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Richard P. Dick

The Oregon Long-Term Soil Quality Project was initiated to identify soil properties that respond rapidly to alternative management practices. Such practices included winter cover cropping, which was implemented at two experimental research stations and several grower fields throughout the Willamette Valley. The goal of this thesis was to identify the major impacts of cover cropping on soil microbial community structure, diversity, and substrate utilization potential. Microbial community structure was assessed by extracting fatty acids from soil and analyzing community fatty acid methyl ester (FAME) profiles. Two different methods for extracting fatty acids were tested; these were the ester linked method and the MIDI method developed by Microbial ID, Inc. (Newark, DE). Both methods discriminated among communities from different soils, but the types and relative amounts of fatty acids extracted differed by method. Microbial communities also were characterized by their potential to utilize a diverse range of carbon substrates using the Biolog assay.

In a laboratory incubation study, the Biolog assay demonstrated that the types and overall diversity of substrates utilized were affected by the complexity of carbon substrate added to soil. In addition, Biolog diversity was significantly correlated with community FAME richness ($r = 0.59$). FAME and Biolog assays were applied to field soils of the Soil Quality Project. Changes occurred in microbial FAME structure and Biolog potential in response to winter cover cropping, but effects often were not observed until incorporation of cover crops to soil. In some soils containing cover crop residues, there were greater amounts of eukaryotic FAME markers compared to winter-fallow soils, suggesting greater fungal and protozoan populations in cover-cropped soil. Biolog diversity also was greater in cover-cropped soil compared to fallow soil, which may reflect the utilization of cover crop residues by soil microorganisms during the summer months. Community patterns across different sizes of soil aggregates also were assessed. Biolog diversity was heterogeneously distributed among aggregates, and winter cover cropping elevated FAME diversity across all but the largest (2.0-5.0 mm) aggregate size class. This thesis also identified season and soil type as strong determinants of microbial community structure.

DETERMINANTS OF MICROBIAL COMMUNITY STRUCTURE AND SUBSTRATE UTILIZATION POTENTIAL IN SOILS OF VEGETABLE CROPPING SYSTEMS

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Mary E. Schutter, Author

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CONTRIBUTION OF AUTHORS

Mary E. Schutter conducted research and wrote each manuscript. Joan M. Sandeno managed all field samplings and assisted in data collection for the project. Dr. Richard P. Dick was engaged in research development and project format and analyzed each manuscript.

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INTRODUCTION

The Oregon Long-Term Soil Quality Project was initiated to identify soil properties that respond rapidly to alternative management practices in commercial vegetable cropping systems of western Oregon. Winter cover cropping treatments were established at two experimental research stations, one in 1989 and the other in 1993. In addition, winter cover cropping and reduced tillage practices were implemented at several commercial grower fields in 1996. Studies conducted during the 1997 and 1998 growing seasons identified several physical and biological soil properties that responded to the alternative practices. Soil properties that responded favorably to winter cover cropping included soil bulk density, water infiltration rate, microbial biomass C, and soil enzyme activities. Improved soil physical properties and changes in microbial biomass and activities suggested opportune conditions for microbial community changes in response to winter cover cropping. The design of this project also allowed for analysis of microbial community structure and diversity across seasons and geographical locations, thus allowing for additional insights regarding seasonal and soil type influences on microbial communities.

In this thesis, microbial communities were analyzed and described according to their fatty acid profiles using methods that extract fatty acids directly from soil. In addition, communities were characterized according to their ability to utilize a diverse

range of carbon substrates using the Biolog assay. Both methods provide a measure of community diversity, both structurally (fatty acids) and metabolically (substrate utilization). Background information on these methods and their application to agriculture and soil research are presented in Chapter 1 of this thesis.

In Chapter 2, an experimental study was conducted to test two different methods for the direct extraction of microbial fatty acids directly from soils. Fatty acids were extracted from four different soils, and the abilities of the two methods to discriminate among the different soil types were compared. Soils also were stored over time under different protocols so that effects of soil storage conditions on microbial community fatty acid profiles could be assessed as well.

In Chapter 3, an incubation study was conducted to evaluate the ability of the Biolog assay to predict substrate quality and availability in soil by analyzing soil microbial communities after substrate amendment. Soils were amended with a variety of substrates associated with plant tissues and incubated over time. Substrates ranged from simple (glucose and cellulose) to complex (lignin and plant residues). In addition, microbial community structure was monitored over time by the fatty acid method. Community Biolog and fatty acid characterizations were conducted to determine if changes in Biolog patterns, in response to substrate amendment and time, would correspond to shifts in microbial community FAME structure.

In Chapter 4, a field study was conducted as part of the Oregon Long-Term Soil Quality Project to identify the major factors influencing microbial communities of vegetable cropping systems of western Oregon. The goals were to determine if microbial communities differed according to management practice and soil type.

Seasonal impacts on microbial communities also were assessed. Previous studies showed that the alternative management practices, which included winter cover cropping, were able to enhance soil structure, microbial biomass, and soil enzyme activities. This suggested that the potential existed for changes in microbial community structure and diversity in response to winter cover cropping.

In the final chapter (Chapter 5), impacts of winter cover cropping on microbial communities were assessed at a finer scale. Microbial communities were studied across different size classes of soil aggregates to determine if winter cover-cropping affected the distribution of soil microorganisms and their activities among aggregates. The study was conducted over the course of one growing season to assess seasonal influences on aggregate distribution and community patterns among aggregate size classes.

CHAPTER 1

LITERATURE REVIEW

Mary E. Schutter

Microbial Communities and Soil Quality

Soil Quality

Soil quality is a concept that integrates physical factors such as aggregate stability and bulk density, chemical factors such as soil pH and cation exchange capacity, and biological factors such as crop productivity and microbial activity (Karlen et al., 1992). In 1995, the Soil Science Society of America defined soil quality as:

> the capacity of a specific kind of soil to function within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation— *Agronomy News, June 1995*

Reliable indices are needed to measure and monitor soil quality. To be acceptable, such an index must integrate physical, biological, and chemical properties of soil while including the environmental and health components as well (Granatstein and Bezdicek, 1992). Soil quality indices must also respond quickly to soil perturbations or restoration efforts. In addition, tests for soil quality parameters must be simple, rapid, and economical in order to be useful.

In recent years, more attention has been focused on the contribution of biological factors to soil quality. Because of the crucial roles microbial populations play (nutrient cycling, aggregate formation, organic matter decomposition, biocontrol of plant pathogens, *etc.*), microorganisms are believed to be important indicators of soil health and fertility (Kennedy and Gewin, 1997; Kennedy and Smith, 1995). Interest in biological soil quality parameters has grown from that of microbial

population sizes and their individual processes to community-level concepts of biological diversity and community functions. Unfortunately, biological diversity in itself has failed so far to serve as a practical indicator of soil quality due to the use of complex, and often expensive, assays which employ non-standardized methodologies. Also, our lack of general knowledge concerning the degree of diversity present in soil ecosystems, as well as our inability to accurately predict the behavior of microbial communities in response to environmental pressures *in situ*, leaves us with few clues regarding the relevance of microbial diversity to soil quality.

Microbial Diversity and Soil Quality

A central concept in ecological theory is that ecosystems containing a high level of organism diversity are inherently resilient to environmental stress. As a result, loss of biological diversity will threaten the ecological integrity and capacity of an ecosystem to recover from perturbations (Odum, 1969; Pimm, 1993). This belief has been extended from the perspective of animal and plant diversity to that of microbial diversity (Atlas, 1984). Unfortunately, as stated above we know little of the breadth of microbial diversity within natural environments to fully understand the roles microorganisms play in maintaining ecosystem stability. This is especially true of soil systems, where the number of bacterial species may be as high as 4000 per gram of soil (Torsvik, 1990). Taking into account that only a few thousand microorganisms *total* have been described in any detail, one begins to realize the near impossibilities of understanding whole microbial communities in the soil environment.

A major confounding problem in studying microbial diversity is the level of functional redundancy that exists within the microbial collective. Although it may be true that diversity does contribute to ecosystem resilience, microbial communities with different levels of diversity may perform similar ecosystem-level processes and function identically. Therefore, it may be more practical in regards to microbial studies to determine the general level of diversity that is required to perform a certain function in order to maintain ecosystem health. A review by Kennedy and Gewin (1997) explores this topic and discusses the importance of identifying a relationship between microbial species richness and function in order to understand the contributions of microorganisms and their processes to ecosystem stability.

Some promising methods do exist, however, that are capable of assessing relative degrees of diversity in microbial communities. One uses fatty acid methyl esters extracted directly from the soil to generate a "fingerprint" of the microbial community structure at a particular moment in time. Another measures the ability of a community to utilize a diverse range of carbon substrate and is thus a measure of the metabolic potential/ functional diversity of the community. Both are sensitive assays capable of detecting changes in community structure and function in response to environmental disturbances and agricultural management practices, and both have the potential to provide information on the relationships between microbial diversity, community function, and soil quality.

Methodology

Fatty Acid Methyl Ester (FAME) Analysis

Analysis of bacterial membrane fatty acids has been used for several years to identify clinical bacterial strains and plant pathogens. Bacterial cultures are grown under defined conditions and analyzed for the kinds and amounts of fatty acids found in their membranes (their fatty acid profile). Each species of bacteria has its own characteristic fatty acid profile, allowing a bacterial isolate to be identified based on the presence and quantity of specific fatty acids. This technique has several advantages over traditional diagnostic methods because it is cheaper, faster, and is capable of identifying most clinical isolates in only 24 hours.

Another approach, however, is to extract fatty acids directly from environmental samples as a means for characterizing microbial communities within natural habitats. For example, fatty acids can be extracted directly from the membranes of bacteria and fungi living in a soil to generate a fingerprint of the structure of the microbial community. Just as individual species contain specific fatty acid patterns, certain populations of soil microorganisms are associated with certain "marker" fatty acids. Examples of signature fatty acid markers and their associated organisms are given in Table 1.1. By looking for "signature" fatty acids, fatty acid profiles can reveal relative abundances of certain microbial biotypes in a natural sample and indicate how community structure may change in response to amelioration efforts or environmental perturbation.

Table 1.1. Signature fatty acid markers in terms of primary origin. Adapted from Ringelberg et al. (1997).

Fatty acid	Functional group	Examples
*i*15:0, *a*15:0, *i*17:0, *a*17:0	Gram-positive	*Arthrobacter, Bacillus*
16:1ω7*c*, 18:1ω7*c*, cy17:0, cy19:0	Gram-negative	*Pseudomonas, Acinetobacter*
10Me18:0	Actinomycetes	*Streptomyces*
10Me16:0, *i*17:1ω7*c*	Sulfate-reducers	*Desulfobacter* (10Me16:0) *Desulfovibrio* (i17:1ω7c)
16:1ω8*c*, 18:1ω8*c*	Methanotrophs	*Methylomonas* (16:1ω8c) *Methylosinus* (18:1ω8c)
18:2ω6*c*, 18:3ω6*c*,	Fungi	*Pisolithus*
20:3ω6*c*, 20:4ω6*c*	Protozoa	*Naegleria*
18:1ω9*c*, 18:1ω11*c*, 18:3ω3*c*, 20:5ω3*c*	Higher plants	

Applications of Community FAME Analysis

Most often, fatty acid analysis is used to indicate the response of microbial communities to environmental stress and disturbance. In subsurface soil, Gram-negative bacteria have show signs of starvation and long-term survival stress, as indicated by higher ratios of *trans-* to *cis-* monounsaturated fatty acids (Kieft et al., 1997). In other studies, subsurface communities were stimulated and shifted towards more Gram-negative microorganisms in response to organic amendments (Smith et al., 1986). In addition, the absence of long chain, polyunsaturated fatty acids indicated that subsurface soils were absent of fungi.

More soil fatty acid research has been in forest soils than in subsurface soils. In forest soils, fungi (marker 18:2ω6*c*) were severely affected by clear-cutting and and burning as a result of damage to mycorrhizae and tree roots (Bååth et al., 1995). However, fungi were not as sensitive to heavy metal pollution as bacteria, as indicated by an increase in the amounts of fungal markers in Zn contaminated soils relative to nonpolluted soils (Frostegård et al., 1996). In addition, liming of forest soils often changed microbial communities towards greater proportions of actinomycetes and Gram-negative bacteria, presumably due to the increased availability of carbon substrates at elevated pH levels (Bååth et al., 1992, 1995; Frostegård et al., 1996). In general, treatments that stimulated rhizosphere development and community growth (liming, addition of substrates, *etc.*) in forest soils led to an overall increase in fungi and Gram-negative bacteria whereas highly polluted soils tend to be dominated by Gram-positive microorganisms (Rajendran et al., 1994; Pennanen et al., 1996).

In agricultural studies, fatty acids have been used to characterize microbial communities in systems differing in soil type, crop type, and cultivation practices. Community fatty acid profiles were shown to differ between rhizosphere and nonrhizosphere soil (Ibekwe and Kennedy, 1999), among rhizospheres of different crop species and cultivars (Ibekwe and Kennedy, 1998; Siciliano et al., 1998), and according to soil type (Bossio et al., 1998), crop rotation, and cultivation practices (Zelles et al., 1995). Changes in microbial community structure also can occur in response to carbon amendments (Bossio and Scow, 1995) and organic and low-input management practices (Bossio et al., 1998). In another study, protozoan markers in manure "hot spots" increased whereas bacterial phospholipids decreased, suggesting

that grazing was an important event (Frostegård et al., 1997). Differences in community structure are not always evident, however. Wander et al. (1995) were unable to detect differences in community profiles between cover-cropped, organic and conventional treatments due to high variability among field replicates (variability among field replicates plots was just as great as variability between the management practices). One explanation is that by increasing vegetation diversity through cover cropping, the heterogeneity of the soil microbial community was subsequently increased.

Substrate Utilization Potential

Microorganisms can also be characterized according to their carbon substrate utilization potentials as a means of measuring diversity. One popular technique employs Biolog MicroPlates™ (Biolog Inc., Hayward, CA). Each plate contains 96 wells, 95 of which contain a different carbon substrate source (the first well, with no carbon substrate, serves as a control). Biolog substrates include various carbohydrates, carboxylic acids, amino acids, amines, and polymers. Each well also contains tetrazolium dye which develops a purple color upon oxidation of the carbon substrate. This method was originally developed as a simple and rapid method to characterize and identify bacterial isolates based on qualitative (growth or no growth) and quantitative (degree of substrate color change) data. More recently, the Biolog method has been applied to soil research in order to bring ecological meaning to studies of heterotrophic microbial communities.

Biolog Applications in Community Studies

Garland and Mills (1991) and Zak et al. (1994) were among the first to use Biolog as a means of measuring the functional diversity of microbial communities in environmental samples. Microbial communities from aquatic, soil, and root zone samples had distinct substrate utilization patterns (Garland and Mills, 1991). The method was sensitive enough to also detect differences in metabolic diversity of microbial communities from soils supporting different plant communities (Zak et al., 1994). In addition, Garland (1996) found that substrate utilization patterns of microbial communities from soybean rhizospheres changed according to the stage of plant development, suggesting a successional effect of soybean growth on microbial diversity. Distinct patterns of C source utilization may be due to vegetation and plant exudation patterns, assuming that shifts in substrate utilization are linked to substrate availability in the environment. Or, they may be due to structural differences in microbial communities, as demonstrated when different inoculation treatments to identical wheat cultures produced different utilization patterns (Garland and Mills, 1994).

Substrate utilization patterns also can be combined with responses to various stresses, such as heavy metals or antibiotics, to characterize microbial communities. Kennedy and Smith (1995) studied the long term effects of agricultural management practices on the metabolic diversity and inherent stress resistance of soil bacteria. Bacterial isolates were collected from cultivated wheat and prairie soils and analyzed for their abilities to utilize a number of carbon substrates. Each isolate was tested as well for its ability to grow in the presence of heavy metals, ethylene glycol, and

antibiotics. When isolates from the two soil systems were plotted according to their substrate utilization and stress rankings, Kennedy and Smith (1995) found that bacteria from the cultivated wheat soil exhibited a broader range of substrate utilization and stress resistance than bacteria isolated from a prairie soil. These results suggested a greater functional heterogeneity in communities from cultivated soil relative to the prairie soil, which exhibited a homogenous or less diverse microbial population.

Community Biolog Diversity and Soil Quality

A review by Konopka et al. (1998) details several important ecological issues that question the practical application of Biolog to community studies. Among these are: (1) Biolog is a culture-based method that reflects the activity of only aerobic heterotrophic populations capable of oxidizing a rich nutrient source; (2) dormant and "viable but non-culturable" populations may not be able to oxidize Biolog substrates and may even die in the presence of high nutrient concentrations, and (3) Biolog substrates may not be ecologically relevant as they generally are simple compounds that do not reflect qualitatively or quantitatively the diversity of substrates found in natural environments.

Despite such biases, however, the Biolog assay has demonstrated an ability to detect changes in community function in response to various soil manipulations, including rice straw incorporation (Bossio and Scow, 1995) and contamination of trichloroethylene (TCE) and toluene (Fuller et al., 1997), known to affect the quality of soil. Although microbial biomass was not affected in either case, microbial activity

was suppressed by TCE and toluene contamination (reduced respiration and fewer substrates utilized) and enhanced with the addition of straw residue (greater respiration and utilization of more substrates). The results of Bossio and Scow (1995) support the hypothesis that microbial communities tend to be limited by carbon and that high inputs of complex carbon substrates, such as crop residues, to the soil will enhance the community's metabolic diversity. This may have important implications concerning the substrate utilization potential of microbial communities when winter cover crops are used as a means for improving the quality of an agricultural soil. Incorporation of cover crop residues may enhance substrate utilization rates by the microbial community, as measured by Biolog. Additional research is required to assess the impact of enhanced substrate utilization on rhizosphere and soil organic matter decomposition.

Conclusions

A critical step in establishing the link between microbial diversity and soil quality is to develop methods capable of demonstrating shifts in microbial communities in response to soil disturbance and restoration efforts. Both FAME and Biolog methods have proven to be successful for this purpose. The next step would be to correlate changes in microbial diversity with changes in microbial activities in response to soil management practices. This could be accomplished in part by combining diversity measurements with measurements of microbial processes (enzyme activity, substrate decomposition rates, nitrification and other nitrogen

cycling activities, *etc.*). However, the underlying environmental factors, and the mechanisms by which they control microbial community fluctuations, also need to be identified. Such factors include season, soil type, and nutrient resource availability. It is possible that the degree to which microbial communities respond to soil quality change may be constrained according to their physical and chemical environment. More research is needed to determine the relative influence of environmental factors compared to management practices on soil microbial communities before a better understanding of the relationship between communities and soil quality can be achieved.

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CHAPTER 2

COMPARISON OF FATTY ACID METHYL ESTER (FAME) METHODS FOR CHARACTERIZING MICROBIAL COMMUNITIES

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Abstract

Fatty acid profiling is a popular method for characterizing microbial communities of natural systems. Direct extraction of microbial fatty acids within natural environments would be useful compared to methods that extract lipids first and subsequently release fatty acids from lipids. In this study, two methods for the direct extraction of fatty acids from soils were compared for three cultivated silt loams and one forested sandy clay loam. Fresh soils were analyzed for their fatty acid methyl ester (FAME) profiles using an ester-linked (EL) method and the method of Microbial ID, Inc. (MIDI). Soils were stored four different ways (moist at 4°C, moist at -20°C, air-dried at 25°C, and partially dry at 4°C) and analyzed for FAME profile changes after one and three months of storage. Eleven and 17 FAMEs were unique to the EL and MIDI method, respectively, but unique FAMEs generally were found in only minute amounts. Soils extracted with the MIDI method yielded more hydroxy FAMEs and short-chain saturated and branched FAMEs. Conversely, EL-extracted soils generally produced more long-chain saturated and branched FAMEs, unsaturated FAMEs, and FAMEs with cyclopropane and methyl groups. Both extraction methods were able to differentiate among communities of different soil types, regardless if soils were fresh or stored. Changes in FAME profiles did occur in stored soils, but the effectiveness of each storage protocol for preserving FAME patterns over time was inconsistent among the four soils. While community analyses should be conducted on fresh soil, overall effects of storage were slight compared to those of extraction method and soil type.

Introduction

The use of microbial lipids to identify microorganisms and characterize microbial communities in natural systems has become increasingly popular. Several methods for the analysis of microbial phospholipid fatty acids (PLFAs) exist and have been in use for over 20 years to estimate microbial biomass and community structure in sediments (White et al., 1979; Guckert et al., 1985; Rajendran et al., 1992). Since its introduction, PLFA methods have been applied to determine the effects of stress on bacterial isolates (Kieft et al., 1994 and 1997), of root exudates on rhizosphere microorganisms (Griffiths et al., 1999), and P on arbuscular mycorrhizal fungi (Olsson et al., 1997). The methods also allowed for the characterization of microbial communities from agricultural soils (Zelles et al., 1992; Wander et al., 1995; Reichardt et al., 1997; Bossio et al., 1998; Ibekwe and Kennedy, 1998b), from soils contaminated with heavy metals, alkaline dust, and acid rain (Pennanen et al., 1996; Bååth et al., 1992; Pennanen et al., 1998), and from other diverse habitats (Sundh et al., 1997; Klamer and Bååth, 1998; Steinberger et al., 1999).

Although analysis of microbial PLFA profiles has proven extremely useful, the methods involved are time consuming. Microbial lipids are extracted from environmental samples in a phase-mixture of chloroform, methanol (MeOH), and water (Bligh and Dyer, 1959). Lipids associated with the organic phase are then fractionated into neutral, glyco-, and phospholipids on silicic acid columns, while the residue at the organic:aqueous interphase can be separated into lipopolysaccharides, teichoic acids, and muramic acid (Vestal and White, 1989). Finally, the

phospholipids are subjected to alkaline methanolysis to produce fatty acid methyl esters (FAMEs) for analysis by gas chromatography (GC).

Recently, a simpler method has been developed to extract microbial fatty acids directly from soils. The protocol of Microbial ID, Inc. (MIDI) was designed to extract fatty acids from pure cultures of bacterial isolates for identification purposes, but it also has been applied to whole-soils. With this method, microbial cells in soil are saponified by heat and the addition of a strong base. Once fatty acids are cleaved from lipids, they are methylated to form FAMEs. The FAMEs are extracted in an organic solvent and analyzed by gas chromatography (Sasser, 1990). Since its introduction, the MIDI technique has been used to assess storage effects on bacterial isolates (Haldeman et al., 1994), to characterize root-associated microorganisms and arbuscular mycorrhizal fungi (Sicilaino and Germida, 1998; Graham et al., 1995), and to describe microbial communities of agricultural soils (Cavigelli et al., 1995; Buyer and Drinkwater, 1997; Ibekwe and Kennedy, 1998a) and soils amended with various organic and inorganic compounds (Fries et al., 1997; Macalady et al., 1998).

While the simplicity of the method is advantageous over that of the PLFA method, it is uncertain whether or not fatty acids extracted by the MIDI method originate only from living microorganisms. It is possible that this extraction procedure also may extract fatty acids associated with soil humic substances and plant roots. Because of this concern, efforts are being made to develop less harsh methods for the direct extraction of fatty acids from soil microorganisms. One potential method is the ester-linked (EL) procedure (Dr. Rhae Drijber, personal communication, 1998). This method uses a mild alkaline reagent to lyse cells and release fatty acids

from lipids once the ester bonds are broken. In theory, only ester-linked and not free fatty acids are extracted with this method. Recently, the EL method successfully characterized microbial communities of several grass seed field soils and placed communities into groupings similar to those generated by a DNA-based method (Ritchie et al. 2000).

The effects of soil storage on FAME profiles also are of great concern to microbial community studies. For example, community structure may change in response to the temperature at which soils are stored (Petersen and Klug, 1994). To date, the effects of air drying or freezing on microbial community FAME structure have not been reported. Earlier studies have shown that microbial biomass and activities, including nitrogen mineralization, are less affected when stored at -20°C compared to 2°C (Stenberg et al., 1998), and that storing air-dried soils at 21°C did not preserve biological activity compared to moist soils stored at cooler temperatures (Zelles et al., 1991). However, effects of storage are complicated by freeze-thaw processes, which can stimulate activity, and by the abilities of soil communities to withstand soil drying and wetting cycles (Stenberg et al., 1998).

The first objective of this study was to compare microbial community FAME profiles of different soils extracted with the MIDI and EL methods and to determine if both methods are able to discriminate between communities of different soil types. The second objective was to determine the effects of soil storage conditions on microbial community FAME structure. To satisfy the second objective, common storage protocols for soil research were chosen, including air-dried storage and cold storage at field capacity.

Materials and Methods

Site and Soil Descriptions

Four soils of varying texture and total organic carbon (TOC) content were sampled in 1998 (Table 2.1). Two samples of a Walla Walla silt loam were obtained from the Residue Utilization Plots (initiated in 1931) at the Columbia Basin Research Center, Pendleton, Oregon. Winter wheat is grown in rotation with a summer fallow, and treatments consist of different nitrogen rates or residue amendments. The two treatments sampled were a 90 kg N ha^{-1} yr^{-1} plot and a 22.4 Mg ha^{-1} 2 yr^{-1} cattle manure plot, where manure is incorporated into the soil during the summer fallow years. Both soils were sampled (0-15 cm depth) in November of the fallow year. The climate is semi-arid Mediterranean with a mean annual precipitation of 416 mm.

The third soil was a Chehalis silt loam from the Oregon State University Vegetable Research Station, Corvallis, Oregon. The soil was sampled (0-15 cm) during the fallow period of a winter fallow-summer vegetable crop rotation treatment. The climate at this site is humid Mediterranean with an average annual rainfall of 1040 mm. The fourth soil was a McKenzie River sandy clay loam collected (0-20 cm depth) from an old growth forest site at the U.S. Forest Service H.J. Andrews Experimental Forest, Blue River, Oregon. The climate is Mediterranean, and the mean annual precipitation ranges from 2300 to 3550 mm depending on the elevation.

Table 2.1. Taxonomy and selected soil properties of the four sites included in this study.

Soil Series	Site History	Soil Family	Soil Subgroup	Sand	Silt	Clay	TOC†
				----------g kg^{-1}----------			
Walla Walla	Agriculture (inorganic N)	coarse-silty, mixed, mesic	Typic Haploxeroll	170	680	150	11.2
Walla Walla	Agriculture (manure)	coarse-silty, mixed, mesic	Typic Haploxeroll	170	680	150	14.5
Chehalis	Agriculture	fine-silty, mixed, mesic	Cumulic Ultic Haploxeroll	220	520	260	16.5
McKenzie River	Old growth forest	fine-loamy, mixed, mesic	Typic Haplumbrept	540	190	270	65.0

†Total organic carbon(TOC) determined by dry combustion.

Sample Preparation and Storage Procedures

After sampling, all soils were stored in coolers for transport to the laboratory, where they were stored at 4°C. Within five days of sampling, the agricultural and forest soils were passed through a 2- and 4.75-mm sieve, respectively. Subsamples of freshly sieved, moist soils were immediately analyzed for their FAME profiles according to the extraction methods described below. The remainder of each soil was then divided into four 200-g portions. Each portion was assigned to one of the following four storage treatments: field-capacity (-33 kPa = 310 g H_2O kg^{-1} soil) at 4°C, field-capacity at -20°C, air-dried at 25°C, and partially-dried at 4°C. The air-dried soils were dried and stored at room temperature. For the fourth storage treatment, soils were slowly dried in a 4°C room until the gravimetric water content was in the range of 60 to 100 g kg^{-1} soil and thereafter stored at 4°C. This particular

storage method was used in previous studies involving soil aggregates, where soil was required to be partially dry during the mechanical sieving/aggregate size separation procedure (Mendes and Bottomley, 1998).

Soils were stored at their respective moisture contents and temperatures for three months. After 1 and 3 months, four subsamples were removed from each treatment and analyzed for their FAME profiles using both extraction methods.

MIDI Extraction Method

Four analytical replications of each fresh and stored soil were extracted by the MIDI procedure (Sasser, 1990; Ibekwe and Kennedy, 1998a). This method uses four reagents and consists of four steps: saponification, methylation, extraction, and a wash. Three grams of soil contained in a 20 × 125-mm teflon-lined, screw-cap test tube were mixed with 3 mL of 3.75 *M* NaOH in MeOH:H_2O (1:1). Test tubes were vortexed and placed in a 100°C water bath for 30 min, during which cells were lysed and saponified (fatty acids cleaved from the cell lipids and converted to sodium salts). To convert fatty acids to FAMEs for increased volatility, 6 mL of 6.00 *M* HCl:MeOH (1:0.85) were added to the tubes. The tubes were then incubated in a water bath for 10 min at 85°C. Next, 2-to-3 mL of hexane:methyl-*tert* butyl ether (1:1) were added to extract the FAMEs from the acidic, aqueous phase into the organic phase. Soil samples were centrifuged at this point to separate soil organic matter from the organic phase. The contents of each tube were transferred to a teflon-lined, screw-cap, 35-mL glass centrifuge tube and centrifuged at 480 × g for 10 min. Afterwards, the clear organic phase was transferred to a 13 × 100-mm teflon-lined, screw-cap glass tube.

The organic phase was washed of residual acidic reagents by adding 3 mL of mild base (0.3 *M* NaOH), followed by gentle mixing for 5 min. Finally, the organic phase was transferred from the glass tube to a vial for gas chromatography (GC) analysis using a Hewlett-Packard 5890 Series II (Palo Alto, CA) equipped with an HP Ultra 2 capillary column (5% diphenyl-95% dimethylpolysiloxane, 25 m × 0.2) and a flame ionization detector. The temperature program ramped from 170°C to 270°C at 5°C per min, with 2 min at 270°C between samples to clean the column. Fatty acids were identified and their relative peak areas were determined using the MIS Aerobe chromatographic program and peak naming table as supplied by MIDI (Microbial ID, Inc., Newark, DE).

In cases where samples were concentrated to improve detection of FAMEs, a portion of the organic solvent was evaporated under a stream of N_2. The remaining organic phase was transferred into a 250-μL glass insert and placed in a GC vial. Because low amounts of total FAME were recovered from the Walla Walla silt loam soils using the MIDI method, Walla Walla FAME samples from fresh and stored soils were concentrated prior to GC analysis. Chehalis soil FAME samples were concentrated after the third month of soil storage, when FAME recovery was low.

A blank (reagents only) and a positive control were included in each set of MIDI extractions. The positive control was *Stenotrophomonas maltophilia* (American Type Culture Collection, # 13637).

EL Extraction Method

The second FAME extraction procedure employs a mild alkaline methanolysis method, which is thought to extract ester-linked fatty acids but not free fatty acids. This particular method was developed by Dr. Rhae Drijber, University of Nebraska, Lincoln, Nebraska (Drijber, personal communication, 1998) and consists of four reagents and four steps. Four analytical replications were extracted per fresh and stored soil. In the first step, 15 mL of 0.2 *M* KOH in methanol were added to a 35-mL teflon-lined, screw-cap glass centrifuge tube containing 3 g of soil. The contents of the tubes were mixed and incubated at 37°C for 1 h, during which ester-linked fatty acids were released and methylated. Samples were vortexed every 10 min during the incubation period. In the second step, 3 mL of 1.0 *M* acetic acid were added to neutralize the pH of the tube contents. FAMEs were partitioned into an organic phase by adding 10 mL of hexane followed by centrifugation at 480 × g for 10 min. No washing step was needed at this point in contrast to the MIDI method, which requires that all acidic residues be removed from the organic phase to prevent damage to the GC column. After the hexane layer was transferred to a clean glass test tube, the hexane was evaporated under a stream of N_2. In the final step, FAMEs were dissolved in 0.5 mL of 1:1 hexane:methyl-*tert* butyl ether and transferred to a GC vial for analysis; results were analyzed by the MIDI system as described above. It was not necessary to concentrate any of the FAME samples from fresh or stored soils with this method.

