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# SOIL QUALITY CHARACTERIZATION AND REMEDIATION IN RELATION TO SOIL MANAGEMENT

A Thesis in

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by

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## ABSTRACT

Concerns have developed over the long-term sustainability and environmental consequences of intensifying agricultural production and its effect on soil quality. Three studies that assess management impacts on indicators of soil quality, the potential remediation of low soil quality, and the use of indicators to monitor soil quality are described in the six chapters of the thesis. The first study evaluated the effects of soil management from a long-term soil fertility and crop rotation experiment (HRE) and Pennsylvania on-farm practices on microbial community levels physiological profiles (CLPP) with gram-negative BIOLOG plates. Principal Component Analysis (PCA) and Cluster Analyses of microbial CLPP distinguished HRE treatments with manure and crop rotations more diverse than continuous corn from the other treatments and farms with routine organic amendments from no manure addition and/or wastewater irrigated farms. Although not without limitation, the CLPP can be used as an exploratory assessment of soil management. The second study evaluated soil remediation effects of compost addition on soil quality indicators and corn silage yields in three different landscape positions at a site irrigated with treated effluent from a municipal wastewater treatment plant and previously identified as having low soil quality. Soil quality indicators including soil enzyme activities, aggregate stability, and microbial biomass C increased with >90 Mg $ha^{-1}$ compost treatment in all landscape positions. CLPP with <45 Mg $ha^{-1}$ compost was different in the summit and backslope from the depression, and from >90 Mg $ha^{-1}$ treatments in all landscape positions. Corn silage yields increased with increasing compost rates in the summit position. Soil quality remediation and site-specific management can be possible using organic amendments where soil quality is impaired. The third study evaluated a multivariate integration of soil quality indicators using principal components analysis (PCA) as a soil quality indexing process of a long-term experiment (HRE) and Pennsylvania farms with different management histories. Soil quality indicators included enzyme activities, soil microbial biomass C, total soil C, total soil N, and aggregate stability. All measured aspects of soil quality were influenced by soil management that included compost or manure additions, and forages and/or small

grains in crop rotation. Through the indexing processes, unknown farms and the soil quality remediation results were assessed and successfully classified. The process of soil management classification and soil quality indexing could be a useful tool for farmers to evaluate farm management, and their farm management decisions.

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## "You should've known better"

Granddad, great-granddad, and his father too,
when working their farms knew just what to do.
They mucked and they ploughed and they harrowed and sowed,
they watched and they tended, and then reaped and mowed,
And when they had finished, and harvest was in,
they held a thanksgiving, and gave praise to Him.
But along came the Chemist, a smart lad was he,
who said, "All you farmers, now listen to me...
You're all too old-fashioned and well out of date;
I can double the crops you've been getting of late".
He went on to prove that by using his wares,
he could fatten the crops and thin out the tares

The mechanical man was soon on the scene,
with his tractor and baler, where horses had been.
And the land became sated with chemicals and smog;
where once were green pastures, there now became bog.
The topsoil was powdered and flushed with the rain,
And deserts sprang up where there once had been grain.
"What shall we do?" cried the farmers aghast,
"Our crops get the wilt, and the grass will not last."
Whilst Granddad in heaven looked down, far from jolly,
and waggled his beard at this grandson's great folly.
His anger increased as he studied in wonder,
and he finally raised in his wrath like the thunder.

"Get horses you clots, and animals many..
You can't make a bob if you don't spend a penny..
And stop burning grass like a blithering fool;
you're burning the litter that keeps the roots cool.
And what about straw that aerates the land,
it looks like that I must take you in hand?
Where's your rake lad? I see none around;
you can't grow good crops if you don't feed the ground.
There's nothing like muck for giving land heart,
and there's always a use for a horse and a cart.
Put your animal droppings into a heap,
and fatten it up with straws from your wheat.
Then get some compost and mix it all in ------
spread your land with it, and not too thin.
And happen it comes to next harvest time,
you'll not need so much of your chemicals and lime.

And as for that tractor --- a smell and a din;
it's packing the land and the rain can't get in.
And where're all the earthworms that wriggled around?
Poisoned no doubt in that chemical ground..
You've gone against nature - that's what you've done.
You should've known better -- or never begun.
So take off your jacket and roll up your sleeves,
and gather your straw and muck and your leaves.
Then get your rake and belt it around
and happen, in time, you may build up the ground".

By S.C.W.
(cited in SANET-MG@LISTS.IFAS.UFL.EDU)

## Chapter 1

# REVIEW OF LITERATURE AND RESEARCH OBJECTIVES

Intensification of agriculture is widely recognized as one of the most significant alterations to the global environment by humans. Concerns have developed, however, over the long-term sustainability and environmental consequences of intensifying agricultural production. One example is the decline of agricultural soil productivity because of intensive management and farming practices (Parr et al., 1990). Soil and water erosion, localized nutrient depletion or excess accumulation, and loss of organic matter are among the severe consequences resulting from unsustainable agricultural practices.

In agriculture, sustainability is defined as meeting current production goals without compromising the future. Sustainable measures are those that enhance the environment, natural resources, and related dimensions of society (Larson et al., 1991). Sustainable agriculture should involve the successful management of agriculture resources to satisfy changing human needs while maintaining or enhancing the natural resource base, and avoiding environmental degradation. According to Hatfield and Karlen (1993), sustainable agriculture encompasses, but is not limited to, farming systems known as biological, ecologically clean, low-input, organic, and alternative agriculture. These systems emphasize the sustainability of the soil resource that is, along with the other essential resources of water, air, and light, sustaining our food production. Thus, a major concern for sustainable societies should be the impact of soil management practices on the physical, chemical, and biological processes of soils that influence the sustainability of agriculture.

Furthermore, a broad goal for the present agricultural management of soil is to sustain and/or increase productivity for an increasing world population. To help achieve this goal, scientists make a significant contribution to sustainable land management by translating scientific knowledge and information on soil function into practical tools and

approaches by which land managers can assess the sustainability of their management practices (Bouma, 1997; Dumanski et al., 1992). The challenge, therefore, is to increase production while avoiding the most serious of the negative consequences. The development of agricultural systems that integrate features of traditional agricultural knowledge and new ecological knowledge into the intensification process can contribute to meeting this challenge.

Indeed, integrated nutrient-organic matter management approaches are receiving attention as pathways to sustainable high-production agriculture and reduction of off-site problems. Strategies that help synchronize nutrient release from organic matter and nutrient supply from inputs with plant demand require more information than conventional high-input management strategies. These improved strategies will require better integration of organic matter inputs (such as crop residues, manure, industrial wastes, etc.), organic matter stocks and turnover, and the biotic community that influences nutrient availability with industrial fertilizers to meet plant demands. The scientific basis of that integration, and the economic and social costs of all such practices that enhance soil quality, must be better understood and incorporated into the development of practices that can be widely implemented.

Important questions concerning the definition and remediation of soil quality still need to be addressed. Developing the knowledge to define a healthy or high quality soil and what are the acceptable ranges of soil quality resulting from agricultural practices will require intensive research. Additionally, the possibilities for crop yield improvement in low soil quality areas following soil quality remediation practices need to be investigated.

Three studies that assess management impacts on indicators of soil quality, and also the use of indicator outcomes as a means of monitoring soil conditions are described in the six chapters of this thesis. The first chapter reviews the current literature pertaining to soil quality, indicators of soil quality, and the effects of agricultural management on these indicators. It characterizes soil quality indicators and justifies the selection of specific indicators for the studies. Finally, specific research objectives are stated in this chapter. Chapter Two describes the experimental setup of the studies, the management

history of the long-term experiment, and Pennsylvania farms that were sampled in 1996 through 2000. Most of the research objectives deal with samples collected from these sites; therefore, references to site descriptions in this chapter will be made throughout the thesis. Chapter Three describes microbial community functional diversity profiling as an indicator of soil quality. These profiles are developed using a microtiter plate and the utilization of carbon substrates by bacterial communities. Chapter Four is a soil remediation study utilizing compost additions to a previously identified low soil quality area. Soil quality indicators were measured and evaluated to determine the extent of remediation success. Chapter Five reports the ability of a multivariate soil quality index of all indicators to differentiate crop and soil practices from Pennsylvania farms and a long-term crop rotation-fertility study (HRE). Finally, Chapter Six consists of overall conclusions from these studies.

## 1.1. Review of Literature

Soil quality

Soil quality is often considered a complex characteristic that cannot be readily defined because it depends on multi-dimensional factors such as land use, soil management practices, and ecosystem and environmental interactions. However, to manage and maintain our soils in an acceptable state for future generations, soil quality must be adequately defined. For instance, Doran and Parkin (1994) defined soil quality as the capacity of the soil to function within ecosystem boundaries, to sustain biological productivity, to maintain environmental quality, and to promote plant and animal health. Larson and Pierce (1991) also defined soil quality as the physical, biological and chemical properties that: 1) provide a medium for plant growth; 2) regulate and partition water flow in the environment; and 3) serve as an environmental buffer in the formation, attenuation, and degradation of environmentally hazardous compounds. Johnson et al. (1997) proposed that: "soil quality is a measure of the condition of the soil relative to the requirements of one or more societies and/or to any human needs or purposes".

Portraying the soil as a living, dynamic entity that functions holistically rather than as an inanimate entity with values defined only by its chemical and physical characteristics and intended use is a major goal of soil quality advocates. Adoption of soil remediation measures can improve soil quality and transform an unsustainable agricultural or land use system into a sustainable system. Furthermore, soil quality assessments could provide environmental impact information and help identify farming practices that will maintain or improve soil resources. If successful, soil quality assessments may help shift public perception regarding the compliance of agriculture with environmental expectations from an emphasis on regulation toward voluntary, results-oriented approaches.

Management effects and indicators

Agricultural practices such as tillage, crop rotation, and inorganic or organic inputs significantly affect the physical, chemical, and biochemical properties of soil (Dick, 1992). These practices can lead to undesirable consequences such as soil erosion and soil organic matter depletion if sustainable management is not implemented. An essential part of sustainable cropping systems is the promotion of a soil ecosystem that can provide a reliable and well-timed nutrient supply to crop plants while maintaining soil organic matter levels that support good soil structure for plant growth and soil conservation. Thus, preserving these soil functions is a proactive measure to avoid concerns about reduced soil productivity and environmental degradation.

Research about the impact of specific farming practices and farming systems on soil properties and soil quality has shown that there are two common factors that determine soil quality in soil-conserving farming systems: the presence of sufficient plant residues and nutrients to maintain the soil organic matter level; and sufficient protection of the soil by crop stubble or permanent plant cover to prevent soil erosion (Wood and Edwards, 1992; Campbell and Zentner, 1993) and create a stable environment for

biological activity (Doran, 1980). Many indicators of soil quality are related to the biological processes that influence nutrient availability, the abundance of trace organics that have enzymatic functions, and the potential impact of diseases and pests on stressed crops.

Conservation of organic matter is essential for the continued productivity of soils and the maintenance of soil quality. Nevertheless, recommendations for soil organic matter (SOM) management are difficult because accepted measures of SOM that reflect soil quality do not exist. Management practices can affect active soil organic matter characteristics, nutrient supply, and soil carbon retention characteristics before total organic matter contents change (Woods and Schuman, 1996; Wander et al., 1994).

Organic management practices modify many aspects of the soil environment, particularly those properties related to C and N cycling (Doran et al., 1987, Wander, 1994; Reganold et al., 1993). Specifically, given similar tillage intensities, the addition of organic residues should lead to greater microbial biomass and organic matter levels in soils of farms managed with organic inputs compared to soils farmed with the addition of only mineral fertilizers (Power et al., 1984). A better understanding of all facets of soil quality is needed in order to develop indicators of trends or changes due to different management systems. An indicator is defined as a pointing or directing device that suggests a given condition (Kennedy and Papendick, 1995). Selected indicators are used to determine the status of a resource and the trend of the resource relative to a specific goal.

Furthermore, the evaluation of long-term soil management experiments provides a basis for evaluating the sustainability of agriculture that cannot be accomplished with the results of typical short-term experiments (Jordan et al., 1995). Physical, chemical, and microbial methodologies might be among the various parameters available to evaluate past and present practices for agricultural ecosystems. Hence, an assessment of soil quality that includes indicators of soil biological, chemical, and physical properties could provide valuable information for determining the sustainability of different soil management practices. However, before practices are evaluated or adopted, indicators

must be identified in order to monitor the consequences of management alternatives as they are implemented.

The use of plants, animals, and microorganisms as indicators of the impact of agricultural practices on soil quality has increased in comparison to other simple physical or chemical techniques used to measure soil conditions. Since changes in the activity of soil organisms may be indicative of changes in soil quality, indicators of activity could be used to describe the status and trends in soil conditions due to management practices. Several authors have also suggested that plant response may provide a more efficient approach for assessing soil quality with respect to crop production. For example, Jensen and Cavalieri (1983) reported that crop growth and development characteristics, especially for a crop such as corn *(Zea mays L.),* were very sensitive to changes in soil resources.

Several others have studied relationships between grain yield and availability of soil nutrients during the growth stages (Denmead and Shaw, 1970). Some of these studies observed that crop yields and nutrient uptake were higher with the addition of organic amendments (manure or compost) than with no organic inputs (NeSmith and Ritchie, 1992; Abrecht and Carberry, 1993). Furthermore, Eghball and Power (1999) found that crop yields from organic input farms were similar to crop yields from farms with fertilizer applications. However, even though grain yield has economic meaning and is often used by farmers as an indicator to establish inherent quality of soils on farms, it is inappropriate as a single indicator of soil quality due to differences in hybrid sensitivity and temporal variation in crop demands (Maddonni et al., 1999).

An example of a microbiological indicator is the activity of the soil microbial communities and the natural processes of respiration, mineralization, and denitrification that transform nutrients in soils and underpin fundamental soil properties such as soil structure (Lee and Pankhurst, 1992). For example, Molope (1987) suggested that the stability of soil aggregates is due to the combined mechanical and biological action of microorganisms and their decomposition products. The determination of enzyme activities, functional activity of microorganisms, and soil organic matter are among other useful indicators (Yakovchenko, 1996; Park, 1995). Bucher (1999) also suggested that

soil enzymes and microbial biomass C were indeed useful tools as soil indicators to assess trends of soil quality.

A major challenge for current and future soil quality research is to identify how individual soil quality indicators should be translated into multifactor soil quality ratings or scores and how they should be used to assess components of soil quality, with respect to the critical functions. Unfortunately, there are no well-defined standards to determine the appropriate ranges for the various indicators. Further investigation is needed to define the ranges of values for a given indicator in a given situation. Despite this, our capacity to understand management impacts on soil functions should not be limited by complexities or by linking cause with effect for individual indicators. Overall trends in soil quality indicators, considered to reflect enhanced or degraded soil functions, could be detected based on our knowledge of basic processes and evaluations of production practices (Leibig et al., 1999).

It is also important to establish in our research that profitability is one of the most important factors governing the adoption of soil conserving practices. If the costs of conservation practices exceed the short-term and possibly, the long-term benefits, farmers will have no incentive to adopt them. However, economically profitable technologies with complementary environment-enhancing characteristics are readily adopted (Camboni and Napier, 1994; Cary, 1994). Thus, successful soil conservation programs must include an analysis of the profitability and the land, labor and capital resource requirements of the proposed conservation system. It is important to consider the possibility of effective soil quality management within a flexible farmer-first approach that includes other management and decision–making criteria that are important to farm survival (Boehm and Burton, 1997). An example of this approach is the adoption of a participatory assistance (Lanyon, 1994). This process emphasizes alternative approaches for technology transfer and farm changes. It focuses on the improvement of the farmer and the farm rather than external interests on specific practices, products or policies (Lanyon, 1994).

## 1.2. Soil Quality Characterization

### 1.2.1. Indicators of Soil Quality

Microbial biomass

Microorganisms make up only 1 to 8 % of the soil organic mass, but they have a great influence on crop production by dominating decomposition through biotransformations (Roder et al., 1988). Soil microorganisms influence the flow of C, N, P, and S, through terrestrial ecosystems by their role in the processes of decomposition, immobilization, and mineralization, Microbes also play a major role in the formation of good soil structure by binding the soil particles together. Hence, microbes help to aggregate the soil to reduce erosion, promote good water infiltration, and maintain adequate aeration of the soil (Carter, 1986; Carter and Kunelius, 1986; Carter and McLeod, 1987).

The usefulness of microbial biomass as a biological indicator of soil quality has already been recognized. For example, Jenkinson and Ladd (1981), Brookes (1995), and Jordan et al. (1995) agree on the use of microbial biomass C as indicator of soil quality owing to its high sensitivity to changes in land use and management practices. Ladd (1994) added that microbial biomass C is a good overall measure of the state of the edaphic environment and that its inclusion in a soil quality index should lead to a reduction in the number of properties that need to be considered. However, the author did not specify a range of acceptable values for soil biomass C measurements.

Soil and crop management practices can greatly influence soil biological activity through their effects on the quantity and quality of organic matter added to the soil and the initial distribution of such material in the soil. For example, those soil systems with the highest organic matter input also tend to have the greatest microbial biomass and activity (Sparling, 1985). The microbial biomass component of soil organic matter has the potential to be a sensitive indicator of organic matter dynamics because the microbial fraction changes comparatively rapidly and differences are detectable before they can be measured in total organic matter (Powlson and Jenkinson, 1981; Powlson et al., 1987).

A good example of microbial biomass differences associated with soil management is a study involving two field experiments in Denmark in which spring barley straw had been burned or incorporated in the soil for 18 years (Powlson et al., 1987). Straw incorporation increased total soil organic C by only 5%, and total soil N by about 10%. However, microbial biomass C increased by about 45%, an easily measurable change. Management of soils that leaves residue on the soil surface often results in higher concentrations of soluble organic carbon compounds (Alvarez et al., 1998), which may result in the enhancement of microbial properties.

Fauci and Dick (1994) also measured microbial biomass C in a long-term crop rotation experiment that since 1931 consisted of a wheat-fallow rotation with manure additions. They observed a significantly higher ($p<0.05$) microbial biomass C in the manured plots (536 µg C $g^{-1}$ soil) than in the control plots (145 µg C $g^{-1}$ soil). Campbell et al. (1991) also observed significantly higher ($p<0.05$) microbial biomass C in the rotations with legumes (1074 kg $ha^{-1}$) than in continuous crops (938 kg $ha^{-1}$). The rotation of one-year fallow, two-years of wheat (*Triticum aestivum L.*), and three-years of bromegrass hay (*Bromus inermis Leyss.),* contained 38% more microbial biomass C than did the two-year rotation fallow-wheat after two years.

According to Bucher (1999), soil microbial biomass C (SMBC) increased with manure addition as compared to industrial fertilizer addition on Pennsylvania farms that had different soil management histories. In addition, the SMBC for continuous corn was significantly greater with manure additions (325 µg C $g^{-1}$ soil) than with industrial fertilizer (156 µg C $g^{-1}$ soil) in a long-term rotation experiment with different crop sequence and nutrient source treatments. Hasebe et al. (1985) and Ritz et al. (1997) also observed greater microbial biomass C in soil treated with organic manure than with inorganic fertilizers or no-fertilizer treatment. Finally, Bucher (1999) measured greater SMBC as small grain and forage crops were added to rotations as compared to continuous corn with either fertilizer or manure treatments. According to Mausbach and Seybold (1998), the range of microbial biomass C is 75-700 µg C $g^{-1}$ soil. This range is based on literature findings and is not soil specific nor for a specific land use.

Microbial biomass measurements, combined with total organic C and soil respiration ($CO_2$), can also provide estimates of soil development or degradation (Insam and Domsch, 1988; Insam et al., 1989). Microbial biomass measurements have been used to detect changes brought about by differential management of sorghum residues (Saffigna et al., 1989), by reduced tillage (Powlson and Jenkinson, 1981; Doran, 1987), and by organic farming (Doran et al., 1987).

Enzyme Activities

Over the past 20 to 30 years, considerable progress has been made in developing methods for measuring the activity of well over 50 enzymes found in soil. Because many are substrate-specific and can be chosen from different functional groupings, there is an opportunity to determine the potential of a soil to carry out a wide range of reactions that may be critical for the functioning of an ecosystem.

Some limitations of soil enzyme assays are due to wide seasonal or year-to-year fluctuations that could mask changes in activity due to soil management. It is also important to select the appropriate soil enzyme for assessing soil quality for a given situation and to know the limitations or confounding factors that might affect interpretations of the results. Particular assays are usually selected based on previous experience with their sensitivity to field management, importance in nutrient cycling and organic matter decomposition, and simplicity of the assay (i.e., potential to be adopted by commercial labs for routine soil testing).

A major advantage of enzyme assays over most other soil biological measurements is that many assays are relatively straightforward and do not require sophisticated instrumentation, or calculation assumptions as for the extraction efficiency coefficient in soil microbial biomass C estimates. Another advantage is that for many enzymes it is possible to run the assay on air-dried samples while retaining the potential to discriminate among soil management effects (Dick, 1994; Bandick, 1999). Air drying greatly facilitates soil sample processing and the use of this pretreatment would

encourage the adoption of soil enzyme activity measurements as part of a soil quality index.

Enzymes are important soil components involved in the dynamics of soil nutrient transformations. Enzyme activity in the soil environment is considered to be a major contributor of overall soil microbial activity (Frankenberg and Dick, 1983) and, more recently, to soil quality (Visser and Parkinson, 1992; Dick, 1994). For the mineralization of an organic substrate to occur, both the synthesis and the activity of a specific enzyme complex are needed. These latter processes may be linked to the presence of countless factors directly implicated in the mechanism of enzyme synthesis and secretion (Martens et al., 1992).

In the soil, part of the microbial population participates in the mineralization of organic P, ester sulfates, and glycosil compounds through the action of phosphatase, arylsulfatase, and ß-glucosidase enzymes, respectively. Specifically, acid phosphatase catalyze the hydrolysis of both esters and anhydrides of $H_3PO_4$, arylsulfatase is important in nutrient cycling because it releases plant available $SO_4$. ß-glucosidase has a role in releasing low molecular weight sugars that are important as energy sources for microorganisms. Enzyme activities hold the potential to indicate the status of microbial activity and the dependent soil biochemical processes.

Among other possibilities, acid phosphatase activity has been proposed as a satisfactory index of microbial activity since it is considered to be derived entirely from the microbial population in soils (Frankenberg and Dick, 1983). Studies on soils exposed to tropical or temperate climates under different cultivation systems showed that phosphatase activity is correlated with the amount of organic P and of organic matter content (Appiah and Thomas, 1982). Management practices such as tillage, rotations and inputs significantly affect the levels of acid phosphatase activity in soils (Doran, 1980; Dick, 1984; Dick, 1994).

Activities of enzymes such as phosphatase (Angers et al., 1993) and arylsulfatase (Dick, 1984) have been used in attempts to describe soil quality. Pankhurst et al. (1995) found greater phosphatase activity (measured using P-nitrophenol (PNP) substrate) in the upper layers of no-till soils and fields with rotations (405 µg PNP $g^{-1}$ soil) when

compared with conventional tillage and continuous cropping practices (239 µg PNP $g^{-1}$ soil). The increased activity values in no-till and crop rotations could indicate enhanced biological activity near the soil surface. Klein and Koths (1980) suggested that increased enzyme activities may promote higher residual nutrient availability and increased fertilizer-use efficiency in soils. However, additional findings suggest that some enzyme activities increase with continuous cropping (Dick, 1984) or do not vary with different management practices such as crop rotation, tillage effects, stubble retention, and fertilizer input (Pankhurst et al., 1995).

Bandick et al. (1999) observed that enzyme activities were greater with organic treatments than those treatments that did not receive organic amendments. In addition, Bucher (1999) found that biological indicators such as enzyme activities (phosphatase, arylsulfatase and ß-glucosidase) increased 40 to 60% in soils with histories of organic inputs (manure additions and crop residues) and crop rotations compared to continuous crops and/or no organic inputs. Specifically, soil enzyme activities for continuous corn were greater with manure (404 vs. 241, 332 vs. 200, and 342 vs. 205 µg PNG $g^{-1}$ soil) than with industrial fertilizer additions (Bucher, 1999). The range of arylsulfatase and phosphatase enzyme concentrations in soils that is considered to be of high quality is between 400-800 µg substrate $g^{-1}$ soil (Jordan et al., 1995; and Miller and Dick, 1995).

At present, assessing specific enzyme activity (e.g., phosphatases, β-glucosidase, etc.) together with the use of some general soil parameters such as aggregate stability, total C and N, etc., seems to be the best approach for evaluating the state of soil microbial activity and for understanding its response to organic amendments, cultivation practices, and environmental factors (Nannipieri et al., 1990). Furthermore, the activity of phosphatase is considered to be an especially useful indicator of both the positive and negative effects of soil management practices on soil quality (Dick, 1994).

Microbial community-level physiological profiles

Measurements of soil microbial biomass give an indication of the standing crop of microbial life in the soil, but provide no indication of community structure. A rapid method for studying microbial communities based on the direct inoculation of mixed microbial samples has been increasingly used with the aid of Biolog microtiter plates (Garland and Mills, 1991; Garland, 1997). The approach uses the patterns of C source utilization generated from respiration of the different sole carbon sources adapted to gram negative bacteria and pigment development in a redox sensitive tretazolium dye within 95 separate wells.

Aggregate Stability

Aggregate stability is an important measure of soil quality for crop establishment, water infiltration, and resistance to erosion and compaction (Beare and Bruce, 1993). An aggregate is a group of primary soil particles that cohere to each other more strongly than to other surrounding soil particles. Size, quantity and stability of aggregates recovered from soils reflect the environmental conditions that enhance the aggregation of soil particles (e.g. wet-dry cycles, organic matter amendments) or cause their disruption (e.g. soil cultivation, biodisturbance). The composition, strength and persistence of the various binding agents responsible for the formation and stabilization of soil aggregates have been discussed in many research and review articles (Harris et al., 1966; Edwards and Bremner, 1967; Tisdall and Oades, 1982; Lynch and Bragg, 1985).

Substantial and rapid changes in the quantity of water-stable macroaggregates (>250 μm) in soils results from changing cropping systems between cropping seasons (Beare and Bruce, 1993). The water-stability of microaggregates (<250 μm) depends on the persistent organic binding agents and appears to be a characteristic of the soil, independent of management (Tisdall and Oades, 1982). The measurement of stable soil aggregates depends on both the forces that bind particles together and the nature and magnitude of the disruptive forces applied. Further, just as the environmental history of a

soil influences the size distribution and stability of aggregates, so can the conditions imposed during soil sampling, preparation and analysis (Arrigo et al., 1993).

Crop rotations, no-till, and other soil management practices such as residue-left (increased organic matter) contribute to a greater aggregate stability (Beare et al., 1994, Arrigo et al., 1993). Accordingly, soil aggregate stability seemed to be the most critical soil property to distinguish soil quality changes in studies of the Argentina Rolling Pampas by Maddonni et al. (1999). In addition, Vazquez et al. (1991) reported that soil aggregate stability was more sensitive to the intensity of land use than to the soil content of labile C and N. Soil aggregation also correlates with soil erodability (Wischmeier and Mannering, 1969). Ranges of aggregate stability measurements for scoring soil quality are 30-100% of stable aggregates (Mausbach and Seybold, 1998)

Total Carbon

There are many agronomically valuable assets of soil associated with total soil C (TSC) content. However, because a direct correlation between TSC and yield or nutrient status is not always evident, TSC content alone may not be an adequate indicator of soil quality (Yakovchenko et al., 1996).

According to results from a long-term experiment in Rothamsted (Johnston, 1994), the TSC in agricultural soils has been constant (approximately 15 g C $kg^{-1}$ soil) for about 100 years on both unmanured plots and those given NPK fertilizers. The amount of C was a little larger in the fertilized soil, because larger crops have been grown and, although straw was removed each year, there have been larger residues from stubble, leaves and roots returned to the soil. Annual additions of fresh farmyard manure had increased soil organic C (up to 19 g C $kg^{-1}$ soil), rapidly at first and then more slowly as a steady state was approached. It is important to note however that the time-span over which this change has occurred is more than 130 years for this medium-texture soil in a temperate climate. Hence, as Johnston (1994) states, animal manure, especially farmyard manure, has the potential of providing a significant increase in soil organic C, except on coarse soils such as sandy loams, where the increase is very small. The contributions of

different arable crops in terms of TSC increase appear to be small compared to effects induced by crop-residue disposal and manure addition.

### 1.3. Objectives

The objectives of the research are to evaluate:

- The cumulative effects of soil management from a long-term experiment and on-farm practices on microbial community functional diversity profiles.

- The impact of soil remediation with compost additions on soil quality indicators and corn silage yields in three different landscape positions of a wastewater irrigated field with below average indicators of soil quality.

- A multivariate integration of biological, chemical, and physical soil quality indicators with principal components analysis (PCA) and its potential use in a soil quality indexing process. The components of this index include the activities of the enzymes acid phosphatase, arylsulfatase and ß-glucosidase, soil microbial biomass C, the microbial community functional diversity, total soil C and total soil N, and aggregate stability, to represent the impact of soil management on soil quality from a long-term experiment and Pennsylvania farms with a range in soil and crop management histories.

