AN ABSTRACT OF THE DISSERTATION OF

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Richard P. Dick

Nitrogen availability in agricultural soils from fertilizer, plant residue inputs, and soil organic matter has important implications beyond crop yield. Legume winter cover crops and one fourth the recommended N rate on sweet corn resulted in yields equivalent to those at the recommended rate in the Willamette Valley of western Oregon. Cereal rye winter crops absorbed an average of 40 kg N/ha that otherwise would have been leached, but did not effectively replace fertilizer N. Cereal rye as a cover crop therefore shows an ability to immobilize N from fertilizer. This was further confirmed in an experiment with ^{15}N labeled urea where results showed that N derived from fertilizer in sweet corn or cereal rye plant residue was less available for crop uptake and loss from the system than inorganic N or N directly immobilized from fertilizer. Losses of N from fertilizer ranged from 40 to 73% of that which was in the soil over winter. Mineralization of organic matter N is an important process in N availability, especially when cover crops are used to replace fertilizer. Finding a general indicator or predictor of N mineralization in soils would help in reducing fertilizer N costs and leaching of inorganic N that is applied in

excess of crop needs. In a screening of 17 biological and chemical properties of 19 differently managed soils from around the state of Oregon, a model using total soil N and β-glucosidase activity provided the best model of mineralized N uptake by ryegrass. Biological activity is primarily responsible for the transformations that result in N availability in soils. Management of soils directly impacts soil biology, and results from multivariate analyses of biological and chemical parameters in differently managed soils showed that disturbance creates an overriding common biochemical state in soils. Beyond disturbance, vegetation and the nature of organic inputs also impart recognizable multivariate patterns in soils managed differently. These results suggest that indicators independent of soil type may be used to discern effects of management on agricultural soils.

Cover Crops and Biochemical Functional Diversity in Relation to Nitrogen Availability in Soil

by

John Z. Burket

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

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John Z. Burket, Author

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CONTRIBUTION OF AUTHORS

Dr. Richard P. Dick was involved in the design, analysis and writing of each manuscript. Dr. Delbert D. Hemphill assisted in data collection and interpretation for parts of the study.

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DEDICATION

I dedicate this work to my mother and my father, no longer on this Earth, who will forever be in my heart.

Cover Crops and Biochemical Functional Diversity in Relation to Nitrogen Availability in Soil

Chapter 1

INTRODUCTION

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Introduction

Approximately 90 % of the nitrogen (N) used for primary plant production in world agriculture comes from the mineralization of N in soil organic matter (Jenny, 1980). How plant residue inputs affect the nature of soil organic N and its availability for future crop production is an increasingly important area of agricultural research for both environmental and economic reasons.

In agricultural as well as natural ecosystems, vegetation exerts the primary influence on the character of soil N, so it is expected that crop and soil management history will have a profound effect on organic N dynamics. Cultivation of soils, especially when organic residues are not returned to the soil, results in the loss of soil organic C and N (Rosswall and Paustian, 1984) and decreases in biological and biochemical properties in soils (Dick, 1992).

Nitrogen dynamics in agricultural soils

Within a given climate, the amount of organic matter that accumulates in aerobic agricultural soils appears to have less to do with the type of organic residue than with the amount of carbon (C) that is returned. In semi-arid regions, organic C levels have been closely correlated with plant residue inputs, regardless of whether the residue was wheat straw, legume green manure, or animal manure (Rasmussen and Collins, 1991; Campbell and Zentner, 1993). In Michigan USA, accumulation of soil organic matter from a variety of crops in rotations such as corn, oats, sugar beets, and navy beans after nine years (Zielke and Christenson, 1986) was closely related to plant biomass additions to soils.

Total N accumulation in soils can be affected by vegetation type and N fertilization. An example of this is seen in Larson et al. (1972) where various organic residues applied to a soil in Iowa USA over 11 years showed no differences in total C accumulation but did show a difference in total N accumulation in soils. In general, N-rich residues such as legumes will cause an accumulation of more soil organic N than non-N_2 - fixing plants (Gupta and Reuszer; 1967). However, the growth of nonlegumes can be stimulated by N fertilizer (thus enriching plant residues with N and increasing the mass of organic N inputs). Also, addition of N fertilizer can contribute directly to soil organic N pools through N immobilization. This has been shown in Canada (Campbell et al.; 1991b) where N fertilizer in combination with continuous wheat, legume green manures, or legume-grass hay crops maintained or increased organic N. Conversely, Gupta and Reuszer (1967), after seven years cropping, found that annual alfalfa harvesting without added fertilizer N had equal or slightly higher soil N levels than corn or bromegrass that received more than 200 kg N $ha^{-1}yr^{-1}$.

The net stabilization of the fertilizer N in soils is likely controlled by climate and soil type. In cooler, moister climates, N reserves tend to increase with N fertilization due to relatively lower rates of organic matter decomposition in these environments. There is relatively little information from long-term studies (>15 yr) on effects of plant residues on N dynamics in tropical agricultural systems, but some generalizations may be made from several shorter term studies. Goyal et al.(1992) found an increase in total soil C after 13 years with only the highest rates of N and P in a pearl millet-wheat rotation in sub-tropical, semi-arid India. But increases in microbial C and N, and total N paralleled yield increases and occurred with N fertilizer alone or with one-half the highest rates of N and P. The

rapid turnover of organic N in tropical systems is also indicated in a two-year study in which N-use efficiency by upland rice in Indonesia was about twice as high with a cowpea residue N source than with fertilizer N (Sisworo et al., 1990).

Nitrogen mineralization of plant residues

From the above discussion, it is clear that plant residue management can affect N accumulation and N mineralization potential. This section will address mineralization of N from plant residues added to soils where the soils vary in total N content and N mineralization potential because of past long-term residue management.

Only two studies have specifically addressed this and both were done under greenhouse conditions. Janzen and Radder (1989) conducted a study on soils obtained from long-term plots (initiated in 1951) in Alberta, Canada that had been under different crop rotations that included continuous spring wheat, wheat-fallow, and wheat in rotations with legumes and hay crops. In the greenhouse, soils from the long-term plots were amended with ^{15}N-labelled Tangier flat pea (*Lathyrus tingitanus* cv. Tinga) and plant N uptake was then determined. Total net N mineralized was highest in soils from the rotations of lentil-wheat, continuous wheat (unfertilized) and native grass and lowest in the rotations with fallow-wheat (fertilized with N) and fallow-wheat with three years of alfalfa/grass forage. Although there were significant differences in net N mineralization of flatpea as a function of soil history, these differences were small and most of the differences observed in total net N mineralization were accounted for by differences in indigenous soil N mineralization.

In a study by Fauci and Dick (1994a,b), soils were collected from the long-term residue utilization plots (wheat-fallow system; Oregon). The four soils received the following amendments since 1931: steer manure (22 T ha^{-1} 2 yr^{-1}); pea (*Pisium sativum* L.) vine green manure (2.2 T ha^{-1} 2 yr^{-1}); 90 kg N ha^{-1} 2 yr^{-1}; or no amendment (control). Each of these soils was amended with pea vine, composted steer manure, poultry manure, or control (organic amendments were added at the rate of 0.5 g N kg^{-1} soil) and had four successive plantings of maize (*Zea mays* L.) over 306 d. In the absence of any greenhouse amendment, long-term applications of steer manure had the highest N mineralization which could be related to its higher N content and biological biomass (Dick et al., 1988; Collins et al., 1992). There was no significant effect of field history on N mineralization of any of the organic residues added in the greenhouse. In other words, a soil that had received a particular amendment over the long-term did not, in the short-term, have a significant advantage in mineralizing N from the same residue over a soil that had never received that amendment.

The type of residue had a much greater effect than did the field history. Pea vine, with the lowest lignin content, had the highest rates of N mineralization. This finding was supported by various biological measures such as microbial biomass C where pea vine added in the greenhouse had a much larger biological response than did the effect of long-term field residue management.

Both of these studies (Janzen and Radder, 1989; Fauci and Dick, 1994a,b) support a hypothesis that field history through residue or cropping management that results in differences in organic matter or biological levels does not significantly affect rates of N mineralization from recently added organic amendments. Long-term applications of

organic residues, though, do appear to affect the mineralization of indigenous soil N. Clearly, studies from other regions are needed (both of these studies were done on soils from the semi-arid regions of the northern parts of North America) under field conditions to verify these findings.

Nitrogen mineralization potential

The ability of a soil to provide plant available N from its store of organic N will be determined by the quantity and quality of organic residue inputs to the soil. The characterization of mineralization was first presented by Stanford and Smith (1972) who developed an aerobic incubation/leaching method to develop cumulative N mineralization curves. Various mathematical models have been fitted to these data in order to calculate N mineralization potential (N_0) and the rate constant (k). The N_0 (mg N kg^{-1}) is defined as the quantity (capacity factor) of the total N in a soil at time zero that is available for mineralization as affected by soil genesis and management whereas k is supposed to be a true rate constant ($week^{-1}$).

Although cross study comparisons of N mineralization constants are not generally appropriate because of differences in soil pretreatment (e.g. air-dried vs. field moist) and use of different models, these constants are sensitive in detecting field treatments. For example, field applications of various manures on a range of soils showed groupings of N_0 as follows: steer manure > poultry manure > sewage sludge (Griffin and Laine, 1983).

Recent and continuous additions of organic residues apparently is important in establishing and maintaining N_0 levels. Evidence for this was shown by Janzen (1987) who found that soils from long-term rotations where the rotation with the highest plant

inputs had the highest N_0 values and total N mineralized. This was closely related to the organic light fraction content of the soils but relatively unrelated to total N or C content of the soils. Furthermore, there was a near perfect (negative) correlation between degree of fallowing and N mineralized. Other long-term studies are consistent with this hypothesis where elevated plant contributions from rotations with legumes, green manures, and hay crops (Campbell et al., 1991b) or soil amendments of steer manure or pea vine residue (Christ, 1993; Dick and Christ, 1995) significantly increased N_0 over systems without these elevated organic inputs. On 19 soil types, cumulative N mineralized and microbial activities were higher in native soil from forest, pasture, wetland, or green manured sites than their pair-wise comparison with the cultivated sites as discussed in Chapters 4 and 5.

Nitrogen mineralization dynamics is also affected by residue quality. Indirect evidence for this was provided by Beauchamp et al. (1986) who found N_0 to be significantly higher in soils under bromegrass or alfalfa than in soils under corn after 15 years of cropping. Yet from this and the other studies mentioned above, it is difficult to separate the effects of qualitative differences in the organic residue from the effect of the amount of biomass and/or N inputs on N mineralization. This was addressed in a unique study by Bonde et al. (1988) where they compared N mineralization from soils of bare ground, cropped, cropped + N fertilizer (80 kg N ha^{-1} yr^{-1}), 1800 kg straw-C ha^{-1} yr^{-1} + N fertilizer (80 kg N yr^{-1} yr^{-1}), and 1800 kg farm yard manure (FYM)-C ha^{-1} yr^{-1} + (80 kg N ha^{-1} yr^{-1}). The highest amount of N mineralization and N_0 values occurred in the 1800 kg C straw or FYM treatments. Using a two-component model they were further able to show that the straw treatment had a lower proportion of N_0 in the recalcitrant fraction than did the FYM treatment. These results are consistent with other studies that have

shown FYM to be resistant to mineralization (Castellanos and Pratt, 1981; Fauci and Dick, 1994a).

Long-term addition of inorganic N in the absence of other organic amendments can increase the amount of N mineralized and N_0 values (El-Harris et al., 1983; Bonde et al., 1988). This effect is due to increased N-rich plant biomass additions to soils and N fertilizer being directly immobilized in the soil over the summer when the microbial activity is highest.

The extent to which cropping or vegetative history influences soil mineralizable N is a function of both a "non-living soil mineralizable N pool" and the size and activity of the soil biomass. A large portion of the easily mineralizable organic N pool is composed of amino acids from both plant and microbial sources.

Amino acids are prevalent and persist in soils as demonstrated by their presence in paleosols (Goh, 1972). Although persistent, amino acids are also dynamic in soils and laboratory incubations have shown that a significant portion of this fraction is readily mineralized under aerobic conditions (Keeney and Bremner, 1966). Stevenson (1956) showed that the amino acid composition of soils from the long-term Morrow plots in Illinois differed with crop rotation and soil amendments, and that soils from which residue is removed and with no organic amendments are depleted in the more labile amino acids.

Analyses of crop residues have shown that alfalfa has three to four times the amino acid content of grasses and about five times that of wheat straw (Campbell et al., 1991c). Long-term application (31 yr) of these materials through crop rotations at a site in Saskatchewan, Canada showed no significant effects on the quality of the N fraction distribution in soils. Unlike Gupta and Reuszer, (1967) who found that alfalfa caused

significantly higher total amino acids in soil than corn or bromegrass, Campbell et al. (1991c) were unable to draw any firm conclusions whether plant quality as indicated by amino N content can have any long-term effect on N fractions.

It seems plausible, as shown by Campbell et al. (1991c), that recent additions of plant residues of varying quality can affect amino-N distributions but this may be seasonal or have only short-term effects. This hypothesis is supported by early work of Kuo and Bartholomew (1966) who showed most organic N in soil is of microbial, not plant, origin which would account for the dynamic nature of soil amino acids. Furthermore, current theories indicate that humic substances are formed largely of microbial decomposition products of lignin and cellulose such as polyphenols or quinones that condense with amino groups of amino acids, peptides, and proteins originating from the cell walls of microorganisms (Stevenson, 1994). These humic substances form over time from recently added plant residue and constitute the build up of more resistant soil organic matter fractions.

Microbial biomass N

A key component of N mineralization is microbial biomass N (MB_N). It is highly correlated to readily mineralizable organic N in soils (Kai et al., 1973; Myrold, 1987) and to rates of N mineralization (Alef et al., 1988; Bonde et al., 1988; Fisk and Schmidt, 1995), and is a significant source of N nutrition for plants (Lethbridge and Davidson, 1983). Nitrogen availability is likely controlled by microbial biomass turnover (Holmes and Zak, 1994). Consequently, long-term effects of plant residues on the activity and size of the MB_N pool is an important consideration in N mineralization.

Physical properties affecting soils such as pore size distribution and wetting and drying regimes are also important for determining the character of soil microbial populations (Van Veen et al., 1984), and within these constraints, vegetation management will also influence soil microbial biomass. Higher soil organic matter levels are directly correlated to higher microbial counts, biomass, and activities (Schnürer et al., 1985). Crop rotations that include legumes or manure amendments have higher microbial activities and microbial biomass C levels than do monoculture soils with little or no organic matter inputs (Dick, 1992).

Crop rotations that include a fallow have significantly lower MB_N than either continuous monocropping or more complex crop rotations (Biederbeck et al., 1984; McGill et al., 1986; Campbell et al., 1991d). It follows that the amount of long-term organic C and N inputs control the MB_N levels. It is less clear whether the quality of plant residues affects MB_N levels. This is partly due to the fact that studies rarely characterize plant residues added to soils for quality beyond C-to-N ratio. However, if one assumes that a legume is a higher quality residue (narrow C-to-N ratio) than a nonlegume, some inferences can be made on the long-term effects of "higher quality" residues such as legumes on MB_N. Studies in Canada (Campbell et al., 1991d) and the southern USA (Franzluebbers et al., 1995) have shown that in the absence of N fertilization, crop rotations that include a legume have substantially higher MB_N. The diminished levels of MB_N with "lower quality" inputs where nonlegumes dominate the cropping systems can, in general, be brought to similar levels as rotations that include legumes by the addition of N fertilizer in temperate regions (Campbell et al., 1991d; Harris et al., 1994; Franzluebbers et al., 1995). Similar results have been found in sub-tropical regions where N fertilization

stimulated MB_N (Goyal et al., 1992; Singh, 1995). The added fertilizer N may increase MB_N by increasing N content of the nonlegume residue that is returned to the soil and by being immobilized directly into soil MB_N.

These results suggest that stimulation of MB_N may have less to do with residue quality than with the total amount of N that is incorporated into the soil as organic or inorganic N.

Summary

A review of the literature shows that in general, addition of plant residues can increase soil organic matter levels and this increase is much more related to the rates of application than to the type or quality of the residue. Accumulation of total N and various N fractions appears to be more complex. Some types of N-rich plant (e.g. legume and forage species) residues have been shown to increase, maintain, or reduce losses of organic N with intensive cultivation of the soil. And in Chapter 2 it is shown how legume winter cover crops can be a highly significant contributor to the N nutrition of a subsequent vegetable crop. But addition of inorganic N in some settings to monoculture cereal or maize plants had the same effect as more complex crop rotations that included hay/legume forage rotations. In the case of inorganic N additions, it is difficult to separate the effects of increased biomass production (i.e. greater N uptake in plant residue) from the effect of immobilizing inorganic N directly in the soil organic matter. Chapter 3 attempts to address the fate of inorganic N in a winter cover crop/vegetable system.

Measuring N mineralization combined with calculations of the N mineralization potential (N_0) and the rate constant (k), is relatively sensitive in discriminating between

various crop rotations (differing in biomass or N contributions) and other organic amendments such as animal manures (Stanford and Smith, 1972). This provides evidence that plant residues (probably in both quantity and quality) do affect N dynamics. Unfortunately, neither N mineralization parameter (N_0 and k) can adequately distinguish between the effects of soil biology vs. the effect of long-term residue management on the readily mineralizable organic N pool.

In conclusion, long-term management and incorporation of plant residues does have a significant effect on N accumulation and mineralization, but to understand the mechanisms that are operating and to develop more efficient use of plant N, more sensitive methods for identifying labile N pools are needed. Furthermore, it is difficult to separate the long-term vegetation effects on the activity, size, or diversity of the soil biomass in relation to N mineralization of indigenous soil N. Experiments such as those in Chapters 4 and 5 that include comparisons of cultivated and non-cultivated soils of the same type help to shed light on factors important to the abundance, diversity, and biochemical functioning of the soil microbial biomass. This knowledge then directly relates to N mineralization and how dependable management tools such as winter cover crops can be for supplying N to subsequent crop plants. A major limitation in answering these questions is a lack of methodologies that can deal with the chemical and biological complexity of soils. Secondly, studies at long-term sites are needed that characterize the quality (e.g. amino acid, cellulose, and lignin content, and C-to-N ratios) of the organic residues in order to relate these inputs to measurable N mineralization parameters and soil organic N pools.

References

Alef, K., T. Beck, L. Zelles, and D. Kleiner. 1988. A comparison of methods to estimate microbial biomass and N-mineralization in agricultural and grassland soils. Soil Bio. Biochem. 20: 561-565.

Beauchamp, E. G., W. D. Reynolds, D. Brasche-Villeneuve, and K. Kirby. 1986. Nitrogen mineralization kinetics with different soil pretreatments and cropping histories. Soil Sci. Soc. Am. J. 50:1478-1483.

Biederbeck, V. O., C. A. Campbell, and R. P. Zentner. 1984. Effect of crop rotation and fertilization on some biological properties of a loam in southwestern Saskatchewan. Can. J. Soil Sci. 64:355-367.

Bonde, T. A., J. Schnürer, and T. Rosswall. 1988. Microbial biomass as a fraction of potentially mineralizable nitrogen in soils from long-term field experiments. Soil Bio. Biochem. 20: 447-452.

Campbell, C. A. and R. P. Zentner. 1993. Soil organic matter as influenced by crop rotations and fertilization. Soil Sci. Soc. Am. J. 57: 1034-1040.

Campbell, C. A., K. E. Bowren, M. Schnitzer, R. P. Zentner, and L. Townley-Smith . 1991a. Effect of crop rotations and fertilization on soil organic matter and some biochemical properties of a thin Black Chernozem. Can. J. Soil Sci. 71: 377-387.

Campbell, C. A., G. P. LaFond, A. J. Letshon, R. P. Zentner, and H. H. Janzen. 1991b. Effect of cropping practices on the initial potential rate of N mineralization in a thin Black Chernozem. Can. J. Soil Sci. 71: 43-53.

Campbell, C. A., M. Schnitzer, G. P. Lafond, R. P. Zentner, and J. E. Knipfel. 1991c. Thirty-year crop rotations and management practices effects on soil and amino nitrogen. Soil Sci. Soc. Am. J. 55: 739-745.

Campbell, C. A., V. O. Biederbeck, R. P. Zentner, and G. P. LaFond. 1991d. Effect of crop rotations and cultural practices on soil organic matter, microbial biomass and respiration in a thin Black Chernozem. Can. J. Soil Sci. 71: 363-376.

Castellanos, J. Z. and P. F. Pratt. 1981. Mineralization of manure nitrogen-correlation with laboratory indexes. Soil Sci. Soc. Am. J. 45: 354-357.

Christ, R. A. 1993. Effect of long-term residue management and nitrogen fertilization on availability and profile distribution of nitrogen, phosphorus, and sulfur. M.S. Thesis, Oregon State University, Corvallis OR, USA.

Collins, H. P., P. E. Rasmussen, and C. L. Douglas, Jr. 1992. Crop rotation and residue management effects on soil carbon and microbial dynamics. Soil Sci. Soc. Am. J. 56: 783-788.

Dick, R. P. 1992. A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. Agric. Ecosys. Environ. 40: 25-36.

Dick, R. P. and R. A. Christ. 1995. Effects of Long-term residue management and nitrogen fertilization on availability and profile distribution of nitrogen. Soil Sci. 159: 402-408.

Dick, R. P., P. E. Rasmussen, and E. A. Kerle. 1988. Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. Biol. and Fert. of Soils. 6: 159-164.

El-Harris, M. K., V. L. Cochran, L. F. Elliott, and D. F. Bezdicek. 1983. Effect of tillage. cropping, and fertilizer management on soil mineralization potential. Soil Sci. Soc. Am. J. 47: 1157-1161.

Fauci, M. F. and R. P. Dick. 1994a. Plant response to organic amendments and decreasing inorganic nitrogen rates in soils from a long-term experiment. Soil Sci. Soc. Am. J. 58: 134-138.

Fauci, M. F. and R. P. Dick. 1994b. Soil microbial dynamics, short- and long-term effects of inorganic and organic nitrogen. Soil Sci. Soc. Am. J. 58: 801-806.

Fisk, M. C. and S. K. Schmidt. 1995. Nitrogen mineralization and microbial biomass nitrogen dynamics in three alpine tundra communities. Soil Sci. Soc. Am. J. 59: 1036-1043.

Franzluebbers, A. J., F. M. Hons, and D. A. Zuberer. 1995. Soil organic carbon, microbial biomass, and mineralizable carbon and nitrogen in sorghum. Soil Sci. Soc. Am. J. 59: 460-466.

Goh, K. M. 1972. Amino acid levels as indicators of paleosols in New Zealand soil profiles. Geoderma 7: 33-47.

Goyal, S., M. M. Mishra, I. S. Hooda, and R. Singh. 1992. Organic matter-microbial biomass relationships in field experiments under tropical conditions: effects of inorganic fertilization and organic amendments. Soil Bio. Biochem. 24: 1081-1084.

Griffin, G. F. and A. F. Laine. 1983. Nitrogen mineralization in soils previously amended with organic wastes. Agron. J. 75: 124-129.

Gupta, U. C. and H. W. Reuszer. 1967. Effect of plant species on the amino acid content and nitrification of soil organic matter. Soil Sci. 104: 395-400.

Harris, G. H., O. B. Hesterman, E. A. Paul, S. E. Peters, and R. R. Janke. 1994. Fate of legume and fertilizer nitrogen-15 in a long-term cropping systems experiment. Agron. J. 86: 910-915.

Holmes, W. E. and D. R. Zak. 1994. Soil microbial biomass dynamics and net nitrogen mineralization in northern hardwood forests. Soil Sci. Soc. Am. J. 58: 238-243.

Janzen, H. H. 1987. Soil organic matter characteristics after long-term cropping to various spring wheat rotations. Can. J. Soil Sci. 67: 845-856.

Janzen, H. H. and G. D. Radder. 1989. Nitrogen mineralization in a green manure-amended soil as influenced by cropping history and subsequent crop. Plant and Soil 120: 125-131.

Jenny, H. 1980. The Soil Resource. Ecological studies, vol. 37. Springer-Verlag, New York.

Kai, H., Z. Ahmad, and T. Harada. 1973. Factors affecting immobilization and release of nitrogen in soil and chemical characteristics of the nitrogen newly immobilized. Soil Sci. Plant Nutr. 19: 275-286.

Keeney, D. R. and J. M. Bremner. 1966. Characterization of mineralizable nitrogen in soil. Soil Sci. Soc. Am. Proc. 30: 714-719.

Kuo, M. H. and W. V. Bartholomew. 1966. On the genesis of organic nitrogen in decomposed plant residue. p. 329-335 *In* The Use of Isotopes in Soil Organic Matter Studies. Pergamon Press, London.

Larson, W. E., C. E. Clapp, W.H. Pierre, and Y.B. Morachan. 1972. Effects of increasing amounts of organic residues on continuous corn, II. Organic carbon, nitrogen, phosphorus, and sulphur. Agron. J. 64: 204-208.

Lethbridge, G. and M. S. Davidson. 1983. Microbial biomass as a source of nitrogen for cereals. Soil Bio. Biochem. 15: 375-376.

McGill, W. B., K. R. Cannon, J. A. Robertson, and F. D. Cook. 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. Can. J. Soil Sci. 66: 1-19.

Myrold. D. D. 1987. Relationship between microbial biomass nitrogen and a nitrogen availability index. Soil Sci. Soc. Am. J. 51: 1047-1049.

Rasmussen, P. E. and H. P. Collins. 1991. Long-term impacts of tillage, fertilizer, and crop residue on soil organic matter in temperate semiarid regions. Adv. in Agron. 45: 93-134.

Rosswall, T. and K. Paustian. 1984. Cycling of nitrogen in modern agricultural systems. Plant and Soil 76: 3-21.

Schnürer, J., M. Clarholm, and T. Rosswall. 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents. Soil Bio. Biochem. 17: 611-618.

Singh, H. 1995. Nitrogen mineralization, microbial biomass and crop yield as affected by wheat residue placement and fertilizer in a semi-arid tropical soil with minimum tillage. J. Appl. Ecol. 32: 588-595.

Sisworo, W. H., M. M. Mitrosuhardjo, H. Rasjid, and R. J. K. Myers. 1990. The relative roles of N fixation, fertilizer, crop residues and soil in supplying N in multiple cropping systems in a humid, tropical upland cropping system. Plant and Soil 121: 73-82.

Stanford, G. and S. J. Smith. 1972. Nitrogen mineralization potentials of soils. Soil Sci. Soc. Am. J. 36: 465-472.

Stevenson, F. J. 1956. Effect of some long-time rotations on the amino acid composition of the soil. Soil Sci. Soc. Proc. 20: 204-208.

Stevenson, F. J. 1994. Humus Chemistry. John Wiley & Sons, New York.

Van Veen, J. A., J. N. Ladd, and M. J. Frissel. 1984. Modelling C and N turnover through the microbial biomass in soil. Plant and Soil 76: 257-274.