Standard nomenclature is used to describe FAMEs detected by both extraction methods. Numbering of carbons begins at the aliphatic (ω) end of the fatty acid

molecule. The number of double bonds within the molecule is given after the colon. *Cis* and *trans* conformations are designated with the suffixes "*c*" and "*t*", respectively. Other notations are "Me" for a methyl group, "OH" for hydroxy, "cy" for cyclopropane groups, and the prefixes "*i*" and "*a*" for *iso*- and *anteiso*-branched FAMEs.

Statistical Analyses

Overall effects of extraction method and storage protocol on relative amounts of FAMEs detected in each soil type were determined by multivariate analysis of variance (MANOVA) and ANOVA procedures (SAS Institute, 1996). All data transformations and multivariate analyses were performed using the PC-ORD program (McCune and Mefford, 1997).

To determine the overall effects of soil type, extraction method, and storage on community structure, non-metric multidimensional scaling (NMS) was performed on a data set containing relative FAME amounts for all four soil types, fresh and stored, extracted with the MIDI and EL methods. FAMEs present in only 1 replicate of 1 soil sample within the entire data set were deleted prior to NMS; the deleted FAMEs were *i*10:0, 11:0 2OH, *i*15:1/ 13:0 3OH, 16:1ω7*c* alcohol, *i*19:0, and *i*19:1. FAME data also were transformed by the arcsine squareroot transformation option of PC-ORD to reduce the non-normality of the data set.

For the above analysis, NMS was performed rather than principal components analysis because of the non-normality of the FAME data set that occurred when EL and MIDI-extracted soils were analyzed together. NMS is an iterative technique that

ordinates samples in a lower-dimensional space so that the distances between objects in this space match as closely as possible the distances between objects in the original *p*-dimensional space, where *p* equals the number of variables (*i.e.*, the number of FAMEs in the data set) (McCune and Mefford, 1997). Examples of NMS applied to ecological studies include Prentice (1977), Field et al. (1982), Oksanen (1983), Tuomisto et al. (1995), and Neitlich and McCune (1997). Distance was measured as Sørensen distance, and 150 iterations were performed to ensure stress was minimized (stress is a measure of the dissimilarity between ordinations in the original *p*-dimensional space and in the reduced dimensional space). Stability of the reduced-dimensional ordination pattern was assessed by plotting values of stress vs. iteration number (not shown), and two dimensions were selected for final analysis.

For each extraction method, fresh and stored soils from the agricultural systems were analyzed by principal components analysis (PCA) to determine the overall effects of management, soil type, and storage on microbial communities. To assess specific effects of storage on community profiles, PCA was performed on FAME data from fresh and stored soils of each site extracted with the MIDI or EL method.

Results

Effect of Extraction Method

Soils were separated mainly by extraction method when the FAME profiles of all soils, fresh and stored, were analyzed by NMS (Fig. 2.1). For all four soil types,

MIDI profiles have lower values on Axis 1 of Fig. 2.1 relative to EL profiles. Interestingly, there was a trend for increased distance between EL and MIDI profiles of same study site as TOC content of the soil increased. The correlation between this distance and TOC was significant at $p < 0.01$ ($r = 0.97$; $n = 3$). Separation of communities by soil type also occurred; communities from the forested McKenzie River soil were unique from communities of the agricultural soils, regardless of extraction method.

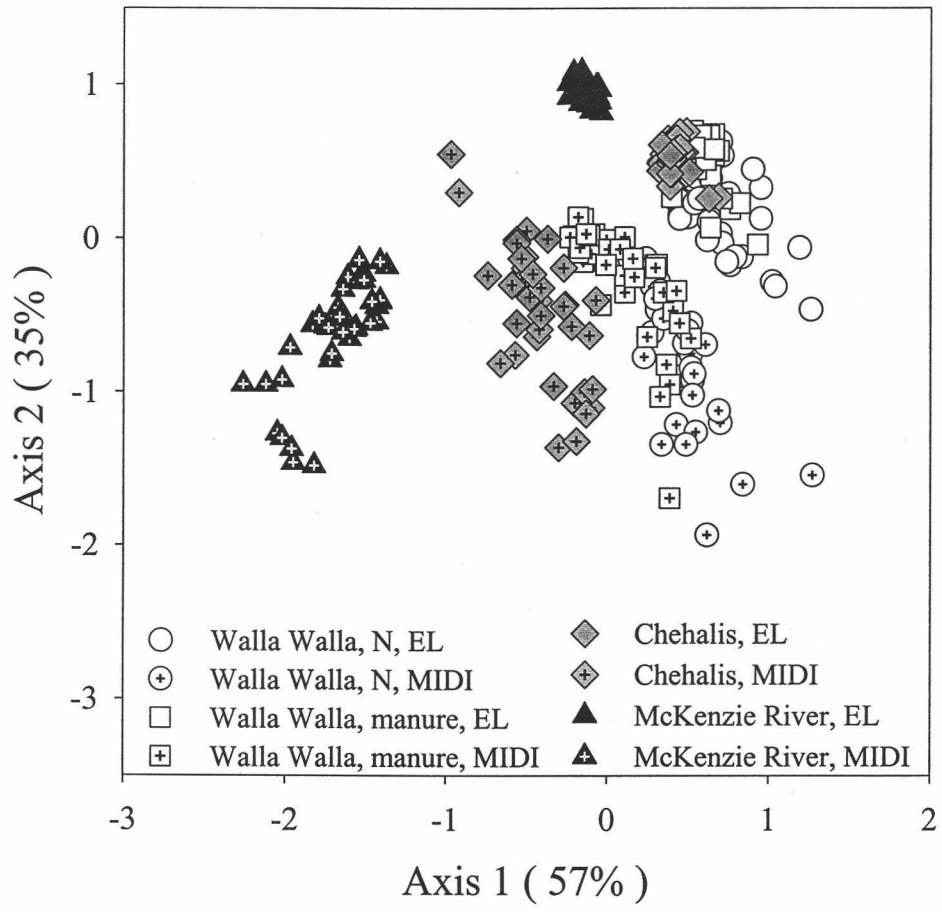

Fig. 2.1. Nonmetric-multidimensional scaling plot of community FAME profiles from four different soils (fresh and stored) extracted with the MIDI or EL method. The proportion of variance explained by each axis is based on the correlation (*R*) between distance in the reduced NMS space and distance in the original space and is reported after each axis heading.

Differences in FAME profiles from soils varying in texture and TOC content were evident based on the NMS. However, we did not test for significant effects of soil type on relative FAME concentrations because the effect of interest was the extraction method. Each sampling site was analyzed separately with MANOVA to determine the main effect of extraction method on individual FAMEs. Significant differences based on extraction method were found for all sites (N-amended Walla Walla, $p < 0.0001$; manure-amended Walla Walla, $p < 0.01$; Chehalis, $p < 0.0001$; McKenzie River, $p < 0.0001$). FAMEs detected in significantly greater amounts in MIDI soil extracts relative to EL extracts are shown in Table 2.2, while FAMEs detected in greater amounts in EL extracts relative to MIDI are listed in Table 2.3. Table 2.4 lists FAMEs not affected or found in significantly greater amounts in either extract, depending on the soil type. For saturated FAMEs, there was a clear effect of method on the detection of FAMEs differing in carbon-chain length. FAME 9:0 was found only in MIDI soil extracts, and other short-chain saturated FAMEs (10:0, 12:0, 13:0, and 14:0) were detected at significantly greater quantities in MIDI than in EL extracts (Table 2.2). Conversely, significantly greater amounts of longer-chain FAMEs (15:0 through 20:0) were detected in EL extracts compared to MIDI extracts (Table 2.3).

Most monounsaturated FAMEs were present in equal or greater quantities in EL soil extracts relative to MIDI extracts (Tables 2.3 and 2.4). Exceptions included 15:1ω8*c*, 16:1ω5*c*, and 17:1ω7*c*. The FAME 17:1ω7*c* was present in especially high amounts in the MIDI extracts McKenzie River soil (Table 2.2). For polyunsaturated FAMEs, 20:4ω6*c* was detected at significantly greater amounts when soils were

extracted by the EL method rather than the MIDI method (Table 2.3), but an opposite trend occurred for 18:3ω6*c* (Table 2.2).

Detection of branched FAMEs of different C-chain lengths also was dependent on the method employed. The MIDI procedure resulted in equal or greater abundances of shorter-chain, *iso*- and *anteiso*-branched FAMEs, including *i*11:0, *a*12:0, *i*13:0, *a*13:0, and *i*14:0 (Tables 2.2 and 2.4). Of these, *i*11:0, *a*12:0, and *a*13:0 were found exclusively in MIDI-extracted soils. All other longer-chain branched FAMEs were more abundant in the EL soil extracts (Table 2.3).

Hydroxylated FAMEs generally were more abundant when soils were extracted with the MIDI method rather than the EL method. Three hydroxylated FAMEs (10:0 3OH, 12:0 2OH, and 12:0 3OH) were detected only in MIDI soil extracts, and 16:0 2OH and 16:0 3OH were present in equal or significantly greater amounts in MIDI versus EL soil extracts (Table 2.2). In addition, 16:0 N alcohol was detected at significantly greater amounts in MIDI extracts (Table 2.2). In contrast, soils extracted with the EL method were more abundant in FAMEs with cyclopropane or methyl groups (Table 2.3), with the exception of 10Me19:0.

There were a total of 11 FAMEs unique to the EL method and 17 unique to the MIDI method. Three of the 11 FAMEs unique to the EL method were present in only one replicate of one soil sample (11:0 2OH, *i*15:1 H/I/13:0 3OH, and i19:1 I) and were deleted from the data sets prior to NMS or PCA analysis. For FAME *i*15:1 H/I/13:0 3OH, MIDI cannot differentiate between the compounds because of identical retention times (such FAMEs are identified as summed features by the MIDI program). The other EL-unique FAMEs were *i*15:1 at 5, *a*15:1 A, 17:0 3OH, *i*18:0, *i*18:1H,

Table 2.2. Relative amounts (%) of FAMEs found in significantly greater quantities ($p < 0.05$) in MIDI than ester-linked (EL) soil extracts ($n = 4$).

	Walla Walla N		Walla Walla Manure		Chehalis		McKenzie River	
FAME	EL	MIDI	EL	MIDI	EL	MIDI	EL	MIDI
Saturated								
9:0	NDa[†‡]	0.31b	NDa	0.21b	NDa	0.21b	NDa	0.32b
10:0	NDa	0.12b	NDa	0.30b	NDa	0.32b	0.06a	0.68b
12:0	0.47a	2.19b	0.68a	2.73b	0.85a	2.91b	0.33a	1.85b
14:0	1.76a	2.33b	1.97a	3.25b	2.37a	4.46b	1.39a	1.62b
Unsaturated								
15:1ω8*c*	ND	ND	ND	ND	ND	ND	NDa	3.26b
16:1ω7*c*/ *i*15:0 2OH[§]	7.69a	10.6b	7.64a	9.56b	6.73a	9.11b	3.16	3.28
17:1ω7*c*	ND	ND	ND	ND	NDa	3.49b	1.85a	27.5b
18:3ω6*c*	1.50a	2.98b	1.27a	2.39b	0.96a	1.90b	1.06a	2.62b
Branched								
*i*11:0	ND	ND	NDa	0.14b	ND	0.03	ND	ND
*a*12:0	ND	ND	ND	ND	ND	ND	NDa	0.05b
*i*13:0	ND	ND	0.05a	0.23b	0.01a	0.02b	ND	ND
*a*13:0	ND	ND	NDa	0.25b	NDa	0.45b	NDa	0.53b
Hydroxy								
10:0 3OH	ND	ND	NDa	0.19b	NDa	0.74b	NDa	0.60b
12:0 2OH	ND	ND	ND	ND	ND	ND	NDa	0.07b
12:0 3OH	ND	ND	NDa	0.24b	NDa	0.24b	ND	0.01
16:0 2OH	ND	0.04	0.03a	0.45b	NDa	0.13b	ND	0.02
16:0 3OH	ND	0.03	0.05a	0.39b	NDa	0.23b	NDa	0.03b
Methylated								
10Me19:0	ND	ND	NDa	0.68b	ND	ND	ND	ND

Table 2.2 (continued). Relative amounts (%) of FAMEs found in significantly greater quantities ($p < 0.05$) in MIDI than ester-linked (EL) soil extracts ($n = 4$).

	Walla Walla N		Walla Walla Manure		Chehalis		McKenzie River	
FAME	EL	MIDI	EL	MIDI	EL	MIDI	EL	MIDI
Cyclopropane								
19:0 cy ω10*c*/ un[§¶]	0.10a	2.33b	0.11a	3.45b	0.11a	7.32b	2.06a	21.2b
Mixed								
*i*16:1 I/ 14:0 3OH/ 12:0 aldehyde[§]	ND	ND	NDa	0.60b	NDa	0.90b	0.01a	0.14b
*i*16:0 3OH	ND	ND	NDa	0.06b	ND	ND	ND	ND
*i*17:1ω10*c*	ND	ND	ND	ND	ND	ND	NDa	0.29b
*i*17:1 I/ *a*17:1 B[§]	ND	0.09	ND	0.06	NDa	0.37b	ND	0.03
*i*17:0 3OH	ND	0.03	NDa	0.21b	0.03a	0.34b	ND	ND
Alcohol								
16:0 N alcohol	NDa	0.27b	0.03a	0.59b	0.07a	0.53b	0.01a	0.60b

[†]ND = Not detected.

[‡]Within each of the four study sites, means of a given FAME followed by the same letter are not significantly different at $p < 0.05$.

[§]MIDI cannot differentiate between these FAMEs because of identical retention times.

[¶]"Un" designates a FAME unnamed by the MIDI system.

Table 2.3. Relative amounts (%) of FAMEs found in significantly greater quantities ($p < 0.05$) in EL than MIDI soil extracts ($n = 4$).

	Walla Walla N		Walla Walla Manure		Chehalis		McKenzie River	
FAME	EL	MIDI	EL	MIDI	EL	MIDI	EL	MIDI
Saturated								
15:0	0.49a[†]	0.26b	0.83	0.80	0.90	0.76	1.02a	0.58b
16:0	16.2	16.1	16.6	16.2	15.6a	14.2b	13.8a	6.11b
17:0	0.34a	0.01b	0.73a	0.25b	0.73a	NDb[‡]	0.78a	NDb
18:0	2.94a	1.27b	3.59a	2.46b	3.42a	1.65b	3.24a	0.73b
19:0	0.11	0.01	0.17	0.10	ND	ND	0.07a	NDb
20:0	1.74a	0.77b	1.82a	0.99b	1.89a	0.18b	5.60a	1.00b
Unsaturated								
16:1ω9*c*	0.06	ND	0.44a	0.06b	0.70a	0.18b	0.09	0.09
16:1ω11*c*	ND	ND	0.02	ND	0.03	ND	0.73a	0.04b
17:1ω8*c*	2.26a	1.00b	2.20a	1.59b	1.71a	NDb	ND	ND
18:1ω7*c*/ 9*t*/12*t*[§]	4.55a	3.39b	5.31a	3.46b	6.00a	3.48b	7.32a	2.39b
18:1ω9*c*	15.7a	10.8b	11.6a	9.48b	12.9a	10.9b	19.5a	9.66b
19:1ω11*c*/un[§¶]	0.52a	NDb	0.47a	NDb	0.02	ND	0.02	ND
20:1ω9*c*	0.06	0.04	0.35a	0.06b	0.74a	NDb	0.49a	NDb
20:4ω*c*	0.73	0.58	0.96a	0.24b	1.05a	0.37b	0.54a	NDb
Branched								
*i*15:0	4.20a	3.08b	4.70a	4.16b	4.86	5.02	3.35a	1.93b
*i*16:0	3.02a	1.60b	2.87a	2.07b	2.70a	2.25b	1.63a	0.58b
*i*17:0	0.86a	0.28b	1.41a	0.78b	1.47a	0.75b	0.99a	0.09b
*a*17:0	1.54a	0.48b	1.78a	0.91b	1.67a	1.32b	1.19a	0.19b
*i*20:0	ND	0.04	0.34a	0.03b	ND	ND	0.11a	NDb

Table 2.3 (continued). Relative amounts (%) of FAMEs found in significantly greater quantities ($p < 0.05$) in EL than MIDI soil extracts ($n = 4$).

	Walla Walla N		Walla Walla Manure		Chehalis		McKenzie River	
FAME	EL	MIDI	EL	MIDI	EL	MIDI	EL	MIDI
Hydroxy								
15:0 3OH	ND	ND	0.03	0.02	ND	ND	0.99a	0.02b
Methylated								
10Me16:0	3.68a	1.27b	4.33a	2.20b	3.56a	2.03b	3.23a	0.74b
10Me17:0	0.74a	0.19b	1.28	1.23	0.86a	0.44b	0.56a	0.02b
10Me18:0	1.26a	NDb	1.62a	0.13b	1.81a	0.03b	0.83a	NDb
Cyclopropane								
17:0 cy	1.24a	0.22b	1.49a	0.64b	1.64a	0.61b	2.32a	0.81b
19:0 cy ω8*c*	2.10a	0.13b	2.42a	0.58b	2.29a	0.15b	6.30a	1.09b
Mixed								
*i*15:1 G	0.03	ND	0.35a	0.20b	0.61a	0.38b	0.08a	NDb
*i*16:1 G	0.04	ND	0.03	0.02	0.08	ND	0.44a	0.01b
*i*16:1 H	0.25a	0.04b	0.73a	0.17b	0.14	0.02	ND	ND
16:1 2OH	0.15a	NDb	0.55a	0.01b	0.53a	NDb	1.05a	0.01b

[†]Within each of the four study sites, means of a given FAME followed by the same letter are not significantly different at $p < 0.05$.

[‡]ND = Not detected.

[§]MIDI cannot differentiate between these FAMEs because of identical retention times.

[¶]"Un" designates a FAME unnamed by the MIDI system.

Table 2.4. Relative amounts (%) of FAMEs not affected or found in significantly greater quantities ($p < 0.05$) in MIDI or EL soil extracts, depending on the site ($n = 4$).

	Walla Walla N		Walla Walla Manure		Chehalis		McKenzie River	
FAME	EL	MIDI	EL	MIDI	EL	MIDI	EL	MIDI
Saturated								
13:0	ND[†]	ND	ND	0.01	ND	ND	0.07a	0.31b
Unsaturated								
16:1ω5*c*	12.0a[‡]	28.0b	7.38a	14.5b	2.66a	3.61b	1.96a	1.43b
17:1ω6*c*	ND	ND	ND	0.02	ND	ND	ND	ND
18:1ω5*c*	0.08	0.04	ND	ND	ND	ND	0.02	ND
19:1ω12*t*	0.12	ND	0.09	0.10	ND	0.21	ND	ND
20:1ω9*t*	0.02	ND	0.08	ND	ND	ND	ND	ND
18:2ω6*c*/ *a*18:0[§]	7.97a	6.30b	6.26	6.01	6.70a	7.96b	8.26a	5.09b
20:3ω6*c*	0.12a	0.69b	0.37	0.52	ND	ND	0.11a	NDb
Branched								
*i*14:0	0.05	0.11	0.54	0.65	0.97a	1.30b	0.15a	NDb
*a*15:0	2.93a	2.00b	3.20	3.10	4.13a	4.79b	1.92a	1.18b
*a*16:0	ND	ND	0.01	ND	ND	0.06	0.01	ND
*i*18:0	0.01	ND	0.07	ND	ND	ND	ND	ND
Hydroxy								
17:0 3OH	0.01	ND	0.01	ND	ND	ND	ND	ND
18:0 2OH	ND	ND	0.13	0.13	0.11	0.31	1.08	0.90

Table 2.4 (continued). Relative amounts (%) of FAMEs not affected or found in significantly greater quantities ($p < 0.05$) in MIDI or EL soil extracts, depending on the site ($n = 4$).

	Walla Walla N		Walla Walla Manure		Chehalis		McKenzie River	
FAME	EL	MIDI	EL	MIDI	EL	MIDI	EL	MIDI
Mixed								
*a*15:1 A	ND	ND	0.02	ND	0.06	ND	ND	ND
*i*15:1 at 5	ND	ND	ND	ND	ND	ND	0.02	ND
*i*15:0 3OH	ND	ND	ND	ND	ND	0.04	ND	ND
*a*17:1ω9*c*	0.31a	NDb	0.93a	0.04b	0.11a	NDb	NDa	0.07b
*i*18:1 H	ND	ND	0.03	ND	ND	ND	ND	ND
18:1 2OH	ND	ND	ND	ND	ND	0.05	0.16	0.23

[†]ND = not detected.

[‡]Within each of the four study sites, means of a given FAME followed by the same letter are not significantly different at $p < 0.05$.

[§]MIDI cannot differentiate between these FAMEs because of identical retention times.

19:1ω11c/unnamed, 20:1ω9*t*, and one unnamed FAME. Most of these FAMEs were present in two of the four soil types at concentrations less than 0.1% (Tables 2.3 and 2.4). Of the 17 FAMEs unique to MIDI, three were present in only one soil sample (*i*10:0, 16:1ω7*c* alcohol, and *i*19:0) and were subsequently deleted from the data sets. Of the remaining 14 FAMEs, two were present in all four soil types (9:0 and *i*17:1 I/*a*17:1 B), and most others were present in multiple replicates of 1 to 3 soil types at concentrations greater than 0.1% (10:0 3OH, *i*11:0, *a*12:0, *a*13:0, 12:0 2OH, 12:0 3OH, 15:1 ω8*c*, *i*15:0 3OH, *i*16:0 3OH, 17:1ω6*c*, *i*17:1 ω10*c*, and 10Me19:0) (Tables 2.2 and 2.4).

Effect of Soil Properties and Storage

It was clear that both the EL and MIDI method could differentiate between forest and agriculture soil communities (Fig. 2.1). Therefore, only the agricultural soils were analyzed further to determine if both extraction methods could separate communities of soils differing in properties and management. Results are shown in Fig. 2.2. Overall, soil type and management had greater influences on community profiles compared to storage effects. Regardless if soils were fresh or stored, the EL and MIDI methods were able to distinguish between communities from the Chehalis and two Walla Walla soils (Fig. 2.2).

Effects of storage protocols on soil FAME profiles were less clear and differed among the four soil types. Results of storage protocols on FAME profiles from Chehalis and McKenzie River soils are presented as examples in Figure 2.3. Changes occurred in soil FAME profiles after one month of storage, regardless of the method

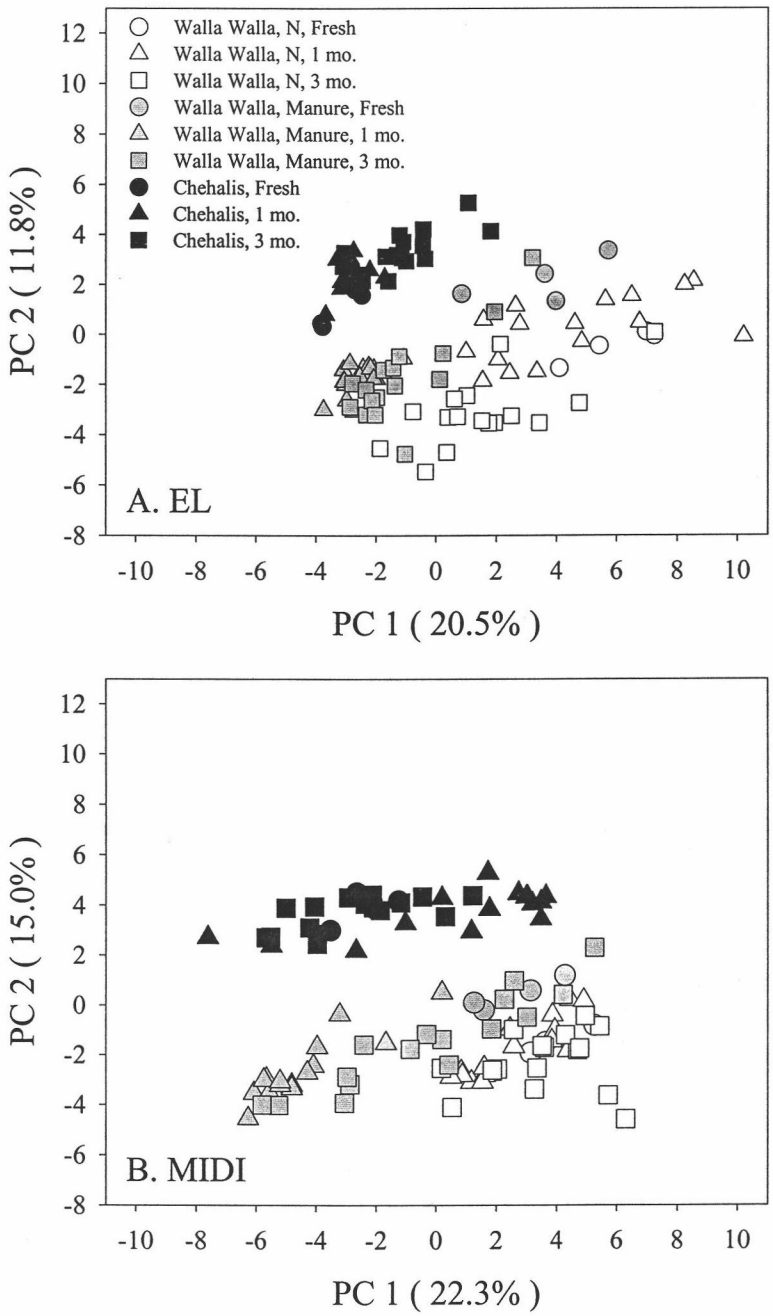

Fig. 2.2. Principal components analyses of community FAME profiles from three agricultural soils, fresh and stored, extracted with the EL method (A) or the MIDI method (B). The variance explained by the each principal component (PC) axis is shown in parentheses.

of soil storage or FAME extraction. Among the FAMEs affected by storage in EL-extracted Chehalis soil (Fig. 2.3A) were 18:1ω7*c*/9*t*/12*t* and 18:1ω9c, which decreased after storage, and *i*11:0 3OH, which increased upon storage (not shown). For some soils, FAME profiles continued to change during the three-month storage period (McKenzie River, Figs. 2.3C and 2.3D; manure-amended Walla Walla soils, data not shown). For other soils, there were few changes in FAME profiles after one month of storage (EL-extracted Chehalis soil, Fig. 2.3A; MIDI-extracted N-amended Walla Walla soils, not shown). For MIDI-extracted Chehalis soil (Fig. 2.3B), the similarity between profiles of fresh soil and soils stored for 3 months is an artifact; after 3 months of storage, MIDI-extracted FAME levels were below detection limits so samples were concentrated prior to GC analysis.

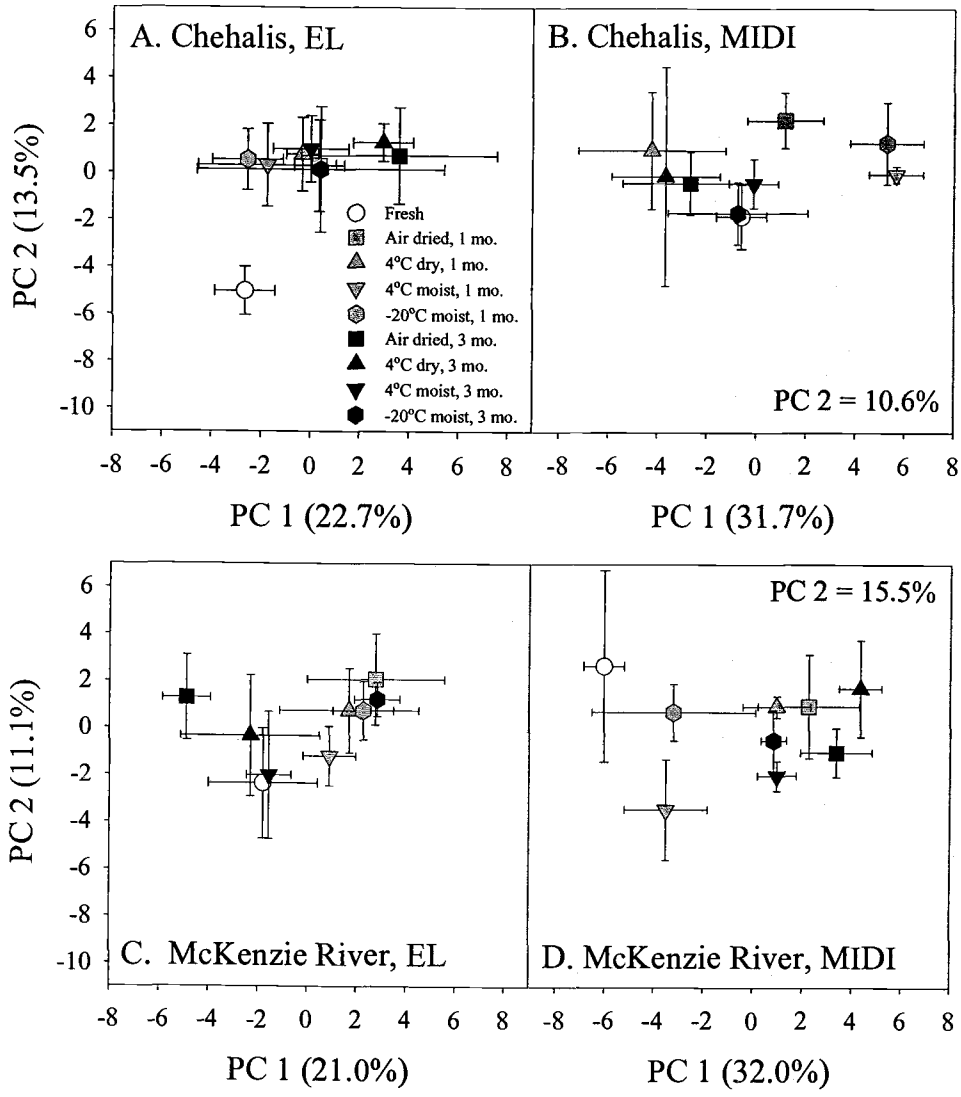

Fig. 2.3. Principal components analyses of FAME profiles from fresh and stored Chehalis soil extracted with EL (A) or the MIDI (B) method and from fresh and stored McKenzie River soil extracted with the EL (C) or MIDI (D) method. Error bars represent the standard deviation from the mean coordinate of FAME profiles per storage protocol ($n = 4$). The variance explained by the first principal component axis (PC) is shown in parentheses.

Changes in FAME profiles for differently-stored soils over time appeared to be related to changes in extractable FAME diversity. For example, the rightward shift in McKenzie River FAME profiles along PC 1 of Fig. 2.3D corresponded to an increase in the number and relative amounts of FAMEs extracted with the MIDI method over time (not shown). FAMEs that increased in relative amounts in McKenzie River soils after three months of storage included 9:0, 13:0, 15:0 18:0, *i*17:0, *a*17:0, and 10Me16:0. Others that were detected only after three months of storage were *a*12:0, 12:0 2OH, 16:0 3OH, 16:1ω9*c*, and *a*17:1ω9*c*. Shifts in FAME structure for the MIDI-extracted Chehalis soils also can be explained by overall changes in extractable-FAME concentration. After one month of storage, the relative amounts of several FAMEs, including 10:0, 15:0, 18:0, *a*13:0, 10:0 3OH, 16:0 N alcohol, and 10Me16:0, declined (not shown), corresponding to the rightward shift observed in Fig. 2.3B. After three months of storage, it was necessary to concentrate the MIDI extracts, thereby increasing FAME diversity and resulting in a shift in FAME profiles towards that of the fresh soils.

