To the Graduate Council:

I am submitting herewith a dissertation written by Michael Joseph Kirchner entitled "The Effect of Conversion from Annual Row Crops to Short-rotation Woody Crops on Soil Microbial Community Structure." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant and Soil Sciences.

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THE EFFECT OF CONVERSION FROM ANNUAL ROW CROPS TO SHORT-ROTATION WOODY CROPS ON SOIL MICROBIAL COMMUNITY STRUCTURE

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Abstract

Short-rotation woody crops (SRWC) are being considered as an alternative to burning coal for power production. Research is being conducted in West Tennessee to examine the environmental impact of converting from a traditional corn silage system to growing sycamore (*Platanus occidentalis* L.) as a SRWC. The objective of this study was to determine what effect this conversion would have on soil microbial community structure, total microbial biomass and activities of soil microorganisms. Since soil microorganisms control the biogeochemical cycling of C, N, O, H and S in the environment, it is essential to understand how changes in crop management systems will influence these organisms.

In recent years a number of authors have expressed concerns about the usefulness of microbiological procedures such as plate-counting to describe microbial populations in the environment. In this study a battery of tests were used to measure soil biological properties. These included such traditional procedures as measuring microbial biomass by chloroform fumigation-extraction, and measuring microbial activities using enzyme assays. Several molecular and biochemical techniques have recently been developed which promise greater information and more accurate measurements of biological properties in environmental samples. Phospholipid fatty acid (PLFA) analysis and nucleic acid hybridization are two such techniques which were used in this study.

Treatments examined to show the effects of this conversion included a corn silage/winter wheat system, a 2-3 year-old plantation of sycamore (called "young sycamore"), a 14-15 year-old plantation of sycamore (called "old sycamore"), and a mature hardwood forest. Soil samples were collected at four dates from April 1997 to August 1998. All tests showed that the old sycamore plantation and the mature forest had significantly more soil microbial biomass and the corn and young sycamore soil. In addition, the conversion from row-cropping to SRWC caused shifts in the soil microbial community structure. The microbial community in the old sycamore and mature forest had a larger population of Gram-negative bacteria, and showed greater microbial activity than the corn or young sycamore systems. Few differences were found in fungi or

actinomycetes between treatments. The older forest systems had significantly more organic C and N, as well as water-extractable C than the corn or young sycamore systems. Although the young sycamore system may become more like the old sycamore once the litter layer is well established and leaves begin to decompose, for the first few years of tree growth the microbial community in the young sycamore plantation is smaller, less diverse, and less active than the community in the old sycamore plantation.

This study showed good correlation between PLFA, chloroform fumigation-extraction, and dehydrogenase as measures of microbial biomass. Although nucleic acid hybridization typically correlated with other measures of microbial biomass, correlation coefficients were smaller. Genetic analysis is, however, ideally suited for identifying specific genes within a complex environmental matrix. Each method has strengths and limitations, and the choice of a methodology depends on the goals of the research. PLFA was found to be a relatively simple method of gaining a broad overview of the microbial community.

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Part 1

General Introduction and Literature Review

Introduction to Short-rotation Woody Crops

Because of concerns about the future of fossil fuel resources, both in terms of supply and their effect on the environment, there has been increasing interest in bioenergy crops. Producers of electrical power, such as the Tennessee Valley Authority (TVA), are examining the potential of woody biomass plantations to produce a substrate to co-fire with coal in steam-generating electrical power plants. Improved technology has been developed for biomass utilization in power generation (Joslin and Schoenholtz, 1997). The biomass power industry in the U.S. grew from 200 MW in 1979 to more than 6000 MW in 1990 (Bain, 1993). The U.S. Department of Energy (D.O.E.) is projecting far greater use of biomass power by 2010. The U.S.D.O.E. (1999) reports that in 1996 the United States used almost 94 quadrillion BTUs of energy. Of that, 38% came from petroleum, 24% from natural gas, 22% from coal, and 2.6% from wood. It is hoped that increased utilization of short-rotation woody crops (SRWC) for bioenergy will reduce net carbon emissions to the atmosphere.

SRWC are fast-growing tree plantations that are grown for a short duration (3 - 8 years) to produce biomass that can be used for power production. Some reasons to consider SRWC include their potential for energy production while reducing net C emissions to the atmosphere compared with burning coal alone. Coal-burning power plants emit carbon dioxide, nitrogen oxides, and sulfur dioxide into the atmosphere. Trees remove CO_2 from the atmosphere while they are growing and then release that CO_2 along with low levels of N oxides and SO_2 when they are burned. Biomass crops may provide utility companies with a cost-effective carbon dioxide mitigation option in case of legislation requiring reduced emissions of CO_2. Biomass crops provide farmers with a new agricultural option. In addition, tree plantations provide a soil cover with their canopies and litter, and their perennial root systems provide a stabilizing effect which functions to conserve and improve soil and water quality, particularly on marginal sites. Bioenergy crops are projected to increase C sequestration in the soils in which they are grown. Conversion of erodible or marginally productive sites to woody crops production

has been identified as having environmental and economic benefits. However, in order to be economically feasible and a viable energy option, there needs to be a dedicated biofuel feedstock, with hundreds of thousands of hectares currently planted to conventional agriculture converted to tree plantations. Tree species that have been proposed as ideal SRWC species for the southeastern U.S. include hybrid poplar (*Populus sp.*), sweetgum (*Liquidambar styraciflua* L.), sycamore (*Platanus occidentalis* L.) and eastern cottonwood (*Populus deltoides* Bartr.) (Joslin and Schoenholtz,1997).

The U.S. Department of Energy (1994) has found SRWC promising for large scale biomass energy production. They have identified a potential land base of 158.6 million ha for production of these crops Of this, 145.3 million ha (92%) is presently being used as cropland or pasture (Graham, 1994). The Northeast, South Central and Southeastern states are considered most suitable for energy crop production. Large scale conversion of cropland and rangeland to SRWC will alter soil physical, chemical, and biological properties affecting erosion, hydrology, water quality and soil quality (OTA, 1993; Ranney and Mann, 1994). Paul and Clark (1996) point out that changes in the crop management system will alter the soil microclimate and affect the soil biota. Curci et al., (1997) add that climatic conditions, crop rotation and crop management systems can cause variations in the turnover of organic C and N, and in microbial activity by influencing soil biological properties. Converting cropland to tree plantations could have profound effects on soil properties and soil sustainability. The soil plays a major role in the long-term sustainability of ecosystems. The goal of this study was to use a battery of biochemical and molecular techniques to describe and quantify changes in soil microbial community structure, microbial biomass, and microbial activities which occur as a result of conversion from annual row crops to SRWC.

Methods of Measuring Soil Biological Properties

Why should we be interested in changes in soil biological properties?

The primary objective of this study was to determine whether changing cropping systems from a corn silage agroecosystem to growing sycamore as a SRWC would alter

the size of the soil microbial biomass, the structure of the microbial community, and the activities of the soil microorganisms. Why was this an important objective? Dr. Norman Pace (1996) states, "Although the chemical balance of the biosphere depends upon microorganisms, we have little understanding of the makeup and dynamics of the microbial ecosystems responsible for maintaining this balance." Through the processes of decomposition, immobilization, and mineralization microbial communities control the flow of C, N, S, O, and H through the biosphere. In order to understand the transformations which occur in a soil system we need to determine what organisms are present and active in the soil microbial community. Microbial communities include viruses, eubacteria, archaebacteria, fungi, protozoa, micrometazoa, and algae (Vestal and White, 1989). These organisms do not function as individuals but as communities. White and Macnaughton (1997) suggest that soil microbial composition is a faithful reflection of the soil's recent history and gives an indication of sustainable crop yields and other measures of soil quality and health.

Problems with traditional methods of measuring microbial populations

The first issue that must be faced in conducting a study of soil biological properties is the choice of microbial techniques that can accurately assess populations and activities of organisms in the soil. Classical microbiological methods used in public health for isolation and culture of clinical specimens are not adequate for environmental samples (White et al., 1997). White and Macnaughton (1997) report that viable counts (plate counts) of organisms are not satisfactory for soils and sediments where they often represent only 0.1% - 10% of the cells detected using acridine orange direct counts. Pace (1996) adds that far less than 1% of the organisms in the environment can be cultivated by standard techniques.

Vestal and White (1989) point out the following concerns about traditional enrichment culture techniques:

1. The method is artificial. Organisms are removed from their habitat and community.
2. Few organisms will grow on artificial media.

3. The procedure is time-consuming.

4. Doesn't show the *in situ* physiological status of the organisms in the environment.

5. It is difficult to quantitatively remove organisms from a soil sample. Most microbial communities are tightly attached to a substratum.

6. Formation of a single colony from bacterial aggregates.

Direct counting techniques also have difficulties when applied to soil systems. Fluorescence of organic debris as well as sediment and clay particles causes over-counting. Cells may be hidden by soil particles or other organisms. It is impossible to tell if an organism is viable. Only viable organisms have potential for metabolic activities (White et al., 1997) Cells can aggregate and be counted as one organism.

Alternatives to enrichment culture

A better approach to determining microbial community structure is to measure a biochemical component of the soil organisms that can be quantitatively extracted from an environmental sample. A component which is found in all microbial cells could give a measurement of their biomass. Components found in a subset of the microbial community could be used to define community structure (Tunlid and White, 1992). Biochemical methods do not depend on the growth of organisms so they do not have the problems inherent to selective enrichment, and they do not remove cells from the soil. These methods have an advantage in comparison with traditional biomass measurements (such as chloroform fumigation methods) in that they can give information on soil microbial biomass as well as community structure and metabolic activity of the soil biota (Tunlid and White, 1992). White et al. (1997) describe the characteristics that a biochemical would need to have in order to give an accurate picture of total microbial biomass:

1. It would have to be universally distributed among all living organisms.

2. It would have to have a short residence time in detrital pools after death so that only viable organisms would be measured.

3. It would have to be expressed at constant levels among the microbial

community and throughout the growth cycle.

4. Scientists would have to be able to extract it and measure it precisely.

Several cellular components have been suggested as meeting most of these criteria. Some of these have included adenosine triphosphate (ATP), microbial membrane components, and components of microbial cell walls. Microbial membrane components that have been utilized to measure soil microbial biomass or components of the microbial community have included phospholipids, lipopolysaccharides (LPS), and ergosterol. Constituents of microbial cell walls that have been used have included muramic acid, D-alanine, diaminopimelic acid (DAP) and glucosamine (Tunlid and White, 1992).

ATP is found in all living cells and can be quantified by the luciferin-luciferase assay. However, it has been found to be difficult to efficiently extract from all soil types (Tunlid and White, 1992). In addition, concentrations of ATP of microbes vary with species, growth rate, and environmental conditions. Lipopolysaccharides are polymers contained in the outer cell membrane of Gram-negative bacteria which consist of a lipid, a core polysaccharide, and side chain. They are unique to Gram-negative bacteria, and can be used to measure Gram-negative populations. Ergosterol is the predominant sterol in most fungi and has been used to provide an estimate of fungal biomass. It can be recovered from soil by extraction with methanol, saponification, and reextraction with hexane. Ergosterol is analyzed by HPLC using a UV detector. This takes advantage of the fact that ergosterol absorbs UV radiation at a wavelength of 282 nm, which is significantly different than other plant and animal sterols. The ergosterol content of fungal mycelia has been found to vary with species and growth conditions (Tunlid and White, 1992).

The peptidoglycan of the bacterial cell wall contains several unique components (including muramic acid and glucosamine) that can be used to estimate the biomass of different components of the microbial community. A molecule of peptidoglycan is made up of alternating units of N-acetylglucosamine and N-acetyl-muramic acid. These units are connected by peptide bridges containing the amino acids D-alanine, and m-diaminopimelic acid (DAP) (Tunlid and White, 1992). DAP is present in Gram-negative

bacteria, but only in some Gram-positive bacteria. Muramic acid has been used to measure bacterial populations, but, like many cell wall components, has been found to persist in the soil after cell death. Glucosamine has been used to indicate fungal populations but is also found in some bacteria and invertebrates.

Phospholipid fatty acid analysis of microbial community structure

Ringelberg et al. (1997) suggest that some of the more descriptive cellular components are the ester-linked phospholipid fatty acids (PLFAs). PLFAs are major components of the cellular membranes of all living organisms. Fatty acids maintain membrane fluidity so that substrates can be assimilated. They are rapidly degraded upon death. They are not found in storage lipids or anthropogenic contaminants (Zelles et al., 1992). They are soluble in organic solvents and can be extracted and measured using capillary gas chromatography. Studies have found that bacteria cells contain a relatively constant proportion of their biomass as phospholipids (Tunlid and White, 1992). The determination of the total PLFA provides a quantitative measure of the viable or potentially-viable biomass (White and Macnaughton, 1997). Measuring the viable biomass of a community gives an indication of that community's capacity for transformations (White et al., 1997). Vestal and White (1989) suggest that the PLFA methodology can be used to study microbial population changes through time brought about by physical or chemical environmental perturbations. Ringelberg et al. (1997) point out that certain PLFA are discriminatory enough to allow various subsets of the microbial community to be identified. In addition to giving evidence of the phenotypic properties of the community, PLFA analysis gives *in situ* indications of starvation, growth rate, aerobic/anaerobic balance, exposure to toxicity, and unbalanced growth (White et al., 1997). Many subsets of the microbial community respond to conditions in their environment with shifts in lipid composition. Ratios of poly β hydroxy alkanoic acid (PHA):PLFA in bacteria provide a measure of nutritional status, as microbes have been shown to form PHAs when lacking some essential nutrient. Anaerobes are associated with high ratios of iso to anteiso fatty acids. Starvation and stationary-phase growth lead

to a conversion of monoenoic fatty acids to cyclopropyl fatty acids. Starvation alters the geometry of the double bond in monounsaturated fatty acids leading to an increase in trans-monoenoic fatty acids compared with cis isomers (Guckert et al., 1986). Other methods of measuring microbial metabolic activity include ATP energy charge, extracellular enzymes, respiration rate measured by oxygen uptake, and incorporation of ^{14}C acetate in lipids (Vestal and White, 1989).

Molecular approaches to analysis of microbial community structure

Recent developments in molecular biology have provided another approach to studying soil microorganisms that doesn't rely on culturing techniques. This procedure involves molecular genetic analysis and the use of nucleic acid hybridization. The recent development of molecular biological approaches into studies in microbial ecology promises a new level of information concerning the structure and function of microbial communities (Sayler, 1992). Torsvik (1994) found that the genetic diversity in the soil microbial community was approximately 200 times greater than what could be isolated from the soil. Sayler et al. (1992) state that gene probe technology based on nucleic acid hybridization is one of the most reliable techniques for detecting genetically defined populations within a complex microbial community. Vestal and White (1989) suggest that while no perfect method for analyzing the role of microbes under natural conditions has been developed, ribosomal RNA (rRNA) isolation and sequencing shows great promise for determining natural community structure. The amount of extracted nucleic acid from an environmental sample should yield reliable information on microbial biomass. Once extracted, the nucleic acid may be targeted with specific gene probes to measure the abundance of an individual population or specific genes. Ribosomal RNA and messenger RNA (mRNA) are, along with DNA, the main targets for nucleic acid hybridization. DNA tends to be more environmentally stable and less prone to enzymatic degradation than RNA (Sayler et al., 1992). Ribosomal RNA offers an increase in sensitivity by several orders of magnitude over DNA sequences since there are so many ribosomes in each cell. Johnston et al. (1996) point out that you can get information about community structure

and, in some cases, function by increasing knowledge at the molecular level. Zhou et al. (1996) comment that the extraction of bacterial nucleic acids from environmental samples has become a useful tool to detect organisms which can not be cultured, to determine the fate of recombinant genes or selected bacteria in the environment, and to show genotypic diversity and its change in natural ecosystems.

Gene probes take advantage of the ability of nucleic acids to be denatured and reannealed (Pepper and Josephson, 1998). Hybridization involves the formation of H bonds between complementary single strands of DNA or RNA to produce double strands. One of the single strands is the "probe" which consists of a known sequence of nucleic acid with a colorimetric, flourescent, or radioactive (^{32}P) label attached. The ^{32}P label is most often used because its high energy yields great sensitivity and shortens autoradiographic exposure times. The other single strand represents the target, and the labeled nucleic acid identifies a specific nucleic acid sequence or gene in the target. The target strand is typically fixed on a nylon or nitrocellulose membrane by vacuum filtration before the probe is added. After hybridization, the unbound probe is washed off the membrane. Radioactive probes are analyzed using X ray film (Stapleton et al., 1998). These methods allow for the study of microorganisms at the molecular level and detection of minute quantities of a particular species or a functional gene. They also allow the classification of organisms into phylogenetic groups. (Pepper and Josephson, 1998). The use of specific oligonucleotides is a powerful tool for the qualitative detection and identification of specific bacteria in environmental samples (Utåker and Nes, 1998).

Initial studies using molecular techniques in microbial ecology were limited by an inability to obtain a representative sample of DNA from an environmental matrix (Johnston et al., 1996). Techniques of removing cells from the sample by centrifugation were limited by extraction efficiency, while DNA:DNA colony hybridization contained a culture bias. To get a representative estimate of the total microbial community in a soil sample, cells are now lysed *in situ* within the soil matrix and the DNA extracted and purified (Pepper and Josephson, 1998).

Kehrmeyer et al. (1996) point out that by directly extracting cellular components

from environmental matrices, nucleic acid and lipid analyses provide complementary information, and each can be used to substantiate the results obtained by the other. The solvent extraction utilized in lipid analysis can liberate cellular nucleic acids which can be used for gene probing (White et al., 1997). However, by using the same samples for both lipid and DNA extraction the yield of DNA has been found to be only 40-50% of that obtained by conventional DNA extraction (Kehrmeyer et al., 1996).

Some of the limitations of nucleic acid hybridization techniques include the following:

1. Soils high in clay adsorb DNA so extraction efficiency varies with soil type and organic matter content.
2. The efficiency of cell lysis is not always high and varies with cell size.
3. Some DNA is lost during purification. White and Macnaughton (1997) suggest that one of the major problems encountered utilizing molecular techniques for analysis of environmental samples is the lack of quantitative recovery of nucleic acids from various complex environmental matrices.
4. Even purified DNA contains some humic material (Pepper and Josephson, 1998).
5. The majority of oligonucleotide probes are made on the basis of a relatively small number of DNA sequences, usually originating from pure cultures. In most cases these are not representative of the true diversity of bacteria in the environment (Utåker and Nes, 1998).
6. Nucleic acid hybridization is limited by the state of current databases and the potential for hybridization with DNA sequences with a high similarity (Stapleton and Sayler, 1998).

Criticism of the PLFA and DNA techniques

Some researchers have been critical of the use of these molecular and biochemical techniques. Degens and Harris (1997) opine "Much of the current work investigating soil microbial diversity has involved measurement of fatty acid profiles or DNA heterogeneity

between soils. These techniques can be complicated to perform and have not been shown to be clearly linked with the functional diversity of the microbial communities in the functioning of soil systems. In most soil studies, diversity is only characterized to the point of determining fungal and bacterial populations. There are currently no techniques that can reliably assess the functional diversity of soil microbial communities in soils". These comments served as an introduction to a study where Biolog® was used to show functional diversity of a soil community. D.C. White (personal communication) responds that the signature lipid analysis has worked well, especially when coupled with gene probes and PCR-rDNA. While these methods are difficult to do properly, they are accurate, quantitative, and provide the best methods to assess the viable counts, community composition and nutritional status of the community.

Zelles et al. (1995) criticized the use of single "signature" fatty acids. They point out that a great deal of data on fatty acid profiles has been obtained by the analysis of bacteria and fungi grown under controlled conditions. They suggest that in view of the complexity of the soil ecosystem, however, the question of whether these fatty acids form a unique signature that can be used to indicate a microbial community is still doubtful. They propose that groups of fatty acids with similar chemical structures serve as bio-indicators for groups of microorganisms. Zelles et al.(1997) also suggest that DNA may be unsuitable for detecting rapid changes in microbial communities because it is stabilized by sorption on clays.

Many scientists are wary of conducting environmental studies using techniques such as PLFA and Biolog® which were developed based on pure culture studies. G.S. Sayler (personal communication) stated that the primary development and strength of PLFA is pure culture taxonomy - not the environment. He adds that while the molecular (DNA/RNA) technology is not yet developed to the point of routine functional analysis for the environment, it is the only new generation technology with true capability for this type of biogeochemical functional analysis of soil biology. D.C. White (1994) points out that while nucleic acid analysis is valuable, it doesn't answer every question about the microbial community. Sometimes mRNA is not translated or the enzymes formed are not

functional due to posttranslational control. White suggests that nucleic acid studies must be supplemented by phenotypic information.

Frostegård et al. (1997) comment that a large number of pure-culture studies have shown that the PLFA composition of cell membranes is influenced by factors such as the metabolic state of the organisms, environmental changes, and toxic substances. Such effects are more difficult to show in complex environmental samples since changes in PLFA patterns have a confounding effect because of species shift. White and Macnaughton (1997) add that the marked changes in PLFA produced in some monoculture studies by shifts in the environment may not be detected in (environmental samples) where competition with better adapted species restricts survival of specific strains to much narrower conditions of growth. Frostegård et al. (1997) suggest that while it is more difficult to show community shifts in environmental samples, they don't think it is impossible. They point to differences that they observed in PLFA profile in a study of manure "hot spots" and comment that some of the alterations observed suggest that changes occurred in membrane composition.

Measuring soil microbial activity by enzyme activities

In addition to describing changes in microbial community structure as a result of conversion of cropland to tree plantations, another objective of this study is to measure changes in the size of the soil microbial biomass and in the activities of soil microorganisms. Enzyme assays have been developed to measure microbial activities in environmental samples. Biochemical reactions are catalyzed by specific proteins known as enzymes which, without being permanently altered themselves, cause chemical reactions to proceed at a faster rate (Tabatabai, 1994). Enzymes are specific for the types of reactions which they catalyze. They combine with their substrate in such a way that they change the electronic configuration around certain bonds allowing reactions to proceed at a lower activation energy than would a non-catalyzed reaction (Tabatabai, 1994).

Extracellular soil enzyme activity and microbial biomass are early indicators of soil biological and biochemical properties (Curci et al., 1997). Changes in the soil biota due to

environmental perturbations will be reflected in some of the more dynamic soil biological characteristics such as microbial biomass, enzyme activities, or respiration, long before total soil organic matter responds (Chandler et al., 1997). Soil microbial biomass and enzyme activity play a crucial role in the storage and cycling of bioelements. Measurement of these parameters is important in studying the soil organic matter transformation processes which are essential for plant nutrition (Curci et al., 1997).

A typical assay to measure the activities of a soil enzyme involves adding a known amount of soil to a solution containing a known amount of substrate and measuring the rate at which the substrate is converted to the final product of the enzyme-catalyzed reaction under a standard set of conditions including temperature and pH. Although the enzymes in soil may be produced by either plant or microbial sources most soil enzymes probably originate from microbial sources (Ladd, 1978; Bolton, 1985). Consequently, levels of soil enzyme activities should give an indication of microbial numbers and activities.

Measurement of the soil microbial biomass

In recent years, studies investigating the effects of management-induced changes on soil biological properties have often measured changes in the soil microbial biomass. Fumigating the soil with chloroform and measuring the bioelements that become extractable as a result has become a common soil microbiological procedure. Soil microbial biomass is an important component of the soil organic matter that regulates the transformation and storage of nutrients. It is a labile component of the soil organic fraction containing 1 - 3% of the total soil C and up to 5% of the total soil N (Smith and Paul, 1990). The size and activity of the soil microbial biomass must be assessed to fully understand nutrient fluxes in managed and natural ecosystems (Horwath and Paul, 1994). Beck et al. (1997) point out that soil microbial biomass is an important indicator of soil fertility providing useful information for soil ecological studies. Investigations into the immobilization and mobilization of bioelements in soil requires information about the amounts of these nutrients stored in the microbial biomass. The increase in extractable N

(Brookes et al., 1985), P, S, and C following fumigation of soil has been used to estimate these elements in the microbial biomass (Vance et al., 1987). Work by Henrot et al. (1994) is representative of a number of studies that have measured changes in microbial biomass following vegetation removal. They suggest that measuring microbial biomass can provide an early indication for slower, less easily detectable soil organic matter changes. Microbial biomass can also provide an index of soil fertility because it represents an important labile pool of soil nutrients. These researchers studied the effect that vegetation removal had on microbial biomass in a humid tropical forest. They found that three years after vegetation removal, the total soil C and N were reduced by 20%, However, changes in microbial biomass C were even more pronounced. In the fallow soil, biomass C declined to 50% of initial values within 6 months.

Because of its importance to soil ecological studies a number of procedures have been developed to measure soil microbial biomass. These have included plate-counting using selective media (Parkinson et al., 1971), direct microscopic counting (Jenkinson et al., 1976), fumigation-incubation (FI) (Jenkinson and Powlson, 1976) fumigation-extraction (FE) (Vance et al., 1987) substrate-induced respiration (SIR) (Anderson and Domsch, 1978) release of ninhydrin-positive compounds (Amato and Ladd, 1988) and measurement of soil levels of adenosine 5'-triphosphate (ATP) (Jenkinson and Oades, 1979).

Using PLFA and nucleic acids to measure soil microbial biomass

Frostegård and Bååth (1996) point out that several methods have been used to estimate the amount of microbial biomass in soil, but few provide information about the structure of the microbial community that makes up that biomass. Extractable phospholipids are capable of providing reliable estimates of the microbial biomass as well as providing information about microbial community structure. Zogg et al. (1997) and Zelles et al. (1997) agree that total PLFA content reflects active microbial biomass. Frostegård et al., (1997) studied microbial community dynamics associated with "manure hot spots". These researchers quantified microbial biomass both as the amount of

phosphate liberated from lipid extracts after digestion (lipid-P) and as the total amount of PLFA. They concluded that total PLFA gives a better indication of living microbial biomass than does lipid-P.

The use of total PLFA as a measure of microbial biomass has been correlated against traditional measures of microbial biomass. Frostegård and Bååth (1996) found direct counts of bacteria were significantly correlated with bacterial PLFA ($r = 0.59$). They extracted fatty acids from soil organisms representing a wide range of organic matter contents and pH values and estimated an average of 1.40×10^{-17} mole of PLFA per bacterial cell. Using this factor and acridine orange direct counts they estimated a range of bacterial biomass of 1.0 to 4.8 mg bacterial C per gram of soil C. Balkwell et al. (1988) found a significant correlation between phospholipid content and ATP content as well as acridine orange direct counts of microorganisms in aquifer sediments. Frostegård et al.,1991) used total lipid phosphate as a measure of biomass and found that it correlated well with SIR ($r = 0.86$) and ATP content ($r = 0.89$). Zelles et al. (1992) found that the amounts of PLFA in agricultural soils correlate well with SIR microbial biomass data and enzyme activity (correlation coefficient > 0.97). They suggested that this is an indication that the fatty acids are closely linked to soil microorganisms and that this method can be used in the study of microbial ecology. They proposed that 28 μg PLFA is approximately equal to 100 μg of biomass C. Zelles et al. (1995) found good correlation ($r > 0.90$) between total ester-linked PLFA and microbial biomass measured with SIR in a variety of forest and agriculturally managed soils.

Zak et al. (1996) used total PLFA as a measure of active microbial biomass in a study where atmospheric CO_2 levels were raised to twice ambient levels. They found that total PLFA was 1.3 times greater under elevated atmospheric CO_2, but this difference was not statistically significant. However, microbial biomass as measured with FI was 1.5X greater under elevated CO_2 conditions. These authors suggest that FI measures both active and inactive (resting and dead) biomass while PLFA only measures active biomass because of the rapid turnover of the phospholipids upon death.

Measurement of nucleic acids in the soil has also been suggested as a measure of

the soil microbial biomass or microbial activity. Sayler et al. (1992) state that since the genetic composition of bacteria is relatively uniform the amount of extracted DNA from an environmental sample should provide a reliable estimate of microbial biomass.

Why might we expect to see increases in microbial biomass and shifts in microbial community structure as a result of conversion from row crops to SRWC?

In most ecosystems the amount of substrate entering the soil from plant litter production is only sufficient to maintain the soil microorganisms, allowing for no net annual growth (Zak et al., 1996). When plant litter production is increased the amount of substrate available for heterotrophic metabolism in soil is also. Since soil microorganisms differ in their ability to convert substrate into biomass, greater plant litter production should shift community composition. During stationary growth, bacterial substrate-use efficiencies range from 20 - 40%. Soil fungi have much greater substrate-use efficiencies (40-75%) than bacteria, so more substrate goes into making new cells and less is lost as CO_2. In spite of these differences in substrate-use efficiency, Zak et al. (1996) found greater biomass but no change in community composition one year after doubling the atmospheric CO_2 concentration.

Griffiths et al. (1999) comment that in most mineral soils the activity and growth of the soil microbial biomass is limited by the availability of substrate, especially C. The addition of substrate is, therefore, a key factor governing the size and activities of the microbial biomass. These researchers examined microbial community structure in a system where synthetic root exudate was applied to a soil. They found that microbial community structure was changed consistently as substrate loading increased. They noted that fungi dominated over bacteria at high substrate loading rates. Frostegård et al. (1997) studied "manure hot spots" and found changes observed in microbial biomass and community composition are mainly attributable to the diffusion of dissolved organic C from the manure.

Bardgett et al. (1997) comment that the abundance and activity of soil microorganisms is strongly related to the quantity and quality of available plant litter.

Active fungi and bacteria constitute some 90-95% of the total heterotrophic metabolism in most soils.

Objectives and Hypothesis

My hypothesis in conducting this study was that changing the crop management system from traditional annual row cropping to growing short rotation woody crops would alter soil biological properties. Although no perfect technique has yet been developed to describe the microbial community structure in environmental samples, new molecular and biochemical procedures offer significant improvements as compared with traditional techniques in accurately describing and quantifying populations and activities of organisms, and determining the structure of microbial communities as they exist in the environment. The objective of this study was to use several of these molecular and biochemical techniques, as well as some of the more traditional procedures to test my hypothesis. An additional objective of this study was to compare these methodologies and assess their suitability for working with the complex and heterogenous medium that soil represents.

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Part 2

Description of the Research Site and Sampling Procedures for Measuring Soil Biological Properties at the Ames Plantation Short-Rotation Woody Crops Project

Introduction

Environmental perturbations or changes in the crop management system will alter the soil environment in an agroecosystem. These changes can influence the structure of the community of microorganisms living in the soil ecosystem and, in turn, affect the soil biogeochemical processes which they control. Dick (1997) points out that there is growing evidence that soil biological properties are sensitive to soil management and environmental stresses. The objective of this chapter is to characterize the site where this research was conducted in order to provide some background to understand how converting a corn silage agroecosystem to a sycamore plantation would influence soil biological properties in that system.

Measurement of soil organic matter fractions

One of the most critical issues to consider when changing crop management systems is how the change will influence levels of soil organic matter (SOM). Microbial processes in the soil are often limited by availability of carbon substrates (Boyer and Groffman, 1996). Soil organic matter directly and indirectly influences numerous soil properties. Some of these properties include cation exchange capacity, soil structure, soil aggregation, water holding capacity, buffering capacity, microbial biomass and activities, color, temperature, porosity, infiltration and percolation (Brady and Weil, 1999; Pritchett, 1989). Organic matter plays a critical role in the cycling of nutrients in terrestrial ecosystems. However, methods commonly used for measuring and describing soil organic matter, such as organic C and humic acids, do not necessarily measure biologically active soil organic matter fractions (Barrios et al., 1996). The mineralization rate of SOM is affected by its bioavailability. Bioavailability of SOM depends on chemical composition and is a function of plant type, growth conditions and age (Boyer and Groffman, 1996). These authors point out that when forests are converted to agriculture there is generally a marked decline in total organic C and microbial biomass C. However, they suggest that the relatively high litter quality (biodegradability) of agricultural crop residues could

increase concentrations of labile soil organic C compared with forest systems.

A variety of procedures have been developed to divide SOM into fractions based on bioavailability. Water-soluble organic matter is considered the most labile and mobile form of SOM (Boyer and Groffman, 1996). These authors compared concentrations of total, water-soluble, and bio-available soil organic matter from temperate forest and agricultural ecosystems located on the same soil type. While they found total soil C was higher in the forest system, concentrations of water-soluble and bioavailable C were higher in agricultural soils largely due to a larger soluble humic acid fraction in the agricultural system. These authors suggest that although agricultural soils may have less total C than forest soils, they may support as much or more microbial activity due to increased production of water-soluble C.

Size and density fractionations have been suggested as promising techniques for physically dividing SOM into pools which differ in composition and biological function. It has been observed that SOM in the sand-size fraction (>53 μm) is often more labile than organic matter in the clay- and silt-size fractions (Gregorich et al., 1988). Size fractionation divides organic matter particles into size fractions. It has been observed that during humification organic matter may become associated with mineral particles and thus occur in particles of higher density. Density fractionation divides SOM into a light fraction consisting of mineral-free organic matter and a heavy fraction consisting of organic matter adsorbed to mineral surfaces. The light fraction typically is considered the biologically-active SOM pool (Jansen et al., 1992). The two fractions can be separated by flotation in solutions such as sodium iodide (density = 1.7 g cm^{-3}) or sodium polytungstate (density = 1.85 g cm^{-3}). A study by Barrios et al. (1996) utilized both size and density fractionation to compare organic matter from different maize and legume cropping systems. Although total C and N were not different among cropping systems, the dry weight, N content and C content of the light fraction differed among cropping systems, with the greatest values for each parameter occurring in systems which included legumes, crop rotations, and retention of crop residues. These authors suggest that these SOM fractions can be used as sensitive indicators of differences in SOM.

Methods and Materials

This study was conducted at the University of Tennessee experiment station at Ames Plantation, located in the loess belt of west Tennessee, near Grand Junction, Tennessee. The site was established as a TVA-sponsored SRWC project designed to examine the environmental impacts of conversion of cropland to SRWC. Prior to establishment of the study, the area was under conventionally-tilled silage corn production for at least 15 consecutive years. The soil type at the study site is a Memphis-Loring silt loam intergrade (fine-silty, mixed, thermic Typic Hapludalf - fine-silty mixed thermic Typic Fragiudalf). These soils are characterized by high silt and low clay content, low CEC, and a variably-developed fragipan between soil depths of 90 - 150 cm. The TVA study site consists of six 0.5 ha plots constructed as mini-hydrological units, with 0.5 m confinement berms (soil brought from other portions of the field) surrounding three corn (Zea mays L.) plots and three sycamore (Platinus occidentalis L.) plots (Figure 2-1). These plots will be referred to as "corn" and "young sycamore" respectively. The young sycamore plots were located within an even-aged 3.5 ha plantation of sycamore. Plots could not be randomly distributed because a wildlife study associated with this project required large unbroken tracts of each treatment. Sycamore has been identified as having a high potential as a biomass tree crop because of its ability to develop a dense canopy and a pervasive root system over a short rotation (Graham, 1994). It is a lowland species that does best on alluvial soils and bottomlands where water is not limiting and flooding is not excessive (Fribourg et al., 1989). The species can be easily regenerated by seed or by coppice, it is relatively free of disease and insect attack, and it lends itself to mechanized harvest.

Each plot was instrumented with four randomly installed tension-free pan lysimeters to monitor leaching. The long axis of the plot was located in downslope direction on each plot with a 0.5 m flume preceded by a 2 m flume approach to monitor runoff. Each flume was equipped with an ISCO (Lincoln, NE) flow meter and an

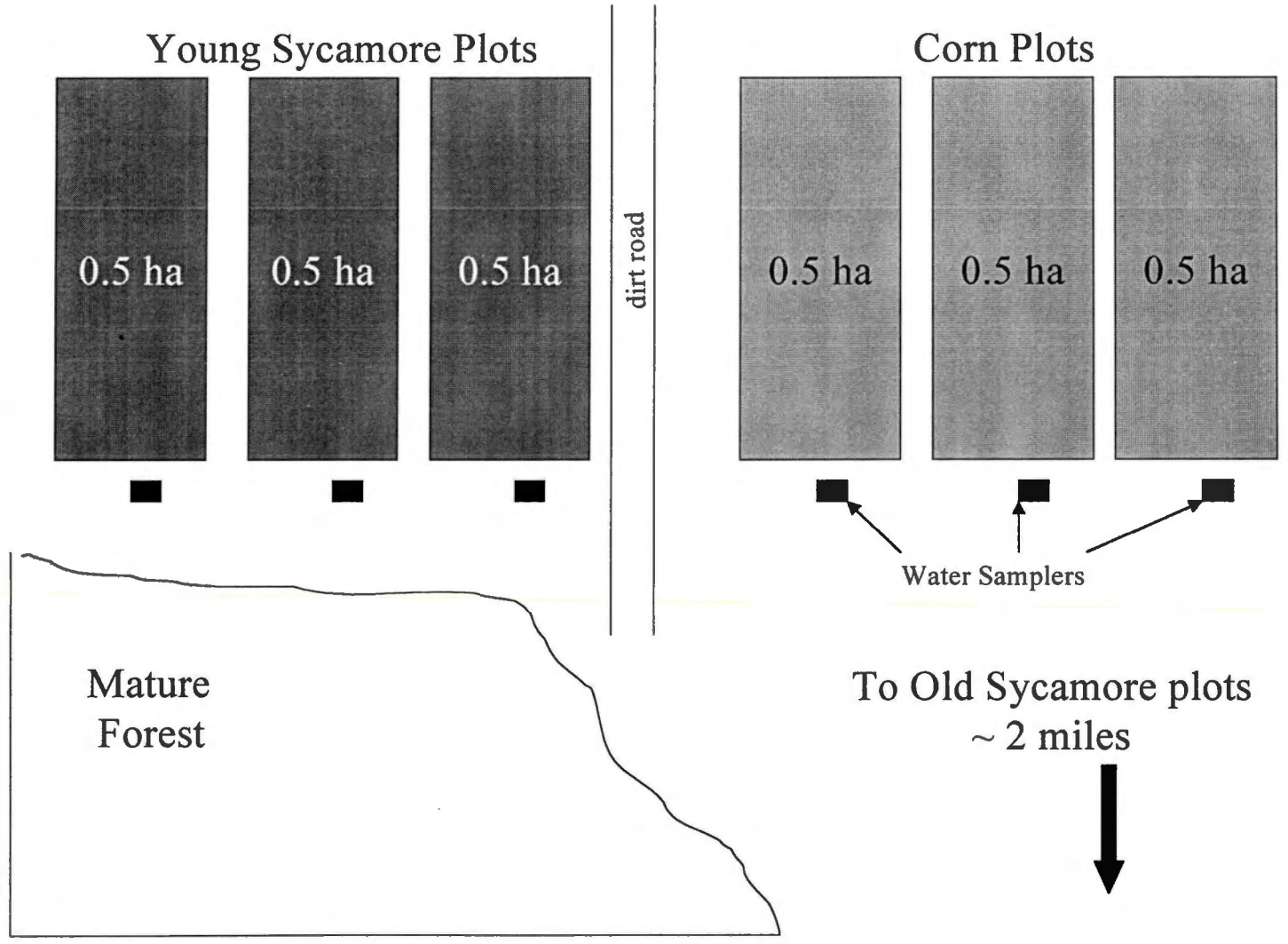


Figure 2-1. Map of Ames short-rotation woody crops site

automated flow proportional sampler, to measure sediment loss and water chemistry of the runoff. Buildings containing solar chargers, computerized flow meters, and water samplers were installed. Sycamore seedlings were planted at 2.4m by 1.2m spacing in February, 1995. After two growing seasons tree survival was more than 97%. The corn plots were maintained in no-till corn silage production. The equipment used on the corn plots have included a John Deere No-till Drill (model JD 750), a John Deere 6-row corn planter (Model JD 100) and a John Deere combine (model 9500). The winter cover crop in 1995 was fescue, but was considered too thick to be managed. For the past three winters the cover crop was wheat. The wheat was killed in the spring, and not harvested. During the growing seasons since establishment, silage corn and sycamore plots were managed as two separate systems, with chemical and fertilization inputs and harvest geared to the best management of each (Tables 2-1 and 2-2).

In 1983, Wells and Fribourg (1992) established three 17m x 17m plots of American Sycamore approximately 2 miles away from the TVA study, but on a similar soil type. These plots were part of a study to compare forage and tree production on marginal soils in Tennessee. This site was intended to represent a control, not a marginal soil. This site will be referred to as "old sycamore". Sycamore seedlings were planted at a spacing of 2.4m between rows and 1.2m within rows on a site that had been planted in conventionally-tilled soybean. The area surrounding the plots was maintained in soybeans. No fertilizer was applied to the trees. Weed growth was controlled by disking or by using glyphosate for one year (Fribourg et al., 1989). At four years of age the trees had a 96% survival rate. Every other row of sycamore was harvested at five years. A second harvest in 1993 collected the coppice and the 10-year-old trees (Fleenor, 1996). The trees which had regrown were cut again in the spring of 1998 and the biomass was left on the site.

