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Micro-scale distribution of microorganisms and microbial enzyme activities in a soil with long-term organic amendment

C. POLL[a], A. THIEDE[a], N. WERMBTER[a], A. SESSITSCH[b] & E. KANDELER[a]
[a]*Institute of Soil Science, Department of Soil Biology, University of Hohenheim, Emil-Wolff-Straße 27, 70599 Stuttgart, Germany, and* [b]*Austrian Research Centers, Division of Life and Environmental Sciences, 2444 Seibersdorf, Austria*

Summary

Microbial ecology is the key to understanding the function of biodiversity for organic matter cycling in the soil. We have investigated the impacts of farmyard manure added over 120 years on organic matter content, enzyme activities, total microbial biomass and structure of microbial populations in several particle-size fractions of a Luvic Phaeozem a few kilometres northeast of Halle, Germany. We compared two treatments: no fertilization (control) and 12 t farmyard manure (FYM) ha^{-1} $year^{-1}$ since 1878. The fine fractions contained most C and N, microbial biomass, total amount of phospholipid fatty acids (PLFAs) and greatest invertase activity. Xylanase activity as well as fungal biomass increased only gradually with diminishing particle size, whereas the relative abundance of fungi decreased with diminishing particle size. The least diversity of the soil microbial community, indicated by the smallest Shannon index based on the abundance and amount of different PLFAs and small number of terminal restriction fragments (T-RFs) of 16S rRNA genes, was in the sand fractions. The results supported the hypothesis that this microhabitat is colonized by a less complex bacterial community than the silt and clay fractions. Addition of FYM had enhanced the amount of organic matter, total microbial biomass, and xylanase and invertase activity, and induced a shift of the microbial community towards a more bacteria-dominated community in the coarse sand fraction. Microbial communities in finer fractions were less affected by addition of FYM.

Introduction

Changes in organic matter input as well as the distribution of litter within the soil profile modify biological, chemical and physical soil properties. Studies over the last decade have focused on soil microorganisms, organic matter dynamics and carbon sequestration mainly at the plot scale (e.g. Saffigna *et al*., 1989; Arshad *et al*., 1990). Recently, knowledge on the micro-scale spatial distribution of organic substrates, microbial biomass and enzyme activities in soils has improved our understanding of the underlying mechanisms driving nutrient cycling (Ladd *et al*., 1993). The physical fractionation of soils has offered a methodological approach to investigate the dynamics of organic matter in various fractions of the soil as well as the role of soil microorganisms for nutrient turnover. For example, Chefetz *et al*. (2002) investigated the composition of organic matter in such fractions. With diminishing particle size the abundance of lignin and polysaccharides decreased, whereas the oxidation products of lignin and microbially derived units increased. Other studies have demonstrated the dynamics and turnover of organic matter in particular fractions (Gregorich *et al*., 1989; Stemmer *et al*., 1999). Less is known about the abundance of microorganisms and their function within fractions. Silt and clay fractions contained the most microbial biomass (Kanazawa & Filip, 1986; Jocteur Monrozier *et al*., 1991; Kandeler *et al*., 2000). Invertase is found mainly in the silt and clay fractions because of the strong binding and protection by clay–humus–enzyme complexes. Xylanase, on the other hand, is bound mainly to particulate organic matter in the coarse sand fraction (Kandeler *et al*., 1999a,b; Gerzabek *et al*., 2002). Although many reports provide insight into the distribution of microorganisms in microhabitats (Chotte *et al*., 1998; Sessitsch *et al*., 2001), it remains unclear whether long-term organic amendments affect the function of soil microorganisms in various size fractions.

Correspondence: E. Kandeler. E-mail: kandeler@uni-hohenheim.de
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Therefore, we have analysed the impact of long-term organic amendment on the microbial enzyme activity and microbial community structure within particle-size fractions of the soil.

Materials and methods

Site and samples

For this study we sampled the soil from the long-term field experiment a few kilometres northeast of Halle in the northeast of Germany, 113 m above sea level, and on a 15-km broad area of sandy loess. The soil, the top 30 cm of a Luvic Phaeozem (FAO), consisted of 8% clay, 20% silt, and 72% sand; additional soil properties are given in Table 1. The mean long-term annual temperature is 9.2°C and the mean long-term annual precipitation is 501 mm. A complete documentation of the experiment and compilation of data can be found in Stumpe *et al.* (1995) and Merbach *et al.* (1999). Treatments have been almost constant since 1878. On all plots chosen for this study, winter rye (*Secale cerale* L.) has been cultivated in monoculture. Two fertilization treatments were chosen: (i) control (no mineral and organic fertilization), and (ii) 12 t farmyard manure (FYM) $ha^{-1}\,year^{-1}$. Since the field experiment was established without any field replicates in 1878, each of these plots (length 25.0 m, width 11.6 m) was divided into three subplots, from which soil samples were taken randomly at a depth of 0–30 cm (about 16 cores per subunit) in autumn 2000. Soil samples were stored at −20°C to preserve microbial biomass and soil enzyme activities, and the soil was allowed to thaw at 4°C for about 2 days before the fractionation. Fractions were treated in the same way before the microbiological analyses started.