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# Chapter 2

# SITE DESCRIPTION AND CROP MANAGEMENT INFORMATION

## 2.1. Site Description

### 2.1.1. Long-term Hunter Rotation Experiment

The Hunter Rotation Experiment (HRE) is located at The Pennsylvania State University R. E. Larson Agricultural Research Center at Rock Springs, PA. This long-term crop rotations x fertility treatment experiment was initiated in 1969. The soil at Rock Springs is classified as Hagerstown (fine, mixed, mesic, Typic Hapludalfs). This series consists of well-drained limestone residual soils of high agricultural productivity (Braker, 1981). The current crop rotations include continuous corn (*Zea mays L.*), corn/soybean (*Glycine max L.*), corn/forage, and corn/small grain/forage. The fertility treatments have changed over the period of the experiment, but now include standard fertilizer recommendations according to soil tests using inorganic fertilizer and combinations of dairy manure applications to meet crop sequence nutrient requirements with inorganic fertilizer supplementation, if needed.

The current experimental plan for the HRE consists of a factorial combination of four crop rotations and three liming and fertilizer treatments (LF1, LF2, LF3). The crop rotations are: Rot1) continuous corn; Rot2) 2-year rotation of corn and soybeans; Rot3) 8-year rotation of 4 years corn and 4 years of alfalfa hay *(Medicago sativa L.*); and Rot5) 5-year rotation of corn-oats *(Avena sativa L.*)-wheat (*Tritucum aestivum L.*)-2 years red clover hay (*Trifolium pratense L.*). Rotations 1 and 2 were established in 1969 and grown every year thereafter. Rotations 3 and 5 were created in 1990, after Rot4 was combined with Rot3.

The current lime-fertilizer treatments are: LF1) lime to maintain soil pH to plow depth at around 7, and to fertilize all crops with N-P-K fertilizer to reach near-maximum production of each crop; LF2) use liquid dairy manure, since 1990**,** in crop rotations 1 and 2 based on P requirements and on N requirements for rotations 3 and 5; and LF3) use liquid manure as primary fertility source, since 1982, based on P requirements of each crop in the cropping system.

Manure application rates in the LF2 and LF3 differ according to each rotation. Manure samples (Table 2.1) were collected at the time of application and analyzed at The Pennsylvania State University Agricultural Analytical Services Laboratory (Doty et al., 1982).

*Table 2.1:* Analysis report of average manure application of Hunter Rotation Experiment from 1992 through 1998.

| N | P | K | Solids |
|---|---|---|---|
| | g $kg^{-1}$ dry wt | | |
| 47.4 | 8.8 | 30.8 | 82.3 |

All crops in each rotation are grown each year. All factorial treatment combinations are replicated four times on 192 plots that are 5.76 m wide and 12.8m long, arranged in a split-split plot experimental design with Year as main plot, LF as sub-plot and rotations as the sub-subplots.

*Table 2.2*: Manure application frequency and rates for the lime-fertility treatments with organic amendments in the Hunter Rotation Experiment.

| LF | Rotation* | Frequency of manure | Average Annual Rate | Total manure received up to 2000 |
|---|---|---|---|---|
| | | *y/y* | *(Mg ha$^{-1}$ dry wt)* | *(Mg ha$^{-1}$ dry wt)* |
| 1 | 1 | 0/10 | 0 | 0 |
| (beginning 1969) | 2 | 0/10 | 0 | 0 |
| | 3 | 0/10 | 0 | 0 |
| | 5 | 0/10 | 0 | 0 |
| 2 | 1 | 10/10 | 8 | 80 |
| (beginning 1990) | 2 | 5/10 | 8 | 40 |
| | 3 | 6/10 | 5.6 | 34 |
| | 5 | 3/10 | 3.2 | 9.6 |
| 3 | 1 | 10/10 | 4.5 | 81 |
| (beginning 1982) | 2 | 5/10 | 6 | 54 |
| | 3 | 6/10 | 7 | 75 |
| | 5 | 3/10 | 3.2 | 17 |

* Rot1 CC
Rot2 CS
Rot3 CCCCAAAA
Rot5 COWRR

Average crop yields for first-year corn grain yields from 1990-2000 (Lanyon, unpublished) are summarized in Table 2.3. In all LF treatments, crop rotation had a positive increase in corn yields. However, in LF2 corn yields of Rot2 were not significantly different from Rot5. Corn yields of Rot5 were not significantly different from all LF treatments.

*Table 2.3*: First-year or continuous corn grain yields from 20 years in the Hunter Rotation Experiment.

| | Crop Rotations | | | |
|---|---|---|---|---|
| LF | 1 | 2 | 3 | 5 |
| | *Mg ha$^{-1}$ dry matter* | | | |
| 1 | 8.6 Bc* | 9.4 Bb | 10.4 Aa | 10.1 Aa |
| 2 | 9.0 Cb | 10.0 Ba | 10.6 A a | 10.3 Ba |
| 3 | 9.3 Ca | 9.9 Ca | 10.8 Ab | 10.4 B a |

*Values within a row sharing the same capital letter or within a column sharing a small letter are not significantly different with pairwise predicted differences at $p<0.05$.

### 2.1.2. Pennsylvania Farms

On-farm test sites were selected from Pennsylvania farms that have a variety of soil and crop management histories (Table 2.4). Crop, tillage, and input histories covering the last three years for each field were collected from the farmers.

Table 2.4. Selected management and soil variables of farm experimental sites.

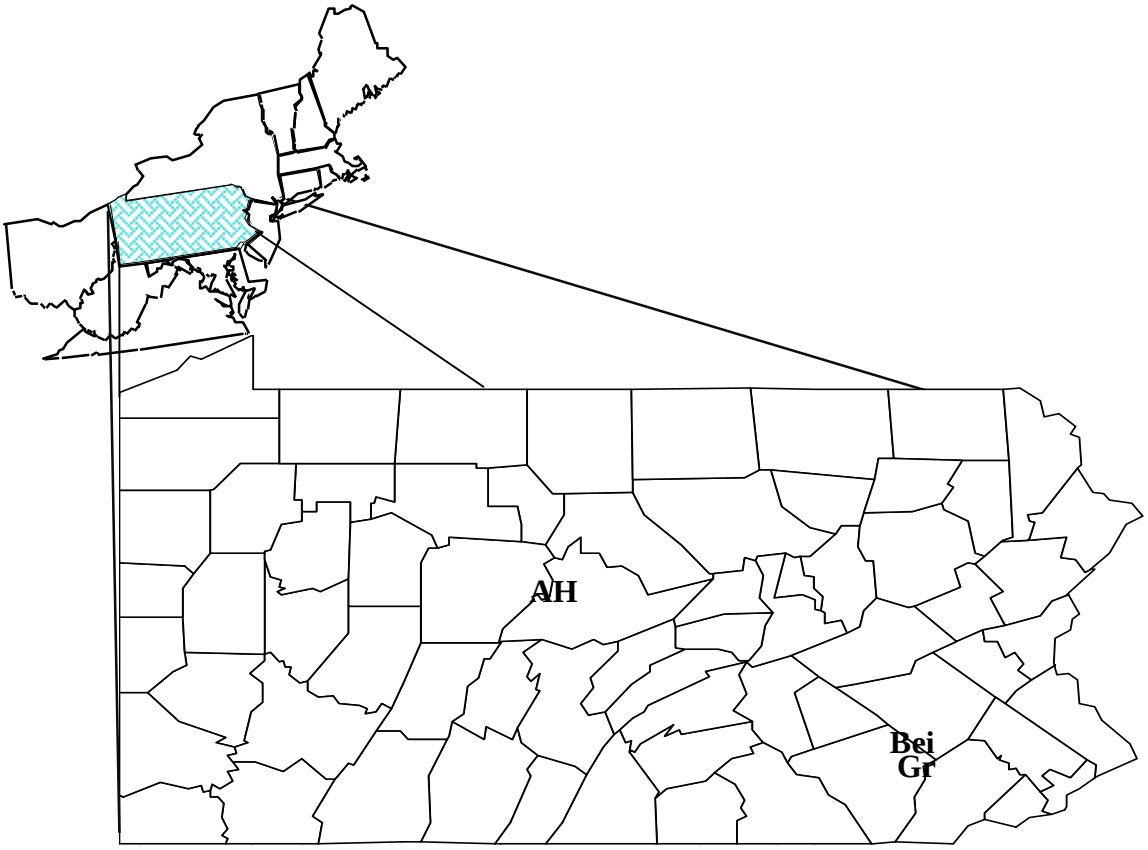
| *Year* | *Farms* | *Tillage* | *Industrial Fertilizer* | *Manure History* | *Management* |
|---|---|---|---|---|---|
| **1996** | 4, 2 | conv. till | no | Steer manure compost | Organic |
| | AH | conservation/ no till | yes | no | Cash crops, no manure |
| | WW | No-till | yes | no | Wastewater Irrigation |
| **1997** | Bro | conservation/ no till | yes | Dairy slurry | Livestock (dairy) |
| | PKE | conservation/ notill | yes | Dairy slurry (beginning 1994) | Livestock (dairy) /cash crop, no manure prior to 1994 |
| | Bai | conv. till | yes | no | Cash crop, no manure |
| | Gr | No-till | yes | Steer and chicken manure | Cash crop with manure |
| | Bei | conv. till | yes | Chicken manure | Cash crop with manure |
| | WW | no-till | yes | no | Wastewater Irrigation |
| **1998** | MY | No-till | yes | Irrigated dairy manure | Livestock (dairy) |
| | W | conservation till | yes | yes | Cash crop with manure |
| | E | conv. till | yes | no | Cash crop, no manure |
| | L | conv. till | yes | no | Cash crop, no manure |
| | WW | No-till | yes | no | Wastewater Irrigation |

Table 2.4. Selected management and soil variables of farm experimental sites (cont.).

| *Year* | *Farms* | *Tillage* | *Ind. Fertilizer* | *Manure History* | *Management* |
|---|---|---|---|---|---|
| **1999** | H1 | No till | yes | no | Livestock |
| | H2 | No till | yes | yes | Livestock |
| | EBM | Conservation tillage | yes | Yes Poultry manure | Cash crops, manure |
| | EBN* | Conservation tillage | yes | no | Cash crop, manure***** |
| | Fur1 | Conventional till/no till | yes | no | Cash crop |
| | Fur2 | Conventional till | yes | Yes Hog manure | Cash crop manure |
| | DB | No till | yes | no | Cash crop |
| | WW | No till | yes | no | Wastewater Irrigation |
| **2000** | HS | Conventional till | no | Yes Dairy manure | Livestock |
| | JP | Conventional till | yes | Yes Dairy manure | Livestock |
| | RF | Conventional till | yes | Yes Dairy manure | Livestock |
| | MW | Conventional till | No | Yes Hog manure | Hog Farm |
| | RM | Conventional till | Yes | no | Cash crop |
| | RB | No till | Yes | no | Cash crop |
| | RH | Conventional till | Yes | no | Cash crop |
| | CC | Conservation till | Yes | no | Cash crop |
| | WW | No till | yes | no | Wastewater Irrigation |

*EBN is considered to be cash crop-manure due to its inadequate management history and possible sampling miscommunication.

2.1.2.2. Pennsylvania farms locations




F*igure 2.1: Sampling locations of* Pennsylvania *farms.* ***Organic farms:*** *4, 2.* ***Livestock farms****: Bro, S, MY, H, RF, HS, MW, JP.* ***Cash Crop, manure****: Gr, Bei, EBN, PKE, Fur, EBM.* ***Cash Crop, no manure****: L, AH, BA, W, E, DB, RB, RM, RH, CC.* ***Wastewater Irrigation farm:*** *WW.*

## 2.2. Soil sampling procedures

Soil samples of the HRE and Pennsylvania farms were gathered in June of 1996 through 2000 for enzyme determination, soil microbial biomass C, aggregate stability, total soil carbon (TSC), and total soil nitrogen (TSN) (Bucher, 1999). Analysis of carbon-substrate utilization profiles in the long-term experiment and Pennsylvania farms include four years (1997, 1998, 1999, and 2000). All of the other soil quality indicators were analyzed over all five years.

Soil samples were collected by compositing 20-25 cores (2 x 15 cm) per unit from each first year corn or continuous corn plot. This was done in order to sample the same crop on every rotation every year. Farm soil samples were collected similarly from first year corn fields when possible, from two or three fields per farm with three to four composite samples per field.

All samples were split and one portion air-dried and ground to < 2 mm for chemical (TSC, TSN, and standard fertility soil test) analysis and biochemical analyses (enzyme activities). Aggregate stability was determined with field moist soils. The remainder of the soil samples was stored at 4 °C for a maximum of 4 weeks for biochemical (community-level physiological profiles and soil microbial biomass C) analysis.

### 2.2.1. Standard Fertility Soil Test

The soil quality samples (air dried and ground to <2 mm) of each year of HRE and the farms were subsampled and submitted to The Pennsylvania State University Agricultural Analytical Services Laboratory for pH, P, K (Table 2.4 and 2.5, respectively). The pH measurements were determined using the 1:1 soil:water method (Eckert and Thomas, 1991), and available P, K, and other cations were measured using the Mehlich-3 extraction procedure (Wolf and Beegle, 1991).

*Table 2.5:* Mean soil test results of the Hunter Rotation Experiment for five sampling years (1996 through 2000).

| LF | Rotations | pH | Mehlich3-P | Mehlich3-K | TSC | TSN |
|---|---|---|---|---|---|---|
| | | | *mg kg$^{-1}$* | | *g kg$^{-1}$ soil* | |
| 1 | 1 | 5.8 | 40 | 98 | 13 | 1.4 |
| | 2 | 6.6 | 41 | 98 | 12 | 1.3 |
| | 3 | 6.2 | 48 | 117 | 14 | 1.2 |
| | 5 | 6.3 | 42 | 102 | 14 | 1.5 |
| 2 | 1 | 6.9 | 67 | 324 | 16 | 1.6 |
| | 2 | 7.0 | 46 | 227 | 15 | 1.3 |
| | 3 | 6.7 | 42 | 156 | 15 | 2.0 |
| | 5 | 6.5 | 37 | 129 | 16 | 1.5 |
| 3 | 1 | 6.5 | 75 | 324 | 16 | 1.6 |
| | 2 | 6.8 | 53 | 223 | 15 | 1.4 |
| | 3 | 6.5 | 57 | 234 | 15 | 1.1 |
| | 5 | 6.6 | 48 | 156 | 16 | 1.5 |

*Table 2.6:* Soil test results from five sampling years (1996 through 2000) for selected fields of Pennsylvania farms.

| Year | Farm | Fields | n* | pH | Mehlich3-P | Mehlich3-K | TSC | TSN |
|---|---|---|---|---|---|---|---|---|
| | | | | | *mg kg$^{-1}$* | | *g kg$^{-1}$ soil* | |
| **1996** | 2 | J, P | 8 | 6.5 | 78 | 70 | 23 | 2.1 |
| | 4 | Q, T, S | 12 | 6.7 | 76 | 156 | 21 | 2.0 |
| | AH | AH | 12 | 6.3 | 38 | 90 | 16 | 1.4 |
| | WW | 15B1 | 4 | 6.8 | 150 | 78 | 14 | 1.2 |
| | WW | 32K, G | 8 | 7.3 | 180 | 74 | 13 | 1.2 |

*Table 2.6:* Soil test results from five sampling years (1996 through 2000) for selected fields of Pennsylvania farms (cont.).

| Year | Farms | Fields | n* | pH | Mehlich3-P | Mehlich3-K | TSC | TSN |
|---|---|---|---|---|---|---|---|---|
| | | | | | *mg kg$^{-1}$* | | *g kg$^{-1}$ soil* | |
| **1997** | Bro | B1, S2, S7 | 12 | 6.4 | 98 | 117 | 21 | 2.3 |
| | PKE | PKE | 4 | 6.7 | 67 | 152 | 15 | 1.4 |
| | Gr | 1, 2 | 8 | 6.7 | 75 | 129 | 15 | 1.9 |
| | Bei | 1, 2 | 8 | 6.8 | 68 | 74 | 15 | 1.7 |
| | Bai | 2, 3, 4, 5 | 16 | 5.9 | 44 | 153 | 14 | 1.4 |
| | WW | 32H3, C4 | 8 | 7.0 | 181 | 98 | 13 | 1.2 |
| | WW | 15B1, C1 | 8 | 7.3 | 149 | 101 | 13 | 1.1 |
| **1998** | MY | H, D | 8 | 7.0 | 303 | 46 | 29 | 2.3 |
| | W | 1, 2 | 8 | 6.5 | 74 | 191 | 22 | 1.6 |
| | E | H, R, D | 12 | 6.8 | 56 | 66 | 16 | 1.6 |
| | L | 1, 2 | 3 | 6.4 | 74 | 63 | 15 | 1.8 |
| | WW | 32A, K | 8 | 7.3 | 141 | 98 | 12 | 1.2 |
| | WW | 15BC, D | 8 | 7.2 | 175 | 113 | 13 | 1.2 |
| **1999** | H1 | 1, 2 | 8 | 7.0 | 93 | 335 | 24 | 2.3 |
| | EB | M, N | 8 | 6.5 | 429 | 340 | 23 | 3.1 |
| | Furn | 1, 2 | 8 | 6.8 | 196 | 151 | 12 | 1.2 |
| | DB | 1, 2, 3 | 12 | 6.5 | 58 | 205 | 16 | 1.5 |
| | WW | 32C1, G13 | 8 | 7.1 | 146 | 173 | 14 | 1.5 |
| | WW | 15B, C | 8 | 7.0 | 259 | 189 | 14 | 1.3 |

*n: number of samples per farm.

*Table 2.6:* Soil test results of five sampling years (1996 through 2000) for selected fields of Pennsylvania farms *(cont.)*

| Years | Farms | Fields | n* | pH | Mehlich 3-P | Mehlich 3-K | TSC | TSN |
|---|---|---|---|---|---|---|---|---|
| | | | | | *mg kg$^{-1}$* | | *g kg$^{-1}$ soil* | |
| **2000** | HS | 1, 2 | 8 | 6.7 | 117 | 454 | 20 | 1.7 |
| | JP | 1, 2, 3 | 12 | 6.1 | 68 | 268 | 29 | 2.2 |
| | RF | 1, 2, 3 | 12 | 5.8 | 310 | 326 | 32 | 3.9 |
| | MW | 1, 2 | 8 | 5.6 | 455 | 315 | 26 | 4.9 |
| | RM | 1, 2 | 8 | 6.0 | 196 | 149 | 23 | 4.6 |
| | RB | 1, 2 | 8 | 7.0 | 148 | 234 | 26 | 2.7 |
| | RH | 1, 2 | 8 | 5.9 | 234 | 169 | 23 | 3.6 |
| | CC | 1, 2 | 8 | 6.0 | 146 | 384 | 18 | 1.4 |
| | WW | 32H, 32K, 15D | 12 | 7.0 | 268 | 117 | 16 | 1.6 |

*n: number of samples per farm.

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# Chapter 3

# EVALUATING SOIL MANAGEMENT BY MICROBIAL COMMUNITY-LEVELS PHYSIOLOGICAL PROFILES

## 3.1. Introduction

Management effects exert strong influence on the biological and biochemical components of soil quality (Doran, 1994). An assessment of the functional diversity in soil microbial communities based on community level physiological profiles (CLPP) can provide greater insight into microbial roles in ecosystems than isolation (Garland and Mills, 1991). The patterns of substrate use in the reaction wells of the Biolog GN microtiter plates (Biolog, Inc., 3938 Trust Way, Hayward, CA 94545, U.S.A.) are related to the microbial activity of the samples analyzed and have shown their potential as a relative method to characterize microbial communities from different soil types (Winding, 1994). Patterns of substrate use have been analyzed as indicators of metabolic diversity despite the fact that the relationship between soil microbial diversity (Zak et al., 1994; Winding, 1994) and the functioning and sustainability of agricultural ecosystems are still unclear (Giller et al., 1997). The CLPP method has been criticized as an absolute measure of soil microbial diversity because it requires outgrowth and bacterial reduction of tetrazolium dyes (Konopka et al., 1998). However, CLPPs continue to be used for research as relative indicators of microbial community functions in different soil.

The Biolog microtiter plate system measures utilization of carbon, the major factor regulating microbial growth in soil (Curl and Truelove, 1986; Wardle, 1992). Biolog plates detect the utilization by bacteria of 95 specific C sources on each of the gram negative (-) microplates. The microplates are designed to provide standardized biochemical tests for identifying a broad range of culturable bacteria (Zak et al., 1994). Thus, each Biolog plate yields a specific pattern of reactions, or community-level physiological profiles (CLPP), representing outgrowth and utilization of specific

substrates by members of the inoculated bacterial community (Bochner, 1989). Color development in each well is interpreted as a measure of microbial activity under the given conditions. Color of the reaction in each well is measured after a determined incubation period. A positive response is identified as an absorbance or optical density value greater than that occurring in the blank well.

The absorbance data for all substrate-containing wells can be analyzed in two different ways. The first approach is to generate binary data based on presence or absence of color development in each of the 95 wells or groups of wells with the same family of substrates (e.g. polymers). The second way is to generate continuous data based on color intensities in each of the 95 wells. Statistical procedures are used to compare soil microbial activity (catabolic potential) under these conditions.

The simplest indicator of functional diversity is the mean absorbance of the microtiter plates. Another indicator is the number of different substrates that are used by the microbial community. Substrate diversity (H) is an index that encompasses both substrate richness (measures the amount of substrates that are positive) and substrate evenness (measures the equitability of activities across all utilized substrates) (Magurran, 1988; Zak et al., 1994).

It must be stressed however, that the metabolic diversity patterns do not necessarily reflect the in situ activity of the soil microbial community since the assay is selective for organisms capable of growing on the media and may represent only a subset of the whole microbial community (Garland and Mills, 1994; Haack et al., 1995; Tate 2000) since frequently less than 1% of soil microorganisms can grow in culture conditions (Domsch et al., 1979). Furthermore, studies in which different combinations of pure microbial cultures were used to inoculate Biolog plates found that the pattern of substrate responses depends more on the mixture rather than the initial relative properties of each organism added (Wünsche et al., 1995; Haack et al., 1995).

Garland and Mills (1991) and Winding (1994) observed that color development appeared to depend only on the growth of any cell in substrate-containing wells. They concluded that it was impossible to determine if all members of the community capable of utilizing the compound had contributed to the profile or if the response in a given well

resulted from growth and activity of a subset, or a single member, of the community. Zak et al. (1994) acknowledged these limitations, but argued that substrate utilization profiles nonetheless provide a “rich data set” for studies of the functional activity and diversity of microbial communities.

The analyses of these variables by univariate (ANOVA) and multivariate statistics such as cluster and principal components analysis (PCA) are common ways of comparing CLPP. Multivariate analysis, such as PCA, is a statistical method used to reduce the number of variables to a smaller number of new variables called principal components (PCs). In other words, it represents an orthogonal rotation of the original test axes to give new axes, the first having maximum variance, the second accounting for a maximum of the remaining variance in a direction orthogonal to the first, and so on. The variances of the axes (components) are given by the eigenvalues. It is often convenient to disregard components which have small eigenvalues, so that the main aspects of variation can be studied in a subspace of fewer dimensions (Khattree and Naik, 2000).

As PCA is an orthogonal rotation, the original inter-sample distances are retained in the total component space, which is Euclidean. Hence, inter-sample distances can be calculated from the component scores (axis co-ordinates) using distance calculation formulas (ex. Pythagorean). Any distances calculated from the scores on the first q components will be an approximation of those in the full component or variable space, but it may happen that the first q components contain all the information that is of interest in a particular study. Following these calculations, a concise summary of the inter-sample distance relationships can lead naturally to the construction of average-linkage cluster analysis, the dendogram of which provides a graphical representation of that information.

Distinctive color patterns in the reaction well array have been reported and described for soils from different ecosystems and plant communities as well as agricultural soil under differing management regimes (Bossio and Scow, 1995). Although Biolog measures only potential C utilization, there is evidence that addition of various carbon sources to soil results in greater utilization of these compounds in the Biolog plates (Grayston et al., 1998). Goodfriend (1998) also compared different saline systems (salt marsh, sand dune, and seawater irrigated agronomic systems) using Biolog plates

and microbial community patterns of carbon substrate utilization. Microbial community substrate utilization reflected similarity in habitat type rather than geographical influences in these systems.

Few studies have been done on soil microbial composition changes due to long-term experimental treatments or comparing different agricultural soil management impacts on Pennsylvania farms. The Hunter Rotation Experiment (HRE) described in Chapter 2, represents an important opportunity to investigate the effect on microbial community C utilization patterns due to crop rotations and nutrient sources maintained continuously for 30 years. Furthermore, the assessment of Pennsylvania farms that include a wide range of conventional and reduced inputs management provides an opportunity to understand changes and effects on the utilization of carbon sources and microbial shifts in these soils. Therefore, the objective of this study was to evaluate soil management impacts on soil microbial community physiological profiles (CLPP) of soils from Pennsylvania farms and a long-term experiment (HRE) using multivariate analysis as an exploratory tool.

## 3.2. Materials and Methods

### 3.2.1. Soil sampling procedures

Samples were collected each June from 1997 through 2000. Soil cores were obtained from first-year corn fields or plots, where available. Crop and soil management of the HRE and Pennsylvania farms are fully described in Chapter 2 of this thesis. Twenty to twenty five core samples (2x15 cm) were composited from each field, three to four fields per farm and in each HRE plot. All samples were moist sieved through a 2mm sieve and stored at 4 °C for a maximum of 4 weeks until analyses.

### 3.2.2. Carbon-Source Utilization Profiles

An assessment of functional substrate utilization differences in microbial

communities from sole-source carbon utilization patterns was performed with the Biolog microplate identification system (Zak et al., 1994). In this method, 150 µl aliquots of a soil suspension diluted 1:5000 with deonized water were added to each of the wells of the microplate. Utilization of specific C sources by bacteria from a set of 95 different C compounds on gram negative (-) microplates was determined by color development (tetrazolium dye). Absorbance was determined after 72 hours incubation at room temperature with a Shimadzu Plate reader at $\lambda$ 590 nm. Absorbance from the control well was subtracted from all 95 wells; however, the reactions in the control well were always colorless.

### 3.2.3. Statistical Analysis

Functional substrate utilization measurements were analyzed by: 1) Analysis of variance (ANOVA) with substrate utilization mean plate values. Mean separation was performed with Duncan's Multiple Range test ($p<0.05$); 2) principal component and cluster analysis based on the presence or absence of color, and on the intensity of utilized substrates; 3) principal component analysis using absorbance values of plate variables such as defined sets of substrate types; and 4) a substrate diversity index (H) that defines substrate richness and evenness on each plate according to the following formula:

$$H = - \Sigma p_i (\ln p_i) \qquad \text{where } p_i = \frac{\text{intensity of each well}}{\Sigma \text{ intensity of all wells}}$$

The statistical analysis was performed using Statistical Analysis System (SAS, 1988) software.

## 3.3. Results and Discussion

### 3.3.1. Long-term Hunter Rotation Experiment

3.3.1.1. Microbial CLPP analyzed by mean plate intensity reactions

Mean microtiter plate intensity in this study was observed to be a potentially useful quantitative tool, because we observed clear differences in this parameter among HRE treatments (Table 3.1). Mean intensity values were significantly different among manure and industrial fertilizers treatments (Table 3.1). Increases in mean plate intensities were also significant as crop diversity and/or the length of the rotations (Rot3 and 5) increased for all the lime-fertility treatments. The LF1 (industrial fertilizer) treatments had generally lower intensities (0.27±0.19), especially for continuous corn (Rot1) and the short rotation (Rot2) (0.39±0.08). Significantly higher intensities were obtained with long-term manure treatments (LF3) and crop rotations that included forage (0.91±0.08) and/or small grains (0.95±0.08) (Rot3 and 5), but not with the LF2-Rot5 treatment (0.75±0.07). Lack of significance in the latter treatment combination indicated a significant treatment interaction. Standard deviations of all treatments are presented in Appendix E.

Table 3.1: Biolog plate mean intensity from 1997-2000 in the Hunter Rotation Experiment.

| **LF** | **Crop Rotations** | | | | |
|---|---|---|---|---|---|
| | **1** | **2** | **3** | **5** | Lsd Rot* |
| | | | *Absorbance* | | |
| **1** | 0.27 | 0.39 | 0.69 | 0.79 | *0.1* |
| **2** | 0.66 | 0.79 | 0.91 | 0.75 | |
| **3** | 0.63 | 0.78 | 0.91 | 0.95 | |
| Lsd LF* | *0.3* | | | | |

* Fisher's protected least significant difference for LF across all rotations and Rot across all lime-fertility treatments at $p<0.05$.

