DIVISION S-3—SOIL BIOLOGY & BIOCHEMISTRY

Dynamics of a Soil Microbial Community under Spring Wheat

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ABSTRACT

In arable systems, seasonal fluctuations of microbiological properties can be significant. We hypothesized that adaptation to soil environmental conditions may contribute to the variation observed, and this was examined by characterization of different microbial community attributes under a range of soil conditions. Soil was sampled from no-till and chisel-tilled fields within a long-term experiment in eastern Washington during growth of spring wheat (*Triticum aestivum*). The range of soil environmental conditions covered was extended by amendment of crop residues. Soil samples were characterized with respect to biomass N and biomass P, substrate utilization dynamics, phospholipid fatty acid (PLFA) profiles and whole-soil fatty acid (MIDI-FA) profiles, and with respect to soil environmental variables (bulk density, soil organic C [SOC], temperature, moisture, and inorganic N and P). Bacterial and fungal lipid biomarkers were negatively correlated ($P < 0.001$), confirming that these subsets of fatty acids are associated with contrasting components of the microbial biomass. Biomass N was closely associated with soil conditions, notably N availability. The proportion of substrates used with no apparent lag phase decreased during summer and was negatively correlated with lipid stress indicators. Cyclopropyl fatty acids accounted for more than 60% of the variation in bacterial PLFA. These observations suggest that adaptation to environmental stresses was partly responsible for the microbial dynamics observed. Tillage practice had little effect on the relationships between soil conditions and microbiological properties. The results showed that MIDI-FA included a significant background of nonmicrobial material and was less sensitive to soil environmental conditions than PLFA.

The microbial community of arable soils is a component with multiple functions of importance to soil fertility, such as catalysis of nutrient transformations, (temporary) storage of nutrients, formation and stabilization of soil structure, and control of plant pathogens (Anderson and Domsch 1989; Oades, 1993; Smith, 1994; Kennedy and Papendick, 1995). Activity and growth of microorganisms is restricted by soil environmental conditions, e.g., temperature, moisture, pore-size distribution and nutrient availability and therefore, indirectly, by cultivation practices.

Seasonal fluctuations of microbial characteristics can be comparable with, or larger than effects of management practices such as spring burn and N fertilization (Ajwa et al., 1999), fertilization and drainage (Bardgett et al., 1999), or organic vs. conventional farming systems (Bossio et al., 1999). Microorganisms are intimately associated with their physical and chemical environment, and it is therefore conceivable that the temporal dynamics observed will partly reflect adaptation to environmental variables rather than competition between different components of the microbial biomass. Many aspects of soil microbial communities are affected by prevailing conditions with respect to substrate quality and stresses. For example, the C/N/P ratio can vary with nutrient availability (Srivastava et al., 1989; Drury et al., 1991), membrane lipid composition can vary with temperature (Petersen and Klug, 1994; Navarrete et al., 2000) and substrate utilization patterns can vary with the number of active organisms (Winding and Hendriksen, 1997). Effects of adaptation should be distinguished from successional changes when the response of microbial communities to management or seasonal changes is interpreted.

The present study described different aspects of a soil microbial community and possible effects of soil environmental conditions on these microbiological properties across a growing season of spring wheat. The range of soil conditions was extended by sampling from field plots under no-till and chisel till, respectively, and with or without residues at the soil surface. Reduced tillage frequently increases soil compaction (Mielke et al., 1986) and will delay the turnover of soil organic matter (Papendick and Moldenhauer, 1995), while crop residues can modify the soil environment by reflecting irradiation, and by creating a boundary layer of stagnant air (Hammel, 1996).

MATERIALS AND METHODS

The study site was the Palouse Conservation Field Station near Pullman, WA. The soil was a Palouse silt loam (fine-silty, mixed mesic, Pachic Ultic Haploxerolls) (Myrold et al., 1981). In the experimental year (1998), May and June were slightly cooler, and July and August were considerably warmer than normal. Precipitation was close to normal (annual precipitation is 500–550 mm). For this study, two fields from a long-term experiment under no-till management and chisel tilled to a 10-cm depth, respectively, were selected. Both had been under spring wheat or winter wheat for the past 20 yr, and 3 Mg ha^{-1} straw had been returned to the soil each fall. Tillage took place ~1 wk prior to fertilization (N/P/S at a rate of 16:20:14

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Abbreviations: 16:1ω7*t*/*c*, trans/cis ratio of hexadecenoic acid; cy17:0/16:1ω7*c* and cy19:0/18:1ω7*c*, cyclopropyl/precursor ratios; 18:2ω6*c*, linoleic acid, a fungal biomarker; bactPLFA, a subset of PLFA found in prokaryotes only; MIDI-FA, fatty acids extracted by the MIDI procedure; PLFA, phospholipid fatty acids; SOC, soil organic C; ΔT, diurnal temperature range at time of sampling.

kg ha^{-1} and liquid urea at a rate of 61 kg N ha^{-1}) and seeding of spring wheat in early May.