Physical fractionation of the soils

The procedure involved dispersing particle-size fractions by a combination of wet-sieving and centrifuging. The use of low-energy sonication for the dispersion should completely disrupt the macroaggregates (< 250 μm), but should prevent the release of stable organic matter that is physically protected within microaggregates in natural sites (Stemmer *et al.*, 1998). Particle-size fractions, namely 2000–250, 250–63, 63–2 and 2–0.1 μm, were obtained by low-energy sonication of soil: water suspension, followed by a combination of wet-sieving and centrifuging. Briefly, 30 g of field-moist soil was dispersed in 100 ml of cooled distilled water by an ultrasonic disaggregator (50 $J\,s^{-1}$ for 120 s). Coarse and medium sand (2000–250 μm) and fine sand (250–63 μm) were separated by manual wet-sieving. Silt-sized particles (63–2 μm) were separated from the clay fraction (2–0.1 μm) by centrifuging at 150 ***g*** for 2 minutes. The supernatants were centrifuged at 3900 ***g*** for 30 minutes to yield clay-sized particles (2–0.1 μm, according to an equispherical diameter and a particle density of 2.65 $g\,cm^{-3}$).

To test the efficiency of low-energy sonication the data were compared with particle-size distribution after chemical dispersion. Recovery rates were calculated by the sum of activities or amounts measured in the fractions referring to the particle-size distributions in the soil.

Carbon and nitrogen analyses

Total C and N were determined by dry combustion in a LECO 2000 CN elemental analyser. Measurements were done in triplicate. Total C was similar to organic C because the soils analysed contained no carbonate.

Enzymatic analyses

Invertase and xylanase activity were measured by the method of Schinner & von Mersi (1990). For the determination of xylanase activity, 0.5–1.0 g of the moist fraction was incubated with 5.0 ml of a substrate solution (1.7% by weight xylan from oats suspended in 2 M acetate buffer, pH 5.5) and 5.0 ml 2 M acetate buffer (pH 5.5) for 24 hours at 50°C. The sugars released during the incubation reduced potassium hexacyanoferrate (III) in an alkaline solution to potassium hexacyanoferrate (II), which was measured colorimetrically according to the Prussian blue reaction (Schinner *et al.*, 1996).

To measure invertase activity, we incubated 0.5–1.0 g of the moist fraction with 5.0 ml of 50 mM sucrose solution and 5.0 ml of 2 M acetate buffer (pH 5.5) for 3 hours at 50°C. Reducing sugars were determined as described for xylanase activity. Enzyme activities were measured in three replicates.

Soil microbial analyses

Microbial biomass N was determined by chloroform–fumigation extraction (CFE) by a modification of the method

Table 1 Biological and chemical properties of the soil (0–30 cm)

Treatment	C_{org} /mg g^{-1}	N_t /mg g^{-1}	C/N	pH	Xylanase /μg GE $g^{-1}\,day^{-1}$	Invertase /μg GE $g^{-1}\,hour^{-1}$
Control	10.4	0.84	12.5	5.6	744	487
FYM	15.9	1.38	11.6	6.2	825	650

C_{org}, organic C; N_t, total N; GE, glucose equivalent; FYM, farmyard manure.

of Amato & Ladd (1988). Briefly, 0.3–0.5 g of the moist fractions was fumigated with 0.1 ml of chloroform (ethanol-free) for 24 hours at 25°C. Subsequently, the chloroform in the samples was removed. Samples and unfumigated controls were extracted with 5.0 ml of 2 M KCl solution for 60 minutes on a rotatory shaker. After filtration, 2 ml of the filtrates was mixed with 0.5 ml of 0.4 M sodium citrate solution. Ninhydrin-reactive N was determined colorimetrically (Schinner *et al.*, 1996).