Higher mean plate intensities in treatments with manure additions and crop rotations could be attributed to the combined effects of increased diversity of crop residues and organic amendments, as was noted in a study by Gunapala and Scow (1997). In our study greater mean plate intensity was observed when industrial fertilizer was used with more diverse crop rotations (LF1-Rot2, 3 and 5) than when it was used with continuous corn (Rot1). Greater mean plate intensity was also observed when corn was fertilized with manure additions (LF3-Rot1) than when it received industrial fertilizer (LF1-1) (Table 3.1). Lack of significance of mean plate intensity between LF2-Rot1 and LF2-Rot5 may be attributed to complementary effects of higher manure applications to continuous corn treatments (Rot1) and of the more diversified cropping system (Rot5) within the short term (LF2) manure treatment (Table 2.2) Therefore, mean plate intensity seems to be enhanced by soil management practices that include either organic additions or a diverse crop rotation. However, there seems to be some synergistic effect as both the quantity and frequency of organic addition and the diversity of crops increase.

Greater mean plate intensity for Rot5 (four crops) compared to Rot1 (continuous corn) suggests the possibility of multiple crops enhancing microbial CLPP, as mentioned above. High crop diversity can have an important influence on microbial communities in their rhizospheres. This phenomenon may be due to the qualitative and quantitative variation of carbon compounds that are incorporated into the soil (Garland et al., 1996). This may be occurring in the HRE, particularly when small grains and forages are included in the rotation. Management of this rotation increases the diversity of organic residues and the frequency in which residues are available to microbes during a growing season in comparison to continuous corn. Increased harvests during the cropping year, such as in the alfalfa rotation, may also increase the amount of organic C from sloughing roots that can be used by microorganisms.

Buyer and Drinkwater (1997) also used mean intensity of microtiter plates to determine differences between treatments during the growing season in a long term experiment (Farming Systems Trial) at Rodale Institute Research Center. Soil management treatments included a conventional (industrial fertilizer) and an organic practice that included a legume green manure for N supply. Both systems were based on

a corn-soybean rotation. However, the legume system, in addition to the green manure, included winter wheat in the rotation, and a rye cover crop was planted after corn harvest in the organic/legume system. Mean plate intensity was greater for organic/legume-based treatment (0.65-1.10) than for conventional treatments (0.45-0.60). As in our experiment, the intensity of CLPP's in the conventional treatment apparently is different from organic/legume-based treatments due to differences in management history and crop residues (Buyer and Drinkwater, 1997).

Lowit et al. (2000) acknowledged that aspects of CLPP methods in need of further study were procedures for sample replication and handling. In our study, treatments are replicated within the HRE. Therefore, four replicates of all lime fertility treatments and crop rotation combinations were sampled. Although some variation was observed among these replicates (standard deviations shown in Appendix E), this sampling procedure agrees with findings which suggest that to best represent a given treatment, it is important to have replicate soil samples or multiple dilutions from samples rather than to replicate CLPP plates from a single dilution. Apparently, most of the variability in the CLPP analyses comes from soil replicates rather than from plate replicates (Balser et al., 2002).

#### 3.3.1.2. Microbial CLPP analyzed by presence/absence reaction

Patterns of absence/presence reactions in the microtiter plates were analyzed by PCA (Figure 3.1). The PC1 accounted for 48% of total variation, while PC2 accounted for 40%. Hence, 88% of the total variance is explained with these two components. The PC1 primarily shows the separation of crop rotations that included limited crop diversity (Rot1) or length of rotation (Rot2) with industrial fertilizers from all other treatments. Within industrial fertilizer treatments (LF1), continuous corn (Rot1) or corn-soybean (Rot2) rotations were separated from crop rotations that included more diverse (forages and/or small grains) crops and longer rotations (Rot3 and 5). Hence, even with lower average annual N additions than with industrial fertilizer treatments, dairy manure treatments influenced the pattern of microbial CLPP for all crop rotations. The CLPP patterns of presence/absence reactions in PC2 clearly distinguished treatments with

manure additions (LF2, and LF3) and all crop rotations from samples with industrial fertilizer (LF1) and long/diverse crop rotations (Rot3 and 5) (Figure 3.1). The microbial CLPP suggests that crop rotation is an important influence on microbial community structure with industrial fertilizer, but not so significant when dairy manure slurry is applied. This result seems to corroborate studies suggesting that high average annual N-fertilizer on continuous cropping or short rotation (like Rot1 and Rot2) may have different effects on soil microbes (Khan et al., 1970) compared to a diverse cropping system with lower overall N fertilizer application (Spiers and McGill, 1979).

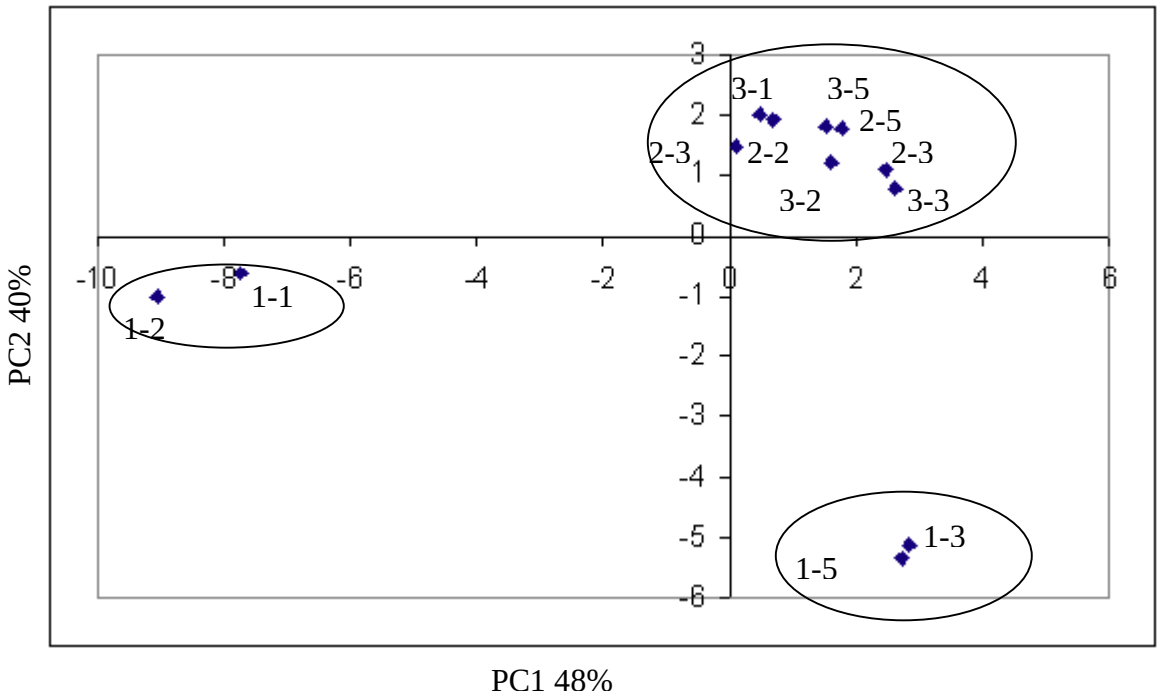


*Figure 3.1:* Distribution of microbial community structure from lime-fertility and crop rotation treatments analyzed by principal components analysis of presence/absence reactions. Lime-fertility treatments: 1 (industrial fertilizer), 2, and 3 (manure additions). Crop rotations: Rot1: CC, Rot2: CS, Rot3: 4C4H; and Rot5: COWHH. (Label: LF#-Rot#). Ellipses represent clusters of manure or industrial fertilizer and different crop rotations treatments. Percentages on principal components represent the amount of variation explained by each PC.

The mean number of carbon sources used by microbial community was calculated for each treatment in HRE (Table 3.2). Continuous corn and corn-soybean rotations under

industrial fertilizer additions (LF1) had the lowest number of utilized C substrates (69-71) compared to rotations with increased crop diversity (89-90) or all crop rotations with manure additions (85-94). This seems to agree with PC1 and the separation of treatments previously described. Treatments with manure additions (LF2 and LF3) had the highest number of C substrates utilized. In addition, treatments with industrial fertilizer and crop rotations that included forages and/or small grains (Rot3 and Rot5) were similar in the number of utilized C substrates to treatments with manure additions LF2 and crop rotations Rot2, Rot3 and Rot5. These treatments were significantly different from manure treatments (LF3) and crop rotations 3 and 5 (Table 3.2).

Table 3.2: Mean number of carbon sources used in CLPP analysis from 1997-2000 in the Hunter Rotation Experiment.

| Treatments | | Mean C substrates |
|---|---|---|
| LF1 | 1 | 69 g* |
| | 2 | 71 g |
| | 3 | 89 cde |
| | 5 | 90 bc |
| LF2 | 1 | 85 f |
| | 2 | 89 cde |
| | 3 | 92 ab |
| | 5 | 90 bc |
| LF3 | 1 | 87 ef |
| | 2 | 92 ab |
| | 3 | 93 a |
| | 5 | 94 a |

*Number with different letters are significantly different at $p<0.05$ with DMR.

For industrial fertilizer treatments, additional substrates utilized with crop rotations that incorporated forages and/or small grains (Rot3 and Rot5) included carbohydrates (L-fucose, lactulose, D-melibiose, β-methylglucoside, D-psicose, D-raffinose, turanose, xylitol, and α-D-lactose), four more carboxylic acids (formic acid, hydroxybutyric, itaconic acid, α-ketobutyric acid), and four more amino acids (glycyl-L-aspartic acid, L-phenylalanine, and L-threonine) than the continuous crop or corn/soybean rotation. Almost all C substrates seemed to be utilized in LF3-Rot5 treatment (94) (Table 3.2). Increase in C substrate utilization suggests that soil management practices that include crop diversity with industrial fertilizer or the additions of organic manure in all cropping systems may positively affect potential microbial C utilization. As previously mentioned in the mean plate analysis, the addition of manure additions to the soil may enhance increased retention of soluble organic C substrates enhancing soil microbial community activity.

In the cluster analysis, there were three primary clusters of average linkage among treatments (Figure 3.2). One with continuous corn or corn/soybean rotations (short and simple) with industrial fertilizers (LF1-Rot1 and LF1-Rot2), and another cluster containing two groups: 1) other than annual or biennial manure additions (LF2, and 3 with Rot3 or 5); and 2) industrial fertilizer (LF1) and diverse/long crop rotations that included forages and/or small grains (Rot3, and 5), and annual or biennial manure additions (LF2, 3 and Rot1, 2) (Figure 3.2). Only the treatment combination LF3-Rot2 was not consistently classified in these clusters. The interchange and misclassifications of this treatment in the cluster analysis may be due to similarities in manure and crop rotation management with only two crops in the rotation (Table 2.2).

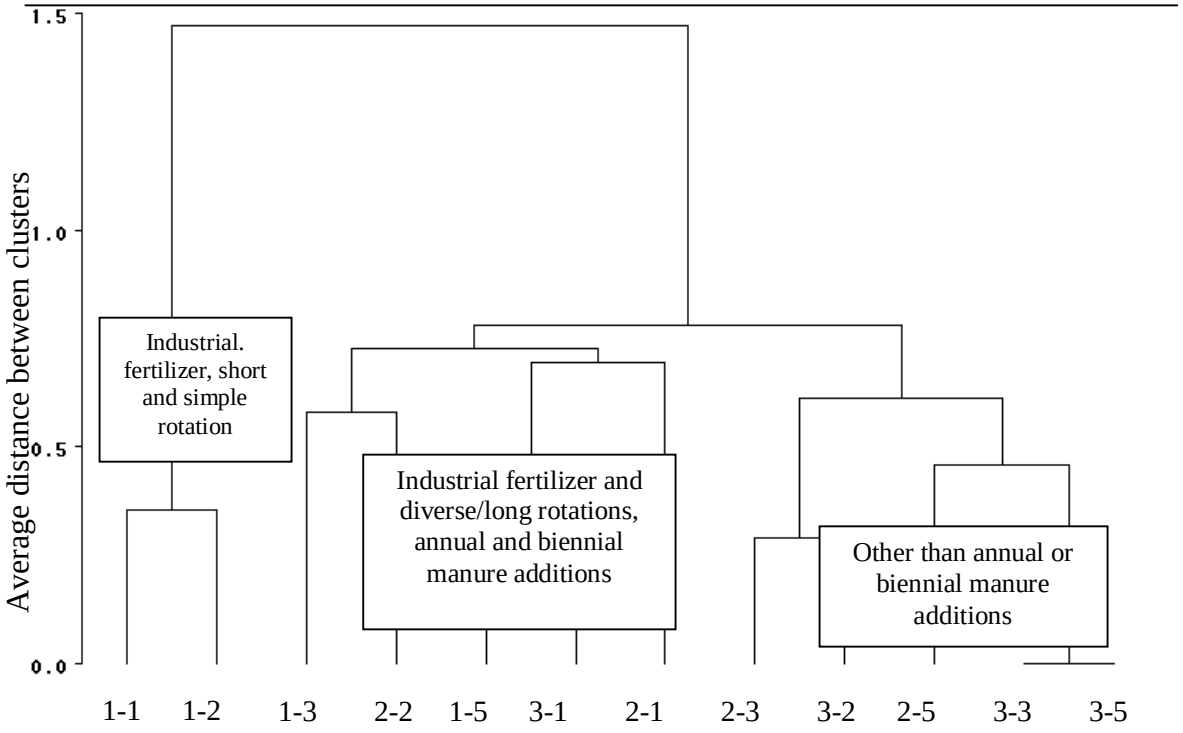


*Figure 3.2:* Distribution of microbial community structure from a lime-fertility and crop rotation treatments analyzed by cluster analysis of presence/absence reactions from a lime-fertility and crop rotation treatments. Lime-fertility treatments: 1 (industrial fertilizer), 2, and 3 (manure additions). Crop rotations: Rot1: CC, Rot2: CS, Rot3: 4C4H; and Rot5: COWHH. (Label: LF#-Rot#).

As previously stated, CLPPs are apparently influenced by both manure additions and crop rotation. This reflects the fairly consistent relationships observed among microbial CLPPs determined by cluster analysis, PCA and the mean number of utilized substrates of the different soil management treatments. In addition, this observation seemed to corroborate previous results observed in the microtiter plate mean intensity (Table 3.1). Bending et al. (2000) reported that CLPP of microbial community following the inoculation of Biolog plates was highly sensitive to management practices. Substrate utilization increased following the addition of soil organic matter (SOM) by ploughing-in of a vetch crop in a barley and clover rotation in a 16 months experiment. They suggested that organic material addition to the soil increased soluble organic C substrates available to the microbial community that contributed to distinct shifts of C utilization patterns in

the microbial community structure. Similarly, Lupwayi et al. (1998) reported that CLPP of wheat crop rotations increased microbial diversity (by increasing substrate utilization) in comparison to a continuous wheat cropping system. Differences were also attributed to varied and increased availability of soluble C substrates with increased diversity of soil crop residues. Likewise, several studies indicate that legumes in rotation cropping systems provide other benefits for maintaining soil C content, and therefore promote microbial biomass (Campbell and Zetner, 1991, Campbell et al., 1991). Drinkwater et al. (1998) suggested that legume-based systems, with their narrow C:N organic residues combined with the relatively greater temporal diversity in cropping systems, significantly increased the retention of soil C and N compared to continuous cropping systems.

In a study of polar lipid fatty acid (PLFA) distribution, Peacock et al. (2001) suggested that soluble organic C was greater in manured soils than chemically fertilized soils. They hypothesized that the stream of substrates available to the microbial community was more stable and readily available in the manured soils and that changes to soil organic matter (SOM) pools in the manure-amended treatments reflecting a balance between microbial synthesis and degradation of the inputs. This relationship would explain the clustering of PLFA assay results from soils receiving dairy manure separately from those receiving industrial fertilizer.

Soil management methods, such as no-tillage (Doran, 1980), use of cover crops (Kirchner et al., 1993; Mullen et al., 1998), and manuring (Dormaar et al., 1998, Drinkwater et al., 1995), are often observed to enhance overall soil C, microbial biomass, populations and activities. According to several authors (Alvarez et al., 1998; Bhogal and Shepard, 1997; Gregorich et al., 1998), these management practices often result in increased concentrations of soluble organic C compounds that can be readily available or promptly broken down by the increase of extracellular soil enzymes. Also, the availability of soluble compounds may stimulate a broader array of organisms than only those that can degrade the more resistant C compounds, both those native to the soil and those also added in the manure. This more diverse group of organisms then can enhance biological utilization and residue degradation capabilities. Such processes may explain

the microbial CLPP differences observed in our treatments, such as the long-term use of manure (LF2 and 3) in HRE.

While animal manures are typically applied to supply crop N, P and K needs, the impact of organic additions to the soil, and consequently, crop growth and microbial activities, goes beyond the application of nutrients. Although not measured in this study, it may be possible that soluble C is available over a longer period, following manure additions, than with industrial fertilizer additions. Consequently, additions of organic material in the LF2 and 3 treatments could provide a more stable and readily available stream of C substrates to the underlying microbial community, either by frequent manure additions to continuous cropping and short/simple rotations, or by less frequent manure additions but including a diverse/long crop rotation in the soil management. Therefore, our results indicate that assessment of CLPP by presence/absence reactions can be a useful tool as a soil quality indicator to determine soil management effects.

3.3.1.3. Microbial CLPP analyzed by intensities of individual reactions

Patterns of substrate utilization intensities in individual wells were analyzed by PCA. The PC1 of the PCA accounted for 39% of total variation, while PC2 accounted for 27%. Hence, 66% of the total variance was explained with these two components (Figure 3.3). The PC1 tended to better separate treatments with inorganic fertilizer (LF1) and continuous cropping or annual rotation (Rot1, and Rot2) from treatments with manure additions (LF2, and 3) and diverse/long crop rotations that included forages (Rot3) and/or small grains (Rot5). For comparison purposes, the same treatment groups separated by PCA of patterns of presence/absence reactions in Figure 3.1 and 3.2 are illustrated by the ellipses in Figure 3.3. The PC2 seemed to separate industrially fertilized and diverse crop rotation treatment (represented by LF1-Rot5) to a greater extent than with manure fertilized rotation treatments for both patterns of presence/absence and intensity reactions.

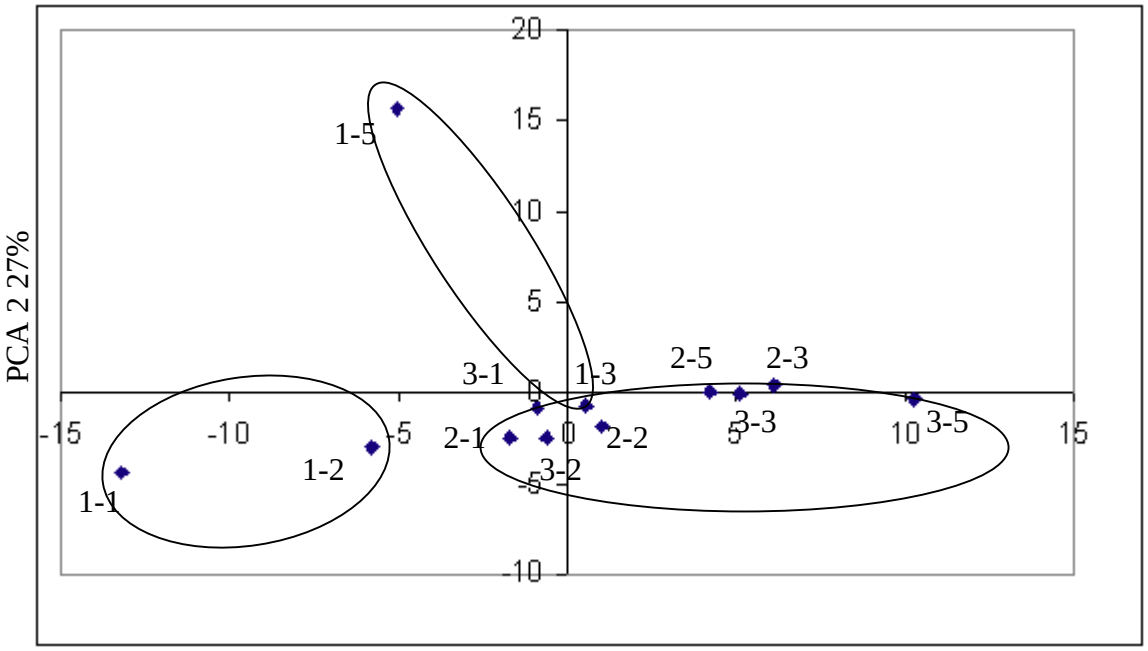


*Figure 3.3:* Distribution of microbial community structure from lime-fertility and crop rotation treatments analyzed by principal components analysis of intensity reactions. Lime-fertility treatments: 1 (industrial fertilizer), 2, and 3 (manure additions). Crop rotations: Rot1: CC, Rot2: CS, Rot3: 4C4H; and Rot5: COWHH. (Label: LF#-Rot#). Ellipses represent clusters of manure versus industrial fertilizer and different crop rotations treatments. Percentages on principal components represent the amount of variation explained by each PC.

Although the presence/absence reactions showed a clear separation of CLPPs in manure and/or diverse/long crop systems, the measured intensities of C utilization in individual wells were not as distinct for all treatments. For example, industrial fertilizer (LF1) and simple cropping systems (Rot2), and forage rotations (Rot3) are not as clearly separated by PCA of reaction intensities (Figure 3.3) as by PCA of presence/absence reactions (Figure 3.1). A possible explanation may be that intensity reactions emphasize the ability of the bacteria to rapidly utilize a particular substrate and grow. Presence/absence patterns reactions only represent the growth of organisms capable of degrading a particular substrate, without considering the kinetics of population growth. Therefore, in order to distinguish the effect of soil management on CLPP using the Biolog procedure and PCA, it is important to clearly specify if a binary

(presence/absence) or a continuous variable (intensity) is analyzed. Future studies are needed to explore which method would be more appropriate to understand microbial dynamics in different soil systems.

Separation of treatments by average linkage in the cluster analysis of intensity data resulted in two different main groups (Figure 3.4). The first cluster included treatments of reaction intensities with industrial fertilizer (LF1) and continuous corn or short and simple rotations (Rot1, and Rot2). The second cluster was divided in two clusters. The first one included treatments with industrial fertilizer and diverse/long crop rotations that included small grains and/or forages (Rot3, and Rot5), as well as annual and biennial manure additions (LF2, 3, with Rot1, 2). The second cluster included other than annual or biennial manure additions (LF2, 3 and Rot 3, 5). Cluster analysis of

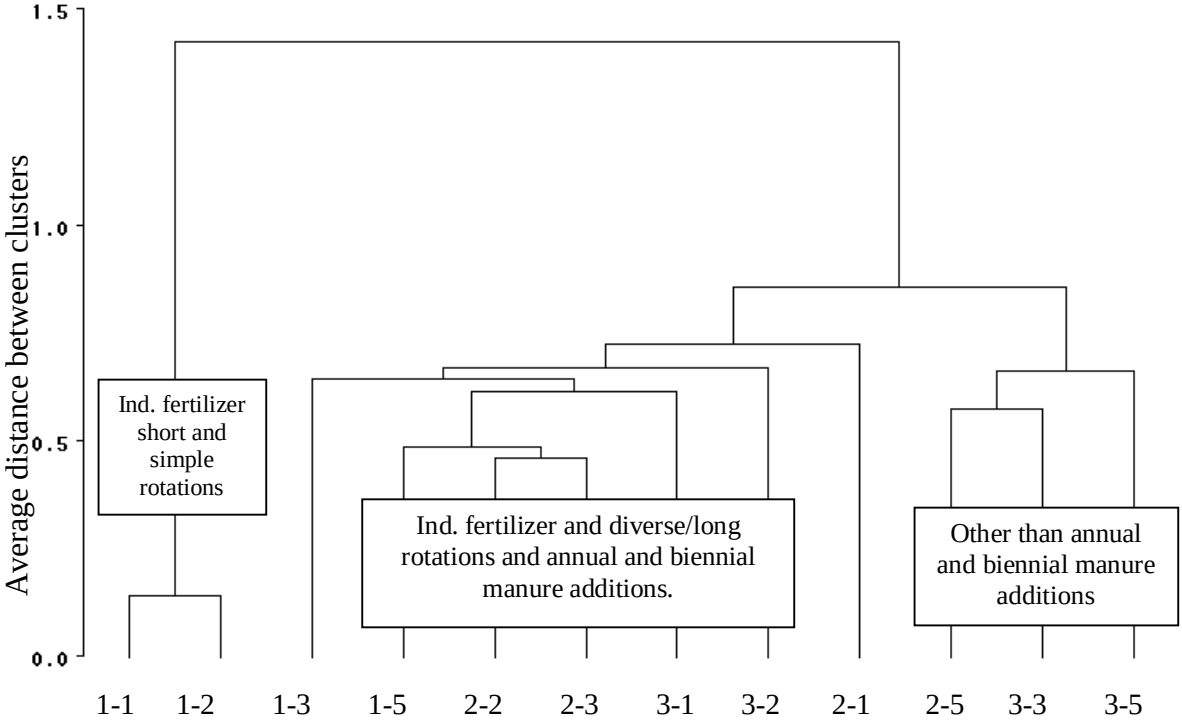


*Figure 3.4:* Distribution of microbial community structure from lime-fertility and crop rotation treatments analyzed by cluster analysis of intensity reactions. Lime-fertility treatments: 1 (industrial fertilizer), 2, and 3 (manure additions). Crop rotations: Rot 1: CC, Rot2: CS, Rot3: 4C4H; and Rot5: COWHH. (Label: LF#-Rot#).

intensity patterns showed patterns similar to those on the presence/absence analysis. In contrast with the presence/absence reactions, only the treatment combination of LF2-Rot3 was not consistently classified in the intensity pattern reactions. As mentioned above, the interchange and misclassifications of this treatment in the cluster analysis (Figure 3.2 and 3.4) may be due to similarities in manure and crop rotation management with only two crops in the rotation.

It is apparent that, as observed and discussed in the PCA, manure additions (LF2, and LF3) and rotations that include forages and/or small grains (Rot3, and Rot5) influence microbial community structure differently than industrial fertilizer and/or continuous corn or corn-soybean treatments (Figure 3.4). As previously stated, treatments of high crop diversity systems and/or with manure treatments are likely to have higher levels of soluble organic C, therefore supporting higher levels of microbial activity.

### 3.3.1.4. Microbial CLPP analyzed by different C guilds

Overall mean value by guilds of C substrate utilization on the Biolog plates were assessed with PCA after their classification among five different guilds (Table 3.3). Although PC1 explained most of the variation (89%), the C utilization by guilds did not differ among treatments, as observed by the same absolute loading value and sign for all guilds. Although it was a relatively small and difference (5% of total variation) the PC2 results suggest that carboxylic acids, amines/amides, and amino acids contrasted with polymers and carbohydrates (Table 3.3). Absolute loading values of PC1 and PC2 are small compared to values of previous PCAs suggesting that the microbial utilization by guilds was relatively uniform compared to the utilization of individual substrates. Therefore, it seems that under the conditions of our study, the analysis of microbial C guild utilization does not provide clear information about soil management impact on microbial CLPPs.

*Table 3.3:* Principal Component Analysis of mean intensity reactions of C guilds of all treatments of the Hunter Rotation Experiment.

| | Principal Components | |
|---|---|---|
| Guilds | PC1 | PC2 |
| | loadings | |
| Carbohydrates | -0.445 | -0.514 |
| Polymers | -0.441 | -0.404 |
| Carboxylic Acids | -0.456 | 0.052 |
| Amines/Amides | -0.436 | 0.744 |
| Amino acids | -0.458 | 0.129 |
| *Variation explained* | *89%* | *5%* |

3.3.1.5. Substrate Diversity Index

The substrate diversity index based on the intensity reactions was greater for manure treatments (LF2, and LF3) and crop rotations with forage and small grains (Rot5) than for the same rotations with industrial fertilizer (LF1) (Table 3.4). Standard deviations of diversity across all years ranged from 0.28-0.74 (Appendix E). Increases in substrate diversity index are similar to the results from the analyses of mean plate intensity patterns and to the results of the number of C substrates utilized (previously reported in Table 3.1 and 3.2). These results suggest that CLPPs are affected by a synergistic relationship of manure additions and type and/or number of crops in the rotations. Increased substrate diversity index in LF3 was different for all rotations compared to LF1 and LF2 reflecting the increase in both substrate utilization potential (substrate richness) and microbial diversity (substrate evenness) possibly due to the long-term selection pressure that promotes the evolution of microbial community diversity with manure additions.

It has been hypothesized that decreases in the diversity of soil organisms will

cause declines in the resistance of soils to stress or disturbance, such as dry-wet periods, intensive erosion, nutrient depletion, etc. (Brusaard et al., 1997; Giller et al.; 1997). Degens et al. (2001) reported that land uses resulting in reduced richness in catabolic substrate utilization in soils, such as long-term cropping, might promote microbial communities with reduced resistance to stress or disturbance. This may be a consequence

*Table 3.4*: Substrate diversity Index of CLPP from 1997-2000 in the Hunter Rotation Experiment.

| LF | Crop Rotations | | | | |
|---|---|---|---|---|---|
| | **1** | **2** | **3** | **5** | Lsd* Rot |
| **1** | 3.8 | 3.9 | 4.4 | 5.3 | *0.2* |
| **2** | 4.3 | 4.5 | 5.0 | 5.8 | |
| **3** | 5.1 | 5.0 | 6.3 | 6.9 | |
| Lsd* LF | *0.3* | | | | |

*Fisher's protected least significant difference for LF across all rotations and Rot across all lime-fertility treatments at p>0.05.

of increased microbial respiration to obtain energy but decreased microbial biomass due to insufficient substrates to increase anabolic and metabolic functions. In contrast, microbial communities in soils with high substrate diversity and catabolic evenness (such as pastures, or diverse/long crop rotations; Degens, 2001) are also more likely to resist acute changes in soil conditions. As indicated by PCA and substrate diversity index, differences in microbial community structure and function were present in the HRE. Therefore, if the previous relationships exist, HRE treatments with manure additions and diverse/long crop rotations could maintain an improved soil quality and confer greater resistance to soil degradation than treatments with industrial fertilizer and continuous or short and simple (C/S) crop production. Thus, substrate diversity index (H) can be a useful tool, as a soil quality indicator, to determine soil management impacts.