Table 2-1. Fertilizer applications, 1995 through 1998, for young sycamore and silage corn plots.

Treatment	Year	Date of application	Rate of application
Young Sycamore	**1995**	April 14	1 lime ton/ac
	1996	April 15	120N-50P-0K
	1997	April 15	60N-0P-0K
			1 lime ton/ac
	1998	April 14	60N-0P-0K

Treatment	Year	Date of application	Rate of application
Corn	**1995**	March 1	62 lime ton/ac
		April 28	90N-50P-50K
		May 30	30N-0P-0K
	1996	April 27	90N-50P-50K
		May 29	40N-0P-0K
	1997	April 3	90N-0P-50K
		May 30	40N-0P-0K
	1998	April 10	90N-0P-50K
		May 27	30N-0P-0K

Table 2-2. Pesticide/herbicide application, 1995 through 1998, for young sycamore and silage corn plots.

Pesticide/herbicide applications to young sycamore trees

Year	Date of application	Treatment	Rate of application	Method of application
1995	April	Poast Plus	24.00 oz/ac	Broadcast
	June 14	Fusilade DX	Spot treatment	Spot treatment
	August 21	Fusilade DX	12.00 oz/ac	Broadcast
	September	Fusilade DX	12.00 oz/ac	Broadcast
1996	April	Roundup	1.00 qt/ac	Broadcast
	May	Fusilade Dx	12.00 oz/ac	Broadcast
	June	Roundup	1.00 qt/ac	Broadcast
	July	Roundup	1.50 qt/ac	Broadcast
1997	May 1	Roundup	1.50 qt/ac	Broadcast
	June 18	Fusilade DX	12.00 oz/ac	Broadcast
1998	-------	No treatments	-------	-------

Pesticide/herbicide applications to corn plots

Year	Date of application	Treatment	Rate of application	Method of application
1995	March 31	Gramoxone Extra	2.50 pt/ac	Broadcast
	April 28	Aatrex 4L	1.00 pt/ac	Overtop--\ mix
		Bicep	4.00 pt/ac	Overtop--/
		Roundup	1.50 qt/ac	Overtop
	May 25	Accent SP	0.66 oz/ac	Overtop
1996	May 1	Atrazine 4L	1.00 pt/ac	Broadcast--\
		Bicep II	2.00 qt/ac	Broadcast---mix
		Roundup Ultra	1.50 qt/ac	Broadcast--/
	May 4	Furidan	24.00 oz/ac	In furrow
	May 6	Dithane DF	2.00 lb/ac	Broadcast
	May 9	Dithane DF	2.00 lb/ac	Broadcast
1997	April 4	Aatrex 4L	1.00 pt/ac	Overtop--\
		Bicep II	2.00 qt/ac	Overtop---mix
		Roundup Ultra	1.50 qt/ac	Overtop--/
	April 9	Furidan 4F	32.00 oz/ac	In furrow
	June 6	Accent SP	0.66 oz/ac	Overtop--\ mix
1998	April 10	Aatrex 4L	1.00 qt/ac	Overtop--/
		Bicep II	2.00 qt/ac	Overtop
		Roundup Ultra	1.50 qt/ac	Overtop
	April 18	Furidan 4F	1.00 qt/ac	In furrow
		Pounce 3.2EC	1.00 oz/ac	In furrow
	June 3	Accent	0.66 oz ac	Broadcast

Immediately adjacent to the young sycamore plots is a mature mixed hardwood forest. This forest is approximately 50 years old, and has a mixed species composition of oak, sweetgum, red maple, black cherry, and an assortment of hardwood trees and shrubs. Soil samples were collected from this site (referred to as "mature forest") in March, May, and August, 1998. These treatments were chosen for study to show soil biological properties in a continuum from a traditional annual row cropping system to variously-aged SRWC systems, to a mature hardwood site.

Soil sampling

Mineral soil cores were collected during June and November, 1997, and March, May, and August, 1998 to a depth of 6 cm using a soil core sampler (inside diameter = 5.3 cm) (SoilMoisture Equipment Corp., Santa Barbara). Organic litter was removed from the soil surface prior to sampling. Aseptic techniques were used to prevent contamination of the samples. These techniques included the following:

1. Nitrile gloves were worn.
2. The soil core sampler was washed with a 1:1 mixture of chloroform:methanol between samplings.
3. The sampling container was lined with aluminum foil and rinsed with isopropanol between samplings.
4. Soil cores had the outer portion, which had come into contact with the soil core sampler, removed with a sterile parring knife.

In June 1997 samples were taken both from within rows of corn or trees as well as between rows, in order to account for any rhizosphere effect (Figure 2-2). Each treatment-plot combination was divided into a grid and a number was assigned to each section of the grid. Three sections were randomly chosen. Three cores were taken within 1 meter of the base of the tree, or within the row of corn and bulked in a plastic bag to represent one "row sample".

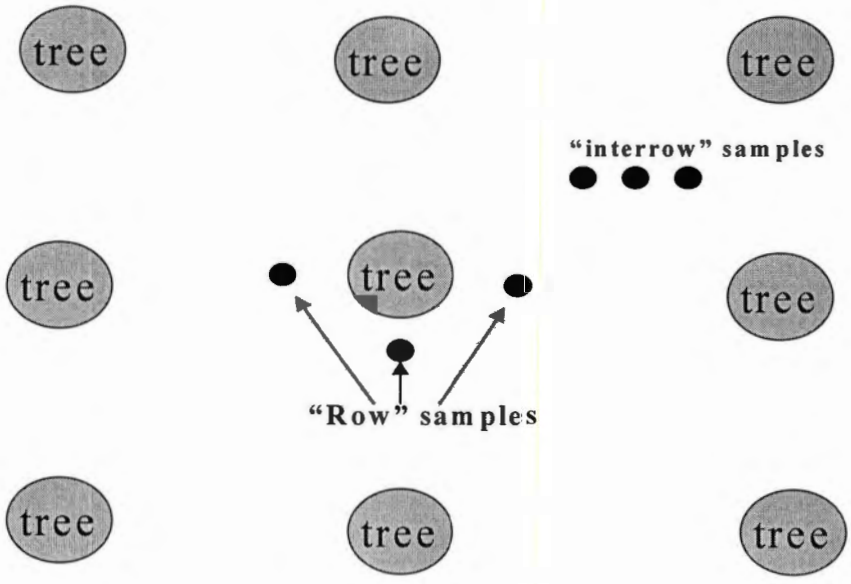


Figure 2-2. Sampling pattern for June 1997

Likewise, 3 or 4 cores were collected from between rows and bulked as an "interrow" sample. The bags were stored in a cooler on dry ice and taken to the laboratory within 24 hours of sampling, where they were stored in a freezer at a maximum temperature of -20°C until they were analyzed.

PLFA analysis showed no significant differences in biomass or community structure due to sampling location (Part 3). For all subsequent sampling events a series of soil cores were collected which included both rhizosphere and interrow samples (Figure 2-3). These were bulked and stored in plastic bags on dry ice. In March, May, and August 1998 a portion of each sample was stored on dry ice to be used for PLFA and DNA analysis. However, the portion to be used for chemical analysis, chloroform-fumigation, and enzyme assays (Part 5) was stored in a cooler on ice, returned to the laboratory, and

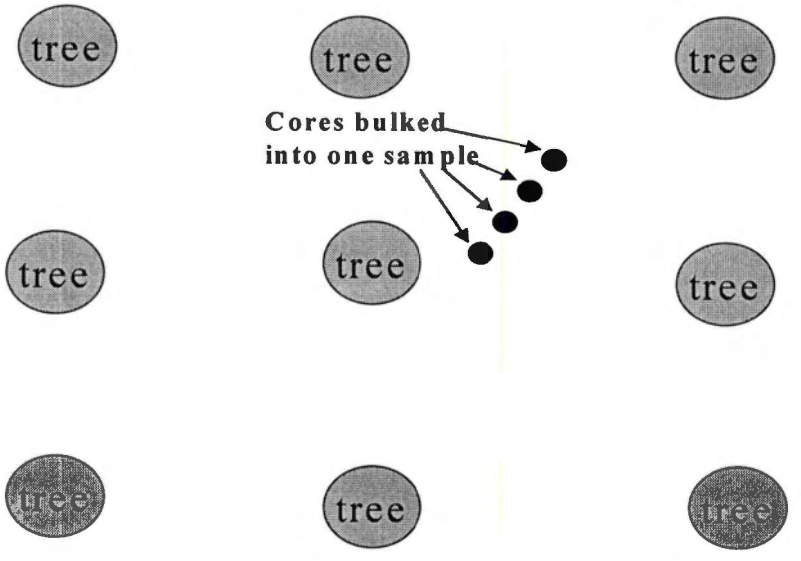


Figure 2-3. Sampling pattern for November 97, March, May, and August. 98

stored at 4°C so as to not kill organisms by freezing. All analyses on these refrigerated samples were conducted within 2 weeks of sampling.

Soil analytical methods

Gravimetric water content was determined by measuring the soil mass before and after oven-drying at 105°C. All soil chemical analyses were conducted on dry soil. Dried soil was crushed and homogenized with a mortar and pestle prior to analysis. Total soil C, S, and N were measured by dry combustion using a LECO CNS2000 analyzer (LECO, St. Joseph, MI) as described by Matejovic (1997). Soil pH was measured with a Corning pH meter 440 (Corning, NY) on a 1:2 soil-water paste. Soil were extracted with 1M KCl and the extracts were assayed for ammonium and nitrate by the indophenol blue method adapted for microtiter plate by Sims et al., (1995).
Color intensity was measured using a 7520 microplate reader (Cambridge Technology, Inc., Watertown, MA) with a 650 nm filter. Soils were also extracted with Mehlich I extractant (Mehlich, 1953) and the extracts analyzed for exchangeable Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn using an inductively-coupled argon plasma-optical emission spectrometer (ICAP61, Thermo Jarrell Ash Corp., Franklin, MA).

At each sampling date, samples of aboveground plant residue were also collected. This was accomplished using a piece of cardboard with a hole of known area cut into it. The cardboard was randomly tossed about the plot, and the plant residue which was found within the hole was collected. This plant residue was dried and weighed. As the area of the hole was known, the mass of dry residue on a per hectare basis could be calculated. The dried plant residue was ground using a cyclone grinder (20 mesh screen), mixed, and analyzed for total C, S, and N by combustion using the LECO CNS2000.

In August 1998 an attempt was made to determine the quality of root biomass in each treatment as a substrate for heterotrophic microorganisms. Roots were collected from the soil cores that were to be analyzed for soil chemical properties. No attempt was made to quantify this root biomass, aside from a simple visual comparison. The roots were washed several times to remove adhering soil. After drying, the roots were chopped

up and analyzed for total C, S, and N using the LECO CNS2000.

Water extractable C was measured in the August 1998 soil samples. Air-dried soil (25 g) was extracted with 75 ml of distilled water. Soils were shaken overnight, centrifuged (Sorvall Superspeed RC-2 centrifuge, 5000g, 15 min), filtered through a Whatman #42 filter paper then filtered with 0.45 μm Acrodisc® syringe filters (Gelman Sciences, Ann Arbor, MI). Total organic C was measured with a Dohrman automatic C analyzer (Santa Clara, CA).

Is the comparison with the "old sycamore" plots valid?

One concern that was considered in the design of this study was whether the comparison between soil properties at the TVA site (corn and young sycamore plots) and the "old sycamore" site was valid. The older sycamore plots are located approximately two miles away, and although the soils at the two sites have been classified as being within the same soil series, an effort was made to demonstrate that differences found between the two sites were truly due to treatments and not due to site differences. Calonne and Dujardin (1987) measured total soil C in the top 0 - 18 cm as 5.6 g kg^{-1} by dry combustion at the establishment of the older sycamore plantation. Soil samples were collected in March, 1998 to a depth of 18 cm to correspond with the sampling of Calonne and Dujardin. Approximately 20 soil cores were gathered using a soil probe from a random selection of sites within each plot. These were bulked into one sample and analyzed as described for the 0-6 cm samples. Samples were again collected in August, 1998 and also included samples from the mature hardwood forest and the soybean field immediately adjacent to the older sycamore plantation. These samples were air-dried, ground with a mortar and pestle, and analyzed for total C, S, and N using the LECO CNS2000.

Statistical analysis

Analysis of variance (ANOVA) were conducted using the type III General Linear Model SAS procedure (SAS Institute, 1990). Effects were considered significant when the null hypotheses, using the residual term as error, were rejected at a $P \leq 0.05$. Fisher's

least significant difference procedure was used for means separation. Values reported for soil analyses are the mean of nine samples (three per plot) at each sampling date. Values from the mature hardwood forest represents means of three samples

Results and Discussion

Perhaps the most obvious change to the soil environment caused by the conversion from annual row crops to a forested system was the accumulation of a litter layer on the soil surface. Certainly, some plant residues accumulated at the soil surface in the no-till corn system, particularly since the winter wheat was not harvested, but a significant amount of the vegetative biomass produced each year in the corn system was removed from the site as corn silage. In the SRWC systems leaves and twigs build up and decompose into soil organic matter. Many researchers have shown that changes in the content and composition of organic matter in the soil are linked to changes in the structure and function of the soil microbial community (Roper and Ophel-Keller, 1997; Beare et al., 1992; Mele and Carter, 1993). Table 2-3 through 2-7 show that levels of total soil C and N were significantly higher in soil from the old sycamore plantation than from the corn or the young sycamore plantation. This represents the accumulation of organic matter in the soil. Table 2-8 shows that the dry weight of plant residue on the soil surface was consistently greater in the old sycamore plots than in the corn system. When samples were collected from the mature hardwood forest they showed that these trends continue as the forest system matures. This increase in soil organic matter will profoundly influence a number of soil properties. Figure 2-4 shows that the older forest systems had significantly greater soil water content than the young sycamore or corn system. Soil organic matter acts as a sponge to absorb and hold water in the soil. Bandaranayake et al. (1996) showed that conversion of annual row crops to SRWC at this site increased soil infiltration rates, water-holding capacity, and lowered bulk density. In addition, this soil organic matter will provide a substrate for microbial metabolism.

Table 2-3. Soil Chemical Properties - June 1997

Treatment	C	S	N	pH	NH_4^+	NO_3^-	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
	g kg^{-1}				mg kg^{-1}										
Corn	11.4 b†	0.17 b	1.2 b	6.1 ab	13.9 b	10.6 a	1750 a	1.1 a	112.1 a	73.1 b	78.1 b	151.9 b	14.0 a	47.7 b	1.0 b
Young Sycamore	9.6 c	0.18 b	1.1 b	5.8 b	13.2 b	6.8 b	1136 b	2.1 a	117.9 a	73.5 b	78.9 b	141.3 b	10.9 b	32.5 b	1.0 b
Old Sycamore	15.3 a	0.21 a	1.4 a	6.4 a	18.1 a	5.8 b	996 b	1.6 a	148.8 a	112.8 a	142.9 a	264.1 a	11.8 b	9.6 c	2.8 a

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table 2-4: Soil Chemical Properties - November, 1997

Treatment	C	S	N	pH	NH_4^+	NO_3^-	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
	g kg^{-1}				mg kg^{-1}										
Corn	10.9 b†	0.15 a	1.2 b	6.0 a	13.9 b	5.4 a	1943 a	6.8 a	123.0 a	83.2 b	84.3 b	200.1 b	18.1 a	38.2 a	2.4 b
Young Sycamore	9.7 b	0.14 a	1.2 b	5.4 a	14.0 b	2.5 a	1225 b	6.2 a	150.1 a	98.8 b	91.6 b	202.4 b	15.8 ab	36.0 a	2.5 b
Old Sycamore	16.4 a	0.14 a	1.4 a	5.7 a	18.9 a	2.6 a	1118 b	5.4 a	152.7 a	140.0 a	178.2 a	352.6 a	14.7 b	13.5 b	3.9 a

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table 2-5. Soil Chemical Properties - March, 1998

Treatment	C	S	N	pH	NH_4^+	NO_3^-	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
	g kg^{-1}				mg kg^{-1}										
Corn	10.6 c†	0.18 ab	1.3 bc	6.0 a	15.9 b	8.7 b	2274 a	0.8 a	118.8 a	66.6 c	80.2 c	195.0 c	17.5 a	37.2 ab	0.7 c
Young Sycamore	9.6 c	0.16 b	1.1 c	5.2 c	14.3 b	7.8 b	1042 b	0.9 a	134.9 a	82.3 c	82.7 c	154.1 c	13.8 b	42.2 a	0.7 c
Old Sycamore	16.6 b	0.16 b	1.4 b	5.9 a	24.5 a	13.6 a	1126 b	0.5 b	122.7 a	121.9 b	167.6 b	270.1 b	13.5 b	12.1 c	1.5 b
Mature Forest	25.0 a	0.20 a	2.4 a	5.7 b	26.5 a	14.8 a	1362 b	0.5 b	89.2 a	177.2 a	252.8 a	367.7 a	12.6 b	16.2 bc	3.0 a

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table 2-6. Soil Chemical Properties - May, 1998.

Treatment	C	S	N	pH	NH_4^+	NO_3^-	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
	g kg^{-1}				mg kg^{-1}										
Corn	11.8 bc	0.17 b	1.3 bc	5.7 a	11.0 c	53.8 a	1956 a	1.5 a	94.0 a	112.8 b	88.8 c	232.5 c	23.4 a	40.6 a	1.3 c
Young Sycamore	10.4 c	0.17 b	1.2 c	4.9 b	11.9 bc	9.8 b	1182 ab	1.8 a	108.0 a	71.4 c	91.6 c	228.0 c	18.2 a	35.5 a	1.5 c
Old Sycamore	14.8 b	0.12 b	1.5 b	5.7 a	13.3 b	6.6 b	1020 b	2.3 a	105.2 a	101.4 b	156.0 b	332.0 b	17.2 a	9.42 b	2.6 b
Mature Forest	30.2 a	0.20 a	2.4 a	5.5 a	21.1 a	10.3 b	1642 ab	2.0 a	70.0 b	136.3 a	275.0 a	441.4 a	16.6 a	17.0 b	4.6 a

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table 2-7. Soil Chemical Properties - August, 1998.

Treatment	C	S	N	pH	NH_4^+	NO_3^-	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
	g kg^{-1}				mg kg^{-1}										
Corn	10.9 c†	0.14 a	1.1 c	5.8 a	5.6 b	17.2 a	1496 a	1.6 ab	77.5 b	104.7 b	93.4 c	87.7 b	18.6 a	24.4 a	1.9 ab
Young Sycamore	9.1 c	0.14 a	1.0 c	5.2 b	5.8 b	5.2 b	1002 a	1.9 a	82.6 b	69.0 c	108.5 c	69.1 b	18.6 a	29.6 a	1.1 b
Old Sycamore	17.4 b	0.13 a	1.5 b	5.8 a	9.2 a	4.5 b	1200 a	1.3 b	124.2 a	98.1 bc	185.0 b	144.6 a	19.8 a	5.5 b	2.4 ab
Mature Forest	23.9 a	0.16 a	1.9 a	5.5 ab	8.2 a	4.3 b	1291 a	1.4 b	48.2 c	151.1 a	263.8 a	154.6 a	19.3 a	6.1 b	3.4 a

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table 2-8: Plant residue collected from the litter layer (± one standard error of the mean).

Date	Carbon	Sulfur	Nitrogen	C/N ratio	Dry Wt
April, 1997	——— g kg^{-1} ———				— kg ha^{-1} —
Corn	266 ± 24	0.99 ± 0.14	8.1 ± 0.9	32.8 ± 2.6	4618 ± 845
Young Sycamore	322 ± 24	2.11 ± 0.14	13.3 ± 0.9	20.6 ± 2.6	7457 ± 845
Old Sycamore	418 ± 24	1.94 ± 0.14	15.8 ± 0.9	31.6 ± 2.6	12429 ± 845
significance	*	**	**	*	**
June, 1997					
Corn	310 ± 29	1.25 ± 0.15	13.9 ± 1.5	22.5 ± 1.7	-
Young Sycamore	274 ± 29	1.06 ± 0.15	15.6 ± 1.5	17.6 ± 1.7	-
Old Sycamore	393 ± 29	1.33 ± 0.15	13.3 ± 1.5	29.7 ± 1.7	-
significance	ns	ns	ns	**	-
November, 1997					
Corn	363 ± 14	1.89 ± 0.14	14.0 ± 1.3	27.4 ± 4.1	1936 ± 1036
Young Sycamore	423 ± 14	2.35 ± 0.14	14.8 ± 1.3	28.6 ± 4.1	7099 ± 1036
Old Sycamore	446 ± 14	1.87 ± 0.14	11.7 ± 1.3	38.6 ± 4.1	6695 ± 1036
significance	*	ns	ns	ns	'*
March, 1998					
Corn	381 ± 8	1.43 ± 0.12	11.3 ± 0.8	33.6 ± 2.4	2952 ± 571
Young Sycamore	375 ± 8	2.06 ± 0.12	16.7 ± 0.8	22.6 ± 2.4	7846 ± 571
Old Sycamore	424 ± 8	1.55 ± 0.12	11.3 ± 0.8	38.0 ± 2.4	7501 ± 571
Mature Forest	394 ± 13	1.28 ± 0.20	12.9 ± 1.5	30.6 ± 2.4	20084 ± 990
significance	*	*	*	*	***
August, 1998					
Corn	339 ± 9	1.85 ± 0.15	10.0 ± 0.7	35.2 ± 3.2	4620 ± 1876
Young Sycamore	404 ± 9	3.10 ± 0.15	18.2 ± 0.7	22.2 ± 3.2	7401 ± 1876
Old Sycamore	404 ± 9	1.97 ± 0.15	12.1 ± 0.7	33.5 ± 3.2	7940 ± 1876
Mature Forest	331 ± 16	1.22 ± 0.26	10.4 ± 1.3	31.9 ± 5.6	15854 ± 3248
significance	**	**	**	ns	ns

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

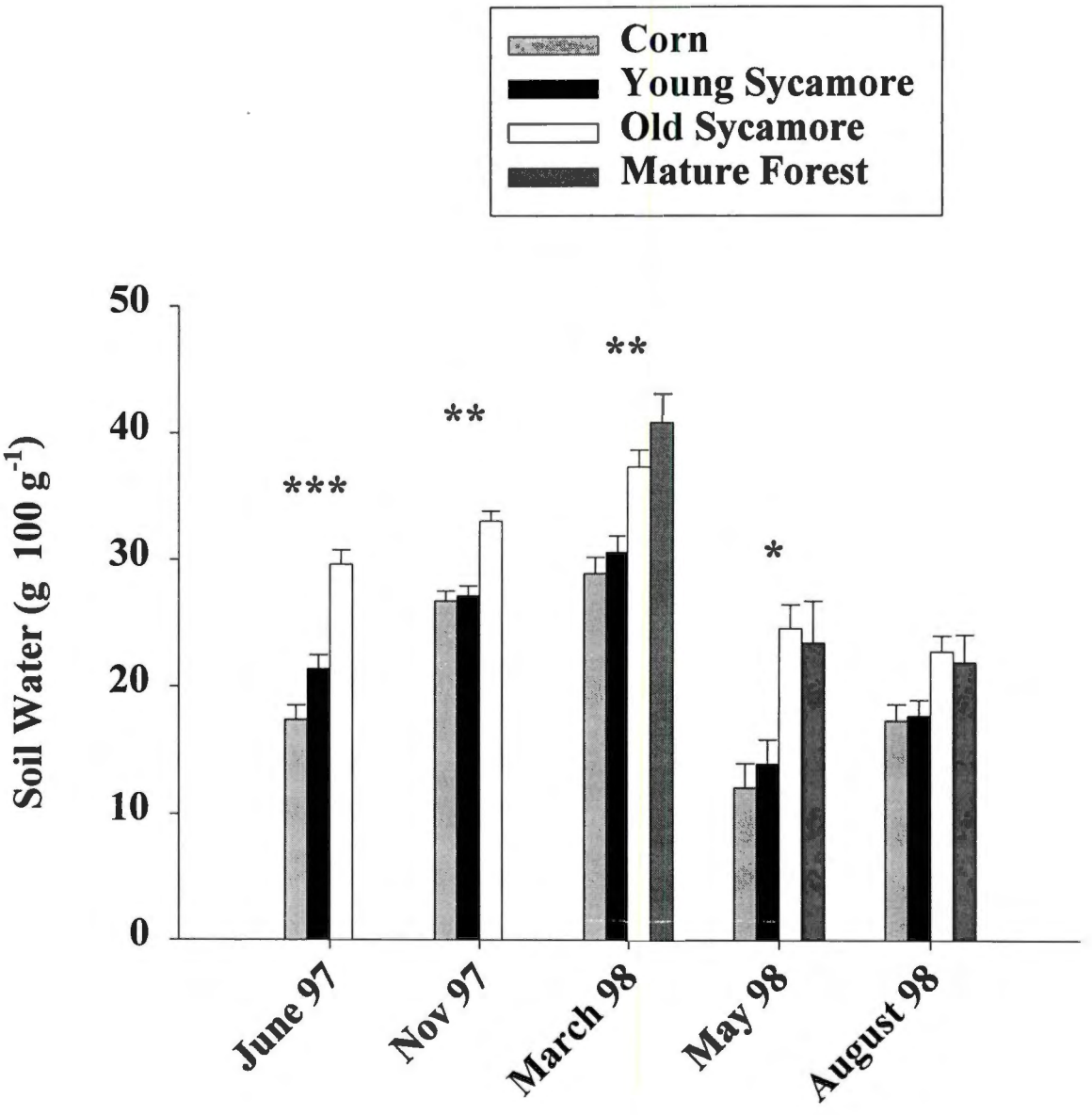


Figure 2-4. Gravimetric water content by sampling date

One question to consider when comparing the older sycamore plantation with the corn and young sycamore plots is whether these results may be due, in part, to reduced tillage. The old sycamore plots have not been tilled for almost 15 years. The corn and young sycamore plots were conventionally tilled until 1995. It has long been established that conversion to a no-till system increases surface residues and increases surface soil organic matter (Brady and Weil, 1996). Tillage aerates the soil, incorporates residues, and speeds the mineralization of organic matter. It is not clear how much of the observed effect is due to tillage and how much is due to the cropping system. In order to have accounted for tillage differences, all of the systems would have had to have the same tillage system for the duration of the study (since 1983).

It is important to note that in the early stages of establishment of the young sycamore plantation few organic residues were cycled back to the soil. This plantation was established in 1995. No cover crop was planted with the trees, and weed competition was reduced through the use of herbicides. The corn system received regular inputs of organic residues in the form of corn and wheat plant debris and root biomass while the young sycamore plantation was more like a fallow system. Plant residue samples collected in April of 1997 (Table 2-8) showed that after two years of growth the young sycamore plots had a more substantial litter layer than the corn plots, but still significantly less than the older sycamore plots. In June of 1997, when the first soil samples for this study were taken, the young trees were well established, but the canopy had not yet closed. Decomposing sycamore leaves were beginning to establish a litter layer under the trees, but few leaves covered the soil in the interrow space. By the November 1997 sampling the forest canopy had closed and fallen leaves covered most of the forest floor. Table 2-8 indicates that by November, 1997 the total biomass of litter in the young sycamore plots was comparable to that found in the older sycamore plots. While this cover would have protected the soil from environmental forces such as rain and wind, the leaves appeared to be slow to decompose, and many of the leaves blew off the site during the winter. As late as the August 1998 sampling, much of the litter layer had only begun to humify. Levels of total soil C and N in the young sycamore plots typically were not significantly different

than the corn plots. As the leaves and twigs humify and are incorporated into the soil organic matter the C/N ratio of the litter in the young sycamore plots will probably increase (Pritchett, 1987).

Figure 2-5 shows water extractable C (WEC) from the soil sampled in August 1998. This should represent a substrate that is readily available to the soil microbiota. Levels of WEC were significantly greater in the mature forest than the other treatments. WEC was also significantly higher in the old sycamore soil than in the young sycamore plots. These samples were collected at the end of the fourth growing season for the young sycamore plantation. It would appear from this data that the newly established litter layer in the young sycamore plantation had not yet begun adding significant amounts of soluble C to the soil organic matter. This data could be confirmed and expanded by doing additional C fractionation procedures such as size and density fractionation (Barrios et al., 1996) to measure the biologically active fractions of the soil organic matter.

Soil pH was consistently lower in soil from the young sycamore plantation than in the other treatments. Tables 2-3 through 2-7 reveal that the soil pH was typically at least one half a pH unit lower in the young sycamore soil in spite of receiving lime applications in 1995 and 1997 (Table 2-1). J.D. Joslin (personal communication) has suggested that perhaps the young trees are taking up Ca from the soil system, but they have not yet begun cycling this nutrient back to the soil since very little organic residue from the trees has been returned to the soil. Indeed, Tables 2-3 through 2-7 show that levels of extractable Ca are significantly lower in the young sycamore soil than in the corn soil. It might be expected that levels of other basic cations would also be lower in the young sycamore soil. This was not necessarily the case, although concentrations of K are confounded by the fact that the corn plots received applications of K fertilizer in April of each year (Table 2-1). In spite of this fact, basic cations tended to be significantly higher in both the older sycamore soil and in soil from the mature forest than in the corn or young sycamore plots. Trees with well established perennial root systems are able to mine nutrients from the soil that an annual crop would not be able to access. These nutrients are eventually cycled back to the soil (Pritchett, 1987).

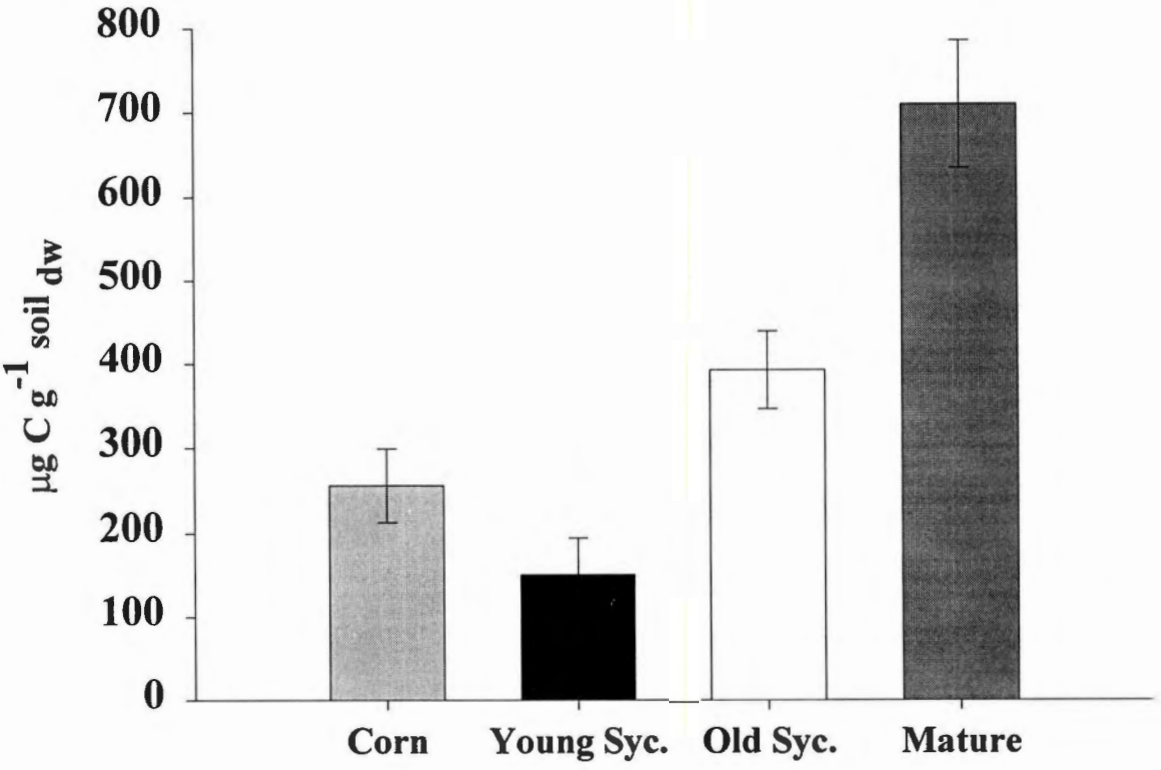


Figure 2-5. August, 1998 water extractable C. Error bars are one standard error of the mean of nine samples (three samples from the mature forest)

The corn system generally had the greatest concentration of extractable nitrate. This should not be surprising since these soils had received split applications of ammonium nitrate fertilizer each spring (Table 2-1). Differences in nitrifying bacteria between sites will be discussed in Part 4. In spite of these fertilizer applications to the corn plots, the more mature forest systems consistently had higher concentrations of ammonium . This may be due to lower rates of nitrification in these soils. This topic will also be discussed in greater detail in Part 4. The residual effect of P fertilizer application could be seen in significantly higher levels of extractable P in the corn and young sycamore systems, although these treatments had not received P fertilizer applications in several years.

Table 2-8 attempts to quantify and qualify aboveground residue additions to soil organic matter. Table 2-9 gives some indication of the belowground biomass that would be available as a substrate for microbial metabolism. Although no attempt was made to quantify root biomass, it should be pointed out that many more roots were found in soil cores from the old sycamore and mature forest plots. While these roots were woody and thick, the roots found in the corn samples were fine and "hairlike." It should be noted that the corn crop had been harvested several weeks before the soil cores were taken in August of 1998, so some roots may have already decomposed. In addition, while the large, woody roots of the forest systems create the impression of significant root biomass, the root hairs of the corn can represent a large surface area and an easily degraded substrate for microbial growth. Table 2-9 shows that the C/N ratio of the roots found in the corn and young sycamore plots was lower than that of the more mature forest plots. In addition, plant residue in the old sycamore plantation tended to have a higher C/N ratio than the corn or young sycamore plots. Although the more mature forests may have a greater quantity of organic material available for microbial metabolism that material seemed to be less easily degraded and probably contained a higher proportion of cellulose and lignin than the corn and young sycamore plots. Additional study could be done to determine the degradability of above and belowground residues using measures such as lignin + polyphenol/N ratio (Barrios et al., 1997).

Table 2-9. Root quality study - August, 1998.

Treatment	Carbon	Nitrogen	C/N Ratio
	——— g kg $^{-1}$ ———		
Corn	348 ± 9.0	15.7 ± 0.6	23.0 ± 1.12
Young Sycamore	382 ± 9.0	14.1 ± 0.6	27.4 ± 1.12
Old Sycamore	365 ± 9.0	9.7 ± 0.6	38.2 ± 1.12
Mature Forest	383 ± 16	9.8 ± 1.0	41.4 ± 1.95
Significance	ns	**	***

,* Significant at the 0.01 and 0.001 probability levels respectively

Tables 2-10 and 2-11 deal with the issue of whether a comparison of these four treatments was valid, given the fact that the old sycamore plots are located approximately two miles from other plots. Are differences found between the treatments caused by the treatments, or are the soils inherently different? One argument for the comparison was that soils from both locations have been classified as belonging to the same soil series. Table 2-10 indicates that at the time that the trees were planted in 1983 the level of total soil C in the top 18 cm was measured by dry combustion to be 5.6 g kg^{-1}. In March, 1998 total soil C in the surface 18 cm was measured by dry combustion to be 7.5 g kg^{-1} in the corn system, 7.4 g kg^{-1} in the young sycamore system, and 10.2 g kg^{-1} in the old sycamore system. Since these soils have been managed with no tillage, levels of soil C would be expected to be higher than in 1983 (Brady and Weil, 1996). Similar values were measured in August 1998. Also, in August samples were taken from the mature forest where soil C in the top 18 cm was measured to be 11.9 g kg^{-1}, and in the soybean field immediately adjacent to the old sycamore plots, where soil C was measured as 7.2 g kg^{-1}. Total soil N was significantly higher in the soybean field than in any other treatment. This is not surprising because of the ability of the soybean plant to fix atmospheric N. These observations present a compelling case that differences found between these two locations are a result of treatment effects and not due to differences inherent in soils.

Conclusions

The four treatments considered at the Ames Plantation site differed significantly in soil chemical properties. For the first few years after conversion of annual row crops to SRWC it appeared that inputs of organic residues and certain plant nutrients were reduced. Although the older sycamore plantation had significantly more total soil C and N than the corn system, the young sycamore plantation seemed to be suffering the effects of reduced organic matter inputs. While the young trees are getting established they take up nutrients but cycle very little back to the soil. As the trees grow and the litter layer becomes established the young sycamore plantation will likely become more like the older sycamore plantation.

Table 2-10. Soil chemical properties (0-18 cm depth) - March 1998

Treatment	C	S	N	pH	NH_4^+	NO_3^-	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
	g kg^{-1}				mg kg^{-1}										
Corn	7.5 b†	0.14 a	0.90 b	6.27 a	4.69 a	2.03 a	1185 a	0.70 a	70.5 a	43.5 b	71.1 a	64.1 b	15.5 a	12.4 a	0.33 c
Young Sycamore	7.4 b	0.15 a	0.95 b	6.02 b	4.50 a	3.56 a	1082 a	0.93 a	76.8 a	57.8 b	91.8 a	59.7 b	15.3 a	15.5 a	0.50 b
Old Sycamore	10.2 a	0.14 a	1.04 a	5.95 b	4.53 a	3.03 a	882 a	0.63 a	74.9 a	85.2 a	109.9 a	91.7 a	15.1 a	2.3 b	0.83 a

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table 2-11. Soil chemical data (0 - 18 cm's depth) - August, 1998

Treatment	C	S	N
	g kg^{-1}		
Corn	7.6c†	0.12a	0.87b
Young Sycamore	7.9bc	0.14a	0.87b
Old Sycamore	9.8b	0.13a	0.94b
Soybean	7.2c	0.12a	1.19a
Mature Forest	11.9a	0.13a	0.78b

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

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Part 3

Measuring Changes in Microbial Community Structure by Phospholipid Fatty Acid Analysis (PLFA) after Conversion of Annual Row Crops to Short-Rotation Woody Crops

Introduction

Because of concerns about the future of fossil fuel resources, both in terms of supply and their effects on the environment, there has been increasing interest in bioenergy crops. Producers of electrical power are examining the potential of woody biomass plantations to produce a substrate to co-fire with coal in steam-generating electrical power plants. The biomass power industry in the U.S. grew from 200 MW in 1979 to more than 6000 MW in 1990 (Bain, 1993). The U.S. Department of Energy is projecting far greater use of biomass power by 2010. It is hoped that increased utilization of short-rotation woody crops (SRWC) for bioenergy will reduce net carbon emissions to the atmosphere.

SRWC are fast-growing tree plantations that are grown for a short duration (3 - 8 years) to produce biomass that can be used for power production. Some reasons to consider SRWC include their potential for energy production while reducing net C emissions to the atmosphere compared with burning coal alone. Trees remove CO_2 from the atmosphere while they are growing. Tree plantations provide a soil cover with their canopies and litter, and their perennial root systems provide a stabilizing effect which functions to conserve and improve soil and water quality, particularly on marginal sites. Bioenergy crops are projected to increase C sequestration in the soils in which they are grown.

The U.S. Department of Energy (1994) has found SRWC promising for large scale biomass energy production. They have identified a potential land base of 158.6 million ha for production of these crops Of this, 145.3 million ha (92%) is presently being used as cropland or pasture (Graham, 1994). Conversion of this land to SRWC will alter the soil physical, chemical, and biological properties affecting erosion, hydrology, water quality and soil quality (OTA, 1993; Ranney and Mann, 1994). Paul and Clark (1996) point out that changes in the crop management system will alter the soil microclimate and affect the soil biota. Converting cropland to tree plantations could have profound effects on soil properties. The soil plays a major role in the long-term sustainability of ecosystems. This

study will describe changes that were found in soil microbial community structure as a result of conversion from annual row crops to SRWC.

The chemical balance of the biosphere depends upon the activities of soil microorganisms (Pace, 1996). Through the processes of decomposition, immobilization, and mineralization microbial communities control the flow of C, N, S, O, and H through the biosphere. In order to understand the transformations which occur in a soil system we need to determine what organisms are present and active in the soil microbial community. Microbial communities include viruses, eubacteria, archaebacteria, fungi, protozoa, micrometazoa, and algae (Vestal and White, 1989). These organisms do not function as individuals, but as communities. White and Macnaughton (1997) suggest that soil microbial composition is a faithful reflection of the soil's recent history and provides clues about future sustainable crop yields and other measures of soil quality and health. However, soil microbiologists must determine how to accurately measure the composition of the soil microbial community. Traditional methods of determining the composition of the microbial community in an environmental sample, such as plate-counting, have been found to measure only a small percentage of the actually community (Pace, 1996).

