NRPP).

The distribution of soil fractions obtained by this gentle procedure confirmed that soils under long term pasture or in regrassed sites exhibited greater soil aggregate stability than the corresponding soil under continuous crop (Haynes and Swift, 1990). From the small differences between rhizosphere and non-rhizosphere soil with respect to the distribution of the size fractions when under permanent pasture (NRPP and RPP), it is likely that the stability of soil macrostructures was less due to the presence of currently active roots (Sparling and Cheshire, 1985) than to the lack of soil disturbance in no-till management (Mahboubi et al., 1993).

Organic C and N

Organic C content of the particle size fractions allowed to distinguish organic C-rich root and FL 2000–200 μm fractions, containing more than 100 mg of C per g, from organo-mineral fractions with less than 50 mg of C per g. With the highest content of organic C (453 mg per g) and the highest C/N ratio (73/1), the elemental composition of the material of the root fraction of the rhizosphere soil (RPP) is close to the composition of oat or of timothy roots (Russell, 1973). It is likely that this fraction contained only a minor part of fine, active roots just as in the root fraction of the non-rhizosphere pasture soil (C/N: 43/1). Still, a high content of organic C (385 mg per g) but lower C/N ratio (24/1) of the root fraction of the cultivated soil correspond to more biodegradable material which would not persist in soil (Duchaufour, 1977; Russell, 1973). The FL 2000–200 μm fractions of the three soils exhibited similar organic C content (112 to 149 mg C per g), evidencing a higher contribution of inorganic material to these fractions than to the root fraction. The narrow C/N ratio (13–14/1) of these light fractions suggests that the inorganic components are clays with N-rich adsorbates (proteins) which could contribute to the small C/N ratio of this fraction. Such clay adsorption on plant pieces has been demonstrated on collapsing roots (Foster et al., 1983) or on fine (200 μm size) living roots (Dorioz and Robert, 1987) by scanning electron microscopy. Hydrophobic interactions would then explain that this clay-organic debris association (FL 2000–200 μm size fraction) would be floating in water.

The more mineral fractions exhibited a lower C/N ratio, decreasing with the decreasing size of the fraction as already described by many earlier reports (Catroux and Schnitzer, 1987; Jocteur Monrozier et al., 1991; Tiessen and Stewart, 1983; Turchenek and Oades, 1979) and could be due to the increasing contribution of microbial tissue to the finest fractions (Catroux and Schnitzer, 1987; Ladd and Amato, 1988; Tiessen et al., 1984).

The main difference between the organic content of the rhizosphere and the non-rhizosphere pasture soil could be attributed to the root fraction (40%) and to the FD 2000–200 μm size fraction (33%). The >2000 μm and the FL 2000–200 μm fractions only contributed for 15% and 9% respectively to the higher amount of organic C in the rhizosphere soil under *Digitaria* compared to the non-rhizosphere soil. None of the finer separates contributed to a specific enrichment of the rhizosphere soil in organic material as recently observed by Angers et al. (1993).

All the fractions of the cultivated soil contained less organic C than the corresponding fractions in the pasture soils. Fourty-one percent of the organic C decrease in the cultivated soil originated from the macroaggregates (> 2000 μm size fraction) which combined a strong decrease of C content with a lower mass than in the rhizosphere pasture soil. Two other fractions, namely the root fraction and the dense fraction of 2000–200 μm size, contributed to the difference of C content between the rhizosphere pasture soil and the cultivated soil. The strong decrease of the mass of the root fraction contributed for 22% of the C decrease in the CC soil, when the reduction of the C content in the FD 2000–200 μm size fraction explained 15% of the C decrease in the continuously cultivated soil. All these results confirmed that both the macroaggregate fraction (Oades, 1988) and the root fraction as particulate organic matter (POM) (Cambardella and Elliott, 1992) contribute in the organic matter content of the grassland soils.

Microbial biomass C

Our results evidenced the concentration of biomass C (μg per g of fraction) in the root fraction and in plant debris from the light fraction of the three soils. Nevertheless the proportion of the soil biomass C found in the organic fractions remained lower than 10% due to the low mass of these fractions in the three soils. On the other hand, the major proportion of soil biomass C (% of recovered biomass C) was found in the > 2000 μm fraction of the permanent pasture soils: 41.5% of the soil microbial biomass C of the rhizosphere soil and 47.5% of the soil microbial biomass C of the non-rhizosphere pasture soil were found in this macroaggregate fraction. In contrast most of the biomass C of the cultivated soil was heavily concentrated in the <2 μm fraction (34.5%) and in the 20–2 μm fraction (19.8%).