Discussion

Qualitative differences in FAMEs extracted by each method should be considered when determining which method to use. We found that the abundances of several important marker FAMEs were dependent on the extraction method. For example, 16:1ω5*c* may be a marker for arbuscular mycorrhizal fungi and specific bacteria such as *Cytophaga* (Olsson et al., 1998; Frostegård et al., 1993). In

agricultural soils, the relative amount of this marker extracted from soils sometimes doubled if the MIDI method was used (Table 2.2). Conversely, the actinomycete marker 10Me18:0 and protozoan-associated 20:4ω6*c* (Vestal and White, 1989; Ringelberg et al., 1997) were more abundant when soils were extracted with the EL method. Also, abundances of several markers for Gram-negative bacteria differed between the two extraction methods. Relative amounts of hydroxylated FAMEs were greater in MIDI extracts, whereas EL extracts contained relatively greater amounts of cyclopropane FAMEs. Although both methods were able to differentiate among the four soil types, inferences regarding community structure clearly may vary according to the method employed. Alternatively, an extraction method may be chosen based on a FAME marker of interest if a particular group of microorganisms is being studied.

There are practical considerations which suggest that the EL-FAME method has advantages compared to the MIDI method. Although approximately equal numbers of samples can be extracted in a given period of time, the EL method lacks the 100°C saponification step and the washing step. The saponification step employed by MIDI can be troublesome as tube contents may boil up and leak out of the test tube. This is of concern, especially if fatty acids are volatilized and escape. Heating a strong alkaline solution also can be hazardous to laboratory personnel. Although the concentration of extracted FAMEs was not quantified, MIDI-extracted samples often had to be concentrated to reach minimal threshold detection limits.

Differences in FAME composition based on extraction method may be due to differences in extraction efficiencies or differences in FAME sources. With the strong saponification and methylation steps, it is conceivable that the MIDI procedure may

yield some FAMEs derived from humic substances. We did find that the NMS distance between MIDI- and EL-extracted soils of a given site increased as the TOC content of the soil increased (Fig. 2.1), suggesting that the effect of extraction method on soil FAME profiles may be related to soil TOC content. However, without further studies we cannot conclude whether or not FAMEs unique to MIDI extracts originated from living microorganisms or soil organic matter. Unfortunately, distinguishing between humic and bacterial fatty acids has proven difficult so far. For example, β-hydroxy and *iso*- and *anteiso*-branched fatty acids have been extracted from purified humin fractions, but because they are also found in bacterial membranes, these fatty acids were considered to originate from bacteria rather than the actual humin (Almendros and Sanz, 1991).

In our study, storing soils for even one month resulted in changes in extractable FAME profiles. It is not clear whether or not these changes were due to changes in microbial populations over time or to autooxidation of microbial lipids. Autooxidation is the process by which unsaturated fatty acids react with O_2, forming labile hydroperoxides that are readily converted to several secondary oxidation products (Gunstone, 1986). In all cases of this study, soils were stored aerobically, and the effects of autooxidation were unknown. Future studies may consider storing soils anaerobically or comparing FAME profiles from stored soils (sterile and unsterile) to distinguish between biological and autooxidation effects on FAME profiles.

There does not appear to be one particular storage method that is best for consistently producing FAME profiles that are highly similar to fresh FAME profiles.

For 1-month stored soils, the percentage of FAMEs significantly affected by storage protocol ranged from 7-26% for EL-extracted soils and 6-45% for MIDI-extracted soils. Corresponding values for soils stored for 3 months ranged from 16-44% and 6-43%. However, there was no one storage protocol that resulted in the fewest changes in FAME numbers and relative amounts among the four soils. For example, after one month of storage, amounts of 19:0 cy ω8*c* was lowest in Chehalis soil stored moist at -20°C compared to the other storage protocols, but in McKenzie River soil, it was lowest in the partially dry soil stored at 4°C. Because of these inconsistencies, it is recommended that community-level analyses be conducted on fresh rather than stored soils.

Another study has examined specifically the influence of storage on microbial community structure, although storage treatments differed from those of this study (Petersen and Klug, 1994). Moist soils were stored at 4.5, 10, or 25°C for three weeks, during which community PLFAs were measured. At the two lower temperatures, changes in PLFA profiles over a 3-week period were inconsistent, with no clear effect of time. However, there was a rapid change in profiles for soils stored at 25°C: PLFAs *i*15:0, *i*17:0, and 18:0 increased but 16:1ω7*c*, 18:1ω7*c*, and 18:2ω6*c* decreased over time. In our study, the method of storage was not as critical as the length of time a soil was stored, and changes in response to storage were due mainly to changes in the number of FAMEs extracted. In contrast to our study, an increase in PLFA numbers was not observed by Petersen and Klug (1994).

In conclusion, both the EL and MIDI methods were able to separate fresh soils of differing texture and TOC content. Certain FAMEs were unique to each method,

but the relative amounts of unique FAMEs generally were minute. Interpretations of community structure may differ depending on the extraction method employed due to differences in the types and relative amounts of fatty acids extracted. Overall, effects of storage method on soil FAME profiles were small compared to effects of extraction method and soil type.

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CHAPTER 3

SHIFTS IN SUBSTRATE UTILIZATION POTENTIAL AND STRUCTURE OF SOIL MICROBIAL COMMUNITIES IN RESPONSE TO CARBON SUBSTRATES

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Abstract

The Biolog assay is a popular tool for estimating the carbon (C) substrate utilization potential of microbial communities within natural environments, but its ecological relevance has been questioned. In this study, impacts of simple and complex C substrates on microbial community Biolog activities were assessed to determine if the resulting utilization potentials reflected the amended substrates. A silt loam soil was amended on an equal C-basis with glucose, cellulose, lignin, hydroxyproline, gelatin, or residues from either triticale (*X Triticosecale* Wittmack) or Austrian winter pea (*Pisum sativum* L.) and incubated for 80 days. Mean utilization of Biolog carbohydrates, amino acids, and polymers were determined at 3, 7, 21, 35, 49, and 80 days following incorporation of each substrate to soil. In addition, microbial biomass C (MB_C) and community fatty acid methyl ester (FAME) profiles were determined at each sampling point to assess community size and structure. Soil amended with lignin maintained similar levels of MB_C, Biolog activities, and FAME profiles relative to a non-amended control soil. Soil amended with glucose had higher MB_C levels relative to non-amended soil and soils amended with cellulose, lignin, and plant residues. Communities from glucose-amended soil utilized Biolog carbohydrates at high rates but exhibited low utilization potentials for amino acids and polymers. Principal components analysis of FAME data revealed greater amounts of fungal markers in soil amended with glucose during the first 21 days of the incubation. In contrast, soils amended with plant residues had relatively high Biolog activities for all three types of substrates and contained a greater diversity of FAMEs compared to

communities from the glucose treatment. Hydroxyproline and gelatin amendments had the greatest impact on the microbial parameters measured. Highest values for MB_C, Biolog activity, FAME richness, and diversity were found in either or both treatments at most sampling dates. Biolog polymer utilization was particularly elevated in soil amended with gelatin, which is similar in structure to the Biolog polymer collagen. Positive impacts of hydroxyproline and gelatin on microbial communities may be due to the N content of these treatments; however, triticale and winter pea residues had very similar impacts on soil communities, despite winter pea legume having 1.5 times more N compared to the triticale residue. Together, the Biolog and FAME data revealed successional patterns in microbial communities over time. For example, an increase in carbohydrate utilization in cellulose-amended soil at day 49 corresponded to an increase in FAME richness and diversity, with elevated amounts of the fungal fatty acids 18:1ω9*c* and 18:3ω6*c* detected in this soil.

Introduction

Biolog microtiter plates have become a popular tool for quickly assessing the metabolic potential of heterotrophic microbial communities of natural environments. Its appeal comes from the large number (95) of carbon (C) substrates contained in wells of each Gram-negative or Gram-positive plate. Oxidation of these substrates by microorganisms can produce utilization patterns that may differ according to microbial community and/or habitat. For example, the Biolog method has been successful for differentiating between microbial communities of aquatic, soil, and

rhizosphere habitats (Garland and Mills, 1991), from rhizospheres of different crops and plant communities (Zak et al., 1994; Garland, 1996; Grayston et al., 1998), and from canola versus wheat rhizoplanes (Siciliano et al., 1998).

In many studies, the substrate utilization patterns generated from Biolog have been used to infer the quality of C substrates available to microorganisms in natural habitats. Insam et al. (1996) measured the Biolog activities of compost microbial communities during an 8-week composting process. Greater utilization of Biolog carbohydrates, such as fructose and galactose, suggested that sugars were the major substrates utilized by microbial communities early in the composting process. Conversely, an increase in Biolog polymer utilization (Tween 40 and Tween 80) at later stages of composting was interpreted as an increase in available polymer substrates in aged composts. Dissimilarities between Biolog patterns of microbial communities among plant rhizospheres were presumably due to variations in plant root exudate compositions or soil organic matter quality (Zak et al., 1994; Garland, 1996; Grayston et al., 1998). However, studies that examined in closer detail the impact of specific substrates on Biolog potentials have produced conflicting results. For example, sucrose additions to soil resulted in higher utilization rates of Biolog sucrose and glucose, which is a product of sucrose hydrolysis (Grayston et al., 1998). Although this suggests that Biolog can detect functional changes in communities, Garland et al. (1997) found no increases in utilization rates of Biolog substrates that corresponded to specific C sources added to a bioreactor.

Applications of the Biolog method to microbial isolate and community studies, as well as concerns regarding its application and interpretation, are described by

Konopka et al. (1998). Ecological issues that must be considered include the physiological state of microorganisms in environmental samples and the metabolic redundancy shared among microbial groups. Because the Biolog system is a culture-based system, there are concerns regarding whether or not ecologically relevant members of the community are represented. Strong growth of community inoculum is often necessary to approach the density (~10^8 CFU ml^{-1}) required for positive utilization response. Faster-growing strains may be at an advantage, making greater contributions to whole-community patterns than expected from the composition of the community (Verschuere et al., 1997). The ecological relevance of the Biolog C substrates also has been questioned. Because Biolog substrates were chosen with the goal of identifying clinical isolates, some have concluded that the substrates do not reflect the diversity of substrates in natural environments (Konopka et al., 1998). However, many of the organic acids, carbohydrates, and amino acids of the Biolog GN plate have been reported as constituents of plant root exudates (Campbell et al., 1997) or are similar in structure to substrates occurring in nature. Garland (1997) reflected that although the Biolog system does not provide a complete view of community function, meaningful information can be gained from studying selective components of the community, just as macrobiologists study selected components of plant or animal communities. Additional experimental studies are needed, however, so that meaningful interpretations can be gained from the assay.

The first objective of this study was to evaluate the ability of the Biolog assay to predict substrate quality and availability in soil by analyzing soil microbial communities after substrate amendment. Soils were amended with substrates that are

associated with plant tissues: glucose was used to model simple plant root exudates; cellulose and lignin are major plant cell wall components; and hydroxyproline and gelatin were selected as models for plant cell wall-associated structural proteins, some of which are abundant in hydroxyproline and, like gelatin, contain repetitive amino acid sequences (Keller, 1993; Showalter, 1993). In addition, we chose the residues of two cover crops, triticale and winter pea, to represent above-ground plant tissues that may be incorporated in soil under agricultural practices. Our hypothesis was that soil amended with glucose should have simple Biolog utilization patterns but high carbohydrate utilization activities. In contrast, soils amended with complex substrates, such as lignin and plant residues, should utilize a greater diversity of Biolog substrates, including polymers.

Our second objective was to determine if changes in Biolog patterns, in response to substrate amendment and time, would correspond to shifts in microbial community FAME structure. The ability to link functional (substrate utilization) with structural (FAME) shifts should be extremely beneficial for elucidating successional changes in microbial communities during decomposition. Although there have been studies in which both Biolog potentials and community structural analyses were performed on the same samples (Ibekwe and Kennedy, 1998; Engelen et al., 1998; Siciliano and Germida, 1998; Siciliano et al., 1998), few have compared directly shifts in microbial community structure with function in response to environmental perturbations or ameliorations (Wenderoth and Reber, 1999).

Materials and Methods

Experimental Design

Soil was collected (0-15 cm) in December, 1997, from the Oregon State University Vegetable Research Station (Corvallis, OR) during the fallow period of a winter fallow-summer vegetable crop rotation treatment. The soil type is a Chehalis silt loam (fine-silty, mixed mesic Cumulic Ultic Haploxeroll) with a soil:water (1:2) pH of 5.9 and a total organic C content of 0.0165 g g^{-1} soil. After transport to the laboratory, the nearly-saturated soil was allowed to dry slowly under sheets of paper at 25 °C until the water content was ~ two-thirds of field capacity (~ 0.205 g g^{-1} soil). Soil was then sieved through a 4-mm mesh screen, mixed, and stored for two weeks at 25 °C, the temperature of the incubation protocol.

After the two-week period, triplicate subsamples of soil were removed for analysis of microbial biomass C (MB_C), substrate utilization potential, and community FAME pattern prior to the start of the experiment. Triplicate moisture contents were determined, and the remaining soil was divided into 400 g (dry weight basis) portions for incorporation of C substrate. There were three replicate soil portions for each substrate and the non-amended control. Carbon substrates were added on an equal-C basis of 2000 μg C g^{-1} soil by adding the appropriate amount of substrate to each soil portion, followed by hand-mixing of the soil until the substrate was evenly incorporated. Each soil portion was then added to 0.946-l glass containers with screw-cap lids. The water content of the soils were adjusted to 0.205 g g^{-1} soil by addition of distilled water.

Carbon substrates included glucose (40% C, EM Science, Cherry Hill, NJ), powdered cellulose (~ 20 μm, 42% C, Aldrich Chemical Co., Inc., Milwaukee, WI), hydrolytic lignin (72.1% C, Aldrich Chemical Co., Inc., Milwaukee, WI), *trans*-4-hydroxy-L-proline (46% C, 11% N; Aldrich Chemical Co., Inc., Milwaukee, WI), and gelatin (~225 bloom from calf skin, 41% C, 16% N; Aldrich Chemical Co., Inc., Milwaukee, WI). The chemical structure of this lignin contains hydroxy and methoxy side groups. Residues of two winter cover crops were also included as substrate amendments. The first was the cereal "Celia" triticale (*X Triticosecale* Wittmack) and the other was Austrian winter pea (*Pisum sativum* L.), a legume. Plant material was collected the previous spring, from winter cover crops growing on research plots at the North Willamette Research and Extension Center (Aurora, OR). Above-ground plant biomass samples were washed, oven-dried, and ground through a 1.0-mm mesh screen using a Tecator 1093 Cyclotec Sample Mill (Höganäs, Sweden). The C content of the residues was ~ 42% C, and N contents were 2.7% for the triticale and 4.1% for the winter pea. Cellulose and lignin contents were 16.6% and 3.1% for triticale, respectively, and corresponding values for the winter pea were 16.2% and 4.3%.

After incorporation of the substrates, incubation chambers were stored at 25°C. Because of the intensive manipulation of soil during substrate amendment, soils were allowed to equilibrate for three days prior to the first sampling. Approximately 45 g of soil were removed carefully from each container and analyzed for MB_C, microbial community substrate utilization potential, and FAME profiles. Soils were sampled again at days 7, 21, 35, 49, and 80 of the incubation period.

Throughout the incubation, lids were removed weekly to aerate the soils and to adjust the moisture content of the soil if needed.

Laboratory Analyses

Microbial biomass C was determined by the chloroform-fumigation incubation method of Jenkinson and Powlson (1976). Ten g of soil were removed from each incubation chamber and fumigated with chloroform for 24 h. After fumigation, soils were incubated for 10 d at 25 °C in acrylic tubes stoppered with rubber septa. The tube headspace was then sampled for CO_2, which was analyzed by gas chromatography (Carle Series 100 AGC, Loveland, CO). A k_C of 0.41 (Voroney and Paul, 1984) was used to calculate MB_C without the subtraction of a control. Non-fumigated controls were not subtracted because when respiration in the fumigated sample was high, so was respiration in the non-fumigated sample. Subtraction of the non-fumigated from the fumigated sample resulted in net differences that did not vary among treatments, implying that there were no effects of substrate amendment on microbial biomass C.

Community substrate utilization activity was measured using Gram-negative (GN) Biolog MicroPlates™ (Biolog Inc., Hayward, CA). Ten g of soil were suspended in 95 mL of sterile 0.145 *M* NaCl. Soil samples were serially diluted to 10^{-3}, and 125 µL of the 10^{-3} suspension were inoculated into each well of a GN-Biolog plate. Plates were incubated at 25 °C, and well absorbance values were read at 595 nm on an automated plate reader (Bio-Tek Instruments, Winooski, VT) every 24 hours for 96 hours total. The 72-h absorbance values were used for calculating the average

well-color development for three types of Biolog substrates (carbohydrates, amino acids, and polymers). Well absorbance values were adjusted by subtracting the absorbance of the control well, which did not contain a substrate.

Community FAME profiles were determined using the ester-linked FAME method (Ritchie et al. 2000; Schutter and Dick, 2000). Three grams of soil were mixed with 15 mL of 0.2 *M* methanolic KOH in a 35-ml glass centrifuge tube. Soil solutions were incubated at 37°C for 1 h, during which tubes were vortexed periodically. Next, 3 mL of 1.0 *M* acetic acid were added to neutralize the pH of the tube contents. Extracted FAMEs were then partitioned into an organic phase by adding 10 mL of hexane. Tubes were centrifuged at 480 × g for 10 min to separate soil organic matter from the hexane layer, after which the hexane layer was transferred to a clean glass test tube. Test tubes were placed in a warm water bath (45°C), and the hexane was evaporated under a stream of N_2. Soil fatty acids were dissolved in 1:1 hexane:methyl-*tert* butyl ether and transferred to a GC vial for analysis using a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA) equipped with a 25 m × 0.2 mm fused silica capillary column (5% diphenyl-95% dimethylpolysiloxane) and a flame ionization detector. The temperature program ramped from 170°C to 270°C at 5°C min^{-1}, with 2 min at 270°C between samples to clean the column. Fatty acids were identified and their relative peak areas were determined using the MIS Aerobe method of the MIDI system (Microbial ID Inc., Newark, DE). In addition to the soil samples, FAME extraction and analysis were performed in triplicate on the each substrate, including the triticale and winter pea residues, using 1-g samples.

Statistical Analyses

Effects of substrate amendment on MB_C, Biolog substrate activity, FAME richness and FAME diversity were determined at each sampling point by PROC ANOVA (SAS Institute Inc., 1996). Richness refers to the number of FAMEs detected in a given soil sample, and diversity was calculated as the Shannon index, where $H' = -\sum (P_i \times \log P_i)$, and P_i is the proportional amount of each FAME. When significant ($\alpha = 0.05$) treatment differences were found, protected LSD values were calculated to separate the means. Shifts in community FAME profiles over time were analyzed by principal components analysis using the PC-ORD program (McCune and Mefford, 1997). Correlations between FAME relative concentrations and PC scores were also calculated by PC-ORD.

Results

Microbial Biomass C

Prior to the start of the experiment, MB_C levels of the soil measured 88.4 μg C g^{-1} soil. For all treatments, MB_C levels were higher at day 3 relative to later sampling dates as biomass levels declined over the course of the incubation to near-background levels (Table 3.1). During the first 21 days, biomass levels were higher in soils amended with plant residues, glucose, hydroxyproline, and gelatin relative to control soil and soils amended with lignin and cellulose. At day 35, MB_C levels of glucose-amended soil declined to 150.5 μg g^{-1} soil. There was one exception to the declining biomass trend: in soil amended with cellulose, there was a small increase in MB_C

levels at day 35, rising from 168 to 198 μg g^{-1} soil. Throughout the incubation study, MB_C was lowest in non-amended soil and soil amended with lignin.

Table 3.1. Microbial biomass C in soils following incorporation of C substrates. Means ($n = 3$) followed by the same letter are not significantly different at $p < 0.05$ for each sampling date.

Treatment	Day 3	Day 7	Day 21	Day 35	Day 49	Day 80
	----------μg C g^{-1} soil----------					
Control	112d	114f	106e	90.5e	83.3e	67.5c
Glucose	721a	515c	350b	150d	139c	85.6bc
Cellulose	229c	200e	168d	198b	173ab	126a
Lignin	116d	109f	98.3e	95.5e	94.5d	70.8c
Hydroxyproline	730a	735a	544a	215a	176a	88.1bc
Gelatin	693a	604b	305b	203ab	171ab	117ab
Triticale	456b	353d	245c	180c	162b	116ab
Winter Pea	468b	340d	252c	194b	166ab	122a

Community Substrate Utilization Potential

Microbial substrate utilization potentials for three types of Biolog substrates (carbohydrates, amino acids, and polymers) were determined using the average well-color development after 72 hours of incubation. The number of well absorbance values averaged were 30 for carbohydrates (Biolog wells A7-C12), 20 for amino acids (wells F5-G12), and 5 for polymers (wells A2-A6). Prior to incorporation of substrates, mean absorbance values for each type of Biolog substrate utilized by

microbial communities were 1.40, 1.19, and 1.05 for carbohydrates, amino acids, and polymers, respectively. In contrast to the MB_C results, the average absorbance values of Biolog guilds for most soils following substrate incorporation remained relatively stable for the first 49 days of the study, after which activity declined (Tables 3.2-3.4).

Throughout most of the incubation period, utilization of Biolog carbohydrates was greatest in the gelatin, hydroxyproline, and plant residue treatments (Table 3.2). Carbohydrate utilization also was significantly higher in glucose-amended soil relative to the control soil and soils amended with cellulose and lignin for most sampling dates. At day 49, however, there was an increase in carbohydrate utilization for soil amended with cellulose, with the mean absorbance value nearly doubling since the previous sampling date. Carbohydrate utilization remained low in the lignin and control treatments, but at day 80 very few significant differences between treatments were found.

Utilization of Biolog amino acids was greatest in soils amended with gelatin, hydroxyproline, and triticale residue, and was significantly lower in the control soil and soils amended with glucose, cellulose, and lignin at days 21-49 (Table 3.3). At day 21, amino acid utilization was highest in soil amended with gelatin, with a mean absorbance value of 2.03. Activity declined dramatically in gelatin-amended soil near the end of the incubation period; at day 80, mean absorbance values were significantly lower in soils amended with gelatin and glucose compared to all other treatments, including the control.

Compared to other treatments, polymer utilization was significantly greatest in soil amended with gelatin (Table 3.4), except for the first two sampling dates when

polymer activity was also high in triticale-amended soils. At day 80, however, polymer utilization in gelatin-amended soil was very low (mean absorbance value of 0.32) relative to all other treatments except glucose. For days 7 through 35, utilization of polymers was relatively low in the control soil and soils amended with glucose, cellulose, and lignin compared to soils amended with plant residues. There were several sampling dates (day 7, 21, and 80) when polymer utilization in glucose-amended soil fell to levels significantly below those of control soil.

Table 3.2. Biolog carbohydrate potential of microbial communities following incorporation of C substrates to soil. Absorbance values are the average of 30 Biolog wells after 72 hours of incubation. Means ($n = 3$) followed by the same letter are not significantly different at $p < 0.05$ for each sampling date.

Treatment	Day 3	Day 7	Day 21	Day 35	Day 49	Day 80
	----------Absorbance (595 nm)----------					
Control	1.45cd	1.36c	0.98d	0.95d	0.74d	0.65ab
Glucose	1.92ab	1.73ab	1.57c	1.77bc	1.42bc	0.58b
Cellulose	1.64bcd	1.38c	1.12d	0.75e	1.39c	0.75ab
Lignin	1.31d	1.43bc	1.18d	1.06d	0.73d	0.71ab
Hydroxyproline	1.85ab	2.00a	2.04ab	1.86b	1.56abc	0.83ab
Gelatin	1.99a	1.94a	2.12a	2.07a	1.83a	0.64ab
Triticale	1.93ab	1.90a	1.76bc	1.67c	1.71abc	1.19a
Winter Pea	1.70abc	1.80a	1.72c	1.72bc	1.74ab	0.95ab

Table 3.3. Biolog amino acid potential of microbial communities following incorporation of C substrates to soil. Absorbance values are the average of 20 Biolog wells after 72 hours of incubation. Means ($n = 3$) followed by the same letter are not significantly different at $p < 0.05$ for each sampling date.

Treatment	Day 3	Day 7	Day 21	Day 35	Day 49	Day 80
	----------Absorbance (595 nm)----------					
Control	1.29c	1.16cd	1.09d	1.02de	0.90de	0.81b
Glucose	1.38bc	0.99d	0.73e	1.06d	0.70e	0.42c
Cellulose	1.29c	1.09d	0.98d	0.85e	0.87de	1.01ab
Lignin	1.34bc	1.31bc	1.09d	0.88e	0.98cd	0.82b
Hydroxyproline	1.71a	1.59a	1.69b	1.67ab	1.66a	0.75b
Gelatin	1.64a	1.59a	2.03a	1.78a	1.70a	0.22c
Triticale	1.57ab	1.55a	1.54bc	1.44c	1.32b	1.11a
Winter Pea	1.22c	1.46ab	1.48c	1.49bc	1.19bc	0.99ab

Table 3.4. Biolog polymer potential of microbial communities following incorporation of C substrates to soil. Absorbance values are the average of 5 Biolog wells after 72 hours of incubation. Means ($n = 3$) followed by the same letter are not significantly different at $p < 0.05$ for each sampling date.

Treatment	Day 3	Day 7	Day 21	Day 35	Day 49	Day 80
	----------Absorbance (595 nm)----------					
Control	0.93d	0.97c	0.95c	0.84c	0.74cde	0.68ab
Glucose	1.26cd	0.59d	0.45d	0.70c	0.60e	0.30c
Cellulose	1.27cd	0.81d	0.70cd	0.73c	0.87bcde	0.67b
Lignin	0.92d	0.91cd	0.65d	0.64c	0.67de	0.75ab
Hydroxyproline	1.51bc	1.54b	1.69b	1.27b	0.95bcd	0.63b
Gelatin	2.03a	2.00a	2.17a	2.08a	1.73a	0.32c
Triticale	1.70ab	1.99a	1.53b	1.27b	1.01bc	0.94a
Winter Pea	1.17cd	1.80ab	1.71b	1.26b	1.12b	0.73ab

Community FAME Profiles

No fatty acids were detected in FAME extracts of pure glucose, cellulose, hydroxyproline, and cellulose substrates. However, FAMEs were detected in the lignin substrate as well as the triticale and winter pea residues. These FAMEs were 16:0, 18:0, 20:0, and 18:2ω6*c*; 18:1ω9*c* also was extracted from lignin. Except for day 49, when lignin communities appear to contain elevated amounts of 18:0 and 20:0 (Fig. 3.1C, Table 3.5), microbial community FAME profiles from soil amended with lignin or plant residue do not appear to be strongly influenced by substrate-associated FAMEs.

Changes in community structure in response to substrate amendment and time, as determined by principal components analysis, are presented in Figure 3.1. Throughout the study, FAME profiles were similar between microbial communities of control and lignin-amended soils, and between communities of the triticale and winter pea treatments (Fig. 3.1A-D). At day 7, separations between communities from soils amended with glucose, hydroxyproline, gelatin, and plant residues were well defined whereas communities from the cellulose treated soil were similar to those from the lignin and control soils. As the incubation period progressed, however, communities from cellulose-amended soil began to differentiate from the control and lignin communities so that maximum separation was achieved by day 49 (Fig. 3.1B-C). At day 49, communities from glucose- and residue-amended soils were relatively similar to each other, and by day 80 were similar to lignin and control soil communities (Fig. 3.1C-D).

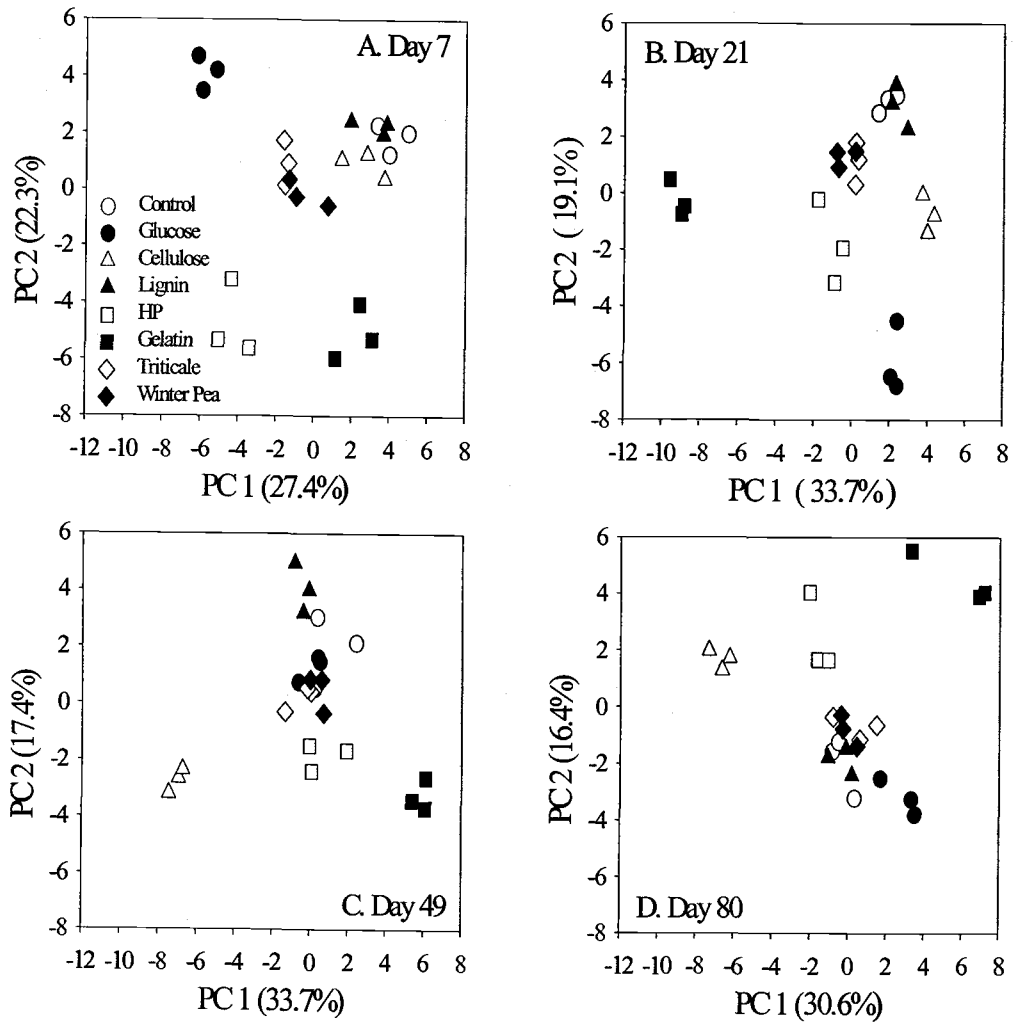

Fig. 3.1. Principal components analysis of microbial community FAME profiles in response to C substrate amendment after 7 (A), 21 (B), 49 (C), and 80 (D) days of incubation. The variance explained by each PC is given in parentheses.