Six microplots (gross area: 1 by 1 m, net area: 0.7 by 0.7 m) were established within each of the two fields. Three of the microplots in each field were amended with an extra 3 Mg ha^{-1} straw on top of the soil to increase surface roughness and shading of the underlying soil. A coarse net (mesh size, 5 by 10 cm) kept the straw in place. The three other microplots in each field were left unamended. At sampling, six soil cores (diam. 2 cm; soil depth 15 cm) were taken from each of the 12 microplots. The soil cores were sectioned into 0- to 5- and 5- to 15-cm depth intervals and each depth interval pooled into duplicate samples representing each of the four basic treatments (i.e., no-till; no-till + extra straw; chisel till; chisel till + extra straw). This gave a total of 16 samples per sampling date (four basic treatments, two depth intervals, two replicates). The number of subsamples was selected after a preliminary analysis of variability (Wollum, 1994; data not shown). The pooled soil samples were sieved (mesh size, 4 mm) and mixed. Root fragments and plant residues were carefully removed by tweezers, leaving predominantly amorphous soil organic matter.

The study included five samplings, i.e., on 12 May (1 wk after seeding and fertilization), 3 June, 26 June, 20 July, and 9 September (~2 wk after harvest). Crop yields were not measured, since there was no physical separation between the 1 by 1 m^2 microplots and the surrounding soil. Inverted white 25-mL plastic vials were used to plug holes left by sampling to minimize the exchange of heat and water between soil and the atmosphere.

Physical Measurements

Bulk density was determined in April, prior to tillage operations, by random sampling within each field. Soil organic C was estimated from measurements of loss-on-ignition (450°C, 3 h) at the first sampling; the C content was assumed to constitute 50% of the loss-on-ignition (Broadbent, 1965). Gravimetric soil moisture was determined for each pooled soil sample (105°C, 24 h) and converted to volumetric soil moisture. Soil water potentials were estimated from an empirical relationship determined for the Palouse silt loam (Myrold et al., 1981). Air temperature at ~75-cm height and soil temperature at 5- and 10-cm depth were recorded during 48-h periods within each sampling week in a selected microplot from each of the four treatments. Temperatures were recorded at 5-min intervals and hourly means stored using a LI-1000 datalogger and corresponding thermistor sensors (Li-Cor; Lincoln, NE).

Chemical Analyses

Field-moist soil was extracted for analysis of inorganic P (P_i), ninhydrin-reactive N, and $NO_2^- + NO_3^-$ within 24 h of sampling. Phosphate was extracted from 5-g portions of soil with 40 mL of 0.5 *M* $NaHCO_3$, pH 8.5, and filtered extracts were analyzed using the ascorbic acid method of Murphy and Riley (1958). Separate extractions were amended with an internal standard to correct for P_i fixation (Brookes et al., 1982). Soluble N was extracted from 5-g soil samples using 40 mL of 1 *M* KCl. Ninhydrin-reactive N of filtered extracts was analyzed immediately (Joergensen and Brookes, 1990), while other subsamples were frozen for later analysis of $NO_2^- + NO_3^-$ using the microplate method of Sims et al. (1995). Using 1 *M* KCl instead of 2 *M* KCl did not influence the colorimetric determination of ninhydrin-reactive N (data not shown). All results are given on a dry weight basis.

Microbiological Analyses

Biomass N was determined by chloroform fumigation-direct extraction as described by Joergensen and Brookes (1990). Biomass P was determined after chloroform fumigation-direct extraction as described by Brookes et al. (1982). For these two assays, background concentrations of ninhydrin-reactive N and P_i, respectively, were determined at the time of sampling, i.e., not after parallel incubations of nonfumigated soil (Smith et al., 1995). At the last three samplings, 1 mL of demineralized water was added to each 5-g soil sample immediately prior to fumigation to ensure efficiency of fumigation (Sparling and West, 1989).

Substrate utilization potentials were determined as described by Kennedy (1994). For the extraction 0.05 *M* K_2HPO_4, pH 6 was used instead of 0.1 *M* $CaSO_4$, pH 6.3, since a preliminary experiment had shown the former to give more reproducible results and an improved precipitation of solids. Five-gram portions of soil were extracted in 40 mL sterile K_2HPO_4 buffer for 30 min. After 30 min settling, a portion of the extract (5 mL) was aseptically transferred to a fresh tube with 40 mL of buffer, vortexed, and the transfer repeated. After vortexing, 150 μL of this second dilution was dispensed into each well of one Biolog GN plate (Biolog, Hayward, CA) per soil sample. The absorbance in the 96 wells of each plate was read at 595 nm after 0, 24, 48, and 72 h using a BIO-RAD 550 plate reader (BioRad, Hercules, CA). Readings were corrected for background prior to data analysis; significant color formation was arbitrarily defined as >0.2 absorption units. Different substrate utilization patterns were identified, i.e., cases with a prolonged lag phase, cases with a sigmoidal substrate utilization pattern, and cases with no apparent lag phase. The latter category was assumed to represent a population of actively metabolizing organisms.