Soil microbial community structure: PLFA analyses

Lipids were extracted from soil, fractionated and quantified as described by Bardgett *et al.* (1996), who used a procedure based on that of Bligh & Dyer (1959) as modified by White *et al.* (1979). Separated fatty-acid methyl esters were identified by chromatographic retention time and mass spectral comparison with a mixture of standard qualitative bacterial acid methyl ester and fatty-acid methyl ester (Fa. Supelco) that ranged from C11 to C20. For each sample the abundance of individual fatty-acid methyl esters was expressed per unit dry weight. The nomenclature for phospholipid fatty acids (PLFAs) is that of Frostegård *et al.* (1993). The fatty acid 18:2ω6 was used as an indicator of fungal biomass (Federle, 1986), and the ratio of fungal to total amount of PLFA was calculated for each particle-size fraction. The diversity of the PLFAs was calculated with the Shannon index H:

$$H = -\sum_{i=1}^{n} p_i \ln p_i,$$

where p_i is the relative abundance of a single fatty acid i, in the total amount of fatty acids, and n is the number of detected PLFAs (Shannon, 1948).

DNA isolation and terminal restriction fragment length polymorphism (T-RFLP)

We isolated DNA as described by Sessitsch *et al.* (2001) based on the procedure published by van Elsas & Smalla (1995). Briefly, 0.15 g of soil was incubated in a lysozyme-containing buffer and, subsequently, cells were lysed by bead-beating. Proteins were removed by phenol–chloroform extraction, and DNA was precipitated with isopropanol. The resulting DNA solution was further purified by passage through sepharose-based spin-columns.

The eubacterial primers 8f (Weisburg *et al.*, 1991) labelled at the 5′ end with 6-carboxyfluorescein (6-Fam; MWG) and 518r (Liu *et al.*, 1997) were used to amplify approximately 530 base pairs (bp) of the 16S rRNA gene. We carried out the reaction with a PTC-100^{TM} thermocycler (MJ Research, Inc.), applying an initial denaturation step of 5 minutes at 95°C followed by 30 cycles of 30 s at 95°C, 1 minute of annealing at 54°C, and 2 minutes of extension at 72°C. The polymerase chain reactions (PCR) (50 μl) contained 1× reaction buffer (Gibco, BRL), 200 μM each dATP, dCTP, dGTP and dTTP, 0.15 μM of each primer, 3 mM $MgCl_2$, 2.5 U Taq DNA polymerase (Gibco, BRL) and 20 ng template DNA. The PCR products (10 μl, approximately 250 ng DNA) were digested for 2 hours in 20 μl with 10 U of a combination of the restriction enzymes *Hha*I and *Hae*III (Gibco, BRL). Aliquots (1 μl) were mixed with 0.16 μl of deionized formamide, 0.83 μl of loading buffer (Perkin-Elmer) and 0.3 μl of DNA fragment length standard (Genescan 500 Rox; Perkin-Elmer). The reaction mixtures were denatured at 92°C for 2 minutes and chilled on ice prior to electrophoresis. Samples (1.5 μl) were run on 5% denaturing polyacrylamide gels, and the fluorescently labelled terminal restriction sizes were analysed on an ABI 373 A automated DNA sequencer (PE Applied Biosystems Inc., Faster City, CA, USA). The lengths of the labelled fragments were determined by comparison with the internal standard.

According to the range of the size marker, T-RF peaks between 50 and 500 bp and peak heights of $\leq$50 fluorescence units were included in the analysis. Generally, the error for determining fragment sizes with our automated DNA sequencer was less than 1 bp, although in some cases more variation was found. Therefore, T-RFs that differed by less than 1.5 bp were clustered, unless individual peaks were detected in a reproducible manner. Fluorescence units of all fragments were summed, and intensities of individual fragments were calculated as percentages. Representative sample profiles show the mean percentage values of each fragment.

Statistical analysis

All analytical results were calculated on the basis of oven-dry (105°C) weight of soil. Results were given as arithmetic means of three replicates and their standard errors. Means of soil characteristics of the control soil and FYM treatment were compared by Student's t-test. The assumptions of normality and homogeneity of variances were tested by the Kolmogoroff–Smirnoff test and the Levene test. The PLFA profiles showed no homogeneity of variances; therefore, we used the Mann–Whitney U-test to characterize significant differences between the different treatments. For multiple comparison of means a two-way analysis of variance was performed. The Kolmogoroff–Smirnoff test and the Cochran test were applied to check the assumptions. Data of invertase, total PLFAs and ninhydrin-reactive N were transformed to logarithms to achieve approximately normal distributions and homogeneity of variances. The variance of the Shannon index seemed inhomogeneous even after transformation, and so we did not do an analysis of variance. Significance between different treatments was accepted at the $P < 0.05$ level of probability.