Zielke, R. C. and D. R. Christenson. 1986. Organic carbon and nitrogen changes in soil under selected cropping systems. Soil Sci. Soc. Am. J. 50: 363-367.

Chapter 2

Fertilizer Nitrogen and Winter Cover Crops in Sweet Corn and Broccoli Rotations

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Abstract

Cover crops hold potential to improve soil quality, to recover residual fertilizer N in the soil remaining after a summer crop that otherwise might leach to the groundwater, and be a source of N for vegetable crops. The objective of this five year study was to determine the N uptake by winter cover crops and its effect on summer vegetable productivity. The experimental design was a split-plot with winter cover crops [red clover (*Trifolium pratense* L.), cereal rye (*Secale cereale* L. var. Wheeler), a cereal rye/Austrian winter pea (*Pisum sativum* L.) mix, or a winter fallow control] as the main plot in a rotation with alternate years of sweet corn (*Zea mays* L. cv. Jubilee) and broccoli (*Brassica oleracea* L. var. *italica* cv. Gem). The sub-plots were N rate (zero, intermediate, and recommended for vegetable crop). Summer relay plantings of red clover or rye were also used to gain early establishment of the cover crop. Rye cover crops took up an average of 40 kg N/ha following the recommended N rates, but after five years of cropping there was no evidence that the conserved rye cover crop N would enable a reduction in inorganic N inputs to maintain yields. Intermediate rates of N applied to summer crops in combination with winter cover crops containing legumes produced vegetable yields similar to those with recommended rates of N in combination with winter fallow or rye cover crops. There was a consistent, trend ($P<0.12$) for cereal rye cover crops to cause a small decrease in broccoli yields as compared to winter fallow.

Introduction

Cover crops have been used for thousands of years to preserve and enhance soil fertility. The first written accounts of cover crops for soil improvement are from the Chou

dynasty in China over 3,000 years ago, and agricultural literature has consistently mentioned the benefits of green manure cover crops (Pieters, 1927). North American agricultural doctrine of the 19th and early 20th century equated financially successful farming with the use of green manures because of their ability to protect fields from erosion and to absorb minerals that would otherwise be leached from the soil (Harlan, 1899; Pieters, 1927).

As use of inorganic N fertilizers increased during the past 60 years, cover cropping declined. Crop yields have risen during this time, but so have soil erosion and nitrate concentrations in groundwater (National Research Council, 1989; Keeney and Follet, 1991). Close monitoring of agricultural soils has revealed evidence of nitrate leaching when crop N use is insufficient to deplete available soil N (Staver and Brinsfield, 1990).

Groundwater pollution by nitrates has been identified as a problem in parts of Oregon's productive Willamette Valley. Of 82 wells tested in Marion County, 28 had nitrate-N concentration greater than the 10 parts per million EPA upper limit for safe drinking water (Petit, 1988). Oregon vegetable growers may apply more than 250 kg inorganic N/ha in a season on sweet corn or broccoli. Wet winters in western Oregon provide optimum conditions for residual soil nitrate to be leached into the groundwater.

Interest in the dynamics of N in agricultural systems has increased in recent years (Keeney and Follet, 1991). Many studies have shown that N can be conserved in agricultural systems by various management strategies (Ditsch and Alley, 1991; Hargrove, 1986; Hesterman et al., 1986; Ebelhar et al., 1984).

A potential method for reducing nitrate leaching from agricultural soils is to replace some of the applied inorganic N with green manure cover crops, because N from

decomposing plant material becomes available over a longer time span than fertilizer N. Green manure residues can supply most or all of crop N needs if mineralization coincides with crop N requirements (Fauci and Dick, 1994; Touchton et al., 1982). Griffen and Hesterman (1991) found that the N content of legumes grown as green manures ranged from 33 to 238 kg ha^{-1}, and the average fertilizer replacement value of these legumes was about 100 kg N/ha. One study showed a recovery of between 89 and 102% of N derived from decomposing ^{15}N-labeled cover crop plant materials buried in mesh bags, and that uptake by barley (*Hordeum vulgare* L.) of the mineralized plant N was correlated to cover crop decomposition (Muller and Sundman, 1988). If mineralization of organic N does not correspond to plant N growth requirements, yields can be reduced. Griffen and Hesterman (1991) did not see a significant yield response of potatoes (*Solanum tuberosum*) to plowed-down legumes, and attributed this to lack of synchrony between N mineralization and plant N needs.

There is little information on agronomic and environmental performance of cover crops in vegetable-based systems located in a Mediterranean climate as is found in western Oregon (Copeland, 1965). This climate is characterized by hot dry summers followed by cool wet winters when conditions are conducive to leaching. The objectives of this study were to investigate the contribution of winter cover crops to the N nutrition of a subsequent crop of either sweet corn or broccoli over five growing seasons, and the ability of winter cover crops to accumulate N after the vegetable harvest.

Materials and Methods

The study area is located at the Oregon State University North Willamette Research and Extension Center near Aurora, Oregon. The soil is a Willamette silt loam (mixed mesic Pachic Ultic Argixeroll). The design of this long-term study (initiated in 1989) is a randomized split-plot complete block with four replications of six winter cover crop treatments and three N rate sub-plots within each main plot (Table 2.1). Main plots are 18 m X 9 m and are divided into three equal sub-plot areas of 54 m^2 except for the spring-seeded red clover winter cover crop treatment which had one half plowed in the late fall and the other half plowed the following spring before summer crop planting; the half plots of clover were then further divided into two sub-plots of 40.5 m^2 and received either zero N or the recommended N rate on subsequent vegetable crops.

Cover Crops

Winter cover crop treatments are 'Wheeler' cereal rye, rye/Austrian winter pea mix, 'Kenland' red clover, and winter fallow (Table 2.1). Cover crops were planted in mid-September of each year. From 1989 to 1992, there were two sets of cover crop rotations where all had the same winter crop treatments (cereal rye or rye/pea mix as described above) but summer crops had either no pesticide inputs (weeds were controlled with cultivation) or recommended pesticide applications (both treatments had 18 X 9 m plots with the same N rate subplots described above and are further described under the Summer Crops subsection below). Because there were no statistical differences between these treatments on either summer crops or cover crop productivity measurements, data

Table 2.1. Timeline of plantings in cover crop-vegetable rotation plots, North Willamette Research and Extension Center.

Fall '89	Spring '90	Fall '90	Spring '91	Fall '91	Spring '92	Fall '92	Spring '93	Fall '93	Spring '94
fallow	corn	fallow	broccoli	wheat	wheat	fallow	broccoli	fallow	corn
red clover	red clover	red clover	broccoli	red clover	red clover	red clover	broccoli	red clover	corn
rye	corn	rye	broccoli	rye	corn	relay rye	broccoli	relay rye	corn
rye/pea	corn	rye/pea	broccoli	rye/pea	corn	relay clover	broccoli	relay clover	corn
rye	corn	rye	broccoli	rye	corn	rye	broccoli	rye	corn
rye/pea	corn	rye/pea	broccoli	rye/pea	corn	rye/pea	broccoli	rye/pea	corn

from these treatments were pooled for statistical analysis and presentation. In the summer of 1992, the treatment receiving no pesticide inputs was converted to a system that had a summer relay establishment of either Wheeler cereal rye (put in plots that previously had a September planting of cereal rye) or Kenland red clover (put in plots that previously had rye/pea mix) into the standing vegetable crop.

Rye was drilled at a rate of 75 kg ha^{-1} in the fall-seeded plots and broadcast at a rate of 85 kg ha^{-1} in summer relay plots. In the rye-pea mix, the rye rate was 40 kg ha^{-1} and the Austrian pea rate was 112 kg ha^{-1}. Fall-seeded red clover plots in rotation with summer crops were drilled at a rate of 20 kg ha^{-1} in 1990 and 1992, harvested for seed in late July, and then allowed to regrow as a ratoon winter cover crop for both years of broccoli. Red clover in summer relay plots was broadcast at a rate of 25 kg ha^{-1}.

In late March of each year, we randomly cut 1 m^2 biomass samples at ground level from each sub-plot of the treatment. In plots planted to a rye/pea mix, the pea plants were counted, weighed and analyzed separately from the rye biomass. Subsamples weighing approximately 0.5 kg were taken from well mixed field samples for dry matter and total N analyses. In April of each year, cover crops were mowed, disked and incorporated with a moldboard plow. Seedbed preparation consisted of disking and rotovating.

Vegetable Crops

The experiment consisted of alternate years of sweet corn (1990, 1992, and 1994) and broccoli (1991 and 1993) fertilized at three rates of N (0, 56, or 224 kg urea-N/ha for sweet corn and 0, 140, or 280 kg urea-N/ha for broccoli). The N treatment sub-plots remained at the same relative rates (zero, low, or recommended) from year to year; for

example, the 56 kg N/ha sub-plot for sweet corn in 1992 and 1994 was also the 140 kg N/ha sub-plot for broccoli in 1991 and 1993. During the 1991 and 1992 growing seasons, half the vegetable plots with winter cover crops received either recommended (atrazine at 2.24 kg ha^{-1} and alachlor at 3.36 kg ha^{-1} for sweet corn, and trifluran at 0.84 kg ha^{-1} for broccoli) or reduced (EPTC at 2.24 kg ha^{-1} for corn and no herbicide for broccoli) pesticide treatments. In the summers of 1992 and 1993, plots with reduced herbicide inputs were interseeded with either red clover or cereal rye into standing sweet corn or broccoli as described in Cover Crop subsection above. Sweet corn and broccoli were planted in 51-cm paired rows spaced 102 cm apart. Sweet corn was seeded to a stand of approximately 62,000 plants/ha. Broccoli stands were thinned to an in-row spacing of 30 cm. All plots received a banded application of 90 kg P/ha as triple superphosphate at planting and, within a week, a surfaced-banded application of one-half the respective N rate for each sub-plot. The other half of the urea N was banded between rows in mid-July.

All vegetable plots were mechanically cultivated and then hand-weeded if necessary. The irrigation facilities available allowed for the use of sprinklers until seedlings were established and subsequent irrigation by drip lines with emitters at 23 cm intervals and a flow rate of 1.9 liters/minute per 30.5 m.

Unhusked ears of sweet corn were harvested and weighed in mid-August from 4.6 m lengths of two inner rows of each sub-plot. Broccoli heads were twice harvested and weighed (mid-August and late August) from 4.6 m lengths of two inner rows in each sub-plot.

Laboratory Analyses and Statistics

Cover crop subsamples were dried in forced air at 60° C for three to five days. Dried subsamples were weighed, ground first in a Wiley mill to a coarse texture, and then ground to pass a 100 mesh size seive. Cover crop plant samples were analyzed for total N by the Kjeldahl procedure.

Data was analyzed by analysis of variance with a general linear model (SAS, 1985) as a randomized complete split-plot block design over years with winter cover crop treatment as main plots and N rate as sub-plots (Table 2.2). This analysis is unbalanced because the low pesticide fall-planted cover crop treatment was converted to relay-planted cover crops in 1992. Means comparisons of sweet corn and broccoli yields at the three N rates and various winter cover crop treatments as well as total N content of cover crops at the three N rates were accomplished using Fisher's Protected LSD (SAS, 1985). The spring-seeded red clover seed crop/vegetable rotation treatments were not included in the statistical analysis because these plots were fertilized at two rates of N rather than three. Statistical analysis shown in Table 2.2 includes the main-plot cover crop rotations that were studied for five years (winter fallow, fall-planted cereal rye, and fall-planted cereal/rye mix) whereas only single year ANOVA was performed for relay established cover crops since these were only tested for one year with broccoli or sweet corn.

Results and Discussion

Winter cover crop treatment and N rate both had highly significant effects on broccoli and sweet corn yield. Sweet corn yields were unaffected by interactions but broccoli head yields were significantly affected by interactions of N rate by cover crop and

N rate by year (Table 2.2). Cover crop N content also had significant interactions of year by cover crop, N rate by cover crop, and N rate by year (Table 2.2). Consequently, data has been pooled only for main effects when there were no significant interactions ($P<0.05$).

Table 2.2. Summary of analyses of variance showing the sources of effects on total cover crop N content, broccoli yield, and sweet corn yield for the years 1990 through 1994.

Source of variation	Cover crop N content		Broccoli head yield		Sweet corn ear yield	
	df	*MS*	*df*	*MS*	*df*	*MS*
Year (Y)	3	18287 (***)	1	49485636 (***)	2	1145794844 (***)
Block (R)	3	572 (NS)	3	8848327 (***)	3	16653263 (NS)
Cover crop (C)	3	8808 (***)	4	12771920 (**)	4	107870994 (***)
Y x C	5	1608 (*)	2	1045833 (NS)	3	3737112 (NS)
Error A	33	459	20	2655776	27	16895447
N rate (N)	2	15479 (***)	2	113352036 (***)	2	905938310 (***)
N x C	6	556 (NS)	8	4860037 (**)	8	3907658 (NS)
N x Y	6	2063 (***)	2	10796853 (**)	4	7084282 (NS)
N x C x Y	10	366 (NS)	4	1067801 (NS)	6	4168392 (NS)
Error B	72	305	46	1604438	60	10380787
Residual error	47	427	27	921900	48	12316583
Total	190		119		167	

NS, *, **, *** Not significant or significant at $P<0.05$, 0.01, and 0.001, respectively.

Cover Crops

Total N uptake was significantly affected by all main effects of time (year), cover crop and N rate (Table 2.2). Cover crop N uptake had a significant year by cover crop interaction which indicates that yearly weather conditions had a differential effect on cover crop N uptake as function of the type of cover crop. Fall precipitation and soil temperature will have a great effect on the establishment and early growth of winter cover crops. Weather data for the North Willamette Research and Extension Center during the months of September and October had large variability over the five years with precipitation ranging from 36 mm in 1993 to 206 mm in 1994. From qualitative observations of cover crop growth, we found that a dry fall and/or excessive rainfall and/or a freeze in November/December severely reduced cover crop growth. However, when we attempted to correlate weather data with cover crop growth and N uptake we had poor and non-significant correlation coefficients which is likely related to the confounding and interacting effects of temperature and moisture conditions each year.

These results indicate that it is important in the Pacific Northwest to design cover crop systems that provide consistent winter growth regardless of weather conditions. Adequate early growth and consistency of growth patterns are needed to reliably recover residual fertilizer N during the time of maximum rainfall and nitrate leaching potential and to develop realistic fertilizer recommendations for subsequent summer crops. These observations led to the development of the relay cover crop treatments. Relay rye and clover grew best during the late summer and early fall in those areas of the plots that were moistened by the drip irrigation for maximum summer crop yield. This suggests that for the typical dry summer of a Mediterranean summer, irrigation facilities are needed for

relay cover crop establishment. All vegetables in Western Oregon are grown under irrigated conditions which would facilitate relay cover crop establishment under farmers' conditions. However, relay establishment of cover crops resulted in amounts of N uptake similar to those of fall established cover crops (Table 2.3).

The N content of the rye cover crop increased with increasing fertilizer N applied to the previous vegetable crop (Table 2.3). An estimate of residual fertilizer N taken up by the cereal rye cover crops may be obtained by subtracting the N uptake of rye grown on unfertilized soil from that grown with fertilizer. This difference, averaged over years, was 51 and 30 kg ha^{-1} for fall-planted rye and relay rye cover crops at the highest N rates, respectively (Table 2.3).

Winter cover crops with a legume component had greater N content at plowdown for all N rates than did a rye cover crop, and at the zero N rates legume cover crops had about twice the N content of the rye cover crop (Table 2.3). The N content of legumes in the winter cover crop did not increase with increasing N rate applied to the previous vegetable crop for either the clover (Table 2.3) or the Austrian winter pea component of the rye and pea mix (Table 2.4). It is interesting to note that the N content of the rye component of the rye/pea mix averaged 28%, 18%, and 12% more N at the zero intermediate and high N rate, respectively, than did the rye cover crop grown alone (data not shown). This higher rate of accumulation of rye in the presence of the legume suggests fixed N from the legume is transferred to the rye either in season or from the accumulated N incorporated into the soil from previous years.

Table 2.3. Total N content of winter cover crops for the years 1991 through 1994.

Winter cover crop treatment	N rate		
	Zero	Intermediate	Recommended
		kg N/ha	
		1991	
clover	61 ab[z] A	--	32 b A
rye	45 b B	65 a AB	138 a A
rye/pea	74 a B	85 a B	124 a A
		1992	
rye	22 b B	31 b AB	49 b A
rye/pea	57 a B	69 a B	85 a A
		1993	
clover	117 a A	--	90 a A
relay clover	73 b A	61 a A	73 ab A
relay rye	33 c B	35 b B	60 ab A
rye	20 c B	24 b B	45 b A
rye/pea	44 bc B	59 a AB	87 a A
		1994	
relay clover	38 a A	35 ab A	41 b A
relay rye	9 b B	16 b B	42 b A
rye	17 b B	40 ab AB	49 ab A
rye/pea	45 a B	58 a AB	66 a A

[z]Within each year, lower case letters show comparisons among cover crops at a specific N rate (columns) and upper case letters show comparisons among N rates for each cover crop (rows). Total cover crop N content means comparisons for each year are separate, and those with the same letter in a column or row do not differ significantly with regard to N rate or cover crop respectively. (Fisher's Protected LSD, $\alpha = 0.05$).

Table 2.4. Nitrogen content of the rye/Austrian winter pea cover crop treatment, 1991 through 1994.

N rate applied to previous crop	Number of pea plants/m^{-2}	N content of pea plants (kg ha^{-1})	N content of rye plants (kg ha^{-1})	Total N content of cover crop (kg ha^{-1})
Zero	71 a[z]	19 a	40 b	59 b
Intermediate	69 a	20 a	51 b	71 b
Recommended	57 b	16 b	79 a	96 a
p value	0.16	0.25	0.05	0.05

[z]Means in a column with the same letter do not differ significantly at specified probability (p) level.

Although it was only significant at $P < 0.16$, increasing rates of N fertilizer applied to the summer crops tended to suppress the number of pea plants in a rye/pea mix (Table 2. 4). This may be due to a greater competitive advantage of rye under high N conditions. This would suggest that to maintain the Austrian pea, intermediate N rates are preferable.

Red clover grown for seed and left as a winter cover crop in 1990 and 1992 contained average N contents of 89 kg ha^{-1} in the zero N rate sub-plots and 61 kg ha^{-1} in the recommended N rate sub-plots (Table 2.3). Total N content of relay red clover, like fall-planted Austrian pea, was unaffected by application of N fertilizer applied to the previous summer vegetable crop (Table 2.3).

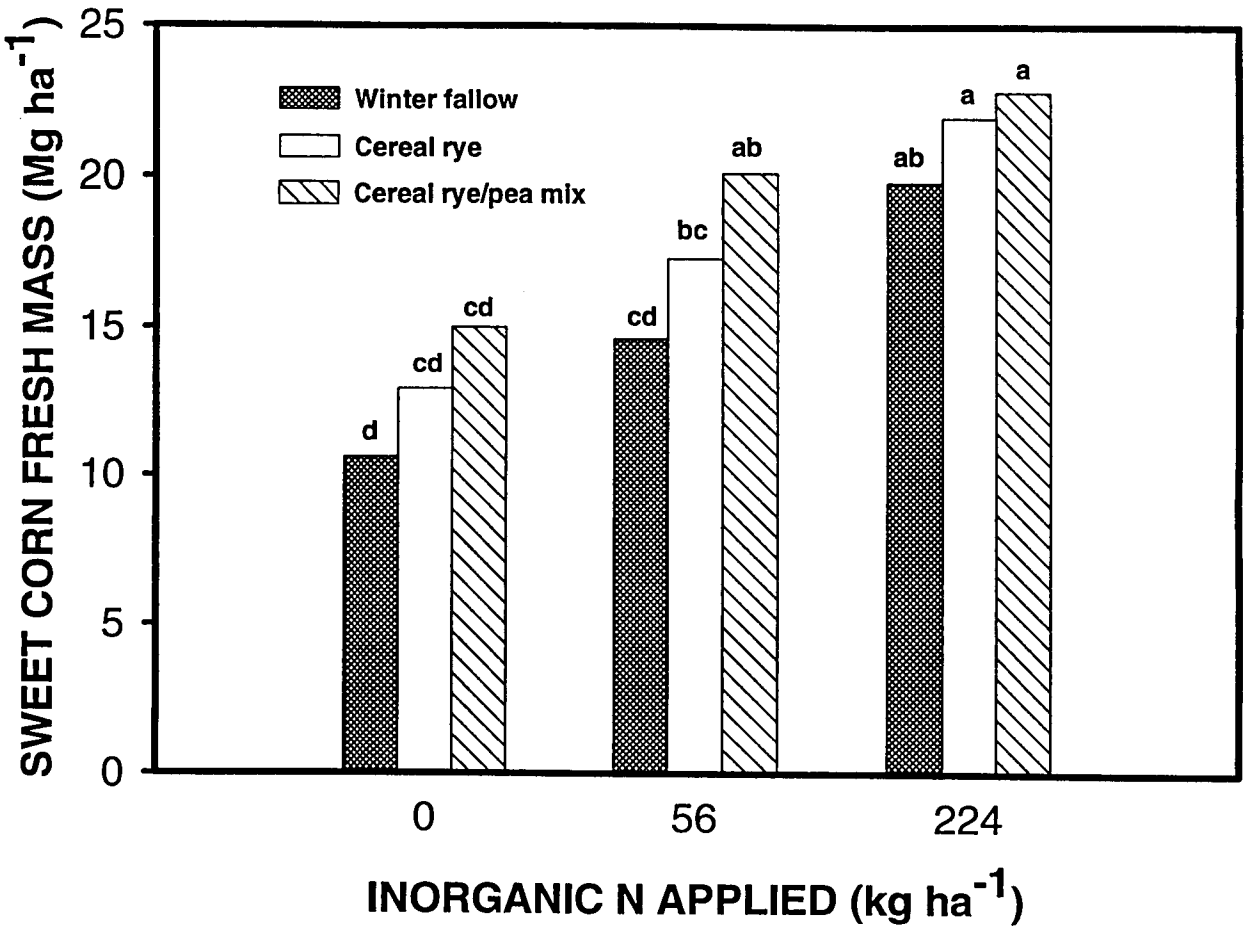
Vegetable Crops

Sweet corn yields by cover crop and N rate are shown in Figs. 2.1 and 2.2. The fallow treatments shown in Fig. 2.1 do not include data from 1992 since there was no fallow treatment for sweet corn that year. Since relay cover crops were in place for only one season prior to sweet corn, yield data for 1994 was analyzed separately in Fig. 2.2.

At all N rates, rye cover crops resulted in a slightly reduced broccoli yield compared to the fallow treatments (Table 2.5). This trend also occurred with sweet corn in 1994 (Fig. 2.2). Averaged over all years and N rates, the relative yield of the vegetable crops from rye cover crop plots compared to fallow plots is 81.3%. Similarly negative field corn responses to a cereal rye cover crop have been reported by McCracken et al. (1989), Mitchell and Teel (1977) and Raimbault et al. (1991). Lettuce (*Latuca sativa* L.) yield and available soil N decreased following an incorporated mature rye cover crop as compared to fallow (Wyland et al., 1995). Cover crops such as rye, with a greater C to N ratio than legumes, may result in net microbial N immobilization and deplete the soil of N available for crop use. Such immobilization may explain the trend seen in our data, but phytotoxic compounds released by the decomposing cereal rye residue may also be an explanation (Raimbault et al., 1991).

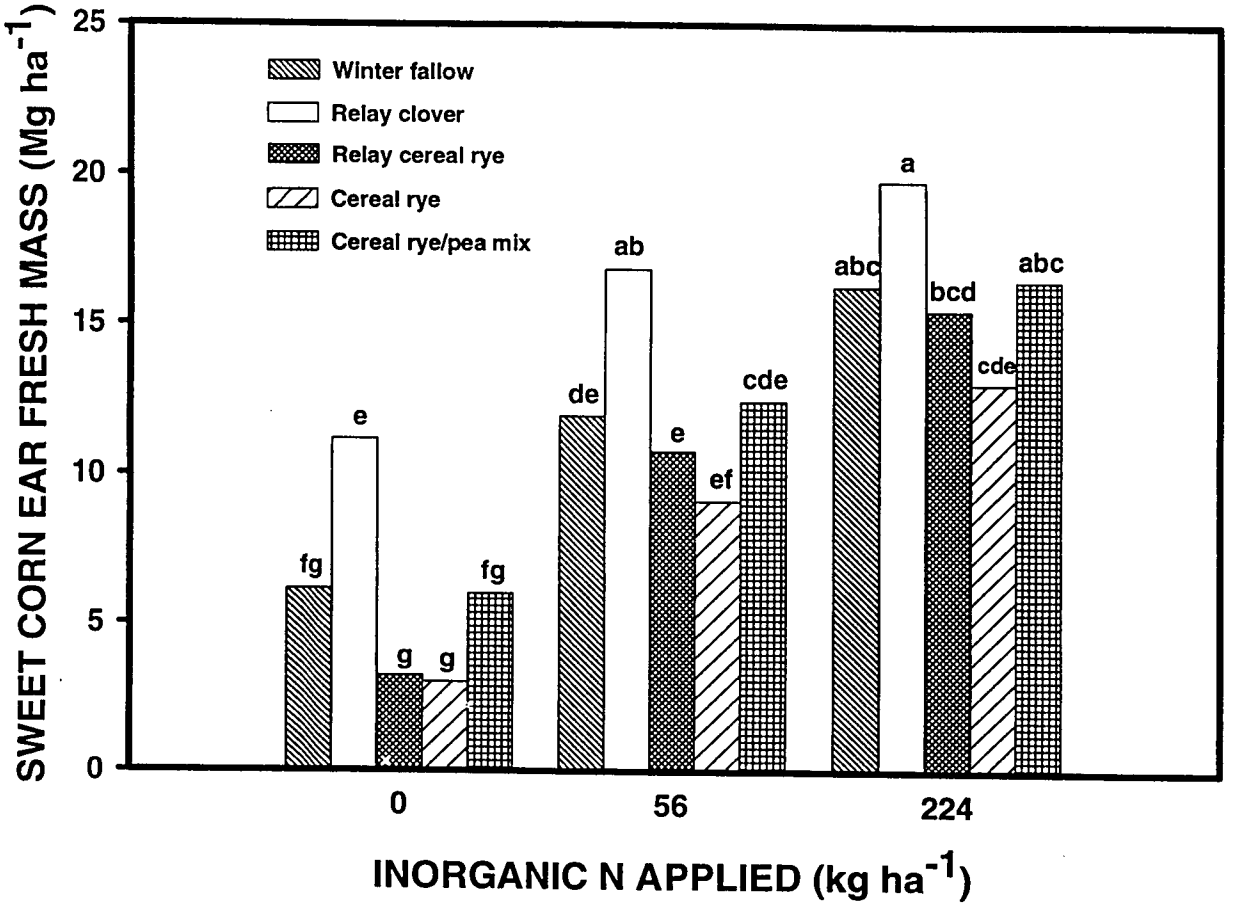
Sweet corn yields at the 56 kg N/ha N rate following a rye/pea mix or relay clover were statistically equivalent to yields at the 224 kg N/ha rate following fallow and rye cover crop treatments (Figs. 2.1 and 2.2). Sweet corn following relay clover in 1994 produced the greatest yields at each N rate, and with no N produced a yield statistically equivalent to that produced with rye and 224 kg N/ha (Fig. 2.2).