Phospholipids are a main constituent of cell membranes, and since phospholipid ester-linked fatty acids are rapidly released upon cell death, this component may be used for characterizing the living biomass (Tunlid and White, 1992). Phospholipid fatty acid methyl esters were prepared as described by Petersen and Klug (1994) with the following modifications. Polar lipids (mainly phospholipids) were isolated from the crude lipid extract by silicic acid columns (100 mg; Varian, Harbor City, CA), and nonadecanoate methyl ester was added during methylation as an internal standard for quantification of PLFA. Fatty acid methyl esters were analyzed by gas chromatography using a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a 25-m fused silica capillary column (Ultra 2) and flame ionization detector. The column temperature was initially 60°C for 1 min, then raised by 20°C min^{-1} to 160°C followed by a 5°C min^{-1} rise to 300°C. Temperatures of injection port and detector were 170 and 300°C, respectively, and H was used as carrier. Peak identification was based on retention times previously confirmed using mass spectrometry.

A second lipid analysis was included for comparison with the PLFA procedure. This so-called MIDI procedure, originally developed by MIDI Inc. (Newark, DE) for analysis of laboratory-grown pure cultures, employs saponification of soil at 100°C, acid methylation at 80°C, an alkaline wash, and an extraction of methyl esters of long-chain fatty acids and other lipid compounds with similar properties into hexane (Kennedy, 1994). At the two last samplings, nonadecanoate methyl ester was included after the methylation step to enable quantification of identified lipids on a molar basis. The gas chromatographic system was the same as described above, but a separate line with split injection was used. Temperatures of injection port and detector were as above, but for this analysis the initial column temperature was 170°C, increasing by 5°C per min to 270°C. Peak identification and integration of areas were car-

ried out by software supplied by MIDI Inc. (Newark, DE), except that a new peak naming table was created which combined all different compounds represented in the default methods.

Fatty acids were designated as X:$Y\omega Z$, where X represents the number of C atoms, Y the number of double bonds, and Z indicates the position of the first double bond from the aliphatic (ω) end of the molecule. Prefixes iso (*i*) and anteiso (*a*) represent branching at C atom No. 2 and 3, respectively, from the ω end, br indicates branching at an unknown position, and cy indicates a cyclopropyl group. The prefix 10Me indicates a methyl group at the tenth C atom from the carboxyl end of the molecule. Cis or trans configuration is indicated by *c* or *t*.

Statistical Analyses

Bulk density differences between depths and tillage treatments were evaluated by one-way ANOVA and Tukey's HSD multiple comparison test. Pearson's product-moment correlations between soil microbiological attributes were calculated; the resulting P values were adjusted according to the step-wise Bonferroni test to control the table-wise error rate (Rice, 1989).

The dependency of microbial dynamics on soil properties and time was examined with a linear mixed model (McCulloch and Searle, 2001) using tillage as class variable. Soil organic C, ΔT, soil moisture, inorganic N, and time were included as independent variables. Based on scatter plots it was decided to log-transform inorganic N concentrations and ΔT, giving the following model:

$$\mu_i = k_{i0} + k_{i1}\,\mathrm{SOC} + k_{i2}\log(\Delta T) + k_{i3}\,\mathrm{Moisture} + k_{i4}\log(N_{\mathrm{inorg}}) + k_{i5}\,\mathrm{Time}, \quad [1]$$

where μ is the response variable and *i* indicates tillage practice. Both untransformed and log-transformed biomass data were tested and results for the best fit presented. The partial type III test (McCulloch and Searle, 2001) was used to evaluate the significance of fixed effects. This test takes all other effects into account before evaluating the significance of the variable in question and so will identify only variation which could not be explained by a combination of other factors in the model.

RESULTS AND DISCUSSION

Physical and Chemical Properties

The experimental treatments covered a wide range of soil conditions with respect to physical and chemical characteristics, albeit with some expected covariation between variables with time (e.g., soil moisture and nutrient concentrations) and depth (e.g., SOC and temperature). Bulk density of the no-till soil was higher than that of chisel-tilled soil, although the difference was not significant at 5- to 10-cm depth (Table 1). Soil organic C showed a stronger stratification in no-till than in tilled soil. Soil under chisel till contained similar or slightly higher levels of SOC by weight (Table 1), but on a volume basis no-till soil contained 15% more SOC than tilled soil in the top layer, and similar concentrations at 5- to 15-cm depth (data not shown). These differences correspond to those expected from previous studies of tillage effects on soil properties (Mielke et al., 1986; Papendick and Moldenhauer, 1995; Rhoton, 2000). Diurnal fluctuations of soil temperature, $\Delta T = (T_{\max} - T_{\min})$, ranged from 3.8 to 24.4°C at a 5-cm depth over the course of this study and was positively correlated with $T_{\max}$ ($P < 0.001$). Amendment of surface residues reduced ΔT by as much as 6°C at the 5-cm depth depending on ambient weather conditions (data not shown). Gravimetric soil moisture ranged from 4.8 to 25.8%, corresponding to water potentials of <-5 to -0.04 MPa, over the course of the experiment. Below ~-0.5 MPa, bacterial movement will be prevented and solute diffusion progressively inhibited (Papendick and Campbell, 1981). Finally, soil concentrations of inorganic N and resin-extractable P_i fluctuated between 0 and 220 mg N kg^{-1} soil, and between 33 and 110 mg P kg^{-1}. For comparison, fertilizer application corresponded to 130 mg N kg^{-1} and 35 mg P kg^{-1} if evenly distributed at 0- to 5-cm soil depth.