### 3.3.2. Pennsylvania Farms

3.3.2.1. Analysis of variance of Pennsylvania farms mean plate intensity

Mean plate intensity was greater for farm soils with a history of organic additions as part of soil management than for farms without this history (Table 3.5). Highest mean plate intensity was obtained with soils from livestock farms (RF, through MW) and from cash crop farms that had a history of manure application as part of their soil management (e.g. Fur). Although sampled as cash crop-no manure, EBN was considered to be a cash crop-manure due to its consistent association with EBM and other farms that received manures. The discrepancy may be due to inadequate crop history or inaccurate sampling location (see Chapter 2). Farms irrigated with wastewater (WW) had very low mean plate intensity, as well as farms that were cash crop-no manure (e.g. CC, W, RM) (Table 3.5). These results agree with differences found in the Rodale FST experiment, in which mean plate intensity was greater for organic practices (0.65-1.10) than conventional systems with no manure or organic additions (0.45-0.60) (Buyer and Drinkwater, 1997).

Although only a small proportion of the microbial community is in an active state at a given time (McGill et al., 1986), the increased availability of labile C source from management practices that include additions of organic matter and crop diversity seems to stimulate the microbial activity. This increased activity may be measured by the mean plate intensity of CLPPs as also described in HRE. Changes in soil microbial biomass composition or its physiological activity due to increased availability of soluble C substrates in soil management has been reported in studies by Alvarez et al. (1998) and Alvarez and Alvarez (2000).

As an example of soil management with limited organic additions, farms irrigated with treated wastewater (WW) received a very low C:N soil amendment (Appendix D) throughout the year. This N-rich amendment may promote the rapid degradation and depletion of almost any readily available C substrate by the microbial community. Therefore, this soil management may lead to a longer-term decrease in the microbial activity and composition due to low C availability.

*Table 3.5*: Biolog plate mean intensity from 1997-2000 in Pennsylvania farms.

| Pennsylvania farms | Management | Mean intensity |
|---|---|---|
| RF | Livestock farm | 1.36a* |
| JP | Livestock farm | 1.23a |
| H | Livestock farm | 1.12a |
| HS | Livestock farm | 0.98b |
| EBN | Cash crop-manure | 0.88bc |
| EBM | Cash crop-manure | 0.75 c |
| S | Livestock farm | 0.74 c |
| MY | Livestock farm | 0.73 c |
| Bro | Livestock farm | 0.72 c |
| MW | Livestock farm | 0.72c |
| Fur | Cash crop-manure | 0.63 c |
| Gr | Cash crop-manure | 0.58c |
| DB | Cash crop-no manure | 0.47d |
| RB | Cash crop-no manure | 0.46d |
| Bei | Cash crop-manure | 0.46d |
| PKE | Cash crop-manure | 0.35 de |
| RM | Cash crop-no manure | 0.34de |
| RH | Cash crop-no manure | 0.30e |
| BA | Cash crop-no manure | 0.29 e |
| CC | Cash crop-no manure | 0.28e |
| W | Cash crop-no manure | 0.28 e |
| WW | Wastewater Irrigation | 0.25 e |
| E | Cash crop-no manure | 0.23 e |
| L | Cash crop-no manure | 0.22e |

*Numbers with different letters are significantly different at $p < 0.05$ with DMR.

3.3.2.2. Microbial CLPP analyzed by presence/absence reactions

Patterns of microbial CLPP based on presence/absence reactions were assessed with PCA. The PC1 explained 47% of total variation, and PC2 13%. Pennsylvania farms with manure additions (livestock farms), except MW, were distinguished in PC1 from cash crop-manure additions (e.g. Bei, Gr), some cash crop-no manure (e.g. E, L, RM) and wastewater irrigation (WW) farms, but not from cash crop-no manure addition farms, such as W, DB, and RH (Figure 3.5). Ellipses separate livestock farms from all cash crop farms (Figure 3.5) for better understanding of patterns in CLPP resulting from the soil management practices. Hog manure in MW may have promoted a different microbial

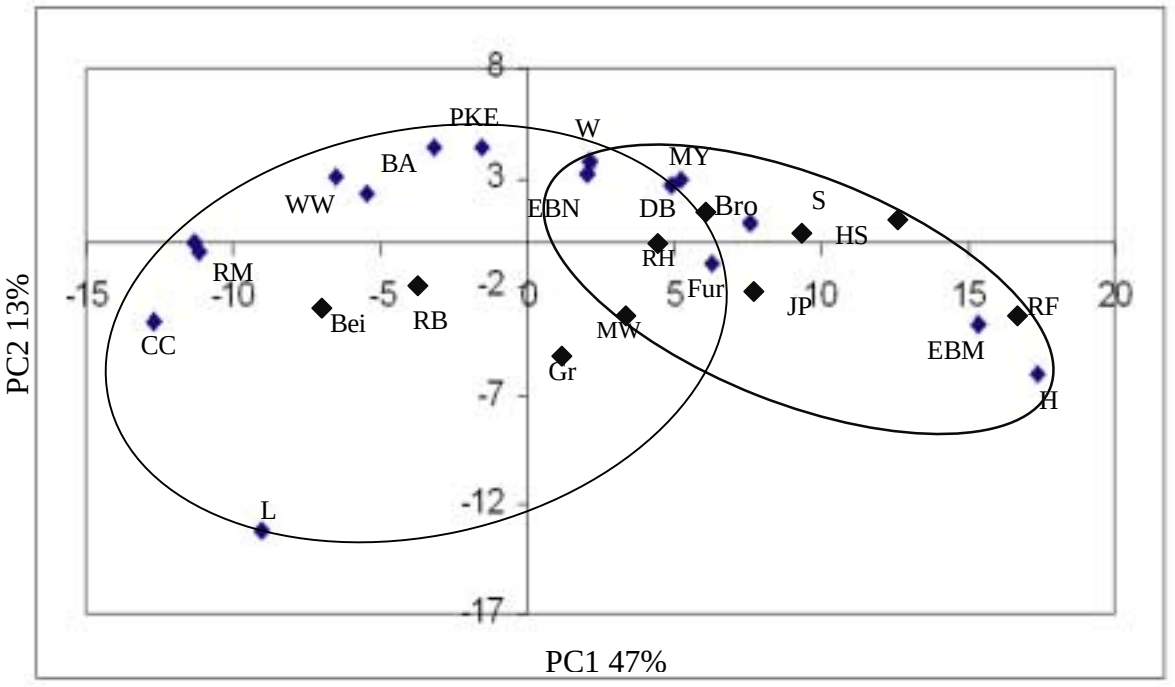


*Figure 3.5*: Distribution of microbial community structure from Pennsylvania farms with different soil management analyzed by PCA of presence/absence reactions. Livestock farms: Bro, S, MY, H, RF, HS, JP, MW. Cash Crop, manure: EBN, EBM, Gr, Bei, PKE, Fur. Cash Crop-no manure: L, BA, W, E, DB, RB, RH, CC, RM. Wastewater Irrigation farm: WW. Ellipses separate groups of livestock farms from cash crop and wastewater irrigation farms. Percentages on principal components represent the amount of variation explained by each PC.

CLPP than additions of dairy manure or compost. Abawi and Widmer (2000), and Pulikeshi et al. (2001) have reported differences in soil microbial responses to different types of manure and compost.

In contrast with the uniform and relatively consistent soil management history at HRE, diversity of farm management (such as different tillage practices) and variable crop histories may be factors influencing CLPP patterns among farms with manure additions. Due to inconsistent soil management over the years, diverse and malleable microbial communities might be present in all these soils, without creating a clear difference in microbial CLPP among the various types of soil management (Figure 3.5).

The mean number of carbon sources used by microbial community was calculated for each Pennsylvania farm (Table 3.6). Livestock farms (e.g. RF, H, Bro) had the highest number of utilized C substrates (94-78) compared to most of the cash crop-manure farms (eg. Fur, Gr, and Bei) (76) and cash crop-no manure additions (71-40). EBN farm although previously considered as cash-crop manure had very low number of utilized C substrates compared to EBM. PKE farm also had very low number of utilized C substrates compared to the other cash crop-manure farms (Table 3.6). Differences in number of utilized C substrates seem to agree with the analyses of mean plate intensity and the separation of treatments previously described with PCA. Based on differences in the number of C substrate utilized, treatments with consistent manure additions seemed to have higher substrate utilization potential than microbial communities in cash crop-no manure and wastewater irrigation farms. As previously stated in the HRE section, manure additions are likely to provide higher levels of soluble organic C (Alvarez et al., 1998) promoting higher levels of microbial activity.

The overall increase in the number of utilized substrates in CLPPs from livestock and cash crop-manure farms included the utilization of additional polymers (glycogen), carbohydrates (N-acetyl-D-galactosamine, adonitol, i-erythritol, L-fucose, gentiobiose, D-melibiose,, D-psicose, turanose), three more carboxylic acids (acetic acid, formic acid, γ-hydroxybutyric acid), amines/amides (succinamic acid, glucuronamide, alaninamide), and amino acids (glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-phenylalanine, and L-threonine) compared to substrates utilized in wastewater irrigation farms. Almost all C

substrates seemed to be utilized in RF, JP and H livestock farms. This result agrees with previous findings of mean plate analysis (Table 3.5) where these farms had the highest

Table 3.6: Mean number of carbon sources used in CLPP analysis from 1997-2000 in Pennsylvania farms from 1997-2000.

| Pennsylvania farms | Number of utilized C substrates |
|---|---|
| RF | 94 a* |
| JP | 93 a |
| H | 93 a |
| EBM | 92 ab |
| Bro | 90 b |
| MW | 87 c |
| HS | 81 d |
| S | 81 d |
| MY | 78 de |
| Fur | 77 de |
| Gr | 76 e |
| RB | 76 e |
| Bei | 76 e |
| RH | 76 e |
| DB | 71 f |
| EBN | 70 f |
| W | 65 g |
| RM | 63 gh |
| CC | 62 h |
| PKE | 59 hi |
| BA | 58 hi |
| WW | 54 j |
| E | 41 k |
| L | 40 k |

*Number with different letters are significantly different at $p<0.05$ with DMR.

mean plate intensity (Table 3.4). Decrease in the number of carbohydrates utilized in soils from wastewater farms seemed to agree with previous suggestions of low C availability in these farms. Differences in the number of substrates utilized in cash crop-no manure farms were inconsistent when compared to livestock and cash crop-manure farms. This may suggest a decrease in overall microbial community activity, rather than a shift in microbial community structure and catabolic function.

Farms with different soil management were not clearly separated in the cluster analysis (Figure 3.6). As in the PCA, most of the livestock farms clustered together, but

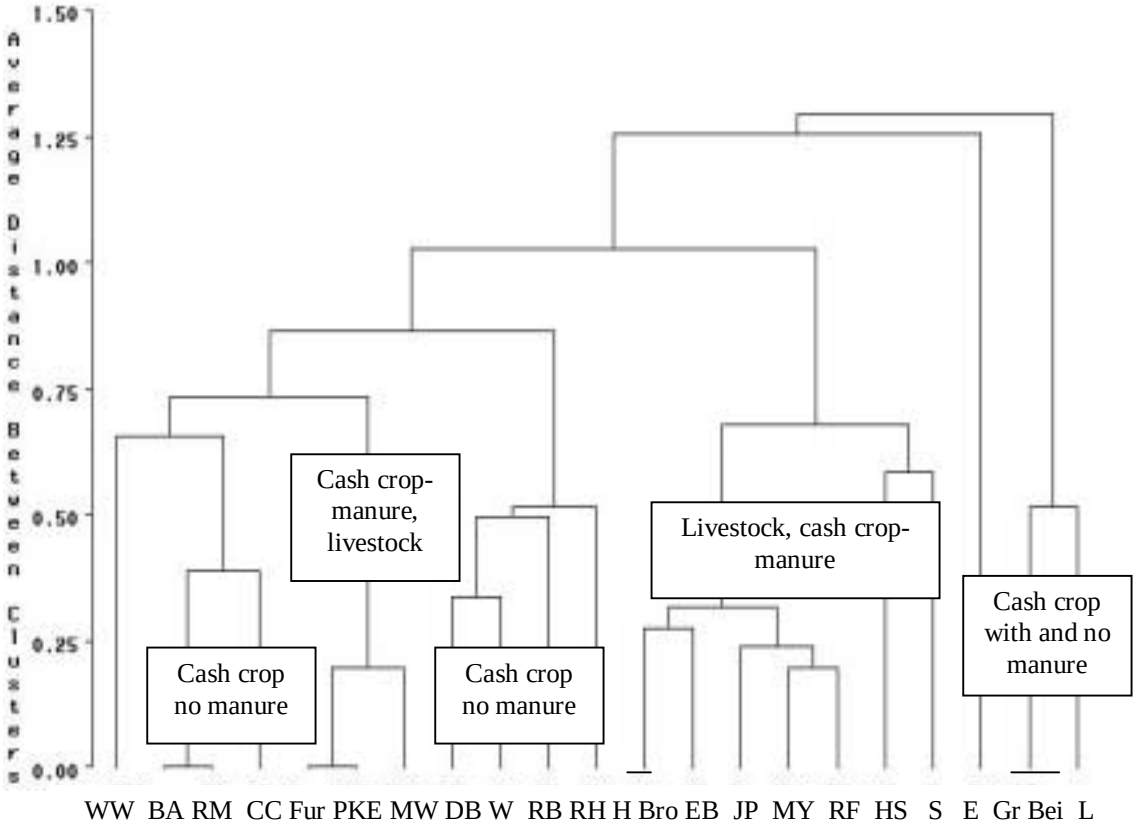


*Figure 3.6.* Distribution of microbial community structure from Pennsylvania farms with different soil management analyzed by cluster analysis of absence/presence reactions. Livestock farm: S, MY, H, RF, JP, HS, MW. Cash Crop, manure: Bei, EB (N/M), Gr, PKE, Fur, RM. Cash Crop, no manure: RB, L, BA, RH, W, E, DB, CC. Wastewater Irrigation farm: WW.

within main clusters that included cash crop-manure and no manure additions. Microbial CLPP of wastewater irrigation farms were separated in a different cluster. In contrast

with HRE, differences in crop rotations were not considered in collecting samples from Pennsylvania farms. Therefore, any additional effects on microbial CLPP due to differences in cropping systems were not evaluated with samples from these farms. As previously discussed in the PCA section, inconsistent soil management and possible variable cropping histories might have diminished the emergence of distinct microbial communities on these farms. Because of the ambiguous results observed in PCA and cluster analysis, we consider that assessment of CLPP by analysis of the presence/absence reactions might not be a very useful tool to clearly determine impacts of soil management on microbial CLPPs in Pennsylvania farms.

3.3.2.3. Microbial CLPP analyzed by assessing intensities of individual reactions

Analysis of intensities of individual reactions were assessed with PCA (Figure 3.7). The PC1 accounted for 63% of the total variation, while PC2 accounted for only 9%. The PCA using patterns of intensity reaction, especially PC1, separated microbial CLPP of livestock farms, and some cash crop farms with manure additions, from cash crop-no manure and wastewater irrigation farms (Figure 3.7). The CLPP based on patterns of intensity reactions separated farm soil management more clearly than CLPPs based on patterns of presence/absence reactions.

Thus, patterns of intensity reactions of microbial CLPPs have a greater potential as soil quality indicators than patterns from the presence/absence reactions of CLPPs in the Pennsylvania farms. This may indicate, in contrast to the analyses of HRE, a change in the potential intensity of C utilization with different soil management, even if the actual microbial community structure is not clearly changed (Buyer and Drinkwater, 1998). Routine additions of organic inputs on livestock farms, or some cash crop farms (EB) probably increased the metabolic activity of the soil microbial communities compared to farms that did not receive the additions.

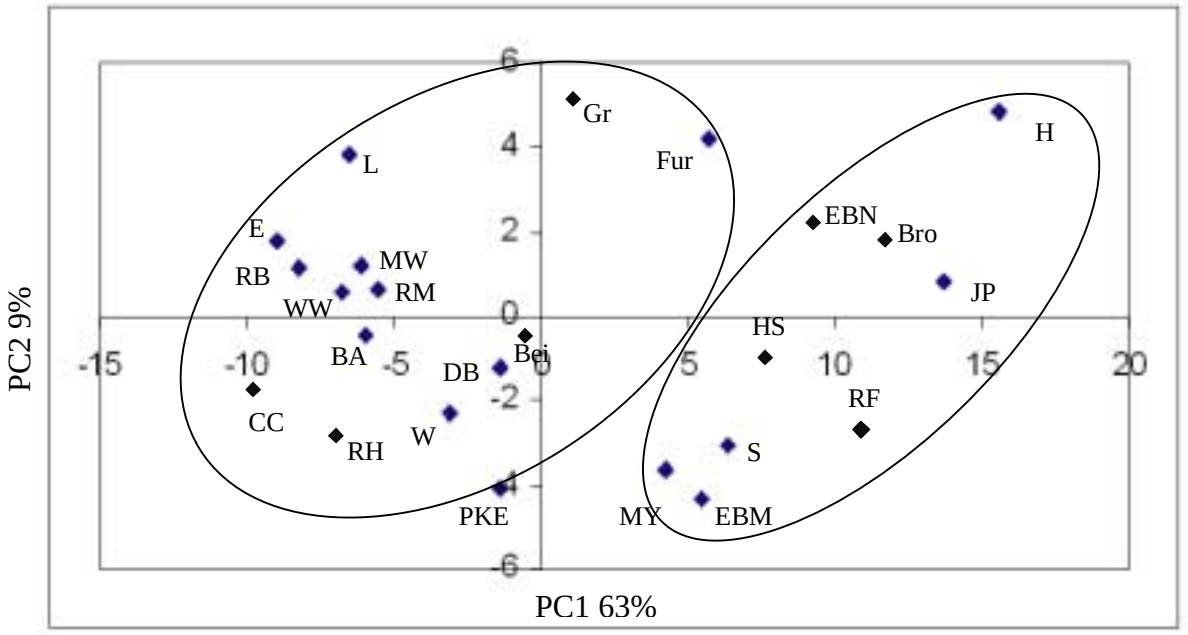


*Figure 3.7:* Distribution of microbial community structure from Pennsylvania farms with different soil management analyzed by PCA of intensity reactions. Livestock farms: Bro, S, MY, H, RF, JP, HS, MW. Cash Crop, manure: EBM, EBN, Gr, PKE, Fur, Bei. Cash Crop-no manure: RM, RH, RB, L, BA, W, E, DB, CC. Wastewater Irrigation farm: WW. Ellipses separates groups of livestock farms from cash crop and wastewater irrigation farms. Percentages on principal components represent the amount of variation explained by each PC.

As in the PCA analysis, farms with different soil management were separated in cluster analysis of CLPPs based on intensity patterns (Figure 3.8). In contrast with CLPPs based on presence/absence patterns (Figure 3.6), intensity patterns distinguished livestock and cash crop manure farms from cash crop-no manure and wastewater irrigation farms. As with CLPPs based on presence/absence patterns, MW livestock farm was misclassified in the cash crop-no manure cluster. MW farm is the only sampled farm that received hog manure. However, it is unclear if the differences in CLPP patterns are attributed to organic addition differences or to a specific unknown management practice on this farm.

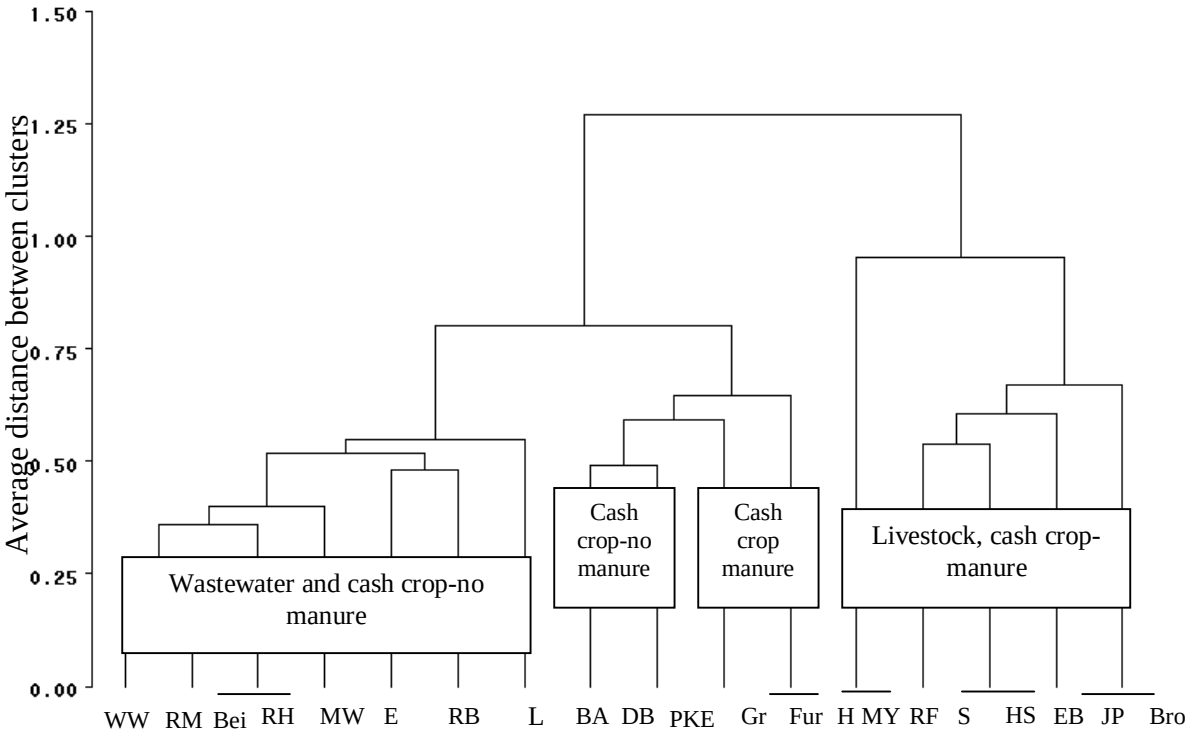


*Figure 3.8:* Distribution of microbial community structure from Pennsylvania farms with different soil management analyzed by cluster analysis of intensity reactions. Livestock farms: Bro, S, MY, H, RF, JP, MW, EB(M/N). Cash Crop, manure: EB (M/N), Bei, PKE, Fur, Gr. Cash Crop-no manure: RB, RM, RH, L, BA, W, E, DB, CC. Wastewater Irrigation farm: WW.

3.3.2.4. Microbial CLPP analyzed by different C guilds.

Mean value by guilds of C substrate utilization on the Biolog plates was assessed with PCA after classification among five different guilds (Table 3.7). Although PC1 explained most of the variation (83%), loading values were similar for each of the C guilds. The PC2 explained only 11% of the total variation with only the loading value for polymers being different from the rest of loadings for the C substrates. Therefore, despite the range in farm soil management, there was no overall difference in C substrate utilization when aggregated at the guild level (Table 3.7). Absolute values of loadings in PC1 and PC2 are small compared to absolute values of the previous PCA of microbial

CLPPs based on intensity data of Pennsylvania farms. Similar to the results from HRE, the lack of differences among C guilds substrate utilization suggest that microbial utilization by guilds is relatively uniform compared to the utilization of all substrates considered separately. Therefore, as also concluded for HRE, assessment of microbial community structure by utilization of different C guilds did not represent a useful tool for CLPP evaluation or an effective indicator of soil quality.

*Table 3.7:* Principal components analysis of mean intensity reactions of C guilds from 1997-2000 in Pennsylvania farms.

| Guilds | *Principal Components* | |
|---|---|---|
| | PC1 | PC2 |
| | loadings | |
| Carbohydrates | -0.447 | -0.180 |
| Polymers | -0.361 | 0.898 |
| Carboxylic Acids | -0.466 | -0.118 |
| Amines/Amides | -0.470 | -0.021 |
| Amino acids | -0.452 | -0.383 |
| *Variation explained* | *83%* | *11%* |

On the other hand, it is also possible that the C substrates represented in the Biolog plates are not the best array of environmental substrates to characterize microbial CLPP patterns and preferences in agriculture management systems. Given the constraints of the Biolog procedure, it is clear that this method provides information only on physiological potential of those organisms that can utilize these substrates under the conditions of the Biolog procedure rather that *in situ* activity (Garland and Mills, 1999).

3.3.2.5. Substrate Diversity Index

There was no significant difference in substrate diversity index (H) from a sample of Pennsylvania farms with different soil management histories. Mean H for all farms was 3.96 ± 0.2 (a complete substrate diversity index table is presented in Appendix E). Therefore, microbial CLPPs from Pennsylvania farms could not be distinguished based on measures of diversity that encompasses both the aggregation of number of utilized C substrates and the measured equitability of activities across all plate substrates (Zak et al., 1994). Moreover, this illustrates the fact that measures of diversity do not necessarily provide information about the composition (or structure) of the microbial community, i.e. two farms can have the same H but still catabolize different substrates (Zak et al., 1994) as observed in Table 3.6.

Conversely, studies of diverse saline systems (salt marsh, sand dune, and seawater irrigation system) by Goodfriend et al. (1998) clearly differentiated among treatments by measuring only substrate richness as measure of microbial diversity index. However, the two agricultural sites (fallow and cultivated) did not differ in substrate richness. Similarly, Degens et al. (2001) found increased catabolic evenness (as microbial diversity index) in soil microbial communities from long-term grass pastures compared to cropping systems when measuring resistance of the microbial communities to soil environmental disturbances (wet-dry and freeze-thaw cycles).

Therefore, substrate diversity indexes may effectively be used to initially assess functional diversity of soil systems such as the HRE. However, this tool was not sensitive enough to establish differences of microbial CLPPs from the Pennsylvania farms where confounding effects of soil management practices may hinder clear differences in microbial CLPPs.

### 3.4. Conclusions

Microbial activity and soil microbial community structure from lime fertility treatments and crop rotations that included manure additions and small grains and/or forages were distinguished from those with industrial fertilizer and continuous corn or corn-soybean rotations in the long-term HRE. The assessment of mean plate intensity, patterns of presence/absence reactions, number of C substrates utilized, and substrate diversity index were evaluated for their usefulness as indicators of soil management impacts on microbial CLPP. The assessment of patterns of intensity reactions did not clearly separate all HRE treatments. The long-term, consistent soil management practices in the HRE research experiment may be a key factor that selects microbial community structure and diversity due to the repetition of practices through time. The probable synergistic effect of crop diversity and manure additions created distinct microbial CLPP patterns. However, crop rotations that included the addition of forages and/or small grains had greater impacts on CLPP patterns with industrial fertilizer than with the addition of dairy manure.

Farms with organic amendment additions in their soil management were generally separated from cash crops-no manure additions, or wastewater irrigated farms by assessment of soil microbial CLPP intensity patterns, mean plate intensity, and number of C substrates utilized. The analysis of patterns of presence/absence reaction did not clearly separate the impact of soil management practices on microbial CLPP. This may result from a lack of clear shifts of microbial communities due to the dynamic nature of actual soil management practices on working farms. Farms with different soil management and less consistent cropping systems or external inputs than the long-term study (HRE) may contain a wide range of C substrates supporting microbial population in community structures that readily adapt to diverse cropping and management conditions.

Most of the soil management practices evaluated in this research were related to addition of organic C. Therefore, the observed effect on the soil microbial community, as seen in the different microbial CLPP reaction patterns, could be attributed to OM influences. Thus, characterization of CLPP could be an important factor for monitoring

the impact of soil management on microbial community activity and community structure, as a soil quality biological indicator. However, differences in CLPP patterns were observed with the use of patterns of presence/absence or intensity reactions in both experiments. Further studies are needed to explore which CLPP analysis approach should be used and in which situation, in order to assess soil management impacts on agricultural sites. On the other hand, future studies may encompass a detailed analysis of the C substrates utilized in each soil management treatment, and of yearly variations of microbial communities in the HRE.

Finally, future studies are necessary to confirm that changes in community structure and catabolic diversity simultaneously or subsequently result in changes in soil processes, and/or may confer soil abilities to resist to stress or disturbance. However, it is more conservative to adopt agricultural practices that preserve or restore microbial functional diversity than to adopt practices that diminish it.