Results

Organic C and total N in particle-size fractions

Physical and chemical dispersion yielded similar amounts of each particle-size fraction (Table 2). Evidently, the ultrasonication

Table 2 Particle-size distribution of soil (0–30 cm) determined after physical and chemical dispersion

	Particle-size distribution /%			
Dispersion	2000–250 μm	250–63 μm	63–2 μm	$< 2\,\mu$m
Chemical	37	32	23	8
Physical	39	33	20	8

completely disrupted the macroaggregates ($> 250\,\mu$m) and totally separated the smaller fractions.

Concentration of organic carbon and total nitrogen (related to fraction dry weight) increased with diminishing particle size of this soil (Figure 1), whereas the largest amounts of organic carbon and total nitrogen were found in the silt fraction. The mean recovery rates of organic C and total N after the fractionation were 100.3% (control), 91.3% (FYM) and 99.2% (control), 83.4% (FYM), respectively. The soil that had received FYM contained more carbon and nitrogen in all particle-size fractions (2000–250, 250–63, 63–2 and 2–0.1 μm) than the unmanured soil; the differences were significant for the silt and clay fractions (Figure 1).

Microbial biomass in particle-size fractions

The summed amount of ninhydrin-reactive N within the particle fractions surpassed the amount in the bulk soil by between 21 and 46%. This large mean recovery rate of microbial biomass N suggests that ultrasonication might expose new colonized surfaces for the fumigation and extraction. The largest concentration of ninhydrin-reactive N was found in the clay fraction and the smallest in the fine and coarse sand particles (Figure 1). Adding FYM greatly influenced microbial biomass in the soil (Figure 1, Table 3). We attribute increase in the bulk soil mainly to the increased microbial biomass in the clay and silt fractions.

Enzyme activities in particle-size fractions

The mean recovery of invertase activity after the fractionation ranged from 87 to 94%. However, the recovery of xylanase exceeded 100% because of the enhanced diffusion of the high-molecular-weight xylan to newly accessible enzyme-bearing surfaces after dispersion. The distribution of invertase and xylanase in the particle-size fractions varied with the enzyme assayed (Figure 1, Table 3). Invertase activity was mainly in the silt and clay fractions, whereas xylanase activity showed only a gradual increase from the coarse to the fine fractions. Adding FYM had promoted invertase activity mainly in the finer fractions, whereas xylanase activity increased significantly in all particle-size fractions, with the largest increase in the sand fractions. The response of the single variables depended strongly on the soil fraction, as is shown by the significant interaction terms for almost all variables (Table 3).

Microbial population structure in particle-size fractions

PLFAs. The amount of the total PLFAs ranged from 7.1 to 16.4 nmol g^{-1} for the bulk soil, and the recovery rate of PLFAs in particle-size fractions was 75% for the control and 73% for FYM. Total PLFAs as an indicator for microbial biomass increased with diminishing particle size and were significant larger in the clay fractions (Figure 1, Table 3). The number of PLFAs, an indicator for microbial diversity, was larger in the smaller fractions (Figure 2). The PLFA patterns in the silt and clay fractions were similar, but differed in the amount of single PLFAs present. In general, more of each PLFA was found in the clay fraction than in the silt. Addition of FYM had increased the amount of PLFAs in finer fractions. In contrast, fewer PLFAs were detected in the sand fraction of the control soil. The FYM increased the number and the content of single PLFAs in the sand fraction. Typical Gram-negative bacteria PLFA biomarkers (16:1ω7, cyc17:0, 18:1ω9c and 18:1ω7) seem to have been introduced into the coarse sand fraction as a result of the addition of FYM. Although PLFA contents were influenced more strongly by particle-size fraction than by the fertilizer treatment (Table 3), a definable shift in the composition of the microbial community was detected due to the addition of FYM (Figure 1). The Shannon index based on the abundance and amount of different PLFAs was likewise greatest in the two smaller fractions and did not increase further as a result of addition of FYM (Figure 1). In contrast, FYM had increased the Shannon index in the sand fractions (Figure 1).

We used the PLFA 18:2ω6c as an indicator for the fungal biomass following Federle (1986) and Frostegård & Bååth (1996). The portion of fungal biomass in the total PLFAs declined with diminishing particle size, whereas the largest concentration of this PLFA was detected in the clay (Table 4). Addition of FYM had decreased this ratio in the coarse sand and in the silt by 50%.