Table 3.5. Community FAMEs[†] associated with Principal Components 1 and 2 of PC plots. Plots for the selected sampling dates are shown in Figure 3.1. Correlations between FAME relative concentrations and PC scores are indicated in parentheses.

Incubation Day	PC 1	PC2
Day 7		
	*i*15:0 (0.95)	16:0 (0.77)
	*i*17:0 (0.93)	18:0 (0.72)
	10Me16:0 (0.91)	18:1ω9*c* (0.66)
	16:1 2OH (0.89)	
	20:4ω6*c* (0.87)	
		17:0 cy (-0.94)
	18:2ω6*c* (-0.85)	18:1ω7*c*/9*t*/12*t*[‡] (-0.93)
	18:1ω9*c* (-0.66)	*a*15:0 (-0.87)
	16:0 N alcohol (-0.62)	*a*17:0 (-0.84)
Day 21		
	16:0 (0.89)	10Me17:0 (0.84)
	10Me16:0 (0.76)	10Me18:0 (0.79)
	18:0 (0.69)	*i*15:0 (0.79)
	18:2ω6*c* (0.66)	20:0 (0.78)
		*i*17:0 (0.73)
	*a*15:0 (-0.93)	16:1 2OH (0.70)
	*i*17:1ω9*c* (-0.91)	
	15:1ω6*c* (-0.90)	
	*i*16:0 (-0.87)	
	15:0 (-0.86)	
	*i*16:1 (-0.84)	
	17:0 cy (-0.83)	15:0 3OH (-0.65)
	*i*13:0 (-0.82)	16:0 N alcohol (-0.65)
	17:0 (-0.80)	18:1ω9*c* (-0.65)
Day 49		
	*a*17:0 (0.90)	20:0 (0.90)
	16:1ω7*c*/*i*15:0 2OH[‡] (0.88)	18:0 (0.81)
	*a*15:0 (0.86)	
	17:0 cy (0.85)	
	*i*16:0 (0.82)	
	16:1ω5*c* (-0.82)	
	18:1ω9*c* (-0.82)	17:0 (-0.62)
	16:0 (-0.76)	18:3ω6*c* (-0.52)

Table 3.5 (continued). Community FAMEs[†] associated with Principal Components 1 and 2 of PC plots. Plots for the selected sampling dates are shown in Figure 3.1. Correlations between FAME relative concentrations and PC scores are indicated in parentheses.

Incubation Day	PC 1	PC2
Day 80		
	17:0 (0.93)	18:1ω7*c*/9*t*/12*t*[‡] (0.76)
	*a*15:0 (0.92)	*i*15:1 (0.66)
	*a*17:0 (0.89)	*i*16:1 (0.66)
	*i*15:0 (0.86)	
	10Me16:0 (0.83)	
	17:1ω8*c* (0.82)	
		20:0 (-0.80)
	18:1ω9*c* (-0.86)	18:0 (-0.79)
	18:2ω6*c* (-0.81)	14:0 (-0.69)
	16:0 (-0.77)	14:1ω5*c*/*t*[‡] (-0.65)

[†]FAMEs are described using standard nomenclature. Numbering of carbons begins at the aliphatic (ω) end of the fatty acid molecule. The number of double bonds within the molecule is given after the colon. *Cis* and *trans* conformations are designated with suffixes "*c*" and "*t*", respectively. Other notations are "Me" for methly, "OH" for hydroxy, "cy" for cyclopropane, and the prefixes "*i*" and "*a*" for *iso*- and *anteiso*-branched FAME's, respectively.

[‡]Due to identical retention times, these FAMEs cannot be differentiated by the MIDI software system.

Community FAMEs with the highest correlations with scores along PC 1 and 2 are presented in Table 3.5. At all time points except day 21, PC 1 consisted of a gradient that ranged from bacterial fatty acids (branched and monounsaturated), which were positively correlated with PC 1 scores, to more saturated and fungal fatty acids (18:2ω6*c* and/or 18:1ω9*c*), which were associated with negative PC 1 scores. For day 21, 18:2ω6*c* was positively correlated with PC 1 whereas the bacterial markers were negatively correlated.

Differences in microbial community FAME richness and diversity in response to substrate amendment are shown in Table 3.6. With the exception of the gelatin treatment, the initial effect of substrate incorporation and soil incubation was to reduce FAME richness from a pre-experiment mean of 55. By day 7, richness in most soils peaked and then began to steadily decline as the incubation continued. For gelatin-amended soil, FAME richness continued to increase until day 21, with 35 fatty acids detected. Later, FAME richness began to increase again in all treatments after 35 (for control and cellulose-amended soils) or 49 days of incubation. Maximum FAME richness was attained at day 80 for the control soil (~ 30 FAMEs) and soils amended with cellulose and lignin (~31 and 30 FAMEs, respectively). For the first part of the incubation period (days 3 - 35), FAME richness was generally highest in soil amended with gelatin and hydroxyproline and lowest in control soil and soils amended with cellulose and lignin. After day 35, richness was highest in the gelatin and cellulose treatments.

For the hydroxyproline and gelatin treatments, FAME diversity was relatively stable throughout the incubation period, ranging from 2.84 to 3.14 (Table 3.6).

Table 3.6. Richness and diversity† of FAMEs extracted from soils following incorporation of C substrates. Within richness or diversity, means ($n = 3$) followed by the same letter are not significantly different at $p < 0.05$ for each sampling date.

Treatment	Day 3	Day 7	Day 21	Day 35	Day 49	Day 80
	----------Richness----------					
Control	25.7d	28.7d	27.3b	22.3b	25.3c	29.7bc
Glucose	30.7ab	34.0abc	30.7b	27.0a	26.7bc	29.7bc
Cellulose	26.7cd	29.3d	27.7b	27.3a	28.7ab	31.3ab
Lignin	26.3cd	29.7cd	26.7b	23.7b	22.3d	30.0bc
Hydroxyproline	28.3bcd	36.3a	35.0a	28.7a	26.7bc	29.0bc
Gelatin	33.0a	34.7ab	35.0a	30.0a	30.0a	34.0a
Triticale	30.0abc	32.7abcd	30.0b	27.7a	25.7c	28.0c
Winter Pea	28.3bcd	31.7bcd	30.3b	30.0a	26.0bc	28.7bc
	----------Diversity----------					
Control	2.83ab	2.90b	2.86bc	2.75de	2.89b	2.99bc
Glucose	2.82ab	2.61c	2.58e	2.73ef	2.86b	3.04b
Cellulose	2.88a	2.92b	2.71d	2.66f	2.72d	2.83e
Lignin	2.84ab	2.90b	2.83c	2.78de	2.75cd	2.98bc
Hydroxyproline	2.86ab	2.90b	2.89b	2.87c	2.84b	2.88de
Gelatin	3.01a	3.05a	3.09a	3.06a	3.04a	3.14a
Triticale	2.56bc	2.83b	2.88bc	2.82cd	2.83bc	2.92cd
Winter Pea	2.43c	2.87b	2.89b	2.96b	2.87b	2.94cd

†Diversity = Shannon's index (H') = $- \sum (P_i \times \log P_i)$, where P_i is the proportional amount of each FAME.

For comparison, mean FAME diversity prior to the experiment was 3.81. For all other treatments, FAME diversity fluctuated over the course of the experiment. For soils amended with plant residues, FAME diversity was lowest at day 3 (2.43 and 2.56 for winter pea and triticale, respectively) and increased soon afterwards. The lowest diversity values for soils amended with glucose (2.58), cellulose (2.66), and lignin (2.75) were observed at day 21, 35, and 49, respectively. Diversity of community FAMEs was significantly greater in soil amended with gelatin compared to all other treatments for incubation days 7 through 80.

Discussion

Effect of Glucose Amendment

Microbial communities from glucose-amended soil were characterized as having high MB_C and FAME richness, at least through day 21 of the incubation study. These communities utilized Biolog carbohydrates quickly and at high rates, but were poor utilizers of Biolog amino acids and polymers, suggesting that this soil was limited in organic N and its community was adapted to simple, rather than complex, C resources. In addition, glucose-amended soil contained relatively low diversity of FAMEs but was enriched in several fungal FAME markers, including 18:1ω9*c* and 18:2ω6*c*. Likewise, Griffiths et al. (1999) measured elevated concentrations of 18:2ω6*c* in soils after incorporation of a synthetic root exudate which included glucose, fructose, and sucrose as constituents. Microbial communities were fungal-dominated especially at high exudate loading rates (> 235 $\mu g\ g^{-1}\ d^{-1}$). In our study, it

is possible that the abundance of glucose, at least initially, prevented competition between bacteria and fungi and encouraged fungal growth. Also, the glucose may have induced an osmotic stress on the bacterial populations, thereby providing an advantage for fungi (Griffiths et al., 1999).

By day 49 of our study, community FAME profiles from glucose-amended soil were more similar to those of control soil and soils amended with plant residues. Similarly, Degens and Harris (1997) reported strong initial differences between communities of glucose-amended and non-amended soils when compared by their abilities to catabolize a variety of organic compounds. After 31 days of incubation, however, the catabolic response of glucose-amended soil converged towards that of the non-amended soil.

Effect of Cellulose and Lignin

Compared to other complex substrates such as gelatin and plant residues, MB_C, Biolog activities, and FAME diversity were very low in soil amended with lignin. In addition, communities from the lignin-amended soil were very similar to those from control soil in terms of MB_C, Biolog substrate utilization, and community FAME profiles. Poor responses to Biolog carbohydrates and amino acids suggests that non-amended and lignin-amended soils were deficient in easily assimilated C and N substrates (Sharma et al., 1998). At days 7 and 21, microbial communities from control and lignin-amended soils were enriched in several fatty acids associated with Gram-positive bacteria (*i*15:0 and *i*17:0), Gram-negative bacteria (16:1 2OH), and actinomycetes (10Me16:0 and 10Me18:0). Lack of differences between communities

of the control and lignin treatments may be due to several factors. Enrichment of lignin-degrading microbes in response to amendment may not have occurred under the conditions of this study. Secondly, the low solubility of lignin may have rendered it inaccessible to microbial communities; thus it was not able to induce functional and/or structural shifts in microbial community that may have occurred if it was utilized. Another possible explanation is that the indigenous microbial community already was adapted to an environment whose predominate source of C is lignin, so that addition of lignin had no effect on the community. However, if this was the case, microbial biomass should have increased in response to lignin input.

Initially, communities from cellulose-amended soil had similar Biolog activities and FAME profiles as communities from control soil and soil amended with lignin; MB_C in soil amended with cellulose was greater, however, compared to the control and lignin treatments. Together, Biolog, and FAME data revealed successional patterns in soil amended with cellulose. At day 35, there was a measurable increase in MB_C and FAME richness, followed by an increase in carbohydrate utilization at day 49. In addition, community FAME profiles in cellulose-amended soil began to shift at day 21, diverging from the profiles of control and lignin-amended soils. This shift was due to an elevation in relative amounts of fungal FAME markers, specifically 18:1ω9*c* and 18:3ω6*c*, in soil amended with cellulose. Our results suggest that communities from cellulose-amended soil were initially bacterial-dominated and were very similar to non-amended soil. As the incubation progressed, fungal components of the community were stimulated and presumably began to degrade the cellulose into simpler units, as suggested by the

increase in Biolog carbohydrate utilization at day 49. Hu and van Bruggen (1997) reported similar successional events in soil amended with ^{14}C-labeled cellulose. The initial stages of cellulose decomposition proceeded at a relatively slow rate and was primarily due to the activity of bacterial populations. The second phase of cellulose decomposition was characterized by a rapid release of $^{14}CO_2$ as fungal populations responded to the cellulose. It was thought that depletion of readily-available C and N by bacteria resulted in the activation of cellulolytic enzymes in fungi.

Effect of Hydroxyproline and Gelatin

Soil amended with hydroxyproline or gelatin tended to have, initially, the highest values for MB_C, Biolog activity, and FAME richness and diversity. This was especially true for MB_C in hydroxyproline-amended soil and for polymer utilization and FAME diversity in the gelatin-amended soil. Interestingly, utilization of Biolog polymers was greatest in soil amended with gelatin through day 49 of the incubation; one of the polymers of the Biolog GN plate is collagen, which when hydrolyzed yields gelatin (Taylor et al., 1998).

As the end of the incubation approached, however, there was a rapid decline in amino acid and polymer utilization potentials for soil amended with gelatin. It is highly possible that by day 80, gelatin, in its original polymeric form, was no longer available for degradation. Mineralization of organic-N during earlier stages may have resulted in the immobilization of inorganic N at later sampling dates, which may explain the reduced utilization of Biolog amino acids at day 80.

Principal components analysis of the FAME data suggests that community composition differed between soils amended with hydroxyproline versus gelatin. Throughout the incubation study, communities from gelatin-amended soil were consistently associated with several bacterial markers, including Gram-positive associated *i*15:0, *a*15:0, *i*17:0, *a*17:0 (day 7) and *i*13:0, *a*15:0, *i*16:0, *a*17:0 (day 21 and 49). Other FAMEs associated with these communities included 16:1 2OH, 10Me16:0, and 20:4ω6*c* at day 7 and 17:0 cy at days 21 and 49. In contrast, communities from the hydroxyproline-amended soil contained elevated amounts of the fungal markers 18:1ω9*c* and 18:2ω6*c* at day 7 of the incubation.

Effect of Triticale and Winter Pea Residues

There were very few differences between the two residue treatments in terms of MB_C and Biolog activities. In addition, communities of triticale- and winter pea-amended soils had similar FAME richness, diversity, and composition. For the first three sampling dates, MB_C was significantly higher in soil amended with either residue relative to control soil and soils amended with cellulose and lignin. Utilization of Biolog carbohydrates, amino acids, and polymers also were elevated in residue-amended soils, compared to the other treatments except hydroxyproline and gelatin.

At most sampling dates, FAME richness and diversity were similar between control soil and soils amended with plant residues. Community FAME profiles from the residue treatments initially were intermediate between those of lignin and control soils and soils amended with glucose, hydroxyproline, and gelatin. Over time, the community profiles of residue-amended soils converged towards those of the non-

amended soil and soils amended with glucose and lignin. In contrast to the glucose treatment, we did not find evidence that fungal populations were enhanced strongly in soil amended with triticale or winter pea residues. This is consistent with the findings of Lin and Brookes (1999), who found no changes in bacteria:fungi ratios in soil amended with ryegrass. In addition, Lundquist et al. (1999) reported greater counts of active bacteria and bacterial-feeding nematodes following incorporation of rye to soil, with little effects of rye on fungi and fungal-feeding nematodes. In their study, it appeared that addition of the rye residue stimulated a largely bacterial response whereas fungal biomass increased as the litter aged.

Similar to our study, Sharma et al. (1998) found that addition of plant residue, specifically maize litter, increased microbial biomass relative to non-amended soil. During a one-year incubation of the amended soil, they also found that utilization of Biolog carbohydrates decreased but amino acid utilization increased. These trends in Biolog patterns presumably were due to the abundance of litter carbohydrates at early stages of the incubation, followed by an abundance of microbial proteins during turnover events after 8-16 weeks of incubation. In our study, carbohydrate utilization remained relatively stable in residue-amended soils, and we observed no increase in amino acid activity over time. However, this discrepancy may be due to our shorter incubation period relative to theirs.

Our study provides an interesting contrast between communities of glucose-amended soil and communities of soils amended with plant residues. Although initial MB_C levels were high in both treatments relative to control soil, MB_C was significantly greater in the glucose-amended soil relative to the residue treatments.

However, soils amended with plant residues had greater utilization potential for Biolog amino acids and polymers, even though carbohydrate utilization was similar between the glucose and residue treatments. Enhanced substrate utilization potentials of microbial communities may be due to the presence of diverse C sources, including hemicellulose, cellulose, and proteins, found in the plant residues. Diversity of FAMEs also was significantly higher in the residue treatments relative to glucose for most sampling dates. In conclusion, although incorporation of glucose to soil resulted in greater MB_C of microorganisms, it did not lead to diversification of the microbial community. Instead, community FAME diversity and Biolog activity were enhanced and maintained throughout most of the incubation period when soils were amended with plant residues.

Perspectives

Very few studies have correlated substrate utilization activity directly with community structure. Wenderoth and Reber (1999) applied sewage sludge to agricultural field soils and measured diversity, using DNA restriction analysis, and catabolic versatility of bacteria isolated from amended soils. At the highest loading rate, heavy metal contamination resulted in reduced diversity of soil isolates, which were characterized with poor ability to utilize aromatic acids. A plot of DNA diversity versus the average number of substrates utilized per isolate produced a saturation curve with a correlation coefficient of 0.86. For our study, the best correlation between FAME and Biolog was achieved when FAME richness was plotted against Biolog diversity (H'), using values for all sampling points except day

80 ($r = 0.59$, $n = 41$; Fig. 3.2). The correlation was lower ($r = 0.50$, $n = 49$; plot not shown) when values for day 80 were included because, at this sampling date, FAME richness appeared to increase while Biolog activity was rapidly declining in most treatments. However, both correlations were significant at $p < 0.001$, and the positive trend does indicate that substrate utilization potentials are greater in communities with greater FAME richness.

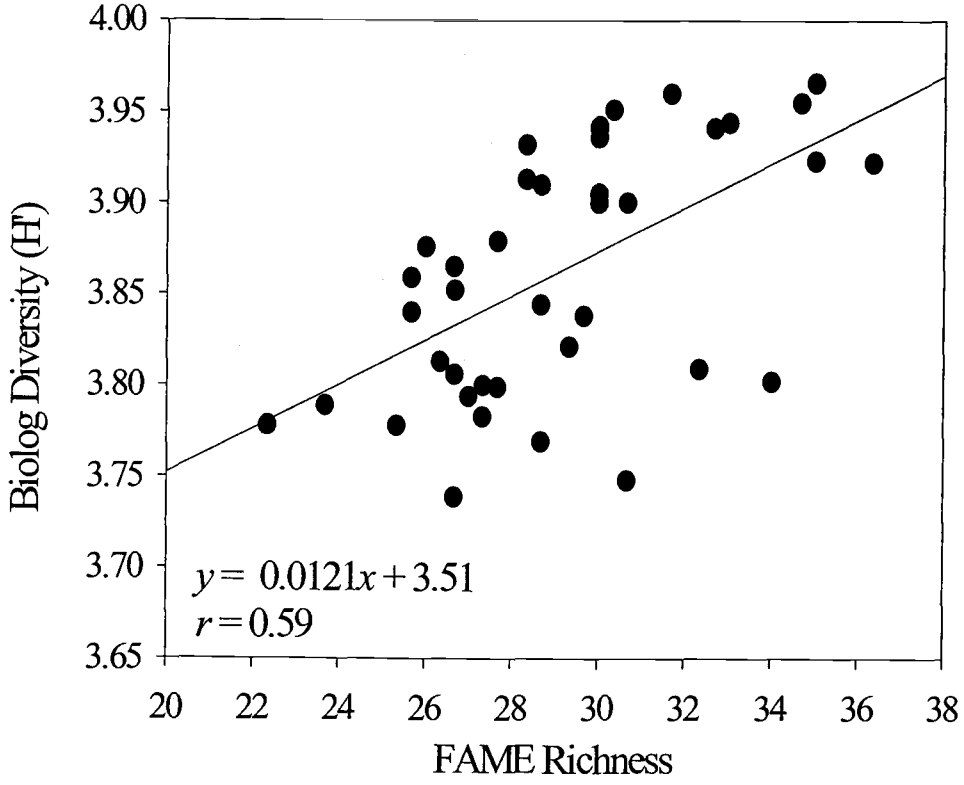

Fig. 3.2. Correlation between community FAME richness and Biolog diversity for all soils analyzed through day 49 of the incubation period. Richness refers to the number of FAMEs detected, and diversity is calculated as the Shannon index (H').

In addition, substrate utilization responses of microbial communities were often related to the quality of C incorporated to soil. For example, soil amended with glucose had high utilization potential for Biolog carbohydrates but not for amino acids and polymers. Soil amended with gelatin, similar in structure to collagen, demonstrated the greatest polymer utilization activities. Thirdly, soils amended with plant residues utilized all three types of Biolog substrates at higher rates than soils amended with simpler substrates (glucose and cellulose). Biolog potentials were also very high in soils amended with hydroxyproline and gelatin. No doubt, the N content of these substrates helped to enhance the overall microbial biomass and activities in this soil. However, other mechanisms may be involved as there were very few differences in microbial parameters between triticale and winter pea-amended soils, despite that the N content of the winter pea residue was 1.5 times that of the triticale. Additional work is needed to determine the impact of N inputs on overall substrate utilization potential of microbial communities.

Conflicting results regarding the success of the Biolog assay in community studies may be a function of the C status of the soil. Smalla et al. (1998) performed genetic analyses on community inocula and compared the DNA patterns to those extracted from Biolog wells. They found that in rhizosphere soil, where no additional substrate was added, Biolog utilization profiles were due to fast-growing members of the community and did not reflect the catabolic potential of the community *in situ* (Smalla et al., 1998). However, if soil was amended with glucose, the resulting Biolog profiles were generated by the dominant populations of the inoculum. Similarly, contamination of soil with oil at a rate of 1.2% (12,000 $\mu g\ g^{-1}$) resulted in

an increase in abundance of hydrocarbon-utilizing bacteria while simultaneously altering the Biolog substrate utilization profile of the soil community (Wünsche et al., 1995). Thus, the Biolog system can be a useful tool as we have found, especially if community members are enriched *in situ* with C sources similar in guild to those of Biolog. Conclusions regarding Biolog potentials and community function must be made cautiously, however, if the quality of nutritional resources available to community microbes are unknown. In addition, to truly link community structure to catabolic function within the environment will require the application of isotope tracers, as demonstrated by Hanson et al. (1999), who coupled the degradation of ^{13}C-labeled toluene to a toluene-metabolizing bacterium whose ^{13}C-enriched lipids were detectable within the whole-soil community PLFA profile.

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CHAPTER 4

SEASONAL, SOIL TYPE, AND ALTERNATIVE MANAGEMENT INFLUENCES ON MICROBIAL COMMUNITIES OF VEGETABLE CROPPING SYSTEMS

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Abstract

Microbial community responses to alternative management may be indicative of soil quality change. In this study, soils were collected from research plots for two years and from commercial grower fields for one year. Treatments at the sites included one to nine years of winter cover cropping. Soils were assayed for microbial fatty acid methyl esters (FAMEs) and Biolog substrate utilization potentials to assess management and seasonal influences on soil communities. The strongest influence was season. Soils in early spring (prior to termination of the cover crop) utilized fewer carboxylic acids and generally were enriched in eukaryotic FAMEs, whereas greater amounts of bacterial FAMEs were detected in soils at canopy closure and harvest of the summer vegetable crop. Within a season, community FAME and Biolog patterns were dependent on field properties. FAME profiles from grower fields in early spring and harvest were correlated significantly with soil texture, CEC, and carbon content. Changes in community structure and Biolog potential occurred in some soils in response to winter cover crops, although effects were not observed until cover crop incorporation. Greater amounts of fungal and protozoan FAME markers were detected in some cover-cropped soils compared to winter fallow soils. Cover crop residues increased FAME diversity at one research station and Biolog diversity at two research stations and the grower fields. Although seasonal and field-dependent factors are major determinants of microbial community structure, shifts in microbial community structure can occur as soil physical and chemical properties change in response to alternative practices, as demonstrated by this study.

Introduction

Soil microorganisms are widely recognized as integrative components of soil quality because of their crucial involvement in many ecosystem processes (*e.g.*, organic matter and nutrient cycling, N_2 fixation, and aggregate formation and stabilization). Examples of potential biological indicators include microbial biomass (Doran, 1987), respiration (Anderson and Domsch, 1978), N mineralization potential (Kennedy and Papendick, 1995), enzyme activities (Dick et al., 1988), and abundance of fungi, nematodes, and earthworms (Lee, 1985; Wall and Moore, 1999). The potential of microbial diversity as an indicator of soil quality has not been realized, even though diversity may be critical for sustaining soil processes as environmental conditions change or for recovery of soil productivity following environmental perturbations (Wardle and Giller, 1996; van der Heijden et al., 1998). Because of the vast diversity of microbes in the soil (Torsvik et al., 1990), and because every species cannot be isolated and studied in pure culture, molecular methods are required to study microorganisms at the community-scale. Such methodologies include analyses of microbial DNA (Borneman et al., 1996) and membrane lipids and fatty acids (Zelles, 1999).

Soil-extracted phospholipids and fatty acids have successfully characterized microbial community structure in agricultural systems. Community fatty acid profiles were shown to differ between rhizosphere and nonrhizosphere soil (Ibekwe and Kennedy, 1999), among rhizospheres of different crop species and cultivars (Ibekwe and Kennedy, 1998; Siciliano et al., 1998), and according to soil type (Bossio et al.,

1998; Ritchie et al., 2000), crop rotation, and cultivation practices (Zelles et al., 1995). Changes in microbial community structure also can occur in response to carbon (C) amendments (Bossio and Scow, 1998) and organic and low-input management practices (Bossio et al., 1998). Similarly, discrimination among microbial communities of different rhizospheres was achieved using the Biolog assay (Zak et al., 1994; Grayston et al., 1998; Siciliano et al., 1998), which provides a measure of the C substrate utilization potential of microbial communities. Community Biolog patterns and substrate use diversity were shown to be sensitive to C inputs (Bossio and Scow, 1998), crop rotation, and tillage practices (Lupwayi et al., 1998).

The Oregon Long-Term Soil Quality Project was initiated to identify soil properties that respond rapidly to alternative management practices in commercial vegetable cropping systems of western Oregon. Winter cover cropping treatments were established at two experimental research stations, one in 1989 and the other in 1993. In addition, winter cover cropping and reduced tillage practices were implemented at several commercial grower fields in 1996. Studies conducted during the 1997 and 1998 growing seasons identified several physical and biological indicators of soil quality change, soil properties that responded to the alternative practices. Reduced soil bulk density and faster water infiltration rates were found in some cover-cropped soils (Buller, 1999). Winter cover-cropped soils also contained higher levels of microbial biomass C and soil β-glucosidase and arylsulfatase activities compared to winter fallow soils (Ndiaye et al., 2000). Improved soil physical properties and changes in microbial biomass and activities suggested

opportune conditions for microbial community changes in response to winter cover cropping.

The objective of this study was to identify the major factors influencing microbial communities of vegetable cropping systems of western Oregon. We hypothesized that microbial communities would differ according to soil type, and that shifts in community profiles would occur in response to seasonal changes. We also hypothesized that microbial communities would be affected by alternative management practices known to enhance microbial biomass and activity. In particular, the added C inputs to soil via cover crops should enhance community substrate utilization potentials. To test these hypotheses, community structure was assessed by extracting fatty acids directly from soil and converting them to fatty acid methyl esters (FAMEs) for analysis. The Biolog assay was used to assess the potential of soil communities to utilize a diverse range of C substrates.

Materials and Methods

Study Sites

The Oregon Long-term Soil Quality Project consisted of two experimental research stations and six fields from commercial vegetable producers, hereafter referred to as grower fields. All sites are located in the Willamette Valley of western Oregon. The climate is Mediterranean, with moist, cool winters and warm, dry summers. The average annual rainfall is 1040 mm. Soils in the Soil Quality Project

are primarily mollisols with loam variant textures. Taxonomy and selected properties of soils from the research stations and grower fields are presented in Table 4.1.

The first experimental station is the Vegetable Research Station (VRS) in Corvallis, Oregon. The design at this station is a randomized complete block with 4 replicate blocks; plots within each block are 30 × 60 m. The two treatments (established in 1993) were a summer vegetable-winter fallow and summer vegetable-winter cover crop rotations. The winter cover crop treatment was established after harvest of the summer vegetable crop and terminated with herbicide in early spring before preparation of plots for the next vegetable crop. In 1997 the winter cover crop was an 80:20 weight mixture of annual ryegrass and red clover with small amounts of oats and buckwheat to attract beneficial insects. In 1998 the winter cover crop was a mixture of winter gray oats (*Avena sativa* L.) and common vetch (*Vicia sativa* L.); winter wheat (*Triticum aestivum* L.) was the cover crop in 1999 (Table 4.2).

The second experimental station is the North Willamette Research and Extension Center (NWREC) in Aurora, Oregon. The design is a randomized split-plot block with four blocks. Treatments were established in 1989. Main plots (18 × 9 m) consist of six cover crop treatments, with three N rates as the split plots (18 × 3 m). Nitrogen fertilizer rates of zero, 56 (medium) or 224 (recommended) kg N ha^{-1} were applied for sweet corn, and rates of zero, 140 (medium), or 280 (recommended) kg N ha^{-1} were applied for broccoli. Cover crops were planted following harvest of the summer vegetable and terminated in early spring with herbicide. Three crop rotation treatments were chosen for this study: summer vegetable-'Celia' triticale (*Triticosecale X* Wittmack) winter cover crop, summer vegetable-triticale/ Austrian

Table 4.1. Taxonomy and selected initial properties of soils from experimental research stations and grower fields.

Site	Soil Classification			Clay	Silt	Sand	TOC†	pH‡	CEC
	Series	Family	Subgroup	g kg^{-1}					cmol kg^{-1}
Research Stations									
NWREC	Willamette	Fine-loamy, mixed, mesic	Pachic Ultic Argixeroll	180	540	280	17.1	5.4	23.1
VRS	Chehalis	Fine-silty, mixed, mesic	Cumulic Ultic Haploxeroll	260	520	220	16.5	5.9	32.7
Farms									
Albany	Chehalis	Fine-silty, mixed, mesic	Cumulic Ultic Haploxeroll	250	560	190	19.2	5.6	35.6
	Newberg	Coarse-loamy, mixed, mesic	Fluventic Haploxeroll	240	530	230	19.3	5.7	34.6
Corvallis	Chehalis	Fine-silty, mixed, mesic	Cumulic Ultic Haploxeroll	270	590	140	14.4	6.1	33.9
Molalla	Aloha	Fine-silty, mixed, mesic	Aquic Xerochrept	200	700	100	12.9	6.7	22.0
Salem	Cloquato	Coarse-silty, mixed, mesic	Cumulic Ultic Haploxeroll	160	380	460	20.2	6.2	32.9
	Newberg	Coarse-loamy, mixed, mesic	Fluventic Haploxeroll	190	500	310	32.6	6.0	38.2
Silverton	Amity	Fine-silty, mixed, mesic	Argiaquic Xeric Argialboll	260	670	70	17.7	5.9	27.2
Stayton	Saturn	Fine-loamy over fragmental, mixed, mesic	Fluventic Haplumbrept	320	470	210	35.1	6.4	30.1

†TOC = Total organic carbon, determined by dry combustion.

‡pH determined by 1:1 soil:water mixture.

Table 4.2. Summer vegetable crop and winter cover crop rotations at the experimental research stations and grower fields.