Table 1. Bulk density (g cm^{-3}, n = 3) and soil organic C (SOC) (g kg^{-1}, n = 4) at different depths in no-till and chisel tilled soil. Letters behind mean values indicate significant differences across both tillage treatment and depth for bulk denisty and SOC, respectively.

	Bulk density		SOC†	
	No-till	Chisel-till	No-till	Chisel-till
0–5 cm	1.25ab	1.02c	30.5ab	32.6a
5–10 cm	1.42a	1.25ab	ND‡	ND
5–15 cm	ND	ND	24.0c	28.6b
10–20 cm	1.37a	1.17bc	ND	ND

† Estimated from loss-on-ignition assuming 50% C.
‡ ND, not determined.

Microbiological Properties

Biomass N concentrations ranged from 8 to 323 mg N kg^{-1} soil; values above ~50 mg N kg^{-1} were restricted to the first sampling 1 wk after fertilization. Biomass P ranged from 0 to 28 mg P kg^{-1}, but measurements of biomass-P were confounded by the relatively high background of P_i and were therefore not further investigated. Concentrations of PLFA varied between 12 and 127 μmol kg^{-1} soil. A total of 35 different PLFAs were consistently present in this study. Concentrations of fatty acids extracted by the MIDI procedure, henceforth MIDI-FA (quantified at the last two samplings only), ranged from 76 to 350 μmol kg^{-1} soil. Almost 120 different lipid compounds were identified by the MIDI system, including 68 long-chain fatty acids ranging from 10 to 30 C atoms.

Substrate utilization dynamics were examined as a qualitative aspect of the microbial community. Figure 1 shows the proportions of color development corresponding to an extended lag phase, a sigmoidal substrate utilization pattern, or substrate utilization with no apparent lag phase. The latter category decreased towards the end of the growing season, while the proportion utilized after an extended lag phase had increased at the last sampling. Seven compounds, i.e., glycogen, N-acetyl-D-glucosamine, β-methyl glucoside, bromosuccinic acid, D,L-α-glycerol phosphate, glucose-1-phosphate, and glucose-6-phosphate, accounted for almost 40% of the cases with no apparent lag phase. None of these compounds have been found in root exudates (Kennedy, 1994; Campbell et al., 1997) and so could not link the change in substrate utilization patterns to crop development. The time course of substrate utilization may vary depending on inoculum density (Garland and Mills,

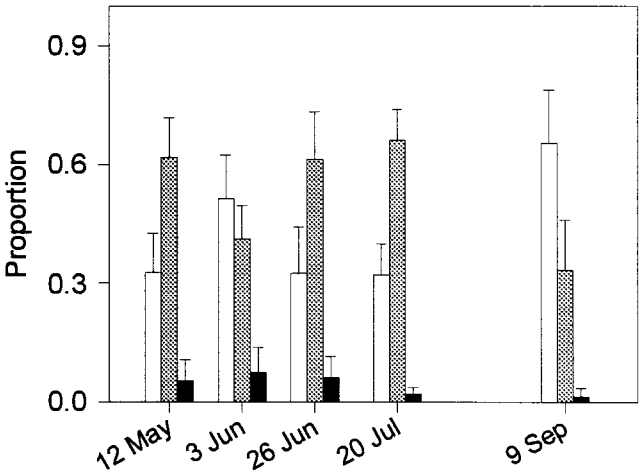

Fig. 1. Frequency distribution of Biolog substrate utilization patterns with sampling time. Patterns of color formation were assigned to one of three categories on the basis of absorbance readings after 0, 24, 48, and 72 h. The three categories were: cases with a prolonged lag phase (white columns); cases with a sigmoidal shape (grey columns); and cases with no apparent lag phase (black columns). Bars indicate standard deviations (n = 16).

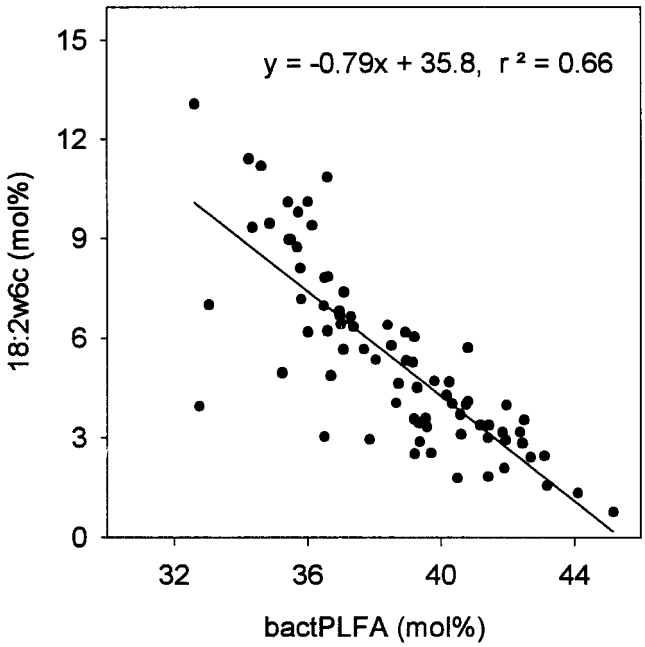

Fig. 2. Mole percentage of linoleic acid (18:2ω6*c*) in relationship to the mol percentage of PLFA phospholipid fatty acids representing bacteria (bactPLFA; see text). Data represent all treatments and sampling dates (n = 80).