T-RFLP. We determined bacterial community shifts in different particle-size fractions receiving fertilizer by T-RFLP analysis of PCR-amplified 16S rRNA genes and detected a total of 39 T-RFs. The number of T-RFs in individual sample profiles ranged from 25 (FYM, fine sand fraction) to 39 (control, clay fraction) (Table 5). In the control treatment, the largest number of dominant bacterial species was detected in the clay fraction, whereas the coarse sand fraction showed the least bacterial diversity. Treatment with FYM had added only two fragments (366 and 388 bp) in the fractions. Several T-RFs were found exclusively in the control treatment, including fragments of 79 bp, 84 bp, 86 bp, 133 bp, 212 bp, 257 bp, 285 bp, 312 bp and 325 bp (Table 5).

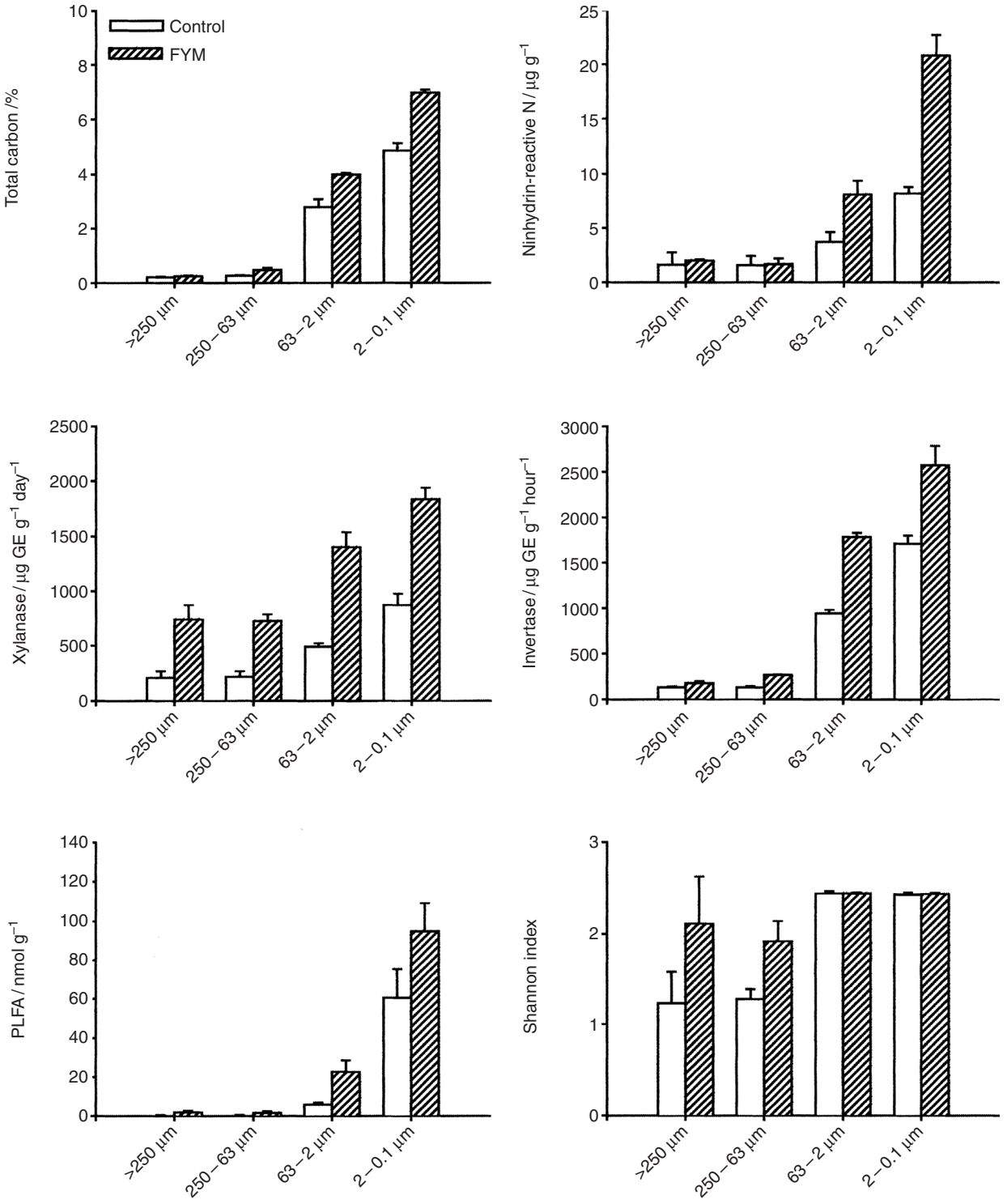

Figure 1 Impact of long-term amendment with farmyard manure (FYM) on microbial biomass, ninhydrin-reactive N content, xylanase and invertase activity (GE, glucose equivalent), total phospholipid fatty acid (PLFA) content and microbial diversity (Shannon index) in particle-size fractions. Values are given as means of three replicate samples; bars indicate standard errors, and asterisks indicate significant differences between treatments at $P = 0.05$.