	1997	1998		1999	
Site	Vegetable	Cover	Vegetable	Cover	Vegetable
Research Stations					
NWREC	Broccoli[†]	Triticale[¶] Triticale + Austrian winter pea[#]	Sweet corn	Triticale Triticale + Austrian winter pea	Green bean
VRS	Sweet corn[‡]	Oat[††] + common vetch[‡‡]	Green bean	Winter wheat[†††]	Green bean
Farms					
Albany	Green bean[§]	Triticale + clover[§§]	Sweet corn		
Corvallis	Green bean	Annual ryegrass[¶¶] + common vetch	Sweet corn		
Molalla	Sweet corn	Barley[##] + common vetch	Cauliflower		
Salem	Sweet corn	Barley + common vetch	Sweet corn		
Silverton	Cauliflower[†]	Barley + oat	Sweet corn		
Stayton	Sweet corn	Oat + common vetch	Green bean		

[†]*Brassica oleracea* L.,[‡]*Zea mays* L., [§]*Phaseolus vulgaris* L., [¶]*Triticosecale X* Wittmack, [#]*Pisum sativum* L.,[††]*Avena sativa* L.,

[‡‡]*Vicia sativa* L.,, [§§]*Trifolium incarnatum* L., [¶¶]*Secale cereale* L., [##]*Hordeum vulgare* L., [†††]*Triticum aestivum* L.

winter pea (*Pisum sativum* L.) winter cover crop mix, and summer vegetable-winter fallow rotations (Table 4.2). Soils were sampled from plots receiving the recommended rate of N for the winter fallow and winter triticale cover crop treatments; plots receiving the medium N rate were sampled for the triticale/ winter pea cover crop mix treatment. Because of frequent flooding, one replication of the summer vegetable-triticale cover crop was removed from the experiment.

Commercial grower fields are identified by the nearest town or city. Fields have been cultivated since the early 1900s except for the Salem field, which was forested until 1996. Two treatments were implemented on each field in the fall of 1997. For all fields except Albany, the treatments were a winter fallow and a winter cover crop, where each treatment was assigned to one-half of the field. Five sampling sites were established on each treatment; sampling sites 1 through 5 were paired across fallow and cover crop sides so that soil type and texture were the same between the two treatments. The location of each soil sampling site was established by the Global Positioning System in 1995 so that researchers could relocate sampling sites throughout the study. At each field, cover crops were planted in early fall, following vegetable crop harvest, and incorporated the following spring prior to planting of the summer vegetable crop. Specific crops grown in the rotation are shown in Table 4.2.

For the Albany field, a winter cover crop was planted across the entire field. Prior to 1998, treatments at Albany consisted of minimum tillage (spring-tooth harrow) versus no-tillage, in which a no-till drill was used to plant the summer crop directly into cover crop stubble. In 1998, a strip tillage planting system was used on the entire field to plant the summer vegetable crop. In the minimum tillage half of the

field, fertilizer was banded in contrast to being broadcasted and incorporated on the conventional tillage side.

The tillage regimes varied between research stations and grower fields. However, the main difference between the treatments was that the winter fallow had intensive fall tillage (moldboard plowing) compared to the cover crop treatment which had a reduced tillage regiment in the fall (disk or strip tillage).

Soil Sampling

Soil samples were taken three times during each growing season (1998 for grower fields and 1998 and 1999 for research stations). The first sampling was in late March, shortly before termination and incorporation of the winter cover crop. Soils were sampled again at canopy closure of the summer vegetable crop, which was approximately 6 weeks after planting. Canopy closure for broccoli and cauliflower was at the eight-to-nine leaf stage of growth; for corn it was the seven-to-eight leaf stage. Canopy closure for green beans was at the two-trifoliate leaf stage. The third sampling occurred within one week before harvest of the summer vegetable crop.

Composite soil samples were obtained by taking 28-36 cores (2.5 cm dia., 0-7.5 cm depth) at each research plot and field sampling site. At the experimental stations, samples were taken from the inner two-thirds of the winter fallow and cover crop plots. For the grower fields, soil samples were taken within a 2-m radius of the designated sample points. When crops were present, equal numbers of soil cores were taken from rhizosphere and nonrhizosphere by sampling perpendicular to planting

rows. After sampling, soils were sieved through a 2-mm mesh screen and stored at 4°C until community analyses were performed.

Soil Analyses

In 1996, chemical and physical soil properties, including total organic C (TOC), cation exchange capacity, soil pH, and particle size analysis, were determined according to standardized methods of the Soil Survey Laboratory (USDA/NRCS, 1996) (Table 4.1). Thereafter, TOC was assessed at each sampling by dry combustion using a Dohrman DC-80 carbon analyzer (Santa Clara, CA).

In 1997, preliminary studies were conducted at the two research stations and two grower fields to determine if bacterial and fungal biomass were affected by winter cover cropping. Active and total bacterial and fungal biomass were estimated from direct counts by Soil Foodweb, Inc. (Corvallis, OR). Total bacteria, including dormant, senescent, and active bacteria, were counted using the fluorescein isothiocyanate method of Babiuk and Paul (1970). Active bacteria were counted after staining with fluorescein diacetate. Biomass estimates were obtained from direct counts following the procedure of van Veen and Paul (1979). Total and active fungal biomass was estimated from lengths and widths of fungal hyphae (Ingham and Klein 1984). In 1998, bacterial and fungal biomass measurements were conducted on VRS samples.

Microbial Community Analyses

Fatty acids were extracted from soil samples collected from research stations and grower fields using the ester-linked FAME method (Ritchie et al. 2000; Schutter and Dick, 2000). Three grams of soil were mixed with 15 mL of 0.2 *M* methanolic KOH in glass centrifuge tubes. Soil solutions were incubated at 37°C for 1 h with periodic mixing. Next, 3 mL of 1.0 *M* acetic acid were added to neutralize the pH of the tube contents. FAMEs were partitioned into an organic phase by adding 10 mL of hexane, followed by centrifugation at 480 × g for 10 min to separate soil organic matter from the hexane layer. The organic phase was transferred to clean glass tubes, and hexane was evaporated under a stream of N_2. FAMEs were dissolved in 1:1 hexane: methyl-*tert*-butyl ether and transferred to a GC vial for analysis using a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA) equipped with a 25 m × 0.2 mm fused silica capillary column (5% diphenyl-95% dimethylpolysiloxane) and a flame ionization detector. The temperature program ramped from 170°C to 270°C at 5°C min^{-1}, with 2 min at 270°C between samples to clean the column. FAMEs were identified and their relative peak areas determined by the MIS Aerobe method of the MIDI system (Microbial ID, Inc., Newark, DE). In addition to soil samples, FAME extractions were performed in triplicate on dried and ground residues from the triticale and winter pea cover crops of NWREC.

FAMEs are described by standard nomenclature. Numbering of carbons begins at the aliphatic (ω) end of the molecule. The number of double bonds within the FAME is given after the colon. *Cis* and *trans* conformations are designated with suffixes "*c*" and "*t*", respectively. Other notations are "Me" for methyl, "OH" for

hydroxy, "cy" for cyclopropane, and the prefixes "*i*" and "*a*" for *iso*- and *anteiso*-branched FAMEs, respectively.

Substrate utilization by microbial communities of research stations and grower fields was measured using Gram-negative Biolog Microplates™ (Biolog, Inc., Hayward, CA). Biolog plates were inoculated with 125 µL of soil suspension which was serially diluted to 10^{-3} in sterile saline. Plates were incubated at 25 °C, and oxidation reactions were monitored every 24 h for 96 h total by measuring well absorbance values with an automated plate reader (595 nm; Bio-Tek Instruments, Winooski, VT). Biolog data from the 72 h reading were chosen for community analyses. Well absorbance values were adjusted by subtraction of a blank control; data were normalized by dividing the absorbance value of each well by the plate's maximum absorbance value.

Statistical Analyses

Community FAME and Biolog diversity were calculated at each sampling period for the two research stations and the grower fields. Diversity was calculated as the Shannon index, where $H' = -\sum (P_i \times \log P_i)$ and P_i is the proportional abundance of a given FAME or the proportional absorbance value of each Biolog well. Analysis of variance (ANOVA) was conducted to determine significant effects of winter cover cropping on community diversity at the VRS and grower fields using SAS statistical software (SAS Institute., 1996); PROC GLM was conducted in the case of NWREC. The overall design of the grower field study was a randomized complete block with two treatments: alternative versus conventional management practices. Each grower

field represented one block of the experimental design. When cover crop effects were assessed on grower fields by ANOVA, the Albany field was removed from the data set so that the design consisted of 5 fields (blocks) and two treatments: winter fallow and winter cover crop.

Community-level effects of season, soil type, and alternative management practices at the grower fields and two research stations were assessed by principal components analyses with the PC-ORD program (McCune and Mefford 1997). Changes in microbial community structure during the course of the growing season were assessed by analyzing FAME data from all three sampling periods of 1998 and again in 1999 for the two research stations. Likewise, Biolog data from the three sampling periods of each growing season were used to determine seasonal effects on community substrate utilization potential. Soil type influences on microbial communities were assessed by principal components analyses of FAME and Biolog data from grower fields at each sampling period of 1998. In addition, correlations between soil properties and principal component scores and between FAME amounts and soil properties were calculated by the PC-ORD program to assess relationships between community structure and field properties. Thirdly, effects of winter cover cropping at the two research stations, as well as individual grower fields, were determined by principal components analyses of the FAME and Biolog data from each sampling period of each growing season. In addition, discriminant analysis was performed on the five grower fields containing the winter cover crop and winter fallow treatments for each sampling season. The purpose of this analysis was to identify relationships between FAMEs that could be used to develop a rule for

discriminating between winter cover-cropped and winter-fallow soils. A step-wise selection procedure was performed first to identify the best FAME discriminators at each sampling season. Canonical discriminant analysis was performed to find a single linear discriminant function which provided the maximum separation between soils of each treatment. The SAS statistical software package was used for the discriminant procedure (SAS Institute, 1996).

Results

Microbial Biomass and Nematode Numbers

Biomass results from the 1997 preliminary study are shown in Table 4.3. Within sites, there were few differences between fallow and cover-cropped soils at each sampling date, with the possible exception of VRS. At canopy closure 1997, total bacterial biomass at NWREC was lower in cover-cropped soil compared to fallow soil, whereas at VRS, active fungal biomass was significantly greater in the cover-cropped soil compared to the fallow treatment. At harvest 1997, treatment differences were observed only at VRS, where active and total bacterial biomass were greater in cover-cropped versus fallow soil. For the two grower fields, biomass levels generally did not increase in response to winter cover cropping. Exceptions were observed at the Salem field, where active bacterial biomass increased in response to cover crops in early spring 1997. In addition, higher nematode counts were observed in the cover-cropped side of the Salem field compared to the fallow side at canopy closure 1997. However, the trend was reversed for active bacterial and fungal

biomass for this field at canopy closure, with lower biomass levels in the cover-cropped soil compared to the fallow soil. Overall, these results indicated that there were few obvious or consistent trends in microbial biomass using the direct count methodology.

Because direct counts of bacteria, fungi, and nematodes revealed few positive responses to winter cover cropping, the method was not applied as extensively in 1998. For the 1998 growing season, biomass estimates by direct counts were performed only on VRS samples. In 1998, there were no significant differences in active and total bacterial biomass, active and total fungal biomass, and nematode numbers between fallow and cover-cropped soil (Table 4.4). As in 1997, biomass levels and nematode numbers changed significantly over time, with greater values of active bacteria, active and total fungi, and nematodes in the spring than at canopy closure or harvest. Total bacterial biomass was greatest at canopy closure compared to early spring or harvest for both 1997 and 1998. In addition, 1998 VRS biomass estimates were not correlated with amounts of fungal or bacterial FAMEs extracted from soil, where 18:2ω6*c* was used as the fungal FAME marker and bacterial FAMEs were the sum of the relative amounts of *i*15:0, *a*15:0, 15:0, *i*16:0, 16:1ω9*c*, *i*17:0, *a*17:0, 17:0 cy, 17:0, 18:1ω7*c*/9*t*/12*t*, and 19:0 cy. Correlations (*r*) between bacterial biomass and bacterial FAMEs were 0.22 and 0.41 for total and active biomass, respectively. Correlations between fungal biomass and 18:2ω6*c* were 0.17 and 0.33 for total and active biomass, respectively.

Table 4.3. Biomass of bacteria and fungi, plus nematode counts, from selected sites in 1997.

Site	Treatment	Active Bacterial Biomass	Total Bacterial Biomass	Active Fungal Biomass	Total Fungal Biomass	Nematodes
		----------μg g^{-1} soil----------				# g^{-1} soil
			Spring 1997			
NWREC	Fallow	36.4a†	84.5a	12.2a	36.1a	3.94a
	Triticale	29.4a	79.0a	16.4a	43.1a	12.2a
	Triticale + winter pea	55.1a	176.8a	15.2a	28.6a	2.93a
VRS	Fallow	49.6a	87.7b	2.96a	12.5b	3.76b
	Cover	59.6a	111.4a	9.36a	15.7a	4.88a
Salem	Fallow	28.1b	55.1a	7.64a	32.3a	2.79a
	Cover	42.7a	56.0a	10.3a	39.5a	3.70a
Silverton	Fallow	51.8a	140.0a	10.2a	25.0a	3.81a
	Cover	36.5a	115.2a	10.2a	39.0a	13.4a
			Canopy Closure 1997			
NWREC	Fallow	7.53a	58.6a	4.31a	7.28a	0.96a
	Triticale	7.40a	46.9b	3.61a	10.3a	1.12a
	Triticale + winter pea	6.83a	36.3c	4.46a	11.3a	0.84a
VRS	Fallow	6.58a	286.3a	2.70b	13.3a	1.74a
	Cover	6.00a	161.3a	3.79a	13.0a	4.77a

Table 4.3 (continued). Biomass of bacteria and fungi, plus nematode counts, from selected sites in 1997.

Site	Treatment	Active Bacterial Biomass	Total Bacterial Biomass	Active Fungal Biomass	Total Fungal Biomass	Nematodes
		----------μg g^{-1} soil----------				# g^{-1} soil
			Canopy Closure 1997			
Salem	Fallow	20.6a	535.6a	9.20a	33.7a	0.35b
	Cover	2.88b	591.0a	2.00b	30.5a	1.34a
Silverton	Fallow	9.73a	256.4a	12.7a	29.2a	3.23a
	Cover	10.2a	617.7a	22.4a	24.8a	3.89a
			Harvest 1997			
NWREC	Fallow	11.2a	50.1a	4.55a	35.7a	0.53a
	Triticale	10.3a	67.9a	13.5a	45.2a	0.27a
	Triticale + winter pea	13.3a	48.8a	7.73a	41.3a	0.56a
VRS	Fallow	9.10b	39.8b	8.93a	9.30a	0.85a
	Cover	13.9a	51.7a	12.5a	16.3a	1.51a
Salem	Fallow	15.4a	44.6a	11.8a	116.3a	2.81a
	Cover	15.3a	38.7a	15.9a	125.8a	3.27a
Silverton	Fallow	5.37a	56.8a	13.6a	20.3a	0.38a
	Cover	5.62a	76.3a	11.3a	28.3a	1.62a

†Within columns of each site and sampling date, means followed by the same letter are not statistically significant at $p < 0.05$.

Table 4.4. Biomass of bacteria and fungi, plus nematode counts, from VRS in 1998.

Treatment	Active Bacterial Biomass	Total Bacterial Biomass	Active Fungal Biomass	Total Fungal Biomass	Nematodes
	----------μg g^{-1} soil----------				# g^{-1} soil
			Spring		
Fallow	20.6a†	172b	21.7a	70.1a	9.15a
Cover	21.5a	195a	21.0a	68.3a	15.2a
			Canopy Closure		
Fallow	12.7a	825a	7.16a	27.6a	3.58a
Cover	16.3a	782a	11.4a	36.7a	6.58a
			Harvest		
Fallow	3.53a	145a	3.93a	19.3a	1.95a
Cover	7.10a	336a	6.78a	41.2a	3.05a
LSD, Date	5.11	287	9.59	23.2	5.92

†Within columns of each sampling date, means followed by the same letter are not statistically significant at $p < 0.05$.

Seasonal Effects on Microbial Community Profiles

Seasonal effects on microbial community FAME profiles were observed in 1998 at the grower fields and in 1998 and 1999 at the research stations; results from principal components analyses of FAME data for 1998 are shown as examples in Figure 4.1. At the VRS site (Fig. 4.1A), community profiles changed from ones

elevated in 18:3ω6*c* and 20:1ω9*c* during the spring sampling period to communities enriched in *i*15:0, *i*17:0, *a*17:0, 10Me16:0, and 16:0 at the canopy closure and harvest dates. NWREC community profiles (Fig. 4.1B) at canopy closure were relatively low in 10Me18:0, 18:3ω6*c*, and 20:4ω6*c* but contained elevated amounts of *i*15:0, *a*15:0, 19:0 cy ω8*c*, and saturated FAMEs 16:0, 18:0, and 20:0 compared to communities sampled in early spring and harvest. For the grower fields (Fig. 4.1C), differences in community structure between early spring and later sampling dates were due to reduced amounts of 17:1ω8*c*, *a*17:1ω9*c*, and 16:0 N alcohol but greater amounts of *i*15:0, *a*15:0, 17:0 cy, 10Me16:0, 10Me18:0, and 16:0 at canopy closure and harvest. Community profiles in the NWREC plots also were affected by sampling season in 1999, but seasonal impacts were less evident at VRS during the second year of the study (PCA not shown). There was poor recovery of FAMEs from VRS samples at canopy closure 1999, requiring FAME extracts be concentrated prior to GC analysis. As a result of concentrating these samples, FAME profiles from canopy closure 1999 were artificially elevated in FAME diversity, and differences between spring and canopy closure profiles in 1999 were fewer than in 1998.

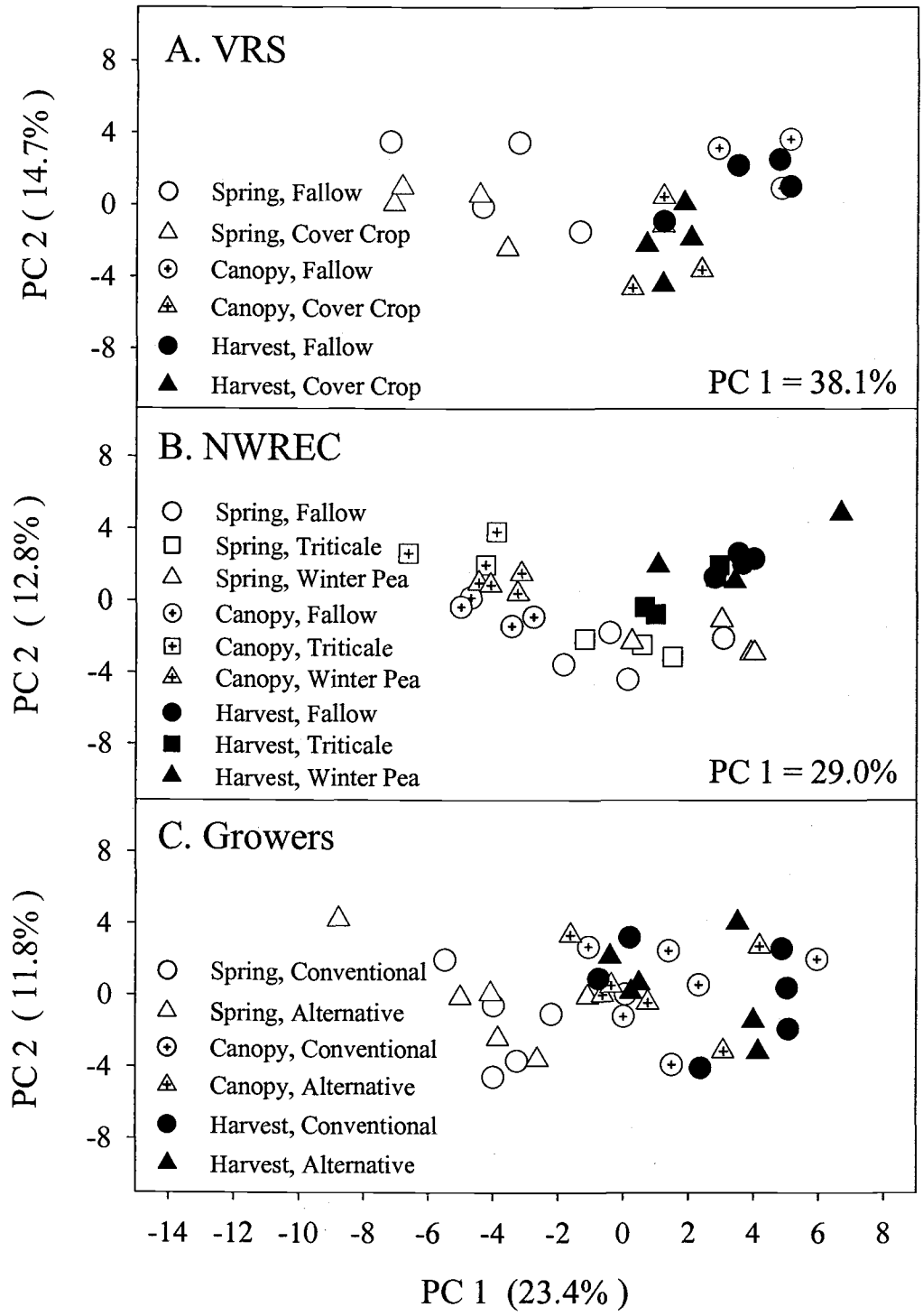

Fig. 4.1. PCA of microbial community FAME profiles from VRS (A), NWREC (B), and grower fields (C). Profiles are from spring, canopy closure, and harvest of 1998. Percent variance explained by each PC is shown in parentheses.

Biolog assays also revealed seasonal impacts on microbial communities from both research stations and the grower fields. Differences in substrate utilization potentials between spring, canopy closure, and harvest samplings in 1998 are shown in Figure 4.2. At the VRS site (Fig. 4.2A), communities at canopy closure and harvest had greater utilization potentials for several carboxylic acids (citric, malonic, succinic, and α-keto glutaric acid). Similarly, NWREC communities utilized succinic and α-keto glutaric acid more at canopy closure and D-gluconic, D,L-lactic, propionic, and α-keto glutaric at harvest than in spring (Fig. 4.2B). Substrate utilization potentials also varied seasonally for communities of grower fields (Fig. 4.2C); differences between canopy closure and harvest were due to greater citric acid, L-histidine, and D,L-carnitine utilization at canopy closure than at harvest. In 1999, seasonal differences in substrate utilization potentials were observed again at NWREC and VRS (not shown).

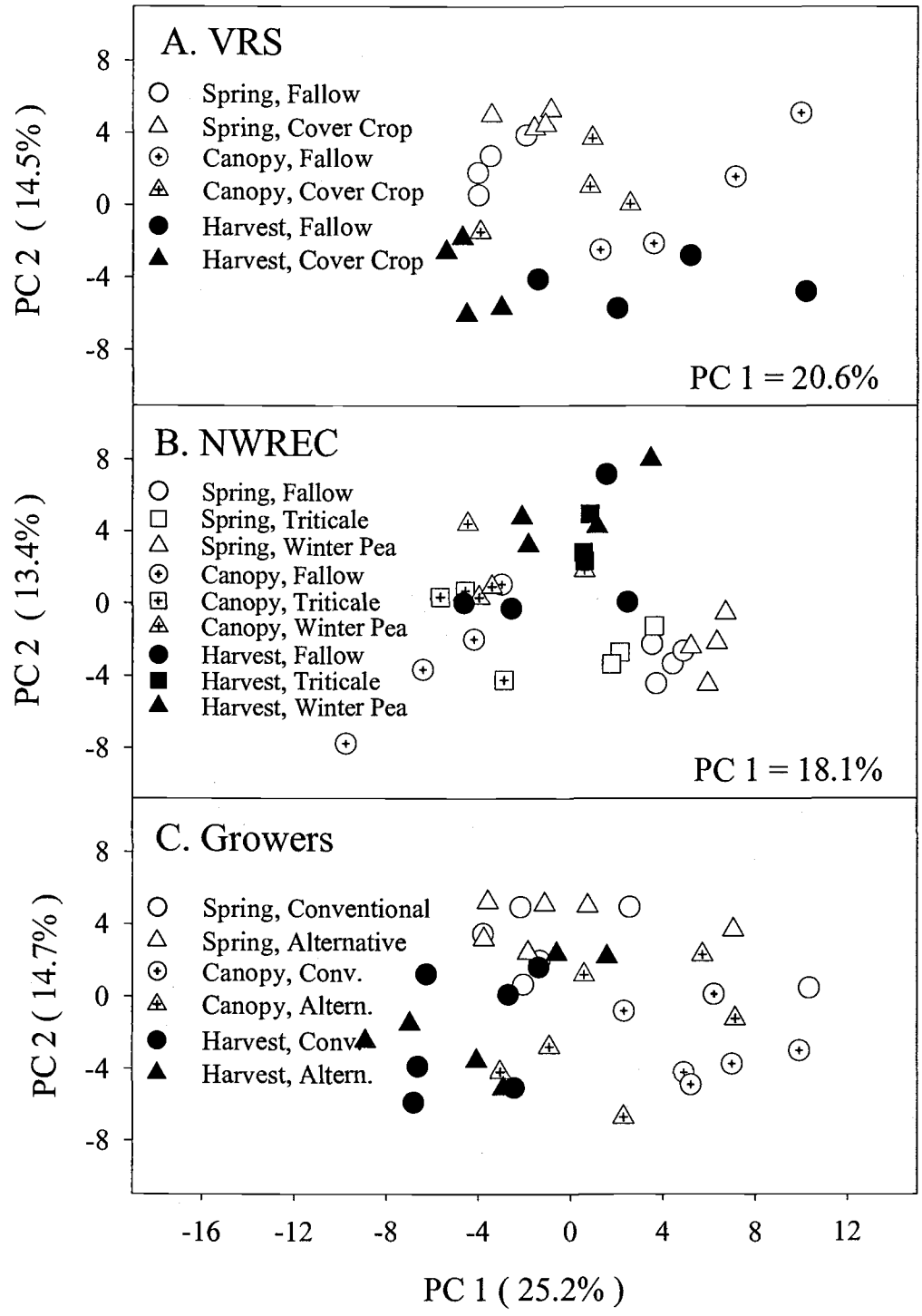

Fig. 4.2. PCA of microbial community Biolog patterns from VRS (A), NWREC (B), and grower fields (C). Patterns are from spring, canopy closure, and harvest of 1998. Percent variance explained by each PC is shown in parentheses.

Effects of Field and Soil Properties.

Community FAME profiles of grower fields generally clustered according to field site rather than alternative management practice when analyzed one season at a time (Fig. 4.3). In early spring, alternative farming practices had little influence on FAME profiles from grower fields, nor were communities ordinated according to crops of the previous summer (Fig. 4.3A). For example, sweet corn was grown on the Molalla, Salem, and Stayton soils in 1997, but microbial communities from these fields were not clustered together in Figure 4.3A. Instead, communities were clustered according to soil properties regardless of management practice. Communities from the Stayton, Silverton, and Molalla fields were separated along principal component (PC) 1; soil from Molalla contained greater amounts of 12:0, 16:0, 18:1ω9*c*, and 18:2ω6*c* compared to Silverton and Stayton soils, which were enriched in 16:1 2OH, 10Me17:0, and 19:0 cy. Also, communities from the Corvallis and Salem fields were relatively abundant in FAMEs 14:0, *a*15:0, *a*17:0, 17:1ω8*c*, and 17:0 cy. Several soil properties were correlated significantly with PC scores for the spring analysis ($p < 0.05$, $n = 12$). Clay and TOC content were negatively correlated with PC 1 ($r = -0.64$ and -0.66, respectively), and CEC was positively correlated with PC 2 ($r = 0.59$). In addition, soil texture, CEC, and TOC were correlated directly with the amounts of 26 of 52 FAMEs detected in the grower fields in early spring (Table 4.5).

At canopy closure, FAME profiles still were site-dependent, although separations between communities of alternative versus conventional practices were observed in some fields, including Albany and Corvallis (Fig. 4.3B). Three of four

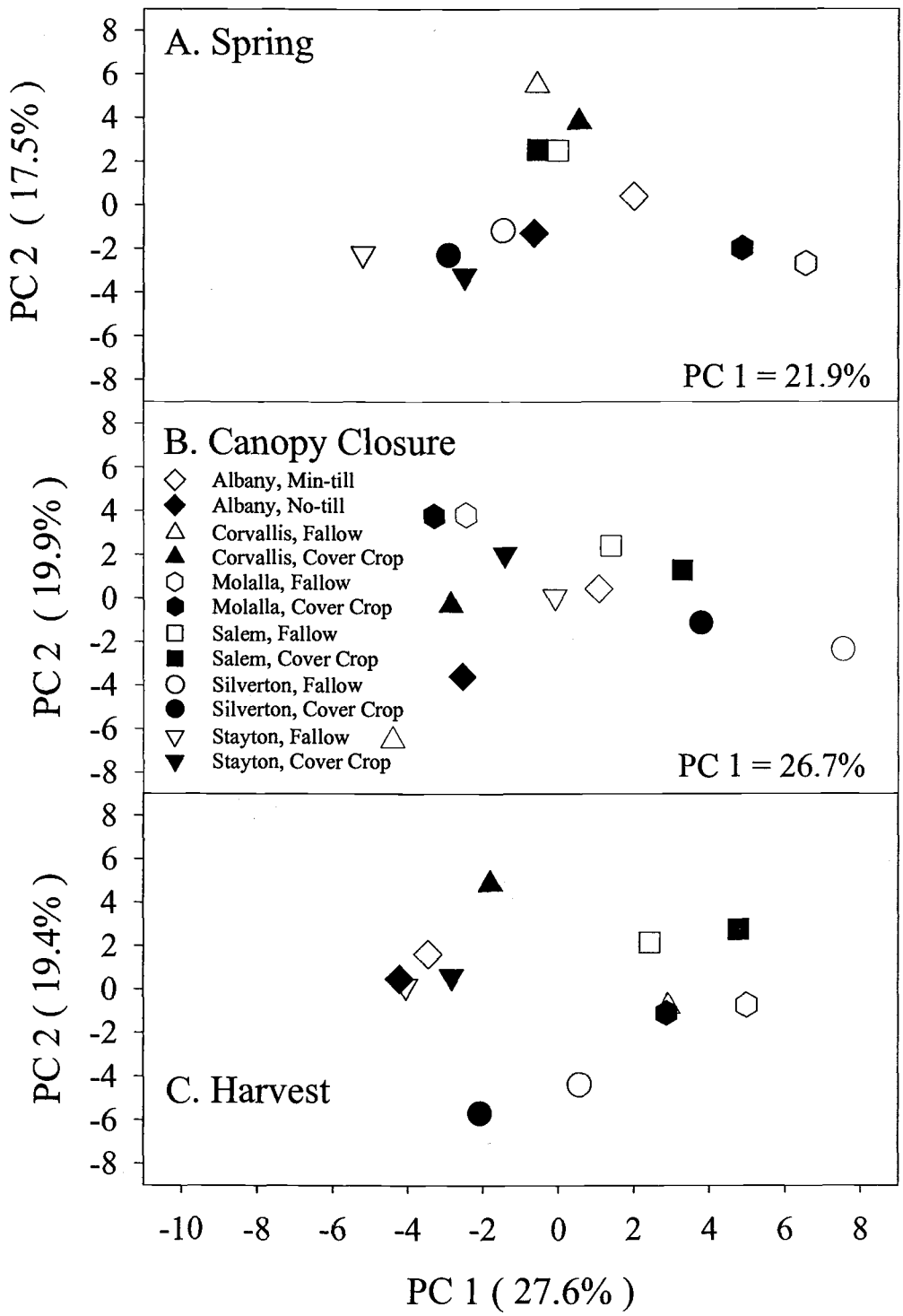

Fig. 4.3. PCA of microbial community FAME profiles from grower fields in spring (A), at canopy closure (B), and harvest (C) 1998. Percent variance explained by each PC is shown in parentheses.