1991; Campbell et al., 1997) which was not standardized in the present study. The patterns observed could therefore reflect either a low level of in situ microbial activity or low numbers of culturable cells in the last part of this study.

Subsets of the PLFA profiles were considered which have been linked with taxonomic groups or cellular functions. These included (i) the summed percentage of the fatty acids *i*15:0, *a*15:0, 15:0, *i*16:0, 16:1ω9, 16:1ω7*t*, *i*17:0, *a*17:0, 17:0, cy17:0, 18:1ω7 and cy19:0 as an index of bacteria (Frostegård and Bååth, 1996), henceforth referred to as 'bactPLFA'; (ii) linoleic acid (18:2ω6*c*) as an index for fungi in root-free soil (Federle, 1986); (iii) ratios between the bacterial fatty acids cy17:0 and cy19:0 and their metabolic precursors, i.e., 16:1ω7*c* and 18:1ω7*c*, as indicators of physiological stress (Grogan and Cronan, 1997); and (iv) the trans/cis ratio of 16:1ω7 (isomers of 18:1ω7 could not be distinguished) as another widely observed response to various stresses (Heipieper et al., 1996).

Intercorrelations between biomass indices and proposed stress indicators are shown in Table 2. Even with the conservative Bonferroni procedure there were several highly significant relationships. The three potential indices of microbial biomass, i.e., biomass N, PLFA, and MIDI-FA, were all strongly correlated. BactPLFA was negatively correlated with both PLFA and MIDI-FA, while 18:2ω6*c* was positively correlated with both lipid indices. This result suggests that fungi dominated the microbial biomass at the higher biomass concentrations. The negative correlation (r^2 = 0.66, $P < 0.001$) between bactPLFA and 18:2ω6*c* (see Fig. 2) corroborates the proposed association of these fatty acids with contrasting subsets of the microbial community.

Concentrations of bactPLFA correlated positively with the bacterial lipid stress indicators, i.e., cyclopropyl-to-precursor ratios and the 16:1ω7*t*/*c* ratio (Tab. 2). These correlations would appear contradictory, since a high proportion of bacteria suggests that conditions for bacterial proliferation were favorable, while elevated levels of stress indicators are supposedly associated with growth inhibition. However, cyclopropyl fatty acids

Table 2. Correlations between quantitative and qualitative microbiological attributes†.

	Biomass N	ΣPLFA	ΣMIDI-FA	Biolog No Lag	BactPLFA	18:2ω6*c*	cy17:0/16:1ω7*c*	cy19:0/18:1ω7*c*	16:1ω7*t*/*c*
Biomass N		**0.46*****	**0.91*****	**0.42****	**−0.28**	**0.30**	**−0.36***	**−0.43****	**−0.29**
ΣPLFA			**0.69*****	**0.07**	**−0.51*****	**0.70*****	**−0.48*****	**−0.57***	**−0.44**
ΣMIDI-FA				**0.37**	**−0.76*****	**0.75*****	**−0.60****	**−0.82*****	**−0.48**
Biolog, No lag					**−0.26**	**0.16**	**−0.41****	**−0.47*****	**−0.40***
BactPLFA						**−0.81*****	**0.67*****	**0.69*****	**0.62*****
18:2ω6*c*							**−0.63*****	**−0.70*****	**−0.55*****
cy17:0/16:1ω7*c*								**0.74*****	**0.89*****
cy19:0/18:1ω7*c*									**0.56*****
16:1ω7*t*/*c*									

*** Significant at the 0.05 probability level.**
**** Significant at the 0.01 probability level.**
***** Significant at the 0.001 probability level.**
† Abbreviations used: PLFA, phospholipid fatty acids; MIDI-FA, fatty acids extracted by the MIDI procedure (see text); bactPLFA, a subset of PLFA found in prokaryotes only; 18:2ω6*c*, linoleic acid, a fungal biomarker; Cy17:0/16:1ω7*c* and cy19:0/18:1ω7*c*, cyclorpopyl-to-precursor ratios; 16:1ω7*t*/*c*, trans/cis ratio of hexadecenoic acid.