Figure 2.1. Mean yields of sweet corn ears at three N rates and five winter cover crop treatments for three growing seasons; 1990, 1992, and 1994.



Treatments with the same letters are not significantly different by Fisher's Protected LSD, $P<0.05$.

Figure 2.2. Mean yields of sweet corn ears at three N rates and five winter cover crop treatments for the 1994 growing season.



Treatments with the same letters are not significantly different by Fisher's Protected LSD, $P<0.05$.

Other studies using legume green manure crops have shown similar results. Decker et al. (1987) reported no fertilizer response in field corn above a rate of 80 kg N/ha following fall-seeded legume winter cover crops. And field corn yields increased significantly compared to a control after three years of a non-harvested alfalfa (*Medicago sativa*) crop that contained about 170 kg N/ha per year (Sheaffer et al., 1991).

Broccoli yields in the rye/pea plots in 1991 were statistically equivalent at the 140 and 280 kg N/ha rates, but in the rye and fallow treatments broccoli yield increased significantly with all N rates. In 1993, however, broccoli yields at the 140 and 280 kg N/ha rates did not differ significantly for all winter cover crop treatments (Table 2.5), a result in contrast to a study without cover crops by Kowalenko and Hall (1987) that showed a greater broccoli yield at 250 kg N/ha than at 125 kg N/ha. At the intermediate rate in 1991, and the zero and intermediate rates in 1993, legume cover crops had a positive effect on broccoli yields (Table 2.5), and the greatest fresh weight yield of 10.2 Mg ha^{-1} was obtained with 140 kg N/ha after the pea/rye mix cover crop (Table 2.5). For both years, broccoli grown with no fertilizer N after a legume-containing winter cover crop had a mean yield of 6.5 Mg ha^{-1}, while a rye or fallow treatment resulted in a mean yield of 4.1 Mg ha^{-1} with no fertilizer N (Table 2.5). In 1993, broccoli grown without N after a relay clover cover crop had a yield equivalent to that obtained at the recommended N rate (Table 2.5), and yields following all winter cover crop treatments except rye reached their maximum yields in this experiment at an N rate of 140 kg ha^{-1}.

Table 2.5. Broccoli head yields by N rate and winter cover crop treatment for the years 1991 and 1993.

Winter cover crop treatment	N rate		
	Zero	Intermediate	Recommended
		1991	
		Mg broccoli heads/ha	
Fallow	3.9 a[z] C	6.3 ab B	8.4 a A
Rye	3.6 a C	5.1 b B	7.4 a A
Rye/pea	4.2 a B	7.1 a A	8.1 a A
		1993	
		Mg broccoli heads/ha	
Fallow	4.2 b B	9.3 a A	8.9 a A
Rye	4.6 ab B	7.8 b A	8.4 a A
Rye/pea	6.6 a B	10.2 a A	8.3 a AB
Relay rye	3.6 b B	7.8 b A	7.4 a A
Relay clover	7.8 a A	8.4 ab A	8.3 a A

[z]Within each year, lower case letters show comparisons among cover crops at a specific N rate (columns) and upper case letters show comparisons among N rates for each cover crop (rows). Means comparisons for each year are separate and those with the same letter in a column or row do not differ significantly with regard to N rate or cover crop, respectively. (Fisher's Protected LSD, $\alpha = 0.5$).

Summary

In theory, cereal winter cover crops that annually conserve fertilizer N and then are repeatedly incorporated into the soil should reduce the amount of N applied to the summer crop. After five years cropping, our results showed that cereal rye did not have this effect because yields of vegetables were similar between fallow and rye plots at zero or intermediate N rates. In contrast, Ditsch and Alley (1991), who had similar N recovery

rates (11 to 81 kg ha^{-1} for wheat (*Triticum aestivum*) and rye cover crops) to our results found that these cereal cover crops could make a significant N contribution to field corn. It may be that more time is needed for this soil and climate before N can accumulate to levels where there is not N mineralization from immobilized N in rye cover-cropped soils. Given that farmers are not likely to continuously grow cover crops on a field year in and year out, results from our study suggest that a legume is needed in a mix or alone for farmers to reduce N levels below the recommended rate.

Legume winter cover crops contributed significant amounts of N to increase sweet corn and broccoli yields. This study showed that winter cover crops containing legumes and an N rate of one quarter of the recommended rate resulted in sweet corn yields that were equivalent to yields obtained at the recommended rate after winter fallow or a cereal rye winter cover crop. In 1991, broccoli yields after a legume winter cover crop and one half the recommended N rate were not significantly different from yields following fallow or rye winter cover crops at the recommended rate. And in 1993, maximum broccoli yields were achieved at the intermediate N rate for all winter cover crop treatments. Cereal rye was able to capture residual soil N that otherwise would have been leached, but its negative effect on subsequent sweet corn and broccoli yields suggests that a different non-leguminous species winter cover crop should be used to recover residual fertilizer N.

References

Bremner, J. M. and C. S. Mulvaney. 1982. Nitrogen-total. p. 595-624. *In* A. L. Page, R. H. Miller, and D. R. Keeney, (ed.) Methods of soil analysis, Part 2. 2nd edition. American Society of Agronomy, Madison, WI.

Copeland, R. D. 1965. The evaluation of cereal grains, legumes, and selected winter annuals as cover crops for the Willamette Valley. MS Thesis, Oregon State University, Corvallis, OR.

Decker, A. M., J. F. Holderbaum, R. F. Mulford, J. J. Meisinger, and L. R. Vough. 1987. Fall seeded legume nitrogen contributions to no-till corn. *In* The role of legumes in conservation tillage systems. (J. F. Powers, editor) Soil Conservation Society of America. pp. 21-22.

Ditsch, D. C. and M. M. Alley. 1991. Nonleguminous cover crop management for residual N recovery and subsequent crop yields. J. Fert. Issues 8:6-13.

Ebelhar, S. A., W. W. Frye, and R. L. Blevins. 1984. Nitrogen from legume cover crops for no-tillage corn. Agron. J. 76:51-55.

Fauci, M. and R. P. Dick. 1994. Plant response to organic amendments and decreasing inorganic nitrogen rates in soil from a long-term experiment. Soil Sci. Soc. Am. J. 58:134-138.

Griffen, T. S. and O. B. Hesterman. 1991. Potato response to legume and fertilizer nitrogen sources. Agron. J. 83:1004-1012.

Hargrove, W. L. 1986. Winter legumes as a nitrogen source for no-till grain sorghum. Agron. J. 78:70-74.

Harlan, C. 1899. Farming with green manures. Alfred J. Ferris, Printer, Philadelphia.

Hesterman, O. B., C. C. Scheaffer, D. K. Barnes, W. E. Lueschen, and J. H. Ford. 1986. Alfalfa dry matter and nitrogen production and fertilizer nitrogen response in legume-corn rotations. Agron. J. 78:19-23.

Keeney, D. R. and R. F. Follet. 1991. Managing nitrogen for groundwater quality and farm profitability: overview and introduction. Soil Sci. Soc. Am., Madison, WI.

Kowalenko, C. G. and J. W. Hall. 1987. Effects of nitrogen applications on direct-seeded broccoli from a single harvest adjusted for maturity. J. Amer. Soc. Hort. Sci. 112:9-13.

McCracken, D. V., S. J. Corak, M. S. Smith, W. W. Frye, and R. L. Blevins. 1989. Residual effects of nitrogen fertilization and winter cover cropping on nitrogen availability. Soil Sci. Soc. Am. J. 53:1459-1464.

Mitchell, W. H. and M. R. Teel. 1977. Winter-annual cover crops for no-tillage corn production. Agron. J. 69:569-573.

Muller, M. M. and V. Sundman. 1988. The fate of nitrogen (^{15}N) released from different plant materials during decomposition under field conditions. Plant and Soil 105:133-139.

National Research Council. 1989. Alternative Agriculture. National Academy Press. Washington, D.C.

Petit, G. 1988. Assessment of Oregon's groundwater for agricultural chemicals. Report to the State of Oregon, Department of Environmental Quality, Portland, Oregon.

Pieters, A. J. 1927. Green Manuring Principles and Practices. John Wiley and Sons, New York.

Raimbault, B. A., T. J. Vyn, and M. Tollenaar. 1991. Corn response to rye cover crop, tillage methods, and planter options. Agron. J. 83:287-290.

SAS Institute, Inc. 1985. SAS user's guide: Statistics. SAS Institute, Inc., Cary, NC.

Sheaffer, C. C., M. P. Russelle, G. H. Heichel, M. M. Hall, and F. F. Thicke. 1991. Non-harvested forage legumes: nitrogen and dry matter yields and effects on a subsequent corn crop. J. Prod. Agric. 4:520-525.

Staver, K. W. and R. B. Brinsfield. 1990. Patterns of soil nitrate availability in corn production systems: Implications for reducing groundwater contamination. J. Soil and Water Cons. 45:318-32.

Touchton, J. T., W. A. Gardner, W. L. Hargrove, and R. R. Duncan. 1982. Reseeding crimson clover a N source for no-tillage grain sorghum production. Agron. J. 74: 283-287.

Wyland, L. J., L. E. Jackson, and K. F. Schulbach. 1995. Soil-plant dynamics following incorporation of a mature rye cover crop in a lettuce production system. J. Agric. Sci. 124:17-25.

Chapter 3

Fate of Applied Fertilizer Nitrogen in a Vegetable-Cover Crop Rotation

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Abstract

Conservation of nitrogen within agricultural systems reduces the need for N applications. This study examined the cycling of fertilizer N within a vegetable /winter cover crop system over the course of 14 months and the N budgets of a larger scale winter cover crop/vegetable rotation study. Sweet corn (*Zea mays* L. cv. Jubilee) was planted in 3 m^2 microplots and fertilized with 5 atom % excess ^{15}N urea at a rate of 240 kg ha^{-1} N. At harvest, half the microplots received labeled corn residue and the other half had labeled residue switched with unlabeled residue. Cereal rye (*Secale cereale* L. var. Wheeler) was then planted in all microplots as a winter cover crop. The following spring, rye grown in labeled plots was switched with rye from an unlabeled area and broccoli (*Brassica oleracea* L. var. *italica* cv. Gem) was planted. Soil samples were taken and analyzed for total, inorganic, and microbial biomass N. At the end of the first growing season, all N applied as fertilizer was accounted for. Sweet corn took up an average of 49% of fertilizer N and the rest remained in the soil, primarily in the form of inorganic N. The following spring, total recovery of the N derived from fertilizer (NDFF) in the various treatments ranged from 40% to 73%, with treatments having a labeled plant residue component showing higher recoveries. The N losses in the microplots are in general agreement with data from both the larger cover crop rotation study and from N leaching data obtained under the same experimental plots. Most of the NDFF that was in the soil in the spring was accounted for after broccoli harvest, but only 2 to 4% was taken up by the broccoli crop.

Introduction

Nitrogen cycling in agricultural systems must be understood in order to help maintain productivity while minimizing environmental impacts. The fate of applied N and how it cycles in cultivated systems is relevant to both environmental and economic concerns for agriculture (Keeney and Follet, 1991). Cover crops have been shown to reduce the need for fertilizer N by contributing significantly to the yield of a subsequent crop (Burket et al., 1997; Ditsch and Alley, 1991). The fate of applied fertilizer N in cover crop systems is therefore of great interest to the above issues. Past work has shown that recently immobilized fertilizer N is more available to plant uptake than the original soil organic N, but over time newly immobilized N also increases in stability (Stewart et al.,1963; Broadbent and Nakashima, 1967; Westerman and Kurtz, 1972; Allen et al., 1973; Kelley and Stevenson, 1985). An important comparison in terms of management is the fate of N recently immobilized directly by the microbial biomass and N immobilized from incorporated plant residue. The relatively few studies that have used labeled plant residue in field situations have shown that N from labeled plant residue is retained in the soil longer and is less available for subsequent crop uptake than other forms of recently added N (Ladd et al. 1981; Wagger et al., 1985; Ladd and Amato, 1986; Muller and Sundman, 1988).

In this study, we report the fate of N originally applied as urea fertilizer and its conversion to other soil-plant N pools. The objective of this study was to determine the potential of a cereal rye winter cover crop to recover fertilizer N applied to a summer

sweet corn crop, and to follow the fate of the fertilizer-derived N through a winter cover crop and into a subsequent broccoli crop.

Materials and Methods

The study area is located at the Oregon State University North Willamette Research and Extension Center near Aurora, Oregon. The soil is a Willamette silt loam (mixed mesic Pachic Ultic Argixeroll). The design of this long-term study (initiated in 1989) is a randomized split-plot complete block (four replications) where the main plots were six winter cover crop treatments and the sub-plots were three N rates within each main plot. Main plots (three replications) were 9 m X 18 m and were divided into three equal sub-plot areas of 54 m^2. An exception was the rotation of a red clover seed crop with vegetables (see Table 2.1 in Chapter 2). In this case, the clover was planted in spring and harvested for seed in July. One half of the red clover plots were plowed in the late fall and the other half was allowed to regrow over the winter and was then plowed the following spring before summer crop planting (ratoon red clover); the half plots of clover were then further divided into two sub-plots of 40.5 m^2 and received either zero N or the recommended N rate on subsequent vegetable crops.

Cover Crops

Winter cover crop treatments were cereal rye as summer relay or fall planted (*Secale cereale* L. var. Wheeler), cereal rye/Austrian winter pea (*Pisum sativum* L.) mix, red clover (*Trifolium pratense* L. var. Kenland), Kenland red clover relay, and winter fallow. Fall cover crops were planted in mid-September of each year after summer crop

residue incorporation and tillage. From 1989 to 1992, there were two sets of cover crop rotations where all had the same winter crop treatments (cereal rye or rye/pea mix as described above) but summer crops had either reduced herbicide inputs (weeds were controlled primarily by cultivation) or recommended herbicide applications (both treatments had 18 X 9 m plots with the same N rate subplots described above and are further described under the Summer Crops subsection below). Because there were no statistical differences between these treatments on either summer crops or cover crop productivity measurements, data from these treatments were pooled for statistical analysis and presentation of the data. In the summer of 1992, the treatment receiving reduced herbicide inputs was converted to a system that had a summer relay establishment of either 'Wheeler' cereal rye (put in plots that previously had a September planting of cereal rye) or 'Kenland' red clover (put in plots that previously had rye/pea mix) into the standing vegetable crop.

Rye was drilled at a rate of 75 kg ha^{-1} in the fall-seeded plots and broadcast at a rate of 85 kg ha^{-1} in summer relay plots. In the rye-pea mix, the rye rate was 40 kg ha^{-1} and the Austrian pea rate was 112 kg ha^{-1}. Fall-seeded red clover plots in rotation with summer crops were drilled at a rate of 20 kg ha^{-1} in 1990 and 1992, harvested for seed in late July, and then allowed to regrow as a ratoon winter cover crop for both years of broccoli. Red clover in summer relay plots was broadcast at a rate of 25 kg ha^{-1}.

In late March of each year, we randomly chose 1 m^2 areas for biomass sampling at ground level from each sub-plot of the treatment. In plots planted to a rye/pea mix, the pea plants were counted, weighed and analyzed separately from the rye biomass. Subsamples weighing approximately 0.5 kg were taken from well mixed field samples for

dry matter and total N analyses. In April of each year, cover crops were mowed, disked and incorporated with a moldboard plow. Seedbed preparation consisted of disking and rotovating.

Vegetable Crops

The experiment consisted of alternate years of 'Jubilee' sweet corn in 1990, 1992, and 1994 and 'Gem' broccoli in 1991 and 1993 fertilized at three rates of N (0, 56, or 224 kg urea-N/ha for sweet corn and 0, 140, or 280 kg urea-N/ha for broccoli), except crops following ratoon clover which were fertilized at zero or recommended rates. The N treatment sub-plots remained at the same relative rates (zero, low, or recommended) from year to year; for example, the 56 kg N/ha sub-plot for sweet corn in 1992 and 1994 was also the 140 kg N/ha sub-plot for broccoli in 1991 and 1993. During the 1991 and 1992 growing seasons, half the vegetable plots with winter cover crops received either recommended (atrazine at 2.24 kg ha^{-1} and alachlor at 3.36 kg ha^{-1} for sweet corn; trifluralin at 0.84 kg ha^{-1} for broccoli) or reduced (EPTC at 2.24 kg ha^{-1} for corn and no herbicide for broccoli) pesticide treatments. In the summers of 1992 and 1993, plots with reduced herbicide inputs were interseeded with either red clover or cereal rye into standing sweet corn or broccoli as described above. Sweet corn and broccoli were planted in 51-cm paired rows spaced 102 cm apart. Sweet corn was seeded to a stand of approximately 62,000 plants/ha. Broccoli stands were thinned to an in-row spacing of 30 cm. All plots received a banded application of 90 kg P/ha as triple superphosphate at planting and, within a week, a surfaced-banded application of one-half the respective N rate for each sub-plot. The other half of the urea N was banded between rows in mid-July.

All vegetable plots were mechanically cultivated and then hand-weeded if necessary. The irrigation facilities available allowed for the use of sprinklers until seedlings were established and subsequent irrigation by drip lines with emitters at 23 cm intervals and a flow rate of 1.9 liters/minute per 30.5 m.

Unhusked ears of sweet corn were harvested and weighed in mid-August from 4.6 m lengths of two inner rows of each sub-plot. Broccoli heads were twice harvested and weighed (mid-August and late August) from 4.6 m lengths of two inner rows in each sub-plot.

^{15}N Microplots

The ^{15}N study took place within the larger vegetable/cover crop rotation study described above. After sweet corn planting in 1992, 3 X 4 m microplots were established within the center of three replicates of a cereal rye winter cover crop treatment. The microplots therefore spanned four rows and extended 48.4 cm beyond the two outside rows in the 3 m direction, and extended 4 m along the rows. The corn was fertilized by means of a backpack sprayer at a rate of 240 kg N/ha with a half application of 4.876 atom % excess ^{15}N urea made to a 3 m X 2 m area within each of the microplots one day after planting. A second application was made 7 weeks after planting. Labeled microplot areas were hand irrigated with a sprinkler can after each fertilizer application in order to move the urea into the soil.

At harvest, sweet corn ears were collected from the labeled microplots and dried for subsequent analysis. Corn plants were cut off at ground level and weighed. Four corn plants from each microplot were collected for shredding in a compost shredder that was

carefully cleaned between each sample. A 1 kg sub-sample was saved for drying and later analysis. Corn stalks were returned to the appropriate microplots after weighing and shredding (Table 3.1). In half of each of the labeled microplot areas now referred to as treatment C+S, for labeled sweet corn and labeled soil (1.5 m by 2 m), the sweet corn stalks and roots that were dug up and collected by hand were carefully incorporated. In the other 1.5 m by 2 m areas, now referred to as treatment S, for labeled soil, labeled stalks and roots were exchanged with the stalks and roots of an equally-sized adjacent unlabeled area, now referred to as treatment C, for labeled sweet corn.

In order to prevent movement of corn residue or soil among the different areas, frames consisting of 5 cm by 25 cm wooden boards were constructed around each sub-microplot (C+S, S, and C) such that the edge of the board was buried 5 cm into the soil.

Microplots were tilled by hand and a cereal rye winter cover crop was broadcast at a rate of 75 kg ha^{-1} and raked into the microplots in early October. The following March, soil samples from the same depth intervals were taken in areas C+S, S, C, and an adjacent equally sized unlabeled area (referred to as R since it received labeled rye cover crop residue prior to broccoli planting).

Within each sub microplot, the rye was dug up such that as much of the root system as possible was preserved. Samples of the rye plants and roots were collected from each area for analysis. Then the entire above and below ground biomass of the rye was handled in the following manner: rye biomass from treatment C+S was switched with rye biomass from the adjacent unlabeled area R. A full schedule timeline for the microplots is shown in Table 3.1 Biomass from treatments S and C was switched with equal amounts of biomass from an unlabeled area in the same treatment but completely outside the

microplot area. Rye biomass was then incorporated by hand into the microplots and the plots planted to broccoli in June. Broccoli was planted on an in-row spacing of 30 cm and was fertilized with urea N at a rate of 280 kg ha^{-1}. Irrigation for both sweet corn and broccoli was by hand until seedlings were established and then by drip lines with emitters at 23 cm intervals and a flow rate of 1.9 liters/minute per 30.5 m. All broccoli heads, stalks, and roots were sampled for ^{15}N analysis in August of 1993.

All plant samples were dried in a forced air oven at 60° C for 3 to 5 days. Dried sub-samples were weighed and ground to pass a 100 mesh seive. Prior to each harvest, soil samples were taken from each of the microplots. Separate samples were taken from the 0-20, 20-40, 40-80, 80-120 cm depth intervals. These samples were kept in a cooler and the next day were split so that one half of the sample could be analyzed for microbial biomass ^{15}N by fumigation-extraction (Brookes et al., 1985) and the other half sieved and dried for inorganic ^{15}N analysis. Samples from all three collection dates were analyzed for total ^{15}N by mass spectrometry, and samples from the first collection (prior to sweet corn harvest) were analyzed for total ^{15}N, NH_4-^{15}N, and NO_3-^{15}N. Inorganic N was determined by the sequential diffusion technique (Brookes et al., 1989; Sorensen and Jensen, 1991) which was modified by using 7 mm diameter discs of Whatman # 3 filter paper that were skewered through their center with an 8 cm length of acid-washed steel welding wire (0.76 mm dia.) that was then carefully pushed into the opening of the plastic cup so that it was slightly bent and held there by means of its own tension. Microbial biomass N was determined by first digesting K_2SO_4 extracts of fumigated and umfumigated labeled soils (Cabrera and Beare, 1993) and then diffusing these samples by the method described above. Digested, unfumigated ^{15}N diffusion samples were subtracted from digested

Table 3.1 Timeline of operations in microplots.

Microplot	Spring 1992	Fall 1992	Spring 1993	Fall 1993
C + S	240 kg ha^{-1} ^{15}N applied as urea	Sweet corn ears harvested, labeled sweet corn residue incorporated within microplot, cereal rye winter cover crop planted	Labeled rye biomass removed, unlabeled rye from outside microplots incorporated, broccoli planted	Broccoli harvested
C	240 kg ha^{-1} ^{15}N applied as urea	Sweet corn ears harvested, unlabeled sweet corn residue incorporated from microplot S, cereal rye winter cover crop planted	Labeled rye biomass removed, unlabeled rye from outside microplots incorporated, broccoli planted	Broccoli harvested
S	240 kg ha^{-1} N applied as urea	Sweet corn ears harvested, labeled sweet corn residue incorporated from microplot C, cereal rye winter cover crop planted	Labeled rye biomass removed, unlabeled rye from outside microplots incorporated, broccoli planted	Broccoli harvested
R	240 kg ha^{-1} N applied as urea	Sweet corn ears harvested, unlabeled sweet corn residue incorporated, cereal rye winter cover crop planted	Unlabeled rye biomass removed, labeled rye from microplot C incorporated, broccoli planted	Broccoli harvested

fumigated ^{15}N samples and the result divided by a K_{en} factor of 0.6 (Horwath and Paul, 1994) to determine microbial biomass ^{15}N.

Data Analyses and Statistics

Data were analyzed by analysis of variance with a general linear model as a randomized complete split-plot block design over years with winter cover crop treatment as main plots and N rate as sub-plots. Means comparisons of sweet corn and broccoli N

contents at the three N rates and various winter cover crop treatments as well as total N content of cover crops at the three N rates were accomplished using Fisher's Protected LSD. The spring-seeded red clover seed crop/vegetable rotation treatments were not included in the statistical analysis because these plots were fertilized at two rates of N rather than three.

Unaccounted for N was calculated by the following equation:

$$FN_x - (CCN_x - CCN_0) - (HN_x - HN_0) = UN_x$$

Where $_x$ is the N rate and FN stands for amount of fertilizer N applied to the vegetable crop, CCN is cover crop N content for the cover crop planted after the summer crop, and HN is the N content of the harvested portion of the summer crop. CCN_0 and HN_0 are the N contents of the respective cover crops and harvested portions of the vegetable crops from the zero N subplots and so represent an estimate of mineralized N in the system. All numbers are in kg ha^{-1}.

Results and Discussion

N Balance Study

The total aboveground N content of the sweet corn and broccoli crops for the years 1991 through 1994 is shown in Table 3.2. This N content does not include root N which can constitute approximately another 25% of the N content of the total N taken up by maize (Anderson, 1986). In broccoli, the root system is not as extensive as corn and analyses of broccoli in the ^{15}N microplots showed that approximately 15% of the total N content of the whole plant was in the roots (data not shown).

In Table 3.3 the total amount of unaccounted for N for each year is shown. The amounts of non-mineralized cover crop and harvested (sweet corn ears or broccoli heads) N is subtracted from the amount of fertilizer N applied. Non-mineralized N is calculated by subtracting the N of cover crops and harvested N in the zero N subplots of each treatment from those N contents in the intermediate and recommended N subplots. The data show for all years significant differences in the amounts of unaccounted for N between the intermediate and recommended N rates. Also of note is the fact that only at the higher N rates do the fallow treatments show significantly more N unaccounted for than other treatments. It is likely that some of the unaccounted for N maintains the soil organic N levels of the soil through immobilization and some is lost from the system through leaching or denitrification. Leaching losses of NO_3-N over three years at the same site (1992-1995) averaged 37 kg ha^{-1} for the intermediate and 69 kg ha^{-1} for the recommended N rates under winter fallow plots, and 26 kg ha^{-1} for the intermediate and 41 kg ha^{-1} for the recommended N rates under cereal rye (Brandi-Dohrn et al., 1997). Denitrification losses of 12.5% of applied fertilizer were found in some grass-seed soils in western Oregon (Horwath et al., 1998), and could therefore constitute a significant portion of the unaccounted for N in the treatments.

^{15}N Microplot Study

The distribution of nitrogen derived from fertilizer (NDFF) and native soil N at the end of the first growing season in the labeled microplots is shown in Table 3.4. The amounts of N listed as derived from soil organic matter is calculated by subtracting the measured components from the total. NDFF remaining in the soil as inorganic N

Table 3.2. Total N content in kg ha^{-1} of aboveground plant parts for summer vegetable crops; broccoli 1991, 1993; sweet corn 1992, 1994, and of winter cover crops ( ) incorporated prior to summer vegetable crops for the years 1991 through 1994.