Table 4.5. Significant correlations ($p < 0.05$) between selected soil properties and FAMEs from grower field soils at Spring 1998 ($n = 12$).

FAME	Clay	Silt	Sand	Total C	CEC
12:0	–	–	–	–	-0.64
14:0	-0.72	–	–	–	–
17:0	–	-0.59	–	–	–
16:1ω5*c*	–	–	–	–	-0.67
16:1ω7*c*/*i*15:0 2OH†	–	0.59	-0.60	–	–
16:1ω9*c*	–	–	–	–	-0.69
16:1ω11*c*	–	–	0.66	–	–
17:1ω7*c*	–	–	0.58	–	–
17:1ω8*c*	–	–	0.68	–	0.60
18:1ω9*c*	–	–	–	-0.63	–
20:1ω9*c*	–	–	–	0.91	–
20:3ω6*c*	–	-0.68	0.67	–	–
*i*15:0	–	–	–	–	0.70
*i*15:1	–	–	–	–	-0.61
*i*16:0	0.78	–	–	0.66	–
*i*16:1	–	–	–	0.90	–
*a*17:0	–	-0.71	0.87	–	–
*i*17:1ω7*c*	–	–	0.63	–	–
*a*17:1ω9*c*	–	-0.64	–	0.65	–
15:0 3OH	0.64	–	–	0.93	–
16:1 2OH	0.69	–	–	–	–

Table 4.5 (continued). Significant correlations ($p < 0.05$) between selected soil properties and FAMEs from grower field soils at Spring 1998 ($n = 12$).

FAME	Clay	Silt	Sand	Total C	CEC
19:0 cy ω10*c*/un[†‡]	–	-0.61	0.67	–	–
19:0 cy ω8*c*	–	–	–	0.60	–
10Me17:0	–	–	–	0.58	–
10Me18:0	0.59	–	-0.58	–	–
16:0 N alcohol	0.62	–	–	–	–

[†]Because of identical retention times, MIDI is unable to differentiate between these two FAMEs.

[‡]Un = unnamed FAME.

fields cropped to corn (Silverton, Corvallis, and Albany) were negatively associated with PC 2, with the Salem field as the exception. In contrast to the spring sampling, community profiles were less influenced by the soil properties measured. No significant correlations were found between the soil properties and PC 1 and 2, and only 13 of the 35 FAMEs detected were correlated significantly with soil texture, CEC, or TOC (data not shown). At the end of the growing season, communities were clustered again according to field site, with little affect of cover cropping except at the Corvallis field (Fig. 4.3C). Clay content was negatively correlated ($r = -0.73$) with PC 1 for the harvest FAME profiles; percentages of silt and sand were negatively ($r = -0.63$) and positively ($r = 0.68$) correlated with PC 2, respectively. In addition, soil

properties were significantly correlated with 20 of the 49 FAME's detected (data not shown).

Similar to FAME, principal components analysis of Biolog substrate utilization profiles revealed site-dependent influences on microbial communities (Fig. 4.4). Community Biolog patterns were influenced by site with little impact of alternative management practice in early spring (Fig. 4.4A). Site influences were obscured at canopy closure due to alternative management practices in some fields, including Silverton, Corvallis, and Albany (Fig. 4.4B). At harvest, however, community Biolog patterns were again field-dependent (Fig. 4.4C), with communities from soil cropped to cauliflower (Molalla) well separated along PC 2 from communities associated with corn or beans.

Winter Cover Crops

Shifts in microbial community FAME profiles occurred in response to winter cover crops at the VRS in 1998 and 1999. Principal components analyses of FAME patterns for the 1999 growing season are shown in Figure 4.5. Differences between communities were slight in early spring (Fig. 4.5A), but communities of the cover cropped and fallow plots diverged later in the growing season after cover crops were incorporated into soil as plant residues (Figs. 4.5B and C). At canopy closure of the summer vegetable crop, microbial communities from cover cropped plots were enriched in FAMEs 16:1ω5*c*, 18:3ω6*c*, and 20:1ω9*c* whereas communities from winter fallow plots contained elevated amounts of *i*15:0, *i*17:0, *a*17:0, 16:1 2OH, 18:0, and 20:0. Separations between communities at harvest sampling were due to greater

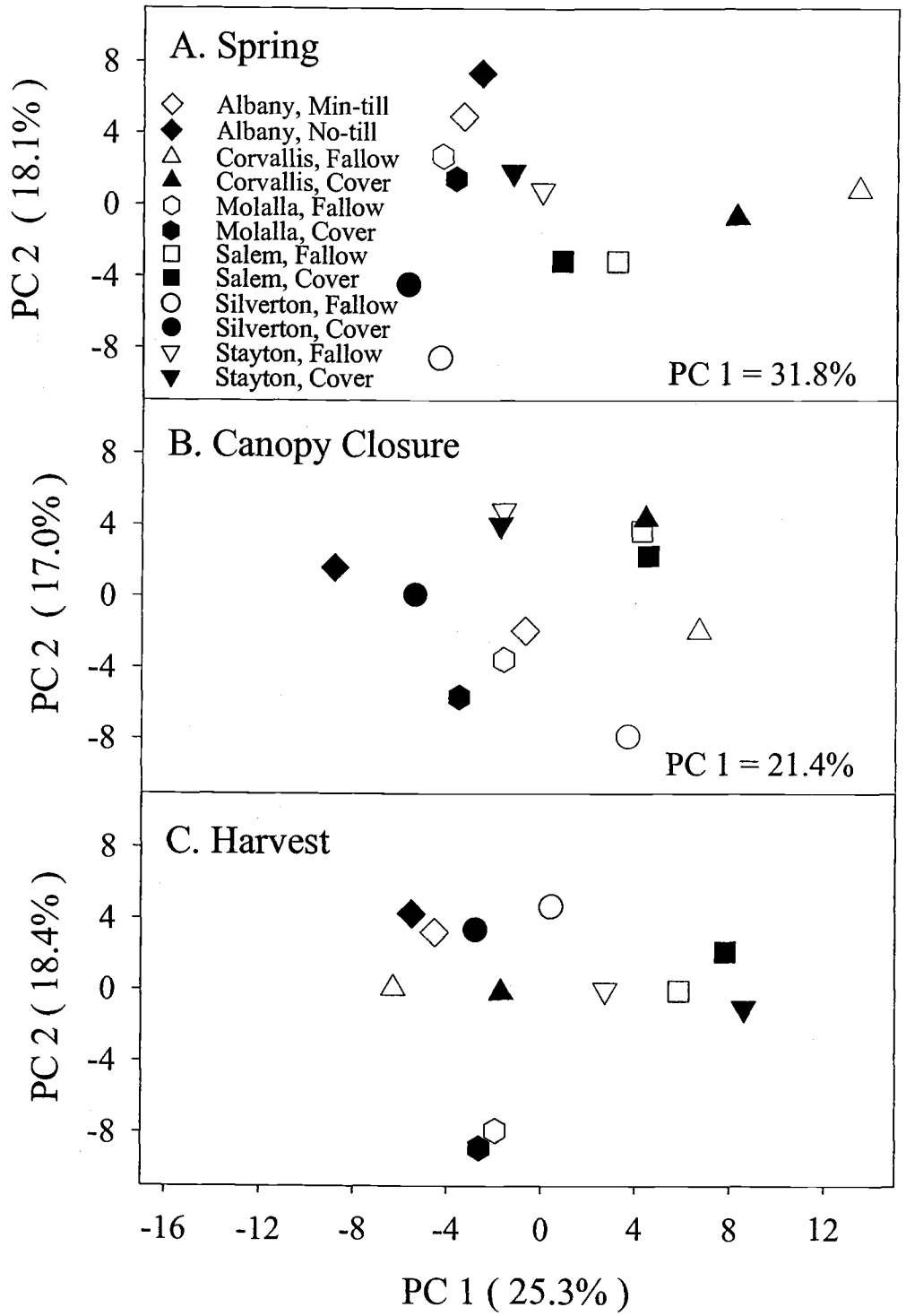

Fig. 4.4. PCA of microbial community Biolog patterns from grower fields in spring (A), at canopy closure (B), and harvest (C) 1998. Percent variance explained by each PC is shown in parentheses.

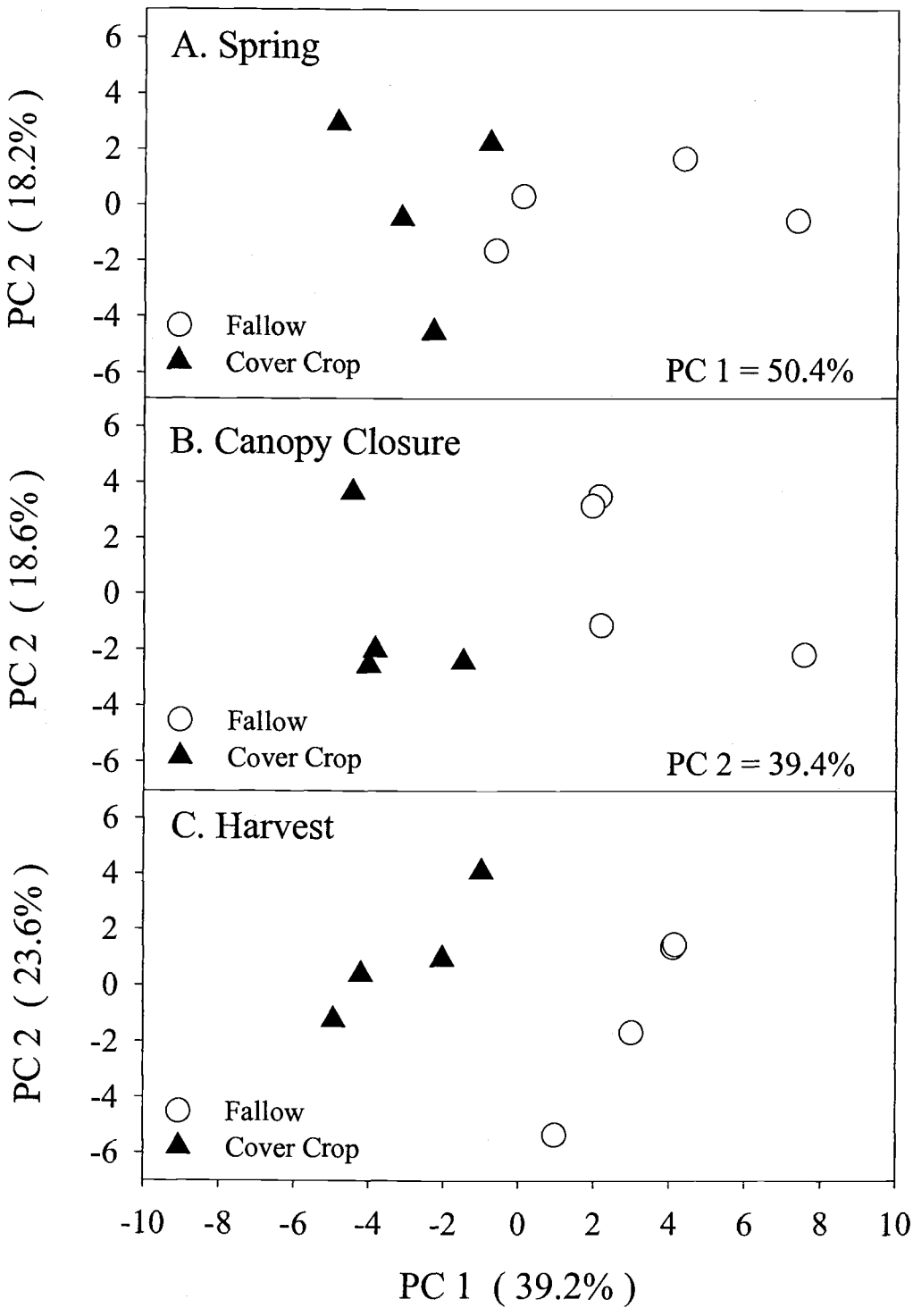

Fig. 4.5. PCA of microbial community FAME profiles from winter fallow and winter cover-cropped soil of VRS in spring (A), at canopy closure (B), and harvest (C) 1999. Percent variance explained by each PC is shown in parentheses.

abundances of 20:4ω6*c* and several saturated FAMEs (12:0, 15:0, and 17:0) in cover-cropped soil compared to the fallow soil, which was enriched in 17:0 cy, 10Me16:0, 10Me18:0, *i*15:0, *i*17:0, and *a*17:0. Trends in 1998 were analogous to those observed in 1999; communities from cover-cropped and fallow soils were similar to each other in early spring of 1998, but diverged at canopy closure and harvest due to enrichment of several FAMEs (10Me16:0, *a*17:0, and 18:2ω6*c*) in the cover-cropped soil (not shown). In addition to causing shifts in community structure, winter cover crops affected the diversity of FAMEs in VRS soil in 1998 and 1999. Diversity was greater in cover-cropped soil at canopy closure and harvest of 1998 and at harvest of 1999 (Table 4.6).

Cover crop effects on community FAME profiles also were observed at NWREC in 1998 after soil incorporation. At the harvest sampling date, FAME patterns differed between treatments due to greater amounts of 18:1ω9*c*, 18:2ω6*c*, and *i*20:0 in plots of both cover crop treatments in contrast to winter fallow plots (not shown). In 1999, however, cover crop influences on soil community FAME patterns were not observed. Also, FAME diversity was not affected consistently by cover crops in 1998 nor 1999 (Table 4.6). For grower fields, cover crops only affected microbial communities of certain fields, specifically Silverton at canopy closure and Corvallis at canopy closure and harvest (Fig. 4.3B and C). Principal components analysis of the Corvallis field soil at harvest revealed greater relative amounts of saturated FAMEs (12:0, 15:0, 17:0), 18:0 2OH, 18:2ω6*c*, and 20:4ω6*c* in cover-cropped soil compared to fallow soil (not shown).

Table 4.6. Effect of winter cover cropping on mean FAME diversity† of soils from the two experimental research stations and five grower fields. The Albany grower field, which consisted of tillage treatments, was not included in the analysis. Values are shown for each sampling period of the 1998 and 1999 growing seasons.

		1998			1999		
Study Site	Treatment	Spring	Canopy	Harvest	Spring	Canopy	Harvest
NWREC	Fallow	2.87a‡	2.77a	2.95a	2.44a	2.90a	2.78a
	Triticale	2.79a	2.61b	2.83a	2.63a	2.90a	2.75a
	Triticale + winter pea	2.83a	2.70ab	2.89a	2.64a	2.92a	2.76a
VRS	Fallow	2.98a	2.58b	2.70b	2.84a	2.97a	2.72b
	Cover	3.05a	2.82a	2.84a	2.95a	3.00a	2.84a
Grower	Fallow	2.98a	2.97a	2.84a			
	Cover	2.98a	2.97a	2.89a			

†Diversity calculated by Shannon's diversity index = H' = $- \sum (P_i \times \log P_i)$, where P_i is the proportional amount of each FAME.

‡Within each sampling season and site in a column, means followed by the same letter are not significantly different at $p < 0.05$.

Discriminant rules for separating fallow and cover-cropped soils at early spring and harvest were successfully obtained from grower field FAME data (excluding Albany). At harvest 1998, the step-wise selection procedure identified 9 soil FAMEs as best discriminators. These were *i*15:0, 15:0, *i*16:1 G, 10Me17:0, 18:0, 19:1ω9*t*, 19:0 cy/unnamed, 18:0 2OH, and 20:4ω6*c*. Cross-validation of the discriminant rule (generated from the 9 FAMEs) showed that samples from cover-cropped soil were correctly classified into the cover-crop treatment group 75% of the time. Samples from fallow soils were correctly classified into the fallow treatment group at a rate of 77%. Canonical discriminant analysis resulted in one significant canonical function ($p < 0.001$) that separated fallow from cover-cropped soils (Fig. 4.6). For soils at spring 1998, 9 FAMEs were selected to generate a discriminate rule that correctly classified cover-cropped soils 80% of the time and fallow soils 70% of the time. These FAMEs were 15:0, *i*16:0, 16:1ω9*c*, 16:1ω11*c*, 16:1 2OH, 18:1ω7*c*/9*t*/12*t* , 18:3ω6*c*, 10Me18:0, and 19:0cy/unnamed. In addition, canonical discriminant analysis resulted in one significant canonical function ($p < 0.001$) that separated fallow from cover-cropped soils (not shown).

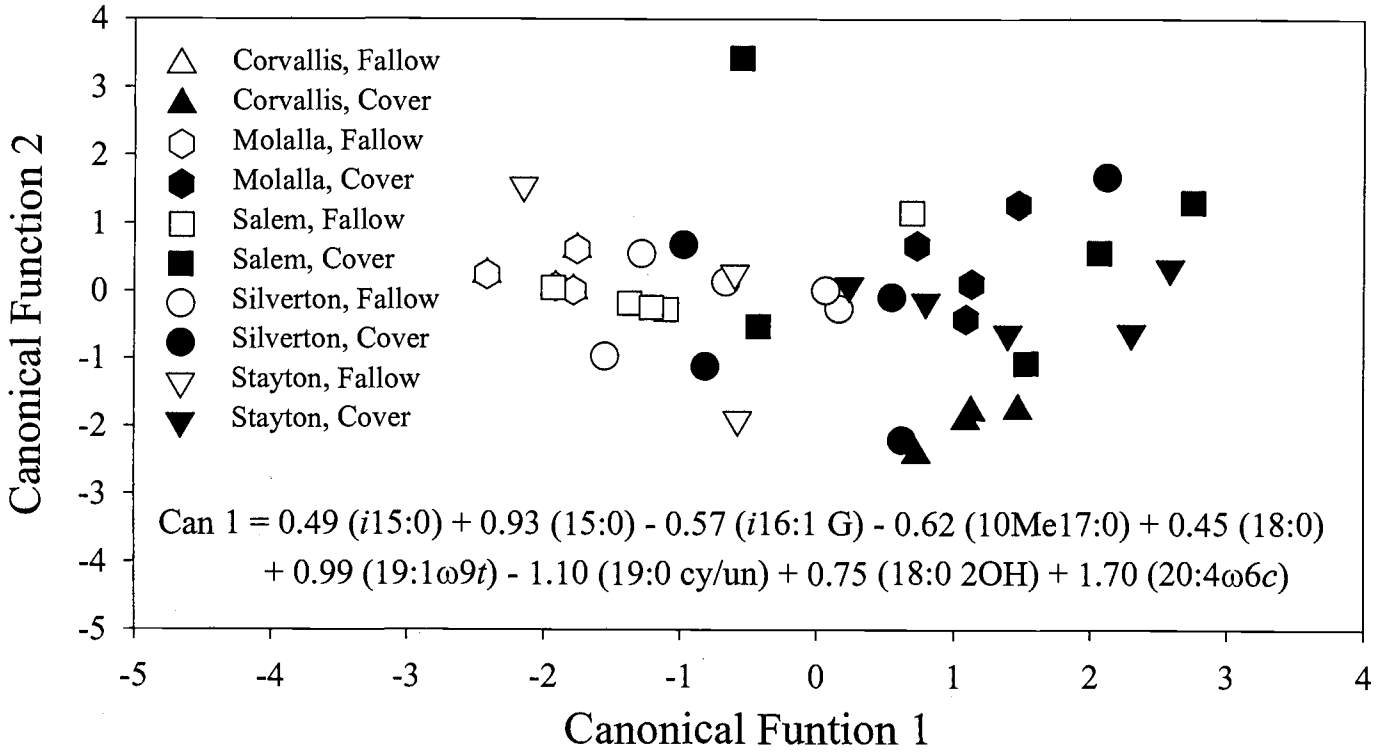

Fig. 4.6. Canonical discriminant analysis of microbial community FAME profiles from grower fields at Harvest 1998. The Albany field was not included in the analysis. The first canonical function is shown and is significant at $p < 0.001$.

An example of the effect of winter cover crops on Biolog substrate utilization potential is shown in Figure 4.7. In early spring 1999, Biolog patterns were highly similar between NWREC plots containing triticale or triticale/Austrian winter pea cover crops (Fig. 4.7A). By harvest, however, Biolog patterns generally differed between soils cropped to the two cover crop treatments (Fig. 4.7B). Principal components analyses of Biolog data did not reveal strong influences of cover crops on community Biolog patterns at NWREC in 1998 nor at VRS in 1998 and 1999. However, cover crops did affect overall diversity of substrates utilized by microbial communities from both research stations. At the VRS site, Biolog diversity was greater in cover-cropped soil during canopy closure and harvest of 1998 and canopy closure of 1999 (Table 4.7). For NWREC in 1999, a greater diversity of Biolog substrates was utilized in cover-cropped plots compared to fallow plots in early spring; at canopy closure, however, Biolog diversity was reduced in cover-cropped soil relative to winter fallow soil.

Substrate utilization potentials of microbial communities also were affected by alternative management practices for some of the grower fields. At canopy closure 1998, separations occurred between communities of minimum-tillage and no-tillage treatments of the Albany field; in addition, Biolog patterns differed at canopy closure between the cover-cropped and fallow soils from Corvallis and Silverton (Fig. 4.4B). For the Corvallis field at canopy closure, communities from cover-cropped soil utilized more sugars (cellobiose, mannose, and trehalose) and amino acids (L-alanine, L-alanyl-glycine, L-leucine, and D-serine) than communities from the fallow soils, which had high utilization potentials for carboxylic acids (succinic, L-aspartic, acetic,

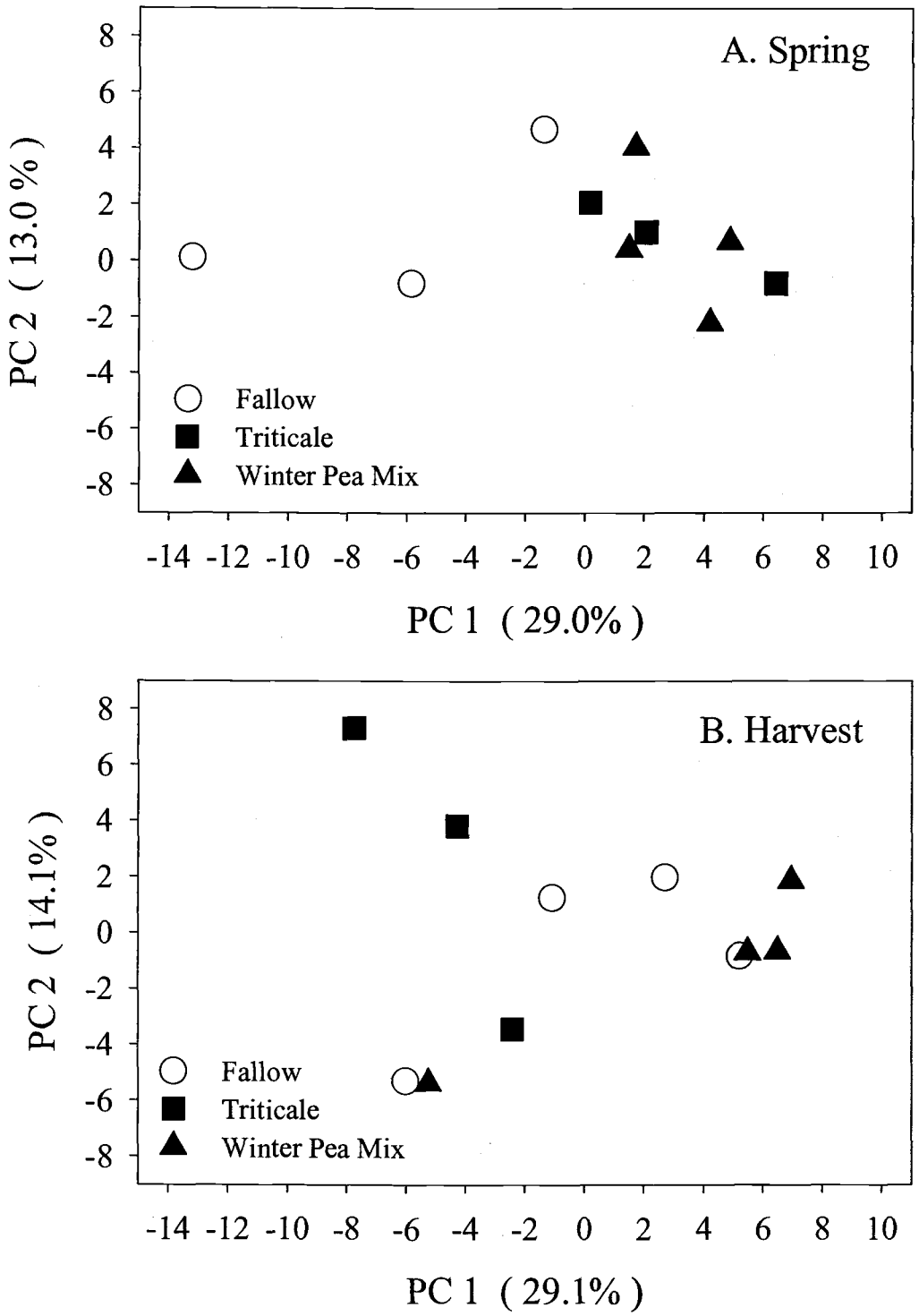

Fig. 4.7. PCA of microbial community Biolog patterns from winter fallow and winter cover-cropped soil of NWREC in spring (A) and at harvest (B) 1999. Percent variance explained by each PC is shown in parentheses.

citric, and D-glucuronic acids). As at VRS, incorporation of cover crop residues into soil resulted in the utilization of a greater diversity of Biolog substrates in grower fields overall at the canopy closure sampling date (Table 4.7).

Table 4.7. Effect of winter cover cropping on mean Biolog diversity[†] of soils from the two experimental research stations and five grower fields. The Albany grower field, which consisted of tillage treatments, was not included in the analysis. Values are shown for each sampling period of the 1998 and 1999 growing seasons.

		1998			1999		
Study Site	Treatment	Spring	Canopy	Harvest	Spring	Canopy	Harvest
NWREC	Fallow	4.29a[‡]	4.20a	4.29a	4.25b	4.27a	4.23a
	Triticale	4.32a	4.20a	4.28a	4.30a	4.12b	4.34a
	Triticale + winter pea	4.33a	4.25a	4.32a	4.33a	4.13b	4.18a
VRS	Fallow	4.36a	4.32b	4.31b	4.20a	4.14b	4.25a
	Cover	4.34a	4.35a	4.40a	4.26a	4.27a	4.29a
Grower ($n = 5$)	Fallow	4.35a	4.33b	4.38a			
	Cover	4.36a	4.36a	4.37a			

[†]Diversity calculated by Shannon's diversity index = $H' = -\sum (P_i \times \log P_i)$, where P_i is the proportional absorbance value of each substrate well.

[‡]Within each sampling season and site of a column, means followed by the same letter are not significantly different at $p < 0.05$.

Discussion

Seasonal and Field-Dependent Influences on Microbial Communities

For soils included in this study, season was the most influential determinant of microbial community structure and substrate utilization potential. Similar results were found by Bossio et al. (1998) for cultivated soils in California, where differently managed plots of the same soil type were analyzed for community PLFA profiles over time. However, when two contrasting soils were compared (sandy loam entisol versus a clay vertisol), influence of season was secondary to that of soil type. In our study, the soils of the grower fields are primarily mollisols and all are loam variants, so differences in soil properties probably were not extreme enough for soil type to be the dominant factor influencing community structure.

FAME profiles from the 1998 growing season suggest fluctuations in eukaryotic and bacterial populations in VRS and NWREC soils over the course of the growing season. In early spring, soils from both research stations were enriched in fungal-associated 18:3ω6*c*; spring soils from VRS and NWREC also yielded greater amounts of 20:1ω9*c* and the protozoan marker 20:4ω6*c*, respectively. In contrast, bacterial markers (*i*15:0, *i*17:0, *a*17:0, 10Me16:0,19:0 cy ω8*c*) dominated soil FAME profiles from both sites at canopy closure. These trends were consistent with direct counts of fungi and bacteria conducted on VRS soil in 1998, which contained significantly greater fungal biomass in spring (69.2 μg g^{-1} soil) than at canopy closure or harvest (32.1 and 30.3 μg g^{-1}, respectively) (Table 4.4). In contrast, greater bacterial biomass was measured at canopy closure (804 μg g^{-1} soil) compared to spring

and harvest (240 and 184 μg g^{-1}, respectively). Greater fungal biomass in spring compared to later sampling dates may be due to greater C availability and/or greater moisture content in soils at early spring. At this time, soils were wetter and contained some residues (above and below ground) of the previous summer crop. Increased moisture and the presence of plant residues have been shown to increase fungal biomass in other systems (Frey et al., 1999; Wienhold and Halvorson, 1998). In addition, tillage events that occurred between the spring and canopy closure samplings may have adversely affected fungi, which can be susceptible to plowing disturbances (Frey et al., 1999).

Within a season, community profiles were influenced more by soil type and field properties than by farm management. Other studies found similar results regarding the importance of soil properties as microbial determinants. Soil type rather than plant species was a major controller of microbial biomass and activity (respiration, denitrification, N mineralization, and nitrification potentials) in soils planted to different grasses (Groffman et al., 1996). Similarly, in community-level studies, soil type differences had greater impacts on microbial community structure and substrate utilization potential than did differences in farm management (Bossio and Scow, 1998; Ibekwe and Kennedy, 1998).

In our study, FAME patterns in early spring were well correlated with soil texture and TOC, and soil properties were often correlated with relative amounts of individual FAMEs (Table 4.5). Meaningful information can be gained when FAME markers are correlated with soil properties; for example, the positive association between the protozoan marker 20:3ω6*c* and sand content indicated that protozoan

populations were enhanced in soils with larger pore structure. However, certain correlations between FAME markers and soil properties could not be explained; for example *i*17:1ω7*c* was positively correlated with sand content even though this FAME is considered a marker for sulfate reducers (Taylor and Parkes, 1983). Discrepancies such as this are inevitable, though, because many FAMEs are common to different groups of microorganisms (*e.g.*, terminally branched fatty acids of Gram-positive and some Gram-negative bacteria) and because the origins of some FAMEs are unknown (Haack et al., 1994).

Winter Cover Crops

Soils from VRS, NWREC, and the Corvallis field became enriched in several fungal (18:2ω6*c* and 18:3ω6*c*) and protozoan (20:4ω6*c*) FAME markers after incorporation of winter cover crops, suggesting that cover crop residues were important for stimulating or maintaining fungal populations over time. Although bacteria may play a significant role in residue decomposition initially, fungal populations have been shown to be dominant members involved in the decomposition of cellulose (Hu and van Bruggen, 1997). In addition, the greater amounts of the protozoan marker in some cover-cropped soils indicates increased predator-prey interactions between protozoa and bacteria (Frostegård et al., 1997). Soil conditions that support higher trophic levels, including predators, could enhance mineralization and availability of inorganic N for plant uptake. However, it is not clear whether or not functional processes in our soils were affected by cover crops despite changes in community structure. For example, N mineralization potential was not affected by

cover cropping in this study (Ndiaye et al., 2000), suggesting that the different communities may have similar N mineralizing capabilities under the conditions of this assay.