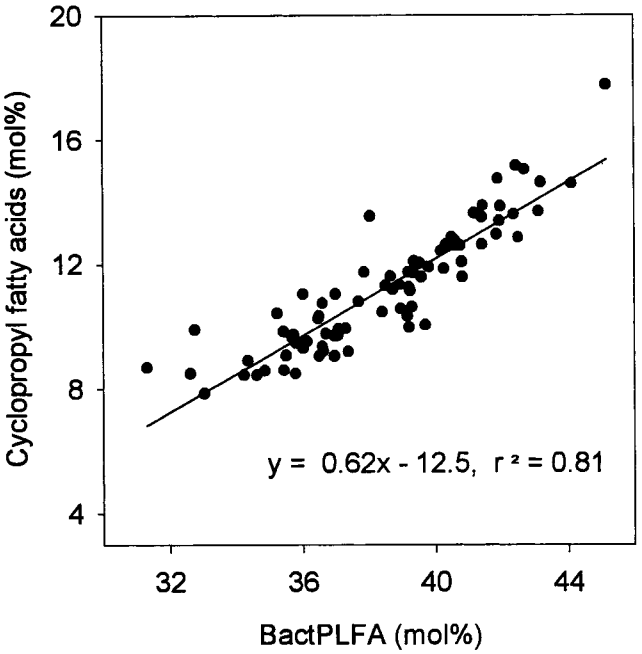

Fig. 3. Cyclopropyl fatty acids (cy17:0 and cy19:0) in relationship to bactPLFA. Data represent all treatments and samplings (n = 80).

alone accounted for >60% of the variation in bactPLFA (Fig. 3). Although bacteria with >50% cyclopropyl fatty acids have been reported in the literature, their share of cellular fatty acids is typically much lower (Wilkinson, 1988). This implies that the high proportions of bactPLFA were at least partly because of adaptations within the extant bacterial biomass rather than successional changes. Cyclopropyl-to-precursor ratios increased with time (see section below) and probably reflected effects of the hot and dry summer period on the soil environment. Accumulation of cyclopropyl fatty acids have been observed in response to various stresses, including nutrient depletion, adverse O_2 conditions, low pH, and osmotic stress (Guckert et al., 1986; Chang and Cronan, 1999; Mazumder et al., 2000; Boumahdi et al., 2001). For example, for a micro-aerophilic *Pseudomonas* strain Mazumder et al. (2000) found a five-fold increase in cy17:0 in response to an O_2 up-shock from 11 to 100% saturation, but mainly under low-substrate conditions. With respect to bactPLFA it should also be noted that 16:1ω7*c*, the metabolic precursor of cy17.0, is not included in bactPLFA as defined by Frostegård and Bååth (1996) since it occurs widely in both prokaryotic and eukaryotic organisms. Hence, membrane modifications involving conversion of 16:1ω7*c* to cy17:0 within existing membranes would show up as an increase in bactPLFA. The relative abundance of Gram positive and Gram negative bacteria could, however, also influence the proportion of cyclopropyl fatty acids, which are mainly found among Gram negatives (Grogan and Cronan, 1997).

The proportion of Biolog substrates used with no apparent lag phase ('Biolog No-lag' in Table 2) correlated positively with biomass N, while it was unrelated to lipid biomass indices, but negatively correlated with lipid-stress indicators. The negative link with stress indicators suggested that the decreasing proportion of substrate utilization with no apparent lag phase shown in Fig. 1 was at least partly because of physiological stresses, and not only related to inoculum density. Physiological stresses would induce organisms to enter a stationary phase or resting stage, corresponding to an extended lag phase during subsequent incubation with the Biolog assay.

Effects of Soil Environmental Variables

Table 3 presents significant effects of soil properties and sampling time on soil microbiological properties, as well as interactions with tillage practice. For main effects, the signs + and − refer to the direction of the relationship. For interactions with tillage, + and − indicate whether the relationship for no-till soil was positive or negative relative to chisel-tilled soil. There were few significant interactions with tillage, however, suggesting that relationships between soil properties and microbiological properties were similar for the two tillage systems.

Biomass N decreased with time and was strongly related to soil inorganic N. This relationship was affected by a transient high concentration of biomass N at 0- to 5-cm soil depth at the first sampling 1 wk after fertilization (data not shown), which may represent a temporary storage of N in microbial cells (Carter and Rennie, 1984;

Table 3. Effects of selected soil properties on microbiological attributes were tested with a linear mixed model using tillage as class variable and a partial test of fixed effects (see text). For main effects, + and − indicate the direction of the association. For interactions with tillage, + and − indicates whether effects with no-till soil were more positive or negative relative to tilled soil.

	Biomass N	Sum, PLFA	Sum, MIDI-FA	BactPLFA	Linoleic acid	No lag	cy17:0/pre	cy19:0/pre	16:17*t/c*
SOC	NS	+***	NS	NS	+***	NS	NS	−***	NS
Δ *T*	NS	NS	NS	NS	NS	NS	−*	NS	NS
Moisture	−*	NS	NS	NS	NS	NS	NS	−*	NS
Inorganic N	+***	+*	NS	NS	NS	NS	NS	NS	NS
Time	−**	NS	NS	+*	NS	−*	NS	+***	+*
SOC × Till	NS	NS	NS	NS	NS	NS	NS	NS	NS
Δ *T* × Till	NS	NS	NS	NS	NS	NS	NS	NS	NS
Moisture × Till	NS	NS	NS	NS	+*	NS	NS	NS	NS
Inorganic N × Till	NS	−*	NS	NS	NS	NS	NS	NS	NS
Time × Till	NS	NS	NS	NS	NS	NS	NS	−*	+*

*** Significant at the 0.05 probability level.**
**** Significant at the 0.01 probability level.**
***** Significant at the 0.001 probability level.**
† Abbreviations used: PLFA, phospholipid fatty acids; MIDI-FA, fatty acids extracted by the MIDI procedure (see text); factPLFA, a subset of PLFA found in prokaryotes only; 18:2ω6*c*, linoleic acid, a fungal biomarker; Cy17:0/16:1ω7*c* and cy19:0/18:1ω7*c*, cyclorpopyl-to-precursor ratios; 16:1ω7*t/c*, trans/cis ratio of hexadecenoic acid; SOC, soil organic carbon; Δ*T*, diurnal temperature range at time of sampling.