Winter cover	N rate		
crop treatment	Zero	Intermediate	Recommended
		kg ha^{-1} N content	
		***1991** (broccoli)*	
fallow	(0) 40 ab[z] C	(0) 69 a B	(0) 106 a A
rye	(45) 31 b B	(65) 52 a AB	(138) 81 b A
rye/pea	(74) 42 a B	(85) 66 a AB	(124) 91 ab A
		***1992** (sweet corn)*	
rye	(22) 69 b B	(31) 97 b B	(49) 187 a A
rye/pea	(57) 92 a C	(69) 122 a B	(85) 192 a A
		***1993** (broccoli)*	
fallow	(0) 80 bc B	(0) 173 a A	(0) 168 ab A
relay clover	(73) 112 a B	(61) 147 ab AB	(73) 161 ab A
relay rye	(33) 70 c C	(35) 124 b B	(60) 148 b A
rye	(20) 90 b B	(24) 159 a A	(45) 165 ab A
rye/pea	(44) 118 a C	(59) 165 a B	(87) 191 a A
		***1994** (sweet corn)*	
fallow	(0) 31 ab B	(0) 47 b B	(0) 76 b A
relay clover	(38) 55 a B	(35) 77 a B	(41) 106 a A
relay rye	(9) 26 b B	(16) 49 b B	(42) 79 b A
rye	(17) 19 bc B	(40) 49 b AB	(49) 59 c A
rye/pea	(45) 29 ab B	(58) 55 ab AB	(66) 80 b A

[z]Within each year, lower case letters show comparisons among N contents across cover crop treatments at a specific N rate (columns) and upper case letters show comparisons among N contents for each cover crop (rows) across N rates. N content means comparisons are separate for each year, and those with the same letter in a column or row do not differ significantly with regard to N rate or cover crop respectively. (Fisher's Protected LSD, $\alpha = 0.05$). Table 2.3 in Chapter 2 shows mean separation statistics for cover crops

Table 3.3 Mass N balances for vegetable crops. Numbers represent the amount of unaccounted for N.

Winter cover	N rate	
crop treatment	Intermediate	Recommended
	Total N unaccounted for , kg ha^{-1}	
	***1991** (broccoli)*	
fallow	131 c[x]	262 a
rye	123 c	233 b
rye/pea	115 c	234 b
	***1992** (sweet corn)*	
rye	32 b	146 a
rye/pea	34 b	166 a
	***1993** (broccoli)*	
fallow	117 cd	258 a
relay clover	137 c	268 a
relay rye	116 cd	229 b
rye	108 d	231 b
rye/pea	113 cd	246 ab
	***1994** (sweet corn)*	
fallow	45 c	196 a
relay clover	45 c	189 ab
relay rye	36 cd	161 b
rye	15 d	168 ab
rye/pea	28 cd	171 ab

[x] Within each year, N amounts with the same letter are not significantly different from each other. N content means comparisons are separate for each year, (Fisher's Protected LSD, $\alpha = 0.05$).

amounted to 53% during the first three months of the experiment (June to August). Interestingly, 8% of NDFF was found in the microbial biomass as opposed to 1.2% of the native soil N being microbial biomass. A substantial amount of applied N, 70 kg ha^{-1} or 44% of the NDFF not taken up by the sweet corn crop, was nitrified over the summer. The average percent recovery of the 240 kg ha^{-1} applied fertilizer by the sweet corn was 49% which is in agreement with many other N balance studies (Legg and Meisinger, 1982). The amounts of N in the sweet corn crop derived from fertilizer and N derived from soil are essentially equal in all components; roots, ears, and stalks (Table 3.4). Balabane and Balesdent (1992) also found equal contributions to the total crop from fertilizer and native soil N in a field study using maize. However, in contrast to our results, lower relative amounts of NDFF in the grain portion were found by both Balabane and Balesdent, (1992) and Gass et al., (1971) which they attributed to NDFF not being present in high amounts in the deeper soil layers that contribute significantly to the N of grain production. In our study, there was no significant difference in the distribution by depth of any of the N fractions (data not shown) in the soil profiles of the microplots, indicating a definite downward movement of the originally added fertilizer N. Another indication of downward movement of inorganic NDFF is soil lysimeter data taken on the same treatment plots. Kowalenko (1989) found a complete leaching of NO_3 from the root zone over winter in British Columbia which has a similar climate to Oregon.

Accounted for NDFF in sweet corn and soil represents a 115% recovery. Recoveries of more than 100% of ^{15}N are not uncommon in tracer studies due to uneven distribution of labeled fertilizer in the soil matrix (Carter, et al., 1967; Hauck and Bremner, 1976).

Table 3.4 Total N and NDFF at sweet corn harvest, August 1992, after an initial application of 240 kg ha^{-1} urea N in June 1992.

	Total N			NDFF		
	kg ha^{-1}	*(SD)*	*% of total*	*kg ha^{-1}*	*(SD)*	*% of total*
Sweet corn total	230	(17)	100	118	(17)	100
ears	103	(18)	45	50	(15)	42
stalks	100	(26)	43	49	(11)	42
roots	27	(11)	12	19	(5)	16
Soil total	7527	(659)	100	160	(70)	100
Soil organic matter	7168	(241)	95	63	(15)	39
NO_3	153	(48)	2	70	(31)	44
NH_4	117	(51)	1.6	14	(9)	9
Microbial biomass	89	(42)	1.2	13	(5)	8

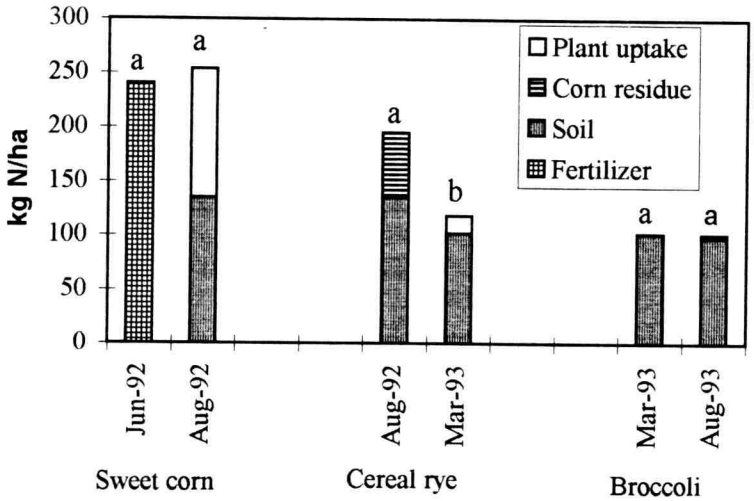
A picture of the fate of NDFF is displayed in Figures 3.1 through 3.4. In treatment C + S (Fig. 3.1), 135 kg ha^{-1} remained in the soil after sweet corn harvest and 60 kg ha^{-1} of N in sweet corn stalks and roots were incorporated prior to planting the cereal rye winter cover crop. The following March, just prior to cover crop incorporation, the soil contained 102 kg ha^{-1} and the rye crop 16 kg ha^{-1} of NDFF. This amount represents a loss of 77 kg ha^{-1} or 40% of the NDFF that had been present at cover crop planting. Measurements at the time of cover crop planting showed that 70 kg ha^{-1} or 44% of the NDFF present in the soil at this time was in the form of NO_3 (Table 3.4). The general agreement between the percentage of measured NO_3 and the percentage of NDFF lost from the system adds to the evidence mentioned above for leaching. In a larger experimenton the same soils, rye absorbed an average of 27 kg ha^{-1} of fertilizer N not taken up by a previous vegetable crop as calculated by the difference method (Burket et al., 1997). The discrepancy between the NDFF measured in the rye cover crop in the ^{15}N

microplots (16 kg ha^{-1} N) and the amount calculated by the difference method (27 kg ha^{-1} N) may be due to more soil N being mineralized in plots with high amounts of inorganic N added.

For the cereal rye winter cover crop in 1992, 146 kg ha^{-1} of N inputs in the form of fertilizer, previous crop residue and rye residue were not accounted for by crop uptake (Table 3.3). This increased unaccounted N may be attributed to addition to soil organic matter, or losses such as denitrification and leaching. A lysimeter study under the same winter rye cover crop system at the same N rate detected an average leaching loss of 41 kg ha^{-1} of NO_3-N for the three year period from 1992-1994 (Brandi-Dohrn, et al., 1997). Denitrification losses of 12.5% of applied fertilizer were found in some grass-seed soils in western Oregon (Horwath et al., 1998), and so could account for up to another 30 kg ha^{-1} of N loss from the treatments.

In the spring of 1993, labeled rye was switched with an equal amount of unlabeled rye so that only soil NDFF was available to the broccoli crop. Of the ^{15}N that was present at broccoli planting, 100% was recovered at broccoli harvest, but only 3% of this was taken up by the broccoli crop (Fig. 3.1). Because the broccoli was fertilized with urea N at a rate of 280 kg ha^{-1}, it is likely that only a small amount of the previous year's NDFF present in the soil would be available to the broccoli. Calculations show however that while this original NDFF in the soil represents only 1.3% of the total soil N (7527 kg ha^{-1}), it contributed 3% of the N present in the broccoli crop. This result is in general agreement with other studies that report an increased availability to plants of recently incorporated N over native soil N albeit at a low rate (Westerman and Kurtz, 1972; Ladd and Amato, 1986; Muller and Sundman, 1988).

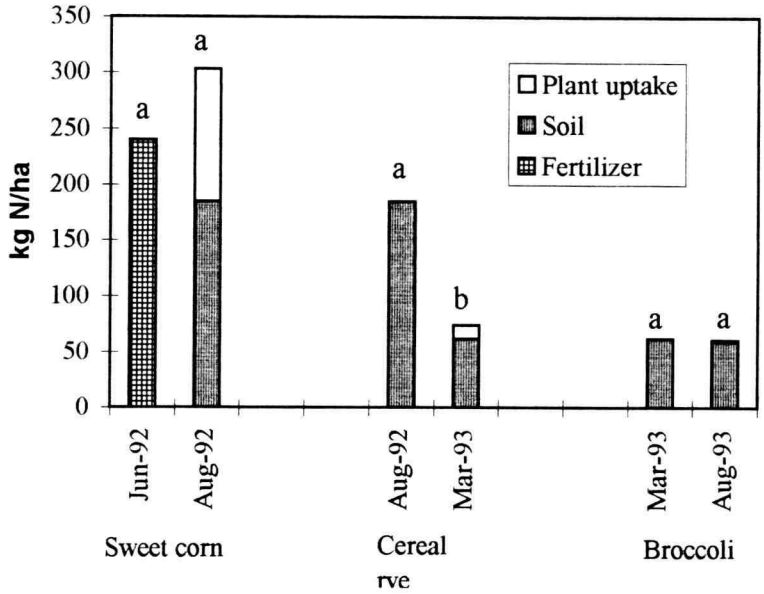
Figure 3.1. Distribution of ^{15}N among soil and plant components within a cropping or cover crop season in the C + S treatment microplots.



*Pairs of columns that have the same letter do not differ significantly from each other ($p<0.05$).

The S treatment microplots (Fig. 3.2) had unlabeled corn residue added to labeled soil that contained 185 kg ha^{-1} NDFF. The following spring, 62 kg ha^{-1} of the soil NDFF remained in the soil and 12 kg ha^{-1} of this was this present in the rye cover crop, indicating a loss of 111 kg ha^{-1} or 60% of the NDFF over winter. Because 44% or 70 kg ha^{-1} of the soil NDFF originally present was in the form of NO_3 (Table 3.4), and the rye winter cover crop took up only 12 kg ha^{-1} of the NDFF, it is easy to see why such a large loss occurred. From this point on the S microplots were treated the same as the C+S microplots and a 100% recovery was again measured between broccoli planting and harvest with 98% of NDFF remaining in the soil and 2% being taken up by the broccoli crop (Fig. 3.2).

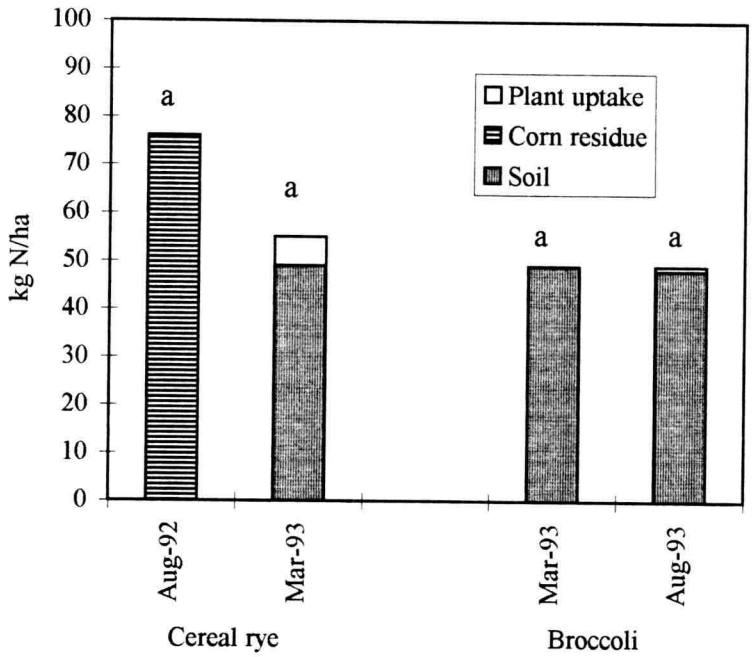
Figure 3.2 Distribution of ^{15}N among soil and plant components within a cropping or cover crop season in the S treatment microplots.



*Pairs of columns that have the same letter do not differ significantly from each other ($p<0.05$).

Treatment C (Fig. 3.3) had 76 kg ha^{-1} of labeled sweet corn residue from treatment S incorporated into unlabeled soil. The following spring, 49 kg ha^{-1} of the NDFF from sweet corn residue was in the soil component and 6 kg ha^{-1} was in the rye crop, representing a loss of 27% of NDFF that had been present in the fall. This substantially lower loss over winter compared to treatments C+S and S, illustrates the persistence of N in the soil when it is in the form of plant residue, a result that has been seen in other studies (Ladd and Amato, 1986; Muller and Sundman, 1988). This treatment had the same regime of incorporating unlabeled rye into the plot and showed the same 100% recovery pattern in broccoli.

Figure 3.3 Distribution of ^{15}N among soil and plant components within a cropping or cover crop season in the M treatment microplots.

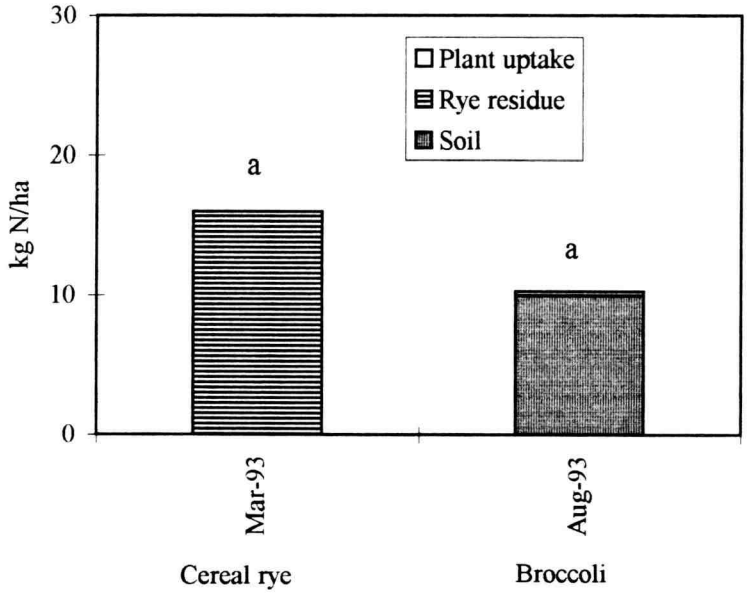


*Pairs of columns that have the same letter do not differ significantly from each other ($p<0.05$).

In treatment R (Fig. 3.4), 16 kg ha^{-1} ^{15}N from labeled rye grown in sub-microplots C+S was added to unlabeled soil and 10 kg ha^{-1} was recovered in the soil (99% of total present) and broccoli crop (1% of total present). This 38% loss of NDFF originally present in the rye residue and compared to the 27% loss from sweet corn residue in treatment C, which was in the soil 4 months longer than the rye residue, seems surprisingly large. Two factors that are likely contributors to the difference are: 1) sweet corn residue was in the soil during the colder winter months when mineralization of plant residue was slow, whereas the rye was in the soil over the summer when mineralization rates would be

at their maximum, and 2) the rye residue was at a younger stage of growth than the sweet corn residue when it was incorporated and so would have a lower C:N ratio and be more prone to breakdown. Abundant inorganic N (280 kg ha^{-1} urea N) was readily available in excess to the broccoli crop, which contained about 165 kg ha^{-1} N in the aboveground portion at harvest (Table 3.2).

Figure 3.4 Distribution of ^{15}N among soil and plant components within a cropping or cover crop season in the R treatment microplots.



* Columns with the same letter do not differ significantly from each other ($p<0.05$).

Summary

Results from this study show that a substantial amount of recently applied N as fertilizer is lost from the system even with a winter cover crop in place, but that NDFF incorporated as plant residue shows strong signs of stability, both by not being lost from the system nor being taken up in plants. Denitrification rates measured in western Oregon soils have ranged from negligible (Myrold, 1988) to over 12% of applied fertilizer N (Horwath et al., 1998). Leaching losses from these same soils have been have been detected at over 100 kg ha^{-1} N under fallow conditions at high N rates, with winter rye cover crops in some cases absorbing only 16 kg ha^{-1} of potentially leached NO_3 (Brandi-Dohrn et al., 1997). The ability of winter cover crops to absorb potentially leached N is dependent on how well the crops become established before winter rains begin.

Our data show that of the NDFF present in the soil at the beginning of winter, 60% or 111 kg ha^{-1} N was lost even with a winter rye cover crop in place. The amount of NDFF remaining in the soil after winter is about 120 kg ha^{-1} N (Fig. 3.2) and the average amount of N leached from this same cover crop and N rate was 41 kg ha^{-1} N (Brandi-Dohrn et al., 1997). These two figures combined with a potential denitrification loss of 2 to 30 kg ha^{-1} N (Myrold, 1988; Horwath et al., 1998) add up to an amount of N that corresponds very closely with the amount of unaccounted for N (146 kg ha^{-1} N) calculated in Table 3.3 for sweet corn at the recommended N rate and with a cereal rye cover crop.

When NDFF was in the form of plant residue and soil N, losses were reduced to 40% of NDFF, and when NDFF was in the form of sweet corn residue only, the loss over winter was only 27%. These numbers are in agreement with NO_3 -N leaching studies on

the same soil, and with N balance studies from the larger cover crop study done on the same site. These data clearly show that N from recently added plant residue is immobilized in a more stable form in the soil than recently added inorganic N that has been immobilized directly into the soil organic matter. Many studies have demonstrated that recently immobilized fertilizer N is predominantly in the form of amino acids (Cheng and Kurtz, 1963; Allen et al., 1973; Smith et al., 1978). Much previous work over the last 30 years has shown that amino acids are readily mineralized from soil organic matter (Burket and Dick, 1997) and would be the first removed from the organic pool by plant uptake or leaching. Our work also confirms other studies that show a progressive stabilization over time of recently incorporated NDFF (Broadbent and Nakashima, 1967; Kelley and Stevenson, 1985). During the second season of the experiment nearly 100% of NDFF that was present in the soil at broccoli planting was also present 3 months later at harvest.

References

Allen, A. L., F. J. Stevenson, and L. T. Kurtz. 1973. Chemical distribution of residual fertilizer nitrogen in soil as revealed by nitrogen-15 studies. J. Environ. Qual. 2: 120-124.

Anderson, E. L. 1986. Maize root response to N in the greenhouse and field. Plant and Soil. 95: 293-296.

Balabane, M. and J. Balesant. 1992. Input of fertilizer-derived labeled N to soil organic matter during a growing season of maize in the field. Soil Biol. and Biochem. 24: 89-96.

Brandi-Dohrn, F. M., R. P. Dick, M. Hess, S. M. Kauffman, D. D. Hemphill, Jr. and J. S. Selker. 1997. Nitrate leaching under a cereal rye cover crop. J. Environ. Qual. 26: 181-188.

Broadbent, F. E. and T. Nakashima. 1967. Reversion of fertilizer nitrogen in soils. Soil Sci. Soc. Am. Pro. 31: 648-652.

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17: 837-842.

Brookes, P. C., J. M. Stark, B.B. Mcinteer, and T. Preston. 1989. Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Sci. Soc. Am. J. 53:1707-1711.

Burket, J. Z. and R. P. Dick. 1997. Long-term vegetation management in relation to accumulation and mineralization of nitrogen in soils. p. 283-296. *In* G. Cadish and K. E. Giller (ed.). Driven by Nature: Plant Litter Quality and Decomposition. CAB International, Wallingford, U.K.

Burket, J. Z., D. D. Hemphill, and R. P. Dick. 1997. Winter cover crops and nitrogen management in sweet corn and broccoli rotations. HortSci. 32: 664-668.

Cabrera, M. L. and M. H. Beare. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci. Soc. Am. J. 57: 1007-1012.

Carter, J. N., O. L. Bennet, and R. W. Pearson. 1967. Recovery of fertilizer nitrogen under field conditions using nitrogen-15. Soil Sci. Soc. Am. Pro. 31: 50-56.

Cheng, H. H. and L. T. Kurtz. 1963. Chemical distribution of added nitrogen in soils. Soil Sci. Soc. Am. Pro. 27: 312-316.

Ditsch, D. C. and M. M. Alley. 1991. Nonleguminous cover cropmanagement for residual N recovery and subsequent crop yields. J. Fert. Issues 8: 6-13.

Gass, W. B., G. A. Peterson, R. D. Hauck, and R. A. Olson. 1971. Recovery of residual nitrogen by corn (*Zea mays* L.) from various soil depths as measured by ^{15}N tracer techniques. Soil Sci. Soc. Am. Pro. 35: 290-294.

Hauck, R. D. and J. M. Bremner 1976. Use of tracers for soil and fertilizer nitrogen research. Adv. Agron. 28: 219-266.

Horwath, W. R. and E. A. Paul. 1994. Microbial biomass. p. 753-773. *In* R. W. Weaver, J. S. Angl, and P. S. Bottomley (ed.) Methods of Soil Analysis. Part 2 Microbiological and Biochemical Properties. Soil Sci. Soc. Am. Book Series : 5. Madison, Wi.

Horwath, W. R., L.E. Elliot, J. J. Steiner, J. H. Davis, and S. M. Griffith. 1998. Denitrification in cultivated and non-cultivated riparian areas of grass cropping systems. J. Environ. Qual. 27: 225-231.

Keeney, D. R. and R. F. Follet. 1991. Managing nitrogen for groundwater quality and farm profitability: overview and introduction. Soil Sci. Soc. Amer. , Madison WI.

Kelley, K. R. and F. J. Stevenson. 1985. Characterization and extractability of immobilized ^{15}N in soil microbial biomass. Soil Biol. Biochem. 17: 517-523.

Kowalenko, C. G. 1989. The fate of applied nitrogen in a Fraser Valley soil using ^{15}N in field microplots. Can J. Soil Sci. 69: 825-833.

Ladd, J. N. and M. Amato. 1986. The fate of nitrogen from legume and fertilizer sources in soils successively cropped with wheat under field conditions. Soil Biol. Biochem. 18: 417-425.

Ladd, J. N., J. M. Oades, and M. Amato. 1981. Distribution and recovery of nitrogen from legume residues decomposing in soils sown to wheat in the field. Soil Biol. Biochem. 13: 251-256.

Legg, J. O. and J. J. Meisinger. 1982. Soil nitrogen budgets. P. 503-566. *In* F. J. Stevenson, (ed.) Nitrogen in Agricultural Soils. Amer. Soc. Agron. Madison Wis.

Muller, M. M. and V. Sundman. 1988. The fate of nitrogen (^{15}N) released from different plant materials during decomposition under field conditions. Plant and Soil 105: 133-139.

Myrold, D. D. 1988. Denitrification in ryegrass and winter wheat cropping systems of western Oregon. Soil Sci. Soc. Am. J. 52: 412-416.

Smith, S. J., F. W. Chichester, and D. E. Kissel. 1978. Residual forms of fertilizer nitrogen in field soils. Soil Sci. 125: 165-169.

Sorensen, P. and E. S. Jensen. 1991. Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene trap for ^{15}N determination. Anal. Chim. Acta 252: 201-203.

Stewart, B. A., D. D. Johnson, and L. K. Porter. 1963. The availability of fertilizer nitrogen immobilized during decomposition of straw. Soil Sci. Soc. Am. Pro. 27: 656-659.

Wagger, M. G., D. E. Kissel, and S. J. Smith. 1985. Mineralization of nitrogen from nitrogen-15 labeled crop residues under field conditions. Soil Sci. Soc. Am. J. 49: 1220-1226.

Westerman, R. L. and L. T. Kurtz. 1972. Residual effects of ^{15}N fertilizers in a field study. Soil Sci. Soc. Am. Pro. 36: 91-94.

Chapter 4

Microbial and Soil Parameters in Relation to N Mineralization in Soils of Diverse Genesis under Differing Management Systems

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Biology and Fertility of Soils

Abstract

Oregon soils from various management and genetic histories were used in a greenhouse study to determine the relationship between soil chemical and biological parameters and the uptake of soil mineralized nitrogen (N) by ryegrass (*Lolium perenne* L.). The soils were tested for asparaginase, amidase, urease, β-glucosidase, and dipeptidase activities and fluorescein diacetate (FDA) hydrolysis. Microbial biomass carbon (C) and N as well as metabolic diversity using Biolog GN plates were measured, as were total soil N and C, pH, and absorbance of soil extracts at 270 nm and 210 nm. Potentially mineralizable N (N_0) and the mineralization rate constant (k) were calculated using a first order nonlinear regression model and these coefficients were used to calculate the initial potential rate of N mineralization (N_0k). Except for metabolic diversity, the other parameters highly correlated to mineralized N uptake and were highly correlated to each other. A model using total soil N and β-glucosidase as parameters provided the best predictor of mineralized N uptake by ryegrass ($R^2 = 0.83$). Chemical and biological parameters of soils with the same history of formation but under different management systems differed significantly from each other in most cases. The calculated values of the initial potential rate of mineralization in some cases revealed management differences within the same soil types. The results showed that management of soils is readily reflected in certain soil chemical and biological indicators and that some biological tests may be useful in predicting N mineralization in soils.

Introduction

Chemical and biological tests have been used for many years in an attempt to predict the nitrogen (N) supplying capacity of soils (Keeney and Bremner, 1966; Gasser and Kalembasa, 1976; Stanford, 1982) but a satisfactory method of predicting N mineralization continues to elude investigators. Better knowledge of the mechanisms and indicators of N mineralization in soils is essential to improve N-use efficiency and lessen environmental impacts from agricultural production on N contamination of surface and groundwater. Soil microbes are the engines of organic N transformations and information is needed on key characteristics of soil biological activity, microbial biomass, and functional diversity in relation to N cycling processes to better predict N mineralization in soils.