Another approach to determining the composition of the soil microbial community is to measure a biochemical component of living cells. Ringelberg et al., (1997) claim that some of the most descriptive cellular components are the ester-linked phospholipid fatty acids (PLFAs). PLFAs are major components of the cellular membranes of all living organisms. Fatty acids maintain membrane fluidity so that substrates can be assimilated. They are rapidly degraded upon death. They are not found in storage lipids or anthropogenic contaminants (Zelles et al., 1992). They are soluble in organic solvents and can be extracted and measured using capillary gas chromatography. Studies have found that bacteria cells contain a relatively constant proportion of their biomass as phospholipids (Tunlid and White, 1992). The determination of the total PLFA provides a quantitative measure of the viable or potentially-viable biomass (White and Macnaughton, 1997). Measuring the viable biomass of a community gives an indication of that community's capacity for transformations (White et al., 1997). Vestal and White (1989)

suggest that the PLFA methodology can be used to study microbial population changes through time brought about by physical or chemical environmental perturbations. Ringelberg et al. (1997) point out that certain PLFA are discriminatory enough to allow various subsets of the microbial community to be identified. In addition to giving evidence of the phenotypic properties of the community, PLFA analysis gives *in situ* indications of starvation, growth rate, aerobic/anaerobic balance, exposure to toxicity, and unbalanced growth (White et al., 1997). Many subsets of the microbial community respond to conditions in their environment with shifts in lipid composition. Ratios of poly β hydroxy alkanoic acid (PHA):PLFA in bacteria provide a measure of nutritional status, as microbes have been shown to form PHAs when lacking some essential nutrient. Anaerobes are associated with high ratios of iso to anteiso fatty acids. Starvation and stationary-phase growth lead to a conversion of monoenoic fatty acids to cyclopropyl fatty acids. Starvation alters the geometry of the double bond in monounsaturated fatty acids leading to an increase in trans- monoenoic fatty acids compared with cis- isomers (Guckert et al., 1986). Other methods of measuring microbial metabolic activity include ATP energy charge, extracellular enzymes (Part 5), respiration rate measured by oxygen uptake, and incorporation of ^{14}C acetate in lipids (Vestal and White, 1989).

Fatty acid nomenclature

Polar lipid fatty acids are designated by the number of carbon atoms in the fatty acid chain, and the number of double bonds, followed by the location of the first double bond from the aliphatic (ω) end of the molecule (Tunlid and White, 1992). The isomeric configuration of the double bond is shown by "c" and "t' which indicate cis- and trans- isomers. For example, 16:1ω6c is a sixteen carbon fatty acid with one double bond located six carbons from the alipathic end, in the cis- configuration. If there are methyl branches off of the chain, "iso" indicates a branch on the second carbon from the methyl end, while "anteiso" indicates a branch on the third carbon from the methyl end. The prefix "br" indicates an unknown methyl branching position. If the methyl branch is mid-chain, it is designated by position from the carboxyl end. 10 Me 18:0 indicates an eighteen

carbon chain with a methyl branch in the tenth position from the carboxyl end. Cyclopropyl fatty acids are indicated with the prefix "cy" (e.g. cy17:0).

Signature fatty acids

Lipid profiles provide insight into microbial community composition (Table 3-1) because the relative abundance of certain PLFAs differs considerably among specific groups of microorganisms (Zogg et al., 1997). Ringelberg et al. (1997) suggest that differences in the end products of the fatty acid biosynthetic pathways provide insight into the microbial community. The branched-chain pathway used by Gram-positive bacteria results in the formation of terminally methyl-branched saturated fatty acids (i15:0, a15:0), whereas the anaerobic desaturase pathway used by Gram-negative bacteria results in the formation of monoenoic fatty acids (16:1ω7c, 18:1ω7c). Gram-positive bacteria tend to be slower-growing, more resilient, and capable of degrading more complex compounds than Gram-negative organisms. Gram-negative bacteria generally are faster-growing, able to utilize many C sources, and adapt quickly to a variety of environments. Tunlid and White (1992) point out that bacteria characteristically contain odd-numbered chains, methyl-branched, and cyclopropane fatty acids. Fungi typically synthesize saturated, even-numbered chains and polyenoic fatty acids.

Much of the literature on signature fatty acids has dealt with extracting lipids from pure cultures of organisms. As an example of this type of study, Zelles (1997) investigated the fatty acids of 33 taxonomically different organisms (bacteria, fungi, and plant cells) which are known to inhabit soil systems. Some of the finding of this study were as follows:

1. 18:1ω11c (cis vaccenic acid) constituted about 50% of the PLFA in selected bacteria from the alpha subclass of proteobacteria, but was not found in other organisms.
2. Cyclopropyl fatty acids are almost exclusively found in Gram-negative organisms.
3. Beta hydroxy fatty acids (from LPS) are found in many bacterial taxa, as well as

Table 3-1. Selected PLFA biomarkers

PLFA	Functional Group	Reference
i15:0, a15:0, i17:0, a17:0	Gram-positive bacteria	Ringelberg et al., 1997
i15:0, a15:0, i16:0, i17:0, a17:0	Gram-positive bacteria	Frostegård & Bååth, 1996
i15:0, a15:0, i16:0, i17:0	Gram-positive bacteria	Bardgett et al., 1996
10me16, i15:0, a15:0, i16:0 i17:0, a17:0	Gram-positive bacteria	Zogg et al., 1997
i15:0, i16:0, 10me16:0	Gram-positive bacteria	Zak et al., 1996
16:1ω7c, 18:1ω7c, cy 17:0, cy19:0	Gram-negative bacteria	Ringelberg et al., 1997
16:1ω9,16:1ω7t, cy 17:0,18:0ω7,cy19:0	Gram-negative bacteria	Frostegård & Bååth, 1996
cy 17:0,18:1ω7,cy19:0	Gram-negative bacteria	Bardgett et al., 1996
16:1ω5c,16:1ω7c,16:1ω7t,16:1ω9c, 18:1ω5c,18:1ω7c,cy17:0,cy19:0	Gram-negative bacteria	Zogg et al., 1997
16:1ω7c, cy17:0, 18:1ω7c, 18:1ω7t cy 19:0	Gram-negative bacteria	Zak et al., 1996
10me18:0	Actinomycetes	Ringelberg et al., 1997
Mid-chain branched fatty acids	Actinomycetes	White et al., 1997
10me16:0	Sulfate-reducers (*Desulfobacter)*	Ringelberg et al, 1997
i17:1ω7c	Sulfate-reducers (*Desulfovibrio)*	Ringelberg et al, 1997
i17:1ω7c, i15:1ω7c, i19:1ω7c	Sulfate-reducers (*Desulfovibrio)*	White et al., 1997
16:1ω8c, 18:1ω8c	Methane oxidizers	Ringelberg et al., 1997
18:2ω6, 18:3ω6, 18:3ω3	Fungi	Ringelberg et al., 1997
18:1ω9, 18:2ω6, 18:3ω3	Fungi	Zogg et al., 1997
18:1ω9, 18:2ω6	Fungi	Zak et al., 1996
20:3ω6, 20:4ω6	Protozoa	Ringelberg et al., 1997
20:2ω6, 20:3ω6, 20:4ω6	Protozoa	White et al., 1997
18:1ω9c, 18:1ω11c, 18:3ω3, 20:5ω3	Higher plants	Ringelberg et al., 1997

fungi and plants. This fact limits their suitability as a signature fatty acid for Gram-negative bacteria.

4. Among the branched-chain fatty acids, iso and anteiso branching occur mainly in Gram-positive bacteria but can be found in some Gram-negative organisms. However, branching at other chain positions (e.g. 10 Me) was almost never found in Gram-negative bacteria.

A number of other studies report on "signature fatty acids" from pure culture studies. Ringelberg et al., (1997) point out that extracting the PLFA of the Gram-positive *Arthrobacter* sp. has shown that some of these organisms contain as much as 80% of the membrane fatty acids as a15:0. Federle (1986) found that in 47 species of fungi the fatty acid 18:2ω6 constituted, on average, 43% of the total PLFA. The use of 18:2ω6 as a fungal biomarker was confirmed by Frostegård and Bååth (1996) who found that the soil content of 18:2ω6 was correlated with the ergosterol content ($r = 0.92$). The methyl-branched PLFA 10 Me16:0 and 10Me18:0 are found in both sulfate reducers and actinomycetes (Sundh, 1997). Frostegård and Bååth (1996) did not include the fatty acid 16:1ω5c in their list of fatty acids which represent bacteria since it has also been found in pure culture studies of arbuscular mycorrhizal fungi.

Physiological status

The signature lipid biomarker analysis can also provide insight into the nutritional and physiological status of the microbial community. Gram-negative bacteria are known to alter their membrane fatty acid components in response to environmental stresses. The Gram-negative signature fatty acids 16:1ω7c and 18:1ω7c are increasingly converted to the cyclopropyl PLFA, cy 17:0 and cy 19:0 as the bacteria move from a logarithmic to a stationary phase of growth (White et al., 1997). An increase in cyclopropyl PLFA formation has been correlated with increased anaerobic metabolism in monoculture studies of facultative heterotrophs (Vestal and White, 1989). Changes in the environment which produce stress (toxicity or starvation) can cause bacteria to produce monounsaturated fatty acids with a "trans-" confirmation. Trans-/cis- ratio of greater than 0.1 have been

shown to indicate starvation is bacterial isolates (White et al., 1997).

Guckert et al. (1986) studied metabolic changes during microbial nutrient starvation. When *Vibrio cholerae* were starved during a 30 day experiment the bacteria decreased their content of the fatty acid 16:1ω7c from 39% of the PLFA profile to 11%, and increased 16:1ω7t from 1% to 17%. Similarly, there was a decrease in 18:1ω7c from 19% to 13% and an increase in 18:1ω7t from 0.2% to 5%.

Kieft et al. (1997) compared starvation survival capabilities of surface and subsurface strains of *Pseudomonas fluorescens* and a novel *Arthrobacter* sp. in natural sediment microcosms. PLFA profiles were used to show PLFA stress signatures. Environmental conditions were shown to strongly affect PLFA stress signatures. While the *Arthrobacter* (Gram-positive) showed few changes in PLFA, both strains of *Pseudomonas fluorescens* (Gram-negative) increased ratios of saturates to unsaturates, increased ratios of trans- to cis- monoenoic fatty acids, and increased ratios of cyclopropyl fatty acids to their monoenoic precursors. Zogg et al. (1997) suggest that changes in lipid composition, such as decreases in unsaturation, usually occur as a result of biosynthesis of new lipids. Since biosynthesis is energetically expensive, portions of the microbial community will experience stress as a result of these shifts.

In some conditions, anaerobic metabolism can be estimated from the ratio of iso-branched to anteiso-branched saturated PLFA. Gram-positive aerobes such as *Arthrobacter* and *Micrococcus* have i17:0/a17:0 of approximately 0.2 while Gram-negative anaerobes such as Desulfovibrio have i17:0/a17:0 of greater than 5.

Shifts in percentages of PLFA groups indicate changes in community structure

The viable biomass can be estimated from the total PLFA measured in the sample. Terminally branched saturates are common to Gram-positive bacteria and some Gram-negative anaerobic bacteria such as the sulfate-reducing bacteria (White et al., 1997). Monoenoics are found in most all Gram-negative bacteria and many microeukaryotes (fungi, yeasts, algae). Zogg et al., (1997) comment that although Gram-positive bacteria can produce monoenoics, these fatty acids contribute very little (typically less than 20%)

to the Gram-positive PLFA profile. Thus, monoenoics are often considered biomarkers for Gram-negative bacteria. In addition, cyclopropane PLFA are common in Gram-negative organisms. Some groups of bacteria form monoenoic PLFA with the double bond in an unusual position such as 18:1ω8c in the type II methane-oxidizing bacteria. Bacteria can use both the anaerobic desaturase pathway forming ω7 monoenoics, or the aerobic desaturase pathway forming ω9 monoenoics. Eukaryotes form ω9 monoenoics. Polyenoics typically indicate the presence of microeukaryotes. 18:2ω6 is common in fungi but also is found in algae and protozoa. Branched monoenoics are common in the anaerobic *Desulfovibrio*-type sulfate-reducing bacteria. They are also found in actinomycetes. Mid-chain branched saturates are prominent in the aerobic actinomycetes (particularly 10 Me 18:0). Samples with large ratios of 10 Me 16:0/10 Me 18:0 often indicate anaerobic gram-negative *Desulfobacter*-type sulfate-reducing bacteria. Normal saturates are common to all classes of microorganisms. Bacteria generally have greater amounts of 16:0 while eukaryotes have greater percentages of 18:0. Normal saturates longer than 20 carbons are typical of microeukaryotes.

Patterns of fatty acids have been used to show differences in soil microbiota as a result of different agricultural practices (Zelles et al., 1992), exposure to alkaline dust (Baath et al., 1992), and with exposure to heavy metal dust (Frostegard et al., 1993b). Baath et al. (1995) used PLFA to study the effects of clearcut, wood ash-fertilized, or prescribed burned coniferous forest on microbial biomass and community structure. Frostegard et al. (1993a) found that liming and wood ash fertilization altered PLFA patterns indicating an increase in gram-negative organisms and actinomycetes with increasing pH. PLFA does not allow for detection of the specific species present in the soil (due to overlap of signature PLFA) but it does provide an overview of the community as a whole (White and Macnaughton, 1997).

The objective of this study was to use patterns of PLFA to describe changes in soil microbial community structure as a result of shifting from a traditional row-cropping system to a system where sycamore trees are grown as a short-rotation woody crops for biomass fuel production.

Methods and Materials

Site description and sampling procedure

This study was conducted at the University of Tennessee experiment station at Ames Plantation near Grand Junction, Tennessee. The site was designed to examine the environmental impacts of conversion of cropland to SRWC. Prior to establishment of the study the area was under conventionally-tilled silage corn production for at least 15 consecutive years. The soil type at the study site is a Memphis-Loring silt loam intergrade (fine-silty mixed thermic Typic Hapludalf - fine-silty mixed thermic Typic Fragiudalf). These soils are characterized by a high silt and low clay content, low CEC, and a variably-developed fragipan between soil depths of 90 - 150 cm. The study site consisted of six 0.5 ha plots constructed as mini-hydrological units, with 0.5m confinement berms (soil brought from other portions of the field) surrounding three corn (Zea mays L.) plots and three sycamore (Platinus occidentalis L.) plots. These plots will be referred to as "corn" and "young sycamore" respectively. The young sycamore plots were located within an even-aged 3.5 ha plantation of sycamore. Plots could not be randomly distributed because a wildlife study associated with this project required large unbroken tracts of each treatment. Sycamore has been identified as having a high potential as a biomass tree crop because of its ability to develop a dense canopy and a pervasive root system over a short rotation (Graham, 1994).

Sycamore seedlings were planted at 2.4m by 1.2m spacing in February, 1995. After two growing seasons tree survival was over 97%. The corn plots have been maintained in no-till corn silage production. The winter cover crop was wheat. The wheat was killed in the spring using glyphosate, and not harvested. During the growing seasons since establishment, silage corn and sycamore plots have been managed as two separate systems, with chemical and fertilization inputs and harvest geared to the best management of each.

In 1983 Wells and Fribourg (1992) established three 17m x 17m plots of American Sycamore approximately 2 miles away but on a similar soil type. This site will

be referred to as "old sycamore". Sycamore seedlings were planted at a spacing of 2.4m between rows and 1.2m within rows on a site that had been planted in conventionally-tilled soybean. The area surrounding the plots was maintained in soybeans. No fertilizer was applied to the trees. Weed growth was controlled by disking or by using glyphosate for one year (Fribourg et al., 1989). At four years of age the trees had a 96% survival rate. Every other row of sycamore was harvested at five years. A second harvest in 1993 collected the coppice and the 10-year-old trees (Fleenor, 1996). The trees which had regrown were cut again in the spring of 1998 and the biomass was left on the site.

Immediately adjacent to the young sycamore plots is a mature mixed hardwood forest. This forest is approximately 50 years old, and has a mixed species composition of oak, sweetgum, red maple, black cherry, and an assortment of hardwood trees and shrubs. Soil samples were collected from this site (referred to as "mature forest") in March, and August, 1998. These treatments were chosen for study to show soil biological properties in a continuum from a traditional annual row cropping system to variously-aged SRWC systems, to a mature hardwood site.

Soil sampling

Mineral soil cores were collected during June and November 1997, and March and August 1998 to a depth of 6 cm using a soil core sampler (inside diameter = 5.3 cm) (SoilMoisture Equipment Corp., Santa Barbara). Organic litter was removed from the soil surface prior to sampling. Aseptic techniques were used to prevent contamination of the samples. These techniques included the following:

1. Nitrile gloves were worn.
2. The soil core sampler was rinsed with a 1:1 mixture of chloroform:methanol between samplings.
3. The sampling container was lined with aluminum foil and rinsed with isopropanol between samplings.
4. Soil cores had the outer portion, which had come into contact with the soil core sampler, removed with a sterile parring knife.

In June 1997, samples were taken both from within rows of corn or trees as well as in between rows, in order to account for any rhizosphere effect. Each treatment-plot combination was divided into a grid and a number was assigned to each section of the grid. Three sections were randomly chosen. Three cores were taken within 1 meter of the base of the tree, or within the row of corn and bulked in a plastic bag to represent one "row sample". Likewise, 3 or 4 cores were collected from between rows and bulked as an "interrow" sample. The bags were stored in a cooler on dry ice and taken to the laboratory within 24 hours of sampling. The samples were lyophilized and stored at a temperature of -20°C or lower until analysis. The samples were ground with a sterile mortar and pestle, and visible organic debris (mostly roots) was removed. Glassware used in analyses was meticulously cleaned and heated in a muffle furnace for a minimum of 4 hours at 450°C before use.

PLFA analysis showed no significant differences due to sampling location. For all subsequent sampling events a series of soil cores were collected which included both rhizosphere and interrow samples. These were bulked and stored in plastic bags on dry ice. In March and August 1998 a portion of each sample was stored on dry ice to be used for PLFA and DNA analysis. However, the portion to be used for chemical analysis (Part 2), chloroform-fumigation (Part 5), and enzyme assays (Part 5) was stored in a cooler on ice, returned to the laboratory, and stored at 4°C so as to not kill organisms by freezing. All analyses on these refrigerated samples were conducted within 2 weeks of sampling.

Description of the phospholipid fatty acid procedure

Fatty acids in lyophilized soil were extracted with a single phase phosphate-buffered mixture of chloroform, methanol (1:2 vol/vol) (Bligh and Dyer, 1959). The solvents dissolved the intact lipids providing a snapshot of the lipids at the time of extraction. Nanopure water and chloroform were added to separate aqueous and organic phases by changing the polarity of the mixture (Vestal and White, 1989). The mixture was shaken and allowed to sit overnight. Lipids remained in the organic phase while the aqueous phase contained LPS-OHFAs (Zogg et al., 1997). The organic phase was

transferred to a silicic acid column and separated into neutral lipid, glycolipid, and polar lipid fractions by elution with solvents of increasing polarity (White et al., 1997). The polar lipid fraction contained the phospholipids. This fraction was subjected to a transesterification by mild alkaline methanolysis to produce fatty acid methyl esters (FAME) which were separated, quantified, and identified by high-resolution fused silica capillary gas chromatography using a Hewlett Packard 5890 Series 2 (Wilmington, DE). Chemical structures are verified by GC-mass spectroscopy using a Hewlett Packard 5971 mass selective detector. Mass spectra were determined by electron impact at 70 eV.

Statistical analysis of PLFA profiles

The relative proportion (percentage mole fraction) of PLFA was used to test the null hypothesis that changing the crop management system from corn to sycamore would not influence the composition of the soil microbial communities. To test that hypothesis, an analysis of variance (ANOVA) using the type III General Linear Model SAS procedure (SAS Institute, 1990) for a completely randomized design with three treatments in June and November of 1997 and four treatments in March and August of 1998 was used. The Values reported are means of 9 samples (3 for the mature forest plots). Differences in the mean proportions of PLFA in each treatment were tested using a Fisher's least-significant-difference procedure. Standard errors of the mean were determined using the lsmeans procedure. MANOVA procedures were also conducted to analyze PLFA profiles. Data (percentage mole fraction) were arc-sine transformed prior to analyses for meet assumptions of normality. Dendrograms from a hierarchical cluster analysis were constructed from arcsin transformed ester-linked PLFA mole percent profiles to analyze similarities between profiles using Ward's method based on euclidean distances. This analysis was utilized to observe relationships between patterns of PLFA, as related to the different cropping systems. The PLFA compositions (expressed in mole percent) of different samples were combined with soil chemical data (Part 2) and subjected to a principal-components analysis (PCA). The hierarchical cluster analysis and the PCA were performed on means of each plots (3 reps per plots) using the statistical package

"Statistica 1997" (StatSoft Inc., Tulsa OK). The plots generated from a PCA illustrate profile differences and also identify which PLFAs and soil properties contribute to the formation of the plots (coefficients of loading). Significance for all statistical tests was accepted at $\alpha = 0.05$.

Results and Discussion

Differences between sampling locations

At the first soil sampling date in June 1997, an effort was made to account for differences on soil biochemical properties as a result of sampling location within the row. Soil was collected from within rows of corn or trees, as well as between rows (Figure 2-2). These samples were analyzed separately. Somewhat surprisingly, no differences were found as a result of sampling location, either in soil chemical or in soil biological properties. Figure 3-1 is representative of the results of this study. It shows total PLFA biomass for the three treatments, separated by sampling location. While there were treatment differences, there were no significant differences due to sampling location. Other studies have shown greater biomass and a different community composition in the soil which adhered to plant roots (considered "rhizosphere") as compared with bulk soil (Ringelberg, et al., 1997). In this study, however, no effort was made to sample "rhizosphere" soil, but simply the soil which was closer to the corn or trees. Both corn and the trees produce extensive root systems which would encompass both the "row" and "interrow" region. Plant litter was fairly evenly distributed in the corn and old sycamore plots. At the June sampling, the litter layer in the young sycamore plantation was not well established and most of the soil cover was located within 1 meter of the base of the tree. However, the leaves had only begun to decompose and probably had contributed little C to the soil organic matter. At the other sampling dates samples were collected from both the "row" and "interrow" locations and combined, but no effort was made to distinguish between the locations. For the June 1997 statistical analysis the two sampling locations were treated as replicate samples.

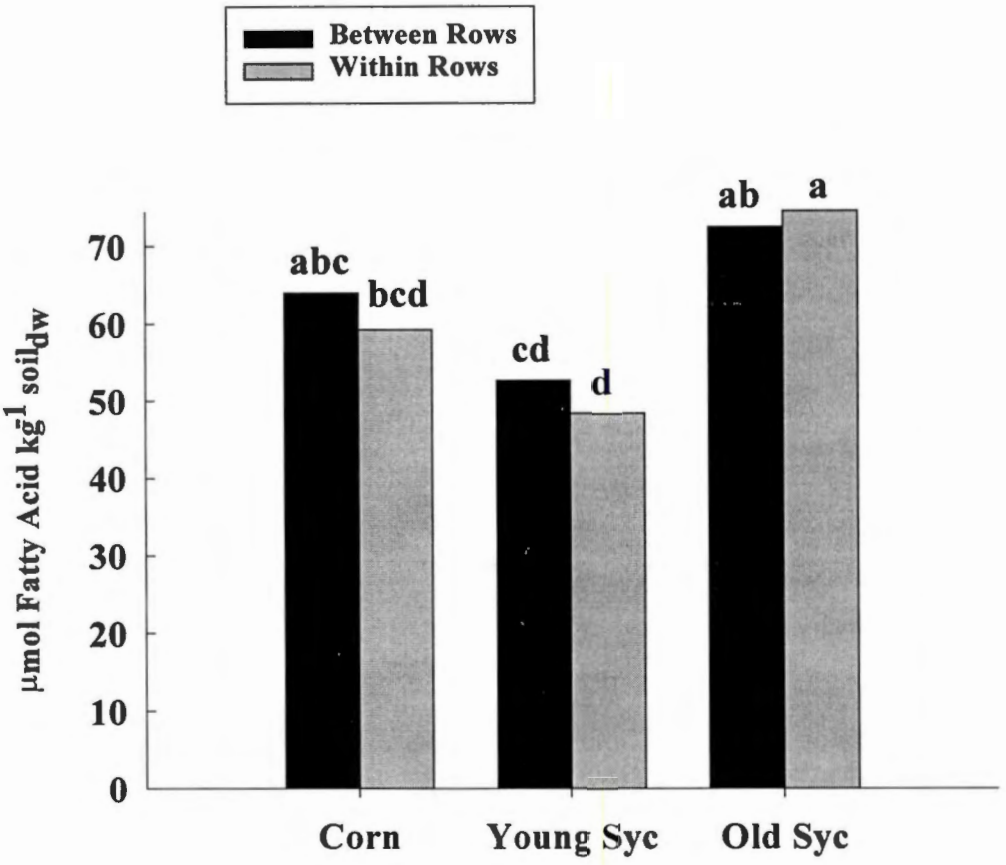


Figure 3-1. June 1997 PLFA biomass by treatment and sampling location

Community structure

Figures 3-2, 3-3, 3-4, and 3-5 show differences between treatments in mole fraction of selected PLFA representing some of the "signature" fatty acid biomarkers (Table 3-1) for the four sampling dates. Mole percentage indicates what percentage of the total PLFA profile is made up by an individual fatty acid. For example, in June 1997 approximately 10% of all the fatty acids extracted from the young sycamore soil were i15:0 (Figure 3-2). Expressing fatty acids on a mole percent basis allows a direct comparison of the community structure of the three treatments. In Figures 3-4 and 3-5, the mature hardwood samples were omitted so as to make the figures more readable.

Although the soil samples were collected at different seasons of the year, there was significant consistency in the community structure at the different sampling dates. The terminally-branched saturated fatty acids are shown to the left of each figure. These are indicative of Gram-positive bacteria (Table 3-1). With the exception of the fatty acid a15:0, these fatty acids were consistently a larger component of the young sycamore soil profiles than they were for other treatments. Gram-positive bacteria tend to be slower-growing, more resilient, and capable of degrading more complex compounds than Gram-negative organisms. Gram-negative bacteria are generally faster-growing, able to utilize many C sources, and able to adapt quickly to a variety of environments. Levels of total C and total N were lower in the young sycamore soil (Figures 2-3 through 2-7), so heterotrophic organisms would have less substrate to mineralize. During the time period that the trees were becoming established the system was more like a fallow system than the other treatments. The trees have been withdrawing nutrients from the soil but cycling very little residue back to the soil. Weeds were controlled with herbicides. The bare soil was exposed to the environment. Lower levels of soil organic matter mean less substrate, lower porosity (lower aeration), higher bulk density, and less water-holding capacity.

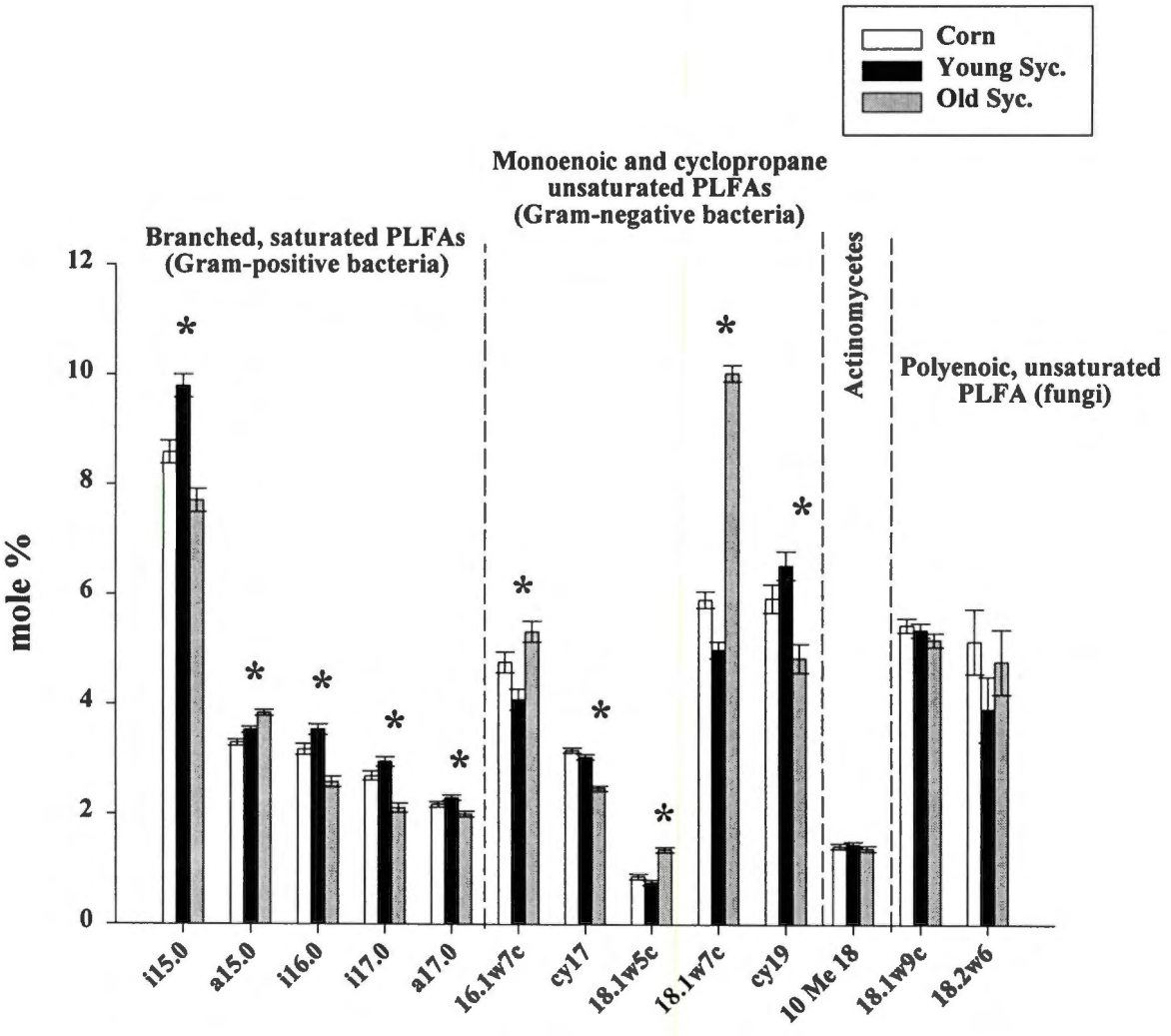


Figure 3-2. June 1997 differences between treatments in mole fraction of selected PLFA extracted from soils. Error bars are one standard error of the mean of nine samples. Asterisks (*) indicate a significant ($P<0.05$) treatment effect using sampling error in an F test

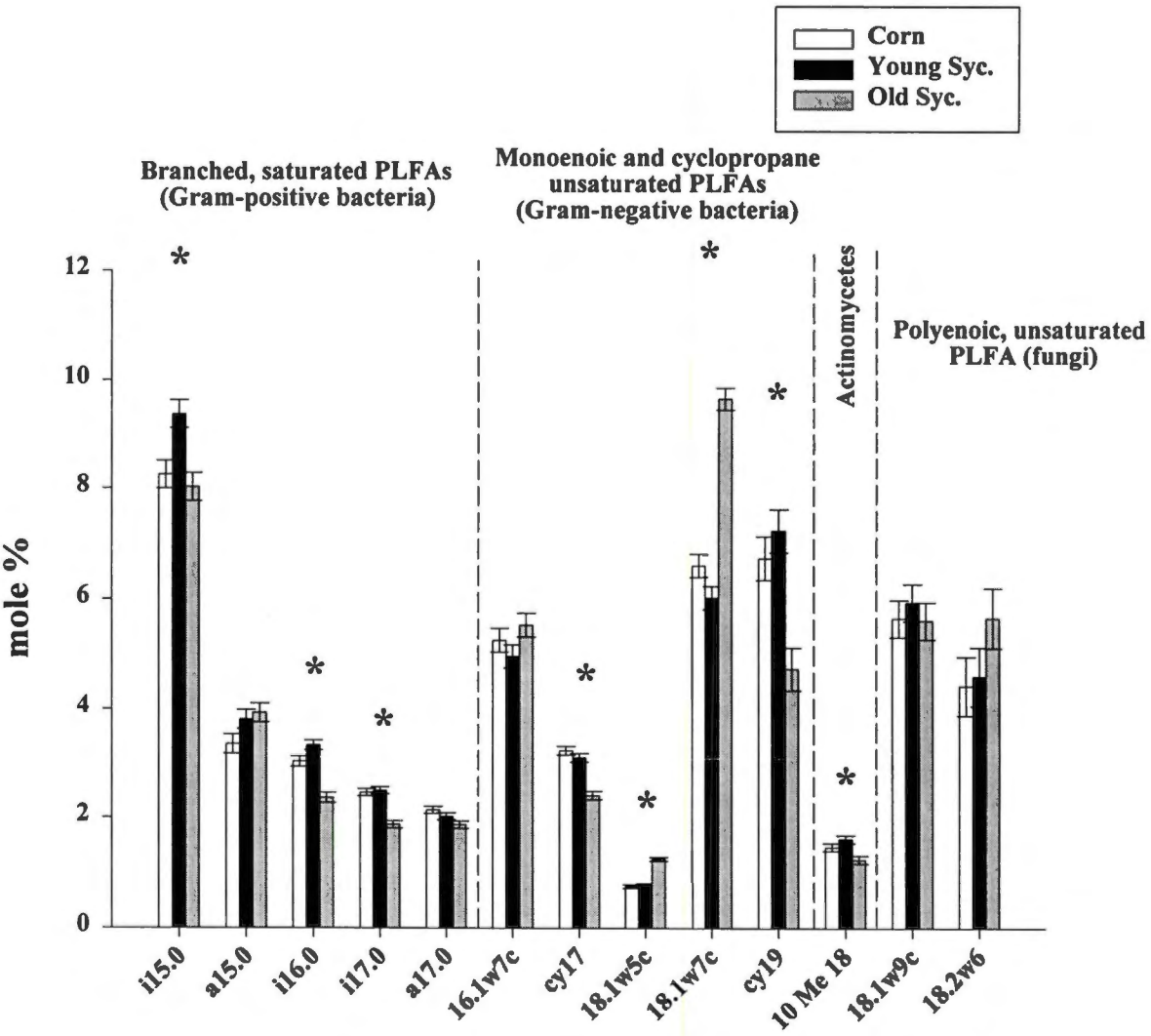


Figure 3-3. November 1997 differences between treatments in mole fraction of selected PLFA extracted from soils. Error bars are one standard error of the mean of nine samples. Asterisks (*) indicate a significant ($P<0.05$) treatment effect using sampling error in an F test

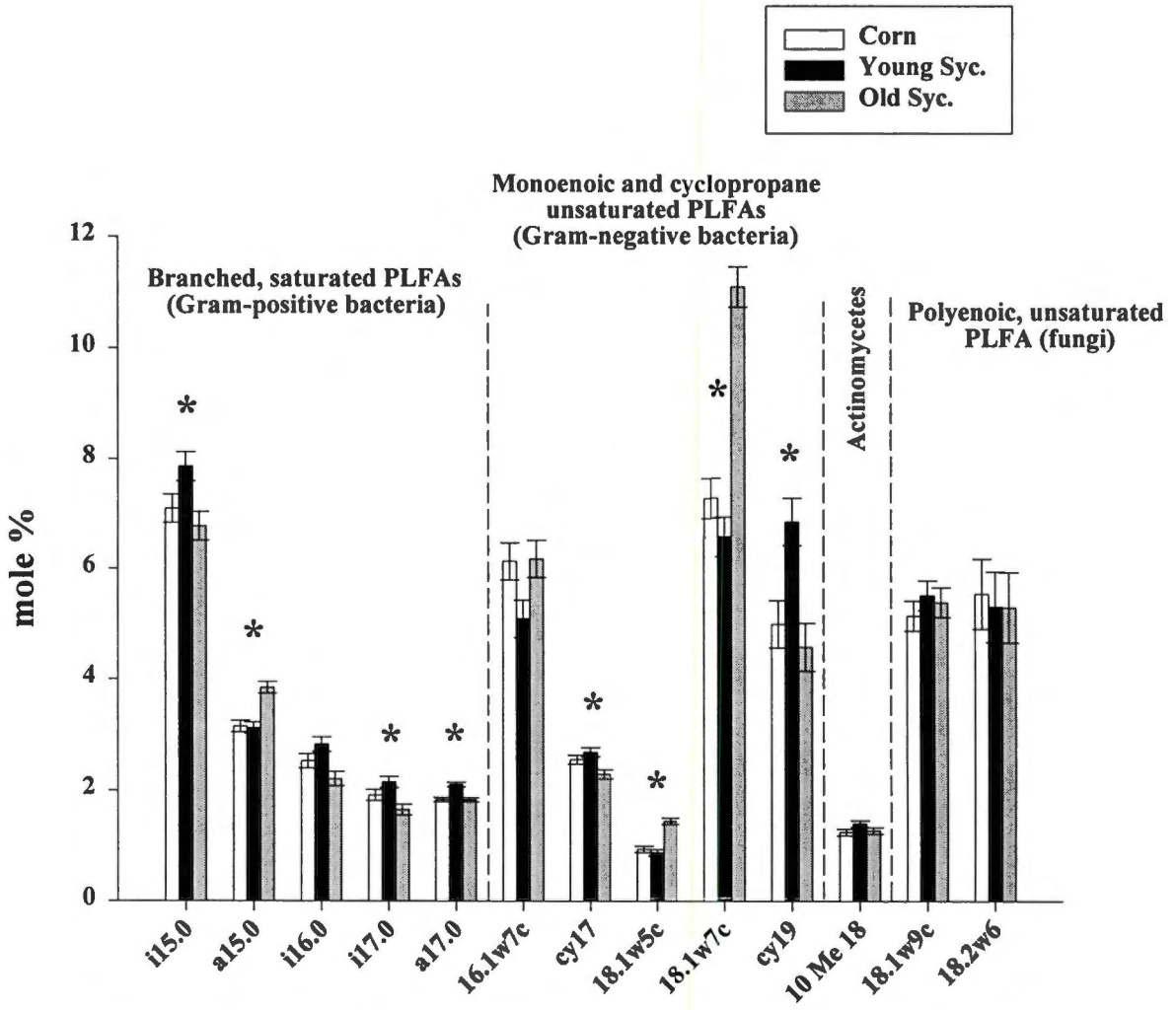


Figure 3-4. March 1998 differences between treatments in mole fraction of selected PLFA extracted from soils. Error bars are one standard error of the mean of nine samples. Asterisks (*) indicate a significant (P<0.05) treatment effect using sampling error in an F test

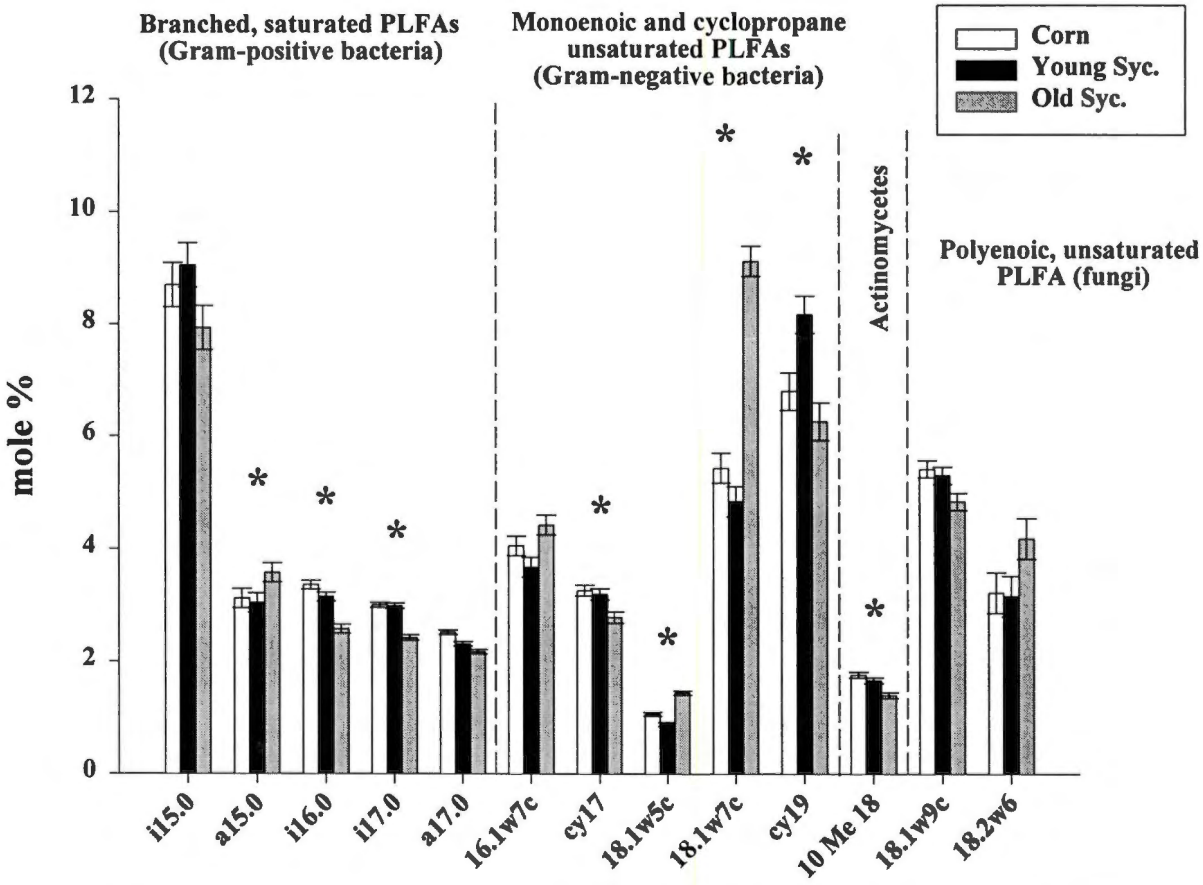


Figure 3-5. August 1998 differences between treatments in mole fraction of selected PLFA extracted from soils. Error bars are one standard error of the mean of nine samples. Asterisks (*) indicate a significant ($P<0.05$) treatment effect using sampling error in an F test

The monoenoic and cyclopropane fatty acids are characteristic of Gram-negative bacteria. The relative amounts of these biomarkers give several clues about the microbial communities in these soils. The Gram-negative fatty acids 16:1ω7c and 18:1ω7c are precursors of the cyclopropyl fatty acids cy17:0 and cy19:0 (White et al., 1997). The slower the growth rate of the bacterial community the greater will be the ratio of cyclopropyl fatty acids to their monoenoic precursors. The fatty acids 18:1ω5c and 18:1ω7c were a significantly greater component of the old sycamore community than the young sycamore community at all four sampling dates. The proportion of 16:1ω7c was consistently larger in the old sycamore community than the young sycamore community, while differences between the old sycamore and the corn tended to be small for this fatty acid. The older sycamore microbial community had significantly less cyclopropyl fatty acids than either the corn or the young sycamore. At no sampling date was there a significant difference between the corn and young sycamore communities in percentage of cy17:0. However, the young sycamore communities had significantly more cy19:0 in March and August, 1998 than did the corn community. This pattern of fatty acids indicates that the old sycamore plantation had a more Gram-negative community of bacteria than the other treatments, and that the organisms in the old sycamore soil were under less nutritional stress than the other treatments.