Table 3 *F*-values for five variables computed by a two-way analysis of variance with factors particle size and treatment

Variable	Particle size with 3 d.f.	Treatment with 1 d.f.	Particle size × Treatment with 3 d.f.
Organic C	764	86	24.25
Biomass	28	10	1.16
Xylanase	42	124	3.41
Invertase	640	88	3.91
PLFA content	109	24	0.75

d.f., degrees of freedom; PLFA, phospholipid fatty acid.

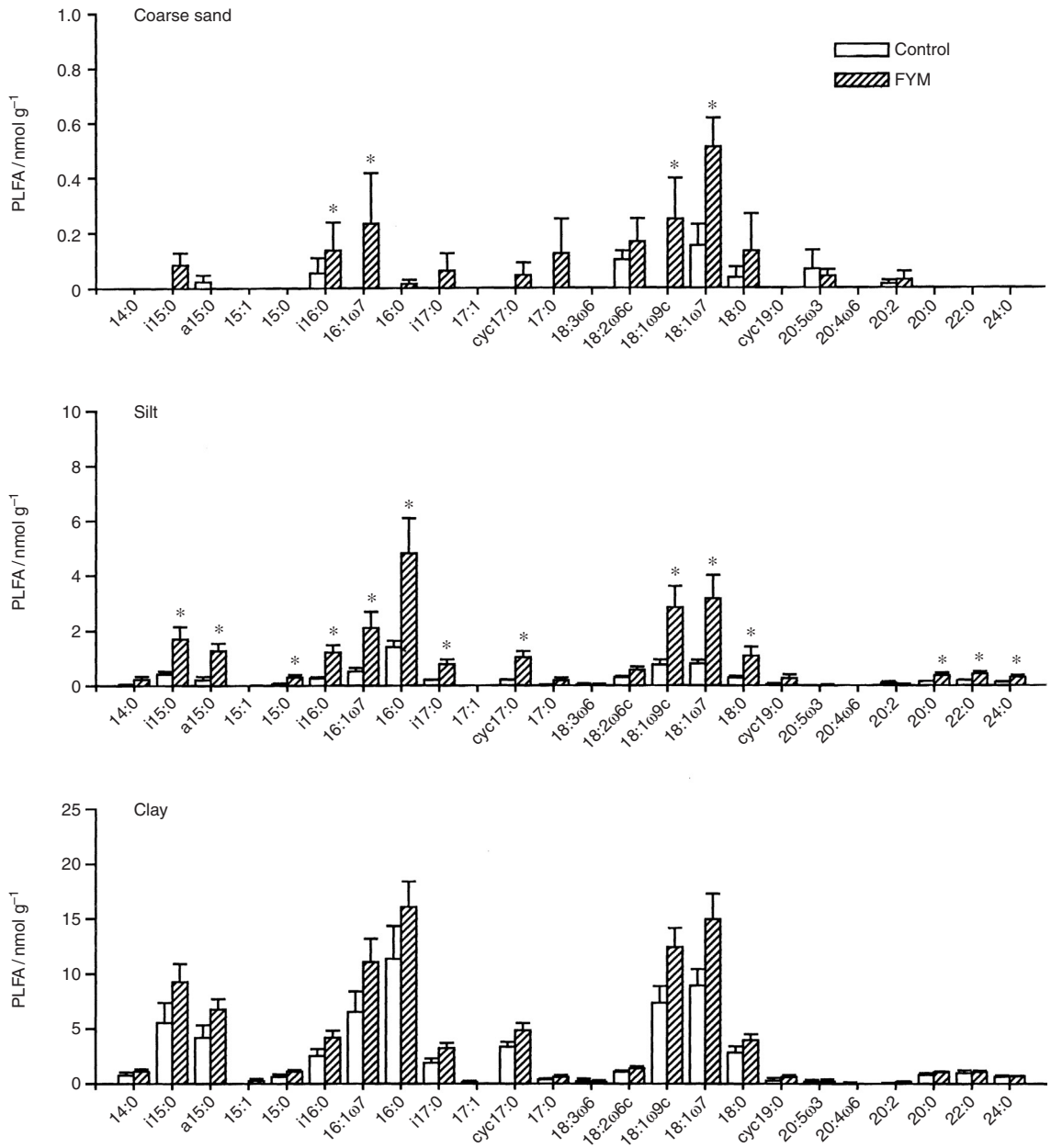

Figure 2 The response of phospholipid fatty acid (PLFA) pattern to long-term amendment with farmyard manure (FYM) in the coarse sand, silt and clay fractions. Values are given as means of three replicate samples; bars indicate standard errors, and asterisks indicate significant differences between treatments at $P = 0.05$.