Enzymes are central to microbial activity and N transformations. Enzyme activities have been shown to be responsive to environmental conditions and agricultural management (Dick, 1992; 1994). For example, Pancholy and Rice (1973) showed that enzymes involved in organic C compound decomposition such as amylase, cellulase, and invertase decreased while dehydrogenase and N-releasing enzymes such as urease increased during plant succession in an abandoned field. In another study, protease, urease, and asparaginase had higher activities in fields with crop rotations than in fields with corn monocultures (Blagoveshchenskaya and Danchenko, 1974). Sparling and Searle, (1993) found that DMSO reduction, microbial biomass C, total soil C and N, and anaerobically mineralized N were all highly correlated to each other in a wide range of New Zealand soils. Soil deaminase activity was higher in forest soils and in grass leys with more available N compared to soil in barley fields, suggesting that this enzyme activity

may be useful in predicting N mineralization (Killham and Rashid, 1986). Urease and phosphatase have been associated with net N immobilization, whereas protease has been related to net N mineralization. (Nannipieri et al., 1983).

Microbial biomass N is not only an important indicator of viable soil microbial populations but also is an important pool of readily mineralizable organic N in soils (Kai et al., 1973; Myrold, 1987; Bonde et al., 1988). Because soil management has a strong influence on microbial biomass in both agricultural (McGill et al., 1986; Fauci and Dick, 1994; Franzluebbers et al., 1995) and forest (Soderstrom et al., 1983) soils, it is a critical component for assessing potentially mineralizable N.

It seems reasonable that the degree of soil microbial functional biodiversity resulting from soil management and/or genesis should be important in controlling N transformations. Although genetic diversity measurements such as DNA characterization provide information on biodiversity, it cannot determine whether genes or functions are expressed. Another approach which may be more useful relative to N transformation is to challenge the microbial population with diverse C substrates to determine functional diversity (Zak et al., 1994). This approach has been used to distinguish differences: in soil moisture regimes and vegetation communities in the Chihuahuan Desert (Zak et al., 1994); among forest, meadow, and agricultural soils in Norway (Torsvik et al., 1990); and among different C inputs and flooding of agricultural plots in California (Bossio and Scow, 1995). There is no information available on the relationship of functional metabolic diversity and N mineralization.

Long-term management of soils, particularly C and N inputs, can have a significant effect on N mineralization (Burket and Dick, 1997) but little is known about the

relationship of soil management relative to soil biology and N mineralization. Therefore, the objective of this study was to examine the relationships between soil biological/ chemical characteristics and soil N mineralization among soils with diverse histories of genesis and management.

Materials and methods

Soils and greenhouse experiment

Soils were collected at 0 to 20 cm depths from five different locations in Oregon and represent three soil orders and 19 different management histories (Table 4.1) in June of 1994. The Walla Walla soils from the long-term Residue Utilization Plots (initiated in 1931) at the Columbia Basin Research Center at Pendleton OR are located in a semi-arid Mediterranean climate region of eastern Oregon (400 mm rainfall yr^{-1}). The other soils are from western Oregon which has a humid Mediterranean climate (1200 mm rainfall yr^{-1}). The Willamette soils are from the long-term Vegetable Cover Crop Plots (initiated in 1989) located at the North Willamette Research and Extension Center, Aurora OR.

After sampling, soils were stored at 4°C prior to sieving to pass a 2 mm mesh screen, distributed into solid-bottom pots (three replications per soil sample) of approximately 2 L volume, and kept at 70-80% of field capacity with deionized water for 21 days to establish an equilibrium. Soil was collected from each replicate pot to perform enzyme, microbial biomass, and other measurements (i.e. all analyses replicated three times) and then the pots were adjusted to contain 1 kg dry soil.

Table 4.1. Description of the 19 Oregon soils used in the N mineralization greenhouse study.

Soil name	Soil classification	Soil abbreviation	Management
Willamette silt loam	mixed, mesic Pachic Ultic Argixeroll	WIL grass, burn	Tall fescue, 125 kg N ha^{-1} applied every year, seed harvested, straw removed
		WIL grass, no burn	Tall fescue, 125 kg N ha^{-1} applied every year, seed harvested, straw burned
		WIL fallow, no N	Alternate years of sweet corn and broccoli, no N, fallow
		WIL fallow, high N	Alternate years of sweet corn and broccoli, 224 or 280 kg N ha^{-1}, winter fallow
		WIL clover, no N	Alternate years of sweet corn and broccoli, no N, red clover cover crop
		WIL clover, high N	Alternate years of sweet corn and broccoli, 224 or 280 kg N ha^{-1}, red clover cover crop
		WIL rye, no N	Alternate years of sweet corn and broccoli, no N, cereal rye cover crop
		WIL rye, high N	Alternate years of sweet corn and broccoli, 224 or 280 kg N ha^{-1}, cereal rye cover crop
Walla Walla silt loam	coarse-silty, mixed, mesic Typic Haploxeroll	WW manure	Wheat, 22.4 Mg ha^{-1} beef manure incorporated every 2 years, straw incorporated
		WW pea vine	Wheat, 22.4 Mg ha^{-1} pea vine incorporated every 2 years, straw incorporated
		WW no N	Wheat, no organic inputs, no fertilizer N, straw incorporated
		WW high N	Wheat, 90 kg N ha^{-1} fertilizer applied every year, straw added
		WW pasture	Mixed grass pasture
Newberg loam	coarse-loamy, mixed mesic Fluventic Haploxeroll	NB sod	Unused area, no soil amendments for past 25 years
		NB corn, high N	Sweet corn past 3 years, 240 kg N ha^{-1}
Jory silty clay loam	clayey, mixed, mesic Xeric Haplohumult	JY forest	Land in preserved forest for past 75 years
		JY orchard	Pear trees, soil under trees kept in bare fallow for past 30 years, no amendments
Bashaw clay	very fine, montmorillonitic, mesic Typic Pelloxerert	BSHW wetland	Natural wetland area, minimal disturbance
		BSHW grass	Perennial ryegrass field past 4 years, 125 kg N ha^{-1} applied

All pots were sown with 1 g of perennial ryegrass (*Lolium perenne* L.) seed. Pots sown to ryegrass were fertilized at the beginning of the experiment and after three months with a liquid solution of K_2SO_4 and $Ca(H_2PO_4)$ such that each pot received 143 mg K, 60 mg S, 175 mg P and 113 mg Ca at each application. All pots were maintained at 70-80% of field capacity, gravimetrically, throughout the experiment by weighing pots every three days and adjusting them to their appropriate weight with deionized water. Greenhouse temperature was kept between 25 ° C and 20 ° C, and day length was kept at 14 hr by means of artificial lighting. Cuttings from the ryegrass plants were taken at a height of 1 cm every 30 days for six months, dried in a forced air oven at 65°C, ground to pass a 0.37 mm sieve, and analyzed for total N (Bremner and Mulvaney, 1982).

Biological measurements

Soils collected for chemical and biological assays were sieved to pass a 2 mm mesh screen and split so that half the sample was dried at room temperature for those analyses requiring air-dried soil. Both moist and dry sub-samples were kept in the cooler at 4°C until analysis. Microbial properties were measured within one week after the ryegrass was planted in the pots.

Enzyme assays for asparaginase (L-asparagine amidohydolase, EC 3.5.1.1), amidase (acylamide amidohydolase, EC 3.5.1.4), urease (urea amidohydolase, EC 3.5.1.5), and β-glucosidase (β-D-glucopyranosidase, EC 3.2.1.21) followed the protocols of Tabatabai (1994) using air-dried soils. The dipeptidase assay was modified from Ladd and Butler (1972) as follows: 1 g of moist soil was placed in a centrifuge tube; 1.8 mL of 0.1 *M*

Tris-sodium borate buffer and 2 mL of 0.002 *M* Z-phenylalanyl leucine substrate were added (except controls where solution minus substrate was added) and shaken to suspend the soil. The tubes were capped tightly, submerged in a 40°C water bath, and shaken lengthwise for 30 min at 150 rpm. Tubes were removed and cooled rapidly to 20°C in another water bath, followed by an addition and mixing of 0.2 mL 5 *N* HCl to each tube. Substrate was then added to controls and all samples were centrifuged at 2000 g for 20 min. Two mL of supernatant were then placed in a small test tube, 1 mL of ninhydrin reagent added, and the samples read at 570 nm. Absorbance of samples compared to prepared leucine standards determined the reported activities of dipeptidase.

The fluorescein diacetate (FDA) assay was modified from Schnürer and Rosswall, (1982) as follows: one g of air-dried soil was shaken with 20 mL of 60 m*M* sodium phosphate buffer in a 125 mL Erlenmeyer flask for 15 min. Then 100 µL of 2 mg mL^{-1} FDA stock solution (substrate) were added to non-controls and all samples were shaken for 2 hr. After shaking, 20 mL of acetone were added to all samples and 100 µL substrate were added to controls. The samples were transferred to centrifuge tubes and spun at 6000 rpm for 5 minutes. Samples were filtered through Whatman #4 filter paper and read at 499 nm. FDA activity was determined from absorbance of samples compared to a standard curve.

Microbial biomass C (MB_C) and N (MB_N) measurements were made according to the fumigation-incubation procedures of Jenkinson and Powlson, (1976) with the following modifications. Fumigated and unfumigated samples in the tubes were incubated in the dark for 10 days at 24°C. The CO_2 produced after 10 days was analyzed with a thermal conductivity gas chromatograph. After CO_2 sampling, 50 mL of 2 *M* KCl were

added to each tube and the tubes were shaken lengthwise for one hour and stored at 4°C until analysis for NH_4^+ by steam distillation. Values for MBc and MB_N were calculated with the following formulas:

$$MB_C = (CO_2 - C_f \text{ minus } CO_2 - C_{uf}) / 0.41 \quad \text{(Voroney and Paul, 1984)}$$

$$MB_N = (NH_4^+ - N_f \text{ minus } NH_4^+ - N_{uf}) / 0.68 \quad \text{(Shen et al., 1984)}$$

The subscripts $_f$ and $_{uf}$ stand for fumigated and unfumigated, respectively.

To determine a measurement of metabolic diversity, 10^{-1} soil dilutions were made by dispersing 10 g of moist soil in flasks containing 95 mL of sterile physiological saline solution (0.15 *M* NaCl) and several sterile glass beads. The suspension was allowed to settle for 5 minutes and then wells of Biolog GN plates were inoculated with 150 µL aliquots taken from the top 10 mm of the flasks. The plates were covered, placed in containers on top of moist paper towels to limit desiccation, and incubated in the dark at 28°C for 48 hr. Microplates were read at 590 nm on an automated microplate spectrophotom. Positive wells were defined as those having an absorbance greater than 0.4 in order to avoid false positives.

Chemical measurements

Inorganic NO_3^- and NH_4^+ were determined by extraction with 2 *M* KCl, steam distillation, and titration (Bremner, 1965). Total soil N was determined by Kjeldahl digestion, followed by NaOH distillation and measured by titration with 25 m*M* H_2SO_4 in boric acid indicator (Bremner and Mulvaney, 1982). Total soil C was measured by dry combustion on a carbon analyzer. Soil pH was determined using a glass electrode on a pH meter with a soil:water ratio of 1:2.

For the ultraviolet absorption procedures, 2.5 g of dry soil were extracted with 50 mL of 0.01 *M* $NaHCO_3$ and the methods followed those of Fox and Piekielek (1978) except that the two wavelengths used for testing the extracts were 210 nm and 270 nm (Norman et al., 1985). The UV method was originally proposed to test for soil NO_3^- which absorbs at 200 nm to 210 nm (Cawse, 1967).

Data analysis

Biological and chemical assay results were analyzed by ANOVA (SAS Institute,1990) and means separation by Fisher's protected LSD ($p<0.05$). Cumulative N uptake by ryegrass was used to calculate total soil mineralized N. Preplant soil nitrate levels (pre-plant soil ammonium levels were negligible) were subtracted from total N uptake by the ryegrass. Nitrogen mineralization potential (N_0) and the mineralization rate constant (k) were calculated from the following exponential equation:

$$N_{min} = N_0[1 - \exp(-kt)] ,$$ where N_{min} = cumulative amount of inorganic N at specific time (t).

The initial potential rate of mineralization was calculated as $N_0 \times k$ (Campbell et al., 1991a). Pearson correlation coefficients were generated among all assays and soil mineralized N uptake by ryegrass (SAS Institute, 1990). Stepwise regression (SAS Institute, 1990) was to find the best model for predicting N mineralization. The North Willamette (Willamette soil) winter fallow, no N vegetable treatment and the Pendleton (Walla Walla soil) pea vine soil amendment were randomly chosen and left out of the original model development to provide two dissimilar soils for model testing. Collinearity

diagnostics were performed to determine the degree of multicollinearity among the variables (Freund and Little, 1986).

Results and Discussion

Biological and chemical measurements

Biological and chemical measurements of the soils are shown in Tables 4.2 and 4.3. In general, soils with long-term additions of plant residues and organic matter had higher levels of the biological parameters (Table 4.2). Native soils or soils under management similar to native conditions (e.g. forests, pastures) showed much higher enzyme activities than more intensively managed soils (Table 4.2). An abundant supply of organic C to soils, either by natural seasonal dieback of vegetation or by addition within a cultural practice, is essential for maintaining biological activity in soils.

Overall, the C to N ratios of the whole soil and the microbial biomass were not significantly correlated to ryegrass N uptake (data not shown). However, the two least disturbed soils in the experiment (Jory forest and Bashaw wetland) had both the widest soil C:N ratios and the highest ryegrass N uptake (Tables 4.3 and 4.4). Soil C:N ratios were similar across soil types and management systems (Table 4.3). Microbial biomass C:N, on the other hand, seems to be much more sensitive to management. Within all soil types, higher N or organic residue inputs and less disturbance resulted in narrower microbial biomass C:N ratios (Table 4.2). This observation emphasizes the responsiveness of soil microbial populations to management systems. One possible explanation for the lower ratios may be higher populations of soil bacteria in soils with residue and high N inputs. Bacteria have the lowest C:N ratio of all soil organisms (Paul and Clark, 1989).

Table 4.2. Measured values of biological tests.

Soil name	Aspara-ginase	Ami-dase	Urease	β-glucosi-dase	FDA	Dipepti-dase	Biolog GN plates	Microbial biomass C	Microbial biomass N	Microbial biomass C:N
	- - - - *mg NH_4-N · kg soil^{-1} · 2 hr^{-1}* - - -			*mg kg soil^{-1}·hr^{-1}*	*mg kg soil^{-1}·hr^{-1}*	*mg kg soil^{-1} hr^{-1}*	*# wells > 0.4 absorb.*	*mg C kg soil^{-1}*	*mg N kg soil^{-1}*	
WIL grass, burn	6.9	87.7	28.0	97.5	82.8	49.5	52.7	144.9	20.1	2
WIL grass, no burn	7.0	91.6	30.8	86.5	83.9	58.2	37.3	193.1	16.0	12
WIL fallow, no N	8.8	84.9	17.5	64.1	47.0	31.7	35.0	142.7	5.6	26
WIL fallow, high N	8.6	35.0	10.1	61.7	39.3	25.2	36.3	64.5	5.7	11
WIL clover, no N	9.1	51.1	15.5	95.1	59.1	51.3	57.7	182.5	8.7	21
WIL clover, high N	6.7	29.6	16.5	82.7	48.9	36.1	51.7	131.4	15.5	8
WIL rye, no N	7.2	40.5	24.5	70.7	51.8	32.9	53.3	168.4	16.3	10
WIL rye, high N	9.5	40.8	17.8	74.7	48.4	19.2	46.0	120.9	13.7	9
WW manure	11.9	141.2	67.7	76.6	61.1	125.2	44.7	268.4	32.6	8
WW pea vine	8.2	130.7	44.6	84.0	51.7	65.8	56.7	171.1	20.7	8
WW high N	3.8	89.8	18.3	67.8	34.4	9.3	47.7	38.2	5.8	39
WW no N	3.3	76.7	26.3	53.0	35.3	26.0	31.0	96.7	3.1	5
WW pasture	43.3	213.3	161.2	115.6	124.7	237.2	51.7	502.3	80.2	6
NB sod	135.3	430.3	111.6	49.7	182.4	314.1	34.7	1705.9	186.0	9
NB corn, high N	11.3	67.2	27.1	102.0	46.2	44.2	48.6	150.6	11.3	13
JY forest	90.0	343.8	131.5	160.2	172.4	226.6	44.0	1352.0	154.0	9
JY orchard	3.8	59.2	13.0	36.0	64.6	11.2	11.7	74.3	4.4	17
BSHW wetland	83.6	323.3	100.9	35.4	200.2	418.2	54.7	2577.5	300.0	9
BSHW grass	26.0	174.9	76.0	73.3	187.9	111.5	52.7	602.0	59.3	11
LSD, $p < 0.05$	7.4	14.7	6.8	7.5	3.6	45.3	1.5	91.7	5.6	

Table 4.3. Measured values of chemical tests.

Soil name	pH	Total soil C	Total soil N	Total C:N	UV 210	UV 270	Ammonium	Nitrate
		mg kg^{-1}	*mg kg^{-1}*		*- - - - - absorbance - - -*		*mg kg^{-1}*	*mg kg^{-1}*
WIL grass, burn	5.4	16124	1164	13.9	0.676	0.209	0.9	8.0
WIL grass, no burn	5.6	16205	1084	14.9	0.586	0.202	1.3	4.4
WIL fallow, no N	5.6	15335	1113	13.7	2.719	0.172	2.4	91.3
WIL fallow, high N	5.3	16073	983	16.4	2.317	0.170	1.1	74.7
WIL clover, no N	5.6	15128	1037	14.6	2.241	0.189	4.4	69.8
WIL clover, high N	5.3	15647	1135	13.8	3.378	0.188	5.7	119.1
WIL rye, no N	5.6	13877	966	14.4	2.669	0.143	2.8	86.5
WIL rye, high N	5.5	14325	1077	13.3	3.426	0.165	4.6	114.8
WW manure	7.1	14000	1136	12.3	0.780	0.156	0.9	21.3
WW pea vine	6.5	11513	963	11.9	0.641	0.146	3.9	20.9
WW no N	6.3	9293	712	13.0	0.393	0.114	1.7	15.9
WW high N	5.8	9440	713	13.2	0.570	0.131	3.0	17.9
WW pasture	6.9	22324	1680	13.2	0.952	0.207	3.9	22.0
NB sod	6.1	34171	2474	13.8	0.916	0.257	3.7	17.0
NB corn, high N	6.1	8184	627	13.1	1.415	0.096	4.4	39.9
JY forest	6.2	45361	1948	23.3	0.433	0.225	7.8	0.9
JY orchard	5.3	10744	782	13.7	1.121	0.231	0.9	31.1
BSHW wetland	5.5	91663	4794	19.1	3.415	0.511	5.4	81.3
BSHW grass	5.1	28138	2050	13.7	1.324	0.276	8.3	32.6
LSD, $p < 0.05$	0.1	3097	156		0.157	0.030	2.0	5.2

Within the same site, those soils which received only long-term N fertilizer additions (Willamette, Walla Walla, and Newberg) tended to reduce the activities of urease, FDA, and dipeptidase, as well as microbial biomass levels, compared to native soils or soils with additional organic matter inputs (Table 4.2). Inorganic N fertilization without additional organic inputs could affect soil biology by several mechanisms. First, there can be a reduction of microbial biomass (Soderstrom et al., 1983) which might be due to a narrowing of C:N ratios in soils. This would likely cause more rapid decomposition of organic inputs which in turn reduces stable C pools for maintaining microbial biomass. Without sufficient organic C inputs to the soil, the microbial biomass will be depressed regardless of the amount of available N present. A second factor is that N ions may have a direct effect on enzyme systems. Dick et al. (1988) showed a negative relationship between increasing rates of N fertilization and amidase and urease activities. They hypothesized that these enzymes were suppressed due to the long-term fertilizer application of the reaction product (NH_4) of these enzymes. This was later confirmed by McCarty et al. (1992) who showed that NH_4 repression of urease activity was real, but the NH_4 effect was indirect and due to by-products of NH_4 assimilation. Lastly, C may become less available with long-term N additions owing to condensation of N-rich compounds (Haider et al., 1975).

Within soil types, management regimes that minimize soil disturbance (such as pasture, sod, wetland and forest) had significantly higher levels of enzyme activities, and microbial biomass (Table 4.2). This is consistent with findings in India on disturbed forest soils that had lower microbial populations and enzyme activities than undisturbed soils (Jha et al., 1992). Klein and Koths (1980) found the highest urease and protease activities

in field soils having less disturbance (no-till) and higher crop residue inputs than conventionally tilled soils with residues removed. Less disturbance of soils results in greater aggregation (Gupta and Germida, 1988) which in turn preserves soil organic matter and allows for a greater variety of microhabitats. Work in soil ecology has shown that the composition of organisms at all trophic levels in the soil is important to ecosystem stability and nutrient transformations (de Ruiter et al., 1995). Some of the differences observed in the N mineralization rates between relatively disturbed and undisturbed soils of the same soil genesis might be attributed to altered trophic structures above the bacteria level.

Biolog plate metabolic diversity was significantly higher in soils with less disturbance and higher organic matter inputs (Table 4.2) with the highest number of positive wells from the Willamette soil with clover and zero N and the lowest number from the Jory orchard site. The Willamette soil in this treatment was one in which yields of broccoli and sweet corn were comparable to other treatments that received over 200 kg ha^{-1} inorganic N fertilizer. The red clover winter cover crop contained about 1200 kg ha^{-1} dry weight and 43 kg N ha^{-1} above ground (Burket et al., 1997). In contrast, the Jory orchard soil was in bare fallow for over 15 years with no soil amendments applied. When the 29 Biolog wells containing amino acids, amides, and amines were examined separately from all 95 wells, results were similar and correlation between this subgroup and the whole plate was high (Pearson correlation coefficient = 0.87, $p<0.0001$). These results are in agreement with Zak et al. (1994) who found that separating substrates into six guilds did not, with the exception of a slight change when looking at only the polymers, change the positional relationships among the environments when compared to overall substrate utilization.

Our method of Biolog analysis did not take into consideration the intensity of color development in the wells as an indication of abundance of any particular ability to metabolize a substrate. Using positive and negative response for individual wells rather than absorbance numbers is a way to ameliorate possible sources of variability among replicates due to innoculum density differences or natural soil heterogeneity (Zak et al., 1994). The positive response threshold has been a standard method for other microplate methodologies in avoiding false positive results (Pscheidt et al., 1992). The presence or absence of a metabolic capability from a soil is the simplest measurement of metabolic diversity, and is analogous to counting species from different environments to compare diversity (Magurran, 1988).

N mineralization parameters

Ryegrass N uptake, N mineralization potentials (N_0), and rate constants (k) data are shown in Table 4.4. Within the four cultivated plots of the Walla Walla soils (Table 4.4), the highest N_0 value is from the manure treatment and the lowest is from the zero N treatment. Janzen (1987) also found that soils from long-term rotations with the highest residue inputs had the highest N_0 values. Except for the winter fallow treatments, N_0 was consistently lower in the Willamette soil plots and the Newberg plots receiving high fertilizer N inputs compared to plots with no inorganic N inputs having the same cover crop and vegetable rotation (Table 4.4). Apparently, a winter cover crop of either rye or clover is more effective in increasing N_0 than fertilizer N inputs. The same relationship among these soils holds true when considering the initial potential mineralization rate (N_0 k) with the fallow plots being exceptions (Table 4.4). These results are in contrast to that

of Campbell et al. (1991a) where fertilizers were shown to be as effective as cover crop residues for increasing N_0k.

Table 4.4. Calculated values of rye N uptake minus preplant soil nitrate, N mineralization potential (N_0), mineralization rate constant (k), and initial potential mineralization rate (N_0 x k).

Soil name	Rye N uptake	N_0	(SE)	k	N_0 x k
	mg kg^{-1}	*mg kg^{-1}*		*wk^{-1}*	
WIL grass, burn	30.4	28.8	(4.1)	0.001432	0.583
WIL grass, no burn	36.8	27.6	(4.6)	0.016168	0.673
WIL fallow, no N	24.9	28.6	(17.2)	0.028223	3.213
WIL fallow, high N	11.3	58.5	(41.9)	0.024141	2.022
WIL clover, no N	35.0	38.5	(2.3)	0.022844	2.369
WIL clover, high N	43.0	34.1	(6.3)	0.028432	4.577
WIL rye, no N	36.4	36.2	(2.2)	0.026217	3.198
WIL rye, high N	20.6	28.5	(22.8)	0.028001	3.524
WW manure	36.3	39.3	(2.4)	0.015799	0.918
WW pea vine	24.0	28.9	(4.6)	0.017037	0.768
WW no N	14.8	24.9	(6.4)	0.016364	0.505
WW high N	23.6	26.3	(7.7)	0.016045	0.639
WW pasture	56.5	93.9	(16.8)	0.010734	0.907
NB sod	50.5	73.3	(35.1)	0.008589	0.662
NB corn, high N	18.2	20.1	(4.5)	0.022689	1.312
JY forest	69.1	76.7	(8.8)	0.009289	0.753
JY orchard	19.7	38.1	(31.0)	0.020545	1.039
BSHW wetland	105.1	279.9	(217.5)	0.012819	2.477
BSHW grass	61.9	63.5	(10.5)	0.016373	1.542

This parameter, N_0k, was found by Campbell et al. (1991a) to be a better index than total N, N_0, or k for assessing the quality of the active fraction of soil organic matter.

The cultivated Walla Walla plots which are from a more arid climate, similar to the Saskatchewan site in the Campbell et al. (1991a) study, had results consistent with that study in which a higher fertilizer N input resulted in a higher N_0k value (Table 4.4). However, among all the five Walla Walla plots, the highest N_0k was from the pasture and is no more useful than N_0, in distinguishing among treatments.

The initial potential rate of mineralization, N_0k, appears to be useful in distinguishing management treatments within, but not between soil type; for instance, the Willamette soil under fallow with zero N and high N, the Walla Walla soil under zero N, Jory orchard, and the Newberg corn with high N treatments all had similar values of N_0k (Table 4.4). Campbell et al. (1991a) compared only soils from cultivated fertilized and unfertilized treatments that had similar organic matter C and N contents, unlike the work presented here which includes relatively undisturbed soils with high levels of soil organic C and N (Table 4.3). Normalizing N_0 by dividing it by total N creates a ratio that shows whether N_0 is in direct proportion to soil N content and is also an indication of the soil organic matter's "active fraction." Except for the Willamette fallow, high N soil, which had an especially high standard error for its N_0, this ratio is highest in the two soils that also had the highest N_0, the Bashaw wetland and the Walla Walla pasture soils (data not shown). This ratio is also very high in the Jory orchard soil, which had a relatively low N_0. Within the Walla Walla soils, the only treatment that stands out in regard to this ratio is the pasture, which had twice the total C as any of the other four treatments (Tables 4.3 and 4.4).