The proportion of organic C found as microbial biomass C was the same (5.5–5.6%) in the unfractionated pasture soils, contrasting with the low value of this proportion (1.2%) in the biomass C-poor cultivated soil. Angers et al. (1993) found that microbial biomass C averaged 1.3% and 4.2% of the total organic C in plowed and minimum tillage situations respectively. In fractions, the proportion of organic C as microbial biomass C ranged from more than 8% in the <2 μm fraction of the non-rhizosphere pasture soil to approximatively 1% or less in the root fraction of the rhizosphere soil and in the FD 2000–200 μm fraction of the cultivated soil. In most fractions, microbial biomass C as % of organic C increased from the cultivated soil to non-rhizosphere soil and to the rhizosphere pasture soil. This reveals a higher immobilization rate of organic C as microbial biomass in the rhizosphere soil, than in the non-rhizosphere pasture soil and still more than in the cultivated soil.

In the non-rhizosphere pasture soil, the FL 2000–200 μm size fraction exhibited the smallest ratio of microbial biomass C to organic C among the three light fractions and among all the fractions of this soil. The organic C found in this fraction could be a residue of decomposed plant material. Contrastingly, the <2 μm fraction of this non-rhizosphere soil exhibited the highest proportion of microbial biomass C to organic C (8.4%) of any of the other fractions. Since the proportion of microbial biomass C in the organic C pool is considered to reflect the microbial turnover (Amato and Ladd, 1992), the dispersible (outer) microorganisms would have a slower turnover rate in the non-rhizosphere soil than in both *Digitaria* and seasonal crop planted soils.

Enumeration and distribution of soil bacteria

In the cultivated soil, AODC data confirmed the biomass C distribution, i.e. that most of the microorganisms were located first in the two finer fractions and then in the >2000 μm size fraction. In the permanent pasture soils the distribution of AODC and of biomass C did not evidence the same similarities than in the cultivated soil: when the maximum of biomass C (more than 40%) was found in the >2000 μm fraction of both RPP and NRPP soils, the maximum AODC were found in the finer fractions of the rhizosphere soil (as observed in the cultivated soil too) and in the 20–2 μm of the non-rhizosphere pasture soil.

From the AODC data, it results that most of the bacterial cells of the planted soils, i.e. the cultivated soil and the rhizosphere soil under *Digitaria*, were found in the finest fractions (20–2 μm and <2μm fractions). Most of the bacteria of the non-rhizosphere bare soil resided in the micro- (20–2 μm) and in macroaggregates (>2000 μm) and not in the dispersible fraction (<2μm). The distribution of bacteria was influenced not only by the abundance of stable macroaggregates but also by the presence of living plants which suggests a shift in the distribution of the bacterial populations toward the dispersible and small aggregate fractions in planted soils. In the absence of a plant, microbial cells mostly reside in aggregates of micron or of millimeter size suggesting either a lack of fresh organic substrates in the dispersible phase (no exudates) or a preferential survival in aggregates as suggested by several authors (Hattori, 1988; Postma et al., 1990; Rutherford and Juma, 1992).

Distribution and location of Azospirillum brasilense

The direct enumeration in soil by fluorescent antibody techniques showed a maximum abundance of *Azospirillum brasilense* in the rhizosphere pasture soil compared to the other studied soils. This difference indicates the important role of the active roots of *Digitaria* in the selection of *A. brasilense* together with the possible role of these active roots in the stabilization of macroaggregates. In our study, in the rhizosphere soil, where the bacterial populations (4.10^9 cells per g of soil) should be stimulated by the exudates of *Digitaria* roots, the proportion of *A. brasilense* on the root fraction represented 10^7 cells g^{-1} dry soil, i.e. 0.6% of

Table 4. The rhizosphere effect expressed as the ratio (R/S) of bacterial numbers in rhizosphere soil (RPP) over those in non-rhizosphere soil (NRPP), for total bacteria (R/S_{total}) and *A. brasilense* ($R/S_{A.\ brasilense}$)

Soil fractions (μm)	Total soil bacteria (AODC)	*A. brasilense* (IF)
	$R/S_{total\ bacteria}$	$R/S_{A.\ brasilense}$
Roots > 2000	1 2	1.5 297
2000–200 d < 1	1	8.3
2000–200 d > 1	2.5	14.5
200–50	2.3	8
50–20	1.5	10
20–2	2	9
< 2	9	37
Unfrac. soil	2	8

the bacterial counts in that fraction. In their study on Kallar grass grown in a Punjab (Pakistan) soil, Reinhold et al. (1986) found 6×10^6 *A. lipoferum* cells per g of grass root and 10^4 in the non-rhizosphere soil. A smaller number of cells and a smaller proportion of *Azospirillum* were enumerated in the non-rhizosphere pasture soil except in the root fraction where the high proportion of *A. brasilense* evidenced the presence of a few active roots of *D. decumbens* in this soil.