Mid-chain branched saturates are characteristic of actinomycetes. The fatty acid 10 Me 18:0 is often considered a signature biomarker for actinomycetes. This fatty acid tended to be found in similar proportions in the corn and young sycamore soil. The old sycamore soil tended to have a lower proportion of this fatty acid, and the difference was found to be significant in November and August. Actinomycetes tend to become a larger component of the microbial community during conditions of prolonged drying because of the ability of actinomycetes to produce conidia which are able to withstand dessication (Alexander, 1998). The August 1998 samples were collected during a prolonged dry spell.

The fatty acids 18:2ω6c and 18:1ω9c are often considered biomarkers for fungi. Several studies have suggested that as the amount of substrate entering the soil increases,

the fungal component of the microbial community should increase because fungi are more efficient than bacteria at converting substrate into biomass (Zak et al., 1996). Nevertheless, the percentage of neither fatty acid was found to be significantly different between these treatments. There was a great deal of "noise" associated with 18:2ω6c which may have prevented detecting significance, particularly in the November and August samplings. In order to test the validity of these fatty acids for the determination of fungal populations another measure of fungal biomass could be used. Frostegard and Baath (1996) describe the use of ergosterol as a measure of fungal biomass. Stahl and Parkin (1996) proposed a method of estimating living fungal biomass from soil ergosterol content which compensates for the variability in fungal ergosterol concentrations.

Tables 3-2 through 3-5 show the mole percent composition of all of the major fatty acids for each sampling date. A comparison of Table 3-1 with these tables will give clues about other components of the microbial community not previously discussed. For example, 10 Me 16:0 is often considered a biomarker for sulfate-reducing bacteria (specifically *Desulfobacter*) (Ringelberg, et al., 1997). This fatty acid tended to be a smaller component of the fatty acid profile of the corn system (significantly lower in June). The fatty acid i17:1ω7c is considered a biomarker for sulfate-reducing bacteria of the genus *Desulfovibrio*. This fatty acid was a significantly larger component of the young sycamore profile. Tables 3-4 and 3-5 also include information about the mature hardwood forest for the March and August samplings. The mature hardwood forest had the highest levels of soil organic matter of any treatment, so it is not surprising that the microbial community tended to have more Gram-negative bacteria than the corn or young sycamore communities. A surprising observation is that while the soil from the mature forest had a very high component of 18:1ω7c, it also had high levels of cy19:0. While both fatty acids are characteristic of Gram-negative bacteria, I would have expected a high growth rate of the organisms in the mature forest because of the high level of substrate. Cyclopropane

Table 3-2. Percent composition (mole %) of PLFA profile (± one standard error of the mean), June 1997

PLFA	Significance	Corn	Young Sycamore	Old Sycamore
i14:0	ns	0.47 ± 0.01	0.46 ± 0.01	0.46 ± 0.01
14:0	**	0.82 ± 0.01	0.83 ± 0.01	0.70 ± 0.01
i15:0	**	8.58 ± 0.21	9.79 ± 0.21	7.70 ± 0.21
a15:0	**	3.29 ± 0.06	3.52 ± 0.06	3.83 ± 0.06
15:0	***	0.64 ± 0.01	0.65 ± 0.01	0.50 ± 0.01
i16:0	**	3.16 ± 0.10	3.53 ± 0.10	2.57 ± 0.10
16:1ω9c	**	1.05 ± 0.04	1.02 ± 0.04	1.29 ± 0.04
16:1ω7c	*	4.75 ± 0.19	4.07 ± 0.19	5.31 ± 0.19
16:1ω7t	**	0.18 ± 0.01	0.16 ± 0.01	0.24 ± 0.01
16:1ω5c	*	3.58 ± 0.10	3.21 ± 0.10	3.83 ± 0.10
16:0	**	15.39 ± 0.23	13.85 ± 0.23	13.73 ± 0.23
i17:1ω7c	**	2.10 ± 0.06	2.54 ± 0.06	2.13 ± 0.06
10 Me 16	*	3.94 ± 0.18	4.67 ± 0.18	4.79 ± 0.18
12 Me 16	ns	0.29 ± 0.02	0.34 ± 0.02	0.32 ± 0.02
i17:0	**	2.68 ± 0.08	2.94 ± 0.08	2.10 ± 0.08
a17:0	*	2.17 ± 0.05	2.29 ± 0.05	2.00 ± 0.05
cy17:0	***	3.15 ± 0.05	3.03 ± 0.05	2.46 ± 0.05
17:0	***	0.59 ± 0.01	0.69 ± 0.01	0.53 ± 0.01
10 Me 17	***	1.18 ± 0.06	1.21 ± 0.06	0.48 ± 0.06
18:2ω6	ns	5.13 ± 0.59	3.90 ± 0.59	4.76 ± 0.59
18:3ω3	ns	0.96 ± 0.05	1.10 ± 0.05	0.95 ± 0.05
18:1ω9c	ns	5.43 ± 0.13	5.34 ± 0.13	5.16 ± 0.13
18:1ω7c	***	5.89 ± 0.15	4.97 ± 0.15	10.01 ± 0.15
18:1ω7t	***	0.21 ± 0.00	0.24 ± 0.00	0.17 ± 0.00
18:1ω5c	***	0.87 ± 0.05	0.76 ± 0.05	1.35 ± 0.05
18:0	**	2.64 ± 0.05	3.00 ± 0.05	2.75 ± 0.05
10:Me 18	ns	1.42 ± 0.05	1.45 ± 0.05	1.38 ± 0.05
12:Me 18	***	0.67 ± 0.02	0.75 ± 0.02	0.49 ± 0.02
19:1ω6c	**	0.26 ± 0.01	0.30 ± 0.01	0.17 ± 0.01
cy 19:0	*	5.92 ± 0.26	6.52 ± 0.26	4.82 ± 0.26
20:4ω6	*	1.37 ± 0.08	1.68 ± 0.08	1.24 ± 0.08
20:5ω3	ns	0.50 ± 0.04	0.63 ± 0.04	0.60 ± 0.04
20:0	**	1.09 ± 0.06	1.22 ± 0.06	0.81 ± 0.06
22:0	ns	0.28 ± 0.02	0.31 ± 0.02	0.33 ± 0.02
24:0	*	0.27 ± 0.02	0.24 ± 0.02	0.19 ± 0.02

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

Table 3-3. Percent composition (mole %) of PLFA profile (± one standard error of the mean), November 1997

PLFA	Significance	Corn	Young Sycamore	Old Sycamore
i14:0	ns	0.38 ± 0.07	0.26 ± 0.07	0.40 ± 0.07
14:0	ns	0.58 ± 0.05	0.40 ± 0.05	0.56 ± 0.05
i15:0	*	8.25 ± 0.26	9.37 ± 0.26	8.02 ± 0.26
a15:0	ns	3.35 ± 0.18	3.80 ± 0.18	3.92 ± 0.18
15:0	*	0.49 ± 0.01	0.46 ± 0.01	0.42 ± 0.01
i16:0	***	3.03 ± 0.09	3.33 ± 0.09	2.36 ± 0.09
16:1ω9c	*	1.15 ± 0.06	1.10 ± 0.06	1.38 ± 0.06
16:1ω7c	ns	5.25 ± 0.21	4.96 ± 0.21	5.52 ± 0.21
16:1ω7t	***	0.02 ± 0.01	0.00 ± 0.01	0.23 ± 0.01
16:1ω5c	ns	4.06 ± 0.18	3.72 ± 0.18	3.98 ± 0.18
16:0	ns	13.01 ± 0.35	13.64 ± 0.35	12.19 ± 0.35
i17:1ω7c	**	2.91 ± 0.09	3.13 ± 0.09	2.36 ± 0.09
10 Me 16	ns	4.54 ± 0.26	4.78 ± 0.26	4.81 ± 0.26
12 Me 16	ns	0.39 ± 0.02	0.34 ± 0.02	0.33 ± 0.02
i17:0	***	2.46 ± 0.06	2.49 ± 0.06	1.87 ± 0.06
a17:0	ns	2.13 ± 0.07	2.02 ± 0.07	1.86 ± 0.07
cy17:0	***	3.23 ± 0.08	3.10 ± 0.08	2.40 ± 0.08
17:0	ns	0.44 ± 0.01	0.50 ± 0.01	0.46 ± 0.01
10 Me 17	***	1.31 ± 0.05	1.26 ± 0.05	0.45 ± 0.05
18:2ω6	ns	4.42 ± 0.54	4.59 ± 0.54	5.65 ± 0.54
18:3ω3	**	1.95 ± 0.10	1.36 ± 0.10	1.31 ± 0.10
18:1ω9c	ns	5.64 ± 0.33	5.93 ± 0.33	5.60 ± 0.33
18:1ω7c	***	6.60 ± 0.20	6.02 ± 0.20	9.65 ± 0.20
18:1ω7t	*	0.16 ± 0.02	0.08 ± 0.02	0.20 ± 0.02
18:1ω5c	***	0.75 ± 0.03	0.77 ± 0.03	1.24 ± 0.03
18:0	*	2.25 ± 0.13	2.85 ± 0.13	2.33 ± 0.13
10:Me 18	*	1.46 ± 0.07	1.61 ± 0.07	1.23 ± 0.07
12:Me 18	*	0.75 ± 0.06	0.73 ± 0.06	0.50 ± 0.06
19:1ω6c	*	0.27 ± 0.02	0.25 ± 0.02	0.16 ± 0.02
cy 19:0	**	6.73 ± 0.39	7.22 ± 0.39	4.73 ± 0.39
20:4ω6	ns	1.16 ± 0.12	0.89 ± 0.12	1.27 ± 0.12
20:5ω3	**	0.56 ± 0.06	0.18 ± 0.06	0.63 ± 0.06
20:0	***	1.24 ± 0.06	1.39 ± 0.06	0.72 ± 0.06
22:0	ns	0.33 ± 0.09	0.59 ± 0.09	0.37 ± 0.09
24:0	*	0.34 ± 0.12	0.79 ± 0.12	0.28 ± 0.12

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

Table 3-4. Percent composition (mole %) of PLFA profile (± one standard error of the mean), March 1998

PLFA	Significance	Corn	Young Syc	Old Syc	Mature
i14:0	ns	0.43 ± 0.05	0.38 ± 0.05	0.40 ± 0.05	0.61 ± 0.08
14:0	*	0.66 ± 0.02	0.59 ± 0.02	0.53 ± 0.02	0.50 ± 0.04
i15:0	*	7.09 ± 0.26	7.86 ± 0.26	6.77 ± 0.26	6.12 ± 0.46
a15:0	**	3.15 ± 0.10	3.12 ± 0.10	3.85 ± 0.10	4.23 ± 0.17
15:0	**	0.54 ± 0.01	0.52 ± 0.01	0.44 ± 0.01	0.40 ± 0.02
i16:0	ns	2.53 ± 0.13	2.83 ± 0.13	2.21 ± 0.13	2.18 ± 0.22
16:1ω9c	ns	1.42 ± 0.10	1.19 ± 0.10	1.57 ± 0.10	1.69 ± 0.18
16:1ω7c	ns	6.13 ± 0.34	5.09 ± 0.34	6.17 ± 0.34	5.97 ± 0.58
16:1ω7t	ns	0.07 ± 0.03	0.07 ± 0.03	0.19 ± 0.03	0.23 ± 0.06
16:1ω5c	ns	4.04 ± 0.29	3.43 ± 0.29	4.06 ± 0.29	3.95 ± 0.50
16:0	**	12.05 ± 0.20	11.49 ± 0.20	10.83 ± 0.20	9.36 ± 0.35
i17:1ω7c	**	2.37 ± 0.10	3.13 ± 0.10	2.39 ± 0.10	2.53 ± 0.17
10 Me 16	ns	3.42 ± 0.31	4.42 ± 0.31	4.26 ± 0.31	4.16 ± 0.53
12 Me 16	ns	0.32 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.43 ± 0.06
i17:0	*	1.91 ± 0.10	2.15 ± 0.10	1.65 ± 0.10	1.52 ± 0.17
a17:0	**	1.83 ± 0.04	2.10 ± 0.04	1.83 ± 0.04	1.78 ± 0.07
cy17:0	*	2.56 ± 0.08	2.69 ± 0.08	2.29 ± 0.08	1.95 ± 0.14
17:0	ns	0.43 ± 0.02	0.47 ± 0.02	0.43 ± 0.02	0.39 ± 0.03
10 Me 17	**	0.97 ± 0.08	0.97 ± 0.08	0.43 ± 0.08	0.41 ± 0.14
18:2ω6	ns	5.54 ± 0.63	5.30 ± 0.63	5.29 ± 0.63	5.03 ± 1.10
18:3ω3	ns	2.42 ± 0.27	1.52 ± 0.27	1.25 ± 0.27	1.12 ± 0.47
18:1ω9c	ns	5.14 ± 0.27	5.51 ± 0.27	5.38 ± 0.27	5.60 ± 0.46
18:1ω7c	***	7.28 ± 0.36	6.57 ± 0.36	11.08 ± 0.36	13.62 ± 0.63
18:1ω7t	ns	0.19 ± 0.03	0.20 ± 0.03	0.18 ± 0.03	0.15 ± 0.06
18:1ω5c	**	0.93 ± 0.06	0.87 ± 0.06	1.44 ± 0.06	1.37 ± 0.10
18:0	ns	2.07 ± 0.07	2.34 ± 0.07	2.22 ± 0.07	2.00 ± 0.13
10:Me 18	ns	1.24 ± 0.06	1.39 ± 0.06	1.27 ± 0.06	1.45 ± 0.11
12:Me 18	*	0.58 ± 0.06	0.72 ± 0.06	0.46 ± 0.06	0.26 ± 0.10
19:1ω6c	ns	0.31 ± 0.04	0.36 ± 0.04	0.20 ± 0.04	0.19 ± 0.06
cy 19:0	*	4.99 ± 0.43	6.84 ± 0.43	4.58 ± 0.43	6.39 ± 0.74
20:4ω6	ns	1.56 ± 0.14	1.12 ± 0.14	1.22 ± 0.14	0.94 ± 0.24
20:5ω3	**	1.18 ± 0.07	0.64 ± 0.07	0.76 ± 0.07	0.62 ± 0.12
20:0	*	1.33 ± 0.10	1.75 ± 0.10	1.00 ± 0.10	1.19 ± 0.18
22:0	**	0.53 ± 0.02	0.65 ± 0.02	0.57 ± 0.02	0.38 ± 0.04
24:0	**	0.63 ± 0.05	0.74 ± 0.05	0.42 ± 0.05	0.26 ± 0.09

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

Table 3-5. Percent composition (mole %) of PLFA profile (± one standard error of the mean), August 1998

PLFA	Significance	Corn	Young Syc	Old Syc	Mature
i14:0	**	0.33 ± 0.03	0.28 ± 0.03	0.36 ± 0.03	0.66 ± 0.04
14:0	ns	0.66 ± 0.05	0.59 ± 0.05	0.61 ± 0.05	0.63 ± 0.08
i15:0	ns	8.70 ± 0.46	9.05 ± 0.46	7.93 ± 0.46	8.20 ± 0.80
a15:0	**	3.12 ± 0.10	3.04 ± 0.10	3.58 ± 0.10	4.35 ± 0.18
15:0	***	0.67 ± 0.01	0.58 ± 0.01	0.53 ± 0.01	0.49 ± 0.02
i16:0	**	3.36 ± 0.07	3.15 ± 0.07	2.58 ± 0.07	2.72 ± 0.12
16:1ω9c	**	0.88 ± 0.04	0.84 ± 0.04	1.03 ± 0.04	1.24 ± 0.06
16:1ω7c	ns	4.05 ± 0.15	3.67 ± 0.15	4.42 ± 0.15	4.28 ± 0.26
16:1ω7t	ns	0.09 ± 0.04	0.08 ± 0.04	0.19 ± 0.04	0.20 ± 0.06
16:1ω5c	ns	3.28 ± 0.18	2.87 ± 0.18	3.31 ± 0.18	3.18 ± 0.30
16:0	ns	13.13 ± 0.26	13.06 ± 0.26	13.68 ± 0.26	11.39 ± 0.44
i17:1ω7c	ns	2.55 ± 0.09	2.90 ± 0.09	2.46 ± 0.09	2.65 ± 0.16
10 Me 16	ns	4.83 ± 0.18	5.45 ± 0.18	5.54 ± 0.18	5.40 ± 0.31
12 Me 16	ns	0.40 ± 0.03	0.41 ± 0.03	0.43 ± 0.03	0.49 ± 0.05
i17:0	***	3.00 ± 0.05	2.98 ± 0.05	2.42 ± 0.05	2.31 ± 0.09
a17:0	ns	2.51 ± 0.08	2.31 ± 0.08	2.17 ± 0.08	2.08 ± 0.14
cy17:0	**	3.26 ± 0.11	3.19 ± 0.11	2.78 ± 0.11	2.22 ± 0.19
17:0	ns	0.67 ± 0.04	0.66 ± 0.04	0.59 ± 0.04	0.51 ± 0.08
10 Me 17	***	1.22 ± 0.04	1.48 ± 0.04	0.35 ± 0.04	0.43 ± 0.08
18:2ω6	ns	3.22 ± 0.44	3.16 ± 0.44	4.18 ± 0.44	2.72 ± 0.76
18:3ω3	ns	0.48 ± 0.09	0.85 ± 0.09	0.60 ± 0.09	0.35 ± 0.16
18:1ω9c	ns	5.41 ± 0.28	5.30 ± 0.28	4.83 ± 0.28	4.96 ± 0.48
18:1ω7c	***	5.43 ± 0.16	4.83 ± 0.16	9.11 ± 0.16	9.93 ± 0.29
18:1ω7t	ns	0.26 ± 0.02	0.28 ± 0.02	0.19 ± 0.02	0.26 ± 0.04
18:1ω5c	***	1.06 ± 0.04	0.88 ± 0.04	1.43 ± 0.04	1.41 ± 0.06
18:0	ns	2.94 ± 0.05	2.93 ± 0.05	3.01 ± 0.05	2.81 ± 0.08
10:Me 18	**	1.76 ± 0.05	1.66 ± 0.05	1.40 ± 0.05	1.42 ± 0.08
12:Me 18	***	0.73 ± 0.04	0.82 ± 0.04	0.50 ± 0.04	0.00 ± 0.07
19:1ω6c	**	0.39 ± 0.02	0.42 ± 0.02	0.21 ± 0.02	0.27 ± 0.04
cy 19:0	**	6.79 ± 0.21	8.16 ± 0.21	6.25 ± 0.21	8.26 ± 0.36
20:4ω6	ns	1.50 ± 0.19	1.14 ± 0.19	1.00 ± 0.19	0.77 ± 0.33
20:5ω3	ns	0.46 ± 0.08	0.33 ± 0.08	0.47 ± 0.08	0.31 ± 0.08
20:0	***	1.65 ± 0.06	2.10 ± 0.06	1.13 ± 0.06	1.62 ± 0.10
22:0	**	0.50 ± 0.02	0.64 ± 0.02	0.54 ± 0.02	0.38 ± 0.03
24:0	**	0.50 ± 0.03	0.61 ± 0.03	0.38 ± 0.03	0.27 ± 0.05

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

fatty acids are typically created during conditions which favor slow growth of the bacterial community. The fatty acid cy17:0 was significantly lower in the mature forest than in the other treatments. This observation shows the danger of making generalizations about a microbial community based on a single fatty acid. Zelles et al. (1995) suggest that in view of the complexity of the soil ecosystem it is better to consider groups of fatty acids with a similar chemical structure as bioindicators for microorganisms

Groupings of fatty acids

In Table 3-6 fatty acids were grouped by mole percentage in an effort to concisely describe the microbial community structure. Normal saturates are common to all classes of microorganisms. The more diverse the microbial community, the more diverse the PLFA profile is likely to be. If a high percentage of the community is represented by normal saturates it could be an indication of less microbial diversity (Zogg, et al., 1997). The corn and young sycamore profiles had significantly greater percentages of normal saturates than did the established forest systems. Mid-branched saturates are typical of actinomycetes. The treatments studied were never significantly different in this grouping. Terminally-branched saturates are common to Gram-positive organisms and were highest in the young sycamore plots. In addition, the corn system typically had a significantly larger component of terminally-branched saturates than did the old sycamore system. Monoenoics are found in almost all Gram-negative organisms and were a significantly larger component of the old sycamore and mature forest systems. Branched-monoenoics are common in sulfate-reducing bacteria (*Desulfovibrio*) and actinomycetes, and percentages tended to be higher in the established forest systems . Polyenoics are characteristic of eukaryotes and were not significantly different in the four treatments.

Table 3-6. Groups of fatty acids (mole % of PLFA profile) ± one standard error of the mean

Sampling Date June 1997	Corn	Young Sycamore	Old Sycamore	Mature Forest	Signif.
Normal Saturates	21.76 ± 0.28	20.82 ± 0.28	19.68 ± 0.28	-	**
Mid-Branched Sats.	11.43 ± 0.43	12.73 ± 0.43	10.98 ± 0.43	-	ns
Term. Branched Sats.	20.51 ± 0.41	22.66 ± 0.41	18.76 ± 0.41	-	**
Monoenoics	32.06 ± 0.32	30.47 ± 0.32	35.97 ± 0.32	-	***
Branched Monoenoics	4.64 ± 0.15	4.99 ± 0.15	5.82 ± 0.15	-	**
Polyenoics	9.26 ± 0.75	8.33 ± 0.75	8.54 ± 0.75	-	ns
November, 1997					
Normal Saturates	18.70 ± 0.72	20.68 ± 0.72	17.43 ± 0.72	-	*
Mid-Branched Sats.	12.25 ± 0.64	11.82 ± 0.64	11.27 ± 0.64	-	ns
Term. Branched Sats.	19.69 ± 0.40	21.32 ± 0.40	18.50 ± 0.40	-	**
Monoenoics	34.51 ± 0.44	33.46 ± 0.44	36.34 ± 0.44	-	*
Branched Monoenoics	5.65 ± 0.20	5.32 ± 0.20	6.38 ± 0.20	-	*
Polyenoics	9.21 ± 0.82	7.40 ± 0.82	10.09 ± 0.82	-	ns
March, 1998					
Normal Saturates	18.59 ± 0.17	18.92 ± 0.17	16.83 ± 0.17	14.78 ± 0.29	***
Mid-Branched Sats.	10.15 ± 0.70	11.94 ± 0.70	10.51 ± 0.70	9.87 ± 1.22	ns
Term. Branched Sats.	17.21 ± 0.55	18.76 ± 0.55	16.87 ± 0.55	16.58 ± 0.96	ns
Monoenoics	34.61 ± 0.85	33.85 ± 0.85	38.55 ± 0.85	42.34 ± 1.47	**
Branched Monoenoics	6.30 ± 0.30	6.56 ± 0.30	7.18 ± 0.30	7.49 ± 0.52	ns
Polyenoics	13.14 ± 0.98	9.97 ± 0.98	10.06 ± 0.98	8.94 ± 1.69	ns
August, 1998					
Normal Saturates	20.98 ± 0.29	21.45 ± 0.29	20.78 ± 0.29	18.39 ± 0.50	*
Mid-Branched Sats.	13.22 ± 0.52	13.72 ± 0.52	11.82 ± 0.52	11.85 ± 0.91	ns
Term. Branched Sats.	21.29 ± 0.49	21.19 ± 0.49	19.25 ± 0.49	20.50 ± 0.85	ns
Monoenoics	32.45 ± 0.35	31.56 ± 0.35	35.10 ± 0.35	37.58 ± 0.61	***
Branched Monoenoics	4.78 ± 0.14	5.36 ± 0.14	5.59 ± 0.14	6.62 ± 0.24	**
Polyenoics	7.27 ± 0.61	6.72 ± 0.61	7.46 ± 0.61	5.06 ± 1.07	ns

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Physiological status

A variety of fatty acid ratios have been proposed as indicators of the nutritional and physiological status of the microbial community (Guckert, 1996; Kieft, 1997). These have included ratios of the cyclopropyl fatty acids cy17:0 and cy19:0 to the monoenoic precursors - 16:1ω7c and 18:1ω7c, respectively (White et al., 1997). This transition has been associated with a decreased growth rate as well as increased anaerobic metabolism (Vestal and White, 1989). Ratios of monoenoic fatty acids with a trans- conformation to those with a cis- conformation has been associated with stress such as starvation or toxicity (Kieft et al., 1997). Ratios of iso-branched to anteiso-branched saturated PLFA (Kieft et al., 1997) and ratios of saturated to unsaturated fatty acids (Zogg et al., 1997) have also been identified as stress indicators for microbial communities.

Table 3-7 summarizes these ratios for the Ames Plantation SRWC project. It is probably best to utilize these ratios with caution, as they were developed based on pure culture studies or on organisms grown in controlled conditions far different than the complex environment of the soil. Nevertheless, the consistency of the ratios in Table 3-7 merits consideration. The ratios are written in such a way that the more starved, stressed, or anaerobic environment should have the highest value. The young sycamore community consistently had the highest ratio or was not significantly different than the corn community. The old sycamore community consistently had lower ratios. The exception to this rule was the trans- to cis- ratio for 16C chains. In many cases there were simply no 16C chains with a trans- conformation found in the young sycamore soils (not enough biomass). Trans- fatty acids have been associated with a response to toxic conditions. There is no reason to expect toxic conditions to exist as this site.

Tables 3-8 through 3-11 show correlation coefficients between various measures of soil microbial biomass, soil C and N, and the ratios of fatty acids which indicate community physiological and nutritional status. Correlation of biomass measures and soil chemical properties will be discussed in Part 5. With the exception of trans- to cis- ratio of 16C chains, (and trans- to cis- ratio of 18C chains in November) the ratios were

Table 3-7. Ratios of fatty acids which indicate physiological status.

Sampling Date June 1997	Corn	Young Sycamore	Old Sycamore	Mature Forest	Signif
15 C iso/antiso ratio	2.62b †	2.81a	2.02c	-	***
17 C iso/antiso ratio	1.24a	1.29a	1.05b	-	**
17cyclopropyl/monoenoic	0.76a	0.69a	0.46b	-	***
19cyclopropyl/monoenoic	1.06b	1.35a	0.48c	-	***
16 C trans/cis ratio	0.038b	0.041ab	0.045a	-	*
18 C trans/cis ratio	0.038b	0.050a	0.017c	-	***
Saturate/Unsaturate ratio	1.47b	1.59a	1.19c	-	***
November, 1997					
15 C iso/antiso ratio	2.47a	2.48a	2.05b	-	*
17 C iso/antiso ratio	1.15a	1.24 a	1.01b	-	**
17cyclopropyl/monoenoic	0.63a	0.63a	0.43b	-	**
19cyclopropyl/monoenoic	1.03a	1.25a	0.50b	-	**
16 C trans/cis ratio	0.003b	0.0007b	0.041a	-	***
18 C trans/cis ratio	0.025a	0.011b	0.022ab	-	ns
Saturate/Unsaturate ratio	1.26a	1.39a	1.11b	-	**
March, 1998					
15 C iso/antiso ratio	2.26b	2.53a	1.76c	1.46d	***
17 C iso/antiso ratio	1.04a	1.02ab	0.90ab	0.86b	ns
17cyclopropyl/monoenoic	0.43ab	0.54a	0.37b	0.33b	*
19cyclopropyl/monoenoic	0.74ab	1.07a	0.42b	0.47b	*
16 C trans/cis ratio	0.014a	0.012a	0.029a	0.037a	ns
18 C trans/cis ratio	0.029a	0.030a	0.017a	0.010a	ns
Saturate/Unsaturate ratio	1.13ab	1.23a	0.97bc	0.83c	*
August, 1998					
15 C iso/antiso ratio	2.79a	2.99a	2.21b	1.93c	***
17 C iso/antiso ratio	1.20ab	1.30a	1.12b	1.12b	*
17cyclopropyl/monoenoic	0.81b	0.88a	0.63c	0.52d	***
19cyclopropyl/monoenoic	1.28b	1.73a	0.69c	0.84c	***
16 C trans/cis ratio	0.024a	0.022a	0.043a	0.050a	ns
18 C trans/cis ratio	0.049ab	0.059a	0.021c	0.030bc	**
Saturate/Unsaturate ratio	1.49a	1.53a	1.28b	1.15c	***

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05)

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 3-8. Correlation coefficients (r) between, microbial biomass C, PLFA biomass, soil chemical properties and physiological indicators, June, 1997

	PLFA	Mic. Bio.	Soil C	Soil N	16 t/c	18 t/c	17 c/m	19 c/m	15 i/a	17 i/a	sat/ un
PLFA	-	0.60 ***	0.68 ***	0.67 ***	ns	-0.76 ***	-0.72 ***	-0.71 ***	-0.59 ***	-0.47 ***	-0.75 ***
Microbial Biomass C	0.60 ***	-	0.71 ***	0.66 ***	ns	-0.58 ***	-0.58 ***	-0.59 ***	-0.68 ***	-0.54 ***	-0.62 ***
Soil C	0.68 ***	0.71 ***	-	0.92 ***	0.28 *	-0.64 ***	-0.66 ***	-0.63 ***	-0.79 ***	-0.60 ***	-0.71 ***
Soil N	0.67 ***	0.66 ***	0.92 ***	-	ns	-0.66 ***	-0.67 ***	-0.63 ***	-0.76 ***	-0.63 ***	-0.74 ***
16 C trans/cis	ns	ns	0.28 *	ns	-	ns	ns	ns	ns	ns	ns
18 C trans/cis	-0.76 ***	-0.58 ***	-0.64 ***	-0.66 ***	ns	-	0.92 ***	0.94 ***	0.77 ***	0.68 ***	0.88 ***
17 cyclopropyl/ 16 monoenoic	-0.72 ***	-0.58 ***	-0.66 ***	-0.67 ***	ns	0.92 ***	-	0.95 ***	0.79 ***	0.74 ***	0.87 ***
19 cyclopropyl/ 18 monoenoic	-0.71 ***	-0.59 ***	-0.63 ***	-0.63 ***	ns	0.94 ***	0.95 ***	-	0.83** *	0.79 ***	0.83 ***
15 C iso/antiso	-0.59 ***	-0.68 ***	-0.79 ***	-0.76 ***	ns	0.77 ***	0.79 ***	0.83 ***	-	0.83 ***	0.76 ***
17 C iso/antiso	-0.47 ***	-0.54 ***	-0.60 ***	-0.63 ***	ns	0.68 ***	0.74 ***	0.79 ***	0.83 ***	-	0.62 ***
Saturate/ unsaturate	-0.75 ***	-0.62 ***	-0.71 ***	-0.74 ***	ns	0.88 ***	0.87 ***	0.83 ***	0.76 ***	0.62 ***	-

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 3-9. Correlation coefficients (r) between, microbial biomass C, PLFA biomass, soil chemical properties and physiological indicators, November, 1997

	PLFA	Mic. Bio.	Soil C	Soil N	16 t/c	18 t/c	17 c/m	19 c/m	15 i/a	17 i/a	sat/ un
PLFA	-	0.54 ***	0.76 ***	0.68 ***	0.81 *	ns	-0.72 ***	-0.76 ***	-0.61 ***	-0.80 ***	-0.85 ***
Microbial Biomass C	0.54 ***	-	0.80 ***	0.68 ***	0.43 *	ns	ns	-0.38 *	-0.46 *	-0.56 **	ns
Soil C	0.76 ***	0.80 ***	-	0.91 ***	0.70 ***	ns	-0.49 **	-0.56 **	-0.62 ***	-0.69 ***	-0.51 **
Soil N	0.68 ***	0.68 ***	0.91 ***	-	0.59 **	ns	-0.44 *	-0.45 *	-0.57 **	-0.56 **	-0.47 *
16 C trans/cis	0.81 *	0.43 *	0.70 ***	0.59 **	-	ns	-0.71 ***	-0.69 ***	-0.61 ***	-0.69 ***	-0.63 ***
18 C trans/cis	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
17 cyclopropyl/ 16 monoenoic	-0.72 ***	ns	-0.49 **	-0.44 *	-0.71 ***	ns	-	0.81 ***	0.68 ***	0.66 ***	0.76 ***
19 cyclopropyl/ 18 monoenoic	-0.76 ***	-0.38 *	-0.56 **	-0.45 *	-0.69 ***	ns	0.81 ***	-	0.71 ***	0.81 ***	0.87 ***
15 C iso/antiso	-0.61 ***	-0.46 *	-0.62 ***	-0.57 **	-0.61 ***	ns	0.68 ***	0.71 ***	-	0.68 ***	0.60 **
17 C iso/antiso	-0.80 ***	-0.56 **	-0.69 ***	-0.56 **	-0.69 ***	ns	0.66 ***	0.81 ***	0.68 ***	-	0.76 ***
Saturate/ unsaturate	-0.85 ***	ns	-0.51 **	-0.47 *	-0.63 ***	ns	0.76 ***	0.87 ***	0.60 **	0.76 ***	-

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 3-10 . Correlation coefficients (r) between PLFA biomass, soil chemical properties and physiological indicators, March, 1998

	PLFA	Soil C	Soil N	16 t/c	18 t/c	17 c/m	19 c/m	15 i/a	17 i/a	sat/ un
PLFA	-	0.64 ***	0.67 ***	0.43 *	-0.42 *	-0.64 ***	-0.58 ***	-0.68 ***	-0.46 *	-0.75 ***
Soil C	0.64 ***	-	0.94 ***	0.46 *	ns	-0.53 **	-0.52 **	-0.70 ***	-0.58 ***	-0.66 ***
Soil N	0.67 ***	0.94 ***	-	0.37 *	ns	-0.50 **	-0.44 *	-0.60 ***	-0.48 **	-0.60 ***
16 C trans/cis	0.43 *	0.46 *	0.37 *	-	ns	ns	ns	-0.47 **	ns	ns
18 C trans/cis	-0.42 *	ns	ns	ns	-	0.61 ***	0.56 **	0.51 **	0.48 **	0.56 **
17 cyclopropyl/ 16 monoenoic	-0.64 ***	-0.53 **	-0.50 **	ns	0.61 ***	-	0.90 ***	0.73 ***	0.66 ***	0.80 ***
19 cyclopropyl/ 18 monoenoic	-0.58 ***	-0.52 **	-0.44 **	ns	0.56 **	0.90 ***	-	0.77 ***	0.77 ***	0.90 ***
15 C iso/antiso	-0.68 ***	-0.70 ***	-0.60 ***	-0.47 **	0.51 **	0.73 ***	0.77 ***	-	0.70 ***	0.75 ***
17 C iso/antiso	-0.46 *	-0.58 ***	-0.48 **	ns	0.48 **	0.66 ***	0.77 ***	0.70 ***	-	0.76 ***
Saturate/ unsaturate	-0.75 ***	-0.66 ***	-0.60 ***	ns	0.56 **	0.80 ***	0.90 ***	0.75 ***	0.76 ***	-

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 3-11. Correlation coefficients (r) between, microbial biomass C, PLFA biomass, soil chemical properties and physiological indicators, August, 1998

	PLFA	Mic. Bio.	Soil C	Soil N	16 t/c	18 t/c	17 c/m	19 c/m	15 i/a	17 i/a	sat/ un
PLFA	-	0.76 ***	0.88 ***	0.86 ***	0.56 **	-0.42 *	-0.86 ***	-0.70 ***	-0.81 ***	-0.57 **	-0.72 ***
Microbial Biomass C	0.76 ***	-	0.84 ***	0.86 ***	ns	-0.42 *	-0.65 ***	-0.63 ***	-0.74 ***	-0.65 ***	-0.63 ***
Soil C	0.88 ***	0.84 ***	-	0.98 ***	0.42 *	-0.48 **	-0.76 ***	-0.63 ***	-0.79 ***	-0.60 ***	-0.75 ***
Soil N	0.86 ***	0.86 ***	0.98 ***	-	0.39 *	-0.47 **	-0.76 ***	-0.62 ***	-0.77 ***	-0.59 ***	-0.75 ***
16 C trans/cis	0.56 **	ns	0.42 *	0.39 *	-	ns	-0.46 *	ns	ns	ns	ns
18 C trans/cis	-0.42 *	-0.42 *	-0.48 **	-0.47 **	ns	-	0.55 **	0.70 ***	0.59 ***	0.46 **	0.74 ***
17 cyclopropyl/ 16 monoenoic	-0.86 ***	-0.65 ***	-0.76 ***	-0.76 ***	-0.46 *	0.55 **	-	0.86 ***	0.85 ***	0.68 ***	0.75 ***
19 cyclopropyl/ 18 monoenoic	-0.70 ***	-0.63 ***	-0.63 ***	-0.62 ***	ns	0.70 ***	0.86 ***	-	0.84 ***	0.74 ***	0.80 ***
15 C iso/antiso	-0.81 ***	-0.74 **	-0.79 ***	-0.77 ***	ns	0.59 ***	0.85 ***	0.84 ***	-	0.74 ***	0.76 ***
17 C iso/antiso	-0.57 **	-0.65 ***	-0.60 ***	-0.59 ***	ns	0.46 **	0.68 ***	0.74 ***	0.74 ***	-	0.59 ***
Saturate/ unsaturate	-0.72 ***	-0.63 ***	-0.75 ***	-0.75 ***	ns	0.74 ***	0.75 ***	0.80 ***	0.76 ***	0.59 ***	-

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

all highly correlated with one another and highly negatively correlated with increasing microbial biomass and soil organic matter. Increased levels of soil C and N should create conditions which favor the growth and health of the microbial community. The correlations were consistent throughout the year. These correlations indicate that these ratios truly are good indicators of stress in soil microbial communities.

Hierarchical cluster analysis

Hierarchical cluster analyses were utilized to show relationships between entire patterns of PLFA, as related to the different treatments. Figures 3-6 through 3-9 show these analyses for the four dates on which PLFA samples were collected. Hierarchical analyses are based on positive correlations between treatments (Ringelberg et al., 1997). The sample coding indicates the sampling date (June, November, March, or August), the treatment (C = corn, YS = young sycamore, OS = old sycamore, or HD = hardwood), and the plot number. The dendrogram for June (Figure 3-6) shows separate "row" and "interrow" samples for each treatment/plot combination. This analysis offers further evidence that soil microbial community structure did not differ significantly with sampling location. Hierarchical cluster analysis showed that the microbial community structure differed for each of these treatments. While the microbial communities in the corn and young sycamore soils had more similarity with each other than with the other treatments, they still were distinct communities. In a like manner, the old sycamore and the mature forest communities (sampled in March and August) had more similarities with each other than with the corn and young sycamore soil communities. Each treatment formed its own cluster group, with the exception of corn plot 3, which clustered with the young sycamore in the March sampling. These results indicate that the greatest difference was between the established forest systems and the corn/young sycamore systems.