When native soils are compared for N_0 or N_0k, the quantity rather than the quality of the soil organic matter seems to become the dominant discriminator according to soil

management. In addition, soils from western Oregon, where there is over twice the annual rainfall of eastern Oregon, appear to create more variability in the calculated parameters N_0 and $N_0 k$ and also result in patterns that do not distinguish among treatments very well (see Willamette soils in Table 4.4). Campbell et al. (1991b) also reported differences in findings when comparing their results to similar treatments from more humid conditions.

Model for N mineralization

Highly significant correlations were found among total and microbial biomass N, UV 270, all enzymes (except β-glucosidase) and mineralized N uptake by ryegrass (Table 4.5). Measurements of microbial biomass would be expected to correlate highly with enzyme activities, since microbes are mainly responsible for production of soil enzymes. Schnürer and Rosswell (1982) found FDA activity to correlate well with soil respiration levels and many enzymes, but enzymes can persist in soils and also reflect past microbial population levels via extracellular enzymes stabilized in the soil matrix (Burns, 1982). The calculated parameters k and $N_0 k$ had no significant correlations with any of the measured parameters. Mineralized N uptake by ryegrass and N_0 were highly correlated to each other ($r=0.97$) as would be expected.

Stepwise regression analysis showed that total soil N and β-glucosidase activity accounted for a significant proportion of the variation of N uptake by ryegrass in the 17 soils used for model development ($R^2=0.82$; $F=114$; $df=2,48$; $p<0.0001$).

The model equation is as follows: Mineralized N uptake by ryegrass = -12.38 + 0.23 (β-glucosidase activity, mg kg^{-1} hr^{-1}) + 0.023 (total N, ppm) with

standard errors of 5.1, 0.05, and 0.002 for y intercept, β-glucosidase activity constant, and total N constant, respectively.

Predicted values of mineralized N uptake by ryegrass and standard errors (SE) from the model equation for the two soils left out of the equation were 27.9 mg kg^{-1} (10.1 mg kg^{-1}) for the Willamette fallow/no N treated soil compared to 24.9 mg kg^{-1} (9.4 mg kg^{-1}) actual mineralized N (Table 4.4) and 28.6 mg kg^{-1} (17.2 mg kg^{-1}) calculated N mineralization potential (N_0 in Table 4.4). The predicted value for the Walla Walla pea vine soil was 29.1 mg kg^{-1} (10.7 mg kg^{-1}) compared to 24.0 mg kg^{-1} (0.6 mg kg^{-1}) actual mineralized N (Table 4.4) and 28.9 mg kg^{-1} (4.6 mg kg^{-1}) calculated N mineralization potential (N_0 in Table 4.4). The general agreement among the calculated and measured values for mineralized N for these two soils further confirms the potential usefulness of the model for predicting mineralized N availability. Total soil N and β-glucosidase activity are not correlated to each other (Table 4.5) which eliminates the problem of multicollinearity that is often severe among soil properties making them less useful for modeling soil processes because collinearity artificially increases R^2 values. In agreement with other studies (Franzluebbers et al., 1995; Frankenberger and Dick, 1983), measurements of organic matter such as MB_N, total N, total C, MB_C, and certain enzyme activities in soils are highly correlated to each other, so inclusion of more than one of these parameters in a model predicting mineralized N uptake would contribute to multicollinearity. This best fit mineralized N uptake model with the parameters total soil N and β-glucosidase activity are measurements that provide a relatively complete picture of readily mineralized soil organic matter that will yield plant available N. β-glucosidase activity represents the soil's potential to hydrolyze low molecular weight carbohydrates (Eivazi and Tabatabai, 1990)

Table 4.5. Matrix of Pearson correlation coefficients for 12 of the measured soil parameters (p values>0.0001).

	UV 270	Asparag-inase	Amid-ase	Urease	FDA	Dipeptid-ase	β-glucosid-ase	Biolog	Total N	Microbial biomass N	Total C	Microbial biomass C
Mineralized N uptake	0.80	0.65	0.71	0.70	0.84	0.81	0.11 (0.41)	0.35 (0.01)	0.87	0.84	0.85	0.83
UV 270	--	0.58	0.60	0.47	0.78	0.75	-0.27 (0.04)	0.10 (0.46)	0.93	0.84	0.91	0.83
Asparaginase	--	--	0.95	0.77	0.83	0.87	0.07 (0.58)	0.01 (0.97)	0.77	0.87	0.71	0.88
Amidase	--	--	--	0.85	0.87	0.83	0.12 (0.39)	0.04 (0.77)	0.74	0.88	0.72	0.88
Urease	--	--	--	--	0.80	0.83	0.36 (0.01)	0.19 (0.16)	0.62	0.73	0.59	0.69
FDA	--	--	--	--	--	0.84	0.10 (0.46)	0.14 (0.29)	0.84	0.86	0.80	0.86
Dipeptidase	--	--	--	--	--	--	0.03 (0.85)	0.19 (0.15)	0.88	0.94	0.87	0.92
β-glucosidase	--	--	--	--	--	--	--	0.41 (0.00)	-0.18 (0.17)	-0.06 (0.67)	-0.09 (0.52)	-0.09 (0.49)
Biolog	--	--	--	--	--	--	--	--	0.23 (0.09)	0.16 (0.25)	0.21 (0.12)	0.12 (0.36)
Total N	--	--	--	--	--	--	--	--	--	0.95	0.96	0.95
Microbial biomass N	--	--	--	--	--	--	--	--	--	--	0.94	0.99
Total C	--	--	--	--	--	--	--	--	--	--	--	0.93

and release glucose, which is an important energy source for soil microbial populations. The combination of total N and β-glucosidase activity may best represent both the pool of mineralizable N in the soil and the potential to release C for energy available for microbes to carry out the mineralization.

Biolog metabolic diversity was significantly correlated with mineralized N uptake, but not with the other parameters except for β-glucosidase. Because the wells of Biolog GN plates contain C sources, correlation of Biolog metabolic diversity with β-glucosidase activity, an enzyme involved in C mineralization, is logical (Table 4.5). Clearly, many types of biological activity in soils are intimately associated with the process of N mineralization. In order to find a useful means of predicting N mineralization in the laboratory that will accurately reflect mineralized N that is also taken up by plants, the measurements should be both biologically relevant to the process of N mineralization and fairly easy to perform. Further work using measurements of total soil N and β-glucosidase activity on field soils to predict plant available mineralized N will determine if the model developed here from a variety of Oregon soils is more broadly applicable.

References

Blagoveshchenskaya, Z. K. and N. A. Danchenko. 1974. Activity of soil enzymes after prolonged application of fertilizers to a corn monoculture and crops in rotation. Sov Soil Sci 5: 569-575.

Bonde, T. A., J. Schnürer, and T. Rosswall. 1988. Microbial biomass as a fraction of potentially mineralizable nitrogen in soils from long-term field experiments. Soil Biol Biochem 20: 447-452.

Bossio, D. A. and K. M. Scow. 1995. Impact of C and flooding on the metabolic diversity of microbial communities in soils. Appl Environ Microbiol 61: 4043- 4050.

Bremner, J. M. 1965. Inorganic forms of nitrogen. p. 1179-1237. *In* C. A. Black (ed.) Methods of soil analysis, Part 2. American Society of Agronomy, Madison, WI.

Bremner, J. M. and C. S. Mulvaney. 1982. Nitrogen-total. p. 595-624. *In* A. L. Page, R. H. Miller, and D. R. Keeney, (ed.) Methods of soil analysis, Part 2. 2nd edition. American Society of Agronomy, Madison, WI.

Burket, J. Z. and R. P. Dick. 1997. Long-term vegetation management in relation to accumulation and mineralization of nitrogen in soils. p.283-296. *In* G. Cadisch and K. E. Giller (ed.) Driven by nature: Plant litter quality and decomposition. CAB International, Wallingford, U.K.

Burket, J. Z., D. D. Hemphill, and R. P. Dick. 1997. Winter cover crops and nitrogen management in sweet corn and broccoli rotations. HortScience 32: 664-668.

Burns, R. G. 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biol Biochem 14: 423-427.

Campbell, C. A., G. P. LaFond, A. J. Letshon, R. P. Zentner, and H. H. Janzen. 1991a. Effect of cropping practices on the initial potential rate of N mineralization in a thin Black Chernozem. Can J Soil Sci 71: 43-53.

Campbell, C. A., V. O. Biederbeck, R. P. Zentner, and G. P. LaFond. 1991b. Effect of crop rotations and cultural practices on soil organic matter, microbial biomass and respiration in a thin Black Chernozem. Can J Soil Sci 71: 363-376.

Cawse, P. A. 1967. The determination of nitrate in soil solutions by ultraviolet spectrophotometry. Analyst 92: 311-315.

de Ruiter, P. C., A. M. Neutel, and J. C. Moore. 1995. Energetics, patterns of interaction strengths, and stability in real ecosystems. Science 269:1257-1260.

Dick, R. P. 1992. A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. Agric Ecosys Environ 40: 25-36.

Dick, R. P. 1994. Soil enzyme activities as indicators of soil quality. *In* p. 107-124. J. W. Doran, D. C. Coleman, D. F. Bezdicek, and B. A. Stewart (ed.) Defining soil quality for a sustainable environment. Soil Sci Soc Amer Special Pub 15, Madison, WI.

Dick, R. P., P. E. Rasmussen, and E. A. Kerle. 1988. Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. Biol Fertil Soils 6: 159-164.

Eivazi, F. and M. A. Tabatabai. 1990. Factors affecting glucosidase and galactosidase activities in soils. Soil Biol Biochem 22: 891-897.

Fauci, M. F. and R. P. Dick. 1994. Plant response to organic amendments and decreasing inorganic nitrogen rates in soils from a long-term experiment. Soil Sci Soc Am J 58: 134-138.

Fox, R. H., and W. P. Piekielek. 1978. A rapid method for estimating the nitrogen-supplying capacity of a soil. Soil Sci Soc Am J 42: 751-753.

Frankenberger, W. T. and W. A. Dick. 1983. Relationships between enzyme activities and microbial growth and activity indices in soils. Soil Sci Soc Am J 47: 945-951.

Franzluebbers, A. J., F. M. Hons, and D. A. Zuberer. 1995. Soil organic C, microbial biomass, and mineralizable carbon and nitrogen in sorghum. Soil Sci Soc Am J 59: 460-466.

Freund, R. S. and R. C. Little. 1986. SAS system for regression. SAS Institute, Cary, NC.

Gasser, J. K. R. and S. J. Kalembasa. 1976. The effects of leys and organic manures on the available N in clay and sandy soils. J Soil Sci 27: 237-249.

Gupta, V. V. S. R. and J. J. Germida. 1988. Distribution of microbial biomass and activity in different soil aggregate size classes as affected by cultivation. Soil Biol Biochem 20: 777-786.

Haider, K., J. P. Martins, and Z. Filip. 1975. Humus biochemistry. p. 195-244. *In* E. A. Paul and A. D. McLaren (eds). Soil biochemistry, vol. 4. Marcel Dekker Inc. New York, NY.

Janzen, H. H. 1987. Soil organic matter characteristics after long-term cropping to various spring wheat rotations. Can J Soil Sci 67: 845-856.

Jenkinson, D. S. and D. S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol Biochem 8: 209-213.

Jha, D. K., G. D. Sharma, and R. R. Mishra. 1992. Soil microbial population numbers and enzyme activities in relation to altitude and forest degradation. Soil Biol Biochem 24: 761-767.

Kai, H., Z. Ahmad, and T. Harada. 1973. Factors affecting immobilization and release of nitrogen in soil and chemical characteristics of the nitrogen newly immobilized. Soil Sci Plant Nutr 19: 275-286.

Keeney, D. R. and J. M. Bremner. 1966. Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. Agron J 58: 498-503.

Killham, K and R. A. Rashid. 1986. Assay of activity of a soil deaminase. Plant Soil 92: 15-21.

Klein, T. M. and J. S. Koths. 1980. Urease, protease, and acid phosphatase in soil continuously cropped to corn by conventional and no-till methods. Soil Biol Biochem 12: 293-294.

Ladd, J. N. and J. H. A. Butler. 1972. Short-term assays of soil proteolytic enzyme activities using protein and dipeptide derivatives as substrates. Soil Biol Biochem 4: 19-30.

Magurran, A. E. 1988. Ecological diversity and its measurement. Princeton University Press. Princeton, NJ. 179 p.

McCarty, G. W., D. R. Shogren, and J. M. Bremner. 1992. Regulation of urease production in soil by microbial assimilation of nitrogen. Biol Fertil Soils 12: 261-264.

McGill, W. B., K. R. Cannon, J. A. Robertson, and F. D. Cook. 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. Can J Soil Sci 66: 1-19.

Myrold, D. D. 1987. Relationship between microbial biomass nitrogen and a nitrogen availability index. Soil Sci Soc Am J 51: 1047-1049.

Nannipieri, P. F., L. Muccini, and C. Ciardi. 1983. Microbial biomass and enzyme activities; production and persistence. Soil Biol Biochem 15: 679-685.

Norman, R. J., J. C. Edberg, and J. W. Stucki. 1985. Determination of nitrate in soil extracts by dual-wavelength ultraviolet spectrophotometry. Soil Sci Soc Am J 49: 1182-1185.

Pancholy, S. K. and E. L. Rice. 1973. Soil enzymes in relation to old field succession: amylase, cellulase, invertase, dehydrogenase, and urease. Soil Sci Soc Am Proc 37: 47-50.

Paul, E. A. and F. E. Clark. 1989. Soil microbiology and biochemistry. Academic Press, Inc., San Diego, CA.

Pscheidt, J. W., J. Z. Burket, S. L. Fischer, and P.B. Ham. 1992. Sensitivity and clinical use of *Phytophthora*-specific immunoassay kits. Plant Dis 76: 928-932.

SAS Institute, Inc. 1990. SAS user's guide: Statistics. SAS Institute, Inc., Cary NC.

Schnürer, J. and T. Rosswall 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl Environ Microbiol 43: 1256-1261.

Shen, S. M., G. Pruden, and D. S. Jenkinson. 1984. Mineralization and immobilization of nitrogen in fumigated soil and the measurement of microbial biomass nitrogen. Soil Biol Biochem 16: 437-444.

Soderstrom, B., E. Baath, and B. Lundgren. 1983. Decrease in soil microbial activity and biomasses owing to nitrogen amendments. Can J Micro 29: 1500-1506.

Sparling, G. P. and P. L. Searle. 1993. Dimethyl sulphoxide reduction as a sensitive indicator of microbial activity in soil: the relationship with microbial biomass and mineralization of nitrogen and sulfur. Soil Biol Biochem 25: 251-256.

Stanford, G. 1982. Assessment of soil nitrogen availability. p. 651-688. *In* F. J. Stevenson (ed.) Nitrogen in agricultural soils. No. 22. Amer Soc Agron, Madison, WI.

Tabatabai, M. A. 1994. Soil enzymes. p. 775-833. *In* R. W. Weaver, J. S. Angle, and P. J. Bottomley (ed.) Methods of soil analysis, Part 2. Microbiological and biochemical properties. Soil Sci Soc Amer book series, no 5. Madison, WI.

Torsvik, V., K. Salte, R. Sorheim, and J. Goksoyr. 1990. Comparison of phenotypic diversity and DNA heterogeneity in population of soil bacteria. Appl Environ Microbiol 56: 776-781.

Voroney, R. P. and E. A. Paul. 1984. Determination of k_c and k_n *in situ* for calibration of the chloroform fumigation incubation method. Soil Biol Biochem 16: 9-14.

Zak, J. C., M. R. Willig, D. L. Moorhead, and H. G. Wildman. 1994. Functional diversity of microbial communities: a quantitative approach. Soil Biol Biochem 26: 1101-1108.

Chapter 5

Soil Management and Functional Microbial Diversity

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Abstract

The role of disturbance and vegetation on soil biological properties and processes is critical for long-term sustainability of agroecosystems. The objective of this study was to use multivariate methods to investigate functional microbial indicators in diverse soils and management systems. Key microbial indicators such as enzyme assays (asparaginase, amidase, urease, β-glucosidase, dipeptidase activities and flourescein diacetate (FDA) hydrolysis, microbial biomass C and N, and metabolic diversity using Biolog GN plates were measured. Additional chemical measurements included total soil N and C, pH, the absorbance of soil extracts at 270 nm and 210 nm, nitrate, and the uptake of soil mineralized N by ryegrass (*Lolium perenne* L.). Principal components analysis of all soils and all variables showed origin to be the primary grouping, with grouping by management to be important only when a managed soil had an uncultivated counterpart. Cluster analysis using the measured parameters as attributes grouped all highly managed (cultivated) soils, regardless of genetic origin, cropping system, or rate of N fertilization, together in one common cluster. Soils that came from minimally disturbed environments formed individual clusters that differed from each other individually as much as they differed from the group of cultivated soils. Intensive cropping seems to create a common biological condition that supercedes genetic origin, vegetation, or environment in these soils. Cluster analysis using the 95 different carbon sources in Biolog GN plates as variables did not create any understandable patterns among the soils In order of importance, disturbance, vegetation, the nature of organic residue inputs, and N fertilization appear as influential in creating clusters within differently managed soils of a common genetic origin.

Introduction

Environmental conditions, including management, will affect the abundance, metabolic activity, and diversity of soil microbial populations. Comparisons of soils in terms of their biological parameters can be useful in revealing the extent to which environmental influences are capable of affecting change in soil ecosystems. In some ecosystems, the composition of the biotic community has been used as an indicator of the stability of the system. Several studies have shown that population diversity declines in freshwater ecosystems (Wassel and Mills, 1983; Atlas, 1984), and soil (Segal and Mancinelli, 1987; Jha, et al., 1992) in the presence of pollutants.

Studies have documented the strong effects of cultivating undisturbed soils on physical, chemical, and biological properties (Chang, 1949; Smith and Young, 1975; Meints and Peterson, 1977; Dick, 1992) as well as organic residue inputs and tillage systems on microbial and enzyme activities (Dick, 1984; McGill et al., 1986; Dick et al., 1988; Collins et al., 1992; Ladd et al., 1994). In a non-agricultural setting, enzymes involved in carbon breakdown such as amylase, cellulase, and invertase decreased while N-releasing enzymes such as urease and dehydrogenase increased during plant succession in an abandoned field (Pancholy and Rice, 1973).

To determine microbial functional diversity, Biolog plates have been used in various soil and water samples (Garland and Mills, 1991) to show that different environments result in different C utilization patterns. Biolog plates were used to distinguish among the soil microbe populations in six different desert plant communities in New Mexico (Zak, et al., 1994), and among nine agricultural, forest, and meadow soils in Denmark (Winding, 1994). In a California rice system study, differences in C utilization

patterns on Biolog GN plates were significantly related to straw and flooding treatments (Bossio and Scow, 1995). A study of hydroponically grown plants by Garland (1996) using Biolog plates was able to distinguish differences among populations of microbes washed from the roots of wheat, sweet potato, and soybean.

The above studies provide little information on consistency of microbial responses to soil management across diverse soil types. Most of the studies on soil microbial biomass, activity, and functional diversity were done on single or similar soil types. Consequently, our study was designed to examine a range of soil types which had been under varying soil management conditions or were under natural vegetation. Multivariate analysis procedures provided a powerful tool to group soils in terms of biologically important parameters and to illustrate how multiple factors discriminate among management and environment in soils. Cluster analysis is an extensively used method for examining the relatedness among objects in terms of a number of selected attributes (Romesburg, 1984). Cluster analyses have been used to confirm existing classifications of soils made by conventional soil survey methods (Gaikawad et al., 1977; Katyal et al., 1985). Principal components analysis has also been used to discover differences among soils in terms of soil biology (Acea and Carballas, 1990; Haack et al., 1995) and can be used to determine which variables are most important in explaining soil groupings.

The objective of this study was to use cluster and principal components analyses to examine diverse soil types with different management histories in terms of an array of biologically important parameters to more fully understand the relative importance of genetic origin, environment, and management on soil biology.

Materials and Methods

Soils were collected from five regions in Oregon and represent three soil orders and 19 different management histories (Table 5.1).

Biological Measurements

Soils collected for chemical and biological assays were sieved to pass a 2 mm mesh screen and split so that half the sample was dried at room temperature for those analyses requiring air-dried soil. Both moist and dry sub-samples were kept in the cooler at 4°C until analysis.

Enzyme assays for asparaginase, amidase, urease, and β-glucosidase followed the protocols of Tabatabai (1994) using air-dried soils. The dipeptidase assay was modified from Ladd and Butler (1972) as follows: 1 g of moist soil was placed in a centrifuge tube;1.8 ml of 0.1 M Tris-sodium borate buffer and 2 ml of 0.002 M Z-phenylalanyl leucine were added and shaken to suspend the soil. The tubes were capped tightly and submerged in a 40°C water bath, and shaken lengthwise for 30 min at 150 rpm. Tubes were removed and cooled rapidly to 20°C in another water bath, followed by an addition and mixing of 0.2 mL 5 N HCl to each tube. For controls, substrate was added at this point and all samples were centrifuged at 2000 g for 20 min. Two mL of supernatant were then placed in a small test tube, 1 mL of ninhydrin reagent added, and the samples read at 570 nm. The fluorescein diacetate (FDA) assay was modified from Schnürer and Rosswall (1982) as follows: 1 g of air-dried soil was shaken with 20 mL of 60 mM sodium phosphate buffer in a 125 mL Erlenmeyer flask for 15 min, followed by an addition of 100 *u*l of 2 mg mL^{-1} FDA stock solution (substrate) and shaking for 2 hr. After shaking,

Table 5.1. Description of the 19 Oregon soils used in the multivariate study.

Soil name	Soil classification	Soil abbreviation	Management
Willamette silt loam	mixed, mesic Pachic Ultic Argixeroll	WIL grass, burn	Tall fescue, 125 kg N ha^{-1} applied every year, seed harvested, straw removed
		WIL grass, no burn	Tall fescue, 125 kg N ha^{-1} applied every year, seed harvested, straw burned
		WIL fallow, no N	Alternate years of sweet corn and broccoli, no N, winter fallow
		WIL fallow, high N	Alternate years of sweet corn and broccoli, 224 or 280 kg N ha^{-1}, winter fallow
		WIL clover, no N	Alternate years of sweet corn and broccoli, no N, red clover winter cover crop
		WIL clover, high N	Alternate years of sweet corn and broccoli, 224 or 280 kg N ha^{-1}, red clover winter cover crop
		WIL rye, no N	Alternate years of sweet corn and broccoli, no N, cereal rye winter cover crop
		WIL rye, high N	Alternate years of sweet corn and broccoli, 224 or 280 kg N ha^{-1}, cereal rye winter cover crop
Walla Walla silt loam	coarse-silty, mixed mesic Typic Haploxeroll	WW manure	Wheat, 22.4 Mg ha^{-1} beef manure incorporated every 2 years, straw incorporated
		WW pea vine	Wheat, 2.24 Mg ha^{-1} pea vine incorporated every 2 years, straw incorporated
		WW no N	Wheat, no organic inputs, no fertilizer N, straw incorporated
		WW high N	Wheat, 90 kg N ha^{-1} fertilizer applied every year, straw incorporated
		WW pasture	Mixed grass pasture
Newberg loam	coarse-loamy, mixed mesic Fluventic Haploxeroll	NB sod	Unused area, no soil amendments for past 25 years
		NB corn, high N	Sweet corn past 3 years, 240 kg N ha^{-1}
Jory silty clay loam	clayey, mixed, mesic Xeric Haplohumult	JY forest	Land in preserved forest for past 75 years
		JY orchard	Pear trees, soil under trees kept in bare fallow for past 30 years, no amendments
Bashaw clay	very fine, montmorillonitic, mesic Typic Pelloxerert	BSHW wetland	Natural wetland area, minimal disturbance
		BSHW grass	Perennial ryegrass field past 4 years, 125 kg N ha^{-1} applied every year

20 mL acetone were added to all samples and 100 *u*l substrate were added to controls. The samples were transferred to centrifuge tubes and spun at 6000 rpm for 5 min. Samples were filtered through Whatman #4 filters and read at 499 nm. Absorbance of samples compared to a standard curve determined FDA activity.

Microbial biomass C (MB_C) and N (MB_N) measurements were made according to the fumigation-incubation procedures of Jenkinson and Powlson (1976) with the following modifications. Ten grams of moist soil were weighed into glass scintillation vials which were then placed in a desiccator containing wet paper towels and a small beaker holding 40 mL of ethanol-free chloroform and some glass boiling beads. The desiccator was put under a vacuum for 24 hours exposing the soil to chloroform vapors. The soil was transferred to 210 mm length by 23 mm diameter polyethylene tubes fitted with rubber septa that allowed sampling for gas chromatography. At the same time that a set of samples was put in the dessicator, a separate set of sample controls was weighed into polyethylene tubes and left to stand unsealed for 24 hours. Fumigated and unfumigated samples in the tubes were sealed at both ends with septa and incubated in the dark for 10 days at 24C. CO_2 produced after 10 days was by gas chromatography. After CO_2 sampling, 50 mL of 2 M KCl was added to each tube and shook lengthwise for one hour and stored at 4 °C until analysis for NH_4^+ by steam distillation. Values for MBc and MB_N were calculated with the following formulas:

$$MB_C = (CO_2 - C_f \text{ minus } CO_2 - C_{uf}) / 0.41 \quad \text{(Voroney and Paul, 1984)}$$

$$MB_N = (NH_4^+ - N_f \text{ minus } NH_4^+ - N_{uf}) / 0.68 \quad \text{(Shen et al., 1984)}$$

The subscripts $_f$ and $_{uf}$ stand for fumigated and unfumigated respectively.

To determine a measurement of metabolic diversity, 10^{-1} soil dilutions were made by dispersing 10 g of moist soil in flasks containing 95 mL of sterile physiological saline (0.15 M NaCl) and several sterile glass beads. The suspension was allowed to settle for 5 minutes and then wells of Biolog GN plates were inoculated with 150 µl aliquots taken from the top 10 cm of the flasks. The plates were covered, placed in containers on top of moist paper towels to limit desiccation, and incubated in the dark at 28 °C for 48 hr. Microplates were read at 590 nm on an automated microplate spectrophotom. Positive wells were defined as those having an absorbance greater than 0.4 in order to avoid false positive results.

Chemical Measurements

Inorganic NO_3^- was determined by extraction with 2 M KCl, steam distillation, and titration (Bremner, 1965). Total soil N was determined by Kjeldahl digestion, followed by NaOH distillation and measured by titration with standardized 25 mM H_2SO_4 in boric acid indicator (Bremner and Mulvaney, 1982). Total soil C was measured by dry combustion on a Leco carbon analyzer. Soil pH was determined using a glass electrode on a pH m with a soil : water ratio of 1: 2.

For the ultraviolet absorption procedures, 2.5 grams of dry soil were extracted with 0.01 M $NaHCO_3$ and the methods followed those of Fox and Piekielek (1978), except that the two wavelengths used for testing the extracts were 210 nm and 270 nm (Norman et al., 1985). The UV method was originally proposed to test for soil NO_3^- which absorbs at 200 nm to 210 nm (Cawse, 1967).