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# Chapter 4

# SOIL QUALITY REMEDIATION IN A TREATED MUNICIPAL WASTEWATER IRRIGATION SITE

## 4.1. Introduction

### 4.1.1. Soil Quality Remediation

Many soil processes are readily affected by human intervention, especially through agricultural practices; therefore soil can be degraded and its inherent quality can be diminished. Soil remediation attempts to undo soil degradation. Therefore, land use and soil management options can be selected to alleviate specific soil and ecological constraints in achieving agricultural sustainability (Lal, 1997) and/or a specific soil function such as crop yield. According to Lal (1997), soil remediation can be achieved even during an intensive agricultural land use.

Effective remediation depends on identification of causative factors that can then be treated. Soil quality indicators may provide an initial assessment of soil degradation and help to identify potential management and remediation procedures. Hence, "identification of possible methods of soil restoration is facilitated by knowledge and possible enhancement of the key soil properties that influence soil quality and their critical limits in relation to the severity of soil degradation" (Lal, 1997). Soil biological and biochemical indicators provide a means for assessing the degree of soil degradation because they act as early and sensitive indicators of soil ecological stress or restoration (Dick and Tabatabai, 1993).

One method to reverse soil quality degradation is the addition of organic matter (Bastian and Ryan, 1986). Soil organic matter (SOM) is linked to desirable soil physical, chemical, and biological properties and is closely related to productivity. Soil C is a major component of SOM that influences the quality of soils and can be influenced by

soil management functions. In recent years compost materials have been used in agricultural lands for both waste disposal and to improve soil conditions and maintain the production system. These materials are rich in energy sources that increase the soil microbial population and its activities, and thus reactivate biogeochemical nutrient cycles (Pascual et al., 1997). Organic wastes also increase the soil water-holding capacity, aggregation and nutrient cycling. Municipal solid waste compost has also been applied for soil quality remediation purposes (Pascual et al., 1998).

### 4.1.2. Soil landscape positions

Hillslopes are ubiquitous landforms that can be described by three components: gradient, slope length, and slope width (Ruhe, 1975). Hillslopes in open and closed systems can be classified as three slope positions: summit/shoulder, backslope, and depression. The variability of soil properties among different positions is often large in complex landscapes (Miller et al., 1988). Soil physical properties such as clay content and distribution with depth, sand content, organic matter and pH have been shown to be highly correlated with landscape position (Ovalles and Collins, 1986).

Soil aggregate stability can also be moderately variable in different landscape positions (Pierson and Mulla, 1990). Difference in soil characteristics were observed in landscape studies on the Penn State Wastewater Irrigation Site (Dadio, 1998). Crop yields on hillslopes are affected by topography and the attendant differences in soil properties (Ovalles and Collins, 1986). Ponding in the bottom of a closed hillslope produces uneven nutrient distribution across the landscape and hinders crop management of the system, reducing crop growth in the shoulder/summit location, and potential nutrient removal (Dadio, 1998). Moreover, lack of good corn stand establishment has also affected crop yield in closed hillslopes of the Penn State Wastewater Irrigation Site.

Corn grain and silage yields can be greater in foot/toeslope positions due to greater soil moisture, nutrient availability, and organic matter content in those positions (Timlin et al., 1998; Wright et al., 1990). The effects of landscape position on grain yield

have been recognized by Mulla et al. (1992), Pan and Hopkins (1991a), and Ovalles and Collins (1986). Kravchenko and Bullock (2000) consistently observed higher corn yields in lower landscape positions than backslope or summit positions on a field scale. However, there are not many descriptions of relationships among yield and soil properties such as microbial characteristics in landscape positions of closed hillslopes. Analysis of soil enzymes and microbial biomass C determinations were previously performed on soil samples from the shoulder/summit and backslope locations of the Penn State Wastewater Irrigation Site (Bucher, 1999). Enzyme activities and microbial biomass C were considerably less than other Pennsylvania farms.

Because farmers tend to manage fields as a single unit, production inputs and management practices are often similar across the various landscape positions represented in a field. Knowledge of the spatial variability and distribution of crop yield can be used to adapt management practices for specific locations (Timlin et al., 1998). Therefore, soil quality indicators could provide insight to the potential basis for differential soil management practices within a complex landscape. The addition of organic matter, such as composted material to low production areas, as a remediation treatment, could be the basis for positive changes in soil quality indicators. Division of fields into smaller units of landscape positions would allow organic amendments inputs to enhance microbial activity and nutrient mineralization, and to promote yield increases within each landscape position.

## 4.2. Materials and Methods

### 4.2.1. Penn State Wastewater Irrigation Site

The Penn State Wastewater Irrigation site is located in Centre County, PA. The soils in the area are largely limestone-derived with common closed hillslope systems. The soil series include (Braker, 1981): Hagerstown (fine, mixed, mesic Typic Hapludalfs), Hublersburg (clayey, illitic, mesic, Typic Hapludults) and Morrison (fine-loamy, mixed, mesic Ultic Hapludalfs). The cropping system before the experiment was corn-wheat-

soybeans with winter rye cover crop and field irrigation with secondary-treated effluent (up to 264 cm $yr^{-1}$) from The Pennsylvania State University Wastewater Treatment Plant since 1983. Chemical analysis from 1997-2000 of the wastewater effluent is in Appendix D. During the experiment, only corn with a rye cover crop was grown in the research plots. Glyphosate (Roundup Ultra) at 1.75 L $ha^{-1}$ was applied to control the rye cover.

The study area was in a closed hillslope system with three landscape positions: the summit/shoulder (S/S), backslope (BS) and depression (D). Twenty-four plots (1.86 x $10^{-3}$ ha $plot^{-1}$) were laid out among the three positions. Chemical and physical characteristics of landscape positions are summarized in Table 4.1. Total soil carbon (TSC) was analyzed by a combustion method (Campbell, 1991). Available soil phosphorous (P) and available soil potassium (K) were determined using the Mehlich-3 extraction procedure (Wolf and Beegle, 1991). All samples were analyzed at the Agricultural Analytical Services Laboratory.

*Table 4.1*: Attributes of landscape positions of a soil quality remediation study

| Landscape Position* | Slope | Relative elevation | TSC | Mehlich3-P | Mehlich3-K |
|---|---|---|---|---|---|
| | % | m | g C $kg^{-1}$ soil | mg $kg^{-1}$ | mg $kg^{-1}$ |
| D | 0.2 | 0 | 22 | 120 | 112 |
| BS | 1.2 | 0.5-1 | 11 | 65 | 70 |
| S/S | 0.8 | 1.2 | 15 | 95 | 80 |

* D: Depression, BS: Backslope, and S/S: Summit/shoulder.

Unscreened wood chip and dairy manure compost from The Pennsylvania State University Office of Physical Plant Compost Facility at 45, 90, and 134 Mg $ha^{-1}$ compost was added. Following manual application of compost with pre-weighed buckets, a field cultivator was used to incorporate the applied compost (corn seeding depth or at a depth of 7.8 cm). Eastland 624 corn seed (108 day relative maturity) was planted at 88400 thousand seeds per hectare with a 4-row John Deere corn planter. Planting depth was

between 3 and 5 cm deep. Corn received 134 kg $ha^{-1}$ of N-P-K starter fertilizer (10-20-10). Prowl and Atrazine herbicides were applied at 3.5 L $ha^{-1}$, and 2.3 L $ha^{-1}$ respectively for weed control. The insecticide permethrin (Pounce) was applied at 350 g $ha^{-1}$ for pest control. The field was side-dressed with N-solution at a rate of 90 N kg $ha^{-1}$. Analysis of compost material was performed by The Pennsylvania State University Agricultural Analysis Laboratory (Table 4.2). The experimental design used for the treatment application was a split-plot design with landscape position as main effect and compost treatment as subplot. There were two replicates per block.

*Table 4.2*: Characteristics of compost used in the soil quality remediation study from 1999-2000.

| Year | Solids | TSN | TSC | C/N | P | K |
|---|---|---|---|---|---|---|
| | % | | g $kg^{-1}$ | | | |
| 1999 | 67.6 | 5.6 | 13.3 | 23.8 | 0.12 | 0.17 |
| 2000 | 65.0 | 3.8 | 7.7 | 20.3 | 0.15 | 0.30 |

4.2.2. Soil sampling and handling procedures

Soil samples were collected in June of 1999 and 2000 for enzyme determination, total soil carbon (TSC), total soil nitrogen (TSN), microbial biomass C, aggregate stability, and community level physiological profiles (CLPP). The samples were collected by compositing 20 to 25 cores (2 x 15 cm) per plot. All samples were split and one portion air-dried and ground to < 2 mm for chemical (TSC, TSN, and standard fertility soil test) analysis and biochemical analyses (enzyme activities). Aggregate stability was determined with field moist soils. The reminder of the soil samples were stored at 4 °C for a maximum of 4 weeks for additional biochemical analysis (community-level physiological profiles and soil microbial biomass C).

4.2.3. Analysis of soil quality indicators

4.2.3.1. Crop population and yields

Silage corn was harvested from the two middle rows of each plot when the grain was at approximately at ½ milk line maturity. Corn plants were weighed, six plants were sub-sampled, chopped, and a sub-sample collected for moisture determination and analysis. Final yield calculation was based on a dry-matter content after corn plants were oven-dried at 105 °C. Yields were reported in Mg $ha^{-1}$ at 65 % moisture content. Plant population by counting corn stands at 30-40 days of age was determined both years in each experimental plot, to assess corn stand establishment. Plants were analyzed for N content at The Pennsylvania State University Agricultural Analytical Services Laboratory (Doty et al., 1982).

4.2.3.2. Biochemical Analysis

4.2.3.2.1. Enzyme determinations

Soil acid phosphatase, arylsulphatase and β-glucosidase activities were determined using P-nitrophenylphosphate (PPNP, 0.5 M), P-nitrophenylsulfate (PNS, 0.5 M) and P-nitrophenyl-β-D-glucoside (PNG, 0.5 M) as enzyme substrates, respectively. These substrates were purchased from Sigma Inc. (Sigma Chemical Co., St. Louis, MO), and stored at -4 °C. Enzyme activity was based on p-nitrophenol (PNP) released by 0.5 g of air-dried ground soil incubated with Modified Universal Buffer (MUB, pH 6.5) solution and the substrate for 30 minutes at 37 °C. The reaction was stopped by the addition of NaOH (0.5M), and $CaCl_2$ (0.5M) to the soil suspension. The suspension was filtered (Whatman No. 42) and absorbance of the filtrate was measured at a wavelength of 410 nm with a Spectronic 1001 split beam spectrophotometer (Tabatabai, 1994). Controls were processed to account for color not derived from p-nitrophenol released by enzyme activity. The same procedure as for the enzyme assay was followed for the controls but the substrate was added to the soil after incubation and immediately prior to

the stopping the reaction. The results were expressed as micrograms of p-nitrophenol released per gram of soil per hour of reaction.

4.2.3.2.2. Soil microbial biomass C determination

Soil microbial biomass carbon (SMBC) was determined by a modified chloroform fumigation and direct extraction method (modified method of Sparling and West, 1988; Hedley and Stewart, 1982; Gregorich et al., 1990; Mele and Carter, 1996). Twelve grams of soil samples were weighed into glass tubes and directly incubated for 24 h with 2-3 ml of liquid ethanol-free chloroform. After that, the glass tubes were opened and left 24h under an extraction hood to allow for maximum evaporation of the chloroform. Soil microbial biomass C was extracted with 0.5 M $K_2SO_4$ with a 2:5 soil weight/extractant volume. The extracted solution was filtered (Whatman filter No. 42) and SMBC was determined using a Shimadzu Carbon Analyzer (TOC-5000). The extracted solution was acidified with 1 M HCl, converting inorganic C (l) to $CO_2$ (g) that was evaporated by sparging the sample with $CO_2$-free air. The sample was then inserted into the furnace and organic C was converted to $CO_2$ in the presence of a platinum catalyst at 680 °C. The $CO_2$ was measured with a non-dispersive gas infrared detector. Soil microbial biomass carbon was calculated as the difference between fumigated and non-fumigated samples, divided by an efficiency constant Kc= 0.17 (Gregorich et al., 1989). The results were expressed as micrograms of soil microbial biomass C per gram of soil.

4.2.3.2.3. Community-level physiological profiles

The Biolog microplate identification system was used to assess the community-level physiological profiles or functional substrate utilization differences in microbial communities from sole-source carbon utilization patterns (Zak et al., 1994). In this method, 150 µl of a 1:5000 distilled water soil suspension were added to each of the wells of the microplate. Utilization of specific carbon sources by bacteria from a set of 95 different carbon compounds on gram negative (-) microplates was determined by color development (tretazolium dye). Absorbance was measured after 72 hours incubation at

room temperature using a Shimadzu Plate reader at λ 590 nm. Presence or absence of color reactions was utilized as the variable to analyze results.

4.2.3.3. Chemical Analyses

4.2.3.3.1. Total Soil Carbon

Air-dried and ground to <2 mm soil samples were used to determine Total Soil Carbon (TSC). This indicator was measured by combustion at 680 °C with a Shimadzu Carbon Analyzer (TOC-5000) using a solid sample module (SSM-5000) and a non-dispersive infrared gas analyzer to detect the emitted $CO_2$. The results were expressed as grams of soil C per kilogram of soil.

4.2.3.3.2.Total Soil Nitrogen

Air-dried and ground to <2 mm soil samples were used to determine Total Soil Nitrogen (TSN). This chemical indicator was measured by total combustion with a Fisons NA1500 Elemental Analyzer at The Pennsylvania State University Agricultural Analytical Services Laboratory (Campbell, 1991). The results were expressed as grams of soil N per kilogram of soil.

4.2.3.3.3. Standard Fertility Soil Test

The soil quality samples (air dried and ground to <2 mm) from each plot were subsampled and submitted to The Pennsylvania State University Agricultural Analysis Laboratory for pH, P, K. The pH measurements were determined using the 1:1 soil:water method (Eckert and Sims, 1991), and available P, K, were measured using the Mehlich-3 extraction procedure (Wolf and Beegle, 1991).

4.2.3.4. Physical Analyses

4.2.3.4.1. Aggregate Stability

Aggregate stability was measured using a standard wet sieving method (Kemper and Rosenau, 1986) with new improvements by Amezketa et al. (1996). Four grams of moist soil sieved through a 2 mm and retained on a 0.4 mm sieve (<2 mm-diam aggregates) were placed on a 60 mesh sieve on a shaker. The sieves were raised and lowered through a 1 cm vertical distance at 36 cycles per minute for 5 minutes in distilled water cans. Material remaining on the sieve after 5 minutes was oven dried (105 ºC) and weighed to give a stable aggregate mass (SA). This was expressed as a percentage of the initial total weight.

4.2.3.4.2. Soil Shock Attenuation

Soil shock attenuation and impact characteristics of the soil surface were measured by dropping a 2.25 kg missile from a specific height onto the surface (Annual Book of ASTM Standards, 1997). A linear accelerometer mounted on the missile monitored the acceleration and the time history of the impact. The maximum gravitational acceleration ($G_{max}$) is detected in order to determine soil compactibility by each impact. An average of 10 impacts per plot were collected.

4.2.4. Statistical Analysis

Analysis of variance (ANOVA) was performed for individual indicators using the Statistical Analysis System (SAS, 1985). CLPP was analyzed using cluster analysis, a multivariate statistical procedure. Mean separation procedure was calculated with Least Square Means for pair comparisons in the interaction tables and Duncan's Multiple Range Test with a p-value of 0.05 for main effect comparisons. The significance of the triple interactions were evaluated by performing F-tests on the sum of squares of the main factors and the triple interaction sum of squares (Steel and Torrie, 1997).

## 4.3. Results and Discussion

4.3.1. Crop yields and plant population

Landscape position, compost treatments, landscape*year, and landscape*compost were significant sources of variation in corn silage yields (Table 4.3). Landscape effect was different each year (significant landscape*year interaction). The most important source of variation was landscape*compost, indicating that compost addition influences yield differently in the different landscape positions. Plant populations had no significant sources of variation (Table 4.3). Corn population (86074 ± 8712 plants per ha, Appendix C) was also not significantly different across all treatments.

*Table 4.3*: Analysis of variance of soil quality indicators from 1999-2000 in the soil quality remediation study from 1999-2000.

| | **Source of Variation** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Variables** | **Year (Y)** | **Rep** | **Landscape (L)** | **LxY** | **Compost (C)** | **LxC** | **CxY** | **LxCxY** |
| **df** | 1 | 1 | 2 | 2 | 3 | 6 | 3 | 6 |
| **Corn yields** | ns | ns | * | * | * | * | ns | ns |
| **Plant Population** | ns | ns | ns | ns | ns | ns | ns | ns |
| **Phosphatase** | ns | ns | * | ns | * | * | * | * |
| **Arylsulfatase** | ns | ns | ns | ns | * | ns | ns | ns |
| **β-glucosidase** | ns | ns | * | ns | * | * | * | * |
| **Microbial Biomass C** | ns | ns | * | * | * | * | * | * |
| **TSC** | ns | ns | ns | ns | * | ns | ns | ns |
| **TSN** | ns | ns | * | * | * | * | * | * |
| **Aggregate Stability** | * | ns | * | ns | * | ns | ns | ns |
| **Soil shock Attenuation** | ns | ns | * | ns | ns | ns | ns | ns |

* significantly different at $p<0.05$.
ns: not significant.

Corn yields with no compost treatment were higher in D than in the S/S or BS (Table 4.4). These results agree with previous findings of greater corn yields in D compared to the BS or S/S positions due to increased moisture, organic matter and nutrient-rich sediment accumulation in the lower positions (Kravchenko and Bullock, 2000; Ovalles and Collins, 1986). Corn silage yields with no compost treatment was significantly different in D from BS or S/S. However, only in the S/S corn silage yields increased (and reached similar yields of the D) with all compost addition treatments (Table 4.4). The lack of yield response in the BS position across all compost treatments may be due to other limiting factors not addressed in this study, such as uneven irrigation distribution on the BS position and a tendency for lower plant populations.

Lack of uniform plant populations across all landscape positions was determined to be a limiting factor for crop yields in previous years (Lanyon, personal communication). Because of no significant differences in plant populations across all treatments, corn stand establishment did not contribute to differences in crop yields across remediation treatments and landscape positions.

*Table 4.4*: Corn silage yields from 1999-2000 in three landscape positions of the soil quality remediation study.

| **Landscape Positions*** | **Compost Rate** (M*g ha*$^{-1}$ wet weight) | | | |
|---|---|---|---|---|
| | **0** | **45** | **90** | **134** |
| | | *(Mg ha$^{-1}$ 65% moisture)* | | |
| D | 21.7 Aa | 21.6 Aa | 22.1 Aa | 22.7 Aa |
| BS | 18.2 Ab | 18.6 Ab | 18.7 Ab | 19.2 Ab |
| S/S | 19.1 Db | 20.9 Ca | 22.6 Ba | 24.3 Aa |

Values within a row sharing the same capital letter or within a column sharing a small letter are not significantly different with pairwise predicted differences at $p<0.05$.

*D: Depression, BS: Backslope, and S/S: Summit/shoulder.

compost application in the summit position may have provided added benefits of increased organic matter, soil structure and biological activity (Pera et al., 1983, Perucci, 1990, Quedraogo et al., 2001) that influenced corn yield increase. Hence, corn silage yield as an indicator of the remediation process, was sensitive to soil management effects in one of the two low soil quality areas in this landscape.

### 4.3.2. Biochemical Indicators

#### 4.3.2.1. Enzyme determinations

Acid Phosphatase

Acid phosphatase activity was different in the three landscape positions as well as with different compost treatments in both years (Table 4.3). The interaction of landscape*compost, as a significant source of variation, indicates that compost additions affect enzyme activities differently in the landscape positions (Table 4.3). Phosphatase activity was equal to or higher in D compared to S/S position for all compost treatments (Table 4.5). Phosphatase activity in the BS position was not different at 0 and 45 Mg ha $^{-1}$. The increase of enzyme activity in response to compost addition was greater in BS and S/S (66% and 108%, respectively) than in D (58%) (Table 4.5). This result can be attributed to compost providing readily utilizable substrates, promoting microbial growth, and increasing enzyme activity in these low quality areas where enzyme activity is most limited (Peacock et al., 2001).

However, changes in enzyme activity in response to compost management in D positions may be small relative to the background activity, in contrast to the other positions. Large amounts of already stabilized enzymes may be present in the D position due to increased OM accumulation (Peacock et al., 2001; Dadio, 1998) (Table 4.1). Elevated enzyme activities appear to be associated with soil management conditions that promotes SOM accumulation, and thus, enhances stabilization and preservation of extracellular enzymes by the increased amounts of enzyme-humus complexes (Bergstrom et al., 1998, Garcia et al., 1994). Bergstrom et al. (1998) also reported greater total carbon

(TC), and measured greater phosphatase enzyme activities compared to BS and S/S positions in coarse textured soils of a poorly drained location at the bottom of a slope. Therefore, according to our results, phosphatase activity was a useful indicator of soil quality remediation treatments with compost addition in the different landscape positions.

*Table 4.5*: Acid phosphatase activity from 1999-2000 in three landscape positions of the soil quality remediation study.

| **Landscape Positions*** | **Compost Rate** *(Mg ha$^{-1}$)* | | | |
|---|---|---|---|---|
| | **0** | **45** | **90** | **134** |
| | *µg PNP g$^{-1}$ h$^{-1}$* | | | |
| D | 243 Da | 330 Ca | 367 Ba | 385 Aa |
| BS | 229 Ca | 255 Cb | 304 Bb | 381 Aa |
| S/S | 181 Cb | 305 Ba | 311 Bb | 376 Aa |

Values within a row sharing the same capital letter or within a column sharing a small letter are not significantly different with pairwise predicted differences at $p<0.05$.
*D: Depression, BS: Backslope, and S/S: Summit/shoulder.

Arylsulfatase

In contrast with phosphatase activity, compost treatment was the only significant source of variation for arysulfatase activity (Table 4.6). Although not significant, the landscape*compost interaction table is presented in Appendix C to provide results in a format similar of the other enzymes. As with previous enzymes, background levels of arylsulfatase with no treatment addition were greater in the D than BS and S/S positions (226, 157, and 153 *µg PNP g$^{-1}$ h$^{-1}$* respectively) (Appendix C). Means of enzyme activity for all locations increased as compost treatment increased, although arylsulfatase activity with 0 Mg ha$^{-1}$ was not significantly different from 45 Mg ha$^{-1}$ treatment (Table 4.6). Highest mean of enzyme activity for all treatments was obtained with 134 Mg ha$^{-1}$ treatment. Arylsulfatase activity is responsible for organic S mineralization in soil (Tabatabai, 1994) and represents an important indirect indicator of fungi, which are the

only soil microorganisms containing ester sulphate (Bandick and Dick, 1999). These authors found that arylsulfatase activity positively correlated with soil organic C content in different soil management practices that included compost addition. Similar compost effect on arylsulfatase activity within all landscape positions suggested that this enzyme was not a useful indicator of soil quality remediation treatment.

*Table 4.6*: Arylsulfatase activity from 1999-2000 in the soil quality remediation study.

| **Compost** | **Arylsulfatase** |
|---|---|
| *Mg ha$^{-1}$* | *$\mu$g PNP g$^{-1}$ h$^{-1}$* |
| 0 | 179 a |
| 45 | 195 ab |
| 90 | 203 b |
| 134 | 250 c |

Values with same letters are not significantly different with DMS at $p<0.05$

β-glucosidase

β-glucosidase activity was different in the three landscape positions as well as with different compost treatments in both years as was phosphatase activity (Table 4.3). The interaction of landscape*compost was a significant source of variation indicating that compost addition affected β-glucosidase activities differently in the landscape positions. Enzyme activity was greater in D and S/S with compost treatment greater than 45 Mg ha$^{-1}$ than BS position (Table 4.7). β-glucosidase activity in the S/S position was not significantly different from the D position when 134 Mg ha$^{-1}$ of compost was applied. This high compost rate could provide sufficient organic matter to promote a high enzyme activity in the most C limiting positions. The enzyme activity increase with compost addition was greater in the S/S than in the B/S or D positions, as previously seen with phosphatase enzyme. Decrease in enzyme activity in the BS position with the 90 Mg ha$^{-1}$ application could not be explained.

*Table 4.7*: β-glucosidase activity from 1999-2000 in three landscape positions of the soil quality remediation study.

| **Landscape Positions*** | **Compost Rate** (Mg ha$^{-1}$) | | | |
|---|---|---|---|---|
| | **0** | **45** | **90** | **134** |
| | *μg PNP g$^{-1}$ h$^{-1}$* | | | |
| D | 187 Da | 202 Ca | 216 Ba | 301 Aa |
| BS | 168 Cb | 194 Ba | 179 Cb | 271 Ab |
| S/S | 144 Dc | 172 Cb | 218 Ba | 294 Aa |

Values within a row sharing the same capital letter or within a column sharing a small letter are not significantly different with pairwise predicted differences at $p<0.05$.
*D: Depression, BS: Backslope, and S/S: Summit/shoulder.

Enzyme activities suggested that soil remediation was most effective in the S/S, as observed by the increase in all enzyme activities. According to previous studies of Pennsylvania farms and on the wastewater irrigation farm (Bucher, 1999), thresholds in this study for enzyme activities, comparable to conditions judged to be of high soil quality, should be ≥300 μg PNP g$^{-1}$ soil for phosphatase, and 200 μg PNP g$^{-1}$ soil for β-glucosidase. These activity levels generally required the application of >90 Mg ha$^{-1}$ of compost in the S/S and BS positions, but only 45 Mg ha$^{-1}$ of compost in the D position. This difference in remediation effort to achieve target enzyme activities reflects the background levels in the different positions.

However, not all the enzymes were equally sensitive to the remediation process and landscape positions, as observed with arylsulfatase activity. It is difficult for enzyme activity alone to reflect the overall state of nutrients and microbial dynamics in soil (Nannipieri et al., 1990). According to Lovell et al. (1995), soil enzyme activity is often influenced by the additions of OM that increase SOM. Cooper and Warman (1997) suggested that initial organic C status of the soil must be considered when assessing soil management impacts on enzyme activity; higher organic C soils may already have higher levels of enzyme activities. This is in agreement with our findings of acid phosphatase

and β-glucosidase activities in the D position. If this is the case, management practices, which might be beneficial to low organic C soils, may be dismissed as ineffectual if tested in high organic C soils. Therefore, SOM could also be used as a first criterion to evaluate site-specific and landscape soil remediation efforts. Compost addition was a useful soil remediation tool that positively affects the activity of two of the three soil enzymes in low soil quality areas.

4.3.2.2. Microbial Biomass C

Compost additions affect soil microbial biomass C (SMBC) in a different way at each landscape position as seen in the significant landscape x compost interaction (Table 4.3). Microbial biomass C was higher in D compared to BS and S/S position with all compost treatments (Table 4.8). In the S/S position, the SMBC was not different from the BS when ≥ 90 Mg $ha^{-1}$ was added (Table 4.8). On the other hand, the increase of SMBC with compost additions in the S/S position was greater than in BS and D, as previously observed with phosphatase enzyme activity. Increased SMBC could be attributed to an increase in soluble carbon and energy sources that can stimulate biogeochemical nutrient

*Table 4.8*: Microbial biomass C from 1999-2000 in three landscape positions of the soil quality remediation study.

| **Landscape Positions*** | **Compost Rate** *(Mg $ha^{-1}$)* | | | |
|---|---|---|---|---|
| | **0** | **45** | **90** | **134** |
| | *μg C $g^{-1}$ soil* | | | |
| D | 258 Da | 314 Ca | 453 Ba | 610 Aa |
| BS | 179 Cb | 194 Cb | 310 Bb | 507 Ab |
| S/S | 141 Dc | 303 Ca | 323 Bb | 521 Ab |

Values within a row sharing the same capital letter or within a column sharing a small letter are not significantly different with pairwise predicted differences at $p<0.05$.
* D: Depression, BS: Backslope, and S/S: Summit/shoulder.

cycles. Microbial biomass C, being the living part of soil organic matter, can be a good index for assessing natural as well as remediated ecosystems (Ross et al., 1982; Sparling et al., 1994). This important soil indicator responds to soil perturbation or restoration over a relatively short time. Pascual et al. (2000) reported that the addition of 65 and 260 Mg $ha^{-1}$ municipal solid compost to abandoned soils after intensive agricultural uses increased SMBC in these fields from 250 µg C $g^{-1}$ soil to 600 and 1000 µg C $g^{-1}$ soil, respectively. The authors believed treatments increased soil microbial population due to the amendments rich in carbon and energy sources. Moreover, their organic additions increased soil structure for plant cover development, which also acted as a carbon and nutrient source, and maintained the levels of organic matter and microbial biomass.

Therefore, low soil quality in different landscapes can be remediated by increasing organic matter content to enhance soil biological and biochemical properties. According to previous studies of Pennsylvania farms and on the wastewater irrigation farm (Bucher, 1999), thresholds in this study for microbial biomass C comparable to conditions judged to be of high soil quality was >400 µg C $g^{-1}$ soil. This SMBC level generally required the application of >134 Mg $ha^{-1}$ of compost in the S/S and BS positions, but only 90 Mg $ha^{-1}$ of compost in the D position. This difference in remediation effort to achieve target SMBC reflects the background levels in the different positions, as previously observed with soil enzymes. Therefore, SMBC was a useful indicator of compost addition as a soil quality remediation tool in different soil landscape positions.