Rhizosphere to non-rhizosphere soil bacteria density ratio has been established to evidence the influence of root vicinity on the concentration of bacteria and on the selection of some microbial species (Balandreau and Knowles, 1978). The bacterial density ratios of rhizosphere to non-rhizosphere soil (R/S) were calculated using AODC counts ($R/S_{total\ bacteria}$) or IF enumeration ($R/S_{A.\ brasilense}$) on the rhizosphere pasture soil (R) and on the non-rhizosphere pasture soil (S) (Table 4). In the unfractionated soils, $R/S_{A.\ brasilense}$ (8/1) was higher than $R/S_{total\ bacteria}$ (2/1). The effect of the rhizosphere situation on the concentration of bacteria was evidenced by $R/S_{total\ bacteria}$ values $\gg 1$.

The highest values of $R/S_{A.\ brasilense}$ were observed in the dispersible clay size fraction (<2 μm $R/S_{A.\ brasilense}$: 37/1) and particularly in the macroaggregate fractions (>2000 μm $R/S_{A.\ brasilense}$: 297/1). Then the presence of fine, active roots of *D. decumbens* in the macroaggregates of the rhizosphere soil is revealed by the relative abundance of *A. brasilense*. Unexpectedly, the $R/S_{A.\ brasilense}$ and the $R/S_{total\ bacteria}$ of the root fraction were similarly low (1.5/1 versus 1/1). This demonstrated that the coarse roots of *Digitaria* in the active rhizosphere did not offer a more suitable niche than the non-rhizosphere soil neither to *A. brasilense* nor to soil bacteria. The selection of the rhizosphere bacteria was more effective in the light fraction of the 2000–200 μm size separate where the $R/S_{A.\ brasilense}$ ratios was 8.3 higher than the corresponding $R/S_{total\ bacterial}$ ratio.

Soil fractions and their biological content as indices of soil changes

Since the higher stability of macroaggregates would involve the cohesion due to soil enmeshment by roots (Tisdall and Oades, 1982), and the soil compaction due to water suction by roots (Haynes and Swift, 1990), fine living roots and their bacterial communities should be found in macroaggregates. The presence of such active roots in the macroaggregates was confirmed by the high proportion of *A. brasilense* in the macroaggregates (>2000 μm) of the rhizosphere soil under *Digitaria* where *A. brasilense* represented 4.7% of the total bacterial counts. The relative abundance of this bacteria, compared to the total microflora, demonstrated that this fraction offered a suitable niche for a microorganism adapted to the rhizosphere. Moreover the abundance of *A. brasilense* revealed the presence of active roots enhancing the proportion of the rhizosphere bacteria and evidencing that fine roots would penetrate these macrostructures in the rhizosphere pasture soil. The relative abundance of the rhizospheric bacteria in other fractions of the rhizosphere soil would evidence the presence of *Digitaria* fine roots in the plant debris of 2000–200 μm size and a contact between active fine roots of *Digitaria* and the dispersible clay size fraction of this soil.

Conversely, the stability of microaggregates involved the role of organic colloids, especially of polysaccharides from bacterial (Foster, 1988) or of root (Haynes and Swift, 1990) origin or of humic compounds (Oades, 1988) and of mineral cements like clay minerals and calcium bridges. Such microaggregates were found to be less sensitive to soil management (Oades, 1988) and would protect surviving microorganisms as well as old organic matter while they offer external sites for bacterial adsorption. The abundance

of microaggregates of less than 200 μm size obtained by the very mild dispersion technique used did not differentiate the three situations. This supports the opinion by Oades (1988) that they are less influenced by soil management than the macrostructures.

Microbiological data revealed that the diversity of the different fractions within the soil was decreasing from the rhizosphere soil to the non-rhizosphere pasture soil and to the continuously cultivated soils. Statistically, all the fractions of the cultivated soil were not different from each other, except the root fraction.

Concluding, the mild technique used to disperse and fractionate a tropical clay soil yielded meaningful soil fractions wherein organic and microbial characteristics were preserved. This allowed to quantify both a rhizosphere effect and a management effect on the main changes in soil aggregate distribution.