Data Analysis

The original data shown in Tables 5.2 and 5.3 were analyzed as a completely randomized design with means separation by Fisher protected Least Significant Difference procedure. Cluster analysis using the Proc Cluster in SAS (1990) with the 19 soils as objects and 16 measured parameters as attributes. Separate analyses for the eight treatments on the Willamette soils and five treatments on the Walla Walla soils were performed to examine a finer site-specific scale of resolution in order to distinguish management differences within the same soil type. Additional cluster analyses were performed using enzyme activities, N parameters, or Biolog results separately on all 19 soils, the eight Willamette treatments, and the five Walla Walla treatments to compare groupings created by these categories of soil attributes. For the Biolog cluster analyses, the binary response matrix (0 for < 0.4 absorbance, 1 for > 0.4 absorbance) for the soils and the 95 different carbon sources was converted to a dissimilarity matrix which was then used in the cluster analysis (SAS, 1990). Principal component analysis of all soils and all variables was performed using Proc Princomp in SAS (1990).

Results and Discussion

Principal components analysis

A plot of the first two principal components is shown in Figure 5.1. The Bashaw wetland soil stands out along both axes and the three other minimally disturbed soils (Newberg sod, Walla Walla pasture, and Jory forest) are the next three soils furthest out along the first principal component axis. The other soils generally fall into two groups: a) the six vegetable rotation Willamette soils along with the Jory orchard and Bashaw grass,

Table 5.2. Measured values of biological tests

Soil name	Asparaginase	Amidase	Urease	Beta-glucosidase	FDA	Dipeptidase	Biolog GN plates	Microbial biomass C	Microbial biomass N
	----*mg NH_4-N · kg soil^{-1} · 2 hr^{-1}*----			*mg p-nitrophenol kg soil^{-1} · hr^{-1}*	*mg flourescein kg soil^{-1} · hr^{-1}*	*mg leucine kg soil^{-1} · hr^{-1}*	*# wells > 0.4 absorbance*	*mg C kg soil^{-1}*	*mg N kg soil^{-1}*
WIL grass, burn	6.9	87.7	28.0	97.5	82.8	49.5	52.7	144.9	20.1
WIL grass, no burn	7.0	91.6	30.8	86.5	83.9	58.2	37.3	193.1	16.0
WIL fallow, no N	8.8	84.9	17.5	64.1	47.0	31.7	35.0	142.7	5.6
WIL fallow, high N	8.6	35.0	10.1	61.7	39.3	25.2	36.3	64.5	5.7
WIL clover, no N	9.1	51.1	15.5	95.1	59.1	51.3	57.7	182.5	8.7
WIL clover, high N	6.7	29.6	16.5	82.7	48.9	36.1	51.7	131.4	15.5
WIL rye, no N	7.2	40.5	24.5	70.7	51.8	32.9	53.3	168.4	16.3
WIL rye, high N	9.5	40.8	17.8	74.7	48.4	19.2	46.0	120.9	13.7
WW manure	11.9	141.2	67.7	76.6	61.1	125.2	44.7	268.4	32.6
WW pea vine	8.2	130.7	44.6	84.0	51.7	65.8	56.7	171.1	20.7
WW no N	3.3	76.7	26.3	53.0	35.3	26.0	31.0	96.7	3.1
WW high N	3.8	89.8	18.3	67.8	34.4	9.3	47.7	38.2	5.8
WW pasture	43.3	213.3	161.2	115.6	124.7	237.2	51.7	502.3	80.2
NB sod	135.3	430.3	111.6	49.7	182.4	314.1	34.7	1705.9	186.0
NB corn, high N	11.3	67.2	27.1	102.0	46.2	44.2	48.6	150.6	11.3
JY forest	90.0	343.8	131.5	160.2	172.4	226.6	44.0	1352.0	154.0
JY orchard	3.8	59.2	13.0	36.0	64.6	11.2	11.7	74.3	4.4
BSHW wetland	83.6	323.3	100.9	35.4	200.2	418.2	54.7	2577.5	300.0
BSHW grass	26.0	174.9	76.0	73.3	187.9	111.5	52.7	602.0	59.3
LSD, $p < 0.05$	7.4	14.7	6.8	7.5	3.6	45.3	1.5	91.7	5.6

Table 5.3. Measured values of chemical tests

Soil name	pH	Total soil C	Total soil N	UV 210 nm	UV 270 nm	Preplant soil ammonium	Preplant soil nitrate
		mg kg^{-1}	*mg kg^{-1}*	*---absorbance---*		*mg kg^{-1}*	*mg kg^{-1}*
WIL grass, burn	5.4	16124	1164	0.676	0.209	0.9	8.0
WIL grass, no burn	5.6	16205	1084	0.586	0.202	1.3	4.4
WIL fallow, no N	5.6	15335	1113	2.719	0.172	2.4	91.3
WIL fallow, high N	5.3	16073	983	2.317	0.170	1.1	74.7
WIL clover, no N	5.6	15128	1037	2.241	0.189	4.4	69.8
WIL clover, high N	5.3	15647	1135	3.378	0.188	5.7	119.1
WIL rye, no N	5.6	13877	966	2.669	0.143	2.8	86.5
WIL rye, high N	5.5	14325	1077	3.426	0.165	4.6	114.8
WW manure	7.1	14000	1136	0.780	0.156	0.9	21.3
WW pea vine	6.5	11513	963	0.641	0.146	3.9	20.9
WW no N	6.3	9293	712	0.393	0.114	1.7	15.9
WW high N	5.8	9440	713	0.570	0.131	3.0	17.9
WW pasture	6.9	22324	1680	0.952	0.207	3.9	22.0
NB sod	6.1	34171	2474	0.916	0.257	3.7	17.0
NB corn, high N	6.1	8184	627	1.415	0.096	4.4	39.9
JY forest	6.2	45361	1948	0.433	0.225	7.8	0.9
JY orchard	5.3	10744	782	1.121	0.231	0.9	31.1
BSHW wetland	5.5	91663	4794	3.415	0.511	5.4	81.3
BSHW grass	5.1	28138	2050	1.324	0.276	8.3	32.6
LSD, $p < 0.05$	0.1	3097	156	0.157	0.030	2.0	5.2

and b) the Walla Walla cultivated soils along with the Newberg cultivated soil. All 15 measured parameters accounted for variability in the first three principal components with

no one of the factors showing a high loading. Consequently, all variables were kept for subsequent cluster analysis.

Within the Walla Walla soils, the pasture stands alone and none of the four other four soils are grouped (Fig. 5.2a). This result may reflect that the same management systems have been in place for over 60 years and have imparted a unique character to each treatment.

Principal components analysis of the eight Willamette soils shows the same parameters accounting for the variability as in the analysis of all the soils. However, only the two grass treatments stand out from the other treatments along the axes (Fig. 5.2b).

Cluster analysis with all chemical and biological factors

An examination of the dendrogram derived from the cluster analysis of all 19 soils (Fig. 5.3) shows that cultivation has a quite strong effect on creating similarities among diverse soils. Where the tree is "cut" determines the number of distinct clusters that are subjectively called different from each other (Romesburg, 1984). If this tree (Fig. 5.3) is cut at the scaled Euclidean distance of 1.0, there are three clusters consisting of: a) the Bashaw wetland soil, b) the other three minimally disturbed soils (Jory forest, Walla Walla pasture, and Newberg sod) and c) the remaining 15 soils that have been cultivated or disturbed. If the tree is cut at a scaled distance of 0.6, eight distinct clusters appear, four of the clusters are the minimally disturbed soils, and the other four clusters consisting of a) the six Willamette treatments cultivated to vegetables, b) the four cultivated Walla Walla treatments, the two grass Willamette treatments and the cultivated Newberg soil, c) the Jory orchard soil, and d) the Bashaw grass soil. The fact that the minimally disturbed

Figure 5.1 Principal components analysis of all 19 soils bases on measured properties.

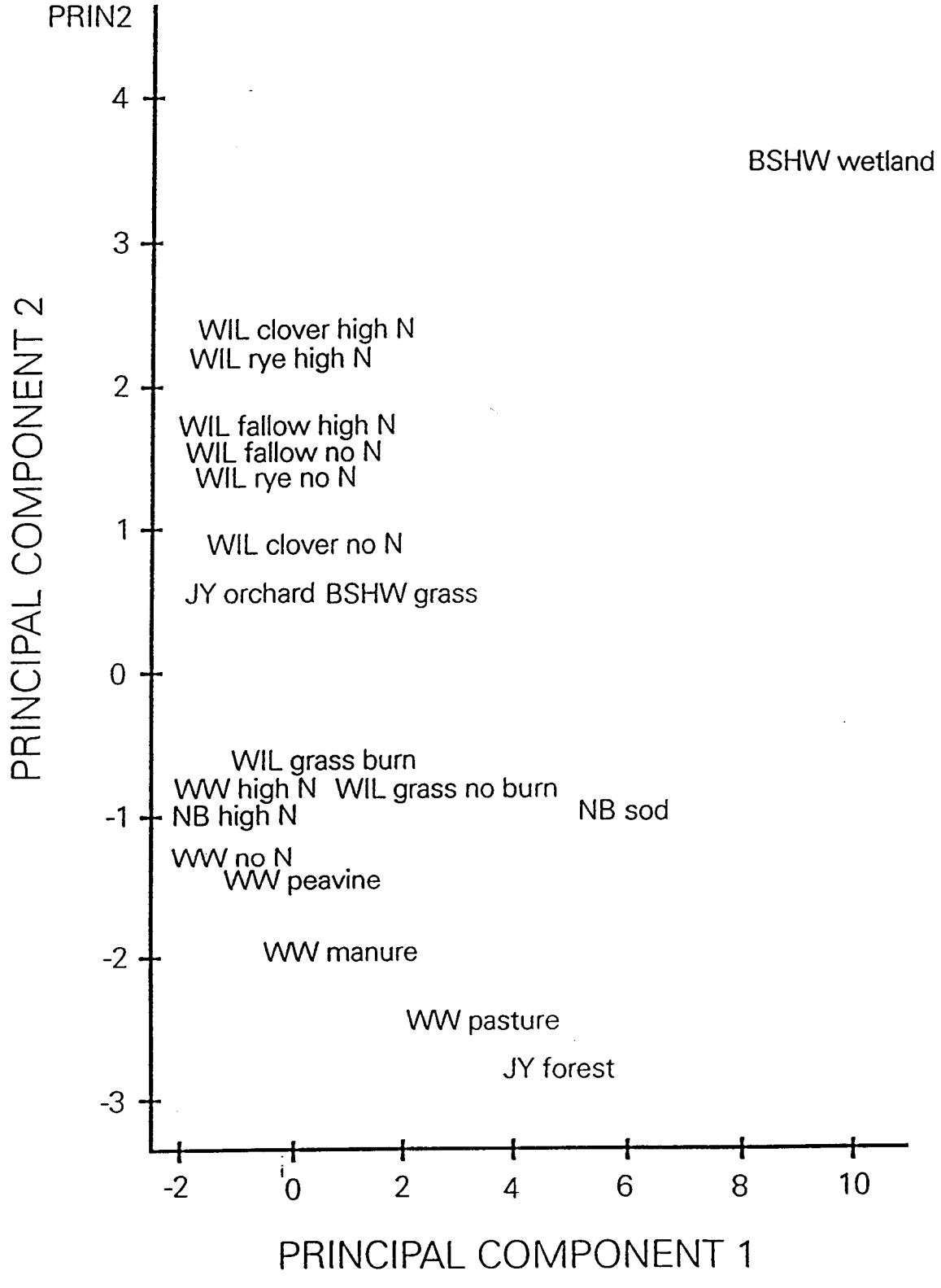
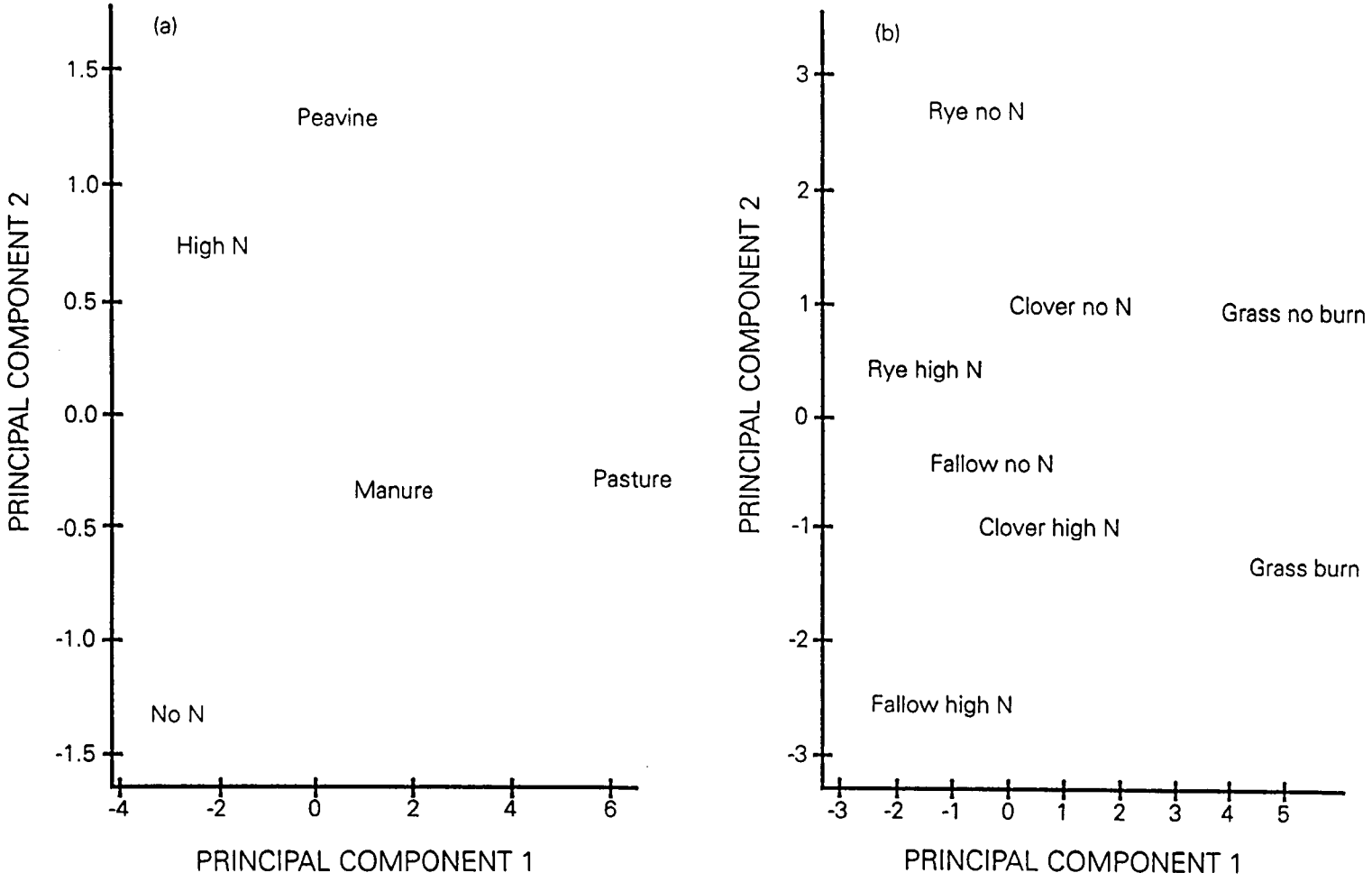


Figure 5.2 (a) Principal components analysis of Walla Walla soils based on all measured variables, and (b) principal components analysis of Willamette soils based on all measured variables.



soils stand out as highly unique from each other and the grouped cultivated soils, indicates that environment and genetic origin are very important factors affecting soil biology, but as soon as disturbance is introduced, genetic and environmental factors become less important. Soil ecosystems that have been in place and undisturbed under specific vegetation for many years would be expected to generate an array of biological characteristics unique to that soil entity.

Within the eight treatments on the Willamette soil, two distinct clusters are present at a scaled distance of 1.0 consisting of: a) the grass seed treatments, and b) the six vegetable rotation treatments (Fig. 5.4). If the cut is made lower on the tree at 0.6, each treatment forms its own cluster. These results suggest that physical disturbance is the strongest factor to create differences within a common soil type treated differently. Studies dealing with soil organic matter have shown that the total amount of residue input, rather than the type, added is often more important to soil organic matter accumulation (Rasmussen and Collins, 1991; Campbell and Zentner, 1993). The cluster analyses done here show that when a measure of many biological parameters is examined as a whole, the effect of residue type on a soil entity's character can be observed. Just as long term above ground vegetation helps to create a unique character for the undisturbed Jory and Bashaw soils, the type of residue in cultivated soils of the same origin imprints a specific set of characteristics that results in unique clusters when examined at a higher resolution.

For the the Walla Walla soils, the pasture stands alone, and the other four treatments form a second cluster at the distance of 1.0 (Fig. 5.5). Lower down on the tree at a distance of 0.6, the manure and peavine treatments form a cluster, and the two treatments without organic inputs forma third cluster. Again, physical disturbance

Figure 5.3 Dendrogram of all 19 soils based on cluster analysis of all chemical and biological parameters.

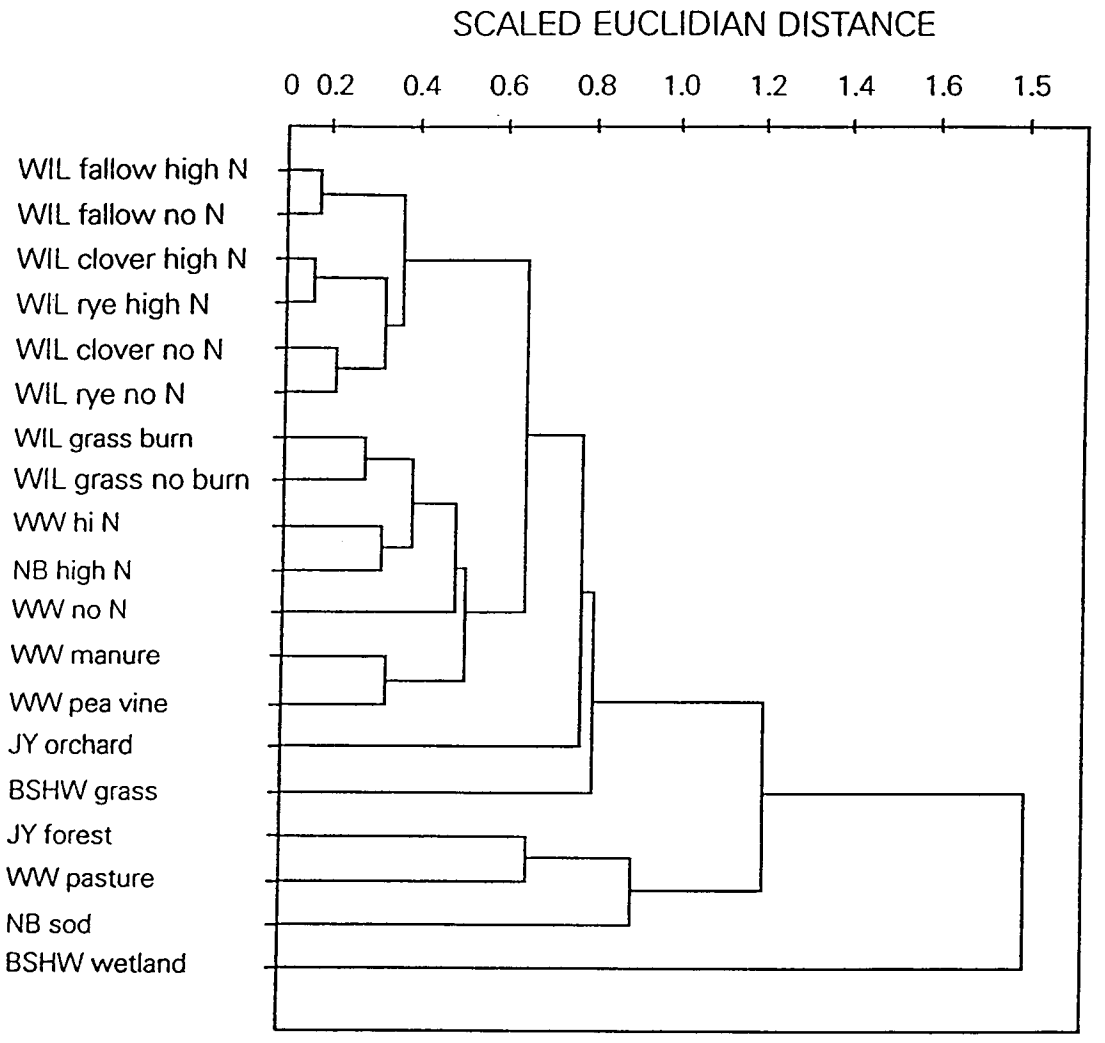
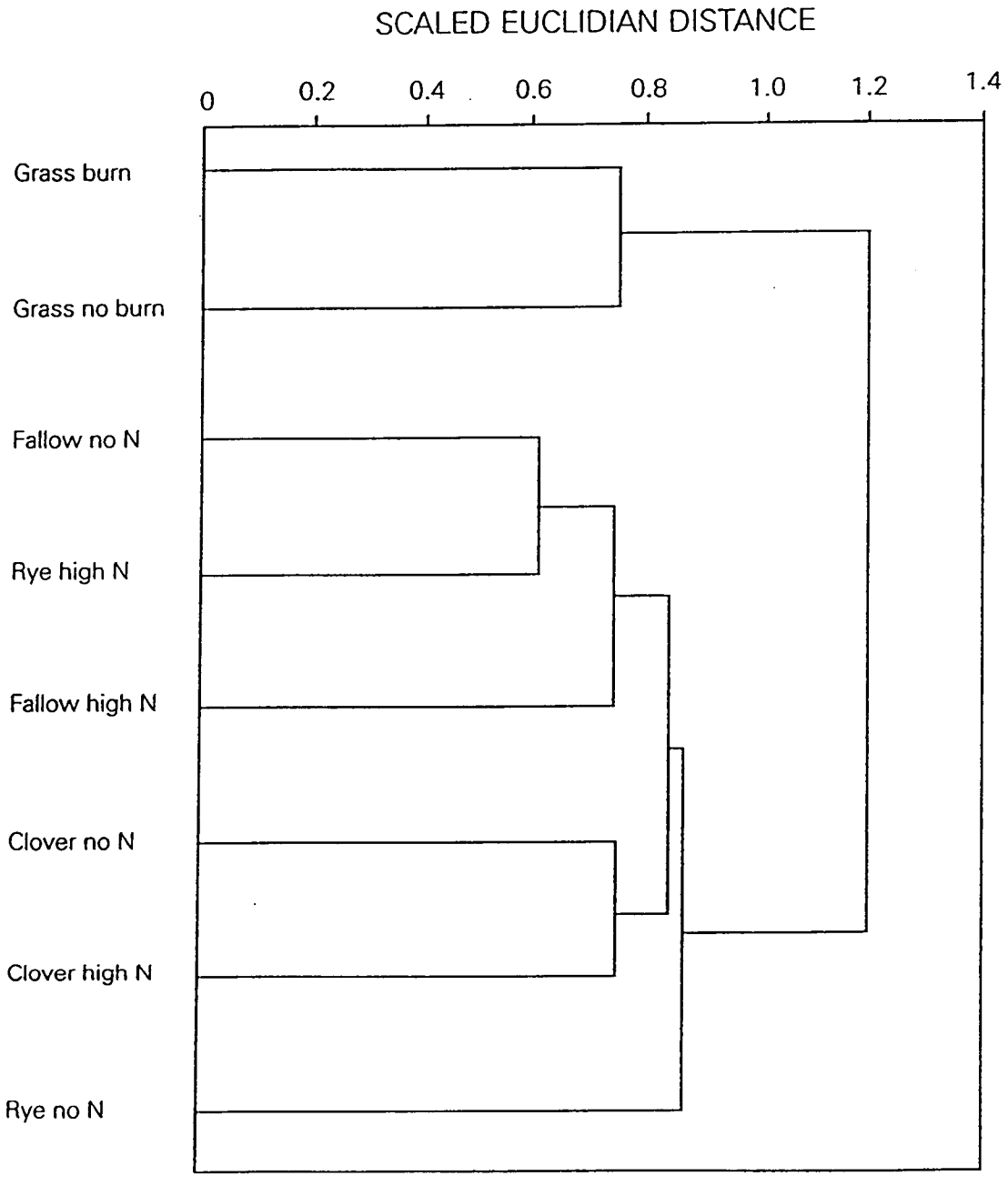


Figure 5.4 Dendrogram of Willamette soil/cover crop treatments based on cluster analysis of all chemical and biological parameters.



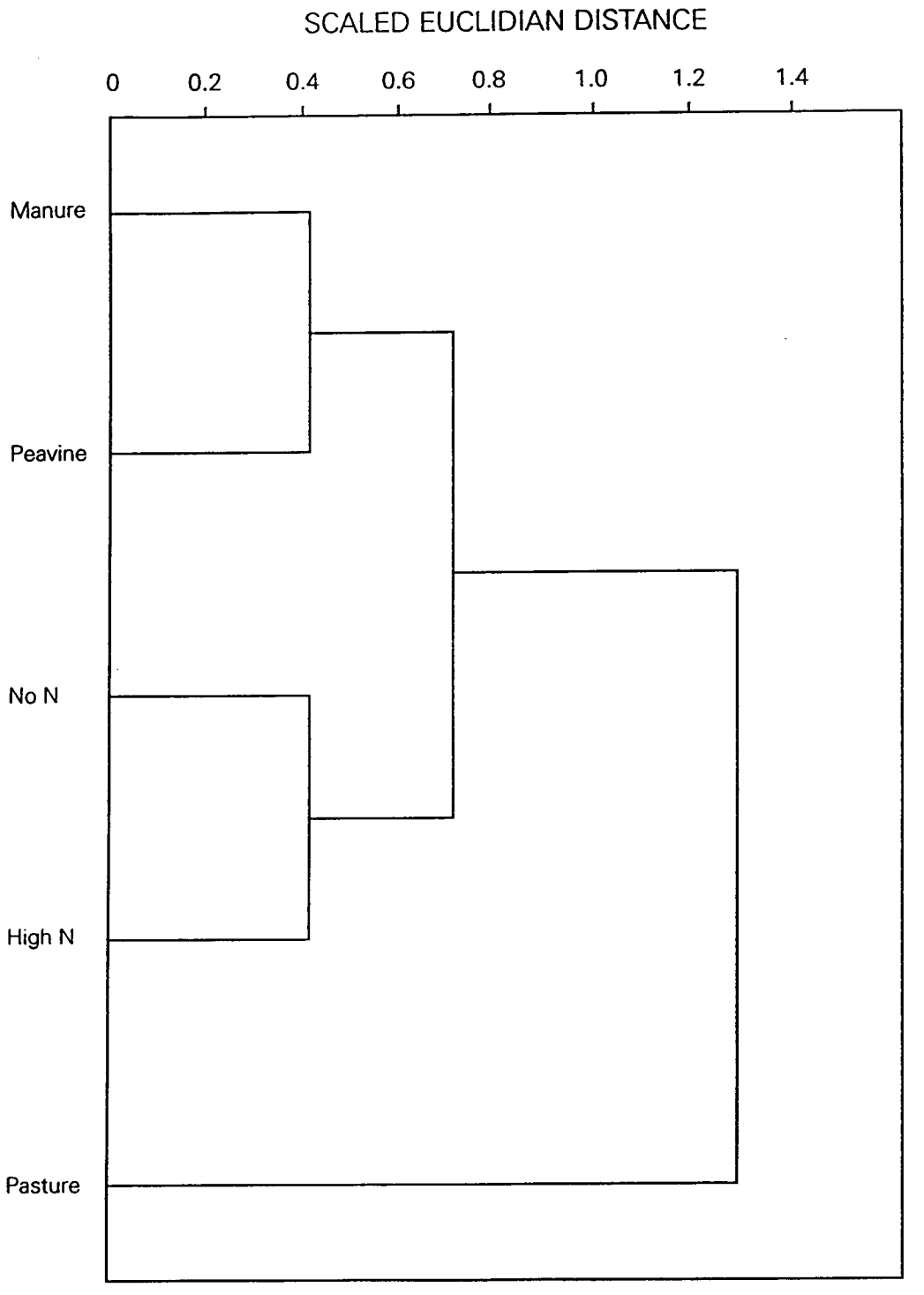
appears to be the primary factor in creating the first two clusters, and whether or not organic residue was added is the factor responsible for the second tier of three clusters, regardless of N fertilization.