Zagal and Persson, 1994). Release of fertilizer N during fumigation would interfere with biomass-N determinations, but it is not likely that nonhydrolyzed urea or insoluble ammonium was present in the soil a week after fertilization (Cabrera et al., 1991; Ouyang et al., 1998).

Concentrations of PLFA were positively linked to SOC, and to some extent to inorganic N (Table 3). The association between biomass and SOC has been described previously by Anderson and Domsch (1989), who concluded that management influenced biomass C/SOC ratios. In the present study, the effect of inorganic N × tillage on PLFA concentrations was weaker for no-till soil, possibly because the liquid urea fertilizer was not as well distributed as in the less compact tilled soil (Table 1). There were no significant effects of soil properties on MIDI-FA concentrations, which were quantified at the last two samplings only.

BactPLFA increased significantly with time according to the model. Potential interferences from adaptation were discussed in the previous section. There were no effects of soil environmental variables on this parameter. The fungal biomarker linoleic acid was strongly related to SOC which, referring to the distribution of SOC (Table 1) implies an accumulation of fungi in the upper soil layer, as previously described for no-till soil (Linn and Doran, 1984). The interaction moisture × till was significant and indicated that the effect of moisture on linoleic acid was more positive in no-till than in tilled soil. The water retention capacity of organic matter is far greater than that of mineral soil (Papendick and Campbell, 1981), and this apparent effect of moisture could therefore reflect the different concentrations of SOC at 5- to 15-cm depth in no-till and tilled soil (cf. Table 1), rather than a direct effect.

The physiological status of the soil microbial community was addressed by the four last columns in Table 3. The decrease with time of substrate utilization with no apparent lag phase, which was described above (see Fig. 1), was also identified by the model. However, a causal relationship with, e.g., moisture or temperature fluctuations, was not observed. The cy17:0/16:1ω7*c* ratio was negatively related to $\log(\Delta T)$, which was mainly a depth effect, i.e., cy17:0/16:1ω7*c* ratios were lower at 0- to 5-cm depth than at 5- to 15-cm depth (data not shown). Low cyclopropyl-to-precursor ratios are characteristic for actively growing cells, suggesting that the more extreme temperature fluctuations near the soil surface lead to enhanced metabolic activity. The ratio cy19:0/18:1ω7 was not related to temperature, but negatively related to soil moisture (an effect of soil moisture on cy17:0/16:1ω7*c* was also suggested, but it was not statistically significant). There was a strong negative effect of SOC on cy19:0/18:1ω7 (Table 3). Since low cy19:0/18:1ω7 ratios indicate enhanced microbial activity, this relationship provides evidence that SOC stimulated microbial activity.

The two cyclopropyl/precursor ratios showed somewhat different links to soil properties. Laboratory studies have shown cy17:0 and cy19:0 to influence membrane properties differently (Chang and Cronan, 1999), and individual populations of soil organisms may also have responded differently to soil environmental conditions. Cyclopropyl 19:0/18:1ω7 increased with time, which provides evidence for increasingly stressful conditions during the course of the summer. A corresponding increase for cy17:0/16:1ω7*c* was seen (data not shown), but it was not statistically significant. The stronger responses for cy19:0/18:1ω7 compared with cy17:0/16:1ω7*c* could be related to the increasing average temperature during the growing season; it is well known that the average chain length of membrane lipid fatty acids will increase with growth temperature (Suutari and Laakso, 1994). Finally, the proposed stress indicator 16:1ω7trans/cis increased with time, especially in no-till soil, probably as a response to the more extreme environmental conditions during the hot and dry summer months.

Relationships between soil properties and microbiological properties could originate from specialization of microorganisms to different ecological niches, or from adaptation within the extant microbial biomass. The observations described above exemplify both mechanisms. The fungal biomarker was associated with soil organic matter and consequently must be relatively more abundant in the upper soil layer (Table 1). Observations which implicate adaptation in the microbial community dynamics observed include (i) a positive correlation between biomass N and N availability over the course of the growing season; (ii) a negative correlation between substrate utilization with no apparent lag phase and lipid stress indicators; (iii) cyclopropyl fatty acids accounted for >60% of the variation in bactPLFA; and (iv) cyclopropyl/precursor ratios were related to environmental variables.

Phospholipid Fatty Acid vs. Fatty Acid Extracted by the MIDI Procedure

Finally, the choice between the two alternative lipid assays should be addressed. Both PLFA analyses (Drijber et al., 2000; Ibekwe et al., 2001) and MIDI-FA analyses (e.g., Cavigelli et al., 1995; Buyer et al., 1997) are widely used for characterizing microbial communities in soil systems. The membrane-derived PLFA represent a functionally more well-defined fraction of soil lipids than MIDI-FA, while the MIDI procedure has an advantage in the ease of sample preparation. The data obtained in this study enabled a direct comparison between the two alternative approaches.