References

Amato M and Ladd J N 1988 Assay for biomass based on ninhydrine reactive nitrogen in extracts of fumigated soils. Soil Biol. Biochem. 20, 107–114.

Amato M and Ladd J N 1992 Decomposition of ^{14}C-labelled glucose and legume material in soils: properties influencing the accumulation of organic residue C and microbial biomass C. Soil Biol. Biochem. 24, 455–464.

Angers D A, N'dayegamiye A and Côté D 1993 Tillage-induced differences in organic matter of particle-size fractions and microbial biomass. Soil Sci. Soc. Am. J. 57, 512–516.

Balandreau J and Knowles R 1978 The rhizosphere. *In* Interactions between non-pathogenic soil microorganisms and plants. Eds. Y R Dommergues and S V Krupa. pp 243–266. Elsevier Scientific Publishing Company, New-York.

Cambardella C A and Elliott E T 1992 Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Sci. Soc. Am. J. 56, 777–783.

Catroux G and Schnitzer M 1987 Chemical, spectroscopic, and biological characteristics of the organic matter in particle size fractions separated from an Aquoll. Soil Sci. Soc. Am. J. 51, 1200–1207.

Chaussod R, Nicolardot B, Catroux G and Chretien J 1986 Relationships between physico-chemical and biological characteristics of a few cultivated soils. Science du Sol 24, 213–221.

Chotte J L, Jocteur Monrozier L, Villemin G and Albrecht A 1992 Soil microhabitats and the importance of the fractionation method. *In* Soil Organic Matter Dynamics and Sustainability of Tropical Agriculture. Eds. K Mulongoy and R Merckx. pp 39–45. Wiley-Sayce Publishers, IITA/KU Leuven, The Netherlands.

Chotte J L, Villemin G, Guilloré P and Jocteur Monrozier L 1993 Morphological aspects of microorganism habitats in a vertisol. *In* International Workshop on Soil Micromorphology (Townsville, Australia, 12–17 July 1992). Elsevier, Amsterdam, The Netherlands.

Christensen B T 1992 Physical fractionation of soil and organic matter in particle size and density separates. Adv. Soil Sci. 20, 1–90.

Collins H P, Rasmussen P E and Douglas jr C L 1992 Crop rotation and residue management effects on soil carbon and microbial dynamics. Soil Sci. Soc. Am. J. 56, 783–788.

Dorioz J M and Robert M 1987 Aspects microscopiques des relations entre les microorganismes ou les végétaux et les argiles. Conséquences sur les microorganisations et sur la microstructuration des sols. *In* Soil Micromorphology. Eds. N Fedoroff, L Bresson and M A Courty. pp 353–361. AFES, Paris, France.

Day J M and Döbereiner J 1976 Physiological aspects of N_2 fixation by a *Spirillum* of *Digitaria* roots. Soil Biol. Biochem. 8, 45–50.

Döbereiner J and Pedrosa F O 1987 Nitrogen fixing bacteria in nonleguminous crop plants. Soil Sci. Soc. Am. Proc. 43, 951–955.

Duchaufour P 1977 Dynamique de la matière organique. *In* Pédologie 1: Pédogénèse et classification. Eds. P Duchaufour et B Souchier. pp 28–70. Masson, Paris, France.

Elliott E T 1986 Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. Proc. 50, 627–633.

Foster R C 1988 Microenviroments of soil microorganisms. Biol. Fertil. Soils 6, 189–203.

Foster R C, Rovira A D and Cock T W 1983 Ultrastructure of the root-soil interface. The American Phytopathological Society, St Paul, Minnesota, USA. 156p.

Gamard P 1991 Etude de populations d'*Azospirillum* dans la rhizosphère: Caractérisation sérologique et activité dénitrifiante. Thèse Univ. Lyon 1, France. 173p.

Gray T R G and Williams S T 1971 Microbial productivity in soils. Gen. Microbiology Soc. Symposium. pp 255–286.

Hart P B S, August J A, Ross C W and Jullian J F 1988 Some biochemical and physical properties of Tokomaro silt loam under pasture and after 10 years of cereal cropping. New-Zeal. J. Agric. Res. 31, 77–86.

Hassink J, Bouwman L A, Zwart K B and Brussaard L 1993 Relationships between habitable pore space, soil biota and mineralization in grassland soils. Soil Biol. Biochem. 25, 47–55.

Hattori T 1988 Soil aggregates as microhabitats of microorganisms. Rep. Inst. Agr. Res. Tohoku Univ. 37, 23–36.