Triple interactions

The components of the triple interaction among landscape*compost*year were analyzed for all indicators by F tests using the interaction mean square as the denominator (F=main effect mean square/interaction mean square). Year (MS=560) and landscape (MS=7946) main effects for phosphatase were not significantly different when tested with the triple interaction (MS=5440). Year (MS=67) and landscape (MS=2533) main effects for β-glucosidase were also not significantly different when tested with the

triple interaction (MS=549), but the compost effect (MS=34097) was significant. Year (MS=202150), landscape (MS=54368), and compost (MS=277839) main effects for SMBC were significantly different when tested with the triple interaction (MS=5332). These results suggest that the compost treatment effect on the soil biological indicators was not the same for each year and each landscape position. This may be explained by the cumulative effects on the plots of compost additions from 1999 to 2000. However, due to the short time frame of the experiment, triple interactions were not considered further.

### 4.3.3. Community Level Physiological Profiles

The CLPP (defined by the ability to metabolize C substrates on the Biolog plates) was analyzed using the presence/absence pattern of color reactions in the Biolog procedure. Separation of treatments by average linkage in the cluster analysis resulted in three different main groups (Figure 4.1). The first cluster included treatments with 0 Mg $ha^{-1}$ in S/S, and 0 and 45 Mg $ha^{-1}$ compost treatment in BS. The second cluster was divided in two clusters. The first one included all landscape positions and compost treatments > 45 Mg $ha^{-1}$, as well as a cluster that included 0 and 45 Mg $ha^{-1}$ compost treatments in the D position. The CLPP patterns suggested that landscape positions and the addition of compost treatments caused changes in microbial community composition. The microbial CLPP with 0 Mg $ha^{-1}$ or 45 Mg $ha^{-1}$ compost treatment in S/S or BS was different from the CLPP in D with 0 or 45 Mg $ha^{-1}$ compost treatment (Figure 4.1). This result corroborates previous findings of different backgrounds levels of SMBC and soil enzymes observed in D compared to the S/S position (Table 4.5 and 4.8).

Compost tended to reduce the landscape position effects on microbial CLPP as illustrated by the separate cluster for all landscape positions with compost treatments ≥90 Mg $ha^{-1}$. The clustering techniques are unlikely to yield perfect groups, but the few outliers in this analysis do not diminish the interpretation of background differences in

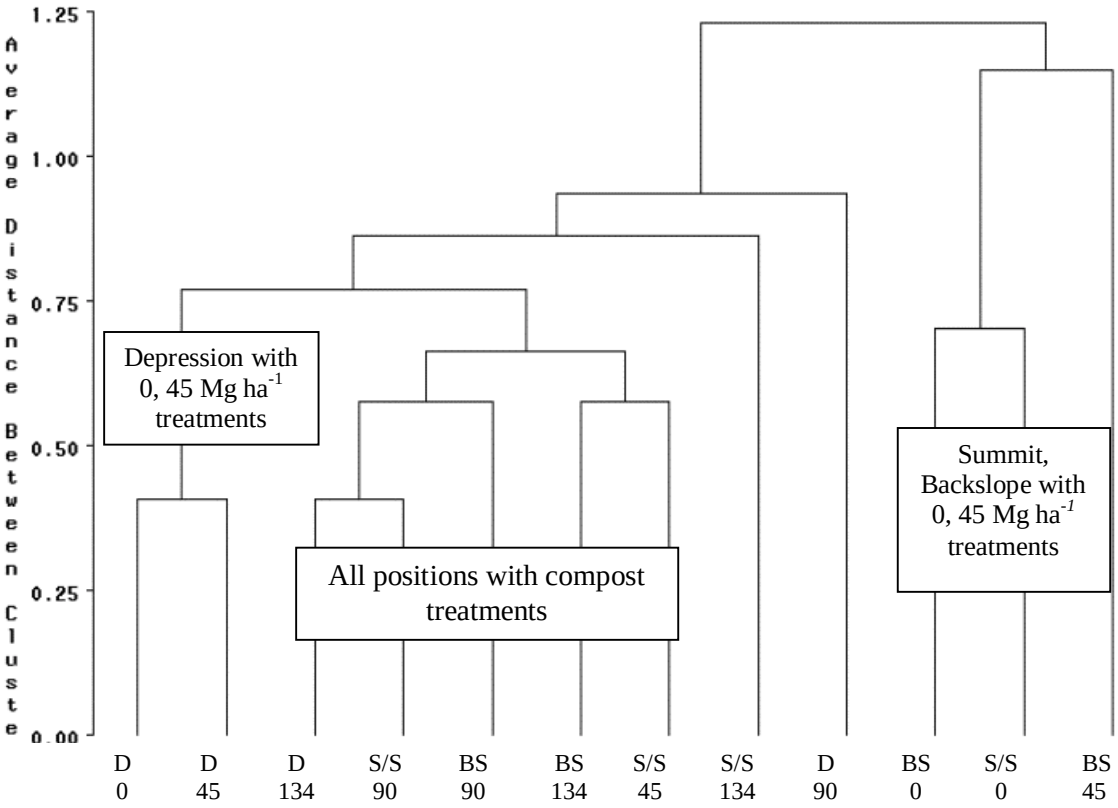


*Figure 4.1*: Community level physiological profile based on presence or absence of reaction in the soil quality remediation study from 1999-2000. Landscape position: Depression (**D**), Backslope (**BS**), and Summit/Shoulder (**S/S**). Compost treatments: 0, 45, 90, and 134 Mg ha$^{-1}$**.**

CLPP due to landscape position and convergence of CLPP with substantial compost additions. According to Peacock et al. (2001), Alvarez et al. (1998), Alvarez and Alvarez (2000), and Bending et al. (2000), organic additions provide a more stable and readily available stream of substrate to the microbial community than management practices with no organic inputs. Moreover, significant differences in microbial CLPPs were reported in the long-term HRE with soil management practices that included organic additions (Chapter 3). Therefore, soil management practices, such as composting or manuring, that result in accumulations of organic C, can promote shifts in microbial CLPP from areas with low microbial biomass backgrounds as S/S and BS (Figure 4.1).

Furthermore, community composition may influence the degradation of soluble C compounds because the enzymatic capacity for the initial steps of degradation occurs in a

comparatively limited number of microbial populations (Hu and van Bruggen, 1997). Although Biolog selects for only a portion of the microbial community (Smalla et al., 1998), the similar outcomes of landscape CLPP, enzyme activities, and microbial biomass in the remediation study suggest that CLPP is also a reliable soil quality indicator by which remediation treatments can be monitored.

### 4.3.4. Chemical Analysis

#### 4.3.4.1. Total Soil Carbon

Compost was the only significant source of variation in total soil carbon (TSC) (Table 4.3). TSC was significantly higher with compost addition > 90 than with 0 and/or 45 Mg $ha^{-1}$ (Table 4.9). On the other hand, higher levels of TSC in soils located in poorly

*Table 4.9*: Total soil C from 1999-2000 in the soil quality remediation study.

| **Compost** | **Total Soil Carbon** |
|---|---|
| *Mg $ha^{-1}$* | *g C $kg^{-1}$ soil* |
| 0 | 16 a |
| 45 | 17 ab |
| 90 | 19 b |
| 134 | 23 c |

Values with same letters are not significantly different with DMS at $p<0.05$

drained locations at the bottom of the slope, compared to BS and S/S positions, have been reported by Bergstrom et al. (1998), Ovalles and Collins (1986), and Changere and Lal (1997). Although remediation treatments did not affect TSC in the landscape positions, the greater microbial biomass C, enzyme activities (Table 4.5 and Table 4.8), and noticeably different CLPP in D position with no compost treatment, suggest that SOM may be more stabilized in this position than in the other landscape positions. Background

level of TSC was indeed higher in D than in the other positions in initial soil fertility tests (Table 4.1).

Therefore, in our remediation experiment, the increase in C with compost treatment seems only to represent increased C from the compost addition and not from differences in stabilized TSC or microbial activity levels in the three landscape positions. This agrees with suggestions that the labile soil quality attributes are more responsive to the management treatments than the TSC (McGill et al., 1988; Biederbeck et al., 1994). Furthermore, in cultivated soil, fertility management practices may not change TSC contents by more than 10% during short time periods (0-10 yr) (Jenkinson et al., 1987; Paustian et al., 1992). Small changes in TSC content may even be overshadowed by natural soil TSC heterogeneity.

Therefore, these results support the fact that TSC may not be an appropriate soil quality or soil remediation indicator due to very slow responses to changes in soil organic matter (Bucher, 1999; Pascual et al., 2000; Parr and Papendick, 1997) or rate of decomposition than SMBC, microbial CLPP, or enzyme activities.

#### 4.3.4.2. Total Soil Nitrogen

Total soil nitrogen (TSN) was higher in the D than in the other landscape positions with all compost treatment rates (Table 4.10). TSN increased as compost treatments increased in the D position. Total soil nitrogen content was significantly different in BS and S/S when 45 Mg $ha^{-1}$ and 134 Mg $ha^{-1}$ compost was added (Table 4.10). In BS, only treatment 134 Mg $ha^{-1}$ compost increased TSN when compared to the other treatments.

Several authors have suggested that TSN, as well as TSC, is less responsive to soil management practices than biological indicators (McGill et al., 1988), however we did observe a positive effect on TSN as compost treatments increased in all landscape positions. Peacock et al. (2001) also documented an increase in TSN after high doses of manure compared to inorganic N fertilizer treatments in a no-till experiment. As with β-

glucosidase, the decrease of TSN in the S/S and 90 Mg $ha^{-1}$ could not be explained. We are unaware of TSN thresholds in agricultural practices cited in the literature. However, because this is a high fertility treatment, increases in TSN may need to be closely monitored in these landscape areas. Excess N application from soil remediation treatments, industrial fertilizer, and wastewater irrigation may increase environmental degradation due to possible N leaching.

*Table 4.10:* Total soil N from 1999-2000 in three landscape positions of the soil quality remediation study.

| **Landscape Positions*** | **Compost Rate** Mg $ha^{-1}$ | | | |
|---|---|---|---|---|
| | **0** | **45** | **90** | **134** |
| | g N $kg^{-1}$ soil | | | |
| D | 1.3 Ca | 1.6 Ca | 1.7 Ba | 2.0 Aa |
| BS | 1.0 Bb | 1.0 Bc | 1.1 Bb | 1.3 Ac |
| S/S | 1.0 Bb | 1.3 Ab | 1.1 Bb | 1.5 Ab |

Values within a row sharing the same capital letter or within a column sharing a small letter are not significantly different with pairwise predicted differences at $p<0.05$.
*D: Depression, BS: Backslope, and S/S: Summit/shoulder.

4.3.4.3. Standard Fertility Soil Tests

Soil test K (STK), and soil test P (STP) were generally greater in the D position than in the BS and in the S/S at all rates of compost application (Table 4.11). Without compost treatment, STP and STK values agreed with the initial background levels of previous soil fertility tests (Table 4.1). The STP in B/S position was the lowest of all the landscape positions. This agrees with previous research (Miller et al., 1988; Dadio, 1998) indicating that fine soil particles, and associated soil nutrients, erode from BS positions and accumulate in lower positions. Additions of compost increased STP and STK in all landscape positions, although STK changes were not consistent with increasing

applications of compost (Table 4.11). Increased STP with compost addition in all landscape positions can also be related to increases in phosphatase activity occuring in these treatments (Table 4.5).

*Table 4.11*: Soil fertility results from 1999 to 2000 in the soil quality remediation study.

| Landscape Positions* | Treatment | pH | Mehlich-P | Mehlich-K |
|---|---|---|---|---|
| | *Mg ha$^{-1}$* | | *mg kg$^{-1}$* | *mg kg$^{-1}$* |
| D | 0 | 7 | 120b | 112b |
| | 45 | 7 | 125b | 99c |
| | 90 | 7 | 136a | 106b |
| | 134 | 7 | 145a | 127a |
| BS | 0 | 7 | 65f | 70f |
| | 45 | 7 | 60f | 80e |
| | 90 | 7 | 79e | 88d |
| | 134 | 7 | 84e | 100c |
| S/S | 0 | 7 | 95d | 80e |
| | 45 | 7 | 100d | 105bc |
| | 90 | 7 | 104cd | 85de |
| | 134 | 7 | 110c | 105bc |

* D: Depression, BS: backslope, and S/S: Summit/shoulder.

4.3.5. Physical Analysis

4.3.5.1. Aggregate Stability

Aggregate stability was not significantly different among all compost treatments in D (Table 4.12), where soil aggregates seemed already highly stabilized due to high background TSC values in this position (Table 4.1). These results seem to corroborate a suggested correlation between aggregate stability and SOM (Cambardella and Elliot, 1993). The stability of soil aggregates did not differ significantly between 0 and 45 Mg ha$^{-1}$, but was significantly higher with 90 and 134 Mg ha$^{-1}$ in BS and in S/S (Table 4.12).

Percentage of aggregate stability with compost treatment of 90 or 134 Mg $ha^{-1}$ did not differ significantly for all landscape positions.

Increased aggregate stability may be correlated with increased microbiological activity (Schjønning et al., 2000; Sparling et al., 1994; Balesdent et al., 2000), and reflected in previous results of enzyme activities and SMBC in D, BS and S/S due to compost additions. The increased aggregation in the S/S and BS could suggest a decrease in runoff, compaction, and even reduced potential for soil erosion from irrigation. Therefore, increased aggregate stability and soil biological indicators after soil remediation treatments may be related to the increased yields in S/S (Table 4.2).

*Table 4.12*: Total aggregate stability from 1999 to 2000 in the soil quality remediation study.

| **Landscape Positions*** | **Compost Rate** Mg $ha^{-1}$ | | | |
|---|---|---|---|---|
| | **0** | **45** | **90** | **134** |
| | | *%* | | |
| D | 61 Aa | 58 Aa | 62 Aa | 67 Aa |
| BS | 42 Bb | 49 Ba | 69 Aa | 59 Aa |
| S/S | 38 Bb | 28 Bb | 54 Aa | 55 Aa |

Values within a row sharing the same capital letter or within a column sharing a small letter are not significantly different with pairwise predicted differences at $p<0.05$.
*D: Depression, BS: Backslope, and S/S: Summit/shoulder.

Changere and Lal (1997) reported the effect of slope position on aggregate stability. However, lower values of aggregate stability in the D position than in the BS or S/S were attributed to deposition of sediment with a high silt content. According to Pierson and Mulla (1990), aggregate stability was greater in lower slope positions than in upper slope positions and correlated with organic C content. They suggested that compost additions in different landscape positions could increase aggregate stability and improve soil condition in areas of low SOM such as summit positions. Aggregate stability was a

consistent soil quality indicator to monitor soil remediation with compost addition effects on different landscape positions.

4.3.5.2. Soil shock attenuation

The shock attenuation characteristic, and therefore soil compactibility, was significant only at the landscape position (Table 4.3). Soil shock attenuation was not affected by the soil remediation treatments. A landscape x compost interaction table is presented in Appendix C for comparison purposes. Soil shock attenuation in the S/S and B/S positions were greater than the D by 56.5% and 34.8%, respectively (Table 4.13). This seems to be related to greater aggregate stability in the D position than in the other

*Table 4.13*: Soil shock attenuation from 1999 to 2000 in the soil quality remediation study.

| Landscape positions | Soil shock attenuation |
|---|---|
| | $G_{max}$ |
| D* | 39 b |
| BS | 53 a |
| S/S | 53 a |

Values with same letters are not significantly different with pairwise predicted differences at $p<0.05$
*D: Depression, BS: Backslope, and S/S: Summit/shoulder.

two landscape positions. This suggests that soils in the backslope and summit are more compacted (higher $G_{max}$ value) than in the D (lower $G_{max}$ value). This result agrees with higher enzyme activities, and distinct CLPP patterns observed in the D positions (Table 4.5, Table 4.7, and Figure 4.1, respectively). It has been hypothesized that the increase in soil quality indicators in the D position may be related to greater soil moisture, nutrient availability, and organic matter content in those positions (Timlin et al., 1998; Wright et al., 1990). In another landscape position study, penetration resistance was the least in the

D position, possibly due to the deposition of loose and unconsolidated material, relatively high soil moisture content, and accumulation of OM that enhanced soil structure in the area (Changere and Lal, 1997).

However, addition of compost as a remediation tool failed to decrease soil shock attenuation values in the BS and/or S/S position (Table 4.13). According to these results, soil resistance did not represent a satisfactory indicator of soil remediation effects on soil quality by compost addition. However, the lack of significance can also be attributed to high data variability in both sampling years. More observation points may be needed in order to better establish the compost effect on soil compactibility or to evaluate the utility of this test.

## 4.4. Conclusions

Soil remediation is a management response in areas where past management practices have diminished agricultural productivity and degraded soil conditions. The sensitivity of soil quality indicator responses to remediation efforts in the experimental time frame of this study supports their use in measuring soil changes due to remediation practices.

The application of compost to areas of low productivity and soil quality resulted in improved corn silage yields and enhanced soil biological and physical indicators, such as soil enzymes, microbial biomass, and aggregate stability. Background levels of enzyme activities and SMBC with no compost application were higher in the landscape position with better background conditions and crop performance (D) than in the other landscape positions. Similarly, the microbial CLPP was different in D from BS and S/S position with zero and with low (45 Mg $ha^{-1}$) compost addition. High compost addition ($\geq$90 Mg $ha^{-1}$) created a noticeable difference in microbial CLPP from low compost addition ($\leq$45 Mg $ha^{-1}$) in all landscape positions. Soil chemical (TSC) and physical indicators (soil shock attenuation) were less responsive to remediation practices than the biological indicators. Responses to the experimental remediation treatments were related

to the quantity of compost added. Thus, future studies of soil remediation practices could evaluate the responses of the soil processes that are influenced by these conditions.

Indicators of soil quality were effective in assessing the initial condition or soil quality and for monitoring the remediation resulting from compost addition. Remediation of a landscape area may be proposed by integrating results from the small research site to a landscape level. Soil quality indicators could be used to integrate site-specific landscape characteristics and soil quality remediation processes. Depending on management goals, different levels of remediation with compost additions in specific areas of the landscape can be achieved to attain desirable thresholds of sensitive soil quality indicators. However, future studies are still needed to refine compost addition thresholds in order to enhance both soil productivity and soil condition.

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# Chapter 5

# INTEGRATION OF SOIL QUALITY INDICATORS

## 5.1 Introduction

### 5.1.1. Integrating multiple soil quality indicators

Sustainability of agricultural management systems has become an issue of public concern and international debate. One result is that soil quality assessment has been suggested as a tool for evaluating sustainability of soil and crop management practices. Larson and Pierce (1994) suggested the evaluation of soil attributes or indicators that controlled or were influenced by the various soil functions. Acton and Gregorich (1995) suggested that soil quality indicators should reflect the capacity of a soil to perform crop productions and environmental functions. Evaluation of individual physical, chemical, and biological parameters of soil is one way to study the impact of soil management on soil quality. However, these parameters are generally interdependent and, more importantly, practices like tillage and rotation diversity may affect each parameter differently, confounding the assessment of overall quality (Weil et al., 1996). Therefore, individual soil biological and biochemical properties may not be adequate measures of soil quality (Skujins, 1978; Nannipieri et al., 1990; Elliott, 1994).

Soil quality assessment might be enhanced if individual parameters were combined in a meaningful way. Thus, integrated soil quality indicators based on a combination of soil properties could better reflect the status of soil quality than individual parameters (Elliott, 1994; Dick, 1994). Many authors have tried to establish relationships among soil quality indicators, in order to create indices to characterize management effects. Proposed soil quality indices include multiplicative (Pierce et al., 1983; Singh et al., 1990) and additive models (Karlen et al., 1994). According to Weil et al. (1996) multiplicative models can exaggerate the importance of any one parameter, especially if

the value for that parameter is near zero. Additive models may be more useful in assessing soil quality, but are very sensitive to the units of each parameter. Therefore individual parameters must be standardized to a common scale.

According to Doran and Parkin (1994) and Karlen et al. (1994), soil quality indicators present different patterns of response to soil quality depending on the relationship they have with the soil environment (e.g. logarithmic, exponential, inverse relation, etc). If the change in an indicator is positive and directly proportional to soil quality, then soil quality can be regarded as improving (Larson and Pierce, 1994; Weil et al., 1996). On the other hand, a negative change in indicators that is inversely proportional to soil quality (e.g., soil strength) would describe an improvement in soil quality (Larson and Pierce, 1994).

Accordingly, investigations of soil quality have continued in a soil management tradition, exploring the effects of selected agronomic practices on individual soil properties. Much of this work demonstrated that residue–returning practices like cover cropping and manure application lead to significant shifts in some soil properties characterized as soil quality indicators (Karlen et al., 1994; Wany et al., 1994; Franco-Vizicano, 1997). Dumontet et al. (2001) reported the use of an index based on the addition of soil enzymes activities. He suggested that even though selected enzymes were not always able to discriminate among treatments when analyzed individually, their sum, as a biological index, showed a strong correlation with soil organic C and soil microbial biomass C, which had increased in a conservation tillage experiment.

Weil et al. (1996) evaluated changes in soil quality and relationships among several parameters comparing the utility of integrated soil quality indicators using principal components analysis (PCA) of a set of variables, including microbial biomass C, basal respiration, anaerobic incubation nitrogen (AIN), available soil nitrogen (ASN), total C and total N, and penetrometer resistance, as means of assessing the effects of different cropping systems. Their results established that effects from a cropping system that included rotations of vetch *(Coronilla varia L.)*, corn *(Zea Mays L.)*, rye *(Secale cereale L.)*, soybean *(Glycine max L.)*, and wheat *(Triticum aestivum L.)* were clearly distinguishable from continuous corn practices in the PCA analysis. Goodfriend (1998)

also determined the microbial relationships among different study sites that ranged from agricultural sites to sand dunes and marshes using PCA analysis.

According to Wander and Bollero (1999), multivariate statistical approaches such as PCA may be an appropriate step toward soil quality assessment processes within regions and cropping systems because it provides an objective means to extract and weight information in data sets of diverse indicators. The PCA is also an appropriate method to examine these data because the analysis produces uncorrelated indices from the linear combination of potentially correlated variables (Manly, 1986). Moreover, analysis of variance on the principal components eigenvalues (PC-ANOVA) evaluates treatments effects by using the output of the multivariate data set as a process to compare unknown treatment to a standard population. One or two indicators can sufficiently represent each soil function; however, indicators may be related to more than one soil function. The studies of Weil et al. (1996) and Goodfriend (1998) suggest that soil quality can be used to characterize land use/soil management and that it is better measured with several rather than by individual indicators.

Therefore, we hypothesized that a multivariate analysis of soil quality microbial indicators, such us microbial community biomass and enzyme activities, physical indicators, such as aggregate stability, and chemical indicators, such as total soil C and total soil N can be integrated. The outcome should distinguish soil quality changes resulting from defined soil management practices in Pennsylvania farms and in a long-term study (HRE). Secondly, we explored PCA as a method for classifying soil quality condition of farms (or “unknown” samples) as an indication of soil management and as a way to monitor the success of a soil quality remediation study in a range of conditions.

## 5.2 Materials and Methods

### 5.2.1. Site descriptions

Soil quality indicators were measured from a long-term crop rotation and lime fertility experiment (HRE), and Pennsylvania farms with a wide range of soil

management in 1996 through 2000. The crop rotations and lime-fertility experiment of the HRE and farm crop history of Pennsylvania farms are fully described in Chapter 2. The success of a soil quality remediation study was monitored in a wastewater irrigated farm treated with additions of 0 to 134 Mg $ha^{-1}$ of wood chip and manure compost (C:N 20:1) as the remediation tool (see Chapter 4). Soil quality indicators resulting from the compost treatment were monitored using multivariate analysis of Pennsylvania farms as the benchmark.

5.2.2. Soil Quality indicators

**Biochemical Analysis**: 1) Enzyme determinations: Acid phosphatase, arylsulfatase, and β-glucosidase activities were determined on all samples using P-nitrophenylphosphate (PNP), P-nitrophenylsulfate (PNS) and P-nitrophenyl-β-D-glucoside (PNG) as enzyme substrates, respectively. These substrates were purchased from Sigma Inc. (Sigma Chemical Co., St. Louis, MO), and stored at -4 °C. Enzyme activity is based on P-nitrophenol released by 0.5 g of air-dried ground soil incubated with Modified Universal Buffer (MUB) solution and the substrate for 30 minutes at 37 °C. The suspension was filtered (Whatmann No. 42) and absorbance of the filtrate was measured at a wavelength of 410 nm with a Spectronic 1001 split beam spectrophotometer (Tabatabai, 1994). Controls were performed for each sample to allow for color not derived from p-nitrophenol released by enzyme activity. Furthermore, soil samples from a known standard soil were used to measure variations between each batch of samples. The results are expressed as micrograms of P-nitrophenol released per gram of soil.

2) Soil microbial biomass carbon determination***:*** Soil microbial biomass carbon (SMBC) was determined by a modified chloroform fumigation and direct extraction method (modified method of Sparling and West, 1988; Hedley and Stewart, 1982; Gregorich et al., 1990; Mele and Carter, 1996). Soil samples were directly incubated with

2-3 ml of liquid ethanol-free chloroform for 24 h and then left open for 24 h under a hood to allow complete evaporation of the chloroform. Soil microbial biomass C was extracted with 0.5 M $K_2SO_4$ with a 2:5 soil weight/extractant volume. The extracted solution was filtered (Whatmann filter No. 42) and SMBC was determined using a Shimadzu Carbon Analyzer (TOC-5000). The extracted solution was acidified with 1 M HCl, converting inorganic C (l) to $CO_2$ (g) that is evaporated by sparging the sample with $CO_2$-free air. The sample was then inserted into the furnace and organic C converted to $CO_2$ in the presence of a platinum catalyst at 680 °C. The $CO_2$ was measured with a non-dispersive gas infrared detector. Soil microbial biomass carbon was calculated as the difference between fumigated and non-fumigated samples, divided by an efficiency constant Kc= 0.17 (Gregorich et al., 1989). The results were expressed as micrograms of soil microbial biomass C per gram of soil.

**Physical Analysis**: 1) Water Stable Aggregates*:* A standard wet sieving method (Kemper and Rosenau, 1986) with new improvements by Amezketa et al. (1996) was used. Four grams of moist soil sieved through a 2mm and a 0.4 mm sieve (1-0.5 mm diameter aggregates) were placed on a 60 mesh sieve in a shaker. The sieves were raised and lowered through a 1 cm vertical distance at 36 cycles per minute for 5 minutes in a distilled water bath. Material remaining on the sieve after 5 minutes was oven dried (105 ºC) and weighed to give a stable aggregate mass (SA).

**Chemical Analysis:** 1) Total Soil Carbon (TSC): The TSC was measured by combustion method at 680 °C with a Shimadzu Carbon Analyzer (TOC-5000) using a solid sample module (SSM-5000) and a non-dispersive infrared gas analyzer to detect the emitted $CO_2$. The results were expressed as grams of soil C per kilogram of soil. 2) *Total Soil Nitrogen* (TSN): The TSN was measured by a combustion method of dry soil samples with a Fisons NA1500 Elemental Analyzer at The Pennsylvania State University Agricultural Analysis Laboratory. The results are expressed as grams of soil N per kilogram of soil (Campbell, 1991).

## 5.3. Statistical Analysis

Multivariate data analysis using PCA was selected to integrate all soil quality indicators for Pennsylvania farms and HRE. ANOVA was performed on the first two PC loadings (eigenvalues) in order to separate management impacts within the range of farms and HRE treatments (PC-ANOVA). Means of PC loadings and standard deviations for each management group were calculated as the soil quality indexing range. Mean separation was performed with Duncan's Multiple Range test ($p<0.05$). In order to cross validate principal components assessment, selected farms were taken out of the analysis and PCA rerun in order to corroborate separation of treatments results. All the analysis was performed with SAS software (SAS, Inc., 1988).

For the second part of our objectives, characterization of soil quality condition of unknown farms was performed by adding soil quality indicator data from randomly selected farms and from a soil remediation study to the PCA of the Pennsylvania farms data set with these farms previously removed. We utilized measurements from a soil remediation study with compost treatments (0, 134 Mg $ha^{-1}$) from two landscape positions, depression (D) and summit (S/S).

## 5.4. Results and Discussion

### 5.4.1. Soil quality indicators

#### Hunter Rotation Experiment

Several sources of variation were significant in the analysis of variance of soil quality indicators from 1996-2000 in the long-term HRE. Year, LF, Rot and LF x Rot were consistently significant sources of variation among all indicators (Table 5.1). Moreover, LF x Year and Rot x Year were also significant sources of variation among some indicators. According to Bucher (1999) the Year x Treatment (LF or Rot)

interaction could be attributed to seasonal variations among years, and the consequences of these variations for the different crops and nutrient sources.