It should be noted that four FAMEs were extracted and detected from the triticale and winter pea residues of NWREC (16:0, 18:0, 20:0, and 18:2ω6*c*). However, this does not imply necessarily that the FAME 18:2ω6*c*, which was elevated in cover-cropped plots of NWREC in 1998, originated from the cover crop residues themselves. In a separate laboratory study, we found no increase in the amounts of extractable 18:2ω6*c* after residues from the same cover crops were incorporated into VRS soil (Schutter and Dick, ____).

Other studies have shown that management practices can affect bacterial and fungal components of the soil community. In contrast to our study, Zelles et al. (1995) reported greater concentrations of bacterial fatty acids in soils under crop rotation, whereas an increase in the fungal marker 18:2ω6,9*c* was associated with organic soils receiving residues from a vetch winter cover crop (Bossio et al., 1998). Although there was an increase in this fungal marker, overall diversity of phospholipid fatty acids was not affected by organic management when compared to conventionally managed systems. However, in our study, diversity of FAMEs was significantly greater in cover-cropped soils at VRS for both the 1998 and 1999 growing season.

Changes in microbial community FAME structure occurred in response to winter cover cropping at VRS, where the cover cropping treatment was established 5 years prior to the 1998 sampling season. For grower fields, where the cover crop treatment was in place for only 1 to 2 years, changes in community FAME profiles

were observed only at the Corvallis field. It is possible that detectable changes in microbial community FAME structure happen only after several years of winter cover cropping. In contrast, Ndiaye et al. (2000) found that microbial biomass and β-glucosidase were sensitive to alternative management practices at the grower fields after only 1 to 2 years of winter cover cropping. Discriminant analysis, however, was useful for identifying several FAMEs that separated soil samples according to treatment. These FAMEs may not have explained much of the variation overall in the data set (hence they were not important FAMEs in the PCA), but they may be useful for generating models for predicting whether or not communities have responded to alternative management practices.

Although winter cover crop treatments were established earlier at NWREC than at VRS, there were fewer community responses to cover crops at NWREC than at VRS. For example, there were no observable changes in community FAME profiles in cover-cropped plots of NWREC in 1999, and cover crops did not enhance the diversity of community FAMEs in either growing season. These findings follow others which demonstrated few changes in soil quality at this site despite 9 years of cover cropping. For example, winter cover cropping did not improve water infiltration and bulk density of NWREC soil (Buller, 1999), nor were microbial biomass and enzyme activities affected by the triticale cover crop (Ndiaye et al., 2000). These results reflect the relatively poor cover crop stands that occurred from 1994 to 1997 as a result from excess rain, cooler temperatures, and some late cover crop plantings (Minshew, 1999).

Our study provides evidence that community Biolog activity may be a sensitive indicator of soil quality. In this study, C substrate utilization diversity proved responsive to winter cover cropping as Biolog diversity was greater in cover-cropped versus fallow soils for the VRS and grower fields. Similarly, Biolog diversity of a soil community increased after incorporation of plant residues in a separate laboratory incubation study (Schutter and Dick, ___). In addition, differences in Biolog patterns between fallow and winter cover-cropped soils of the Corvallis field suggested utilization of different substrates by microbial communities. At canopy closure, communities from the cover-cropped soil utilized more Biolog sugars and amino acids, whereas communities from fallow soils utilized more carboxylic acids. This paralleled a seasonal trend, where greater utilization of carboxylic acids were observed at canopy closure rather than spring for several soils. A possible explanation for these patterns is that carboxylic acid use may be indicative of communities utilizing labile substrates from plant rhizospheres, including root exudates, to a greater extent than decomposing residues. Although sugars, amino acids, and carboxylic acids are all found in root exudates (Kraffczyk et al., 1984), carboxylic acids are less-associated with decomposing plant residues, which were present in soils in early spring (from previous summer crop) as well as in cover-cropped soils at canopy closure. In a laboratory study, Sharma et al. (1998) found that utilization patterns of Biolog carbohydrates and amino acids were successful for characterizing soil communities during decomposition of maize litter whereas carboxylic acids were not.

Conclusions

Although season and soil type were dominant factors influencing microbial communities, alternative management practices also affected communities under certain conditions. Changes in microbial community FAME structure were detected in research plots with 5 or more years of winter cover cropping and at one grower field with only one year of winter cover cropping. Diversity of FAMEs also was greater in cover-cropped soil at one research station, whereas diversity of Biolog substrates utilized by microbial communities was greater in cover-cropped soils at one research station and grower fields overall. Cover crop residues appear to have enhanced soil fungal and protozoan populations, at least in some soils, and may have increased the potential of microbial communities to utilize a diverse range of C substrates. Although this study demonstrates that microbial community shifts can be indicative of changes in soil quality due to alternative management, additional studies are necessary to confirm that changes in community structure simultaneously or subsequently result in changes in soil processes. In particular, it would be beneficial to determine whether community shifts result in enhanced rates of nutrient mineralization at key stages of crop development.

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CHAPTER 5

MICROBIAL COMMUNITY PROFILES AND ACTIVITIES AMONG AGGREGATES OF WINTER FALLOW AND COVER-CROPPED SOIL

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Abstract

Microbial biomass and activities are heterogeneously distributed among soil aggregates, but little is known if community structure or diversity differs across aggregates. Also, winter cover crops can affect aggregate distribution by promoting their formation, which may affect microbial distribution patterns. In this study, aggregates of a winter fallow and winter cover-cropped soil were analyzed for microbial biomass, respiration, N mineralization potential, and community diversity. Community fatty acid methyl ester (FAME) profiles and substrate utilization potentials were assessed as diversity indicators. In addition, the soil was sampled over the course of the growing season to determine seasonal impacts on aggregate and microbial distribution patterns. Although winter cover cropping did not affect total organic carbon or aggregate distribution significantly, the presence of cover crop residues in soil enhanced microbial biomass, N mineralization, FAME diversity, and substrate utilization potential. In the summer, total Kjeldahl N, microbial biomass, and microbial diversity were generally highest in the intermediate (0.25-0.5 and 0.5-1.0 mm) and sometimes smallest (<0.1 mm) aggregate size classes of the fallow treatment. In soils containing cover crop residues, microbial biomass, FAME diversity, and FAME richness were more evenly distributed across all but the largest aggregate size class (2.0-5.0 mm), where values were lower compared to other aggregate sizes. The ratio of bacterial:fungal FAMEs differed among aggregates at some sampling dates, with wider ratios in the smallest aggregates (< 0.1 mm). Wider ratios were associated with the cover-cropped treatment as well. Effects of cover

crops on the protozoan FAME marker (20:4ω6*c*) were observed in the summer, with greater amounts of this marker in aggregates and whole-soils from the cover-cropped treatment compared to the fallow. Overall, community FAME profiles differed between the fallow and cover crop treatment, but profiles were heterogenous among and within aggregate size classes, with no clear pattern based on aggregate size.

Introduction

Microorganisms appear to be heterogeneously distributed across soil aggregates. Evidence indicates that proportions of fungi and bacteria differ among aggregate sizes, for example, with lower bacterial:fungal ratios associated with macroaggregates than microaggregates (Gupta and Germida, 1988; Singh and Singh, 1995; Monreal and Kodama, 1997). Fungal hyphae, but not spores, are restricted from the interparticle spaces of microaggregates (Monreal and Kodama, 1997), whereas bacteria may survive longer in the smaller pores of microaggregates by escaping predators (Heijnen et al., 1991). Alternatively, bacteria are more intimately associated with microaggregates as clay platelets bind to polysaccharide-coated bacterial cells, whereas fungal hyphae enmesh microaggregates to form larger aggregates (Tisdall and Oades, 1982).

In addition to differences in fungal and bacterial proportions, the overall microbial biomass can vary among aggregates. Gupta and Germida (1988) reported lower microbial biomass in microaggregates (<0.25 mm) compared to macroaggregates. Similar results were found by Miller and Dick (1995), who found

low biomass values in aggregates smaller than 0.25 mm and greater biomass in the 0.5-1.0 mm aggregate size class. Microbial mineralization and enzyme activities also have been found to vary among different sizes of soil aggregates (Mendes et al., 1999), but some distribution patterns changed over time. For example, in June, N mineralization for one soil was greatest in the 2.0-5.0 and <0.25 mm aggregate size classes, but in September the rate was highest in the 1.0-2.0-mm aggregates (Mendes et al., 1999).

Another factor that may affect microbial distribution patterns across soil aggregates is the presence of a winter cover crop. In Oregon's Willamette Valley, winter cover crops often are grown as a means for capturing leachable nutrients and preventing soil erosion during the rainy winter months (Burket et al., 1997). Incorporation of cover crops in the spring also adds organic matter to soil. Additional C inputs to soil from cover crops can have important impacts on soil biological properties as well as aggregation processes (Miller and Dick, 1995). By providing greater root activity and C inputs, cover crops were shown to improve soil aggregation and maintain organic C pools in a soil compared to conventionally managed (winter fallow) soil (Miller and Dick, 1995). In addition, the distribution of microbial biomass and activities can be affected by winter cover crops as microbes within a particular aggregate size class respond to cover crop residue inputs (Mendes et al., 1999).

Other than differences between fungal and bacterial biomass, there is little information regarding whether or not microbial community structure varies among aggregate size classes. However, population sizes of denitrifiers and *Rhizobium* can

differ among aggregate sizes (Seech and Beauchamp, 1988; Mendes and Bottomley, 1998), suggesting that community structure may be heterogenously distributed. A better understanding of the community structure associated with aggregate size classes may help explain whether heterogenous activities are due to differences in nutrient availability, predation pressures, or due to different populations associating with different sizes of soil aggregates. Although community distribution patterns may be determined by aggregate pore sizes, there also is evidence that microbial distribution patterns may be related to clay and organic carbon (C) content rather than porosity (Van Gestel et al., 1996).

To gain insights to the spatial distribution patterns of microbial communities across aggregates, microbial communities were studied across a range of aggregate size classes. Aggregates were obtained from winter-fallow and a winter cover-cropped soil to determine the impact of cover cropping on microbial distribution patterns among aggregates. Communities were characterized according to their fatty acid methyl ester (FAME) profiles as well as their ability to utilize a diverse range of C substrates using the Biolog assay. The objectives of this study were to assess the impacts of winter cover cropping on the distribution (1) of aggregate sizes in a silt loam soil, (2) of microbial biomass and mineralization activities among aggregate size classes, and (3) of microbial community FAME structure and Biolog activities among aggregate size classes. Our fourth objective was to assess the influence of season on aggregate distribution and spatial patterns of microbial communities and activities among aggregate size classes.

Materials and Methods

Experimental Site and Soil Sampling

The study was conducted with soil from the Oregon State University Vegetable Research Station (Corvallis, OR). The soil at this site is a Chehalis silt loam (fine-silty, mixed, mesic Cumulic Ultic Halpoxeroll) with 260 g kg^{-1} clay, 520 g kg^{-1} silt, and a soil pH (1:2 soil:water) of 5.9. The design at this station is a randomized complete block with four replicate blocks. Each block consists of two treatments (30 × 60 m plots), which are a summer vegetable-winter cover crop and a summer vegetable-winter fallow rotation. Treatments were established in 1993. Winter cover crops were planted after harvest of the summer vegetable crop and incorporated into soil the following spring. In the fall of 1997, a mixture of gray oats (*Avena sativa* L.) and common vetch (*Vicia sativa* L.) were planted as the winter cover crop. Green beans (*Phaseolus vulgaris* L.) were planted as the summer vegetable crop in 1998, followed by winter wheat as the cover crop from fakk 1998 to spring 1999.

Soil was collected from each plot during the course of a growing season. The first sampling was in March 1998, one week prior to the termination of the oats/vetch cover crop. Soils were collected again in July 1998, which corresponded to canopy closure of summer vegetable crop (green beans). For green beans, canopy closure was at the two-trifoliate leaf stage and occurred approximately six weeks after planting. The third sampling occurred in August 1998, one week prior to harvest of the green beans. The fourth sampling was in spring 1999, just prior to the termination of the

winter wheat cover crop. Soil was collected from each plot with a shovel (0-15 cm) and transported in 1-gallon (3.8 L) plastic bags to the laboratory.

Soil Sieving and Aggregate Distribution

Preparation and sieving of soils were done according to procedures described by Mendes et al. (1999). After sampling, large soil clods were gently broken by hand, and then soils were laid out between sheets of brown paper to slowly dry for several days at 4 °C. Soils were allowed to dry until a gravimetric water content of 60 to 100 g kg^{-1} was reached. Soils collected in spring were near field capacity (-33 kPa = 310 g kg^{-1}), whereas soils at canopy closure and harvest soil were drier, ranging in water content from 180 to 280 g kg^{-1} at canopy closure and 140 to 210 g kg^{-1} at harvest.

To prepare aggregates, 200-g portions of the dried soil were placed on nested sieves (20-cm diameter) mounted on a Tyler Ro-Tap sieve shaker (Combustion Engineering Inc., Mentor, OH). Sieves were mechanically shaken (200-250 oscillations min^{-1}) for 2 minutes to separate soil into the following aggregate size classes: 2.0-5.0, 1.0-2.0, 0.5-1.0, 0.25-0.5, 0.1-0.25, and <0.1 mm. Preliminary experiments showed that shaking for 2 min was adequate for separating soil aggregates without causing mechanical destruction of large aggregates (data not shown). Aggregate distribution was determined by weighing soil from each aggregate size class. Soil aggregates were stored at 4 °C until analyzed for chemical, physical, and biological properties.

Soil Chemical and Physical Analyses

Samples of whole soils and aggregates from the cover-cropped and fallow plots were air-dried for C and N analyses. Whole soils and aggregates larger than 0.25 mm were ground with a mortar and pestle. Total organic carbon (TOC) of whole soils and aggregates was measured by combustion to CO_2 with a Leco C determinator (model WR12, St. Joseph, MI). Total Kjeldahl N (TKN) (organic N and NH_4^+) of aggregates and whole soils was determined following the method of Bremner and Mulvaney (1982). Particle size analysis was conducted on three aggregate fractions (1.0-2.0, 0.25-0.5, and < 0.1 mm) by the Central Analytical Laboratory (Oregon State University, Corvallis, OR). The method of Gee and Bauder (1986) with pretreatment for organic matter removal was followed.

Microbiological Analyses

Prior to microbiological analyses, 50-g samples of whole soils and aggregates were moistened with distilled water until a water content of 205 g kg^{-1} (two-thirds field capacity) was obtained. Samples then were allowed to equilibrate for 4 days in the dark at 25 °C.

Microbial biomass C (MB_C) of whole soils and aggregates was determined by the fumigation-incubation method of Jenkinson and Powlson (1976). Pre-moistened soil (10 g) was fumigated with chloroform for 24 h. After fumigation, soils were incubated for 10 days at 25 °C in acrylic tubes stoppered with rubber septa. The tube headspace was sampled for CO_2, which was analyzed by gas chromatography (Carle

Series 100 AGC, Loveland, CO). A k_C of 0.41 (Voroney and Paul, 1984) was used to calculate MB_C without the substraction of a nonfumigated control. Non-fumigated controls were not subtracted because when respiration in the fumigated sample was high, so was respiration in the non-fumigated sample. Subtraction of the non-fumigated from the fumigated sample resulted in net differences that did not vary among aggregate sizes or between cover crop treatments, implying that there were no effects of aggregate size or cover crop on microbial biomass C.

Microbial respiration was calculated from the amount of CO_2-C evolved from 10 g of nonfumigated soil, which was incubated in septa-stoppered acrylic tubes for 10 days at 25°C. The headspace was sampled and analyzed for CO_2 as described above. Microbial N mineralization potential was determined by the anaerobic production of NH_4^+ as described by Bundy and Meisinger (1994).

Community FAME profiles of whole soils and aggregates were determined by the ester-linked method (Ritchie et al., 2000; Schutter and Dick, 2000). Three g of soil were added to 35-mL glass centrifuge tubes and mixed with 15 mL of 0.2 *M* KOH in methanol. Soils were incubated at 37°C for 1 h with periodic vortexing. Next, 3 mL of 1.0 *M* acetic acid were added to neutralize the pH of the tube contents. Extracted FAMEs were partitioned into an organic phase by adding 10 mL of hexane. Tubes then were centrifuged for 10 min at 480 × g to separate organic matter from the hexane. The hexane layer was transferred to a clean glass tube and evaporated under a stream of N_2. FAMEs were dissolved in 1:1 hexane: methyl-*tert*-butyl ether and were transferred to a GC vial for analysis by a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA) equipped with 25 m × 0.2 mm fused silica capillary

column (5% diphenyl-95% dimethylpolysiloxane) and a flame ionization detector. FAMEs were identified and their relative peak areas determined by the MIS Aerobe method of the MIDI system (Microbial ID, Inc., Newark, DE).

FAMEs are described by standard nomenclature. Numbering of carbons begins at the aliphatic (ω) end of the molecule. The number of double bonds within the FAME is given after the colon. *Cis* and *trans* conformations are designated with suffixes "*c*" and "*t*", respectively. Other notations are "Me" for methyl, "OH" for hydroxy, "cy" for cyclopropane, and the prefixes "*i*" and "*a*" for *iso*- and *anteiso*-branched FAMEs, respectively. For determination of bacterial:fungal FAME ratios across aggregates, fungi were represented by the relative amount of 18:2ω6*c*; bacteria were represented by the sum of the following FAMEs: *i*15:0, *a*15:0, 15:0, *i*16:0, 16:1ω9*c*, *i*17:0, *a*17:0, 17:0 cy, 17:0, 18:1ω7*c*/9*t*/12*t*, and 19:0 cy. Protozoa were represented by their associated marker, 20:4ω6*c* (Frostegård et al., 1997).

Microbial community substrate utilization potential of whole soils and aggregates was determined with Gram-negative Biolog Microplates™ (Biolog Inc., Hayward, CA). Biolog plates were inoculated with 125 µl of soil suspension (10^{-3} strength) made by serially diluting 2-g soil samples in sterile saline. Plates were incubated at 25°C, and reactions were monitored every 24 h for 96 h total by measuring well absorbance values with an automated plate reader (595 nm; Bio-Tek Instruments, Winooski, VT). Biolog data from the 72 h reading were chosen for community analyses. Well absorbance values were adjusted by subtraction of a blank control; data were normalized by dividing the absorbance value of each well by the plate's maximum absorbance value.

Statistical Analyses

For each sampling date, principal components analysis (PCA) was performed on community Biolog and FAME data to characterize microbial communities across aggregate sizes. The PC-ORD program was used (McCune and Mefford, 1997). Biolog diversity, FAME diversity, and FAME richness were determined for each sample; diversity was measured as the Shannon's index (H'), where $H' = -\sum (P_i \times \log P_i)$, and P_i is the proportional amount of each FAME or the proportional absorbance of each Biolog well. Richness refers to the number of FAMEs detected in each sample.

All univariate analyses were performed with the SAS statistical package (SAS Institute, Cary, NC). For each sampling date, data were analyzed by a repeated measures analysis of variance, with aggregate size as the repeated term. The repeated term consisted of seven levels, six corresponding to the aggregate size classes and the whole soil sample as the seventh. When the sphericity test was satisfied, the data were treated as a split-plot design, with aggregate size as the subplot. When the sphericity test failed, significance levels were determined using the Huynh and Feldt epsilon adjustment (Huynh and Feldt, 1976). To assess effects of sampling date, the data were analyzed by a two-factor repeated measures analysis of variance. Repeated terms were sampling date (four levels) and aggregate size (seven levels). Protected LSD values were calculated to separated means at the level of $p < 0.05$.

Results

Aggregate Physical and Chemical Properties

Although the main effect of sampling date on aggregate size was not significant, there were some aggregate size fractions whose distributions were affected over time ($p < 0.0001$). From March to August 1998, the amounts of intermediate aggregate sizes (1.0-2.0 and 0.5-1.0 mm) increased as the 2.0-5.0 mm size class decreased (Fig. 5.1A), although this trend was reversed by March 1999. Throughout the study, there was no effect of winter cover cropping on the aggregate size distribution. In addition, variations in soil texture among aggregates were insignificant. Clay contents for the 1.0-2.0, 0.25-0.5, and < 0.1-mm aggregate size classes were 279, 284, and 251 g kg^{-1}, respectively. Corresponding values for silt content were 626, 617, and 652 g kg^{-1}.

The average value of TOC in soil was 17.0 g kg^{-1}. Levels of TOC did not change significantly over the course of the growing season, nor did levels differ among size classes of soil aggregates (data not shown). Although TOC was not significantly affected by winter cover cropping, there was a trend in July 1998 for higher levels of TOC in cover-cropped soil (17.7 g C kg^{-1}) compared to the winter-fallow treatment (15.5 g C kg^{-1}) ($p = 0.12$). In contrast to TOC content, there was a significant effect of aggregate size on TKN levels. In July 1998, TKN levels varied significantly among aggregate sizes (Table 5.1), with highest levels of N occurring in the smallest aggregate size class (< 0.1 mm). There also was a significant interaction between sampling date and aggregate size. Compared to other sampling dates, TKN

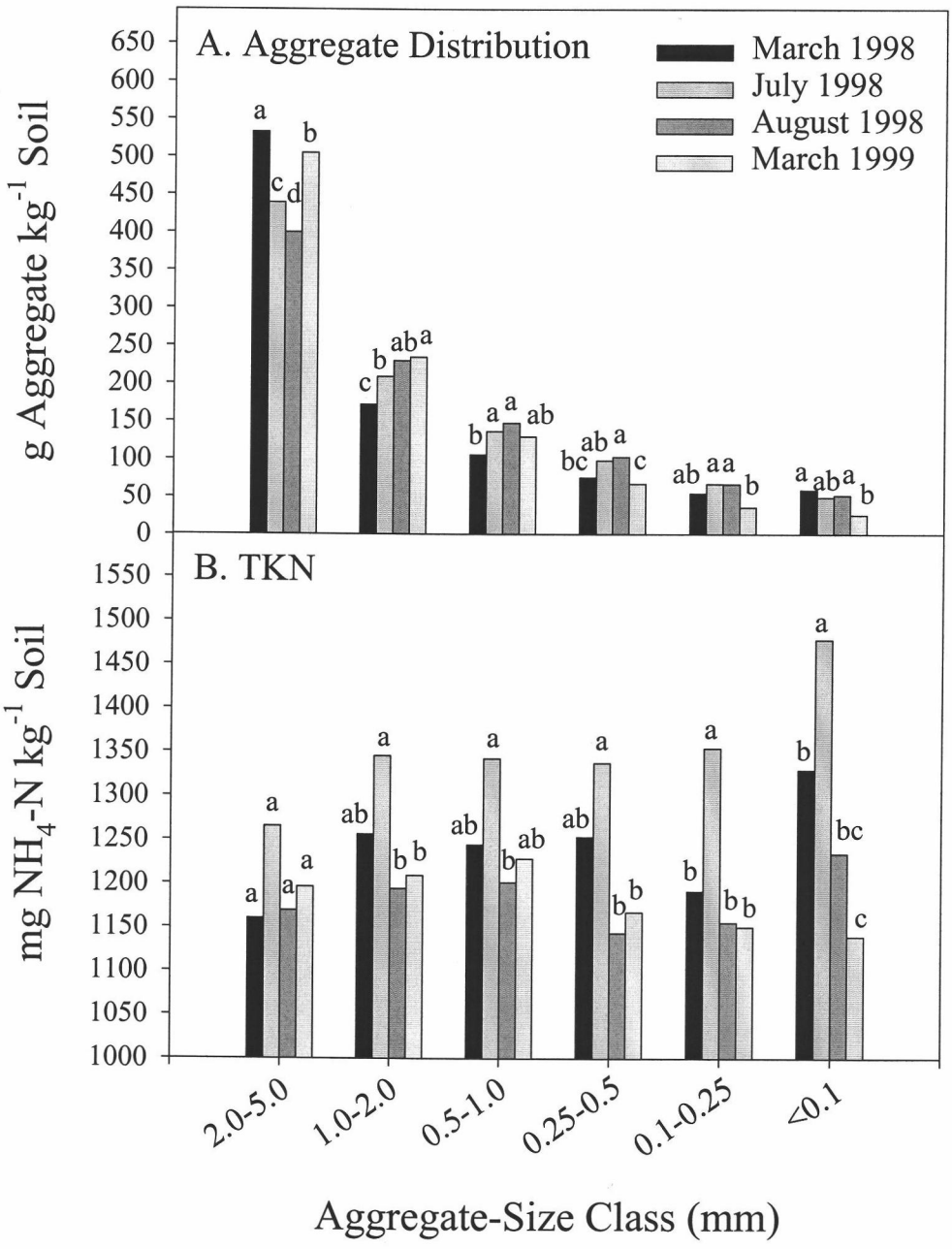

Fig. 5.1. Change in aggregate distribution of soil mass (A) and total Kjeldahl N (TKN) (B) among aggregate size classes over the course of one growing season. For each aggregate size class, means followed by the same letter are not significantly different at $p < 0.05$.

Table 5.1. Effect of aggregate size on total Kjeldahl N in winter-fallow and winter cover-cropped soil in March, July, August 1998, and March 1999.

	March 1998		July 1998		August 1998		March 1999	
Aggregate	Fallow	Cover	Fallow	Cover	Fallow	Cover	Fallow	Cover
mm	----------mg N kg^{-1} soil----------							
2.0-5.0	1147a[†]	1173a	1274b	1357b	1100a	1239a	1152a	1240a
1.0-2.0	1240a	1273a	1269b	1422b	1125a	1263a	1169a	1249a
0.5-1.0	1232a	1256a	1275ab	1409b	1119a	1282a	1214a	1242a
0.25-0.5	1266a	1239a	1233b	1443b	1065a	1221a	1143a	1190a
0.1-0.25	1137a	1245a	1254b	1455ab	1066a	1244a	1090a	1210a
<0.1	1267a	1392a	1392a	1565a	1155a	1313a	1098a	1180a

[†]Within a column, values followed by the same lowercase letter are not significantly different at $p < 0.05$.

levels were higher in July 1998 in all aggregate classes except for the 2.0-5.0 mm size class (Fig. 5.1B). Although not statistically significant, TKN levels of whole soils were generally higher in cover-cropped soil compared to fallow soil in July ($p = 0.06$) and August ($p = 0.09$) 1998 (Table 5.1).

Microbial Biomass Carbon

Microbial biomass C in whole soil was significantly affected by winter cover cropping, with higher biomass levels in cover-cropped soil compared to fallow soil in July and August 1998 (Table 5.2). Sampling date and the interaction between date and cover cropping treatment also were significant factors affecting MB_C. Although MB_C tended to decline after March 1998, biomass levels were maintained and even stimulated in cover-cropped soil in July and August 1998 (Table 5.2). In addition, MB_C differed significantly among aggregate size classes for all sampling dates except March 1999 (Table 5.3). In March 1998, MB_C was highest in the 0.5-1.0 mm aggregate size class for both cover-cropped and fallow treatments. In July 1998, MB_C increased in the smallest aggregate size class for the fallow soil, but did not differ significantly among aggregates in the cover-cropped soil. For each aggregate size class in July 1998, MB_C was significantly higher in cover-cropped soil compared to fallow soil (Table 5.3). A similar trend occurred in August 1998, with higher MB_C in cover-cropped soil in each aggregate size class. The effect of aggregate size was significant at this sampling date as well, with the lowest levels of MB_C occurring in 2.0-5.0 mm aggregate size class for both treatments.

Table 5.2. Effects of winter cover cropping on whole-soil biological properties in March, July, August 1998, and March 1999. Significant effects of sampling date also are indicated.

Treatment	MBC[†]	Respiration	N min.	FAME H'	FAME Richness	Bacteria: Fungi	Protozoa 20:4ω6*c*	Biolog H'
	mg C kg^{-1}	mg CO_2-C kg^{-1}	mg NH_4-N kg^{-1}				Relative%	
				March 1998				
Fallow	231a[‡]	57.2b	29.8a	2.93b	29b	3.41b	2.18a	4.28a
Cover	253a	73.1a	31.6a	3.04a	32a	4.39a	1.67b	4.29a
				July 1998				
Fallow	133b	33.7b	15.2b	2.79b	23b	3.29b	1.19b	4.06b
Cover	222a	53.1a	31.4a	2.95a	29a	4.31a	1.41a	4.23a
				August 1998				
Fallow	188b	34.1b	12.6b	2.86b	25b	3.63b	1.24a	4.20b
Cover	315a	51.6a	20.9a	2.94a	28a	4.79a	1.20a	4.29a

Table 5.2 (continued). Effects of winter cover cropping on whole-soil biological properties in March, July, August 1998, and March 1999. Significant effects of sampling date also are indicated.

Treatment	MBC†	Respiration	N min.	FAME H'	FAME Richness	Bacteria: Fungi	Protozoa 20:4ω6*c*	Biolog H'
	mg C kg^{-1}	mg CO_2-C kg^{-1}	mg NH_4-N kg^{-1}				Relative%	
				March 1999				
Fallow	153a	15.5b	21.7b	2.84b	28a	5.52b	1.11a	4.24a
Cover	184a	29.7a	34.5a	2.95a	28a	8.06a	1.18a	4.19a
LSD, Date	42	9.8	6.7	0.07	3	0.88	0.21	0.07

†MB_C, microbial biomass C; N min., N mineralization potential; FAME H', FAME diversity; Bacteria:Fungi, ratio of bacterial to fungal FAMEs; Protozoa, relative percent of FAME marker 20:4ω6*c*.

‡Within columns of each season, values followed by same letter are not significantly different at $p < 0.05$.

Table 5.3. Effect of aggregate size on microbial biomass C in winter-fallow and winter cover-cropped soil in March, July, August 1998, and March 1999.

	March 1998		July 1998		August 1998		March 1999	
Aggregate	Fallow	Cover	Fallow	Cover	Fallow	Cover	Fallow	Cover
				Microbial biomass C				
mm	---mg C kg^{-1} soil---							
2.0-5.0	242bc[†]	258bc	121c B[‡]	207a A	158b B	276c A	160a	169a
1.0-2.0	273b	300ab	134bc B	216a A	196ab B	326ab A	151a	199a
0.5-1.0	321a	312a	157ab B	240a A	211a B	351a A	159a	199a
0.25-0.5	270b	273abc	146b B	231a A	186ab B	326ab A	189a	184a
0.1-0.25	240bc	255c	159ab B	237a A	200ab B	291bc A	168a	202a
<0.1	220c	250c	183a B	225a A	205a B	327ab A	161a	174a

[†]Values followed by the same lowercase letter within a column are not significantly different at $p < 0.05$.

[‡]Within rows of each sampling date, values followed by the same uppercase letter are not significantly different at $p < 0.05$.

Microbial Respiration and N Mineralization

For all sampling dates, microbial respiration in whole soil was significantly greater in winter cover-cropped treatment compared to the fallow treatment (Table 5.2). Microbial respiration also changed significantly over time, with lower respiration activities in the July and August sampling dates compared to March 1998. In addition, microbial respiration varied significantly among aggregate size classes at all sampling dates (Table 5.4). In March 1998, respiration was greater in the intermediate sizes of aggregates (0.5-1.0 and 0.25-0.5 mm). In July and August, there was a shift towards greater activity in the smaller aggregate size classes, with greatest activities in the <0.10 mm fraction occurring in July 1998. In addition, microbial respiration was greater in aggregates of cover-cropped plots than in the fallow plots at the July and August sampling dates (Table 5.4). This trend continued in March 1999 ($p = 0.08$).