Haynes R J and Swift R S 1990 Stability of soil aggregates in relation to organic constituents and soil water content. J. Soil Sci. 41, 73–83.

Jocteur Monrozier L, Ladd J N, Fitzpatrick R W, Foster R C and Raupach M 1991 Components and microbial biomass content of size fractions in soils of contrasting aggregation. Geoderma 49, 37–62.

Ladd J N and Amato M 1988 Relationships between biomass ^{14}C of a range of fumigated soils. Soil Biol. Biochem 20, 115–116.

Ladd J N and Foster R M 1987 Role of soil microflora in nitrogen turn-over. *In* Advances in Nitrogen Cycling in Agricultural Ecosystems. Ed. J R Wilson. pp 113–133. CAB International, Wallingford, Oxon, UK.

Mahboubi A A, Lal R and Faussey N R 1993 Twenty-eight years of tillage effects on two soils in Ohio. Soil Sci. Soc. Am. J. 57, 506–512.

Oades J M 1988 The retention of organic matter in soils. Biogeochemistry 5, 35–70.

O'Hara G W, Davey M R and Lucas J A 1983 Association between the nitrogen fixing bacterium *A. brasilense* and excised plant roots. Z. Pflanze. Physiol. Bd. 113, 1–13.

Patriquin D G, Döbereiner J and Jain D K 1983 Sites and processes of association between diazotrophs and grasses. Can. J. Microbiol. 29, 900–915.

Postma J, Hok-a-hin C H and Van Veen J A 1990 Role of microniches in protecting introduced *Rhizobium leguminosarum* biovar *trifolii*

against competition and predation in soil. Appl. Environ. Microbiol. 56, 495–502.
Ramsay A J and Bawden A D 1983 Effects of sterilisation and storage on respiration, nitrogen status and direct counts of soil bacteria using acridine orange. Soil Biol. Biochem. 15, 263–268.
Reinhold B, Hurck T, Niemann E G and Fendrik I 1986 Close association of *Azospirillum* and diazotrophic rods with different root zones of kallar grass. Appl. Environ. Microbiol. 52, 520–526.
Rutherford P M and Juma N G 1992 Influence of texture on habitable pore space and bacterial-protozoan populations in soil. Biol. Fertil. Soils 12, 221–227.
Russel E W 1973 Soil Conditions and Plant Growth. Longman, London, 849 p.
Santruckova H and Straskraba M 1991 On the relationship between specific respiration activity and microbial biomass in soils. Soil Biol. Biochem. 23, 525–532.
Schank C, Bouton J H, Weiser G C, Zuberer D A, Tyler M E, Milam J R and Littell R C 1979 Fluorescent antibody technique to identify *A. brasilense* associated with roots of grasses. Soil Biol. Biochem. 11, 287–295.
Scherba G, Weige R M and O'Brien Jr W D 1991 Quantitative assessment of the germicidal efficacy of ultrasonic energy. Appl. Environ. Microbiol. 57, 2079–2084.
Schmidt E 1974 Quantitative autoecological study of microorganisms in soil by immunofluorescence. Soil Science 118, 141–149.
Smith M S and Tiedje J M 1979 The effect of roots on soil denitrificaton. Soil Sci. Soc. Am. Proc. 43, 951–955.
Sparling G P, Ord B G and Vaughan D 1981 Changes in microbial biomass and activity in soils amended with phenolic acids. Soil Biol. Biochem. 13, 455–460.
Sparling G P and Cheshire M V 1985 Effect of periodate oxidation on the polysaccharide content and microaggregate stability of rhizosphere and non-rhizosphere soils. Plant and Soil 88, 113–122.
Stotzky G and Burns R G 1982 The soil environment: clay -humus microbe interaction. *In* Experimental Microbial Ecology. Eds. R G Burns and J H Slater. pp 105–133. Blackwell Scientific, Oxford, UK.
Tiessen H and Stewart J W B 1983 Particle size fractions and their studies of soil organic matter. II. Cultivation effects on organic matter composition in size fractions. Soil Sci. Soc. Am. J. 47, 509–514.
Tiessen H, Karamanos R E, Stewart J W B and Selles F 1984 Natural ^{15}N abundance as an indicator of soil organic transformations in native and cultivated soils. Soil Sci. Soc. Am. J. 48, 312–315.
Tisdall J M and Oades J M 1982 Organic matter and water stable aggregates in soil. J. Soil Sci. 32, 141–163.
Turchenek L W and Oades J M 1979 Fractionation of organo-mineral complexes by sedimentation and density techniques. Geoderma 21, 311–343.

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