Principle components analysis

Principle components analysis (PCA) of the 38 most abundant PLFAs and 16

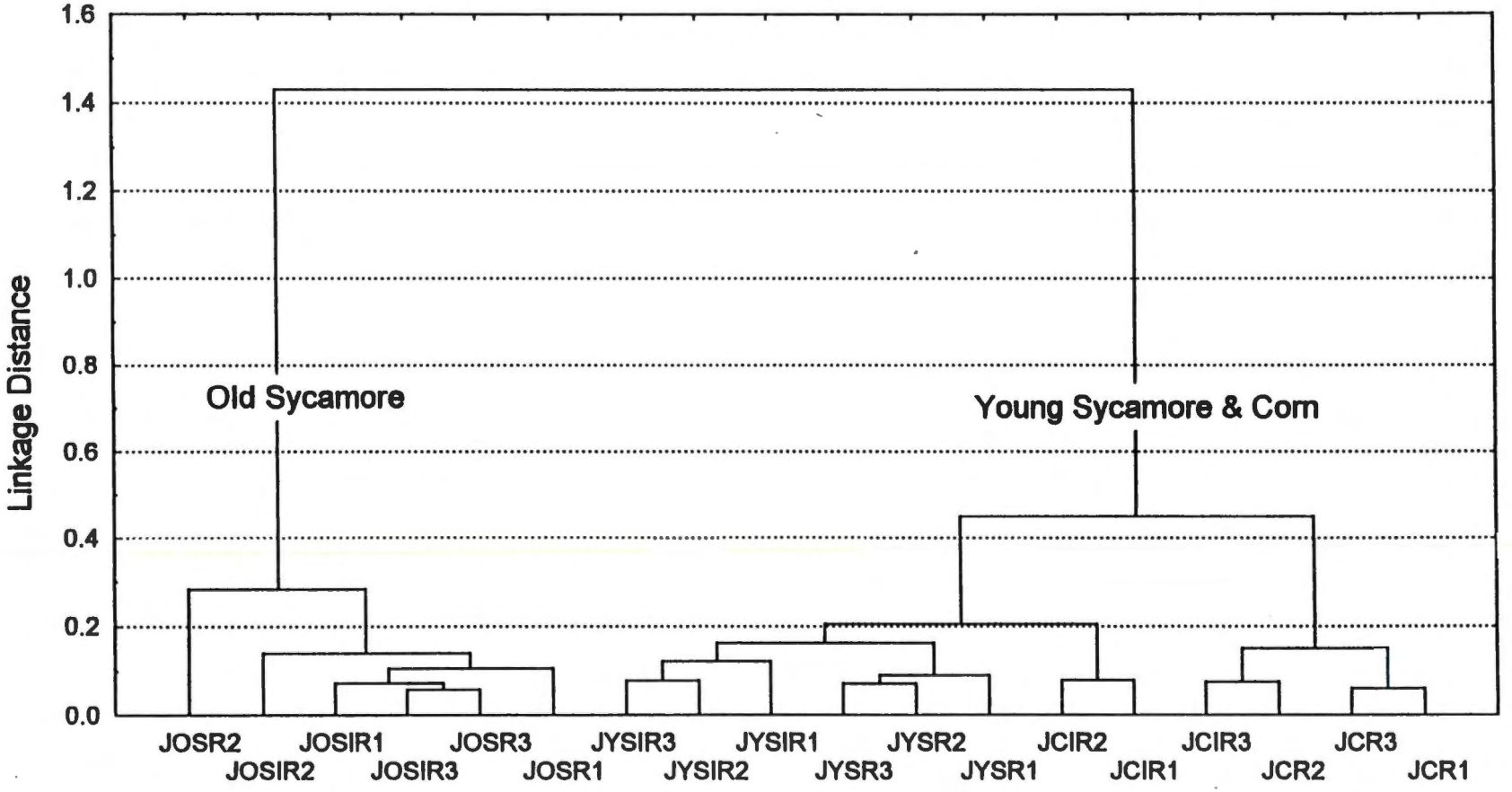


Figure 3-6. A dendrogram representing the results of a hierarchical cluster analysis (Ward's method) of averaged PLFA mole percentages (arcsin transformed) from the June 1997 sampling. Similarity shown by linkage at a lower euclidean distance

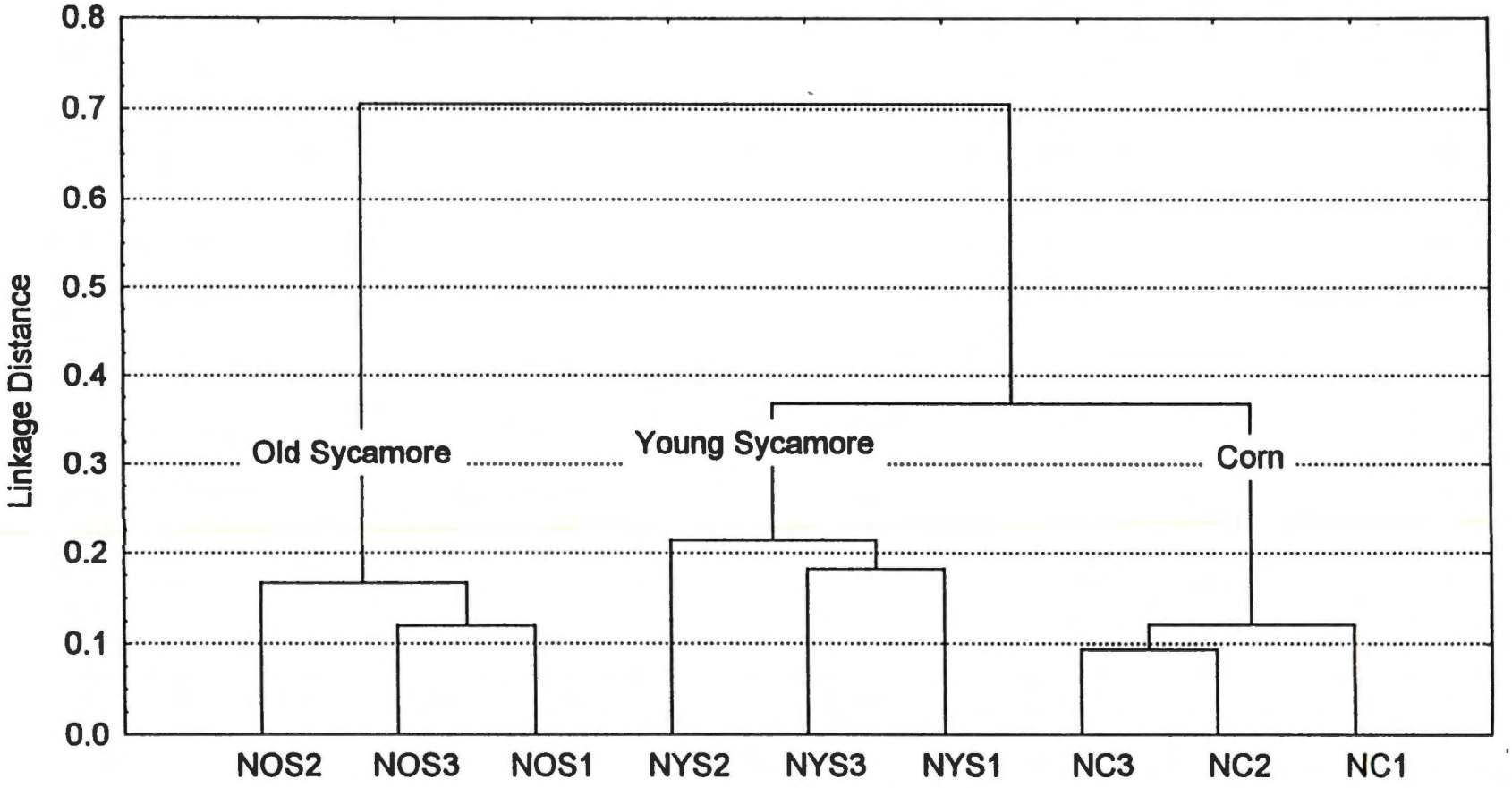


Figure 3-7. A dendrogram representing the results of a hierarchical cluster analysis (Ward's method) of averaged PLFA mole percentages (arcsin transformed) from the November 1997 sampling. Similarity shown by linkage at a lower euclidean distance

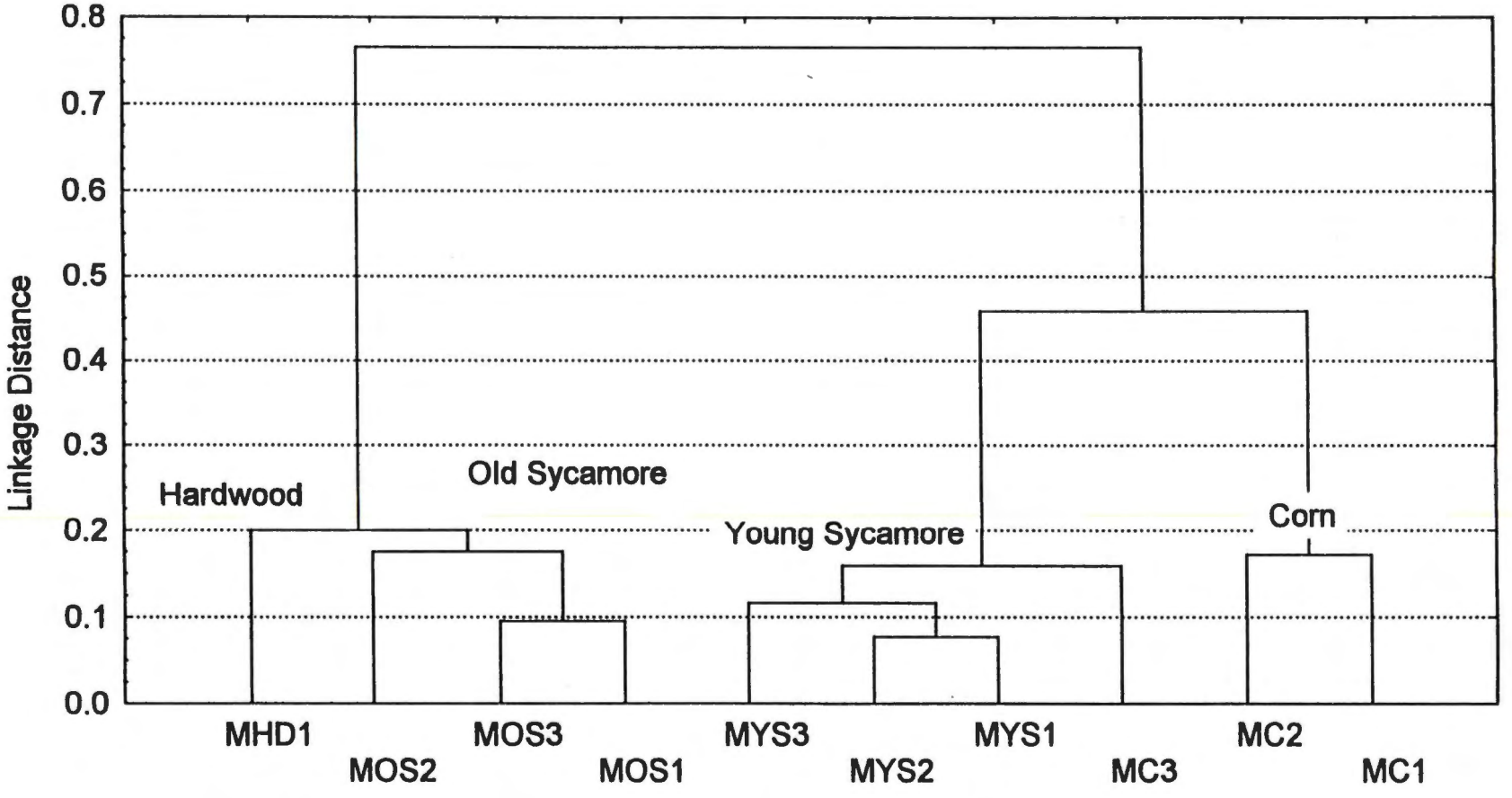


Figure 3-8. A dendrogram representing the results of a hierarchical cluster analysis (Ward's method) of averaged PLFA mole percentages (arcsin transformed) from the March 1998 sampling. Similarity shown by linkage at a lower euclidean distance

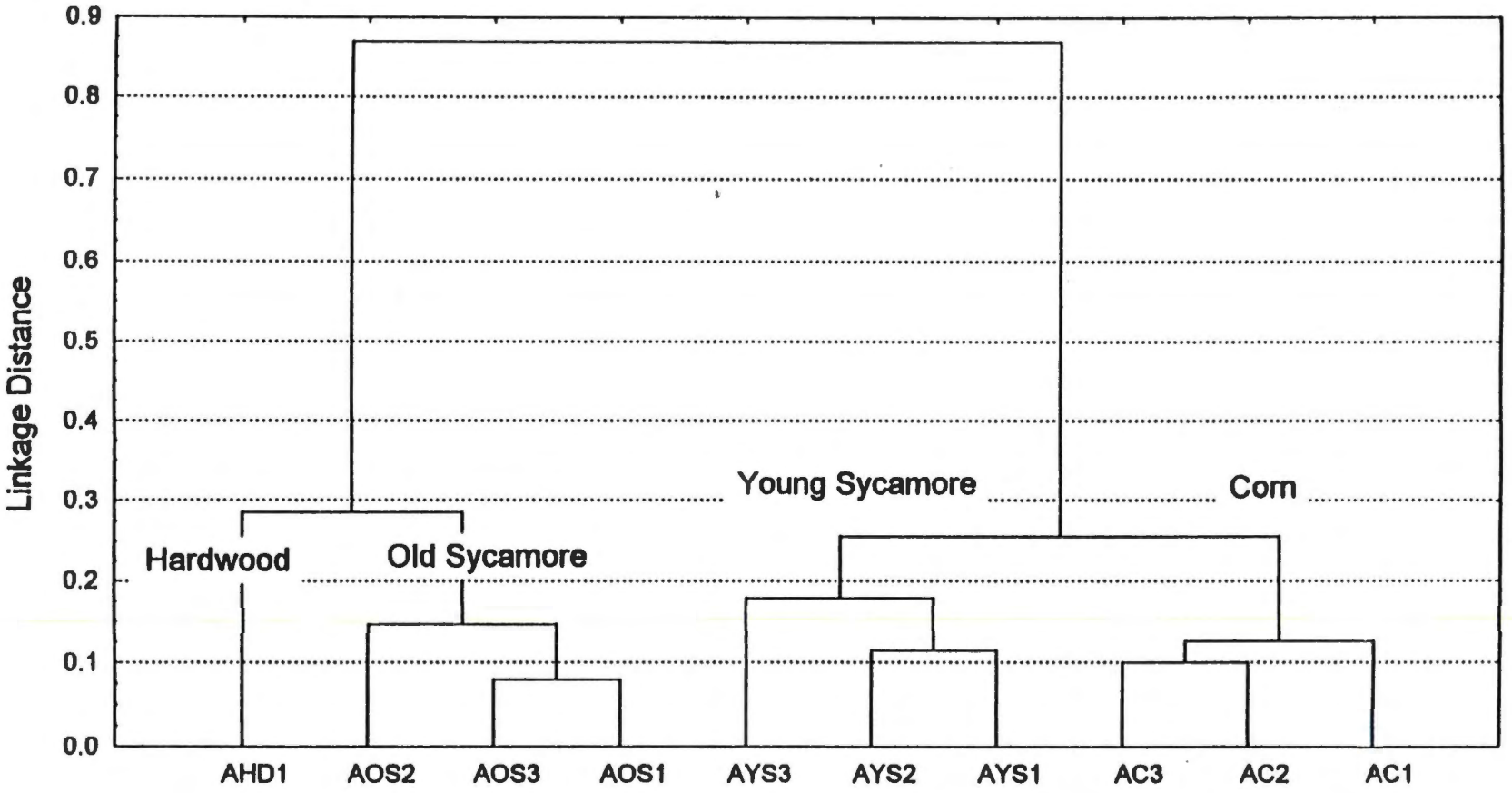


Figure 3-9. A dendrogram representing the results of a hierarchical cluster analysis (Ward's method) of averaged PLFA mole percentages (arcsin transformed) from the August 1998 sampling. Similarity shown by linkage at a lower euclidean distance.

measured soil chemical properties showed clear differentiation among treatments (Figure 3-10 through Figure 3-13). The old sycamore plots and the mature forest plots were particularly well separated. One of the useful aspects of PCA is that, in addition to showing differentiation between treatments, the analysis gives some perspective on the factors which cause this differentiation. Not surprisingly, factors that commonly caused differentiation between treatments were fatty acids characteristic of Gram-negative or Gram-positive bacteria, measures of microbial biomass, factors associated with soil organic matter (total soil C, total soil N, moisture) and fatty acids characteristic of eukaryotes. For example, in the PCA for the June sampling 46% of the variance was explained by the first factor. Fatty acids characteristic of Gram-negative organisms (monoenoics), total PLFA biomass and total soil C and N were heavily negatively loaded while fatty acids characteristic of Gram-positive bacteria heavily positively loaded. In Figures 3-10 through 3-13, axes are labeled to indicate which fatty acids or factors contributed to that loading. If an axis is not labeled it is an indication that no single factor could be identified as causing the loading. Figures 3-12 and 3-13 are three-dimensional because three factors were useful in explaining the differentiation between treatments.

Conclusions

The conversion of a corn silage agroecosystem to a short-rotation woody crop plantation significantly altered soil microbial and chemical properties. The microbial communities in these treatments were clearly different from each other. PLFA analysis was used to show how the structure of the microbial community differs between a no-till corn silage/winter wheat system, a 2-3 year-old plantation of sycamore, a 14-15 year-old plantation of SRWC sycamore, and a mature hardwood forest. The young sycamore system supported a smaller community of microorganisms that was more Gram-positive, and showed more signs of nutritional stress than the other treatments. The older sycamore plantation and the mature forest had a more Gram-negative community of bacteria which showed few signs of stress. Populations of fungi and actinomycetes were typically not significantly different

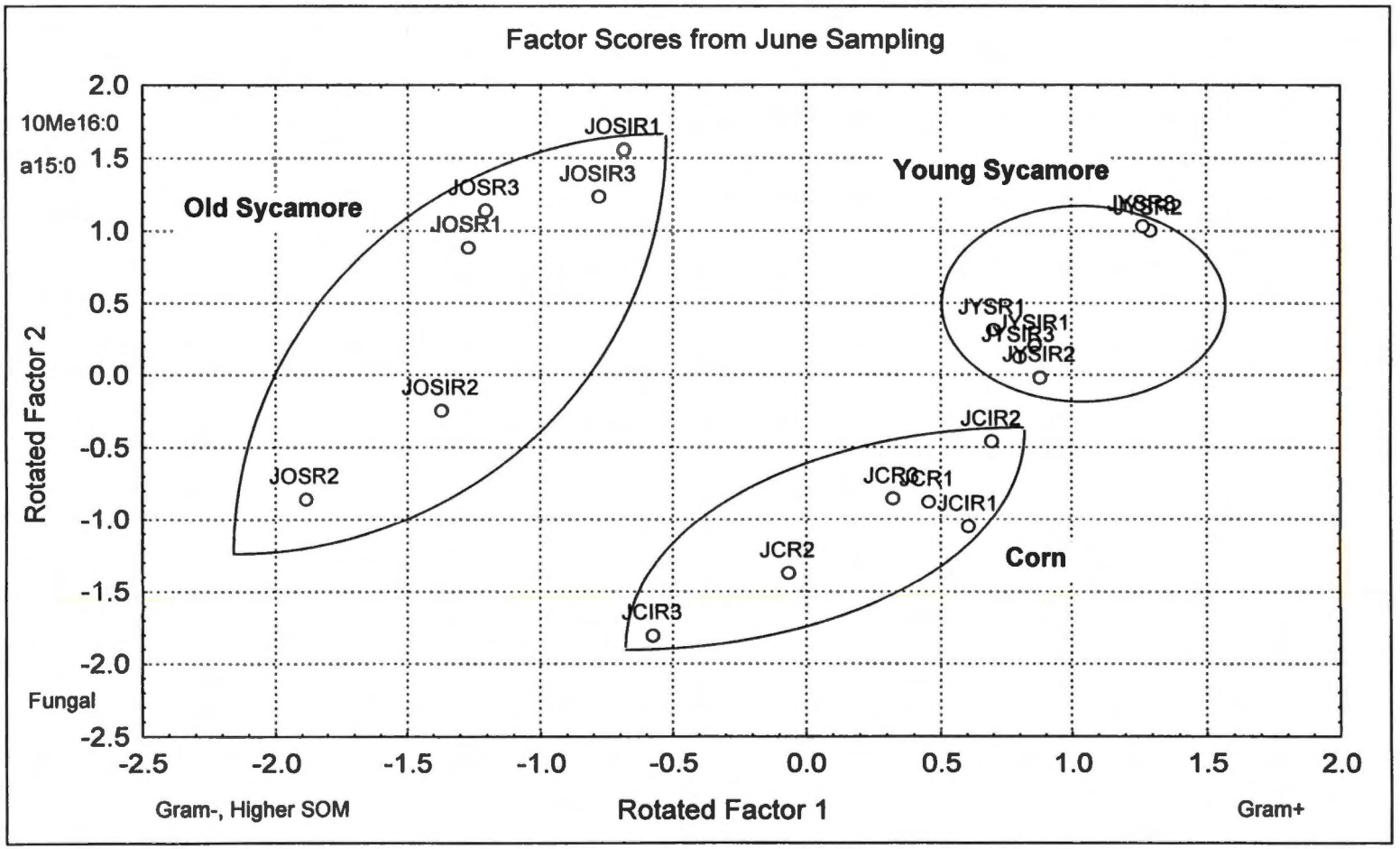


Figure 3-10. Principle components analysis (PCA) of the mole percentage of 38 fatty acids and and 16 soil chemical properties for the June sampling. Variance explained by the two rotated factors were 46.4% and 17.0% respectively

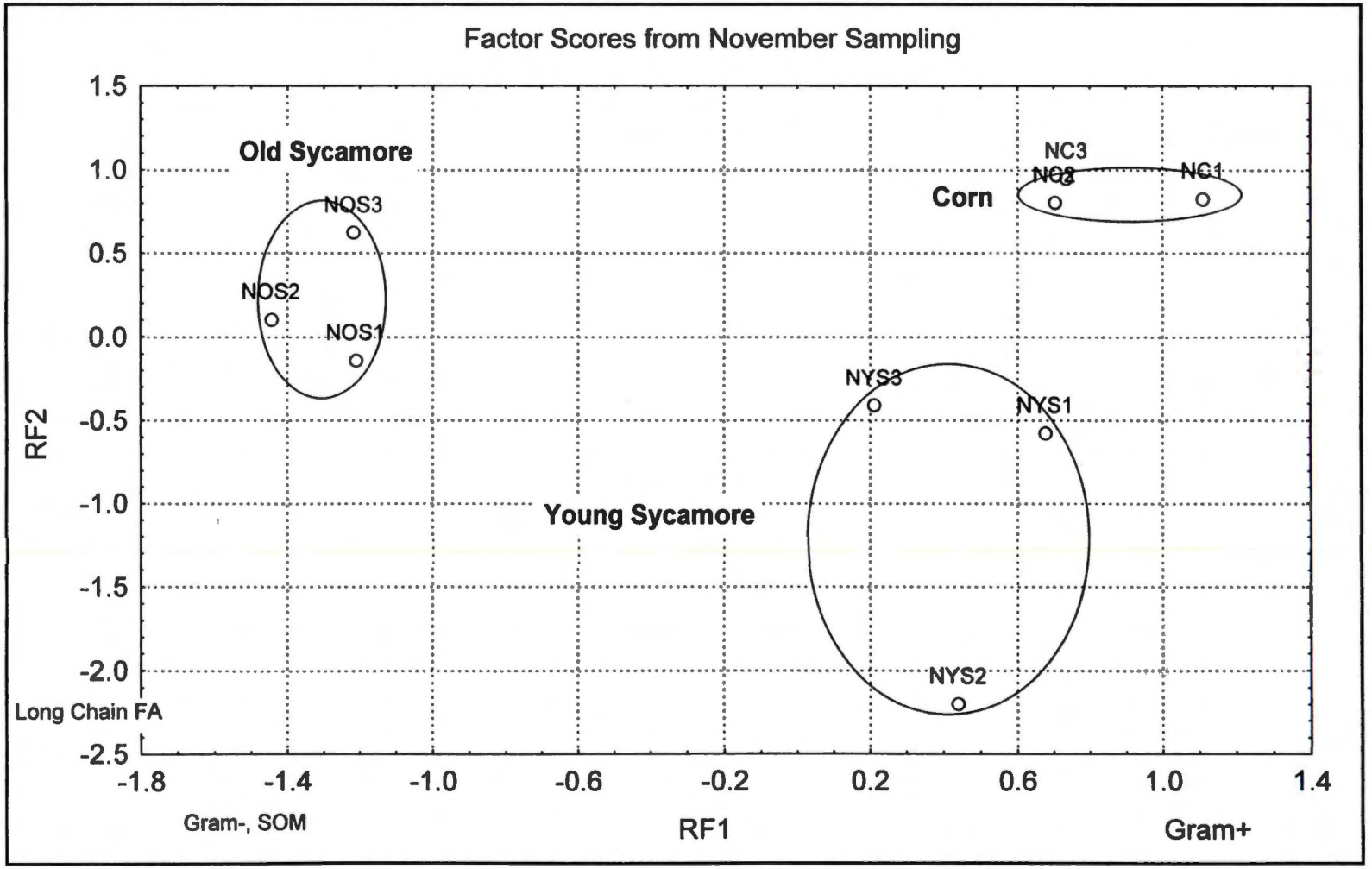


Figure 3-11. Principle components analysis (PCA) of the mole percentage of 38 fatty acids and and 16 soil chemical properties for the November sampling. Variance explained by the two rotated factors were 43.5% and 26.0% respectively

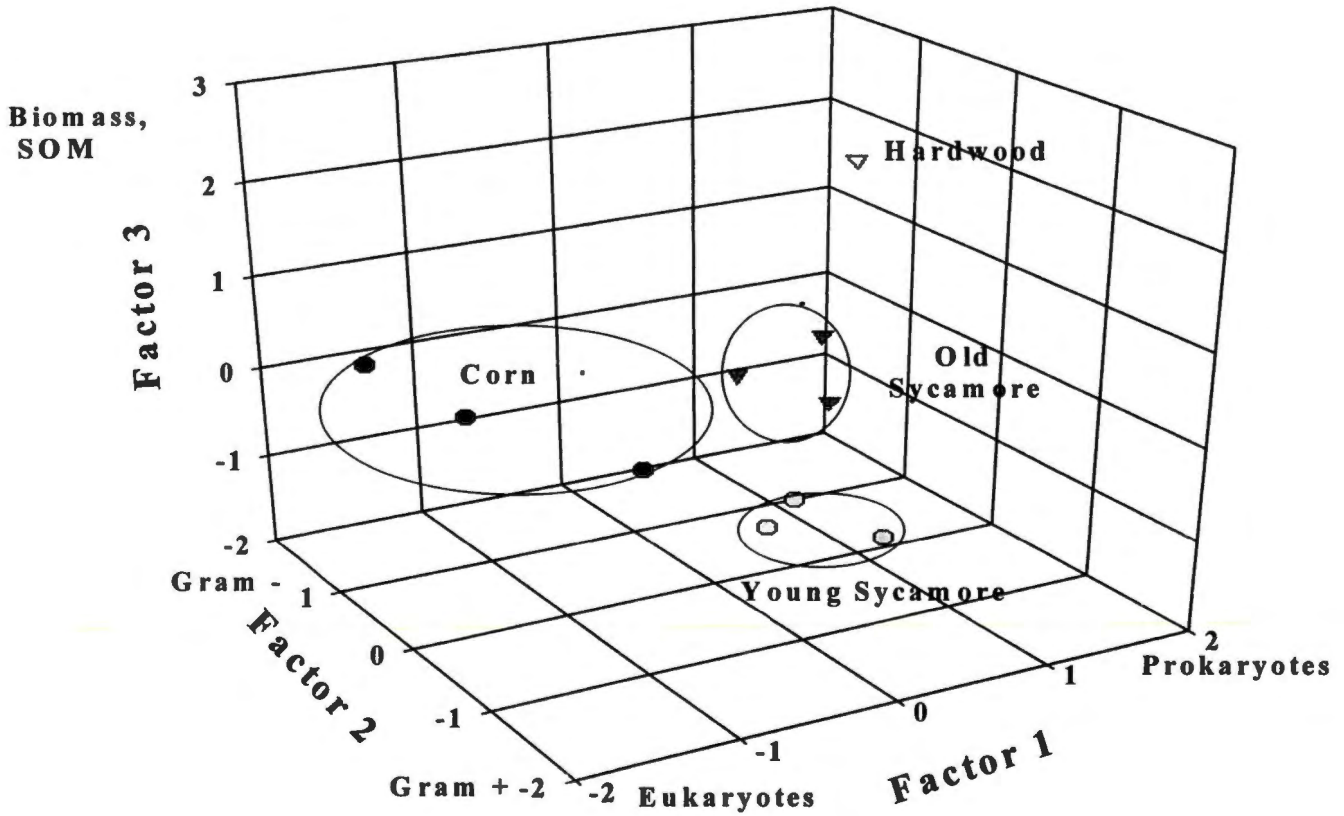


Figure 3-12. Principle components analysis (PCA) of the mole percentage of 38 fatty acids and 11 soil chemical properties for the March 98 sampling. Variance explained by the three rotated factors were 30.2%, 28.1% and 24.3 % respectively.

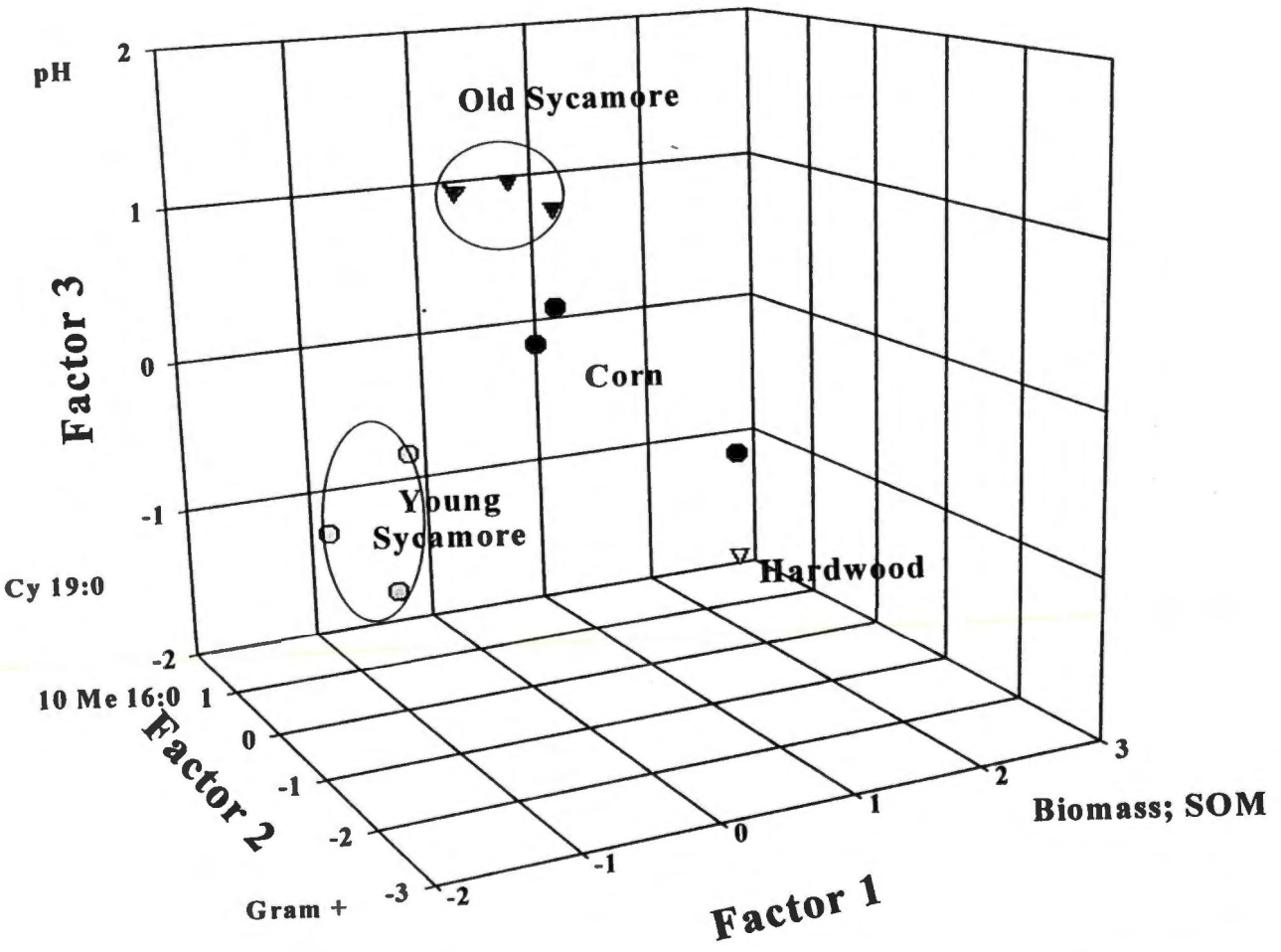


Figure 3-13. Principle components analysis (PCA) of the mole percentage of 38 fatty acids and 16 soil chemical properties for the August 98 sampling. Variance explained by the three rotated factors were 32.5%, 25.5% and 15.3 % respectively.

between treatments. It seems that the primary environmental factor influencing these community shifts was soil organic matter. The young plantation of sycamore took up nutrients from the soil but cycled very few bioelements back to the soil during the first years after plantation establishment. This lack of substrate for microbial growth was reflected in a smaller population of organisms and more stress bioindicators in the young sycamore soil than in the other treatments. In older sycamore plantation the litter layer was well established and the level of soil organic matter was significantly higher than the corn and young sycamore systems. This soil was found to support a larger and less stressed community of microorganisms. It should be pointed out that while the young sycamore plantation supported a smaller community of microorganisms than the old sycamore system, it would not be correct to assume that the young sycamore system was "unhealthy" or "unsustainable". These comparisons are relative to the other treatments at this site. As the litter layer becomes established in the younger sycamore plantation it is expected that the system will become more like the older sycamore plantation.

PLFA analysis was found to be a relatively easy and accurate method for obtaining certain information about the structure of the microbial community in environmental soil samples. More significance should be given to groupings of fatty acids rather than individual "signature" fatty acids. Finally, most ratios of fatty acids reported in the literature to give information about community nutritional and physiological status gave consistent information in this study, although the ratio of trans- to cis- fatty acids was less consistent.

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Part 4

Measuring Changes in Microbial Community Structure using Molecular Genetic Analysis after Conversion of Annual Row Crops to Short-Rotation Woody Crops

Introduction

Because of concerns about the future of fossil fuel resources, both in terms of supply and their effect on the environment, there has been increasing interest in bioenergy crops. Producers of electrical power are examining the potential of woody biomass plantations to produce a substrate to co-fire with coal in steam-generating electrical power plants. The U.S. Department of Energy (1994) is projecting far greater use of biomass power by 2010. It is hoped that increased utilization of short-rotation woody crops (SRWC) for bioenergy will reduce net carbon emissions to the atmosphere.

SRWC are fast-growing tree plantations that are grown for a short duration (3 - 8 years) to produce biomass that can be used for power production. Some reasons to consider SRWC include their potential for energy production while reducing net C emissions to the atmosphere compared with burning coal alone. Trees remove CO_2 from the atmosphere while they are growing. Tree plantations provide a soil cover with their canopies and litter, and their perennial root systems provide a stabilizing effect which functions to conserve and improve soil and water quality, particularly on marginal sites. In order for bioenergy crops to be economically feasible and a viable energy option, there needs to be a dedicated biofuel feedstock, with hundreds of thousands of hectares currently planted to conventional agriculture converted to tree plantations.

Large scale conversion of cropland and rangeland to SRWC will alter soil physical, chemical, and biological properties affecting erosion, hydrology, water quality and soil quality (OTA, 1993; Ranney and Mann, 1994). Paul and Clark (1996) point out that changes in the crop management system will alter the soil microclimate and affect the soil biota. Converting cropland to tree plantations could have profound effects on soil properties and soil sustainability. The soil plays a major role in the long-term sustainability of ecosystems (Curci et al.,1997) .

A major concern of environmental microbiologists is developing methodologies which can be used to examine microbial communities *in situ*. Classical microbiological

methods used in public health for isolation and culture of clinical specimens are not adequate for environmental samples (White et al., 1997). White and Macnaughton (1997) report that viable counts (plate counts) of organisms are not satisfactory for soils and sediments where they often represent only 0.1% - 10% of the cells detected using acridine orange direct counts. Torsvik (1994) reported that the genetic diversity in the soil microbial community was approximately 200 times greater than what could be isolated from the soil. Recent developments in molecular biology have provided an approach to studying soil microorganisms that doesn't rely on culturing techniques. This procedure involves molecular genetic analysis and the use of nucleic acid hybridization. The recent development of molecular biological approaches for studies in microbial ecology promises a new level of information concerning the structure and function of microbial communities (Sayler, 1992). Sayler et al. (1992) state that gene probe technology based on nucleic acid hybridization is one of the most reliable techniques for detecting genetically-defined populations within a complex microbial community. Vestal and White (1989) suggest that while no perfect method exists for analyzing the role of microbes under natural conditions, ribosomal RNA isolation and sequencing shows great promise for determining natural community structure. The amount of extracted nucleic acid from an environmental sample should yield reliable information on microbial biomass. Once extracted, the nucleic acid may be targeted with specific gene probes to measure the abundance of an individual population or specific genes. Ribosomal rRNA (rRNA) and messenger RNA (mRNA) are, along with DNA, the main targets for nucleic acid hybridization. DNA tends to be more environmentally stable and less prone to enzymatic degradation than RNA (Sayler et al., 1992). Ribosomal RNA offers an increase in sensitivity by several orders of magnitude over DNA sequences since there are so many ribosomes in each cell. Johnston et al. (1996) point out that you can get information about community structure and, in some cases, function by increasing knowledge at the molecular level. Zhou et al. (1996) comment that the extraction of bacterial nucleic acids from environmental samples has become a useful tool to detect organisms which can not be cultured, to determine the fate of recombinant genes or

selected bacteria in the environment, and to show genotypic diversity and its change in natural ecosystems.

Gene probes take advantage of the ability of nucleic acids to be denatured and reannealed (Pepper and Josephson, 1998). Hybridization involves the formation of H bonds between complementary single strands of DNA or RNA to produce double strands. One of the single strands is the "probe" which consists of a known sequence of nucleic acid with a colorimetric, flourescent, or radioactive (^{32}P) label attached. The ^{32}P label is most often used because its high energy yields great sensitivity and shortens autoradiographic exposure times. The other single strand represents the target, and the labeled nucleic acid identifies a specific nucleic acid sequence or gene in the target. The target strand is typically fixed on a nylon or nitrocellulose membrane by vacuum filtration before the probe is added. After hybridization, the unbound probe is washed off the membrane. Radioactive probes are typically analyzed using X ray film (Stapleton et al., 1998). These methods allow the examination of microorganisms at the molecular level and detection of minute quantities of a particular species or a functional gene. They also allow the classification of organisms into phylogenetic groups. (Pepper and Josephson, 1998). The use of specific oligonucleotides is a powerful tool for the qualitative detection and identification of specific bacteria in environmental samples (Utaker and Nes, 1998).

Initial studies using molecular techniques in microbial ecology were limited by an inability to obtain a representative sample of DNA from an environmental matrix (Johnston et al., 1996). Techniques of removing cells from the sample by centrifugation were limited by extraction efficiency, while DNA:DNA colony hybridization contained a culture bias. To get a representative estimate of the total microbial community in a soil sample, cells are now lysed *in situ* within the soil matrix and the DNA extracted and purified (Pepper and Josephson, 1998).

Some of the limitations of nucleic acid hybridization techniques include the following:

1. Soils high in clay adsorb DNA so extraction efficiency varies with soil texture Soil organic matter content can also influence extraction efficiency.

2. The efficiency of cell lysis is not always high and varies with cell size.

3. Some DNA is lost during purification. White and Macnaughton (1997) suggest that one of the major problems encountered utilizing molecular techniques for analysis of environmental samples is the lack of quantitative recovery of nucleic acids from various complex environmental matrices.

4. Even purified DNA contains some humic material (Pepper and Josephson, 1998). This inhibits amplification and hybridization.