*Table 5.1*: Analysis of variance of soil quality indicators for the Hunter Rotation Experiment from 1996-2000.

| | Soil quality indicators | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source of variation | df | TSN | TSC | Phosphatase | Arylsulfatase | β-glucosidase | Microbial Biomass C | Aggregate Stability |
| Year (Y) | 4 | * | * | * | * | * | * | * |
| Rep | 3 | ns | ns | ns | ns | ns | ns | ns |
| RepxYear | 12 | ns | ns | ns | ns | ns | ns | ns |
| LF | 2 | * | * | * | * | * | * | * |
| LFxYear | 8 | ns | ns | * | ns | * | * | ns |
| Repx LFxYear | 30 | ns | ns | ns | ns | ns | ns | ns |
| Rot | 4 | * | * | * | * | * | * | * |
| LFxRot | 8 | * | * | * | * | * | * | * |
| RotxYear | 11 | ns | ns | * | * | * | * | ns |
| LFxRotx Year | 22 | ns | * | ns | ns | * | ns | ns |
| RepxLFx RotxYear | 133 | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* |

*Statistically significant at $p<0.05$.
ns :non-significant

All chemical, physical and biological indicators were greater in the manure treatments than in industrial fertilizers treatments for all crop rotations (Table 5.2, Appendix B). Increases in soil quality indicators were also significant as crop diversity and/or length of the rotations (Rot3 and Rot5) increased for all fertility treatments compared to continuous corn (Rot1) or short rotations such as C/S (Rot2) (Table 5.2). This may be due to the increase in different soluble C compounds released in root

exudates and in the manure additions that can be promptly broken down, which would contribute to an increase in microbial biomass (Alvarez et al., 1998, Garland et al., 1996). Similarly, crop rotations that included forages and small grains may increase the diversity of organic residues and the frequency in which residues are available to microbes during a growing season in comparison to continuous corn. Soil management practices, such as crop rotations and manuring, are often observed to enhance overall soil C, microbial biomass, and enzyme activities, as well as aggregate stability (Gregorich et al., 1998; Dormaar et al., 1988, Campbell et al., 1991).

*Table 5.2*: Soil quality chemical, physical and biological indicators of Hunter Rotation Experiment from 1996-2000.

| Treatments | Soil Quality Indicators | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | Enzyme Activity | | | | |
| | TSN | TSC± | Phosphatase | Arylsulfatase | β-glucosidase | Microbial Biomass C | Aggregate Stability |
| | *g kg$^{-1}$ soil* | | *µg PNP# g$^{-1}$ h$^{-1}$* | | | µg C g$^{-1}$ soil | *%* |
| **LF1** | | | | | | | |
| Rot1 | 1.4 b | 15 bc | 201 e | 86 d | 140 e | 163 g | 11 e |
| Rot2 | 1.3 bc | 13 c | 230 de | 194 c | 181 d | 190 f | 13 e |
| Rot3 | 1.4 b | 14 c | 256 d | 214 c | 196 cd | 294 e | 17 d |
| Rot5 | 1.5 ab | 15 bc | 273 d | 266 bc | 246 bc | 396 c | 32 c |
| **LF2** | | | | | | | |
| Rot1 | 1.6 a | 17 ab | 338 bc | 318 a | 357 a | 373 d | 24 d |
| Rot2 | 1.3 bc | 14 c | 286 d | 289 b | 199 cd | 208 f | 32 c |
| Rot3 | 1.5 ab | 18 a | 323 c | 330 a | 262 b | 414 c | 29 cd |
| Rot5 | 1.5 ab | 16 b | 371 b | 348 a | 268 b | 340 d | 37 c |
| **LF3** | | | | | | | |
| Rot1 | 1.6 a | 17 ab | 406 a | 285 b | 283 b | 476 b | 42 b |
| Rot2 | 1.4 b | 16 ab | 345 b | 280 b | 226 c | 370 d | 33 c |
| Rot3 | 1.5 ab | 17 a | 399 a | 310 a | 233 c | 465 b | 44 b |
| Rot5 | 1.5 ab | 17 a | 464 a | 287 b | 276 b | 557 a | 60 a |

*Results followed by the same letter within a column are not significantly different by Duncan's Multiple Range Test at $p<0.05$.

± Total Soil C

# P-nitrophenylphosphate

A significant correlation was present among most of the HRE soil quality indicators (Table 5.3). However, aggregate stability was not significantly correlated with TSN or β-glucosidase enzyme activity. In addition, TSN and SBMC were not correlated with arylsulfatase activity. Klose and Tabatabai (1999) reported that arylsulfatase activity was significantly correlated with TSN. However, their study only compared samples from different surface soils with no distinction in soil management practices. Consistent increases in soil quality indicators with treatments that included diverse crop residues and/or additions of dairy manure may suggest and corroborate the sensitivity of the indicators to diverse and/or readily available C sources (Table 5.3).

*Table 5.3*: Correlation of soil quality chemical, biological and physical indicators of HRE since 1996-2000.

| | TSC | Phosphatase | Arylsulfatase | Glucosidase | Microbial Biomass C | Aggregate Stability |
|---|---|---|---|---|---|---|
| Phosphatase | 0.735* | - | | | | |
| Arylsulfatase | 0.602* | 0.735* | - | | | |
| Glucosidase | 0.695* | 0.670* | 0.694* | - | | |
| Microbial Biomass C | 0.787* | 0.888* | 0.548$^{ns}$ | 0.689* | - | |
| Aggregate stability | 0.596* | 0.929* | 0.584* | 0.498$^{ns}$ | 0.849* | - |
| TSN | 0.812* | 0.620* | 0.412$^{ns}$ | 0.824* | 0.746* | 0.463$^{ns}$ |

*Statistically significant at $p<0.05$ with Pearson correlation.
ns :non-significant

Pennsylvania farms

Individual soil quality indicators for Pennsylvania farms are presented in Table 5.4. Soil quality indicators were significantly greater for farms with livestock (largely dairy) or cash crop with manure additions in their soil management. Soil quality indicators from wastewater irrigation farms had in general the lowest values of all farms.

As previously reported for the HRE, soil management practices that included organic additions tended to increase biological, chemical, and physical indicators of soil quality.

*Table 5.4*: Soil quality chemical, biological and physical indicators of Pennsylvania farms since 1996-2000.

| | | Soil Quality Indicators | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Enzyme Activity | | | | |
| Farm | Management Description | TSN | TSC± | Phosphatase | Arylsulfatase | Glucosidase | Microbial Biomass | Aggregate Stability |
| | | *g kg$^{-1}$ soil* | | ——— | *µg PNP$^{\#}$ g$^{-1}$ h$^{-1}$* | ——— | µg C g$^{-1}$ soil | *%* |
| **1996** | | | | | | | | |
| 4 | Organic | 2.1 b* | 20 c | 572 e | 448 d | 400 bc | ND | 63 bc |
| 2 | Organic | 2.0 b | 19 c | 584 de | 469 c | 387 c | ND | 68 b |
| AH | Cash Crop-no manure | 1.4 de | 15 d | 286 i | 311 f | 281 ef | ND | 50 c |
| RL | Cash Crop-no manure | 1.8 c | 15 d | 302 i | 308 f | 306 de | ND | 44 d |
| WW | Wastewater Irrigation | 1.2 ef | 12 g | 239 k | 244 g | 251 fg | ND | 35 ef |
| **1997** | | | | | | | | |
| Bro | Livestock | 2.3 b | 19 c | 876 b | 734 b | 548 a | 889 a | 70 ab |
| Bei | Cash Crop-manure | 1.7 cd | 14 e | 650 c | 445 d | 447 b | 517 bc | 64 b |
| Bai | Cash Crop-no manure | 1.4 de | 13 f | 443 f | 447 d | 274 ef | 467 cd | 44 d |
| Gr | Cash Crop-manure | 1.9 c | 15 d | 660 c | 447 d | 379 c | 572 b | 68 b |
| PKE | Cash Crop-manure | 1.4 de | 14 e | 416 g | 385 e | 316 de | 435 d | 69 b |
| WW | Wastewater Irrigation | 1.1 f | 13 f | 276 i | 240 g | 248 fg | 245 f | 22 g |
| **1998** | | | | | | | | |
| MY | Livestock | 2.3 b | 27 a | 1340 a | 977 a | 611 a | 966 a | 84a |

*Table 5.4*: Soil quality chemical, biological and physical indicators of Pennsylvania farms since 1996-2000 (cont.).

| Farm | Management Description | TSN | TSC± | Phosphatase | Arylsulfatase | Glucosidase | Microbial Biomass C | Aggregate Stability |
|---|---|---|---|---|---|---|---|---|
| | | *g kg$^{-1}$ soil* | | ——— | *µg PNP# g$^{-1}$ h$^{-1}$* | ——— | µg C g$^{-1}$ soil | *%* |
| L | Cash Crop-no manure | 1.8 c | 22 b | 602 d | 457 cd | 257 fg | 463 cd | 35ef |
| W | Cash Crop-no manure | 1.6 d | 15 d | 678 c | 382 e | 359 cd | 570 b | 47d |
| E | Cash Crop-no manure | 1.6 d | 16 d | 383 h | 303 f | 223 g | 316 e | 41de |
| WW | Wastewater Irrigation | 1.2 e | 14 e | 249 j | 235 g | 228 g | 251 f | 32f |
| **1999** | | | | | | | | |
| H | Livestock | 2.0 bc | 25 c | 684 a | 580 a | 467 a | 825 a | 74 ab |
| EBM | Cash Crop-manure | 2.6 ab | 23 d | 641 b | 475 b | 475 a | 795 a | 44 d |
| EBN | Cash Crop-manure | 2.2 b | 23 d | 555 c | 415 c | 345 b | 598 c | 39 e |
| Fur | Cash Crop-manure | 1.5 d | 13 h | 368 e | 186 e | 177 d | 271de | 45 d |
| DB | Cash Crop-no manure | 1.5 d | 16 f | 404 d | 244 d | 247 c | 199 e | 59 c |
| WW | Wastewater Irrigation | 1.5 d | 14 g | 271 f | 182 e | 175 d | 221 de | 32 f |
| **2000** | | | | | | | | |
| HS | Livestock | 1.8 c | 18 e | 609 d | 425 c | 358 c | 550 b | 88 a |
| JP | Livestock | 2.2 b | 29 a | 770 c | 587 a | 405 b | 547 b | 74 ab |
| RF | Livestock | 3.0 a | 26 b | 997 a | 475 b | 456 a | 995 a | 68 b |
| MW | Livestock | 4.0 a | 26 b | 894 b | 373 d | 333 c | 530 bc | 52 c |

*Table 5.4*: Soil quality chemical, biological and physical indicators of Pennsylvania farms since 1996-2000 (cont.).

| Farm | Management Description | TSN | TSC± | Phosphatase | Arylsulfatase | Glucosidase | Microbial Biomass C | Aggregate Stability |
|---|---|---|---|---|---|---|---|---|
| | | *g kg$^{-1}$ soil* | | ——— | *µg PNP$^{\#}$ g$^{-1}$ h$^{-1}$* | ——— | µg C g$^{-1}$ soil | *%* |
| RM | Cash Crop-no manure | 4.0 a | 23 d | 546 e | 283 e | 253 de | 394 d | 44 d |
| RB | Cash Crop-no manure | 1.8 c | 25 c | 471 fg | 409 d | 260 de | 398 d | 32 f |
| RH | Cash Crop-no manure | 3.0 a | 24 c | 510 ef | 350 d | 277 d | 480 c | 34 ef |
| CC | Cash Crop-no manure | 1.2 e | 14 g | 420 g | 171 f | 242 e | 341 d | 31 f |
| WW | Wastewater Irrigation | 1.5 d | 16 f | 345 h | 205 f | 198 f | 232 e | 38 e |

*Results followed by the same letter within a column are not significantly different by Duncan's Multiple Range Test at $p<0.05$.
± Total Soil C
# P-nitrophenylphosphate,
ND: No Determination

Soil quality indicators from cash crop-no manure farms were intermediate between livestock and wastewater irrigation farms (Table 5.4). Similar findings were observed in a three-year study of soil enzymes and microbial biomass determinations in the Pennsylvania farms sampled in 1996 through 1998 (Bucher, 1999). Enzyme activities and microbial biomass C were greater in active livestock and organic farms for all years (e.g. MY, Bro, 4, 2, HS) than for cash crops-no manure additions (e.g. Bai, AH, E, RH). Bulluck et al. (2001) also demonstrated that alternative soil amendments, such as manure or compost additions, could enhance soil biological, chemical, and physical attributes of a soil compared with inorganic amendments. The authors reported that organic amendments improved plant yield, plant disease suppression, and overall soil quality in three organic vegetable farms when compared to farms with conventional practices in Virginia and Maryland from 1996-1997.

Under temperate climatic conditions, organic amendments increase organic C, which stimulates enzyme activities, increases microbial biomass, and increases aggregate stability (Schnurer et al., 1985; Sparling et al., 1986). The response can be attributed to the increased availability of soluble and easily degradable C compounds in addition to the formation of rapidly cycled C sources including root exudates and microbial cells with a transient existence. Increased SMBC in Pennsylvania farms with manure additions can enhance biotransformations of organic matter, and improve the flow of C, N, P and other nutrients in these farm ecosystems. Addition of organic residues has been suggested as a practice to maintain organic matter and microbial biomass activity levels in cultivated soils (Boyle and Paul, 1989). Perucci et al. (1984) also observed that changes in SMBC depend on the type and amount of buried crop residues and/or organic amendments. Studies among the soil quality indicators indicated that soil enzymes and SMBC were highly correlated in Pennsylvania farms (Bucher, 1999). Similarly, Klose and Tabatabai, (1999), reported significantly correlations among arylsulfatase activity and SMBC, TSC, and TSN in studies of 10 different surface soils.

Soil chemical indicators of Pennsylvania farms were also affected by different soil management practices (Table 5.4), even though these indicators are considered to be less sensitive to rapid organic matter changes (McGill et al., 1986). Active livestock farms had higher values of TSC and TSN than cash crop-manure farms and cash crop-no manure farms (Table 5.4). Ritz et al. (1997) also found increased levels of TSC in farms fields with crop rotations and manure additions as part of their soil management practices. Soil aggregate stability has been correlated with increase of SOM in several tillage and crop studies (Yang and Wander, 1998) as well as with additions of organic amendments (Jordahl and Karlen, 1993). Contrary to the HRE results, all biological, chemical, and physical indicators of the sampled Pennsylvania farms were positively correlated to each other (Table 5.5). Discrepancies between Pennsylvania farms and HRE may be attributed to a wider range in the magnitudes of Pennsylvania soil quality indicators than the HRE. On the other hand, a larger number of farms were sampled compare to HRE plots, so smaller values of a correlation coefficient ($r^2$) can indicate a significant correlation. Long-term and consistent soil management in HRE may help

distinguish subtler variations among soil quality indicators than in the Pennsylvania farms with a range in diverse crop and soil management histories. Nevertheless, the increase in soil quality indicator values due to consistent manure additions in Pennsylvania may suggest and corroborate the sensitivity of the indicators to diverse and/or readily available C sources, as in the HRE (Table 5.5).

*Table 5.5*: Correlation of soil quality chemical, biological and physical indicators of Pennsylvania farms since 1996-2000.

| | TSC | Phosphatase | Arylsulfatase | Glucosidase | Microbial Biomass C | Aggregate Stability |
|---|---|---|---|---|---|---|
| Phosphatase | 0.636* | | | | | |
| Arylsulfatase | 0.678* | 0.859* | - | | | |
| Glucosidase | 0.734* | 0.853* | 0.903* | - | | |
| Microbial Biomass C | 0.721* | 0.883* | 0.852* | 0.920* | - | |
| Aggregate stability | 0.627* | 0.603* | 0.653* | 0.717* | 0.624* | - |
| TSN | 0.518* | 0.629* | 0.637* | 0.609* | 0.622* | 0.593* |

*Statistically significant at $p<0.05$ with Pearson correlation.

The analysis of soil management impacts on soil quality indicators from 1996-2000 provides important information about suites of properties collected simultaneously and within a long-term time frame. These results suggest the relatedness among soil properties and soil management (Halvorson et al., 1995). These types of data sets provide the basis for a better understanding of soil condition than single-year or shorter-term studies for implementing different soil management strategies and applying soil quality information to specific regions and situations (Hussain et al., 1999).

5.4.2. Principal Components Analysis

5.4.2.1. Hunter Rotation Experiment

Complex relationships among all soil quality indicators are summarized with the PCA Eighty four percent of the variance in all soil quality indicators from 1996 through 2000 in the HRE plots was explained by the first two PCs (Figure 5.1). Loadings of all variables were similar (Appendix A, Table A.3). Among treatments, lime fertility treatments with manure additions (LF2 and LF3) were separated by PC1 from lime-fertility treatments with additions of industrial fertilizers (LF1). Crop rotations did not

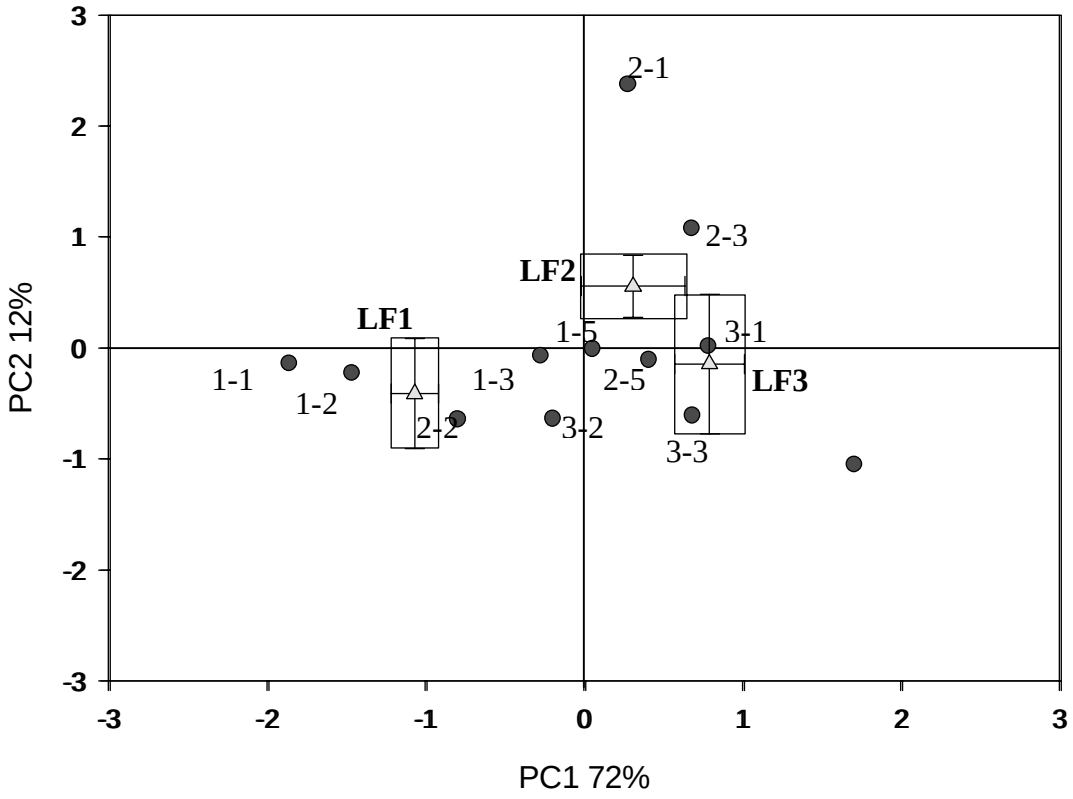


*Figure 5.1:* Principal Components Analysis of soil quality indicators of HRE lime-fertility and crop rotation treatments. *Solid circle* ●: Lime-fertility treatments: 1-industrial fertilizer, 2, and 3-manure addition. Crop rotations: Rot 1: CC, Rot2: CS, Rot3: 4C4H, and Rot5: COWHH (Label: LF#-Rot#). *Solid triangle* ▲ *and box*: Mean values and standard deviations for each lime fertility treatment. Percentages on each axis are the variation explained by each PC.

seem to contribute to the separation of treatments with manure additions, but PC1 separated continuous cropping (Rot1) or short rotations (Rot2) from crop rotations that included forages (Rot3) and/or small grains (Rot5) with industrial fertilizer (Figure 5.1). These results suggest that soil management practices that include organic additions, such as manure additions or increased crop residue diversity, may influence most of the biological, chemical, and physical aspects of soil quality differently than treatments with industrial fertilizers and continuous corn. Separation of treatments by PCA also is consistent with the trends observed within the individual soil quality indicators (Table 5.2).

5.4.2.2. Pennsylvania farms

Eighty two percent of the variance in all soil quality indicators from 1996 through 2000 was explained by the first two PCs (Figure 5.2). Loadings of all variables were similar (Appendix A). The PC1 ranked and separated farms in the following soil management groups after ANOVA: Livestock > Cash crop-manure > Cash crop-no manure > wastewater irrigation farms. Means and standard deviations of the PC-ANOVA of the farms are in Appendix A. These results are consistent with the results from HRE in which treatments that included organic additions influenced most of the biological, chemical, and physical aspects of soil quality differently than treatments using industrial fertilizers and continuous cropping systems.

The positive influence on soil quality indicators of soil management practices with organic matter additions is also observed in a biplot of the Pennsylvania farms (Figure 5.3). In the biplot, vectors of soil quality indicators (representing loadings of similar sign and magnitude) are added to the PCA plot. The biplot indicated that all indicators are mostly affected by livestock or cash crop-manure farms and that they are closely related to each other in agreement with the previous correlation result. Wander and Bollero (1999) also utilized PCA to determine management practice effects on soil

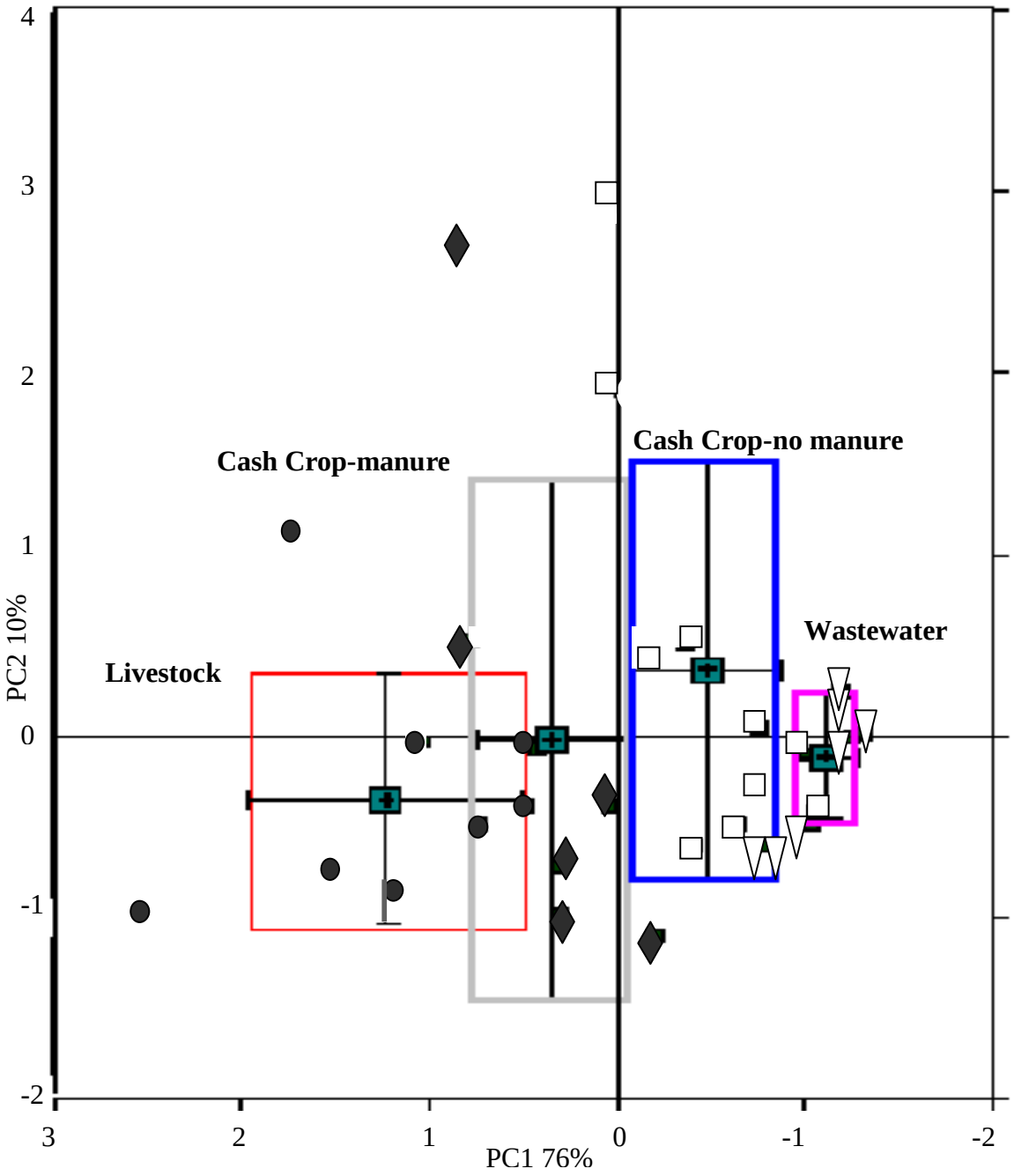


*Figure 5.2*: Principal Components Analysis of soil quality indicators of Pennsylvania farms with different managements. *Solid circle* ●: Livestock farms. *Solid diamond* ♦: Cash Crop, manure. *Open rectangle* □: Cash Crop, no manure. *Open triangle* ∇: Wastewater Irrigation. *Solid square* ■ *and boxes*: Mean values and standard deviations for each Pennsylvania farm management group. Percentages on each axis are the variation explained by each PC.

quality in Illinois. They found that tillage management affected most of the soil variables in the study. They observed that biological and physical aspects of soil quality were the most sensitive indicators of soil quality. In addition, PCA showed that the biological and physical aspects of soil influenced by organic matter were the properties most altered by the Illinois agronomic practices.

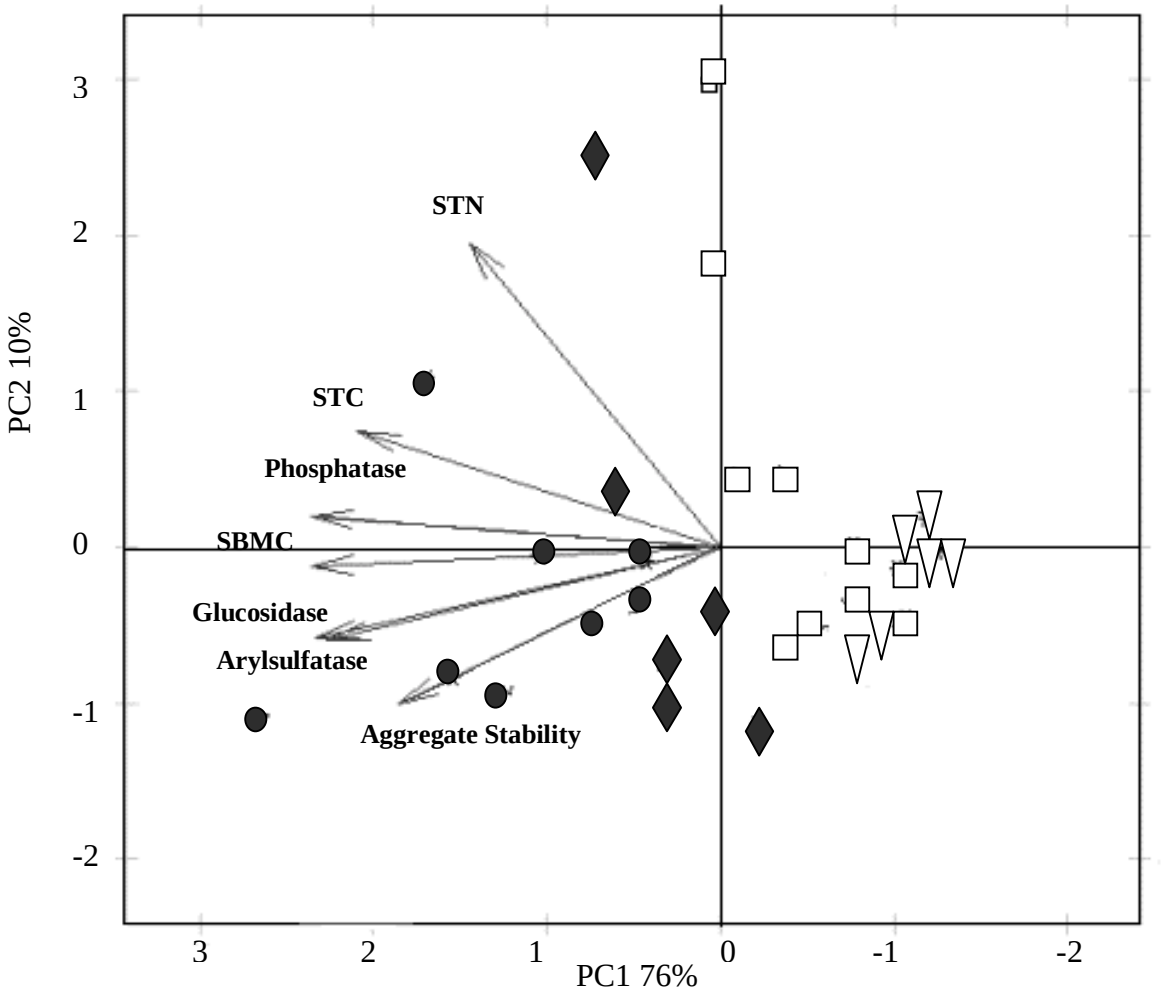


*Figure 5.3*: Biplot of soil quality indicators of Pennsylvania farms with different managements. *Solid circle* ●: Livestock farms. *Solid diamond* ♦: Cash Crop, manure. *Open rectangle* □: Cash Crop, no manure. *Open triangle* ∇: Wastewater Irrigation. Arrows indicate direction and loadings of vectors representing soil quality indicators. Percentages on each axis are the variation explained by each PC

5.4.3. Development of soil quality indexing process

5.4.3.1. Pennsylvania farms

In order to demonstrate the process of classifying soil quality status of an unknown sample or farm, data from three farms were selected from each soil management group (one livestock, cash crop, and wastewater irrigation farm) and extracted from the original data set (30 farms). A new PCA was run on the depleted data set (27 farms) and ANOVA of PC1 and 2 loadings were conducted. Then, the three unknown farms were added again to replenish the data set (30 farms) and a new PCA was completed. The PC1 and PC2 of the unknown farms were compared against the means and standard deviations of the PC1 and 2 from the analysis of the depleted farm data set (27 farms) (Table 5.6). The PC1 and PC2 of the three "unknown farms" could be most closely associated with the means of PC1 and PC2 from the expected soil management farm groups. The farms were correctly classified as livestock (MY), cash crop-manure (W), and as a wastewater irrigation farm (WW).