Twenty-two fatty acids ranging from 14C to 20C were common to the MIDI procedure and the PLFA procedure, and ratios between yields of PLFA and MIDI-FA were calculated for these 22 fatty acids (Table 4). Concentrations were on average 4.5 times higher with the MIDI procedure than with the PLFA procedure. Phospholipids typically constitute 50 to 90% of total cell lipids (O'Leary and Wilkinson, 1988; Wilkinson, 1988; Lösel, 1988), so this difference could not be accounted for by, e.g., cell wall or storage lipids. In particular, straight-chain saturated fatty acids and γ-linolenic acid (18:3ω6*c*) were enriched in the MIDI procedure, corresponding to a fatty acid composition with a more eukaryotic character (Harwood and Russell, 1984). In contrast, there was almost no enrichment of cyclopropyl fatty acids of bacterial origin. These observations provide evidence that a background of material from non-

Table 4. Ratios ± standard error (SE) between quantitative yields of 22 fatty acids (FA) consistently found with both the MIDI procedure and the PLFA procedure. Fatty acid nomenclature: See text.

Fatty acid†	MIDI-FA:PLFA‡ Ratio ± SE
Saturated FA	
14:0	11.5 ± 1.31
15:0	7.5 ± 0.60
16:0	8.1 ± 1.25
17:0	7.8 ± 0.81
18:0	9.3 ± 1.63
20:0	11.2 ± 1.47
Mono-unsaturated FA	
16:1ω7*c*	2.7 ± 0.34
16:1ω5*c*	4.0 ± 0.70
17:1ω8*c*	4.6 ± 0.37
18:1ω9*c*	3.4 ± 0.52
18:1ω7	2.0 ± 0.25
Poly-unsaturated FA	
18:2ω6*c*	3.6 ± 0.65
18:3ω6*c*	11.4 ± 2.37
Cyclopropyl FA	
cy17:0	1.6 ± 0.24
cy19:0	1.6 ± 0.46
Branched-chain FA	
i15:0	3.1 ± 0.37
a15:0	3.8 ± 0.46
i16:0	7.1 ± 0.96
i17:1	5.5 ± 0.65
i17:0	4.7 ± 0.62
a17:0	5.3 ± 0.70
10Me18:0	4.5 ± 0.46

† Fatty acids were designated as *X*:*Y*ω*Z*, where *X* represents the number of C atoms, *Y* the number of double bonds, and *Z* indicates the position of the first double bond from the aliphatic (ω) end of the molecule. Prefixes iso (*i*) and anteiso (*a*) represent branching at C atom No. 2 and 3, respectively, from the ω end, br indicates branching at an unknown position, and cy indicates a cyclopropyl group. The prefix 10Me indicates a methyl group at the tenth C atom from the carboxyl end of the molecule. Cis or trnas configuration is indicated by *c* or *t*.

‡ Midi-FA, fatty acids derived from the Midi procedure; PLFA, phospholipid fatty acid.

bacterial sources is extracted with the MIDI procedure, as previously suggested (Macalady, 1998).

It was recently demonstrated that even the mildly alkaline extraction used with the PLFA method dissolves a small background (probably 5–10%) of lipid material derived from soil organic matter (Nielsen and Petersen, 2000). It is therefore not surprising that considerably more lipid material was released, and possibly modified by oxidative reactions (Stevenson, 1982), during the harsh initial saponification step of the MIDI procedure. The subsequent methylation also represents a potential problem for the MIDI procedure in studies of adaptation, since acidic conditions may disrupt cyclopropyl functional groups (Christie, 1982). The inclusion of lipid material associated with soil organic matter, the inclusion of cellular lipids other than membrane lipids, and the potential modifications of fatty acids may alle have confounded adaptational responses with the MIDI-FA profiles, and it is therefore not surprising that no associations were found between soil environmental conditions and this variable.

CONCLUSION

Soil microbiological properties and selected soil properties were characterized across a growing season of spring wheat.The analysis of microbial community attributes under a variety of realistic growth conditions provided evidence that the microbial community was modified by environmental conditions during the growing season. The importance of sampling time in the statistical analysis showed that the changes in community composition was a long-term process occurring on a timescale of months. There was evidence that changes were at least partly caused by microbial adaptation. Biomass N was positively, though transiently, affected by fertilization. Substrate utilization patterns changed during the growing season, and the correlation with lipid stress indicators suggested that this was related to a decrease in microbial activity. The dynamics of fungal and bacterial biomarker fatty acids indicated that these compounds were indeed associated with contrasting subsets of the soil microbial community, but that biomarker fatty acids can be affected by changes related to physiological stress. Hence, adaptation must be taken into consideration when microbial dynamics are interpreted in systems with strong environmental gradients. There were only limited effects of tillage practice on relationships between soil conditions and microbiological properties, indicating that common mechanisms regulate microbial dynamics in the two systems.

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