Table 4 Proportion of the fungal phospholipid fatty acid (PLFA) 18:2ω6 to total amount of PLFAs. Values in parentheses are standard errors

	Proportion of fungal PLFA /%			
	Coarse sand	Fine sand	Silt	Clay
Control	22.2 (2.9)	10.0 (7.6)	4.8 (0.2)	1.8 (0.2)
FYM	9.3 (4.6)	10.7 (5.2)	2.5 (0.2)	1.5 (0.0)

Discussion

Organic C, total N and biomass of the particle-size fractions

We found most C and N in the silt fraction, whereas the largest concentration was in the clay fraction. In general, the finer is the particle size the larger is the concentration of organic matter (Christensen, 1996; Stemmer *et al*., 1999), and our results are in accordance. Nevertheless, because the soil we studied contained such a large proportion of silt, this fraction contained the most C and N. Addition of FYM over the 120 years seemed to have increased the amounts of organic C and total N in the fine sand and silt fractions (Figure 1). A similar tendency was found with the microbial biomass (Figure 1). In the finer fractions, adding FYM had induced an increase of ninhydrin-reactive N, whereas in both sand fractions no significant effects were detected. These results accord with several studies of microbial biomass in particle-size fractions (Jocteur Monrozier *et al*., 1991; Kandeler *et al*., 1999a,b, 2000). Therefore, addition of FYM induced mainly microbial growth in smaller fractions over the long period of organic amendment. The preferential growth of soil microorganisms in the finer fractions may be attributed not only to the availability of carbon sources but also to the protection of soil microorganisms from faunal predation, as shown by Ladd *et al*. (1996).

Enzyme activity

Invertase showed significantly greater activity in the fine fractions, further confirming the assumption that this enzyme is bound to the fractions with dense organic matter and large microbial biomass, as expected. Our results contrast with data of Stemmer *et al*. (1999), in that we found a considerable increase in invertase activity in the fine fractions but not in the sand fractions (Figure 1). This difference could be explained by the different experimental conditions. We investigated a soil of a long-term experiment, whereas Stemmer *et al*. (1999) followed the short-term decomposition of straw after a single amendment. In general, the extent of increased invertase activity in the finer fractions depends on the amount and quality of the added substrates (Gerzabek *et al*., 2002). Microbial residues might be an important substrate for invertase activity in the finer fractions.

Xylanase is involved in the decomposition of the high-molecular-weight substrate xylan (Stemmer *et al*., 1999), and its activity increased only slightly in the finer fractions. The relatively small enzyme activity in the coarser fractions may reflect a dilution effect. Farmyard manure delivers new substrates into the sand fraction for xylanase to act on, and in the long term this had greatly increased xylanase activity in this fraction.

Microbial community structure

The contents of ninhydrin-reactive N and total PLFAs, indicators for microbial biomass, were heterogeneously distributed among particle-size fractions. They were greatest in the finest fractions, as was the diversity of PLFAs (Figure 1). The larger biomass in silt and clay might be attributed to a greater diversity rather than to better colonization by particular species (Sessitsch *et al*., 2001). The coarse and fine sand fractions of control soils were characterized by a rather simple microbial community (few PLFAs in small quantities). We attribute the large abundance of linoleic acid (18:2ω6c) in the coarse sand to fungal-specific membrane component (Frostegård & Bååth, 1996). Additional evidence for the preferential colonization of the coarser fraction by fungi came from the high xylanase activity produced by fungi (Stemmer *et al*., 1999) and from larger amounts of fungal hyphae, relative to other community components, in larger aggregates produced by a dry-sieving procedure that yielded different aggregate-size classes (5.0–2.0, 2.0–1.0, 1.0–0.5, 0.5–0.25, 0.25–0.1 and <0.1 mm) (Schutter & Dick, 2002).