*Table 5.6:* Means of Principal Component Analysis of Pennsylvania farms with different soil management.

| Farm Group | | PC1 | | PC2 | | Unknown Farms | | |
|---|---|---|---|---|---|---|---|---|
| | N | *Mean* | *Std* | *Mean* | *Std* | | PC1 | PC2 |
| | | loadings | | | | | loadings | |
| Livestock farms | 8 | 1.223 a* | 0.733 | -0.353 a | 0.693 | MY | 2.129 | -1.077 |
| Cash crop-manure | 6 | 0.336 b | 0.400 | -0.020 a | 1.443 | W | 0.038 | -0.418 |
| Cash crop–no manure | 10 | -0.500 c | 0.397 | 0.365 b | 1.164 | | - | - |
| Wastewater irrigation | 6 | -1.132 d | 0.178 | -0.116 a | 0.346 | WW | -0.806 | -0.632 |

*Means with the same letter are not significantly different with DMR at $p<0.05$.

These results suggest that the soil management of unknown farms could be classified based on the status of the soil quality indicators measured in this study. Thus, PCA with ANOVA of the sample of Pennsylvania farms could be a foundation for an effective process of indexing soil quality. Limitations of this process would be that the same number of indicators as the standard data set is required. Furthermore, as more farms are added to the standard data set, a more refined classification of farm management groups might be necessary.

### 5.4.4. Using a soil quality indexing process to evaluate soil quality remediation

The results from a soil remediation study (see chapter 4) were also considered to be unknown farms to be classified in relation to other Pennsylvania farms. Remediation treatments of 0 and 134 Mg $ha^{-1}$ compost addition in the depression (D) and summit (S/S) landscape positions were selected as test entries. The PCA was performed to include soil quality indicator data from the remediation treatments with the original Pennsylvania farm data set.

Remediation treatment of 0 Mg $ha^{-1}$ compost addition in the depression (D) position was identified as resembling farms characterized as cash crop-no manure (Figure 5.4). When no treatment (0 Mg $ha^{-1}$ compost) was applied to summit (S/S) position, it was classified as most closely resembling a wastewater irrigation farm. Most of the wastewater irrigation farm samples for soil quality analyses had always been collected from the S/S and BS positions. The D position was generally excluded as not reflecting characteristics of the more extensive area. This fact corroborates results in Chapter 4, where values of soil quality indicators in the depression (D) positions were greater than the indicators of the summit (S/S) position with no compost additions. When compost (134 Mg $ha^{-1}$) was added to both depression (D) and summit (S/S) positions, PCA of soil quality indicators from D was grouped with farms classified as cash crop-manure (Figure 5.4). The PCA of soil quality indicators classified the compost treatment, 134 Mg $ha^{-1}$, in the S/S position as resembling the group of cash crop-no manure farms. This implies a

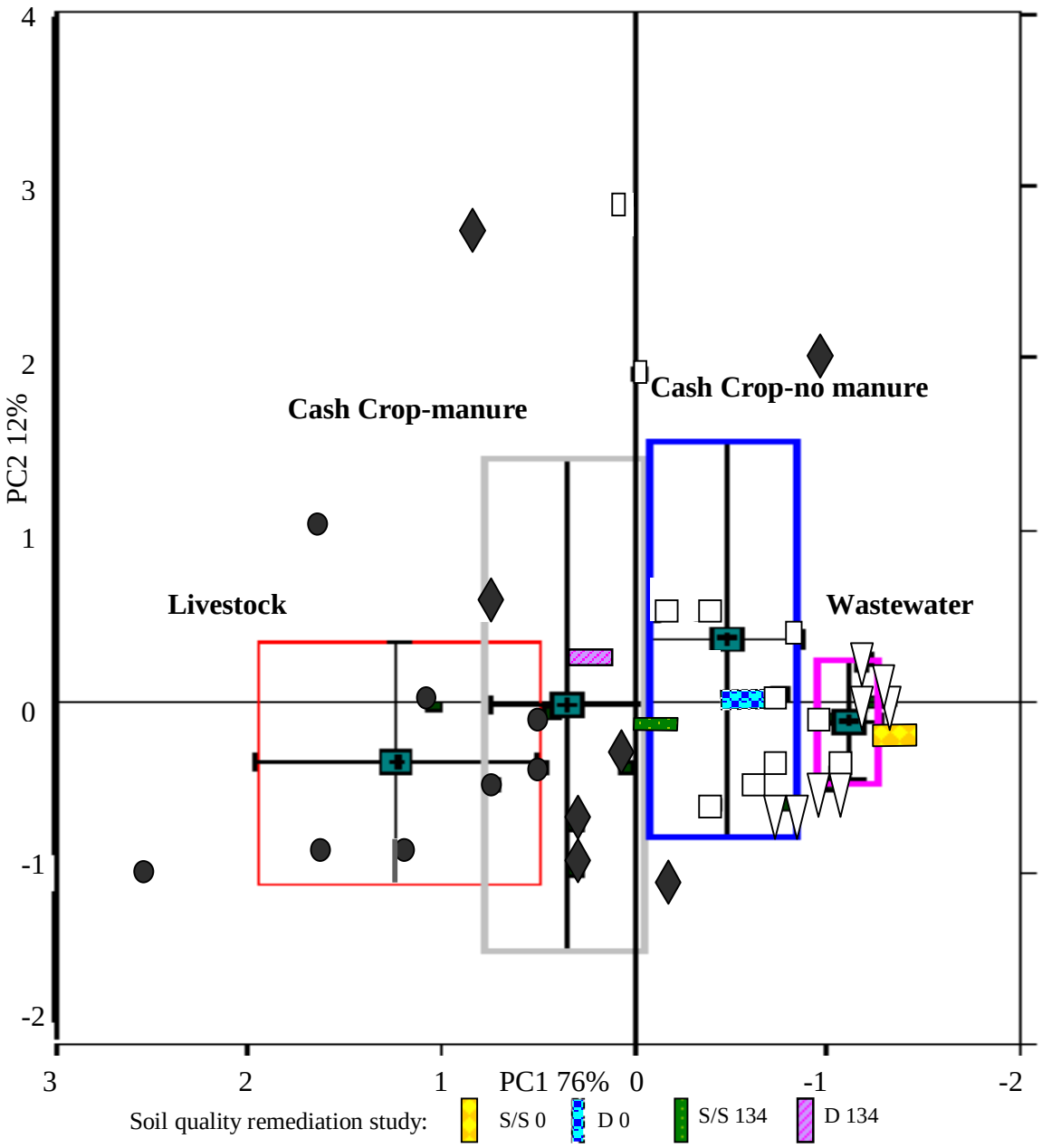


*Figure 5.4*: Principal Components Analysis of soil quality indicators of Pennsylvania farms with different managements. *Solid circle* •: Livestock farms. *Solid diamond* ♦: Cash Crop-manure. *Open rectangle* □: Cash Crop-no manure. *Open triangle* ∇: Wastewater Irrigation. *Solid square* ■: Mean values and standard deviations (boxes) for Pennsylvania farm management group. *Soil quality remediation study:* Landscape positions: Depression (D), and Summit (S/S). Compost treatments 0 and 134 (Mg $ha^{-1}$). Percentages on each axis are the variation explained by each PC.

positive response of soil quality to compost addition. Hence, the soil quality indexing process based on PCA can be a useful tool for characterizing and monitoring soil quality initial conditions and/or trends.

## 5.5. Conclusions

Multivariate analysis provides a robust and comprehensive way of integrating soil quality indicators to evaluate soil management effects on Pennsylvania farms and in the long-term experiment (HRE). Additions of manure and crop rotations that included small grains and forages affected soil quality indicators differently than soil management with only industrial fertilizers and continuous corn in the HRE. The PCA of soil quality physical, chemical and biological indicators separated and identified farm management treatments that included livestock, cash crop-manure additions, cash crop-no manure additions, or those receiving wastewater irrigation. Future studies are needed in order to determine the number of effective indicators to be incorporated in the data set to effectively characterize soil management impacts.

Assessment of soil quality indicators by PCA was a useful tool to develop a process of soil quality indexing among Pennsylvania farms. Selected farms were predictably classified and soil quality remediation progress (and/or success) from a degraded farm was effectively tracked. Evaluation of soil quality indicators could help farmers interpret the overall condition and status of their soils relative to other farms and different management treatments. The process of soil quality indexing could provide farmers with a foundation for management decisions to maintain or improve soil quality according to their farm management goals. This procedure could represent an appropriate first step toward soil quality assessment within regions and soil management systems. Moreover, soil quality indexing could also be a process offered by soil testing laboratories in the future.

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# Chapter 6

## CONCLUSIONS

Monitoring soil quality has the potential to contribute to the sustainability of land management systems. Long-term experiments are valuable resources for evaluating the effects of diverse soil management practices, especially on biological indicators of soil quality. In the HRE, CLPP of biological communities clearly distinguished soil microbial activity and community structure in treatments with both manure additions and corn-based rotations including small grains and/or forages rotations, from industrial fertilizer and continuous crops or corn-soybean rotations as soil management practices. The long duration, consistent application and diverse nature of the soil management practices may be key factors differentiating soil microbial community structure. Farms with organic amendment additions in their soil management also established different soil microbial community patterns than in cash crops with no manure additions, or wastewater irrigated farms. However, shifts in microbial communities were not as clear as in the HRE probably due to increased variability of management and cropping history in these farm systems. Characterization of CLPP could have great value for monitoring soil quality and impacts due to different soil management. The challenge is to make better use of this biological community diversity and resiliency with continued organic inputs and crop rotations to maintain a quality ecosystem that may foster sustainability.

On a broader scale, monitoring soil quality as a "process" was determined with a multivariate integration of all our soil quality indicators, as an all-encompassing soil quality indexing process. The method of integrating chemical, physical, and chemical indicators with a principal components analysis could represent a more systems-like description of overall soil quality. Use of multivariate scores (principal components 1 and

2) as system descriptors, allowed us to effectively evaluate and distinguish both Pennsylvania farms and a long-term lime-fertility and crop rotation treatments with organic additions and crop rotations from no manure and continuous cropping practices. Therefore, evaluation of soil management effects on soil quality could be considered as an option for the farmers to be included in their routine soil testing. Farmers could use the comprehensive description to interpret the condition of their soils and evaluate their management practices relative to other farms and management.

Remediation of low soil quality areas of a wastewater-irrigated farm was achieved with addition of manure and wood chip compost as the remediation tool. Soil quality, expressed by the indicator outcomes, was greatest and least responsive in depressions areas of the landscape, due to a possible greater accumulation of nutrients and organic matter than in BS or S/S positions. Microbial indicators were more sensitive to remediation than chemical or physical indicators of soil quality, as observed in soil enzymes activities and microbial biomass C reactions to the different compost additions. The sensitivity of soil quality indicators, especially the biological indicators, and their response to management effects in our studies supported the use of the soil quality indicators as a measure of soil remediation success. The application of different compost rates to areas of low crop productivity and low soil quality enhanced soil quality as measured by soil quality indicators and improved crop production. Sustainable soil management requires action and not just conceptual definitions of sustainability or soil quality. The assessment of soil quality, and its change in time, can be a measure of the sustainable management of our land.

# Appendix A

## PRINCIPAL COMPONENTS ANALYSIS OF PENNSYLVANIA FARMS AND HUNTER ROTATION EXPERIMENT

Table A.1: Principal Components of Pennsylvania farms with different soil management of 1996 trough 2000.

| Farms | Management | PC1 | PC2 |
|---|---|---|---|
| 4 | Organic | 0.4318 | -0.086 |
| 2 | Organic | 0.4766 | -0.4176 |
| Bro | Livestock | 1.5453 | -0.8302 |
| MY | Livestock | 2.6292 | -1.0772 |
| H | Livestock | 1.2324 | -0.938 |
| HS | Livestock | 0.7320 | -0.5114 |
| JP | Livestock | 1.0419 | -0.0521 |
| RF | Livestock | 1.6964 | 1.0890 |
| Bei | Cash Crop Manure | 0.3079 | -1.0088 |
| Gro | Cash Crop Manure | 0.3121 | -0.7493 |
| PKE | Cash Crop Manure | -0.2156 | -1.1297 |
| W | Cash Crop Manure | 0.0384 | -0.4181 |
| EBM | Cash Crop Manure | 0.7878 | 0.5132 |
| EBN | Cash Crop Manure | 0.7078 | 0.4913 |

| | | | |
|---|---|---|---|
| MW | Cash Crop Manure | 0.7882 | 2.6685 |
| AH | CashCrop No-manure | -0.6364 | -0.5088 |
| BA | Cash Crop No-manure | -0.4004 | -0.6249 |
| E | Cash Crop No-manure | -0.7672 | 0.02646 |
| L | Cash Crop No-manure | -0.0991 | 0.47189 |
| DG | Cash Crop No-manure | -1.0400 | -0.5039 |
| DB | Cash Crop No-manure | -0.7148 | -0.3620 |
| RM | Cash Crop No-manure | 0.0557 | 2.9078 |
| RB | Cash Crop No-manure | -0.3624 | 0.4848 |
| RH | Cash Crop No-manure | -0.0265 | 1.8904 |
| CC | Cash Crop No-manure | -1.0183 | -0.1306 |
| WW1 | Wastewater Irrigation | -1.0645 | -0.4443 |
| WW2 | Wastewater Irrigation | -0.8060 | -0.63175 |
| WW3 | Wastewater Irrigation | -1.2460 | -0.0271 |
| WW4 | Wastewater Irrigation | -1.1887 | 0.2251 |
| WW5 | Wastewater Irrigation | -1.3055 | -0.0078 |
| WW6 | Wastewater Irrigation | -1.1842 | 0.1846 |
| *Variation explained* | | *72%* | *14%* |

Table A.2: Mean values of Principal Components of Hunter Rotation Experiment of 1996 through 2000.

| Treatments | Year | PC1 | PC2 |
|---|---|---|---|
| LF1 Rot1 | 1996 | -1.7873 | -1.3133 |
| LF1 Rot1 | 1997 | -1.9288 | 0.5531 |
| LF1 Rot1 | 1998 | -1.8644 | 0.3495 |
| LF1 Rot1 | 1999 | -1.7898 | 0.4354 |
| LF1 Rot1 | 2000 | -1.8435 | 0.4765 |
| LF1Rot2 | 1996 | -1.5520 | -0.2212 |
| LF1Rot2 | 1997 | -1.4732 | 0.0606 |
| LF1Rot2 | 1998 | -1.3754 | -0.5161 |
| LF1Rot2 | 1999 | -1.2987 | 0.0122 |
| LF1Rot2 | 2000 | -1.4124 | -0.3245 |
| LF1Rot3 | 1996 | -0.9978 | -0.5497 |
| LF1Rot3 | 1997 | -0.7978 | -0.5123 |
| LF1Rot3 | 1998 | -0.8986 | -0.4456 |
| LF1Rot3 | 1999 | -0.9377 | -0.3722 |
| LF1Rot3 | 2000 | -0.4534 | -1.0051 |
| LF1Rot5 | 1996 | -0.1473 | -0.3380 |
| LF1Rot5 | 1997 | -0.3551 | -0.5621 |
| LF1Rot5 | 1998 | -0.0925 | -1.0070 |
| LF1Rot5 | 1999 | -0.3765 | -0.3212 |
| LF1Rot5 | 2000 | -0.2335 | -0.2365 |
| LF2Rot1 | 1996 | 0.4320 | 2.4958 |
| LF2Rot1 | 1997 | -0.0292 | 2.2758 |
| LF2Rot1 | 1998 | 0.4332 | 2.3668 |
| LF2Rot1 | 1999 | 0.1292 | 2.9435 |
| LF2Rot1 | 2000 | 0.0134 | 2.0643 |
| LF2Rot2 | 1996 | -0.7722 | 0.0031 |
| LF2Rot2 | 1997 | -0.2790 | 0.1150 |
| LF2Rot2 | 1998 | -0.6546 | 0.0234 |
| LF2Rot2 | 1999 | 0.3643 | -0.2343 |
| LF2Rot2 | 2000 | 0.2299 | -0.3260 |
| LF2Rot3 | 1996 | 0.4260 | 0.2120 |
| LF2Rot3 | 1997 | 0.3296 | -0.4016 |
| LF2Rot3 | 1998 | 0.2560 | -0.3515 |
| LF2Rot3 | 1999 | 0.2666 | -0.4414 |
| LF2Rot3 | 2000 | 0.5371 | -0.0843 |

| | | | |
|---|---|---|---|
| LF2Rot5 | 1996 | 0.4178 | 0.5237 |
| LF2Rot5 | 1997 | 0.6545 | -0.1078 |
| LF2Rot5 | 1998 | 0.6787 | -0.2343 |
| LF2Rot5 | 1999 | 0.7229 | -0.3559 |
| LF2Rot5 | 2000 | 1.2110 | -0.1133 |
| | | | |
| LF3Rot1 | 1996 | 0.7300 | -0.1945 |
| LF3Rot1 | 1997 | 0.4876 | 1.7643 |
| LF3Rot1 | 1998 | 0.8176 | -0.1885 |
| LF3Rot1 | 1999 | 0.7310 | 1.8350 |
| LF3Rot1 | 2000 | 0.4921 | 1.5895 |
| | | | |
| LF3Rot2 | 1996 | 0.1616 | -0.3067 |
| LF3Rot2 | 1997 | -0.0683 | 0.6762 |
| LF3Rot2 | 1998 | -0.0615 | -0.3483 |
| LF3Rot2 | 1999 | -0.0283 | 0.7761 |
| LF3Rot2 | 2000 | 0.2541 | -0.4523 |
| | | | |
| LF3Rot3 | 1996 | 0.6061 | -0.3483 |
| LF3Rot3 | 1997 | 0.7281 | 0.7761 |
| LF3Rot3 | 1998 | 0.6341 | -0.8764 |
| LF3Rot3 | 1999 | 0.6602 | -0.0346 |
| LF3Rot3 | 2000 | 0.7566 | -0.9081 |
| | | | |
| LF3Rot5 | 1996 | 1.1594 | -0.8168 |
| LF3Rot5 | 1997 | 1.9087 | -1.0600 |
| LF3Rot5 | 1998 | 2.0446 | -1.2644 |
| LF3Rot5 | 1999 | 2.0098 | -1.0325 |
| LF3Rot5 | 2000 | 1.7645 | -1.2434 |

*Table A..3:* Means of Principal Component Analysis of Hunter Rotation Experiment from 1996-2000.

| Treatments | N | PC1 | | PC2 | |
|---|---|---|---|---|---|
| | | *Mean* | *Std Dev* | *Mean* | *Std Dev* |
| | | loadings | | loadings | |
| **LF1** Rot1 | 5 | -1.850g | 0.0701 | -0.1368c | 1.0239 |
| Rot2 | 5 | -1.466g | 0.0884 | -0.2255c | 0.2884 |
| Rot3 | 5 | -0.796f | 0.2984 | -0.6423c | 0.3264 |
| Rot5 | 5 | -0.198ed | 0.1385 | -0.6357c | 0.3405 |
| **LF2** Rot1 | 5 | 0.278bcd | 0.2666 | 2.3794a | 0.1104 |
| Rot2 | 5 | -0.273e | 0.5011 | -0.069c | 0.2292 |
| Rot3 | 5 | 0.409bc | 0.1359 | -0.1047c | 0.3271 |
| Rot5 | 5 | 0.783b | 0.4001 | 0.0181c | 0.4543 |
| **LF3** Rot1 | 5 | 0.680b | 0.1686 | 1.0786b | 1.1042 |
| Rot2 | 5 | 0.054cde | 0.1734 | -0.0081c | 0.6812 |
| Rot3 | 5 | 0.683b | 0.0644 | -0.606c | 0.4954 |
| Rot5 | 5 | 1.704a* | 0.4766 | -1.047c | 0.2240 |

*Means with the same letter are not significantly different with DMR at $p<0.05$.

# Appendix B

## ANOVA OF SOIL QUALITY INDICATORS OF PENNSYLVANIA FARMS

*Table B–1*: Analysis of variance of soil quality indicators Pennsylvania farms from 1996-2000.

| Source of variation | **df** | **Phosphatase** | **Arylsulfatase** | **β-glucosidase** | **Microbial Biomass C** | **TSC** | **TSN** | **Aggregate Stability** |
|---|---|---|---|---|---|---|---|---|
| Rep | 3 | ns | ns | ns | ns | ns | ns | ns |
| Field (farm) | 39 | * | * | * | * | * | * | * |
| Farm | 20 | * | * | * | * | * | * | * |

# Appendix C

## PLANT POPULATION IN WASTEWATER REMEDIATION STUDY

*Table C-1*: Corn plant population in three landscape positions and compost treatment of the soil quality remediation study from 1999-2000.

| **Year** | **Corn population** (plants per plots*) |
|---|---|
| 1999 | 86 a |
| 2000 | 79 a |

Values with same letters are not significantly different with DMR at $p<0.05$.
*Plot dimension: 100 $ft^2$

| **Landscape Positions** | **Corn population** (plants per plots) |
|---|---|
| D | 86 a |
| BS | 79 a |
| S/S | 85 a |

Values with same letters are not significantly different with DMR at $p<0.05$.

| **Compost treatment** *Mg $ha^{-1}$* | **Corn population** (plants per plots) |
|---|---|
| 0 | 80 a |
| 45 | 84 a |
| 90 | 84 a |
| 134 | 86 a |

Values with same letters are not significantly different with DMR at $p<0.05$.

*Table C-2*: Arylsulfatase activity from 1999-2000 in three landscape positions of the Penn State Irrigation Site remediation study.

| **Landscape positions** | **Compost Rate** *(Mg ha$^{-1}$)* | | | |
|---|---|---|---|---|
| | **0** | **45** | **90** | **134** |
| | *µg PNP g$^{-1}$ h$^{-1}$* | | | |
| D | 226 | 215 | 217 | 273 |
| BS | 157 | 170 | 211 | 243 |
| S/S | 153 | 180 | 199 | 236 |

*D: Depression, BS: Backslope, and S/S: Summit/shoulder.

*Table C-3*: Soil shock attenuation from 1999-2000 in three landscape positions of the Penn State Irrigation Site remediation study.

| **Landscape Positions*** | **Compost Rate** **Mg ha$^{-1}$** | | | |
|---|---|---|---|---|
| | **0** | **45** | **90** | **134** |
| | $G_{max}$ | | | |
| D | 38 | 38 | 37 | 45 |
| BS | 54 | 52 | 53 | 52 |
| S/S | 58 | 57 | 51 | 58 |

*D: Depression, BS: Backslope, and S/S: Summit/shoulder.

# Appendix D

## CHEMICAL ANALYSIS OF WASTEWATER EFFLUENT USED FOR IRRIGATION AT THE SOIL QUALITY REMEDIATION SITE

*Table D 1: Chemical analysis of treated wastewater effluent from 1997-2000.*

| Date Sampled | 1997 | 1998 | 1999 | 2000 | 2001 |
|---|---|---|---|---|---|
| Alkalinity pH 8.3 mg $L^{-1}$ as $CaCO_3$ | 15 | 30 | >1 | 1.1 | - |
| Alkalinity pH 4 mg $L^{-1}$ as $CaCO_3$ | 223 | 247 | 178 | 180 | 202 |
| Ammonia N mg $L^{-1}$ | 13 | 9 | 2.7 | 1.5 | 2.3 |
| Chloride mg $L^{-1}$ | 210 | 241 | 215 | 207 | 180 |
| Nitrate N mg $L^{-1}$ | 2.0 | 2.7 | 6.6 | 6 | 5.2 |
| Nitrite N mg $L^{-1}$ | 0.4 | 0.1 | 0.9 | 0.06 | 0.21 |
| pH | 7.8 | 7.8 | 7.6 | 7.4 | 7.7 |
| Phosphate-Ortho mg $L^{-1}$ | 4 | 3.8 | 4.4 | 3.8 | 3.2 |
| Total Solids mg $L^{-1}$ | 668 | 666 | 613 | 643 | 593 |
| Sulfate mg $L^{-1}$ | 24 | 30 | 27 | 24 | 29 |
| Hardness mg $L^{-1}$ as $CaCO_3$ | 192 | 232 | 206 | 190 | 230 |
| Total Organic Carbon mg $L^{-1}$ | 18 | 19 | 5 | 3 | 5 |
| Chemical Oxygen Demand mg $L^{-1}$ | 86 | 47 | 15 | 22 | 37 |
| Biochemical Oxygen Demand mg $L^{-1}$ | 12 | 14 | 4 | 6 | 4 |

## Appendix E

## MICROBIAL CLPP ANALYSIS OF HRE AND PENNSYLVANIA FARMS

Table E.1: Biolog plate mean intensity in the Hunter Rotation Experiment from 1997-2000.

| LF | Crop Rotations | | | |
|---|---|---|---|---|
| | **1** | **2** | **3** | **5** |
| | *Absorbance* | | | |
| **1** | 0.27 (0.19) | 0.39 (0.08) | 0.69 (0.03) | 0.79 (0.10) |
| **2** | 0.66 (0.14) | 0.79 (0.14) | 0.91 (0.11) | 0.75 (0.07) |
| **3** | 0.63 (0.08) | 0.78 (0.13) | 0.91 (0.08) | 0.95 (0.08) |

Number in parenthesis is standard deviation.

*Table E.2*: Substrate diversity Index (H) of CLPP in the Hunter Rotation Experiment from 1997-2000.

| LF | Crop Rotations | | | |
|---|---|---|---|---|
| | **1** | **2** | **3** | **5** |
| **1** | 3.8 (0.3) | 3.9 (0.1) | 4.4 (0.1) | 5.3 (0.2) |
| **2** | 4.3 (0.2) | 4.5 (0.5) | 5.0 (0.1) | 5.8 (0.6) |
| **3** | 4.2 (0.1) | 5.0 (0.4) | 6.3 (0.7) | 6.9 (0.7) |

Number in parenthesis are standard deviations.

*Table E.3:* Substrate diversity Index (H) sampled in 1997-2000 of Pennsylvania farms with different managements. Livestock farms: Bro, S, MY, EB, H, RF, HS, JP. Cash crop-manure: Gr, Bei, PKE, Fur, MW. Cash crop-no manure: L, BA, W, E, DB, RB, RM, RH, CC. Wastewater Irrigation farm: WW.

| Pennsylvania farms | Management | H |
|---|---|---|
| EBM | Cash crop, manure | 4.34* |
| Fur | Cash crop, manure | 4.28 |
| H | Livestock farm | 4.24 |
| JP | Livestock farm | 4.21 |
| DB | Cash crop no manure | 4.18 |
| HS | Livestock farm | 4.10 |
| EBN | Cash crop, manure | 4.01 |
| MY | Livestock farm | 4.03 |
| MW | Livestock farm | 4.02 |
| Bro | Livestock farm | 3.98 |
| S | Livestock farm | 3.95 |
| Gr | Cash crop, manure | 3.95 |
| BA | Cash crop no manure | 3.88 |
| PKE | Cash crop, manure | 3.98 |
| RB | Cash crop no manure | 3.97 |
| Bei | Cash crop, manure | 3.91 |
| RH | Cash crop no manure | 3.68 |
| CC | Cash crop no manure | 3.88 |
| WW | Wastewater Irrigation | 3.88 |
| W | Cash crop no manure | 3.70 |
| RM | Cash crop no manure | 3.67 |
| E | Cash crop no manure | 3.67 |
| L | Cash crop, manure | 3.67 |

*Not significantly different at $p < 0.05$ with DMR.

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