5. The majority of oligonucleotide probes are made on the basis of a relatively small number of DNA sequences, usually originating from pure cultures. In most cases these are not representative of the true diversity of bacteria in the environment (Utaker and Nes, 1998).

6. Nucleic acid hybridization is limited by the state of current databases and the potential for hybridization with DNA sequences with a high similarity (Stapleton and Sayler, 1998).

Ammonia Oxidizing Bacteria - An example of utilizing molecular techniques to study microbial communities

Past studies of microbial communities responsible for biogeochemical transformations have been limited by a lack of tools for cultivating and identifying the members of the community. It is now possible to identify ribosomal RNA sequences that are conserved within phylogenetic groups. These sequences can serve as hybridization sites for nucleic acid probes which can be used to examine the structure and diversity of these microbial communities (Devereux et al. 1996: Amman et al., 1995). One such phylogenetically-linked function that has been extensively studied is ammonia oxidation.

Nitrification is carried out by two phylogenetically unrelated groups of autotrophic Gram-negative bacteria - the ammonia oxidizers and the nitrite oxidizers. Ammonia oxidizing bacteria are difficult to study by traditional culture-based techniques or MPN techniques because of their low growth rates and low biomass yields (Kowalchuk et al., 1999). Most studies involving conventional techniques for isolation of

pure cultures have focused on a single isolate of *Nitrosomonas europaea*. However, many studies have found *Nitrosospira* sp. to be common in terrestrial ecosystems (Kowalchuk et al., 1997). Most nitrifying bacteria are chemolithotrophs (organisms that obtain their energy from the oxidation of inorganic compounds). These organisms play a critical role in the global N cycle. Nitrifiers obtain their energy by oxidizing reduced forms of nitrogen (NH_3, NH_4^+).

The conversion of ammonia to nitrite is a two step reaction that proceeds as follows:

$$NH_3 + O_2 + 2\ H^+ + 2 \text{ electrons} \Rightarrow N\ H_2OH + H_2O \text{ (Myrold, 1998)}$$

The conversion of ammonia to hydroxylamine is catalyzed by the enzyme ammonia monooxygenase. The second step of the reaction involves several undefined steps whereby hydroxylamine is converted to nitrite:

$$NH_2OH + H_2O \Rightarrow NO_2^- + 5\ H^+ + 4 \text{ electrons}$$

Ammonia oxidation involves the transfer of 6 electrons and yields 271 kJ (65 kcal) mol^{-1} NH_3 (Myrold, 1998). The oxidation of nitrite to nitrate proceeds as follows:

$$NO_2^- + H_2O \Rightarrow NO_3^- + 2\ H^+ + 2 \text{ electrons}$$

This reaction is catalyzed by nitrite oxidoreductase and yields 77 kJ (18 kcal) mol^{-1} NO_2^- (Myrold, 1998). Nitrification involves the releases of protons and can lead to significant soil acidification (Stephen et al., 1999).

Inorganic N can have many fates in the soil environment. Ammonium can be taken up by plants, fixed on clay particles, lost via ammonia volatilization, or can serve as a substrate for nitrification. Nitrate can be taken up by plants or microorganisms, leached into the groundwater, or lost via denitrification. Plant roots take up nitrogen from the soil solution as ammonium or nitrate. The form of nitrogen that plants preferentially take up depends upon the plant species. It has been reported (Smith et al., 1971; McFee and Stone, 1968; Pharis et al., 1964; and Benzian, 1965) that ammoniacal-N is superior to nitrate-N in stimulating the growth of young conifers. Some authors have concluded that because they don't see evidence for nitrification, temperate forest trees must take up

ammonium (Nadelhoffer et al., 1984). Others have suggested that ammonium is preferentially taken up by trees because of the high energy cost of reducing nitrate, and because ammonium is formed first in the process of nitrification. Conversely, Nadelhoffer et al. (1984) studied N uptake from nine temperate forest sites representing a variety of tree species and found that nitrate supplied most of the N at 8 of the 9 study sites. They found that nitrate pools were generally smaller than ammonium pools, but that nitrification was still a significant process.

Myrold (1998) points out that nitrifiers have been shown to increase as a result of nitrogen fertilization. This same increase has been shown after perturbation of undisturbed soils. Soils of climax communities in natural ecosystems have been reported to have a low population of nitrifiers and low nitrate concentrations. Several theories have been proposed to explain this observation (Myrold, 1998). One states that as ecosystems mature, losses of N are reduced and N cycling becomes more conservative. It has also been theorized that climax vegetation produces allelochemicals (tannins, polyphenols) which inhibit nitrification. However, additional research (Davidson, et al., 1992) counters this theory and suggests that low concentrations of nitrate are due to plant uptake and microbial immobilization. This study, as well as Stark and Hart (1997), utilized a ^{15}N isotope-dilution technique to measure gross rates of nitrification and microbial assimilation of nitrate. They found that net nitrification rates were low because soil microbial communities assimilated almost all of the nitrate produced. Gross nitrification rates were high in all of the forests examined. They suggest that current models greatly underestimate the role of the microbial community in preventing nitrate losses.

Ammonia oxidizers are responsible for the conversion of ammonia to nitrite. Five genera are recognized based on their morphology. They are *Nitrosomonas, Nitrosolobus, Nitrosovibrio, Nitrosococcus, and Nitrosospira.* Myrold (1998) comment that ammonia-oxidizing bacteria are thought to be a relatively defined and coherent group. Recent phylogenetic work based on 16S rRNA sequences has largely confirmed this close phylogenetic relationship. Except for 2 strains of *Nitrosococcus oceanus*, all

autotrophic ammonia-oxidizers are tightly clustered together phylogenetically. Head et al. (1993) studied the gene sequences of 11 PCR-amplified 16S rRNA genes from autotrophic ammonia-oxidizers and found that *Nitrosomonas* spp., *Nitrococcus mobilis*, and strains of *Nitrosovibrio, Nitrosospira, and Nitrosolobus* fit within the β-subdivision of proteobacteria. *Nitrosococcus oceanus* strains are a deep branch in the gamma subdivision. The β-subdivision ammonia oxidizers form a family. All are obligate chemoautotrophs with an essentially identical central metabolism. They fix carbon dioxide via the Calvin cycle and oxidize ammonia to nitrite to produce energy and reducing power. Their electron transport systems appear to be identical as determined by difference spectroscopy (Head et al., 1993). The genera *Nitrosovibrio, Nitrosospira, and Nitrosolobus* have very high homology in their sequences and Head et al. suggested they be considered a single genus called *Nitrosospira* .

Ammonia oxidizers utilize a monooxygenase enzyme with NADH as an electron donor. The oxidation of nitrite to nitrate is accomplished via a nitrite oxidase system, with electrons transported to oxygen via cytochromes. Teske et al. (1994) used comparative 16S rRNA sequencing to evaluate relationships among ammonia- and nitrite-oxidizing bacteria. They point out that these chemolithotrophs have traditionally been viewed as one coherent group within the family *Nitrobacteriaceae* defined by their ability to grow as lithotrophs by oxidizing ammonia to nitrite or nitrite to nitrate. These researchers found that strains of *Nitrobacter* formed a closely related assemblage within the alpha subdivision of proteobacteria, while all ammonia-oxidizing genera except *Nitrosococcus oceanus* form a monophyletic assemblage within the β subdivision of the proteobacteria.

Environmental studies of ammonia oxidizing bacteria

Nitrifying bacteria are central to the global nitrogen cycle. While nitrification can lead to nitrate leaching, eutrophication, and loss of N by denitrification, nitrifiers can also play an environmentally-positive role by reducing wastewater content of ammonia (which is often toxic to aquatic life). By combining nitrification with denitrification, N can be

removed from wastewater. However, nitrification is difficult to maintain in engineered systems due to the organism's sensitivity to toxic shock, as well as pH and temperature fluctuations (Mobarry et al., 1996). Better process control requires a better understanding of nitrifying bacteria. Nitrifiers are difficult to study using traditional cultivation techniques. They are slow-growers and have poor counting efficiency because they tend to form clusters within sludge flocs. Since the number and activity of ammonia-oxidizers is usually the rate-limiting step in nitrification, researchers need a rapid and reliable method of identifying these organisms in activated sludge (Wagner et al., 1995). Comparative 16S rRNA sequencing has provided a better way of studying environmental populations of nitrifiers. Phylogenetic techniques have provided a framework for evaluating the diversity and abundance of nitrifying environmental populations (While I am focusing on ammonia-oxidizers, these techniques have also been valuable in quantifying nitrite-oxidizers. Mobarry et al. (1996) found that *Nitrosomonas* and *Nitrobacter* occurred in clusters and frequently were in contact with each other within sludge flocs). Phylogenetically-based hybridization probes target the 16S rRNAs and enable the researcher to quantify the population of organisms. The presence of the organisms has been correlated with their activities.

Wagner et al. (1995) developed a fluorescently-labeled, rRNA-targeted oligonucleotide probe that can be used for cultivation-independent *in situ* identification and enumeration of bacteria in complex microbial communities such as activated sludge. They used this procedure in several activated sludges and a trickling filter biofilm and found dense cell clusters of ammonia-oxidizers in samples coming from sewage treatment plants that have stable nitrification. This was confirmed using transmission electronmicroscopy. In sludge samples from an animal-waste processing treatment plant up to 20% of total bacteria were identified *in situ* as ammonia-oxidizers. Using this *in situ* hybridization procedure in combination with digital image analysis and scanning confocal laser microscopy, the rRNA content and spatial distribution of ammonia-oxidizers within their natural environment can be analyzed. These researchers are involved in developing other oligonucleotide probes to learn about the structure and

function of the nitrifying community within wastewater treatment.

One observation about these environmental studies is that while phylogenetic probes are well suited for identification of genes in the environment, and may be used with fluorescent microscopy to show the structure of a bacterial communities, they seem to be less appropriate for quantitative assessments of community structure. One major concern, at least in studies where rRNA is extracted from the environment, seems to be recovery of rRNA from a variety of environments and with different types of organisms. Without knowing whether quantitative recovery has occurred and whether that recovery is representative of total rRNA, it is better to simply make statements like "rRNA from this species represent 20% of the total rRNA extracted".

By using 16S rDNA, specific changes in β-subgroup ammonia oxidizer populations have been found with changing pH (Stephen et al., 1996), additions of swine manure to soil (Hastings et al., 1997), additions of salmon farm waste to marine sediment (Stephen et al., 1996) and with contamination of soils with toxic metals (Stephen et al., 1999).

Objective

The objective of this study was to utilize molecular genetic techniques to examine whether changes occur in microbial community structure and microbial biomass after conversion of a traditional corn silage agroecosystem to a system where sycamore was grown as a short-rotation woody crop. These techniques included the use of nucleic acid hybridization probes to determine the structure of the microbial communities as well as PCR amplification techniques to examine one specific component of these communities - the ammonia oxidizing bacteria. Populations of ammonia oxidizing bacteria were quantified as an example of applying molecular techniques to the examination of a phylogenetically-linked function.

Methods and Materials

This study was conducted at the University of Tennessee experiment station at

Ames Plantation near Grand Junction, Tennessee. The study was designed to examine the environmental impacts of conversion of cropland to SRWC. Prior to establishment of the study the area was under conventionally-tilled silage corn production for at least 15 consecutive years. The soil type at the study site is a Memphis-Loring silt loam intergrade (fine-silty mixed thermic Typic Hapludalf - fine-silty mixed thermic Typic Fragiudalf). These soils are characterized by a high silt and low clay content, low CEC, and a variably-developed fragipan between soil depths of 90 - 150 cm. The study site consists of six 0.5 ha plots constructed as mini-hydrological units, with 0.5m confinement berms (soil brought from other portions of the field) surrounding three corn (Zea mays L.) plots and three sycamore (Platinus occidentalis L.) plots. These plots will be referred to as "corn" and "young sycamore" respectively. The young sycamore plots were located within an even-aged 3.5 ha plantation of sycamore. Plots could not be randomly distributed because a wildlife study associated with this project requires large unbroken tracts of each treatment. Sycamore has been identified as having a high potential as a biomass tree crop because of its ability to develop a dense canopy and a pervasive root system over a short rotation (Graham, 1994).

Sycamore seedlings were planted at 2.4m by 1.2m spacing in February, 1995. After two growing seasons tree survival was over 97%. The corn plots have been maintained in no-till corn silage production. The winter cover crop was wheat. The wheat has been killed in the spring, and not harvested. During the growing seasons since establishment, silage corn and sycamore plots have been managed as two separate systems, with chemical and fertilization inputs and harvest geared to the best management of each.

In 1983 Wells and Fribourg (1992) established three 17m x 17m plots of American Sycamore approximately 2 miles away, but on a similar soil type. This site will be referred to as "old sycamore". Sycamore seedlings were planted at a spacing of 2.4m between rows and 1.2m within rows on a site that had been planted in conventionally-tilled soybean. The area surrounding the plots was maintained in soybeans. No fertilizer was applied to the trees. Every other row of sycamore was harvested at five years. A

second harvest in 1993 collected the coppice and the 10-year-old trees (Fleenor, 1996). The trees which had regrown were cut again in the spring of 1998 and the biomass was left on the site.

Immediately adjacent to the young sycamore plots is a mature mixed hardwood forest. This forest is approximately 50 years old, and has a mixed species composition of oak, sweetgum, red maple, black cherry, and an assortment of hardwood trees and shrubs. Soil samples were collected from this site (referred to as "mature forest") in March and August 1998. These treatments were chosen for study to show soil biological properties in a continuum from a traditional annual row cropping system to variously-aged SRWC systems, to a mature hardwood site.

Soil sampling

Mineral soil cores were collected during June and November, 1997, and March, and August, 1998 to a depth of 6 cm using a soil core sampler (inside diameter = 5.3 cm) (SoilMoisture Equipment Corp., Santa Barbara). Organic litter was removed from the soil surface prior to sampling.

Soil samples consisted of a series of cores taken to include both rhizosphere and interrow samples. Each treatment-plot combination was divided into a grid and a number was assigned to each section of the grid. Three sections were randomly chosen. Three or four cores were taken across the row and bulked in a plastic bag to represent one sample. The bags were stored in a cooler on dry ice and taken to the laboratory within 24 hours of sampling, where they were stored in a freezer at a maximum temperature of -20°C until they were analyzed. In March, and August, 1998 a portion of each sample was stored on dry ice to be used for PLFA (Part 3) and DNA analysis. However, the portion to be used for chemical analysis (Part 2), chloroform-fumigation, and enzyme assays (Part 5) was stored in a cooler on ice, returned to the laboratory, and stored at 4°C so as to not kill organisms by freezing.

Methodology of extracting DNA from soil

The procedure of isolating DNA from soil was pioneered by Torsvik and Goksoyr (1978) Their method involved homogenization and fractionated centrifugations of separate bacterial cells and fungal mycelia from the soil. The cells were lysed and the DNA was recovered. Ogram et al. (1987) developed a method that directly extracts soil DNA *in situ* which involved a ballistic disintegration of the cell envelope by bead-mill homogenization and dissolution by the detergent sodium dodecyl sulfate (SDS), followed by centrifugation and DNA clean-up procedures. The lysis efficiency is greater than 90% for sediments. This procedure fails to lyze the smallest size fraction of bacteria (0.3 - 1.2 μm) which are generally Gram-positive cocci. Nevertheless, it is currently the most efficient method for isolating DNA from the environment. (Johnston et al., 1996).

A large soil sample is used for this procedure since Lame' and Defize (1993) determined that a sample size of less than 10 g resulted in unreasonable variance. A 35 g (dry weight basis) sample was incubated at 70°C for one hour with an ionic detergent (5% w/v sodium dodecyl sulfate) to solubilize the bacterial cell walls. Sodium phosphate (0.12 M dibasic, pH 8.0) was used as an alkaline buffer for the extraction of DNA. In order to isolate the DNA from environmental samples the cells must be lysed. A number of procedures have been suggested including bacteriolytic enzymes, freeze-thawing, ultra-sonic probes, and ballistic disintegration. This procedure lysed cells using the shear stresses produced by blending the sample in a Bead-Beater (Bio Spec; Bartlesville, OK) with 25 g of 0.1 mm diameter glass beads for 5 minutes. Nucleic acids which were released were recovered in the aqueous phase by successive alkaline extractions and centrifugations of the soil.

The DNA from the sample was concentrated by means of precipitation in isopropanol. Isopropanol and ethanol purification caused DNA to precipitate away from contaminants (Ogram, 1988). Precipitation occurred because the alcohol decreased the polarity of the solution enough that the DNA was no longer soluble. The precipitate was centrifuged and the DNA pellet dissolved in TE buffer (10mM Tris-HCl, 1mM EDTA, pH 7.5-8.0). Because of the high concentration of salts in this procedure, exhaustive

dialysis was undertaken using dialysis tubing (Spectropore; Spectrum Medical Industries, Los Angeles, CA) with a molecular weight cutoff of 6000 - 8000.

After dialysis, purification steps were required to remove protein, other cellular debris, and many contaminants such as humic materials. Extraction of DNA from soils always results in coextraction of humic substances which interfere with DNA detection and measurement (Zhou et al., 1996). This can inhibit Taq polymerase in PCR and DNA hybridization specificity. Purification of DNA following direct extraction is important to obtain DNA of sufficient purity. A number of purification techniques have been developed including size-exclusion chromatography, hydroxyapatite chromatography, cesium chloride - ethidium bromide gradient centrifugation, and the use of polymers such as polyvinylpolypyrrolidone (PVPP) and cetyltrimethyammonium bromide (CTAB) to complex and remove humic acids from DNA. (Ogram, 1988; Zhou et al., 1996). The number of purification steps may dramatically reduce the efficiency of DNA recovery (Johnston et al., 1996). However, Zhou et al. (1996) found no difference in DNA yield among treatments with or without CTAB. Neither PVPP nor CTAB is able to completely remove humic compounds. Ogram (1988) comments that CTAB will remove many high molecular weight impurities that ethanol precipitation will not.

Phenol extraction acted to remove proteins bound to DNA. Extraction with a 24:1 mix of chloroform/isoamyl alcohol removed any remaining phenol from the sample. After purification, samples were once again concentrated via alcohol precipitation using ethanol overnight at -20°C. The DNA obtained by this method was pure enough for slot blot hybridization.

Slot-blot hybridization

The DNA which was extracted from the soil was quantitatively analyzed by nucleic acid hybridizations using radiolabelled gene probes. A slot-blot manifold (BioRad, Hercules, CA) was carefully scrubbed and rinsed. It was then soaked for a minimum of 1 hour in a solution of 1 M NaOH. Afterwards it was rerinsed and assembled as per the manufacturer's directions. The membranes used were 0.45 μm

Biotrans (+) Nylon Transfer Membrane (ICN Biomedicals Inc., Costa Mesa, CA). The extracted DNA was thawed, vortexed and 10 µl was added to a 1.5 ml Eppendorf tube and the volume was made up to 50 µl with 0.5 M NaOH. This small volume was used because humic substances remaining in the purified sample plugged the membrane at higher volumes. The membrane also was wetted with NaOH before the DNA was affixed. A set of DNA standards of the appropriate target sequence was included on the membrane. These were prepared by mixing 100 µl of standard with 400 µl of 0.5 M NaOH. The DNA samples were denatured by boiling the tubes for 10 minutes. The samples and standard solutions were applied to the membrane, loading each sample into a separate well on the slot-blot apparatus. Empty wells were filled with TE (10 mM Tris-HCl, 1 mM EDTA) buffer. A slight vacuum, not exceeding 4 psi, was applied to the membrane and the blotting solution passed through leaving the DNA affixed to the membrane. Several drops of 0.4M NaOH were used to wash the membrane.

The apparatus was disassembled and the membrane baked for 1 hour at 80°C to bind the DNA to the membrane. The membrane was rinsed with 2X SSC (g $liter^{-1}$; NaCl 17.53; Na citrate 8.82, pH 7.0 - Stair, 1998) solution and baked again for an hour. The membranes were stored in Kapak/Scotchpak Heat Sealable bags, 10 ml of hybridization solution (0.5M NaH2PO4, 1mM EDTA, 7% SDS (pH 7.2) were added, air bubbles were removed and the bag was sealed. Nucleic acid extracts were hybridized with two 16S rDNA oligonucleotide probes. A 15 bp Universal probe with the sequence (5' to 3') ACGGGCGGTGTGRC was used to determine total microbial biomass (Stahl et al., 1988). A eubacterial probe (Eub 338) with the sequence GCTGCCTCCCGTAGGAGT was used to determine eubacterial biomass (Amman et al. 1990).

The bags were subjected to a "pre-hybridization wash" overnight at 42°C for the Universal probe and at 55°C for Eub338 (Stair, 1998) The next day the oligonucleotide probe with a ^{32}P-ATP end was made and added to the bag. This was incubated overnight at 42°C (55°C for Eub338) to allow for hybridization. To make the universal oligonucleotide probe, 200 ng of the oligonucleotide fragment were mixed with 10 µl of 10X Forward Reaction Buffer (Gibco BRL), 2.5 µl of ^{32}P gamma ATP (415 µCi μl^{-1})

(ICN Biomedicals, Inc.), 2 µl of T4 Polynucleotide Kinase (Gibco BRL), and 25.5 µl of sterile water. For the eubacterial probe, 50 ng of oligonucleotide was used. This mixture was centrifuged and incubated for 30 minutes at 37°C in a Perkin-Elmer Cetus DNA Thermal Cycler set on a soak cycle. This time allowed for the nucleotide to attach to the fragment.

The probe was purified to remove any radiolabel that was not attached to the oligonucleotide fragment. A Stratagene (LaJolla, CA) nuc-probe column was pre-wetted with 70 µl of STE buffer (0.1M NaCl, 10 mM Tris-HCl, 1mM EDTA, pH 8.0). All of the probe mixture was added and carefully pushed through the column using air pressure from a 10 ml lure-loc syringe. The column was then washed with 70 µl of STE buffer to remove any remaining probe. Probes with a specific activity of at least 10^5 cpm/µl, as determined by scintillation counting of 5 µl of purified probe in 10 ml of Beckman Ready-Safe scintillation cocktail, were added to the membranes.

After incubating overnight, the membrane was washed with high stringency washing buffer, three times for 20 minutes each, to remove any unhybridized probe. Membranes were air-dried. For the June samples the membrane was exposed to X ray film for visualization of positive samples on the DNA from the June sampling. A computer image of the autoradiogram was generated and a regression curve produced for the known standards on the membrane using a Hewlett Packard ScanJet IIC flat bed scanner and Sigma Gel imaging software from Jandel Scientific. Unknown sample values were inserted into the regression equation and the amount of target sequences calculated as nanograms of DNA. To visualize the blots from the November and August samplings, the Molecular Dynamics Storm 840 phosphor-imaging system was used. The blots were placed in one cassette and one Kodak Storage Phosphor-imaging Screen SO230 was used. The cassette sat at room temperature overnight. The screen was then scanned using the Storm 840 and each blot was quantified using Molecular Dynamic's ImageQuaNT imaging software (Version 4.2a).

16S ribosomal RNA analysis to measure shifts in microbial community structure

In March 1998 J. O. Stair, a M.S. student from the Department of Microbiology at the University of Tennessee, assisted with soil sampling. Samples were collected in the usual fashion, but the bulked samples were split into two plastic bags. One sample was analyzed as previously described and the other bag was taken to the Department of Microbiology for analysis. 16s rRNA was directly extracted from the soil and, without further amplification, the RNA was blotted onto a membrane and hybridized with a hierarchical array of rRNA probes, and quantified by comparison to standard concentrations of RNA (Stair, 1998).

Methods of extracting RNA from the soil

Fifteen grams of soil was homogenized with a sterilized mortar and pestle and added to a 30 ml Oak Ridge tube that had been autoclaved to degrade RNases. Eight ml of extraction buffer (0.2M Sodium Phosphate Buffer, 0.1M EDTA, pH 8.0) was added and mixed throughly. To this tube, 1.0 ml of 10% SDS was added and mixed by shaking briefly. The tube was then placed in a boiling water bath for 5 minutes. Periodically, the tube was removed and carefully shaken to ensure even warming. Next, the tube was placed in the -80°C freezer for 30 minutes. Afterwards, the sample was thawed for 15 minutes at 65°C, mixing occasionally to ensure total thawing. The sample was then centrifuged at 10,000 rpm for 15 minutes at 10°C. The lysate was decanted into a fresh tube to which 10 ml of denaturing solution (4M guanidium isothiocyanate, 0.5% sarcosyl, 25mM sodium citrate, pH 7.0) had been added to inactivate any remaining nucleases.

The remaining soil pellet was resuspended in 5 ml of extraction buffer and again boiled for 5 minutes. After centrifuging as before, the resulting supernatant was combined with the aqueous portion from the first lysis step. To remove proteins and other cellular components, 10 ml of phenol (pH 5.2) and 5 ml of chloroform:isoamyl alcohol (24:1 v/v) was added and the sample carefully mixed by shaking. This solution was centrifuged at 6,000 RPM for 10 minutes at 4°C. The aqueous portion was transferred to a fresh tube using a sterile transfer pipette, taking care not to disturb the

interface. To ensure any remaining phenol was removed, the aqueous solution was reextracted with 10 ml of chloroform:isoamyl alcohol (24:1 v/v). After mixing and centrifuging, as above, the aqueous portion was transferred using a sterile transfer pipette to a 30 ml Corex tube (baked 4 hours at 180°C),

The nucleic acids were precipitated with 0.1 volume 3M NaAc (pH 5.2) and an equal volume of isopropanol at room temperature. Precipitation occurred overnight at 4°C . It is important that the isopropanol be at room temperature to limit the amount of SDS and salts that might co-precipitate along with the nucleic acids in this method.

The nucleic acid-containing solution was centrifuged at 10,000 RPM for 30 minutes at 4°C to pellet the precipitate. The isopropanol was then very carefully decanted and pipetted away from the pellet. The nucleic acids were then resuspended and transferred using 400 µl of DEPC-treated dH2O to an RNase-free eppendorf tube. The extract was then stored at -80°C until analysis.

To ensure that RNA extracts were free of DNA, all extracts were enzymatically treated with DNAse. Affixing the RNA to the membrane and hybridization protocol were similar to that described for DNA. More detail can be found in Stair (1998). RNA was detected and quantified using a Molecular Dynamics Storm 840 phosphor-imaging system. A comparison of each group probe relative to the total RNA, obtained using the Universal probe, was made. This comparison gave a value of the relative percentage of each group of organisms relative to the total microbial biomass.

Methods of studying ammonia oxidizers in soils

Quantifying ammonia oxidizers from soil samples involved the use of the polymerase chain reaction (PCR). The PCR is a method for amplifying DNA *in vitro*. Oligonucleotide primers complementary to nucleotide sequences in target genes are used to copy the target sequence through the use of a heat stable DNA polymerase and magnesium chloride (Stapleton, et al., 1998). Extraction of nucleic acids from soil for PCR amplification of ammonia-oxidizing bacteria involved a procedure similar to, but with some modifications on the procedure previously described. It was essential that

these extracts be as clean as possible since humic material can interfere with amplification. Soil (0.5 g), 0.12 M sodium phosphate buffer (pH 8.0, 425 µl), chaotropic reagent (CRSR, Bio-101, Vista, Calif., 175 µl) and 0.17 mm glass beads (0.5 g) were agitated in a 1.5 ml microcentrifuge tube by using a high-speed Crescent WIG-L-BUG bead beater (Crescent Dental Mfg. Co., Lyons, Ill.) for 1.5 minutes. The sample mixtures was centrifuged at 13,000 X g for 5 minutes, and the supernatant was collected. Chloroform (300 µl) was added to the soil pellet, mixed throughly, and centrifuged at 13,000 X g for 5 minutes. The aqueous supernatant was collected and combined with the first supernatant fraction. The chloroform extraction was repeated. DNA was precipitated from the aqueous phase with an equal volume of isopropanol in an ice bath for 30 minutes. The DNA was pelleted by centrifugation at 13,000 X g at 4°C for 15 minutes, washed with 1 ml of 80% ethanol twice, air dried, and redissolved in 100 µl of Tris-EDTA (TE) buffer (pH 8.0). The DNA extract was purified by extracting twice with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1, vol/vol/vol) followed by a glass milk DNA purification protocol with a Gene Clean kit (Bio-101) as described by the manufacturer.

PCR amplification of *amoA*

The *amoA* gene was amplified using the forward primer *amoA*-1F* with two ambiguities to improve matching with the target sequences of various cultured representatives of the β-subgroup of ammonia oxidizers (GGGGHTTYTACTGGTGGT; H = not G; Y = C or T; altered bases are underlined). This primer targets a stretch corresponding to positions 332 to 349 of the open reading frame of for the amoA gene sequence of Nitrosomonas europaea (McTavish et al, 1993). The reverse primer used (*amoA*-2R: CCCCTCKGSAAAGCCTTCTTC; K = G or T; S = G or C) targets a stretch corresponding to positions 802 - 822. These primers generated a specific PCR product 491 bp long (Rotthauwe et al., 1997). The reaction conditions were 35 cycles of 92°C for 60 seconds, 55°C for 60 seconds, and 68°C for 45 seconds with 1.225 U of Expand HF polymerase, the supplied buffer (Boehringer Mannheim, Indianapolis, Ind.) and 10

pmol of each primer in a total volume of 25 µl on a Robocycler 96 thermocycler (Stratagene, La Jolla, Calif.).

Competitive PCR was carried out as described by Sambrook et al. (1989) in order to quantify the number of amoA copies in each treatment. This procedure involved coamplification of two templates: the amoA sequence from the samples and a competitor template which is added in known amounts to a series of PCR reactions. The competitor was distinguished from the target following amplification by a size difference caused by deletion of a small segment of the sequences that lie between the two oligonucleotide primers. A molar-equivalent conversion factor of 1.11 was used to account for the size difference between the competitor and the target molecules. An amoA internal control standard was generated as follows: 3' truncation of the amoA sequence carried by clone NAB_8_23 was achieved by amplification with amoA-1F and amoA-2R DEL (CCCCTCTGCAAAGCCTTCTTCCCTTCACGTAGAAGAAG). The 5' 20 bases (underlined) correspond to amoA-2R (Rotthauwe et al., 1997), and the 3' 17 bases correspond to positions 535 to 553 of the coding sequence of *Nitrosomonas europaea* amoA (McTavish et al., 1993). The amplification product (428 bp) was cloned with the vector PCR2.1 TOPO and *E. Coli* TOP10F' to generate p428-NAB_8_23. Plasmids were isolated with a Wizard mini-prep kit (Promega Corp., Madison, WI) and linearized with *Eco*RI (Promega), for which the amplified control sequence did not carry a recognition site. Linearized products were gel purified on 0.8% agarose with TAE buffer and extracted with a Gene-Clean kit prior to quantification by using a Hoefer DyNA-Quant 200 fluorometer and Hoechst H33258 dye binding assay (Pharmacia Biotech. Inc., Piscataway, N.J.).

In the competitive PCR reaction the relative amounts of the two templates which were amplified reflected the relative concentrations of the sequences in the original reaction mixture. The relative abundance of amoA and amoA deletion fragments were assayed by ethidium bromide fluorescence and quantified with alpha imager software (Alpha Innotech).

Statistical analysis

Differences between treatments were determined using analysis of variance (ANOVA) using the type III General Linear Model SAS procedure (SAS Institute, 1990) and Fisher's least significant difference procedure. Effects were considered significant when the null hypothesis, using the residual term as error, was rejected at a $P \leq 0.05$. Values reported are the mean of nine samples at each sampling date. Standard errors of the mean were calculated using the lsmeans procedure.

Results and Discussion

Total microbial biomass as measured using a universal 16S rDNA oligonucleotide probe is shown in Table 4-1. Hybridizations were performed on DNA extracted from soil sampled in June and November of 1997, and August 1998. The older sycamore plantation contained a significantly larger microbial biomass in June and November 1997. Differences between treatments were not significant in August 1998. The old sycamore plantation has been accumulating a litter layer for the last 15 years, and this probably accounts for the larger microbial biomass in the top 6 cm of soil in this treatment. In addition to providing a readily-available substrate for heterotrophic organisms, the higher levels of soil organic matter in the old sycamore system increase soil water-holding capacity, porosity (increasing aeration), and the litter layer provides a protective and insulating layer over the soil. In addition, the 15 year-old trees have a well established perennial root system providing C as the roots decay and root exudates as the trees grow. At no sampling date was the difference in total DNA between the corn system and the young sycamore system significantly different.

While the relative comparison of DNA biomass within a sampling date shows differences between treatments, I would be uncomfortable making comparisons between microbial biomass levels at the different sampling dates, because of differences in the handling of the samples. The DNA from the June and November samples were extracted as discussed above (Methodology of extracting DNA from soil). However, the June

Table 4-1. Total DNA biomass as measured using a universal 16S rDNA oligonucleotide probe

Date	Treatment	DNA biomass
		ng DNA g^{-1}
June 1997	Corn	6.16b†
	Young Sycamore	9.32b
	Old Sycamore	18.06a
November 1997	Corn	0.60b
	Young Sycamore	0.65b
	Old Sycamore	1.10a
August 1998	Corn	29.18a
	Young Sycamore	34.95a
	Old Sycamore	39.33a

†Within columns, means followed by the same letter are not significantly different according to LSD ($\alpha = 0.05$).

samples were analyzed immediately. The November samples were extracted in June of 1998 and not analyzed until March of 1999. There may have been some degradation of the DNA during that time of storage at -20°C. In spite of this concern, the relative comparison of treatments within the two sampling dates should be accurate. The DNA from the August samples was extracted by the methodology explained in the ammonia-oxidizers portion of the Methods and Materials. This procedure was similar to that used for the other samples, but involved the use of a Gene-clean kit (Bio-101). This yielded a DNA extract with far less humic material co-extracted. Two areas of concern about this procedure were the small amount of soil used (0.5 g) and the question of how much DNA was lost during the Gene-cleaning step. Nevertheless, the relative amounts of DNA biomass in each treatment were similar in the August sampling as at the other sampling dates. To make comparisons at the different sampling dates, uniform procedures for nucleic acid extraction and hybridization would need to be utilized.

Total eubacterial biomass was measured in the November 97 DNA extracts using the Eub 338 probe. Because of high levels of background hybridization, plotting the standard curve was impossible. However, a relative comparison of samples was possible, reported in counts per gram of soil (Table 4-2). While the old sycamore plots appeared to have the highest levels of eubacterial biomass, differences between treatments were not significant.

Results of 16S ribosomal RNA analysis (Stair, 1998)

Some discussion of the results of the 16S ribosomal RNA analysis conducted by J.O. Stair on these same samples in March 1998 seems appropriate. Table 4.3 lists the probes that were used in the analysis. Tables 4-4 through 4-6 show the microbial community structure of these treatments as determined by rRNA. Stair (1998) utilized domain-level probes to measure the domains of Eubacteria, Eukarya and Archaea. The

Table 4-2. Relative eubacterial DNA biomass as measured using the Eub 338 probe

Date	Treatment	Eubacterial biomass
		Counts g^{-1}
November 1997	Corn	1.34a†
	Young Sycamore	1.55a
	Old Sycamore	1.95a

†Within columns, means followed by the same letter are not significantly different according to LSD ($\alpha = 0.05$).

Table 4.3 Probe target sequences and references for probes used in 16S rRNA analysis (Data from Stair, 1998)

Probe	Target	Sequence (5' to 3')	Reference
Univ1390	All Domains 16S rRNA, position 1390-1408	GACGGGCGGTGTG TACAA	Zheng et al. (1996)
Eub338	Bacteria 16S rRNA, position 338-355	GCTGCCTCCCGTAG GAGT	Amann et al. (1990)
Euk	Eukarya 16S rRNA, position 1195-1209	GGGCATCACAGAC CTG	Giovannoni et al. (1988)
Arch915	Archaea 16S rRNA, position 915-934	GTGCTCCCCCGCCA ATTCCT	Stahl and Amann (1991)
Alf1b	α-subdivision of Proteobacteria 16S rRNA, position 19-35	CGTTCGYTCTGAGC CAG	Manz et al. (1992)
Bet42a	β-subdivision of Proteobacteria 23S rRNA, position 1027-1043	GCCTTCCCACTTCG TTT	Manz et al. (1992)
Gam42a	γ-subdivision of Proteobacteria 23S rRNA, position 1027-1043	GCCTTCCCACATCG TTT	Manz et al. (1992)
SRB385	δ-subdivision of Proteobacteria 16S rRNA, position 385-402	CGGGCGTCGCTGCG TCAGG	Amann et al. (1990)
HGC69a	Gram-positive bacteria with high DNA G+C content. 23S rRNA, position 1901-1918	TATAGTTACCACCG CCGT	Roller et al. (1994)
LGCa,b,c	Gram-positive bacteria with low DNA G+C content. 16S rRNA, position 979,982,982 for a,b,c	Sequences have not yet been published	R. Amann Unpublished
CF319a	*Cytophaga-Flavobacterium* cluster of the CFB phylum 16S rRNA, position 319-336	TGGTCCGTGTCTCA GTAC	Manz et al. (1992)

Table 4-4. Total microbial biomass (± one standard deviation) as determined by the Universal rRNA probe (Data from Stair, 1998)

Corn Plots	Young Sycamore	Old Sycamore	Mature Hardwood
ng RNA g^{-1}			
158.53 ± 63.25	86.11 ± 23.80	206.00 ± 88.97	196.81 ± 6.99

Table 4-5. Microbial community structure as determined by rRNA. Percentage (± one standard deviation) of domain relative to the total community RNA as measured by the Universal probe (Data from Stair, 1998)

Domain	Corn Plots	Young Sycamore	Old Sycamore	Mature Hardwood
Eubacteria	69.62 ± 25.68	69.53 ± 18.97	53.04 ± 16.69	49.88 ± 4.97
Eukarya	18.68 ± 9.58	6.40 ± 4.74	14.59 ± 7.08	1.15 ± 1.03
Archaea	0.27 ± 0.16	0.38 ± 0.21	0.25 ± 0.21	0.16 ± 0.01
Total of 3 Domains	**88.57 ± 32.47**	**76.31 ± 20.67**	**65.97 ± 19.42**	**51.19 ± 4.10**

Table 4-6. Eubacterial community structure as determined by rRNA. Percentage (± one standard deviation) of eubacterial group relative to the total eubacterial RNA as measured by the Eubacterial probe (Data from Stair, 1998)

Eubacterial Groups	Corn Plots	Young Sycamore	Old Sycamore	Mature Hardwood
Alpha	21.64 ± 7.56	27.09 ± 11.09	35.01 ± 10.33	16.81 ± 8.14
Beta	7.63 ± 3.52	6.04 ± 1.58	10.35 ± 6.95	4.35 ± 1.34
Gamma	2.86 ± 1.76	4.61 ± 2.48	3.82 ± 2.76	1.26 ± 0.03
Delta	8.73 ± 5.62	12.70 ± 8.43	9.78 ± 5.72	10.40 ± 5.97
High G+C	1.36 ± 3.07	5.21 ± 6.63	9.31 ± 8.73	12.82 ± 7.04
Low G+C	2.46 ± 2.64	0.75 ± 1.69	0.94 ± 1.22	3.93 ± 3.34
Cytophaga/ Flavobacterium	0.87 ± 0.96	0.77 ± 0.59	0.76 ± 0.75	0.71 ± 0.16
Sum Total of all Eubacterial Groups	**47.48 ± 12.77**	**53.26 ± 12.26**	**69.96 ± 15.96**	**50.28 ± 13.29**

values obtained for each of these domains were expressed as a relative percentage of the total 16S rRNA (Table 4-4). In theory, these should sum to 100%, if the domain-level probes detected all organisms. As Table 4-5 shows, the domain-level probes were able to identify 88.6% of the total biomass in the corn plots, but only 66% and 51% of the total biomass in the old sycamore and mature hardwood plots, respectively. This gives an indication of higher genetic diversity in these forested systems. Table 4-6 shows groupings within the Eubacterial domain and gives their percentage relative to the Eubacterial domain probe. Again, in theory these should sum to 100%. Fisher's LSD test was used with the Universal probe data to determine that the young sycamore soil had significantly less total biomass than the other treatment. Other treatments were not significantly different than one another. Stair used the summation of the α, β, γ, and δ subdivisions of Proteobacteria as a measurement of Gram-negative organisms. Using this approach, nearly 60% of the total microbial community in the old sycamore plots was represented by Gram negative organisms, compared with 42% for corn, 48% for young sycamore, and 33% for the mature forest. The summation of the High G+C, Low G+C, and CFB groups was used as a measurement of the Gram-positive organisms. Using this measure, 17.5% of the mature forest community was Gram-positive compared with 11% for the old sycamore plots, 5% for the corn plots and 7% for the young sycamore plots. Using this approach to define community structure seems suspect as it would mean that the old sycamore community is more Gram-positive and Gram-negative than the corn and young sycamore communities. Obviously, the large percentage of unknown organisms in these communities prevents us from making definitive statements about the microbial community structure.