Trends of N mineralization potential in whole soils and aggregates were similar to those of microbial respiration. At all sampling dates except March 1998, N mineralization potential of whole soils was significantly greater in cover-cropped than in fallow plots. Impacts of sampling date were significant as well, with greater net N mineralization occurring in spring than in the summer. Mineralization potentials also differed across aggregate size classes for all sampling dates except March 1999 (Table 5.5). In general, N mineralization was lowest in the two largest aggregate sizes (1.0-2.0 and 2.0-5.0 mm). Mineralization was greatest in the intermediate size fraction (0.5-1.0 mm) for fallow soils in March 1998, but at other sampling dates, N mineralization was also high in the smaller aggregate size classes (Table 5.5). As with

Table 5.4. Effect of aggregate size on microbial respiration in winter-fallow and winter cover-cropped soil in March, July, August 1998, and March 1999.

	March 1998		July 1998		August 1998		March 1999	
Aggregate	Fallow	Cover	Fallow	Cover	Fallow	Cover	Fallow	Cover
				Microbial respiration				
mm	----------	----------	----------	mg CO_2-C kg^{-1} soil	----------	----------	----------	----------
2.0-5.0	57.9c[†]	53.0c	27.5c B[‡]	38.2c A	27.3bc B	42.0d A	20.0b	26.1c
1.0-2.0	87.3bc	91.8ab	29.6bc B	52.8b A	25.9c B	49.6cd A	21.3b	27.7bc
0.5-1.0	131a	120a	36.9b B	56.9ab A	38.6a B	71.1a A	21.5ab	30.0abc
0.25-0.5	110ab	105ab	34.0bc B	56.4ab A	39.7a B	70.5a A	24.6ab	35.1a
0.1-0.25	76.4c	76.9bc	35.1bc B	57.2ab A	34.3ab B	61.2b A	27.3a	32.2a
<0.1	83.4bc	77.6bc	47.6a B	64.3a A	33.2ab B	56.5bc A	25.6ab	31.4abc

[†]Within columns, values followed by the same lowercase letter are not statistically significant at $p < 0.05$.

[‡]Within rows of each sampling date, values followed by the same uppercase letter are not statistically significant at $p < 0.05$.

Table 5.5. Effect of aggregate size on microbial N mineralization potential in winter-fallow and winter cover-cropped soil in March, July, August 1998, and March 1999.

	March 1998		July 1998		August 1998		March 1999	
Aggregate	Fallow	Cover	Fallow	Cover	Fallow	Cover	Fallow	Cover
				N mineralization potential				
mm	----------	----------	----------	mg NH_4-N kg^{-1} soil	----------	----------	----------	----------
2.0-5.0	26.3c[†]	24.9b	13.4b B[‡]	27.1b A	10.3b A	15.5c A	21.1a	28.3a
1.0-2.0	32.1abc	35.7a	13.1b B	27.6b A	10.9b B	19.1c A	23.1a	29.6a
0.5-1.0	38.2a	36.7a	18.2ab B	35.1a A	12.0b B	26.3b A	26.6a	32.7a
0.25-0.5	35.8ab	33.9a	20.6a B	33.4a A	15.8ab B	32.5ab A	28.4a	32.3a
0.1-0.25	30.2abc	33.4ab	19.1a B	38.3a A	15.7ab B	30.6b A	25.9a	33.0a
<0.1	28.8bc	29.9ab	22.8a B	38.4a A	20.2a B	38.7a A	27.7a	32.0a

[†]Within columns, values followed by the same lowercase letter are not statistically significant at $p < 0.05$.

[‡]Within rows of each sampling date, values followed by the same uppercase letter are not statistically significant at $p < 0.05$.

microbial respiration, N mineralization potential was greatest in aggregates from the cover-cropped treatment compared to the fallow in July and August 1998.

Microbial Community FAME Profiles

In whole soils, winter cover cropping resulted in greater FAME diversity and richness and wider bacterial:fungal FAME ratios (Table 5.2). This trend was observed at most sampling dates, except for FAME richness in March 1999. Effects of time were significant as well, with greater FAME richness and diversity in March 1998 compared to other sampling dates. The protozoan marker was also significantly affected by winter cover cropping and by sampling date. Relative amounts of the protozoa FAME marker was greater in the fallow soil compared to cover-cropped soil in March 1998 (Table 5.2). However, by July 1998, levels were significantly greater in cover-cropped soil than in fallow soil, despite a general decline in levels over the course of the growing season.

FAME diversity differed among aggregate size classes at the July 1998 and March 1999 sampling dates (Table 5.6). In July, FAME diversity in the fallow treatment was greatest in the intermediate size fraction (0.5-1.0 mm), whereas FAME diversity in the cover-cropped treatment was greatest in the smaller aggregate size classes (0.1-0.25 and <0.1 mm). In March 1999, differences in FAME diversity based on aggregate size were observed only in the cover-cropped plots, where FAME diversity was lower in the largest aggregate size class (2.0-5.0 mm) compared to other size classes. In addition, winter cover-cropping significantly increased FAME diversity in soil aggregates in July 1998 and March 1999. In March 1998, the trend

Table 5.6. Effect of aggregate size on FAME diversity (H')[†] in winter-fallow and winter cover-cropped soil in March, July, August 1998, and March 1999.

	March 1998		July 1998		August 1998		March 1999	
Aggregate	Fallow	Cover	Fallow	Cover	Fallow	Cover	Fallow	Cover
				FAME Diversity (H')				
mm								
2.0-5.0	2.89a[‡]	3.02a	2.69bc B[§]	2.84b A	2.78a	2.91a	2.84a B	2.93b A
1.0-2.0	2.93a	3.04a	2.81ab A	2.94ab A	2.82a	2.94a	2.84a B	2.97ab A
0.5-1.0	2.99a	3.05a	2.88a A	2.94ab A	2.79a	2.90a	2.86a B	3.02a A
0.25-0.5	2.93a	3.02a	2.72bc B	2.88ab A	2.90a	2.95a	2.85a B	2.99a A
0.1-0.25	2.93a	3.03a	2.65c B	3.01a A	2.86a	2.97a	2.82a B	2.97ab A
<0.1	2.97a	3.05a	2.74bc B	2.99a A	2.87a	2.86a	2.81a B	2.99a A

[†]FAME diversity = Shannon's index (H') = $-\sum (Pi \times \log Pi)$, where Pi is the proportional amount of each FAME.

[‡]Within columns, values followed by the same lowercase letter are not statistically significant at $p < 0.05$.

[§]Within rows of each sampling date, values followed by the same uppercase letter are not statistically significant at $p < 0.05$.

for greater FAME diversity in aggregates of the cover-cropped treatment was significant at $p = 0.10$.

FAME richness changed significantly across aggregate size classes in July 1998 (Table 5.7). For the winter-fallow treatment, FAME richness was highest in the 0.5-1.0 and <0.1 mm size classes, whereas in the cover-cropped treatment, FAME richness was highest in the two smallest aggregate size classes (0.1-0.25 and <0.1 mm). Also in July 1998, there was a cover crop effect on FAME richness for the two smallest aggregate fractions (0.1-0.25 and <0.1 mm), where FAME richness was significantly higher in cover-cropped versus fallow treatment. In addition, FAME richness varied in March and August 1998 among aggregate size classes from the fallow treatment, with lower numbers of FAMEs detected in the 2.0-5.0 mm size aggregate (Table 5.7). In the cover-cropped treatment in March 1999, FAME richness varied significantly among the aggregates, with lower richness in the largest size class.

The ratio of bacterial:fungal FAME markers was affected significantly by aggregate size at all sampling dates except July 1998 (Table 5.8). In March 1998, this ratio was highest in the <0.1 mm size class. In August 1998 and March 1999, differences based on aggregate size were observed only in the cover-cropped plots, where again the ratio was widest in the <0.1 mm size class. Effects of winter cover crops on bacterial:fungal FAME ratios were statistically significant only at the last sampling date, with wider ratios occurring in aggregates from the cover-cropped treatment compared to the fallow treatment (Table 5.8). This trend was also observed in the July and August sampling dates and was significant at $p < 0.10$.

Table 5.7. Effect of aggregate size on FAME richness in winter-fallow and winter cover-cropped soil in March, July, August 1998, and March 1999.

	March 1998		July 1998		August 1998		March 1999	
Aggregate	Fallow	Cover	Fallow	Cover	Fallow	Cover	Fallow	Cover
				FAME Richness				
mm								
2.0-5.0	29c[†]	31a	21b A[‡]	24b A	22c	26a	27a A	27c A
1.0-2.0	30bc	32a	25ab A	29ab A	23bc	28a	27a A	29bc A
0.5-1.0	34a	33a	29a A	29ab A	23b	27a	28a B	32a A
0.25-0.5	32abc	32a	23b A	27ab A	28a	29a	29a A	30ab A
0.1-0.25	32abc	33a	20b B	32a A	27ab	29a	28a A	30ab A
<0.1	33ab	32a	24a B	30a A	27ab	25a	29a A	31ab A

[†]Within columns, values followed by the same lowercase letter are not statistically significant at $p < 0.05$.

[‡]Within rows of each sampling date, values followed by the same uppercase letter are not statistically significant at $p < 0.05$.

Table 5.8. Effect of aggregate size on the ratio of bacterial-to-fungal FAMEs[†] in winter-fallow and winter cover-cropped soil in March, July, August 1998, and March 1999.

	March 1998		July 1998		August 1998		March 1999	
Aggregate	Fallow	Cover	Fallow	Cover	Fallow	Cover	Fallow	Cover
				Bacteria:Fungi				
mm								
2.0-5.0	3.08b[‡]	4.08b	2.73a	3.54a	3.64a	5.28ab	5.17a B[§]	8.48b A
1.0-2.0	3.30b	3.80bc	3.11a	4.00a	3.73a	4.86bc	5.79a B	9.01b A
0.5-1.0	3.02b	3.61c	3.04a	4.02a	3.42a	4.34d	5.83a B	8.81b A
0.25-0.5	2.86b	3.65bc	2.60a	3.85a	3.20a	4.49c	5.31a B	8.94b A
0.1-0.25	3.23b	3.92bc	2.64a	4.26a	3.31a	5.01bc	5.27a B	8.65b A
<0.1	3.82a	5.11a	3.09a	4.76a	3.78a	5.81a	5.99a B	10.1a A

[†]Bacterial FAMEs were the sum of *i*15:0, *a*15:0, 15:0, *i*16:0, 16:1ω*c*, *i*17:0, *a*17:0, 17:0 cy, 17:0, 18:1ω7*c*/9*t*/12*t*, and 19:0 cy. The fungal FAME was the relative amount of 18:2ω6*c*.

[‡]Within columns, values followed by the same lowercase letter are not statistically significant at $p < 0.05$.

[§]Within rows of each sampling date, values followed by the same uppercase letter are not statistically significant at $p < 0.05$.

Effects of winter cover cropping or aggregate size on the protozoan marker were relatively few. The FAME 20:4ω6*c* was affected by aggregate size only at the July 1998 sampling date (Table 5.9). In the fallow treatment at this time, this marker was not detected in the 0.1-0.25 mm aggregate size class. When the other aggregate size classes were compared, relative amounts of 20:4ω6*c* was lowest in the largest aggregate size class (2.0-5.0 mm). Winter cover crops also significantly affected this FAME marker at the July sampling date. In the fallow treatment, relative amounts of 20:4ω6*c* were lower in the 2.0-5.0, 0.25-0.5, and 1.0-0.25 mm size classes compared to the cover-cropped treatment.

Principal components analysis was performed on aggregate community FAME data for each sampling date to determine if winter cover cropping and aggregate size affected the microbial community structure. Results from July 1998 are shown in Figure 5.2. Overall, the cover-crop treatment was more influential than aggregate size in separating microbial communities. In Fig. 5.2, communities from cover-cropped soils generally were ordinated to the left of communities from the winter-fallow soils. Similarly, PCA of FAME data from the other sampling dates resulted in the separation of communities according to cover-crop treatment (plots not shown). To assess if community structure was affected by aggregate size within a treatment, aggregate FAME data from fallow soils were analyzed separately from FAME data of cover-cropped soils for each sampling date. Results from July 1998 are shown in Figure 5.3 as an example. For either treatment, no clear pattern was observed in community FAME profiles based on aggregate size class, and variability among plot replicates was often as great as the variability among aggregate size classes.

Table 5.9. Effect of aggregate size on protozoan FAME marker (20:4ω6*c*) in winter-fallow and winter cover-cropped soil in March, July, August 1998, and March 1999.

	March 1998		July 1998		August 1998		March 1999	
Aggregate	Fallow	Cover	Fallow	Cover	Fallow	Cover	Fallow	Cover
				20:4ω6*c*				
mm	----------Relative %----------							
2.0-5.0	1.96a[†]	1.84a	0.73b B[‡]	1.81a A	0.56a	1.10a	1.23a	1.15a
1.0-2.0	1.87a	1.57a	1.22a A	1.52a A	0.72a	0.87a	1.13a	1.13a
0.5-1.0	1.82a	1.77a	1.21a A	1.52a A	0.33a	0.54a	1.08a	1.05a
0.25-0.5	1.25a	1.57a	0.91a B	1.32a A	1.18a	1.09a	1.06a	1.03a
0.1-0.25	2.15a	1.48a	0.00c B	1.37a A	0.86a	0.87a	1.14a	1.06a
<0.1	1.25a	1.62a	1.32a A	1.46a A	0.97a	0.67a	1.16a	1.06a

[†]Within columns, values followed by the same lowercase letter within columns are not statistically significant at $p < 0.05$.

[‡]Within rows of each sampling date, values followed by the same uppercase letter are not statistically significant at $p < 0.05$.

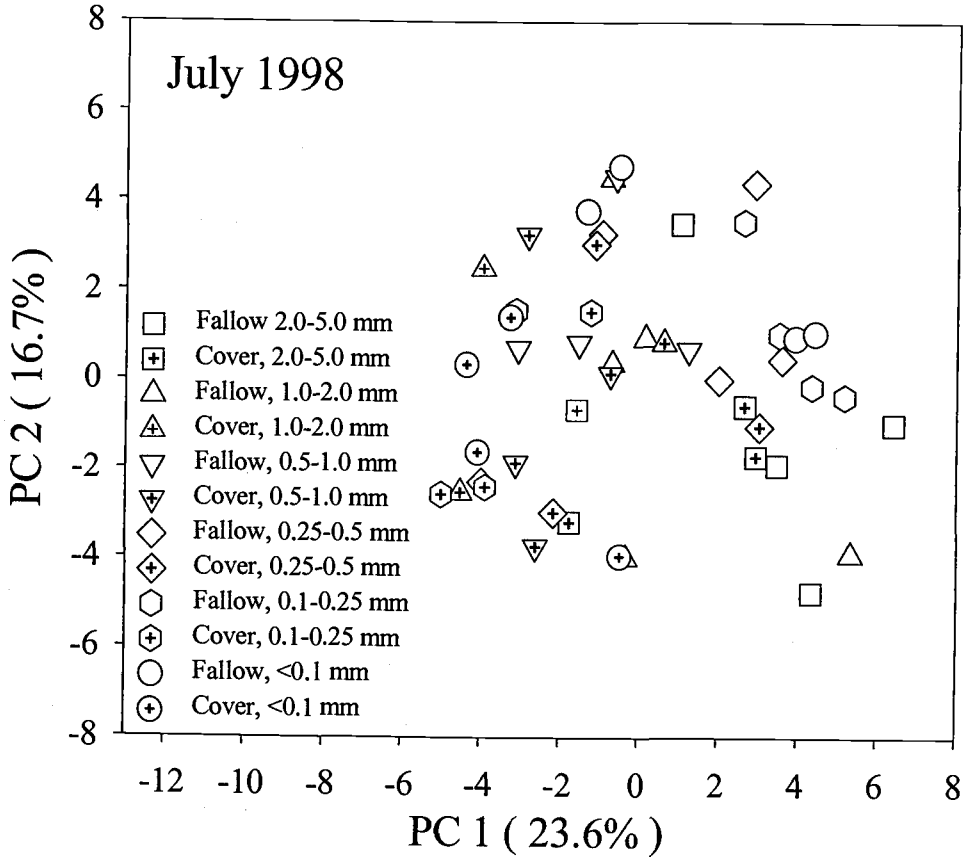

Fig. 5.2. Principal components analysis of microbial community FAME profiles across aggregates of winter-fallow and winter cover-cropped soil in July 1998. Percent variance explained by each axis is shown in parentheses.

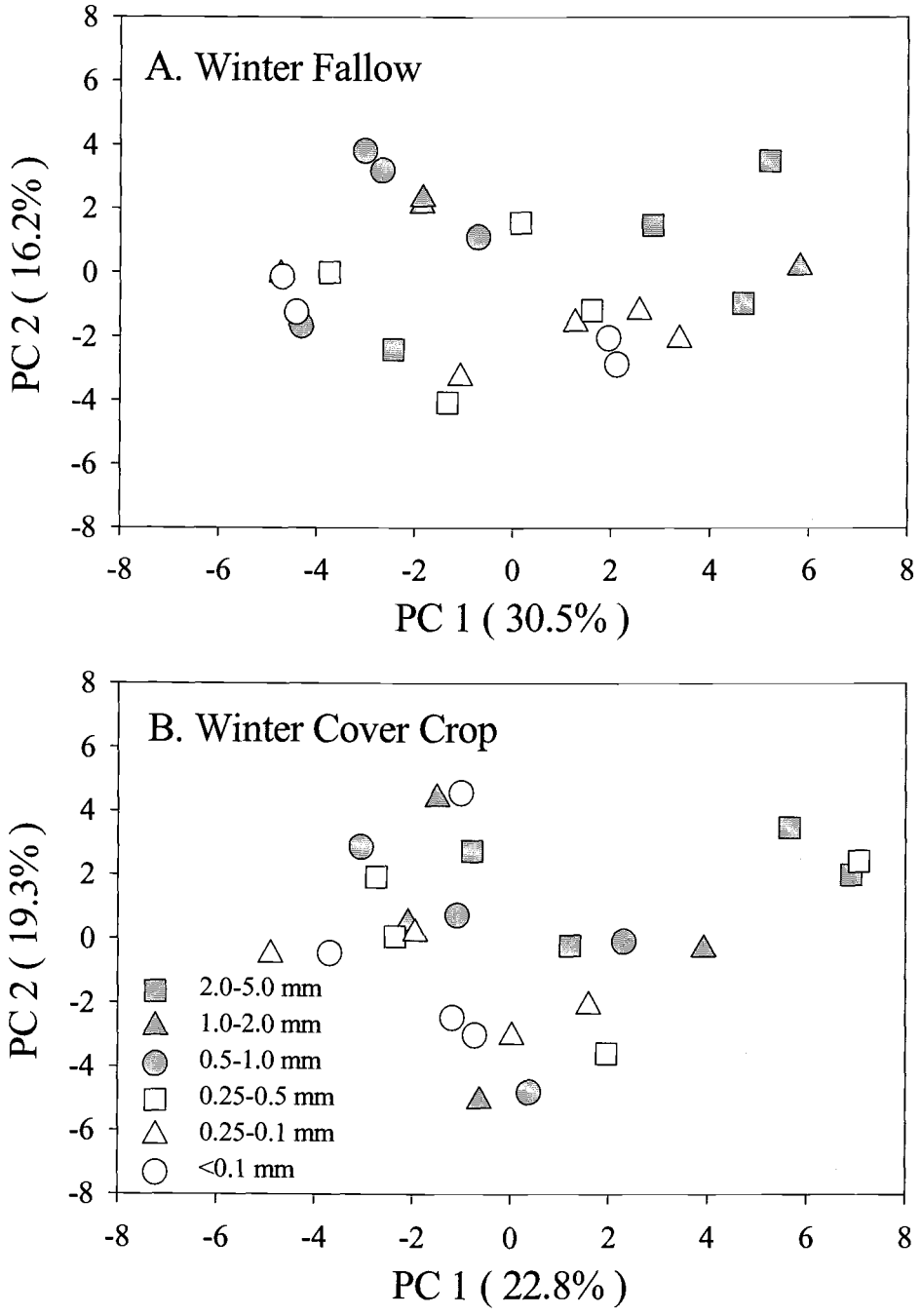

Fig. 5.3. Principal components analysis of microbial community FAME profiles across aggregates of winter-fallow (A) and winter cover-cropped (B) soil in July 1998. Percent variance explained by each axis is shown in parentheses.

Microbial Community Substrate Utilization Patterns

Substrate utilization in whole soils was significantly affected by winter cover crops after residues were incorporated into soil. In July and August 1998, a greater diversity of Biolog substrates was utilized by microbial communities from cover-cropped soil compared to fallow soil (Table 5.2). Biolog activity also changed over time, with the greatest diversity of substrates utilized in March 1998 compared to the July 1998 and March 1999 sampling dates. In addition, Biolog activity differed significantly among aggregate size classes for all sampling dates except August 1998 (Table 5.10). In general, greater diversity of substrates were utilized in the intermediate aggregate size classes (0.25-0.5 and 0.5-1.0 mm), but there was an exception in July 1998, where diversity was greatest in the 0.1-0.25-mm fraction for winter-fallow soils. In August 1998, there was a significant effect of winter cover cropping on Biolog activity, with greater substrate use diversity in the 1.0-2.0 mm size aggregates of the cover-crop treatment compared to same-size aggregates of the fallow treatment.

Table 5.10. Effect of aggregate size on Biolog diversity (H')[†] in winter-fallow and winter cover-cropped soil in March, July, August 1998, and March 1999.

	March 1998		July 1998		August 1998		March 1999	
Aggregate	Fallow	Cover	Fallow	Cover	Fallow	Cover	Fallow	Cover
				Biolog Diversity (H')				
mm								
2.0-5.0	4.26ab[‡]	4.26ab	4.12bc	4.04c	4.17a A[§]	4.26a A	4.16ab	4.21a
1.0-2.0	4.25ab	4.30ab	4.23ab	4.19ab	4.19a B	4.32a A	4.12ab	4.18a
0.5-1.0	4.30a	4.32a	4.24ab	4.19ab	4.23a A	4.31a A	4.16ab	4.11ab
0.25-0.5	4.30a	4.28ab	4.22ab	4.28a	4.21a A	4.29a A	4.22a	4.22a
0.1-0.25	4.22b	4.24b	4.27a	4.24ab	4.23a A	4.24a A	4.18ab	4.16ab
<0.1	4.20b	4.24b	4.03c	4.10bc	4.17a A	4.26a A	4.09b	4.05b

[†]Biolog diversity = Shannon's index (H') = $-\sum (Pi \times \log Pi)$, where Pi is the proportional absorbance value of each Biolog well.

[‡]Within columns of each sampling date, values followed by the same lowercase letter are not statistically significant at $p < 0.05$.

[§]Within rows of each sampling date, values followed by the same uppercase letter are not statistically significant at $p < 0.05$.

Discussion

Microbial Distribution Patterns Across Aggregates

In this study, microbial biomass, respiration, N mineralization potential, and community diversity indices were heterogeneously distributed among soil aggregates of different sizes. For example, carbon substrate utilization activities differed among soil aggregates, with greater activities often associated with intermediate aggregate sizes (0.25-0.5 and 0.5-1.0 mm). Winding (1994) also reported differences in community Biolog patterns across soil aggregates, with strong differences in utilization activity between micro- and macroaggregate communities. Although FAME richness and diversity fluctuated across aggregates, there were no consistent differences in FAME profiles between aggregate sizes. Petersen et al. (1997) also found few differences in microbial community phospholipid patterns among different sizes of soil macroaggregates. They suggested that cultivated soils which experience frequent tillage events quickly reach an equilibrium where microbial communities become evenly distributed among macroaggregates.

In this study, distribution patterns for some microbial properties changed over the course of the growing season. For example, in March 1998, biomass C and N mineralization potential were greatest in intermediate size aggregates (0.25-0.5 and 0.5-1.0 mm), but in July and August, levels in aggregates <0.1 mm were as great, if not greater, than in the intermediate aggregates. Similarly, greater amounts of CO_2 were evolved from aggregates <0.1 mm compared to larger aggregates in July 1998. High biomass and respiration rates associated with particular size classes of

aggregates usually are attributed to the presence of labile C substrates and a physical habitat which provides protection from predators (Elliott, 1986; Gupta and Germida, 1988; Beauchamp and Seech, 1990; Mendes et al., 1999). Although TOC levels did not differ significantly among aggregate sizes, TKN concentrations were significantly greater in the <0.1-mm aggregates compared to larger aggregates in July. Similarly, Miller and Dick (1995) reported greater biomass C values in aggregates with greater total N content.

Although microaggregates may protect offer protection from predators, the habitats provided by microaggregates generally have been described as harsh, containing low levels of biologically recalcitrant organic matter with turnover rates of hundreds of years (Elliott, 1986; Angers and Giroux, 1996; Jastrow et al., 1996; Monreal et al., 1997). Poor substrate quality has been used to explain lower microbial biomass and activities in microaggregates compared to macroaggregates (Elliott, 1986; Gupta and Germida, 1988; Franzluebbers and Arshad, 1997). Such trends often were observed when soils were separated into aggregates by wet-sieving. However, other studies have reported high microbial biomass levels and activities in microaggregates when soils were dry sieved to separate aggregate size classes (Seech and Beauchamp, 1988: Mendes and Bottomley,1998; Mendes et al., 1999). Seech and Beauchamp (1988) suggested that low levels of activity were found in wet-sieved microaggregates because water-soluble C may have been removed from microaggregates during the wet-sieving process. In a later study, they found that microbial denitrification activity decreased as the dry-sieved aggregate size increased but that the rate increased as the wet-sieved aggregate size increased. In both cases,

however, denitrification activity was correlated with mineralizable C, and presumably thus labile C substrate (Beauchamp and Seech, 1990). In our study, high respiration and TKN levels in the smallest aggregates are indicative of the presence of labile substrates in microaggregates during the July sampling period.

Greater biomass in microaggregates also has been attributed to absorption of bacterial cells to clays in microaggregate size fraction (Van Gestel et al., 1996), but this seems unlikely in our study as clay content did not differ significantly between <0.1, 0.25-0.5, and 1.0-2.0 mm aggregate size classes. It also is possible that the porosity and texture of microaggregates may protect microorganisms from dessication, but such protection did not occur in several studies (Van Gestel et al., 1991; West et al., 1992). Alternatively, it is possible that in this study, some of the < 0.1-mm microaggregates were loosely held to the surfaces of macroaggregates and disassociated from the surface during the sieving process. A substantial portion of the soil biomass is thought to live on or near the aggregate surface (Oades, 1984), which may explain the high biomass in microaggregates <0.1 mm if they originated on macroaggregate surfaces. In addition, protozoan grazing may be restricted to surfaces of microaggregates because of pore size exclusion (Hattori, 1988). In our study, the protozoan FAME marker was detected in aggregates <0.1 mm but not in the 0.1-0.25 mm aggregate size class. It is possible that protozoa were restricted from the pores of the 0.1-0.25 mm size microaggregate but were detected in <0.1 mm size microaggregates that coated the surface of larger aggregates.

Impact of Winter Cover Cropping on Microbial Distribution Patterns

In general, winter cover crops increased microbial biomass, activities, and diversity indices in whole soils, but for many microbial properties, this effect was not observed until July or August 1998, after cover crops were incorporated into soil. For at least one sampling date, the presence of cover crop residues increased MB_C, respiration, N mineralization potential, FAME diversity, FAME richness, and the amounts of the protozoan FAME marker in most aggregate size classes. These positive responses are indicative of a substrate limitation in whole soils and aggregates which was ameliorated by the addition of labile cover crop residues. Similar findings were reported by Miller and Dick (1995) and Mendes et al. (1999) at a different experimental research station in Oregon, where establishment of a legume cover crop shortly was followed by increases in microbial biomass C and labile organic matter pools (particulate and dissolved organic C) (Miller and Dick, 1995). Distributions of microbial biomass C, C and N mineralization, and enzyme activities among soil aggregates also were affected by cover crop residues (Mendes et al., 1999) as in our study.

In conclusion, the effect of winter cover cropping on microbial spatial distribution patterns can be complex. In some cases, the presence of cover crop residues can increase soil microbial biomass and activities across all aggregate size classes, as shown by Mendes et al. (1999) and this study. Cover crop residues can also affect microbial populations within certain aggregate size classes. For example, Mendes and Bottomley demonstrated that the distribution of *Rhizobium* serotypes among aggregate size classes was differentially affected by winter cover cropping

treatment. In this study, cover crop residues increased the number of extractable FAMEs only in aggregates <0.25 mm in July and in the 0.5-1.0 mm aggregate size class in March 1999. Also, increases in the protozoan FAME marker in response to winter cover cropping occurred only specific aggregate size classes (2.0-5.0, 0.25-0.5, and 0.1-0.25 mm). Such results are indicative of the complex relationships among environmental factors (time, physical habitat, and the presence of decomposing residues) and their impacts on microbial distribution patterns in soil. Nevertheless, aggregate studies are useful for identifying sources of heterogeneity in soil biological properties and are particularly beneficial in studies involving alternative management practices which may alter the distribution of soil aggregate sizes.

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SUMMARY

The goal of this thesis was to identify the major impacts of cover cropping on soil microbial community structure, diversity, and substrate utilization potential. Of interest was determining whether microbial communities respond quickly to winter cover cropping practices. If so, then it may be possible to monitor shifts in microbial community structure as means to indicate changes in soil quality. The studies presented here suggest that residues of cover crops generally resulted in greater microbial FAME and Biolog diversity in soil, although several years of cover cropping were required before strong shifts in community structure are observed. Although the types of FAMEs that responded to cover crops differed between fields, there was a general trend for increases in amounts of fungal and protozoan FAME markers in soils containing cover crop residues. In addition, Biolog diversity was greater in cover-cropped versus fallow soils of the experimental research stations and the grower fields overall. Overall, cover crop residues were important for maintaining levels of diversity in whole soils and among aggregates over time, and eukaryotic FAME markers and Biolog diversity may be useful indicators of soil quality change as a result of cover crop residue inputs.

This thesis also identified the major determinants of soil microbial community structure and Biolog activity in the soils studied. The strongest influence was that of season. Soils in early spring, just prior to termination of the cover crop, utilized fewer carboxylic acids and generally were enriched in eukaryotic FAMEs. At canopy closure, which was approximately six weeks after planting of the vegetable crop,

bacterial FAME markers dominated community FAME profiles. The next strongest influence, after season, was that of soil type. Within a season, community FAME and Biolog patterns were often correlated with soil textural properties, CEC, and carbon content. This was especially true for the early spring and harvest seasons. The effect of winter cover cropping was observed when FAME profiles from one soil type within a season were analyzed. For individual grower fields, there were relatively few effects of winter cover cropping on community FAME profiles, except for the Corvallis field. However, cover cropping was implemented only one or two years prior to the study. Stronger impacts of winter cover crops were observed at the Vegetable Research Station, where the cover cropping treatment was established five years ago. Soil aggregates at the Vegetable Research Station were also examined to determine the influence of aggregate size on microbial communities. In the fallow treatment, FAME diversity was heterogeneously distributed among aggregates, with diversity greatest in the intermediate (0.25-0.5 and 0.5-1.0 mm) and smallest (<0.1 mm) aggregate size classes. Ratios of bacterial:fungal FAMEs and relative amounts of the protozoan FAME marker (20:4ω6c) also were heterogeneously distributed among aggregates at some sampling dates. In cover cropped soils, however, FAME diversity was elevated in all aggregate size classes except for the largest. When FAME profiles were analyzed to determine the overall impacts of aggregate size on community structure, it was found that although aggregate communities could be separated according to winter cover cropping or fallow treatment, there was no clear trend of aggregate size influences on community structure.

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