The amendment of FYM changed the microbial community structure mainly in the coarse sand fraction. The decrease in fungal abundance here (Table 4) may be due to the smaller C/N ratio of the substrates, which favoured bacterial growth (Eiland *et al*., 2001). In contrast, typical Gram-negative bacteria PLFA biomarkers (monounsaturated plus cyclopropyl fatty acids) were detectable in the soil that had received FYM (Figure 2). Several studies of soil PLFAs have documented an increase in monounsaturated fatty acids with increased availability of organic substrates and manures (Bossio & Scow, 1998; Peacock *et al*., 2001). Gram-negative bacteria seemed to be not only very abundant, but also very active in the coarse sand fraction after addition of FYM. This evidence came from the small abundance of cyclopropyl fatty acids (cyc17:0, cyc19:0), which are produced from the corresponding monounsaturated fatty acids (16:1ω7c, 18:1ω7c) by many Gram-negative bacteria as a step-down procedure in response to depletion of substrate or stress (Petersen *et al*., 1997; Lundquist *et al*., 1999).

Recently, Lukow *et al*. (2000), Dunbar *et al*. (2000) and Kuske *et al*. (2002) used T-RFLP to compare bacterial communities in environmental samples. In our study, particle size

Table 5 Response of soil microbial community composition to farmyard manure (FYM) assessed by representative sample T-RFLP profiles of particle-size fractions and the bulk soil. Percentage of total fluorescence of each fragment which is characterized by its number of base pairs (bp) is given

	FYM					Control				
bp	Bulk soil	Clay	Silt	Coarse sand	Fine sand	Bulk soil	Clay	Silt	Coarse sand	Fine sand
59	□	□	□	□	□	□	□	□	□	□
62	□	□	■	□	□	□	■	□	□	□
66	■	■	■	■	□	■	□	□	■	□
67	□	□	□	□	□	■	□	□	□	□
75	□	□	□	□	□	□	□			□
76	□	□	□	□	□	□				□
79										■
81	■	■	■	□	▲	▲	■	■	▲	▲
84						□		□	□	
86								□		□
90	□	□	□	□	□		□	□		□
92	□	□	□			□	□	□	□	□
94	□	□	□	□	□	□	■	□	□	□
95	□	□	□	□	□	□	□	□	□	□
101	□	□	□	□		□	□	□	□	
133						□	□			
140	□	□	□	□	□	□	□	□	□	□
148							□			
194	□	□	□	□	□	□	□	□	□	□
195	□	□	□	□	□	□	□	□	□	□
199	□		□			□	□	□		□
205	□	□	□	□	□	□	□	▲	□	▲
209	■	□	□	□	□	□	□	■	□	□
212							□		□	□
217	□	□	□	□	□	□	□	□	□	□
220	□	□	□	□	□	□	□	□	□	□
224	□	□		□	□	□	□	□	□	□
227	■	□	□	□	□	□	□	■	□	□
230	■	□	□	■	□	■	□	□	■	□
233	□	□		■		□	□	□	□	□
236	□	□	□	□	□	□	□	□	□	□
240	□	□		□	□	□	□		□	□
257						□	□		□	
262	□	□	□	□	□	□	□	□	□	□
285						□				
294	□	□	□	□		□	□	□	□	
298	□	□				□	□			
301							□			
305		■	▲				□	▲		■
309	□			□		□			□	
312								□		
325							□	□	□	
330	□	□	□	□			□	□	□	
366				□						
378		□	□	□			□		□	
388			□							
414	□	□	□	□	□	□	□	□	□	□
∑	31	31	29	30	25	34	39	32	32	31

□, 1–5%; ■, 6–10%; ▲, >10% of total fluorescence; ∑, sum of fragments of each treatment.

and organic amendments did not appear to have the same strong influence on the microbial community based on T-RFLP analyses as on PLFAs. The pattern of T-RFLP was also observed to be remarkably similar among plots that shared a long-term history of agricultural management (Buckley & Schmidt, 2001), despite recent differences in the composition of the plant community and land management on the plots. Although our T-RFLP data revealed no significant differences in bacterial diversity of the soil microbial community, the fewer fragments in the coarse sand support the hypothesis that this microhabitat is colonized by a less complex bacterial community than the silt and clay fractions.

Conclusion

Our small-scale study revealed that coarse sand fractions are colonized mainly by a fungal-dominated community and a rather simple bacterial community, whereas silt and clay fractions are colonized by a complex bacterial community. Application of farmyard manure shifted the microbial community towards a more bacterial-dominated community in the coarse sand. Growth and activity of Gram-negative bacteria, measured based on their corresponding PLFA biomarker, was greater in the coarse sand fraction. Therefore, our results clearly demonstrated that our knowledge of microbial biomass, enzymes involved in carbon cycling, and microbial community composition has improved the understanding of how soil microorganisms are distributed and function within microhabitats.

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