Table 4-7 shows the total biomass measured by each probe. Whereas, Table 4-5 and 4-6 showed microbial community structure for each treatment, Table 4-7 shows that conversion from row cropping to tree plantations often increased the biomass of the microbial groups examined in this study.

Table 4-7. Results of 16S rRNA dot blot hybridization (Stair, 1998)

Probe	Corn	Young Sycamore	Old Sycamore	Mature Forest	Significance
	ng rRNA dot-blot^{-1}				
Univ1390	31.71 ± 12.6	17.22 ± 4.76	41.20 ± 17.8	39.36 ± 1.4	**
Euk	5.11 ± 1.9	1.24 ± 0.9	6.12 ± 4.5	0.46 ± 0.4	**
Eub	19.44 ± 3.3	12.20 ± 2.6	19.61 ± 7.1	19.59 ± 1.4	*
Arch915	0.07 ± 0.03	0.06 ± 0.04	0.08 ± 0.03	0.06 ± 0.00	ns
Alf1b	6.14 ± 1.4	4.87 ± 2.9	14.00 ± 7.2	6.54 ± 3.0	***
Bet42a	2.04 ± 0.8	1.06 ± 0.4	4.41 ± 3.6	1.70 ± 0.5	*
Gam42a	0.87 ± 0.6	0.79 ± 0.5	1.56 ± 1.4	0.50 ± 0.0	*
SRB385	2.34 ± 0.9	1.80 ± 0.4	3.47 ± 1.8	4.05 ± 2.2	*
HGC69a	0.51 ± 1.1	1.02 ± 1.5	4.10 ± 4.3	5.06 ± 2.8	*
LGCa,b,c	0.79 ± 0.7	0.11 ± 0.2	0.29 ± 0.4	1.54 ± 1.3	**
CF319a	0.25 ± 0.2	0.11 ± 0.1	0.30 ± 0.4	0.28 ± 0.1	ns

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

Ammonia-oxidizing bacteria

Figure 4-1 shows the results of the competitive PCR analysis conducted on the August 1998 samples. A value of 70% DNA extraction efficiency was used to calculate the number of copies of amoA gene per gram of soil (Leung et al., 1999). No attempt was made to measure ammonia-oxidizing bacteria (AOB) in the mature hardwood forest. Ammonia oxidizers as a percentage of the community was calculated following Stapleton et al. (1998) and the results are presented in Figure 4-2. For this calculation 1 amoA gene per chromosome was assumed. Also, 7 copies of 16S rDNA was assumed (Stapleton and Sayler, 1998). The corn system had a significantly larger community of AOB than did the young sycamore system. This observation agrees with other studies which have shown increased populations of AOB after N fertilization (Myrold, 1998). In 1998 the corn system received two applications of ammonium nitrate fertilizer totaling 120 kg N ha^{-1}, while the young sycamore system received only one application of 60 kg ha^{-1} (Table 2-1).

AOB were not detectable in the old sycamore plots. This could be interpreted in three ways.

1. It could be that the old sycamore system contained a community of AOB that was different than the other systems, and was not amplified by the primers that were used. The structure of the community of AOB could be analyzed by denaturing gradient gel electrophoresis (DGGE) as described by Kowalchuk et al. (1997). DGGE provides information about sequence variation in a mixture of PCR fragments of identical length using differential mobility in an acrylamide gel matrix of increasing denaturant concentration. This information can be used to show differences in the composition of the microbial community. DGGE patterns can be compared with the migration of reference clones of known sequence. The major bands can be sequenced to further investigate their identities (Kowalchuk, 1997).
2. Another option is that the populations of AOB truly were so small in the old sycamore system as to be undetectable. This would explain why the old sycamore

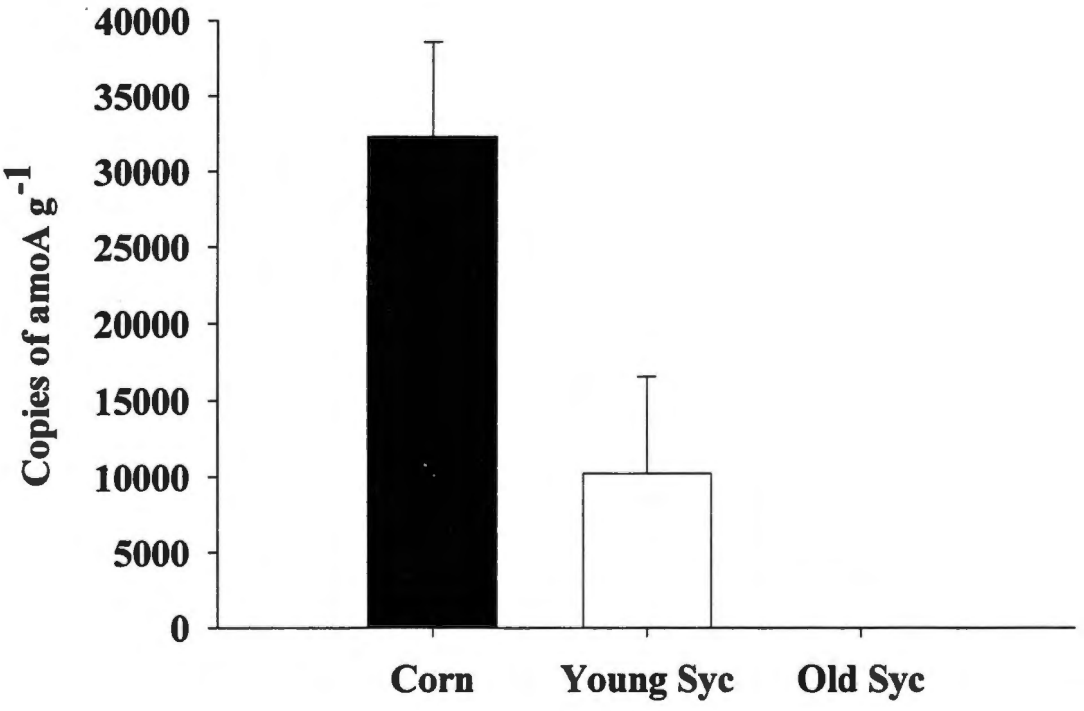


Figure 4-1. Copies of the amoA gene (+/- std error of the mean)

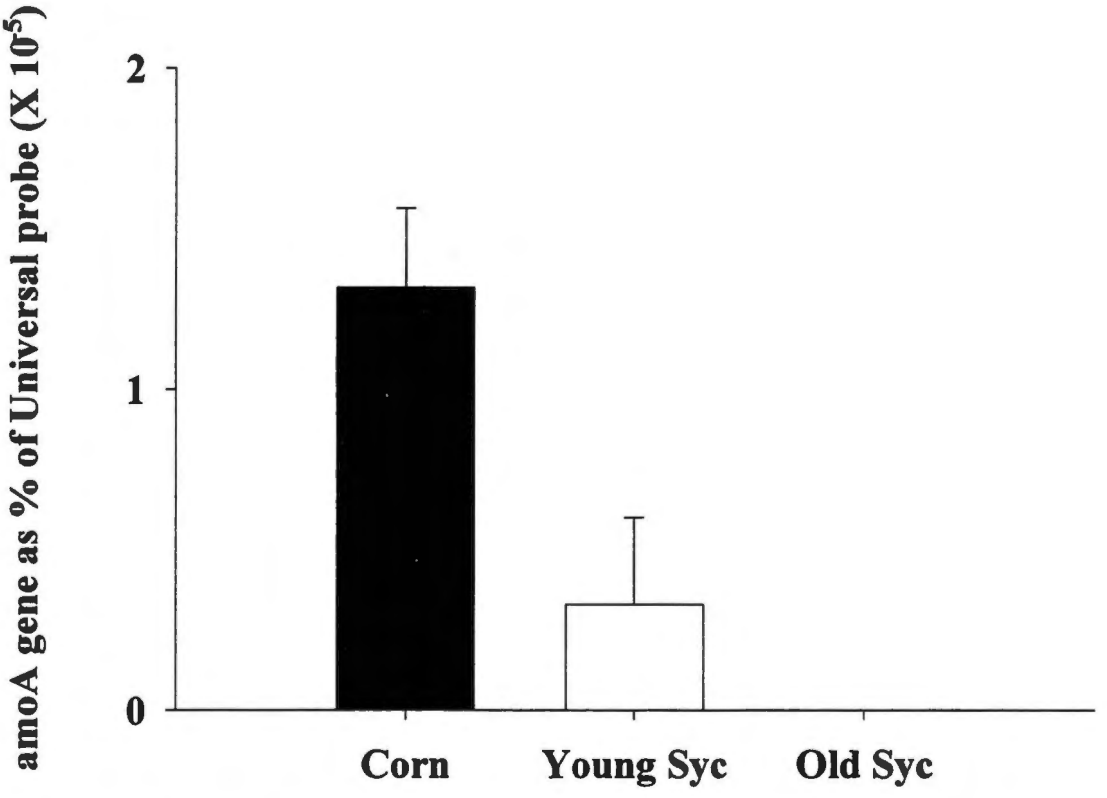


Figure 4-2. Percentage of total DNA made up by amoA gene (+/- std error of the mean)

soil consistently had the highest levels of ammonium-N of any treatment and lower levels of nitrate-N (Part 2).

3. A third possibility is that there was some compound in the old sycamore system (probably humic material, which has been shown to inhibit amplification (Pepper and Josephson, 1998)) which caused inhibition of the PCR. Options 2 and 3 could be tested by measuring gross nitrification and nitrate immobilization using the ^{15}N isotope-dilution procedure described by Stark and Hart (1997). Utilization of this procedure would give an indication of whether significant nitrification is occurring in the old sycamore system. If various concentrations of the deletion fragment were added to the PCR reaction with the old sycamore samples to determine whether amplification took place. If a humic substance were inhibiting amplification then the deletion fragment would not amplify either. Myrold (1998) proposed a calculation to determine the number of AOB in an environmental sample based on nitrification rate assuming a net nitrification rate in soil of 1 mg N kg^{-1} day^{-1}. However, this calculation is based on pure culture studies with ammonia oxidizers.

Conclusions

The recent development of molecular genetic analysis has provided soil microbiologists with an approach to studying soil microorganisms *in situ*, and without relying on culturing techniques. Several molecular techniques were utilized to study changes in soil microbial community structure which occurred as a result of converting a traditional corn silage agroecosystem to a plantation of short-rotation woody crops. Nucleic acid hybridization techniques demonstrated that this conversion led to greater microbial biomass in the forested system and shifts in the structure of the microbial community. Quantitative assessment of these communities is complicated by incomplete extraction of nucleic acids and a large number of "unknown" organisms that fail to bind with probes designed for their group. Competitive PCR techniques were used to show a decline in populations of ammonia-oxidizing bacteria populations after conversion from

row crops to tree crops, although the status of the old sycamore system needs additional study.

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Part 5

Shifts in Microbial Biomass and Activities after Conversion from Annual Row Crops to Short-Rotation Woody Crops

Introduction and Literature Review

Because of concerns about the future of fossil fuel resources, both in terms of supply and their effects on the environment, there has been increasing interest in bioenergy crops. Producers of electrical power are examining the potential of woody biomass plantations to produce a substrate to co-fire with coal in steam-generating electrical power plants. The U.S. Department of Energy is projecting far greater use of biomass power by 2010. It is hoped that increased utilization of short-rotation woody crops (SRWC) for bioenergy will reduce net carbon emissions to the atmosphere.

SRWC are fast-growing tree plantations that are grown for a short duration (3 - 8 years) to produce biomass that can be used for power production. Some reasons to consider SRWC include their potential for energy production while reducing net C emissions to the atmosphere compared with burning coal alone. Trees remove CO_2 from the atmosphere while they are growing. Tree plantations also provide a soil cover with their canopies and litter, and their perennial root systems provide a stabilizing effect which functions to conserve and improve soil and water quality, particularly on marginal sites. Bioenergy crops are projected to increase C sequestration in the soils in which they are grown.

The U.S. Department of Energy (1994) has found SRWC promising for large scale biomass energy production. They have identified a potential land base of 158.6 million ha for production of these crops Of this, 145.3 million ha (92%) is presently being used as cropland or pasture (Graham, 1994). Large scale conversion of this land to SRWC will alter the soil physical, chemical, and biological properties affecting erosion, hydrology, water quality and soil quality (OTA, 1993; Ranney and Mann, 1994). Paul and Clark (1996) point out that changes in the crop management system will alter the soil microclimate and affect the soil biota. Curci et al., (1997) add that climatic conditions, crop rotation and crop management systems can cause variations in the turnover of organic C and N, and in microbial activity by influencing soil biological

properties. Converting cropland to tree plantations could have profound effects on soil properties and soil sustainability. The soil plays a major role in the long-term sustainability of ecosystems. However, changes in soil properties such as total organic C can occur slowly and be hard to quantify (Curci et al. 1997). Enzyme activities and microbial biomass are responsible for transformations of soil organic matter and cycling of plant nutrients (Curci et al. 1997). These parameters have been suggested as early indicators of soil biological and biochemical changes (Frankenberger and Dick, 1983). Studying soil enzyme activities and microbial biomass can provide useful information about soil organic matter dynamics and transformation processes that are essential for plant nutrition. The objective of this study was to use measures of soil microbial biomass and enzyme activities to measure changes that occurred as a result of conversion from annual row crops to SRWC. Several methods of measuring soil microbial biomass were employed, including the chloroform fumigation-extraction method, total PLFA biomass, and nucleic acid hybridization.

Soil Microbial Biomass

In recent years, studies investigating the effects of management-induced changes on soil biological properties have often measured changes in the soil microbial biomass. Several authors have suggested that trends in the microbial biomass content of soils will predict long term trends in total organic matter content (Sparling, 1997; Powlson et al., 1987). Fumigating the soil with chloroform and measuring the bioelements that become extractable as a result has become a common soil microbiological procedure. Soil microbial biomass is an important component of the soil organic matter that regulates the transformation and storage of nutrients. It is a labile component of the soil organic fraction containing 1 - 3% of the total soil C and up to 5% of the total soil N (Smith and Paul, 1990). Curci et al. (1997) contend that the concentration of microbial biomass is controlled by the level of soil organic matter. The size and activity of the soil microbial biomass must be assessed to fully understand nutrient fluxes in managed and natural ecosystems (Horwath and Paul, 1994). Beck et al. (1997) point out that soil microbial

biomass is an important indicator of soil fertility providing useful information for soil ecological studies. Investigations into the immobilization and mobilization of bioelements in soil requires information about the amounts of these nutrients stored in the microbial biomass. The increase in extractable N (Brookes et al., 1985), P, S, and C following fumigation of soil has been used to estimate these elements in the microbial biomass (Vance et al., 1987). Work by Henrot et al. (1994) is representative of a number of studies that have measured changes in microbial biomass following vegetation removal. They suggest that measuring microbial biomass can provide an early indication for slower, less easily detectable soil organic matter changes. Microbial biomass can also provide an index of soil fertility because it represents an important labile pool of soil nutrients. These researchers studied the effect that vegetation removal had on microbial biomass in a humid tropical forest. They found that three years after vegetation removal the total soil C and N were reduced by 20%, However, changes in microbial biomass C were even more pronounced. In the fallow soil, biomass C declined to 50% of initial values within 6 months.

Because of its importance to soil ecological studies a number of procedures have been developed to measure soil microbial biomass. These have included plate-counting using selective media (Parkinson et al., 1971), direct microscopic counting (Jenkinson et al., 1976), fumigation-incubation (FI) (Jenkinson and Powlson, 1976), fumigation-extraction (FE) (Vance et al., 1987), substrate-induced respiration (SIR) (Anderson and Domsch, 1978), release of ninhydrin-positive compounds (Amato and Ladd, 1988), and measurement of soil levels of adenosine 5'-triphosphate (ATP) (Jenkinson and Oades, 1979).

Numerous studies have been conducted utilizing these techniques under different environmental conditions and with different soil types. In general, the literature has shown good correlation between methods:

1. SIR correlated with FI (Ocio and Brookes, 1990).
2. SIR correlated with FE (Anderson and Joergensen, 1997).
3. Correlation of SIR, FI, and FE (Sparling et al., 1990).

4. SIR, FE, and ATP correlated (Lin and Brookes, 1996).

5. SIR, FI, FE and direct microscopy (Sparling and Williams, 1986).

6. FE and ATP (Ocio and Brookes, 1990).

In spite of these good correlations, several areas of concern have been raised:

1. SIR may overestimate microbial biomass (Ross and Sparling, 1993).

2. Correlations have not been as good in soils that have been air-dried and rewetted, or have received recent amendments with carbon substrates (Ross et al., 1984).

3. FI does not work with acid soil (pH<5.0) or with those that have received recent amendments of carbon substrate (Ocio and Brookes, 1990).

4. SIR must be calibrated against one of the other techniques.

5. The FE procedure requires the use of a correction factor to account for incomplete C extraction. A wide range of values have been reported for extraction efficiency and this factor may not be the same for every soil type and environmental condition.

6. Zelles et al. (1997) suggests that since methods for estimating soil microbial biomass are least reliable in situations where the biomass is in rapid change, in such situations it is wise to use a battery of methods.

Description of chloroform fumigation procedures

The precursor of most of these methods of estimating soil microbial biomass was the chloroform fumigation-incubation (FI) method developed by Jenkinson and Powlson (1976). Fumigating the soil with ethanol-free chloroform and measuring the bioelements contained in the microbial biomass has become a common soil microbiological procedure. The fumigation kills and lyses microbial cells with the release of the cytoplasm into the soil environment. The microbial biomass is measured as the flush of CO_2-C caused by fumigation during an aerobic incubation of 10 days at 25 °C. Chloroform is used because it is an effective biocide and does not solubilize non-microbial organic matter or render it decomposable (Vance et al., 1987). Researchers

have found that the chloroform FI method should be restricted to work on well-drained soils, with a pH above 4.8, that have not received recent additions of easily decomposable materials (Beck et al., 1997). The fumigation-extraction (FE) method (Vance et al., 1987) does not have these restrictions. In this procedure, biomass C is estimated from the increase in 0.5 M K_2SO_4-extractable organic C following chloroform fumigation. K_2SO_4 is used as the extractant because the K flocculates the soil and prevents absorption of ammonium released by fumigation. In addition, the high salt concentration inhibits microbial decomposition of decomposable microbial material extracted after fumigation (Joergensen, 1995).

Using PLFA and nucleic acids to measure soil microbial biomass

Frostegård and Bååth (1996) comment that a variety of methods have been used to estimate the amount of microbial biomass in soil, but few differentiate between the different groups of microorganisms that make up that biomass. Extractable phospholipids are capable of providing reliable estimates of the microbial biomass as well as providing information about microbial community structure (Part 3). Zogg et al. (1997) and Zelles et al. (1997) agree that total PLFA content reflects active microbial biomass. Frostegård et al., (1997) studied microbial community dynamics associated with "manure hot spots". These researchers quantified microbial biomass both as the amount of phosphate liberated from lipid extracts after digestion (lipid-P) and as the total amount of PLFA. They concluded that total PLFA gives a better indication of living microbial biomass than does lipid-P.

The use of total PLFA as a measure of microbial biomass has been correlated against traditional measures of microbial biomass. Frostegård and Bååth (1996) found direct counts of bacteria were significantly correlated with bacterial PLFA ® = 0.59). They extracted the fatty acids of organisms from soils representing a wide range of organic matter contents and pH values and estimated an average of 1.40×10^{-17} moles of PLFA per bacterial cell. Using this factor and acridine orange direct counts they estimated bacterial biomass as 1.0 - 4.8 mg bacterial C per gram of soil C. Balkwill et al.

(1988) found a significant correlation between phospholipid content and ATP content as well as acridine orange direct counts of microorganisms in aquifer sediments. Frostegård, et al. (1991) used total lipid phosphate as a measure of biomass and found that it correlated well with SIR ® = 0.86) and ATP content ® = 0.89). Zelles et al. (1992) found that the amounts of PLFA in agricultural soils correlate well with microbial biomass data obtained with SIR and enzyme activity (correlation coefficient > 0.97). They suggested that this is an indication that the fatty acids are closely linked to soil microorganisms and that this method can be used in the study of microbial ecology. They proposed that 28 μg PLFA is approximately equal to 100 μg of biomass C. Zelles et al. (1995) found good correlation ® > 0.90) between total ester-linked PLFA and microbial biomass measured with SIR in a variety of forest and agriculturally managed soils.

Zak et al. (1996) used total PLFA as a measure of active microbial biomass in a study where atmospheric CO_2 levels were raised to twice ambient levels. They found that total PLFA was 1.3 times greater under elevated atmospheric CO_2, but this difference was not statistically significant. However, microbial biomass as measured with FI was 1.5X greater under elevated CO_2 conditions. These authors suggest that FI measures both active and inactive (resting and dead) biomass while PLFA only measures active biomass because of the rapid turnover of the phospholipids upon death.

Measurement of nucleic acids in the soil has also been suggested as a measure of the soil microbial biomass or microbial activity. Sayler et al. (1992) suggest that since the genetic composition of bacteria is relatively uniform the amount of extracted DNA from an environmental sample should yield reliable information on microbial biomass.

Soil microbial activity measured by enzyme activity

Biochemical reactions are catalyzed by specific proteins known as enzymes which, without being permanently altered themselves, cause chemical reactions to proceed at a faster rate (Tabatabai, 1982). Enzymes are specific for the types of reactions which they catalyze. They combine with their substrate in such a way that they

change the electronic configuration around certain bonds allowing the reaction to proceed at a lower activation energy than would a non-catalyzed reaction (Tabatabai, 1982). Enzymes are the catalysts of important soil biogeochemical functions including:

1. Decomposition and transformation of organic matter.
2. Mineralization of inorganic nutrients.
3. Nitrogen fixation.
4. Detoxification of xenobiotics.
5. Nitrification and denitrification. (Dick, 1997)

Management practices which influence microbial ecology and enzyme activities can have a significant impact on these biogeochemical functions (Farrell et al., 1994).

Extracellular soil enzyme activity and microbial biomass are considered early indicators of soil biological and biochemical properties (Curci et al., 1997). Changes in the soil biota due to environmental perturbations will be reflected in some of the more dynamic soil biological characteristics such as microbial biomass, enzyme activities, or respiration, long before total soil organic matter responds (Chandler et al., 1997). Soil microbial biomass and enzyme activity play a crucial role in the storage and cycling of bioelements. Measurement of these parameters is important in studies of the soil organic matter transformation processes which are essential for plant nutrition (Curci et al., 1997).

Frankenberger and Dick (1983) studied 11 enzymes in 10 different soils and found that alkaline phosphatase, amidase, and catalase activities correlated well with microbial respiration and total biomass. However, other studies have shown no correlation between enzyme activities and microbial biomass or activities. This may be due to the presence of enzymes produced by non-microbial sources. The ideal enzyme to study is one that is directly associated with microbial activity and that is rapidly inactivated upon cell death (Dick, 1997). Dick (1997) suggests that the most widely studied enzyme indicator of soil biological activity is dehydrogenase because it should only exist in viable cells and it is clearly linked with microbial activity. Alkaline phosphatase in soil appears to be derived totally from microbial origin and, as such, is a

very good indicator of microbial activity (Tabatabai, 1982). Deng and Tabatabai, (1997) report that alkaline phosphatase has not been found in higher plants. Frankenberger and Dick (1983) also found alkaline phosphatase to be one of the best enzymes for determining the relative activity and size of the microbial populations in soils. Acid phosphatase, however, is produced both by plants and microbes (Dick et al., 1983). These non-microbial sources can confound the interpretation of enzyme assays.

Curci et al. (1997) found glucosidase and dehydrogenase to be significantly affected by tillage. They noted significant correlations between extractable DNA and β-galactosidase, and between extractable RNA and β-glucosidase, β-galactosidase, alkaline phosphatase. The enzymes α-glucosidase, β-galactosidase, alkaline phosphatase, and phosphodiesterase were highly correlated with biomass C measured by the fumigation-extraction method. Curci et al. (1997) also found significant correlations between organic C and alkaline phosphatase (r = 0.90***) and arylsulfatase (r = 0.77*), as well as between total N and alkaline phosphatase (r = 0.85**) and arylsulfatase (r = 0.77*). Frankenberger and Dick (1983) also reported a relationship between organic C and activities of acid phosphatase, alkaline phosphatase, arylsulfatase, and urease.

Deng and Tabatabai (1997) found that enzyme activities increased with mulching and decreased with tillage. The effect of mulching on phosphatase was not as significant as it was on activities of arylsulfatase. Activities of phosphatase and arylsulfatase were significantly correlated with organic C. These authors suggest one reason for this correlation could be that organic matter protects and maintains enzymes in their active forms.

Numerous studies have shown that cropping systems that include green manures or which leave high levels of crop residues on the soil surface significantly increase the activity of a wide range of soil enzymes as compared with unamended soil (Dick et al., 1988; Gauger, 1987; Jordan et al., 1995; Martens et al., 1992). Kirchner et al., (1993) found that incorporation of crimson clover as a green manure in a corn system significantly increased soil levels of alkaline phosphatase, arylsulfatase, and β-glucosidase as compared with a corn/fallow rotation.

A typical assay to measure the activities of a soil enzyme involves adding a known amount of soil to a solution containing a known amount of substrate and measuring the rate at which the substrate is converted to the final product of the enzyme-catalyzed reaction under a standard set of conditions including temperature and pH. Although the enzymes in soil may be produced by either plant or microbial sources most soil enzymes probably originate from microbial sources (Ladd, 1978; Bolton, 1985). Consequently, levels of soil enzymes should give an indication of microbial numbers and activities.

Phosphatases: A significant portion of the soil P is typically in the form of soil organic compounds. Before this P becomes plant-available these compounds must be mineralized to HPO_4^{-2} and $H_2PO_4^-$ (Gauger, 1987). Thus, the enzymes known as phosphatases are very important in plant nutrition. Phosphatases catalyze the hydrolysis of the esters and anhydrides of H_3PO_4 (Tabatabai, 1994). The phosphatases that were measured in this study were the phosphomonoesterases known as acid phosphatase and alkaline phosphatase (indicating their optimum pH range). Alkaline phosphatase in soil appears to be derived totally from microbial origin and thus, is a very good indicator of microbial activity (Tabatabai, 1994). Frankenberger and Dick (1983) found alkaline phosphatase to be one of the best enzymes for determining the relative activity and size of the microbial population in soils. Acid phosphatase is produced both by plants and microbes (Dick et al., 1983). Levels of phosphatase activity in the soil have been found to be related to levels of soil organic matter, moisture content, total N, texture, pH, and C/P ratio (Drobnikova, 1961; Halstead, 1964; Ross, 1970; and Harrison, 1983). Phosphatase activity also has been found to vary with season and depth in the soil profile (Harrison, 1979). Nannipieri et al. (1978) and Nannipieri et al. (1979) showed a correlation between the size of the microbial biomass and acid phosphatase activity.

Sulfatases: Since plants take up sulfur as inorganic sulfate the mineralization of organic sulfur is important to plant nutrition. Sulfatases are classified by the type of organic sulfate esters which they hydrolyze. Arylsulfatase catalyzes the hydrolysis of an arylsulfate anion by breaking the O-S bond:

$$R\text{-}OSO^{-}_{3} + H_20 \rightarrow R\text{-}OH + H^{+} + SO4^{-2}$$ (Tabatabai, 1994)

This enzyme is partly responsible for S cycling in soil. Arylsulfatase has been found in plants, animals, and microorganisms, although Farrell et al. (1994) report that microorganisms and plant roots are the major sources of arylsulfatase in the soil. Whalen and Warmen (1996) point out that abiotic sources of arylsulfatase exist, and this enzyme is a relatively stable and persistent component of soil. Arylsulfatase becomes bound in stable complexes with clay particles and humic colloids resulting in stable and persistent enzyme activity. Like many soil enzymes, arylsulfatase activity has been highly correlated with soil organic matter. Soil levels of arylsulfatase are likely affected by the many of same soil factors as phosphatases. Tabatabai and Bremner (1970) found sulfatase activity decreased with soil depth. Farrell et al. (1994) found that clearing and cultivation of native grassland and forest soils resulted in significant decreases in arylsulfatase activity.

β glucosidase: The terms glycosidase and glucosidase have been used interchangeably to describe the enzymes which act on glycosyl compounds catalyzing the hydrolysis of different glycosides:

$$Glycosides + H_2O \rightarrow Sugar + aglycons$$ (Tabatabai, 1994)

These enzymes are important because their hydrolysis products (sugars) are important energy sources for microorganisms (Eivazi and Tabatabai, 1990). β glucosidase catalyzes the hydrolysis of β-D-glucopyranosides. β glucosidase is found in yeasts, fungi and higher plants and is important in providing energy sources for microbes (Tabatabai, 1994). Eivazi and Tabatabai, (1990) found β glucosidase concentration decreased with depth and was significantly correlated with organic C.

Dehydrogenase: Dick (1997) reports that the most widely studied enzyme indicator of soil biological activity is dehydrogenase because it should only exist in viable cells. Biological oxidation of organic compounds is generally a dehydrogenation process (Tabatabai, 1994). The microbial oxidation of organic substances in aerobic conditions depends on a membrane-bound electron transport chain with oxygen as the final electron acceptor. This is coupled to the synthesis of ATP by "oxidative phosphorylation"

(Camiña et al., 1998). The electron transport chain uses dehydrogenases which play an essential role in the initial stages of oxidation of soil organic matter by transferring electrons or protons from substrates to acceptors. Dehydrogenase activity serves as an indicator of the microbiological redox system in soils and can be considered a good measure of microbial oxidative activity. Curci (1997) claims that dehydrogenase in soil derives entirely from the microbial population. Zelles et al. (1997) add that dehydrogenase activity is an indicator for potential non-specific intracellular enzyme activity of the total microbial biomass. It has been used as an indicator of active microbial biomass. Dehydrogenase and alkaline phosphatase activity have been shown to correlate well with measures of soil microbial biomass (Chandler et al., 1997). This fact supports the use of these enzymes as measures of microbial activity.

Methods and Materials

Description of the site and sampling procedures

This study was conducted at the University of Tennessee experiment station at Ames Plantation, near Grand Junction, Tennessee. The study was designed to examine the environmental impacts of conversion of cropland to SRWC. Prior to establishment of the study the area was under conventionally-tilled silage corn production for at least 15 consecutive years. The soil type at the study site is a Memphis-Loring silt loam intergrade (fine-silty mixed thermic Typic Hapludalf - fine-silty mixed thermic Typic Fragiudalf). These soils are characterized by a high silt and low clay content, low CEC, and a variably-developed fragipan between soil depths of 90 - 150 cm. The study site consisted of six 0.5 ha plots constructed as mini-hydrological units, with 0.5m confinement berms (soil brought from other portions of the field) surrounding three corn (Zea mays L.) plots and three sycamore (Platinus occidentalis L.) plots. These plots will be referred to as "corn" and "young sycamore" respectively. The young sycamore plots were located within an even-aged 3.5 ha plantation of sycamore. Plots could not be randomly distributed because a wildlife study associated with this project required large unbroken tracts of each treatment. Sycamore has been identified as having a high

potential as a biomass tree crop because of its ability to develop a dense canopy and a pervasive root system over a short rotation (Graham, 1994).

Sycamore seedlings were planted at 2.4m by 1.2m spacing in February, 1995. After two growing seasons tree survival was over 97%. The corn plots have been maintained in no-till corn silage production. The winter cover crop was wheat. The wheat was killed in the spring using glyphosate, and not harvested. During the growing seasons since establishment, the silage corn and sycamore plots have been managed as two separate systems, with chemical and fertilization inputs and harvest geared to the best management of each.

In 1983 Wells and Fribourg (1992) had established three 17m x 17m plots of American Sycamore approximately 2 miles away from the corn and young sycamore plots, but on a similar soil type. These plots were part of a study to compare forage and tree production on marginal soils in Tennessee. This site was intended to represent a control, not a marginal soil. This site will be referred to as "old sycamore". Sycamore seedlings were planted at a spacing of 2.4m between rows and 1.2m within rows on a site that had been planted in conventionally-tilled soybean. The area surrounding the plots was maintained in soybeans. No fertilizer was applied to the trees. Weed growth was controlled by disking or by using glyphosate for one year (Fribourg et al., 1989). At four years of age the trees had a 96% survival rate. Every other row of sycamore was harvested at five years. A second harvest in 1993 collected the coppice and the 10-year-old trees (Fleenor, 1996). The trees which had regrown were cut again in the spring of 1998 and the biomass was left on the site.

Immediately adjacent to the young sycamore plots is a mature mixed hardwood forest. This forest is approximately 50 years old, and has a mixed species composition of oak, sweetgum, red maple, black cherry, and an assortment of hardwood trees and shrubs. Soil samples were collected from this site (referred to as "mature forest") in March, May, and August, 1998. These four treatments were chosen for study to show soil biological properties in a continuum from a traditional annual row cropping system to variously-aged SRWC systems, to a mature hardwood site.

Soil sampling

Mineral soil cores were collected during June and November, 1997, and March, May, and August 1998 to a depth of 6 cm using a soil core sampler (inside diameter = 5.3 cm) (SoilMoisture Equipment Corp., Santa Barbara). Organic litter was removed from the soil surface prior to sampling. Each treatment-plot combination was divided into a grid and a number was assigned to each section of the grid. Three sections were randomly chosen. Three soil cores were collected across the row to include both rhizosphere and interrow samples. These were bulked and stored in plastic bags on dry ice. In June and November of 1997 the bags were stored in a cooler on dry ice and taken to the laboratory within 24 hours of sampling, where they were stored in a freezer at a maximum temperature of -20°C until they were analyzed. In March, 1998 a study was conducted to determine whether storing samples on dry ice during transport from the field reduced levels of microbial biomass (data not shown). Deeply frozen samples were found to contain lower levels of microbial biomass C. In March, May, and August 1998 a portion of each sample was stored on dry ice to be used for PLFA and DNA analysis. However, the portion to be used for chemical analysis, chloroform-fumigation, and enzyme assays was stored in a cooler on ice, returned to the laboratory, and stored at 4°C so as to not kill organisms by freezing. All analyses on these refrigerated samples were conducted within 2 weeks of sampling.

Methodology of measuring soil microbial biomass

Microbial biomass C was measured in freshly collected soils. However, Stenberg et al. (1998) claim that storage at -20°C for up to 13 months does not affect the microflora in a significant way. Moist soils were sieved (< 4 mm) and recognizable plant material was removed. Triplicate samples of moist soil containing 20 g was weighed into 50 ml glass beakers. Other researchers have suggested that the water content of the soil should be greater than 30% of water-holding capacity as microorganisms are less affected by chloroform in dry samples (Joergensen, 1995). Samples were placed into a desiccator with a beaker containing 40 ml of ethanol-free

chloroform and a few boiling chips. A beaker of soda lime was added to the desiccator to absorb CO_2 evolved during the fumigation period (Joergensen, 1995). The samples were covered by a moist paper towel to maintain humidity. The desiccator was evacuated until the chloroform boiled vigorously for 2 minutes. The desiccator was kept in the dark for 24 hours at 25°C.

Triplicate samples of the same soils were extracted immediately with 0.5 M K_2SO_4. These represented the "unfumigated" samples and were extracted to determine background levels of soluble organic C. Most authors recommend an extractant to soil ratio of at least 4:1 (v/w). Beck et al. (1997) recommended a larger ratio for soils with large contents of organic material. Eighty ml of 0.5 M K_2SO_4 was used to extract soluble organic C from these samples. Samples were shaken for 30 minutes and filtered through Whatman 42 filter paper. Vance et al., (1987) suggested that, while it would be best if the extracts were analyzed immediately, they can be stored at 1 - 2°C for 1 - 2 weeks. All samples in this study were analyzed within one week of extraction. Joergensen (1995) proposed that samples be stored at -15°C prior to analysis. A white precipitate formed during storage. Joergensen (1995) suggests that this was hydrated $CaSO_4$ and claims that it will not interfere with the analysis. K_2SO_4-extractable organic C was measured by an automated u.v.-persulfate oxidation method by a Dohrman automatic carbon analyzer (Santa Clara, CA).

After 24 hours the chloroform was removed from the desiccator and residual chloroform vapor was removed from the soil by repeated evacuation (6 fold). These "fumigated" samples were extracted and analyzed as described for the "unfumigated" samples. Ec is the difference in C extracted from the fumigated and the unfumigated samples.

After chloroform fumigation, the additional organic C extracted from the fumigated samples is converted to microbial biomass using proportionality constants which correct for incomplete release or extraction of microbial C (Beck et al., 1997). For chloroform FE a Kec value of 0.45 has been obtained empirically, mainly by calibration against the FI method. Hintze et al. (1994) point out that since Kec for FE

was calibrated by the FI method, problems with the FI method may cause errors in the calibration of the FE method. They suggest that an overall conversion factor from respiration to biomass for all soil types seems questionable.

Formerly K_2SO_4 soil extracts were digested with potassium dichromate and back-titrated with ferrous ammonium sulfate to determine organic C. Vance suggested that biomass C should equal 2.64 Ec (where Ec = organic C extracted from fumigated soil - organic C extracted from unfumigated soil). Wu et al., (1990) pointed out that reliable automated techniques to measure organic C in aqueous solutions have become available. These systems are more efficient than the former digestion, so they suggest that biomass C be calculated as 2.22Ec (i.e. 45% of cellular C is extracted by K_2SO_4).

Methodology of measuring enzymatic activity:

The activities of the soil enzymes acid and alkaline phosphatase (phosphomonoesterases), arylsulfatase, and β glucosidase were measured following the procedure described by Tabatabai (1994). The labels "acid" and "alkaline" for the phosphatases refer to the pH ranges where these enzymes show optimum activity. The assay to estimate the activities of these enzymes involved colorimetric estimation of the amount of p-nitrophenol released when soil was incubated with buffered p-nitrophenol substrate solutions and toluene.

To measure phosphatase activity, 50 ml Erlenmeyer flasks containing 1 gram of moist soil (<2 mm), 40 ml of modified universal buffer (pH 6.5 for assay of acid phosphatase or pH 11 for assay of alkaline phosphatase), 1 ml of 0.05 M p-nitrophenol phosphate solution (Sigma 104 sodium salt; Sigma Chemical Co., St. Louis) made in the same buffer, and 0.2 ml of toluene were fitted with no. 2 stoppers, swirled to mix contents, and incubated for one hour at 37° C. Toluene was used to stop the synthesis of enzymes by the living cells and to prevent assimilation of the reaction products (Tabatabai, 1982). After 1 hour the stopper was removed and 1 ml of 0.5 M $CaCl_2$ and 0.5 M NaOH was added to stop the reaction and develop color, and the soil suspension was filtered through a Whatman no. 42 filter paper. Alkaline solutions of p-nitrophenol

have a yellow color which is quantitatively related to the amount of p-nitrophenol which has been released by the enzymes. The absorbence of the assay solution was read on a spectrophotometer (Milton Roy Spectronic 401, Rochester, NY) at 410 nm. The p-nitrophenol content of the filtrate was calculated by reference to a standard curve created from standards containing known amounts of p-nitrophenol. Samples typically needed to be diluted with water before being quantified. Controls were performed to account for color not derived from p-nitrophenol released by phosphatase activity (Tabatabai, 1994).

For arylsulfatase, 1 gram of moist soil was incubated with 0.05 M p-nitrophenol sulfate (Sigma Chemical Co., St. Louis) and 0.25 ml of toluene. The system was buffered with 0.5 M acetate buffer (pH 5.8) and analyzed as described for the phosphatases. For the assay of β glucosidase, the substrate used was 0.05 M p-nitrophenol-β-D glucoside (P.N.G.) (Sigma Chemical Co., St. Louis) and the system was buffered with modified universal buffer, pH 6. In this assay system NaOH was replaced with Tris(hydrometry)aminomethane (THAM) at pH 12 since PNG is hydrolyzed over time in the presence of excess NaOH. The remainder of the assay system was the same as that used for the other enzyme assays.

Dehydrogenase was determined by measuring the concentration of the reduced form of an artificial electron acceptor which was reduced by microbial activity. The assay measured the reduction of 2,3,5 triphenyltetrazolium chloride (TTC) by microorganisms to 2,3,5 triphenyl formazan (TPF). TPF is an intensely-colored, methanol soluble compound. Twenty grams of air-dried soil (<2 mm) was mixed with 0.2 grams of $CaCO_3$. Six grams was placed in each of three test tubes (for a triplicate reaction). 1 ml of 3% TTC (Sigma Chemical Co., St. Louis) was added along with 2.5 ml of water. The contents of the tube were mixed and incubated for 24 hours at 37°C. After 24 hours, 10 ml of methanol was added and the tube shaken for 1 minute. The soil was quantitatively transferred to a funnel containing a Whatman #42 filter paper. The suspension was filtered into a 100 ml volumetric flask. The tube was washed with methanol and additional methanol was added to the funnel until the red color was removed from the filter. The volume was made up to 100 ml. The red color is the TPF.

This reduced formazan was quantified by measuring the intensity of the red color using a Milton Roy Spectronic 401 spectrophotometer (Rochester, NY) set at a wavelength of 485 nm. Methanol was used as a blank. The amount of TPF was calculated by comparison to a standard curve prepared from TPF standards (Tabatabai, 1994).

Methodology of measuring biomass by PLFA and nucleic acid hybridization

Microbial biomass was estimated by total phospholipid fatty acids using the procedure described in Part 3. Nucleic acid hybridization was also used to estimate total microbial biomass utilizing the procedure described in Part 4.

Statistical analysis

Differences between treatments were determined using analysis of variance (ANOVA) using the type III General Linear Model SAS procedure (SAS Institute, 1990) and Fisher's least significant difference procedure. Effects were considered significant when the null hypothesis, using the residual term as error, were rejected at a $P \leq 0.05$. Values reported are the mean of nine samples at each sampling date, with the exception that only three samples were collected in the mature hardwood forest at each sampling date. Standard errors of the mean were calculated using the lsmeans procedure. The relationships between each enzyme activity, and each measure of microbial biomass were determined by simple linear correlation coefficients.

Results and Discussion

Microbial biomass

Table 5-1 shows values of microbial biomass as measured by total PLFA, chloroform fumigation-extraction (FE) as well as total RNA and total DNA. Not all measurements were conducted at each sampling event. Curci et al. (1997) report that the size of the soil microbial biomass is dependent on the level of soil organic matter. This makes sense since the soil organic matter provides substrate for

Table 5-1. PLFA biomass, total nucleic acids, and microbial biomass by chloroform-fumigation extraction

Treatment	PLFA Biomass	FE Microbial Biomass	RNA	DNA
	μmol kg^{-1}	μg C g^{-1} soil	ng g^{-1} soil	ng g^{-1} soil
		June, 1997		
Corn	61.63b†	261.8b	-	6.16b
Young Sycamore	50.46c	237.0b	-	9.32b
Old Sycamore	73.35a	380.7a	-	18.06a
		November, 1997		
Corn	27.13b	146.8b	-	0.60b
Young Sycamore	15.22c	130.0b	-	0.65b
Old Sycamore	48.92a	241.7a	-	1.10a
		March, 1998		
Corn	18.13b	-	158.5a	-
Young Sycamore	10.51c	-	86.1a	-
Old Sycamore	20.01b	-	206.0a	-
Mature Forest	46.22a	-	196.8a	-
		May, 1998		
Corn	-	323.4b	-	-
Young Sycamore	-	206.5c	-	-
Old Sycamore	-	339.5b	-	-
Mature Forest	-	732.2a	-	-
		August, 1998		
Corn	12.23c	313.0b	-	29.18a
Young Sycamore	9.34d	145.8c	-	34.95a
Old Sycamore	18.81b	386.1ab	-	39.33a
Mature Forest	27.48a	469.9a	-	-

†Within columns, means followed by the same letter are not significantly different by LSD(0.05).

heterotrophic metabolism.. Griffiths et al. (1999) comment that in most mineral soils the microbial biomass is limited in growth and activity by substrate availability. Therefore, the input of substrate to the system plays a major role in determining the size and activity of the microbial biomass. The soil organic matter affects soil properties such as bulk density, water-holding capacity, porosity, and structure, which have direct and indirect influences on the soil microbial community. The presence or absence of organic matter affects the availability of substrate, water, and oxygen for microbial metabolism. In this study, the older sycamore plantation and the mature hardwood forest had the greatest levels of soil microbial biomass by every measure for each sampling event. These treatments also had the greatest levels of total soil C and total soil N (Tables 2-3 through 2-7). Tables 5-2 through 5-6 show correlation coefficients between measures of microbial biomass and soil chemical and biochemical properties. Microbial biomass as measured by FE was significantly correlated with total soil C for every sampling event, with r values ranging from 0.71 in June 1997 to 0.94 in May 1998. In addition, microbial biomass by FE was significantly correlated with total soil N for every sampling event, with r values ranging from 0.65 in June 1997 to 0.93 in May 1998. Microbial biomass measured as total PLFA was also significantly correlated with soil C, with r values ranging from 0.64 in March 1998 to 0.88 in August 1998. Correlations between soil N and PLFA biomass were significant and r values ranged between 0.66 in March 1998 and 0.86 in August 1998. Obviously, values of total soil C and total soil N were very highly correlated with each other with r values always greater than 0.9.

One theory to account for significantly less microbial biomass in the young sycamore plots is related to soil pH. The young sycamore soils consistently measured about one half a pH unit less than the other treatments. Perhaps the lower pH reduced the size of the microbial biomass. Soil pH correlated with FE microbial biomass (r = 0.35*) and PLFA biomass (r = 0.38**) in June 1997. However, pH did not correlate with either measure of microbial biomass at other sampling dates. This could mean either that soil pH didn't play a major role in determining the size of the microbial biomass, or that if one component of the microbial community was reduced by

Table 5-2. Correlation coefficients (r) between PLFA biomass, microbial biomass, and soil chemical properties, June, 1997.

	PLFA biomass	Microbial Biomass C	Soil C	Soil N	Soil pH	DNA
PLFA biomass	-	0.60***	0.68***	0.68***	0.38**	ns
Microbial Biomass C	0.60***	-	0.71***	0.65***	0.35*	ns
Total soil C	0.68***	0.71***	-	0.94***	0.45***	0.47***
Total soil N	0.68***	0.65***	0.94***	-	0.47***	0.44**
Soil pH	0.38**	0.35*	0.45***	0.47***	-	ns
DNA	ns	ns	0.47***	0.44**	ns	-

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 5-3. Correlation coefficients (r) between PLFA biomass, microbial biomass, and soil chemical properties, November, 1997.

	PLFA biomass	Microbial Biomass C	Soil C	Soil N	Soil pH
PLFA biomass	-	0.54**	0.76***	0.68***	ns
Microbial Biomass C	0.54**	-	0.80***	0.68***	ns
Total soil C	0.76***	0.80***	-	0.91***	ns
Total soil N	0.68***	0.68***	0.91***	-	ns
Soil pH	ns	ns	ns	ns	-

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 5-4. Correlation coefficients (r) between PLFA biomass, RNA and soil chemical properties, March, 1998.

	PLFA biomass	RNA	Soil C	Soil N	Soil pH
PLFA biomass	-	0.39*	0.64***	0.66***	ns
RNA	0.39*	-	0.40*	ns	0.53**
Total soil C	0.64***	0.40*	-	0.94***	ns
Total soil N	0.66***	ns	0.94***	-	ns
Soil pH	ns	0.53**	ns	ns	-

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 5-5. Correlation coefficients (r) between enzymatic activities, microbial biomass C, and soil chemical properties, May, 1998.

	Acid Phos.	Alk Phos.	Arylsul.	β-Gluco	Dehy	Mic. Bio.	Soil C	Soil N	pH
Acid Phosphatase	-	0.43 *	ns	0.72 ***	ns	0.61 ***	0.50 **	0.47 **	ns
Alkaline Phosphatase	0.43 *	-	0.53 **	0.72 ***	0.40 *	0.68 ***	0.57 **	0.59 ***	0.70 ***
Aryl-sulfatase	ns	0.53 **	-	0.60 **	0.61 ***	0.86 ***	0.94 ***	0.93 ***	ns
β-Glucosidase	0.72 ***	0.72 ***	0.60 **	-	ns	0.85 ***	0.77 ***	0.78 ***	ns
Dehydro-genase	ns	0.40 *	0.61 ***	ns	-	0.52 **	0.56 **	0.59 ***	0.51 **
Microbial Biomass C	0.61 ***	0.68 ***	0.86 ***	0.85 ***	0.52 **	-	0.94 ***	0.93 ***	ns
Total soil C	0.50 **	0.57 **	0.94 ***	0.77 ***	0.56 **	0.94 ***	-	0.98 ***	ns
Total soil N	0.47 **	0.59 ***	0.93 ***	0.78 ***	0.59 ***	0.93 ***	0.98 ***	-	ns
Soil pH	ns	0.70 ***	ns	ns	0.51 **	ns	ns	ns	-

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 5-6. Correlation coefficients (r) between enzymatic activities, microbial biomass C, PLFA biomass and soil chemical properties, August, 1998.

	Acid Phos.	Alk Phos.	Arylsul.	β-Gluco	Dehy	Mic. Bio.	PLFA	Soil C	Soil N	pH
Acid Phosphatase	-	ns	ns	0.41 *	ns	ns	ns	ns	ns	ns
Alkaline Phosphatase	ns	-	0.63 **	0.55 **	0.39 *	0.66 ***	0.60 ***	0.67 ***	0.70 ***	0.56 **
Aryl-sulfatase	ns	0.63 **	-	0.47 **	0.72 ***	0.77 ***	0.90 ***	0.93 ***	0.91 ***	ns
β-Glucosidase	0.41 *	0.55 **	0.47 **	-	0.57 **	0.67 ***	0.40 *	0.58 ***	0.64 ***	ns
Dehydro-genase	ns	0.39 *	0.72 ***	0.57 **	-	0.81 ***	0.64 ***	0.72 ***	0.73 ***	0.56 **
Microbial Biomass C	ns	0.66 ***	0.77 ***	0.67 ***	0.81 ***	-	0.76 ***	0.84 ***	0.86 ***	ns
PLFA Biomass	ns	0.60 ***	0.90 ***	0.40 *	0.64 ***	0.76 ***	-	0.88 ***	0.86 ***	ns
Total soil C	ns	0.67 ***	0.93 ***	0.58 ***	0.72 ***	0.84 ***	0.88 ***	-	0.98 ***	ns
Total soil N	ns	0.70 ***	0.91 ***	0.64 ***	0.73 ***	0.86 ***	0.86 ***	0.98 ***	-	0.37 *
Soil pH	ns	0.56 **	ns	ns	0.56 **	ns	ns	ns	0.37 *	-

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

the lower soil pH in this system, another component (such as fungi) was favored and increased in biomass. If such shifts in community structure did occur they would have likely been detected by PLFA analysis (Part 3). A more likely scenario is that pH values in the young sycamore system were not low enough to affect levels of microbial biomass as significantly as shifts in soil organic matter did. Differences in soil microbial biomass between the corn system and the young sycamore system are more likely to be related to soil pH since there are only small differences in soil organic matter between these treatments. A correlation was run between just the corn and young sycamore systems for the variables pH, microbial biomass by FE, and PLFA biomass for the August 1998 sampling. In this case, pH was correlated with FE microbial biomass (r = 0.58*) but was still not correlated with PLFA biomass.

Although PLFA biomass and microbial biomass by FE represent very different approaches to measuring microbial biomass, they were significantly correlated with each other. The correlation coefficient in June 1997 was 0.60***, in November 1997 was 0.54**, and in August 1998 was 0.76***. Using soil that had not been stored on dry ice for the May and August samplings seemed to improve the accuracy of the microbial biomass procedure, although the results from June and November were significantly correlated with PLFA biomass.

Extractable RNA was measured in March 1998 (Stair, 1998) and was found to be correlated with PLFA biomass with an r value of 0.39*. However, extractable DNA measured from the June 1997 sampling was not significantly correlated with either PLFA biomass or microbial biomass by FE. While nucleic acid levels showed greater microbial biomass in soil from the old sycamore plots and the mature forest as compared with the corn and young sycamore plots, high variability between samples prevented better correlation between nucleic acids and other measures of microbial biomass.

Levels of soil microbial biomass varied across the year, although the more mature forest systems had the greatest biomass at each sampling. In a study by Bardgett et al. (1997) microbial biomass and microbial activity showed a summer maxima and a winter minima. This was thought to be related to changes in soil temperature and moisture

conditions and increased availability of root exudates as root growth and turnover increased in the spring and summer. This study also showed levels of microbial biomass to decrease during the winter, with levels lower in November and March than they had been in June. The lowest levels of PLFA biomass was found during the August 1998 sampling. I would suggest two possible reasons for this observation. First, the late summer of 1998 was a very dry period. The corn crop was harvested in late July because of insufficient rain. Figure 2-4 shows that both the May and August samplings were done during dry periods. This lack of soil water would have reduced soil microbial populations and activities. A second possible explanation for low values for PLFA biomass has to do with the PLFA methodology. A 50 pmol internal standard had been included with each fatty acid sample to quantify fatty acids by comparison to the standard. I used the same internal standard for June, November, and March. However, I ran out of this internal standard and had to make a new internal standard for the August samples. A difference in concentration of the internal standard could account for some difference in total biomass measurement between sampling dates. It is interesting to note that values of microbial biomass as measured by FE in August 1998 are comparable to those of the June 1997 sampling, while PLFA biomass values were significantly less. This observation supports the conclusion that there was a problem with the internal standard. However, PLFA biomass and the FE procedure are measuring different components of the microbial community, and the two measures could be affected differently by the dry conditions present in August.

Enzyme Activities

The activities of selected soil enzymes were assayed in May and August of 1998 in an attempt to correlate the activities of soil microorganisms with the other measures of microbial biomass and activity considered in this study. Table 5-7 summarizes the results of these assays. Correlations between enzyme activities and other measures of soil chemical and biochemical properties can be found in Tables 5-5 and 5-6. In an actively-functioning agroecosystem it would be expected that microbial activities would increase

Table 5-7. Results of Enzyme Assays

Treatment	Acid Phosphatase	Alkaline Phosphatase	Arylsulfatase	β Glucosidase	Dehydrogenase
	— μg p-nitrophenol g^{-1} soil (dry wt.) hr^{-1} (±std error) —				μg TPF g^{-1} soil
May, 1998					
Corn	6060 ± 321 b†	1367 ± 112 ab	754 ± 128 c	1296 ± 109 b	88.98 ± 14 b
Young Sycamore	4798 ± 321 bc	750 ± 112 c	608 ± 128 c	965 ± 109 b	42.34 ± 14 b
Old Sycamore	3828 ± 321 c	1122 ± 112 Bc	2538 ± 128 b	1042 ± 109 b	160.75 ± 14 a
Mature Forest	7809 ± 556 a	1713 ± 195 a	5928 ± 221 a	2018 ± 189 a	143.70 ± 25 a
August, 1998					
Corn	6057 ± 305 a	1105 ±135 ab	440 ±171 c	819 ±105 a	83.77 ± 13 Bc
Young Sycamore	4838 ± 305 ab	916 ±135 b	382 ±171 c	670 ±105 a	32.76 ± 13 c
Old Sycamore	4466 ± 329 b	1295 ±146 ab	2404 ±184 b	991 ±113 a	157.79 ± 14 a
Mature Forest	5648 ± 528 a	1571 ±234 a	3427 ±296 a	920 ±182 a	117.29 ± 22 ab

†Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

as the size of the microbial biomass increases. At the May sampling all five soil enzymes were highly correlated with microbial biomass C. Not surprisingly, they also correlated with levels of total soil C. In August both microbial biomass C measured by FE and PLFA biomass correlated with all enzymes except acid phosphatase. Acid phosphatase has been found to have numerous sources in addition to microorganisms. It is notable that in August acid phosphatase was correlated with no other enzyme or soil property except β-glucosidase. As Table 5-7 shows the old sycamore plots had the lowest activities of acid phosphatase of any treatment at both sampling dates (although the values were not significantly lower than the young sycamore plots). If enzymes truly give an indication of microbial activity, it might be expected that they should correlate with one another, assuming that their respective substrates were present. All of the other enzymes correlate with one another as well as with total soil C and N. Only alkaline phosphatase and dehydrogenase correlated with pH.

Alkaline phosphatase is considered a good indicator of soil microbial activity because it is only produced by microorganisms. Interestingly, soils from the corn plots had alkaline phosphatase activities that were not significantly lower than the mature hardwood or the old sycamore at the May and August samplings. Also, levels of β-glucosidase were similar to levels of alkaline phosphatase. At both of these sampling dates the corn plots had levels of microbial biomass C measured by FE that were not significantly lower than the old sycamore plots (Table 5-1). This was not so surprising for the May sampling. The corn system had just received applications of fertilizer and the young corn crop would be adding root biomass and root exudates to the soil. I was surprised that there was not a greater difference between corn and old sycamore in August. The corn crop was removed in late July, so there would be corn roots to decompose, but with the dry period that this site was experiencing in August it would seem that the ability of the forested soils to hold moisture would give the microbial communities a distinct advantage.

Arylsulfatase activities were significantly higher in the forested systems than in the corn or young sycamore soil. Whalen and Warman (1996) point out that there are

abiotic sources of arylsulfatase, and arylsulfatase is a relatively stable enzyme which can become bound in complexes with clay particles and humic colloids, resulting in stable and persistent enzyme activity. These authors also point out that arylsulfatase has been highly correlated with organic matter. Indeed, at the May sampling arylsulfatase and total soil C correlated with an r value of 0.94, while in August the r value was 0.93. Probably the high levels of arylsulfatase activities seen in these soils were caused by retention of the enzyme in soil organic matter, which would occur to a higher degree in the established forest systems.

Dehydrogenase activity was also significantly greater in the old sycamore and mature forest systems than in the corn and young sycamore soil at both samplings. Zelles et al. (1997), state that dehydrogenase activity is an indicator for potential non-specific intracellular activity of the total microbial biomass. Whereas the other enzymes considered give an indication of the microbial community's ability to metabolize a specific substrate, dehydrogenase is an indicator of the activity of the biomass as a whole. At both sampling dates, levels of dehydrogenase indicate that the microbial community in the old sycamore system is much more active than either the young sycamore community or the corn community. In fact, all of the soil enzymes indicate a lower level of microbial activity in the young sycamore plantation compared with the other treatments.

Conclusions

This research showed seems that the conversion from annual row crops to SRWC eventually leads to a larger and more active soil microbial community. Using various measures of soil microbial biomass and different assays to measure soil enzyme activities, we found that the old sycamore and the mature forest plots had more microbial biomass and greater activities of some enzymes. However, during the time that the trees are getting established, the total soil microbial biomass was smaller and less active. This would appear to mainly a function of reduced inputs of substrate into the soil during the time of early tree growth. For the first few year after the trees are planted they take up

nutrients from the site, but return very little plant residue to the soil. Once the litter layer is established and levels of soil organic matter begin to increase at the soil surface, the soil biological properties in the young sycamore plantation should become more like the older forest systems. If one of the goals of converting to SRWC is soil conservation, it might make sense to plant a cover crop with the trees for the first few years. This would serve to protect the soil, reducing runoff and increasing infiltration, and it would provide inputs of C to the soil system. This would speed the development of the litter layer and promote the development of an active and healthy community of soil microorganisms.

This research also showed good correlation between microbial biomass values measured by PLFA, chloroform FE, and dehydrogenase activity. It appears that more work needs to be done to improve nucleic acid hybridization as a measure of soil microbial biomass.

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Part 6

Summary of the Study of Changes in Soil Biological Properties Resulting from Conversion of Annual Row Crops to Short-Rotation Woody Crops

In the course of this study of soil biological properties at the Ames Plantation short-rotation woody crop project I intended to answer a series of research questions:

1. Does the conversion from the corn silage agroecosystem to the sycamore plantation causes shifts in the structure of the microbial community?
2. Does this conversion in crop management systems alter the size of the microbial biomass?
3. Does this conversion affect the activity and health of the microbial community, thus influencing the ability of the community to cycle nutrients through the ecosystem?
4. If this conversion in crop management systems does alter the soil microbial community and/or the size of the microbial biomass, what factors in the ecosystem influenced this shift?
5. If the soil microbial community does change after conversion from row cropping to tree plantations, when does this shift occur? Is it a rapid change or does it take some years for the young sycamore plantation to become more like an established forest?
6. If differences are found between management systems, can these differences be seen throughout the year, or only during the growing season? How would these microbial communities change during the different seasons of the year?
7. A supplemental goal of this study was to compare and contrast the microbial and biochemical techniques used to study these ecosystems.

A battery of molecular and biochemical techniques were employed in this study, partly to increase confidence in the results obtained, and partly to compare the different techniques. Every measure used showed that the four treatments studied differed in microbial community structure and in the size of the microbial biomass. After the corn system was converted to a sycamore plantation the microbial biomass decreased slightly and the bacterial community became more Gram-positive. There was some discrepancy

on this point between the PLFA analysis and the rRNA procedure performed by J.O. Stair. However, the rRNA procedure classified a large component of each community as "unknown", leaving significant doubt as to the true structure of the community. The PLFA procedure consistently showed that the young sycamore system had the most Gram-positive community of any of the treatments. The old sycamore system represented the next stage in the continuum from annual row cropping to short-rotation woody cropping. This system showed a significantly larger microbial biomass by every measure used. The microbial community in the old sycamore system had a larger component of Gram-negative bacteria. These observations were highly correlated with levels of soil organic matter. Soil organic matter appeared to be the environmental factor which most influenced the size and structure of the microbial community. More soil organic matter means more substrate for heterotrophic metabolism, more bioelements cycling through the system, more soil water-holding capacity, better structure and porosity which influence soil aeration. The old sycamore plantation and the mature hardwood forest had significantly higher levels of soil organic matter and significantly more microbial biomass.

During the early stages of development of the young sycamore plantation very little organic matter is returned to the soil. Although a large volume of biomass is removed from the corn system as silage each year, crop residues and winter wheat residue is left on the soil surface and considerable root biomass provides a readily-available source of substrate for microbial growth. In the young sycamore system, a continuous litter layer was not established until the fall of 1997 (after three seasons of growth). Even then, the sycamore leaves were slow to decompose, so that in August of 1998 the young sycamore plantation showed significantly less water-extractable C than any other treatment. The old sycamore plantation had a well established litter layer and significantly more soil organic matter than the corn or young sycamore systems. Most of the PLFA ratios which have been reported to show the health and activity of the microbial community, showed that the community of organisms in the old sycamore plantation was under less stress and was growing faster than the corn or young sycamore systems. The enzymes arylsulfatase and dehydrogenase showed more microbial activity in the older sycamore and the mature

forest systems than the corn and young sycamore systems.

The answer to the question of when the young sycamore community will become more like the old sycamore community has not yet been found. As the litter layer begins to be mineralized and levels of soil organic matter increase I would expect the microbial community to shift to become more like the old sycamore community. If there is one recommendation that I would make to land owners who are considering shifting from annual row crops to short-rotation woody crops, it would be to plant a cover crop with the SRWC. If a soil cover could be used that would reduce weed growth, reduce erosion, provide organic residue to the system, while not reducing tree growth, I would expect a larger and healthier soil microbial community to develop in the young sycamore system. What benefits could the land owner expect to derive from a larger and healthier community of soil microorganisms? Most of these benefits would relate to increased cycling of nutrients through the soil system increasing nutrient availability for plant growth. Increased soil aggregation would improve soil porosity and aeration, reduce bulk density and increase soil infiltration and percolation. These factors, combined with increased plant residue on the soil surface would reduce overland flow of water, and thus reduce erosion.

Although the level of soil microbial biomass changed with the seasons, with a smaller biomass during the winter months, the structure of the microbial community stayed very much the same throughout the year.

I have concluded that the perfect technique for measuring soil microbial community structure and microbial biomass in environmental samples has not yet been developed. Each of the methods used in this study has strengths and weaknesses, and the choice of methodology will depend upon the question being asked. Certainly, nucleic acid hybridization is excellent for determining the presence of specific genes in the environment. However, it has not yet developed to the point of being able to quantify the microbial structure of a whole soil community. Although PLFA is limited by the overlap of fatty acid profiles between microbial groups, the technique does provide valuable information about the community. I found correlations between all of the techniques used

to determine soil microbial biomass. Although we are not yet able to perfectly describe the soil microbial community, we are getting closer to the day when we will be able to.

Appendices

Appendix A. Example Calculations

Most examples will be done for Corn plot 1 rep 1 at the August sampling date.

Moisture Content

Wet weight of soil = 18.00 g
Dry weight of soil = 16.06 g
Weight of water = 2.74 g
Moisture content = water wt./dry wt. of soil
2.737g / 16.063 g = 0.170 g water g^{-1}

Microbial Biomass by Fumigation-Extraction

Fumigated soil = 46.43 μg C ml^{-1}
Unfumigated soil = 18.94 μg C ml^{-1}
Microbial C = 27.49 μg C ml^{-1} * 5 (dilution factor = 20 g soil in 100 ml solution)
* 2.22 (Kec) = 305.14 μg C g^{-1}
Put it on a dry weight basis: With a moisture content of 0.17, 1 g of wet soil equals 0.854 g dry soil, 305.14 μg C g^{-1}/0.854 g soil = 357.3 μg C g^{-1}

Enzyme assays - All of the enzyme assays involve reading a value off of the spectrophotometer and comparing that value to a standard curve. I will show acid phosphatase as an example calculation:

By comparison to the standard curve: 58.74 μg p-nitrophenol g^{-1} hr^{-1}
Samples were diluted 1:10 before reading: 587.4 μg p-nitrophenol g^{-1} hr^{-1}
Subtract control used to measure color developed without soil: 586.04 μg
1 gram of soil was added to 10 ml of solution: 5860.4 μg p-nitrophenol g^{-1} hr^{-1}
Put the value on a dry weight basis: 5860.4/0.854 g soil = 6862.3 g^{-1} hr^{-1} p-nitrophenol g^{-1} hr^{-1}

Dehydrogenase - Slightly different than other enzyme assays.

272.81 μg TPF $100ml^{-1}$ measured by comparison with the standard curve
Divide by 6 g of soil per reaction and put it on a dry wt basis = 45.48 μg TPF g^{-1}

DNA - Nucleic acid hybridization also used a standard curve. The dilution factor was based on how much of the total nucleic acid was applied to the membrane.

A blot was measured as 1.30 ng DNA
The sample DNA was contained in 100 μl of TE buffer
Of that, 10 μl was applied to the membrane for a dilution of 10
That 1.3 ng of DNA came from 0.5 gram of soil.
1.3 * 10 = 13. Double that to put in on a per gram basis = 26 ng DNA g^{-1}

Ammonia oxidizing bacteria

Using the competitive PCR it was determined that there were **1000 copies** of the amoA gene in a sample. That sample was taken from 0.5 g of soil, so there were **2000 copies g^{-1}.**

That value was multiplied by a molecular equivalent conversion factor of 1.1 to account for the fact that the competitor was smaller than the target. **2,200 copies**

Studies have shown that the extraction method we used has a 70% extraction efficiency, so 2,200 was divided by 0.7 = **3142.8 copies.**

The PCR procedure was done on samples that had been diluted 1:10, so the final value was **31,428 copies amoA g^{-1}**

Percent Community

Each µl of the amoA stock contained 6 ng of the amoA gene. Using the M.W. of 330,000 for the molecule, we determined that this was equivalent to 1.09×10^{10} copies of the amoA gene. Thus, 1 ng of amoA = 1.816×10^{9} copies. I measured 31,428 copies or 1.73×10^{-5} ng of amoA.

According to Stapleton and Sayler (1998):

$$\text{Percent Community} = \frac{\text{(ng DNA target/copy number target)}}{\text{(ng 16S rDNA target/copy number 16S rDNA)}}$$

$$\text{Percent community} = \frac{(1.73 \times 10^{-5}\ \text{ng}/1)}{(26.0\ \text{ng DNA}/7)} = 4.66 \times 10^{-5}$$

This value was multiplied by 3 because the size of the 16S rDNA gene is 3 times larger than the amoA gene:

$$\text{Percent community} = 4.66 \times 10^{-5} * 3 = 1.4 \times 10^{-5}$$

Appendix B. Factor Loadings for Principal Components Analysis

June, 1997 - Factor Loadings for Principal Components Analysis

Factor Loadings (Varimax normalized)
Extraction: Principal components
(Marked loadings are > .700000)

	Factor 1		Factor 2
Negative Loadings			
N	-0.954	**C16_0**	-0.828
C	-0.953	**CA**	-0.734
C18_1W7C	-0.946	**C18_2W6**	-0.727
PLFA Biomass	-0.898		
K	-0.885		
C18_1W5C	-0.878		
C16_1W9C	-0.845		
MN	-0.836		
MG	-0.835		
C16_1W5C	-0.799		
CBR18_1	-0.788		
C16_1W7C	-0.787		
Ammonium	-0.723		
Soil Moisture	-0.715		
PH	-0.709		
Positive Loadings			
C17_0	0.726		
C14_0	0.760		
CY17_0	0.822		
A17_0	0.836		
CY19_0	0.861		
C15_0	0.864		
C20_0	0.870		
BR16_0	0.875		
I15_0	0.898		
C10ME17	0.925		
BR18_0	0.934		
C12ME18	0.946		
I17_0	0.966	**A15_0**	0.751
I16_0	0.967	**C10ME16**	0.905
Expl.Var	25.550	Expl.Var	9.366
Prp.Totl	0.465	Prp.Totl	0.170

June, 1997 - Factor Loadings for Principal Components Analysis

Factor Loadings (Varimax normalized)
Extraction: Principal factors (MINRES)
(Marked loadings are > .700000)

	Factor 1		Factor 2
Negative Loadings			
N	-0.931	C16_0	-0.811
C	-0.930	CA	-0.712
C18_1W7C	-0.925	C18_2W6	-0.691
PLFA Biomass	-0.872		
K	-0.864		
C18_1W5C	-0.855		
C16_1W9C	-0.822		
MG	-0.814		
MN	-0.813		
C16_1W5C	-0.773		
CBR18_1	-0.763		
C16_1W7C	-0.762		
Positive Loadings			
CY17_0	0.806		
A17_0	0.806		
CY19_0	0.834		
C15_0	0.844		
C20_0	0.845		
BR16_0	0.853		
I15_0	0.873		
C10ME17	0.905		
BR18_0	0.911		
C12ME18	0.922		
I17_0	0.944	A15_0	0.730
I16_0	0.944	C10ME16	0.879
Expl.Var	24.149	Expl.Var	8.622
Prp.Totl	0.439	Prp.Totl	0.157

November, 1997 - Factor loading for Principal Components Analysis

Factor Loadings (Varimax normalized)
Extraction: Principal components
(Marked loadings are > .700000)

	Factor 1		Factor 2
Negative Loadings			
MN	-0.962	**C18_0**	-0.966
C18_1W5C	-0.960	**C22_0**	-0.946
Soil Moisture	-0.943	**C24_0**	-0.893
C18_1W7C	-0.927	**CBR18_1**	-0.838
K	-0.901	**C17_0**	-0.759
C	-0.887		
Ammonium	-0.867		
MG	-0.834		
PLFA Biomass	-0.831		
C16_1W9C	-0.824		
N	-0.791		
BR17_0	-0.716		
Positive Loadings			
C15_0	0.769		
C10ME18	0.789		
I17_1W7C	0.801		
A17_0	0.805		
I16_0	0.886	**C12ME16**	0.752
C20_0	0.893	**C14_0**	0.759
CY19_0	0.899	**I16_1**	0.761
BR16_0	0.916	**C18_3W3**	0.765
CY17_0	0.926	**I15_1C**	0.785
P	0.931	**I14_0**	0.836
I17_0	0.964	**PH**	0.868
C10ME17	0.980	**C20_5W3**	0.887
Expl.Var	23.911	Expl.Var	14.298
Prp.Totl	0.435	Prp.Totl	0.260

November, 1997 - Factor loading for Principal Components Analysis

Factor Loadings (Varimax normalized)
Extraction: Principal factors (MINRES)
(Marked loadings are > .700000)

	Factor 1		Factor 2
Negative Loadings			
MN	-0.916	**C18_0**	-0.926
C18_1W5C	-0.915	**C22_0**	-0.904
Soil Moisture	-0.898	**C24_0**	-0.857
C18:1W7C	-0.884	**CBR18_1**	-0.794
K	-0.860	**C17_0**	-0.702
C	-0.847		
Ammonium	-0.820		
PLFA Biomass	-0.790		
MG	-0.786		
C16_1W9C	-0.781		
N	-0.749		
Positive Loadings			
C15_0	0.727		
C10ME18	0.749		
I17_1W7C	0.760		
A17_0	0.768		
I16_0	0.842		
C20_0	0.853	**I16_1**	0.716
CY19_0	0.855	**C18_3W3**	0.718
BR16_0	0.872	**C14_0**	0.721
CY17_0	0.881	**I15_1C**	0.750
P	0.891	**I14_0**	0.790
I17_0	0.919	**PH**	0.829
C10ME17	0.936	**C20_5W3**	0.847
Expl.Var	21.537	Expl.Var	12.800
Prp.Totl	0.392	Prp.Totl	0.233

March 1998 - Factor loadings for Principal Components Analysis

Factor Loadings (Varimax normalized)
Extraction: Principal components
(Marked loadings are > .700000)

Negative Loadings					
	Factor 1.000		Factor 1		Factor 3
C14_0	-0.873	CY19_0	-0.959	C22_0	-0.938
NA	-0.847	C20_0	-0.867	C17_0	-0.746
CA	-0.844	I16_0	-0.832		
C18_3W3	-0.838	C10ME18	-0.828		
C15_0	-0.799	BR18_0	-0.817		
C18_3W6	-0.778	A17_0	-0.808		
C20_5W3	-0.765	I17_1W7C	-0.761		
C16_0	-0.740	I17_0	-0.742		
CU	-0.704				
		Positive Loadings			
C10ME16	0.724	PH	0.773	MN	0.718
A15_0	0.746	C16_1W9C	0.798	C	0.758
Soil Moisture	0.781	C16_1W5C	0.858	ZN	0.776
I16_1	0.911	I15_1C	0.860	PLFA Biomass	0.891
BR17_0	0.972	C16_1W7C	0.929	N	0.935
Prp.Totl	0.303	Prp.Totl	0.281	Prp.Totl	0.243

March 1998 - Factor loadings for Principal Components Analysis

Factor Loadings (Varimax normalized) (mikes.sta)
Extraction: Principal factors (MINRES)
(Marked loadings are > .700000)

Negative Loadings					
	Factor 2		Factor 1		Factor 3
C14_0	-0.85	CY19_0	-0.92	C22_0	-0.89
NA	-0.82	C20_0	-0.83		
C15_0	-0.78	C10ME18	-0.79		
CA	-0.78	I16_0	-0.78		
C18_3W3	-0.77	BR18_0	-0.76		
C18_3W6	-0.72	A17_0	-0.76		
C20_5W3	-0.71	I17_1W7C	-0.74		
C16_0	-0.71				
		Positive Loadings			
C10ME16	0.69	C16_1W9C	0.72		
A15_0	0.71	PH	0.73	C	0.71
Soil Moisture	0.76	I15_1C	0.77	ZN	0.75
I16_1	0.87	C16_1W5C	0.78	PLFA Biomass	0.87
BR17_0	0.93	C16_1W7C	0.86	N	0.90
Prp.Totl	0.28	Prp.Totl	0.25	Prp.Totl	0.22

August 1998 - Factor loadings for Principal Components Analysis

Factor Loadings (Varimax normalized) (mikes.sta)
Extraction: **Principal components**
(Marked loadings are > .700000)

	Factor 1		Factor 2		Factor 3
Negative Loadings					
C22_0	-0.90	NITRATE	-0.88	CY19_0	-0.88
C12ME18	-0.82	C18_3W6	-0.88	C20_0	-0.77
C24_0	-0.81	A17_0	-0.85	I17_1W7C	-0.71
CY17_0	-0.77	I16_0	-0.84		
		Acid Phosphatase	-0.82		
		C20_4W6	-0.81		
		C10ME18	-0.80		
		BR16_0	-0.78		
		C15_0	-0.78		
		I17_0	-0.74		
		C17_0	-0.72		
Positive Loadings					
C18_1W7C	0.70				
MN	0.71				
C18_1W5C	0.71				
MG	0.74				
A15_0	0.76				
I14_0	0.78				
C16_1W9C	0.78				
BR17_0	0.78				
Arylsulfatase	0.78				
Alkaline Phos.	0.85				
PLFA Biomass	0.86				
C	0.86				
N	0.89			C16_0	0.72
Microbial Biomass	0.91			FE	0.75
K	0.92	C10ME16	0.73	PH	0.75
Var. Expl.	0.32	Var. Expl.	0.25	Var. Expl.	0.15

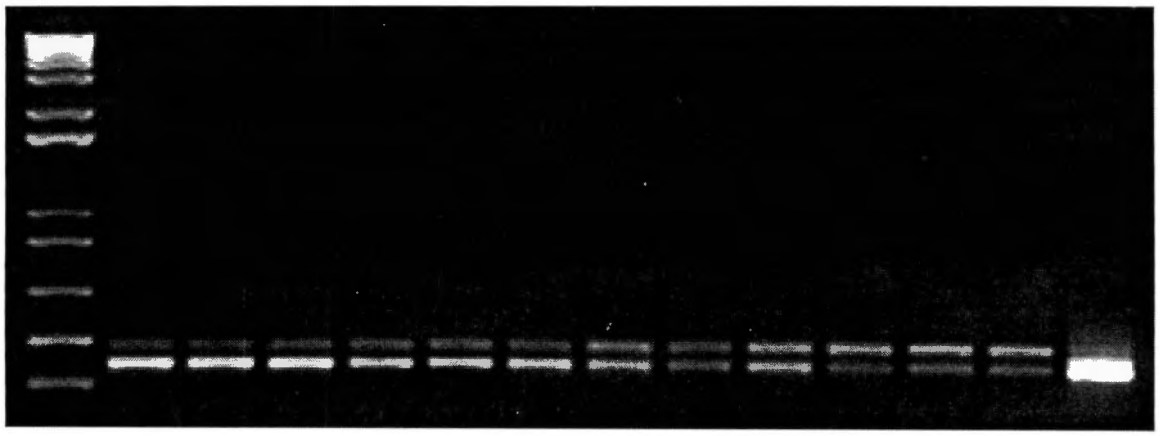
August 1998 - Factor loadings for Principal Components Analysis

Factor Loadings (Varimax normalized) (mikes.sta)
Extraction: **Principal factors (MINRES)**
(Marked loadings are > .700000)

Negative Loadings	Factor 1		Factor 2		Factor 3
C22_0	-0.85978	NITRATE	-0.83937	CY19_0	-0.83726
C12ME18	-0.78528	C18_3W6	-0.83322	C20_0	-0.74871
C24_0	-0.77367	I16_0	-0.80385		
CY17_0	-0.72932	A17_0	-0.80352		
		Acid Phosphatase	-0.7819		
		C20_4W6	-0.76061		
		C10ME18	-0.75862		
		BR16_0	-0.74538		
		C15_0	-0.74126		
		I17_0	-0.70103		
Positive Loadings					
A15_0	0.729108				
BR17_0	0.731365				
C16_1W9C	0.742074				
I14_0	0.743438				
Arylsulfatase	0.745903				
Alkaline Phosphatase	0.798363				
PLFA Biomass	0.822249				
C	0.825469				
N	0.847765				
Microbial Biomass C	0.867458			FE	0.70263
K	0.879733			PH	0.713984
Var. Expl.	0.291465	Var. Expl.	0.226135	Var. Expl.	0.135954

Appendix C. Example of Competitive PCR

Competitive PCR to quantify amoA gene copies. Corn Plot 1 Rep 3. The upper line is the amoA gene while the lower line is the deletion competitor. Caption shows how many copies of the competitor were added to the PCR reaction. Bands of equal intensity show that the number of target and competitor genes are equivalent



10000 copies 10000 10000 5000 5000 5000 2000 2000 2000 1000 1000 1000

Positive control

VITA

Michael Kirchner was born on September 9, 1962 in Havre De Grace, Maryland. He was the youngest of five children of Dr. Francis G. Kirchner and Virginia A. Kirchner. He graduated from Aberdeen High School in June, 1980. Mike attended North Carolina State University in Raleigh, NC where he earned his B.S. degree in Forestry in 1984.

Shortly after graduating from NCSU, Mike married Caroline W. Borden on December 22, 1984. Together, they attended Columbia Graduate School of Bible and Missions in Columbia, SC. After earning a Certificate of Biblical Studies, the Kirchners moved to Atlanta where they served as campus staff members for InterVarsity Christian Fellowship at Georgia Tech and Oglethorpe University from 1985 - 1989. In January, 1987 they became the parents of twin daughters - Katherine Winslow and Elizabeth Virginia. In 1989, Mike returned to North Carolina State University, earning a M.S. degree in Soil Science in 1991. His research dealt with soil microbial properties in low-input sustainable agricultural systems.

The Kirchners moved to Clewiston, Florida in 1991 where Mike worked in the Research Department of The United States Sugar Corporation. In 1994, Mike took a research associate position in the Plant and Soil Science Department at The University of Tennessee. He worked with Dr. Michael Mullen on research related to the effects of animal wastes on soil and water quality. In October, 1998 the Kirchner family again expanded with the birth of Jennifer Caroline. Mike received his Ph.D. in May, 1999.

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