The strong homogenizing effect of cultivation on biological characteristics of soils from different environments and origins is seen clearly in this study. One possible explanation is the alteration of the soil's physical structure due to cultivation and the consequent effect on microbial habitats and energy sources. Gupta and Germida (1988) showed that labile soil organic matter, arylsulfatase, acid phosphatase, and microbial respiratory activities decline, and soil fungal populations decline relative to bacterial populations as the proportion of macroaggregates (0.25 mm to 1.0 mm size class) is reduced by cultivation. Increasing intensity of tillage in a Nebraska study reduced soil organic matter, microbial biomass, and carbon dioxide respiration (Follet and Schimel, 1989). In the present study, if the cut is made at a scaled distance of 0.2 on the dendrogram of all 19 soils, the Newberg grass, the Bashaw grass seed, and Walla Walla pasture soils separate out into their own cluster (Fig. 5.3). These three soils are the least disturbed of the 17 managed soils that formed their own cluster when the tree was cut at a higher level (Fig. 5.3). The position of these three soils in relation to the other more intensively managed soils confirms the importance of physical disturbance to soil biology.

Enzyme activities and N factor cluster analyses

When enzyme activities and N factors (total N, MB_N , nitrate, UV 210, and UV 270) are used separately to create clusters among all soils, the soils form groups similar to the patterns seen when all the data are used (Fig. 5.6 and 5.7).

Figure 5.5 Dendrogram of Walla Walla soil with organic residues or N treatments based on cluster analysis of all chemical and biological parameters.



With N factors as the only variables at the distance of 1.0, two clusters are formed- the Bashaw wetland and all the other 18 soils (Fig.5.6). At the distance of 0.6 the cultivated soils, except for the Bashaw grass, form two clusters, the Jory forest and the Newberg sod form a cluster, the Walla Walla pasture and Bashaw grass form a cluster, and the Bashaw wetland stands alone (Fig. 5.6).

When enzyme activities are used as the only variables (Fig. 5.7), clustering resembles more closely the complete analysis (Fig. 5.3). This result is in accord with other studies that have shown enzyme activities to be responsive to changes in management (McGill et al., 1986; Dick, 1988; Collins et al., 1992; Ladd et al., 1994). This would suggest that enzyme assays are advantageous as a integrative soil quality indicator because they offer the potential to reduce the number of analyses needed to be sensitive to soil management effects. The only case where multivariate analysis of all factors was superior to enzyme analysis was in distinguishing the unique character of the Bashaw wetland soil (Figs 5.1 and 5.3)

Biolog cluster analysis

The comparison of metabolic capabilities in a number of soils as measured by the Biolog GN plates is a simple measurement of metabolic diversity for those soils. Counting the number of utilized substrates can be seen as analogous to counting species as a method of estimating diversity within different ecosystems (Magurran, 1988). More accurately, this method simply indicates the ability of the soil to hydrolyze a diverse set of substrates and provides no evidence whether this is accomplished by many or a few species.

Figure 5.6 Dendrogram of all 19 soils based on N factors.

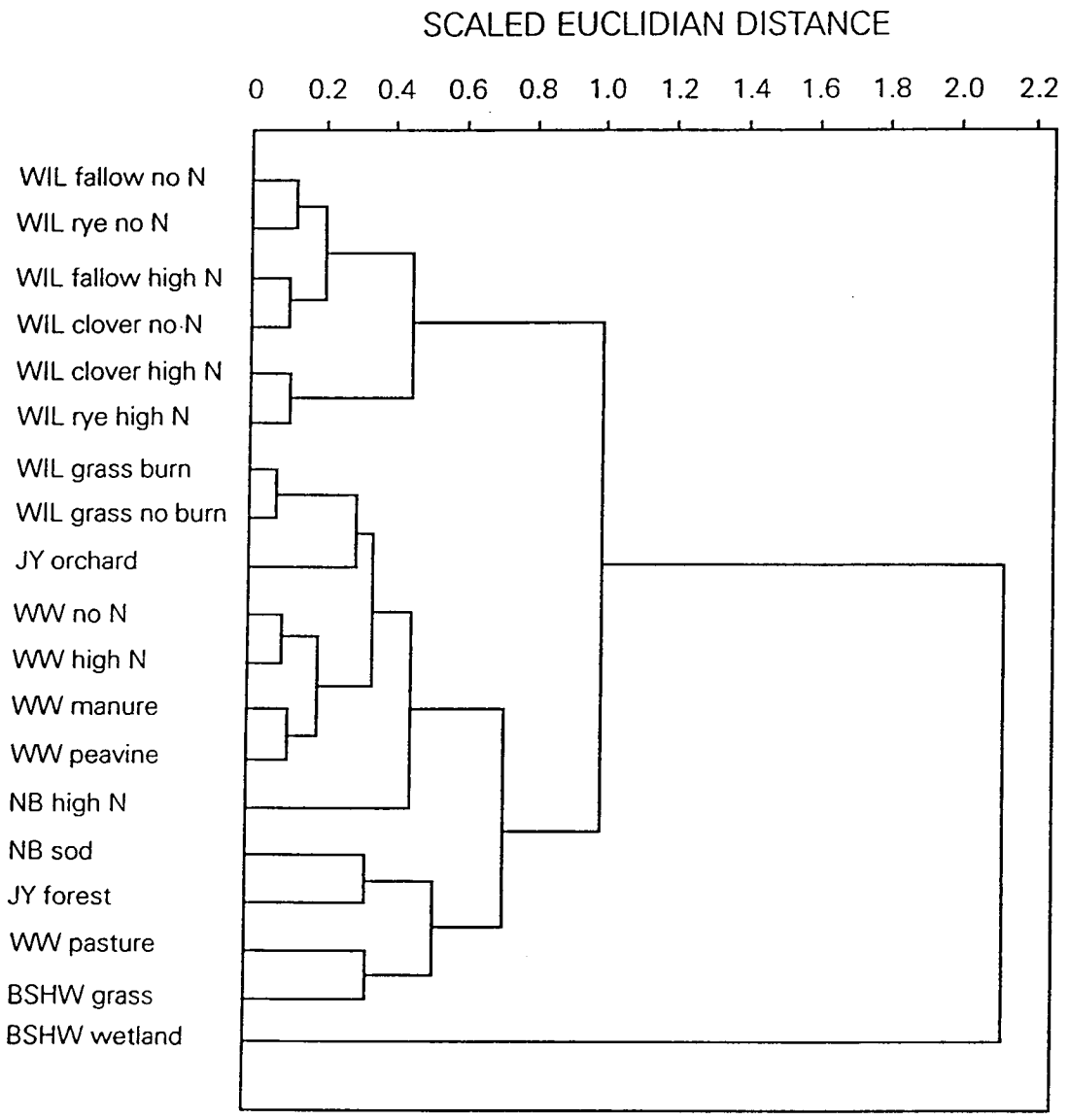
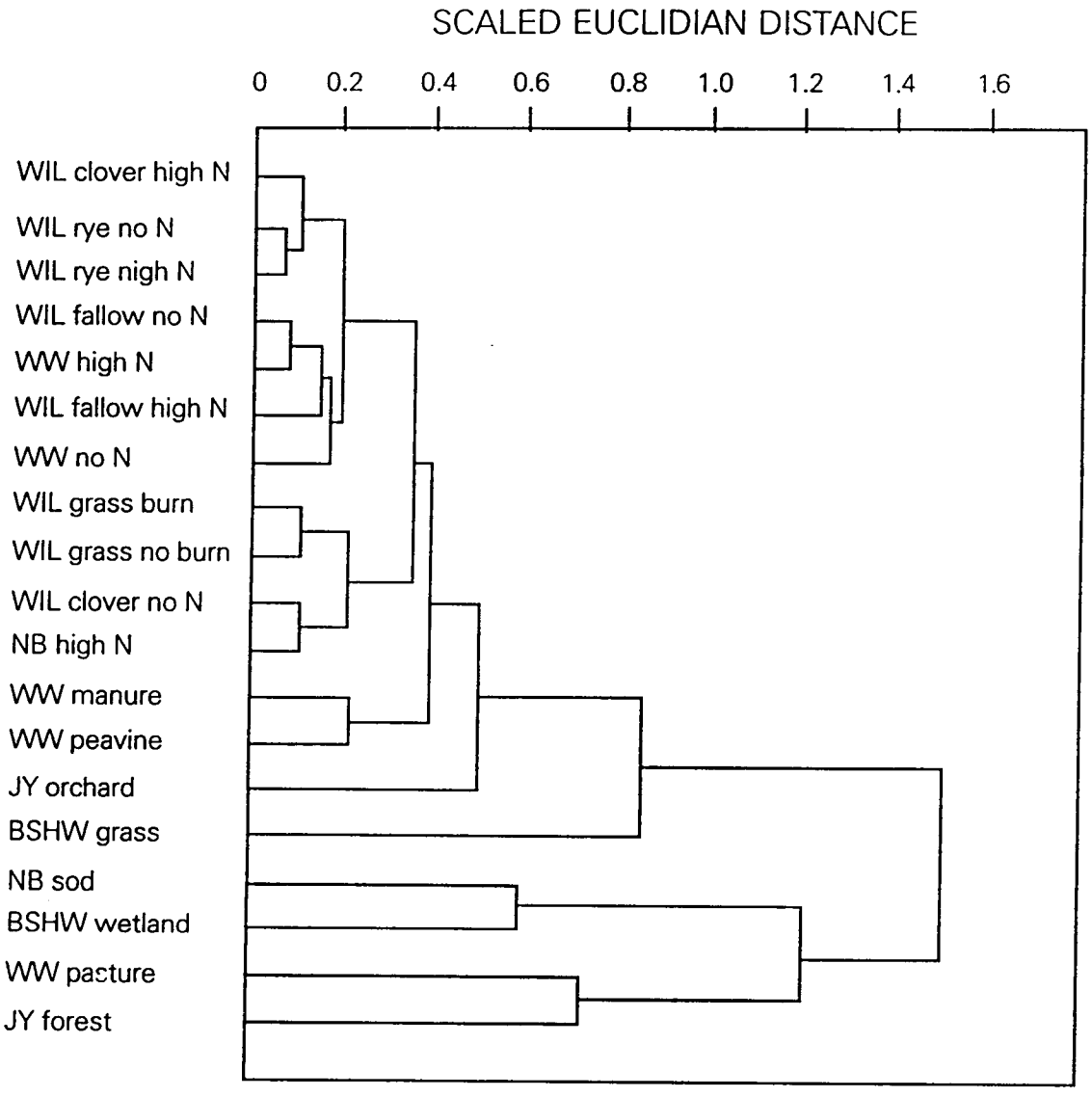


Figure 5.7 Dendrogram of all 19 soils based on enzyme activities.



Biolog plate diversity was in most cases higher in soils that had less disturbance, higher organic matter inputs, and lower inorganic N inputs (Table 5.2). The lowest number of positive wells was seen in the Jory orchard soil whereas the Willamette soil under clover with no added N had the highest. The Willamette soil in this treatment is one in which the red clover cover crop contained an average of 72 kg ha $^{-1}$ N and yields of broccoli and sweet corn were equivalent to other treatments that received over 200 kg ha $^{-1}$ inorganic N (Burket et al., 1997). In contrast, the Jory orchard soil had been kept in bare fallow for over 15 years without soil amendments. Exceptions to this pattern were the high N plots of the Walla Walla and Newberg soils which had higher counts than their zero N counterparts (Table 5.2). For these soils, the increased plant mass returned to the soil resulting from N fertilization may explain the increased metabolic diversity seen in the plates.

When only the Biolog data is used in cluster analysis, the 19 soils do not form groups according to either management or origin (data not shown). Similarly, examination of separate Biolog cluster analyses from both the Willamette and Walla Walla sites fails to produce any logical groupings according to management (data not shown). The apparent random groupings from the Biolog data may allow for too many chance similarities among the 19 soils.

This analysis did not take into consideration the intensity of color development in the wells as an indication of the relative abundance of organisms with the ability to metabolize particular substrates, as have other studies (Garland and Mills, 1994; Winding, 1994; Bossio and Scow, 1995). The high variability of absorbances for the same substrate among replicate wells convinced us that a relatively high threshold response value such as

0.4 would give a more discernible indication of a soil's ability to metabolize a particular substrate. Our use of positive or negative response based on a rigorous threshold is a way to ameliorate possible sources of variability among replicate wells such as differences in inoculum density and soil heterogeneity. Haack et al. (1995) were unable to distinguish between bulk and rhizosphere soil with Biolog plates and attributed the result to the lack of similarity among replicate plates due to microscale heterogeneity in soils. Using a response threshold to avoid false positive results has been a standard method in other microplate detection procedures (Ali-Shtayeh et al., 1991; Pscheidt et al., 1992).

Innoculum density was not taken into consideration in this study as it has been in others (Haack et al., 1995; Garland, 1996). Previous work has also shown that most wells with color development have absorbances greater than 0.4 after 48 hours of incubation (Garland and Mills, 1991; Bossio and Scow 1995). Allowing the wells to develop color for 48 hours and using the 0.4 absorbance threshold for a positive response reduces the importance of innoculum density as a factor in the Biolog plates.

Biolog plates have many limitations as a means to compare whole soil communities. Substrates may select for only a fraction of the aerobic microbes present, and the heterogeneity within soil samples creates high degrees of variability. In reality, Biolog plates can only assess the ability of soils to hydrolyze a wide array of organic substrates. This ability could be related to either community diversity or to a few types of organisms that have the ability to hydrolyze many substrates. For these reasons, Biolog plates in their present form should not be used as a sole means to compare the imprints of environment or management systems on soils.

Summary

Origin, environment, and vegetation have a profound effect on the characteristics of a particular soil which develop over long periods of soil genesis (Jenny, 1980). Surprisingly, our study has shown that cultivation or other disturbances have an effect on soil biological characteristics that can over shadow origin and environment when examined with multivariate analysis. At a finer scale of resolution within the soils of a common origin, the type of organic residue added to a soil had a distinguishable effect on the array of biological parameters studied.

References

Acea, M. J. and T. Caraballas. 1990. Principal components analysis of the soil microbial population of humid zone of Galicia (Spain). Soil Biol. and Biochem. 22:749-759.

Ali-Shtayeh, M. S., J. D. Macdonald, and J. Kabashima. 1991. A method for using commercial ELISA tests to detect zoospores of *Phythophthora* and *Pythium* species in irrigation water. Plant Dis. 75:305-311.

Atlas, R. M. 1984. Diversity of microbial communities. pp. 1-47. *In* K. C. Marshall (ed.) Advances in microbial ecology. Plenum Press, New York.

Bossio, D. A. and K. M. Scow. (1995) Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. Appl. Environ. Microbiol. 61:4043-4050.

Bremner, J. M. 1965. Inorganic forms of nitrogen. pp. 1179-1237. *In* C. A. Black (ed.) Methods of soil analysis, Part 2. American Society of Agronomy, Madison, WI.

Bremner, J. M. and C. S. Mulvaney. 1982. Nitrogen-total. pp. 595-624. *In* A. L. Page, R. H. Miller, and D. R. Keeney (eds.) Methods of soil analysis, Part 2, 2nd edition. American Society of Agronomy, Madison, WI.

Burket , J. Z., D. D. Hemphill, and R. P. Dick. 1997. Winter cover crops and nitrogen management in sweet corn and broccoli rotations. HortSci. 32:664-668.

Campbell, C. A. and R. P. Zentner. 1993. Soil organic matter as influenced by crop rotations and fertilization. Soil Sci. Soc. Am. J. 57:1034-1040.

Cawse, P. A. 1967. The determination of nitrate in soil solutions by ultraviolet spectrophotometry. Analyst 92:311-315.

Chang, C. W. 1949. Effect of long-time cropping on soil properties in northwestern New Mexico. New Mexico Agric. Exp. Sta. No. 57. pp. 359-368.

Collins, H.P., P. E. Rasmussen, and C. L. Douglas, Jr. 1992. Crop rotation and residue manage-ment effects on soil carbon and microbial dynamics. Soil Sci. Soc. Am. J. 56:783-788.

Dick, R. P. 1992. A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. Agric. Ecosys. Environ. 40:25-36.

Dick, R. P., P. E. Rasmussen, and E. A. Kerle. 1988. Influence of long-term residue manage-ment on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. Biol. Fertil. Soils 6:159-164.

Dick, W. A. 1984. Influence of long-term tillage and crop rotation combinations on soil enzyme activities. Soil Sci. Soc. Am. J. 48:569-574.

Follet, R. F. and D. S. Schimel. 1989. Effect of tillage practices on microbial biomass dynamics. Soil Sci. Soc. Am. J. 53:1091-1096.

Fox, R. H., and W. P. Piekielek. 1978. A rapid method for estimating the nitrogen-supplying capacity of a soil. Soil Sci. Soc. Am. J. 42:751-753.

Gaikawad, S. T., S. K. Goel, and N. G. Raghumohan. 1977. Numerical classification of some Indian soils. J. Indian Soc. Soil Sci. 25: 288-296.

Garland, J. L. 1996. Patterns of potential C source utilization by rhizosphere communities. Soil Biol. Biochem. 28:223-230.

Garland, J. L. and A. L. Mills. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57:2351-2359.

Garland, J. L. and A. L. Mills. 1994. A community-level physiological approach for studying microbial communities. British Soc. Soil Sci. Wiley-Sayce Pub. pp. 77-83.

Gupta, V. V. S. R., and J. J. Germida. 1988. Distribution of microbial biomass and activity in different soil aggregate size classes as affected by cultivation. Soil Biol. Biochem. 20:777-786.

Haack, S. K., H. Garchow, M. J. Klug, and L. J. Forney. 1995. Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Appl. Environ. Microbiol. 61:1458-1468.

Jenkinson, D. S. and D. S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol. Biochem. 8:209-213.

Jenny, H. 1980. The soil resource. Ecological studies, vol. 37. Springer-Verlag, New York.

Jha, D. K., G. D. Sharma, and R. R. Mishra. 1992. Soil microbial population numbers and enzyme activities in relation to altitude and forest degradation. Soil Biol. Biochem. 24:61-767.

Katyal, J. C., S. P. Doshi, and P. K. Malhotra. 1985. Use of cluster analysis for classification of benchmark soil samples from India in different micronutrient availability groups. J. Agric. Sci., Camb. 104:421-424.

Ladd, J. N., and J. H. A. Butler. 1972. Short-term assays of soil proteolytic enzyme activities using protein and dipeptide derivatives as substrates. Soil Biol. Biochem. 4:19-30.

Ladd, J. N., M. Amato, Zhou Li-Kai, and J. E. Schultz. 1994. Differential effects of rotation, plant residue and nitrogen fertilizer on microbial biomass and organic matter in an Australian Alfisol. Soil Biol. Biochem. 26:821-831.

Magurran, A. E. 1988. Ecological diversity and its measurement. Princeton University Press. Princeton, NJ. 179 p.

McGill, W. B., K. R. Cannon, J. A. Robertson, and F. D. Cook. 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. Can. J. Soil Sci. 66:1-19.

Meints, V. W. and G. A. Peterson. 1977. The influence of cultivation on the distribution of nitrogen in soils of the Ustoll suborder. Soil Sci. 124:334-342.

Norman, R. J., J. C. Edberg, and J. W. Stucki. 1985. Determination of nitrate in soil extracts by dual-wavelength ultraviolet spectrophotometry. Soil Sci. Soc. Am. J. 49:1182-1185.

Pancholy, S. K. and E. L. Rice. 1973. Soil enzymes in relation to old field succession: amylase, cellulase, invertase, dehydrogenase, and urease. Soil Sci. Soc. Am. Proc. 37:47-50.

Pscheidt, J. W., J. Z. Burket, S. L. Fischer, and P. B. Ham. 1992. Sensitivity and clinical use of *Phytophthora*- specific immunoassay kits. Plant Dis. 76: 928-932.

Rassmussen, P. E. and H. P. Collins. 1991. Long-term impacts of tillage, fertilizer, and crop residue on soil organic matter in temperate semiarid regions. Adv. Agron. 45:93-134.

Romesburg, C. A. 1984. Cluster Analysis for Researchers. Lifetime Learning Publications, Belmont, CA.

SAS Institute, Inc. 1990. SAS user's guide: Statistics. SAS Institute, Inc., Cary, NC.

Schnürer, J., and T. Rosswall. 1982. Flourescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl. Environ. Micro. 43:1256-1261.

Segal, W., and R. L. Mancinelli. 1987. Extent of regeneration of the microbial community in reclaimed spent oil shale land. J. Environ. Qual. 16: 44-48.

Shen, S. M., G. Pruden, and D. S. Jenkinson. 1984. Mineralization and immobilization of nitrogen in fumigated soil and the measurement of microbial biomass nitrogen. Soil Biol. and Biochem. 17:11-16.

Smith, S. J. and L. B. Young. 1975. Distribution of nitrogen forms in virgin and cultivated soils. Soil Sci. 120:354-360.

Tabatabai, M. A. 1994. Soil enzymes. *In* R. W. Weaver, J. S. Angle, and P. J. Bottomley (eds.) Methods of soil analysis, Part 2. Microbiological and biochemical properties. Soil Sci. Soc. Amer. book series, no. 5. Madison, WI. pp. 775-833.

Voroney, R. P., and E. A. Paul. 1984. Determination of k_c and k_n *in situ* for calibration of the chloroform fumigation incubation method. Soil Biol. Biochem. 16:9-14.

Wassel, R. A., and A. L. Mills. 1983. Changes in water and sediment bacterial community structure in a lake receiving acid mine drainage. Microb. Ecol. 9:155-169.

Winding, A. 1994. Fingerprinting bacterial soil communities using Biolog microtitre plates. *In* K. Ritz, J. Dighton, and K. E. Giller (eds.) Beyond the Biomass. British Society of Soil Science, Wiley-Sayce Pub. pp. 85-94.

Zak, J. C., M. R. Willig, D. L. Moorhead, and H. G. Wildman. 1994. Functional diversity of microbial communities: a quantitative approach. Soil Biol. Biochem. 26:1101-1108.

Chapter 6

SUMMARY

Cover Crops and Biochemical Functional Diversity in Relation to Nitrogen Availability in Soil

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Many factors are involved in the N dynamics of agricultural soils. Finding tools that will increase the knowledge of how N availability in the soil is controlled by the management of soil organic matter, plant residue, cover crops, and fertilizer will be an ongoing focus in agricultural research for many years to come.

Winter cover crops that annually absorb fertilizer N and are incorporated into the soil would be expected to reduce the amount of N applied to the summer crop. After four years of winter cover cropping, our results showed that cereal rye did not have this effect because yields of vegetables were similar between fallow and rye plots at zero or intermediate N rates. A winter cover crop of cereal rye was not reliable as a mineralizable N source possibly because the C to N ratio of the rye residue kept it immobilized in the soil system. In contrast, legume winter cover crops, either alone or in a mix with cereal rye, contributed significant amounts of N to sweet corn and broccoli yields. This study showed that winter cover crops containing legumes and an N rate of one quarter of the recommended rate resulted in sweet corn yields that were equivalent to yields obtained at the recommended rate after winter fallow or a cereal rye winter cover crop. Results were less dramatic for broccoli in that equivalent yields following legume winter cover crops were achieved at one half the recommended rate. Our study suggests that a legume is needed in a mix or alone for farmers to reduce N levels below the recommended rate.

The fate of fertilizer N in cropping systems is important to economic and environmental concerns. We found that a substantial amount of recently applied N as fertilizer is lost from the system between cropping seasons, even with a winter cover crop in place. On the other hand, N from incorporated plant residue appears to be relatively

stable, but because of this stability is also not very available for uptake by subsequent crop plants.

Mineralization of organic N in agricultural soils that receive plant residue inputs is an area of research that can yield information on ways to reduce the input of inorganic N fertilizers. Leaching of applied fertilizer N is a large contributing factor to nitrate pollution problems in groundwater. But the predictability of N mineralization for crop plant uptake is not yet well understood. We found a biological indicator, β-glucosidase activity, and total soil N to constitute a good mineralized N uptake model. Clearly, many types of biological activity in soils are intimately associated with the process of N mineralization. In order to find a useful means of predicting N mineralization in the laboratory that will accurately reflect the mineralized N taken up by plants, the measurements should be both biologically relevant to the process of N mineralization and fairly easy to perform. Further work using measurements of total soil N and β-glucosidase activity on field soils to predict plant available mineralized N will determine if the model developed here from a variety of Oregon soils is more broadly applicable.

Soil biology is the driving force of N transformations in agricultural soils. Factors such as the history of a soil's formation and climate in turn superimpose their control over the functioning of the soil biota. Cultivation and other disturbances also have profound effects on soil biological characteristics and can actually overshadow the influences of origin and environment. Furthermore, when soils of a common origin are compared in terms of different plant residue management regimes, the type of organic residue added to a soil can be seen to have a recognizable effect on a wide array of biological parameters.

Monitoring soils in terms of their biological components holds the potential to be a useful tool in soil quality assessments because of their responsiveness to changes in management.

In conclusion, the incorporation of plant residues in agricultural systems has significant positive effects on N accumulation and subsequent mineralization, but to understand the mechanisms that are operating so that these processes can be channeled to increase sustainable soil fertility, more knowledge of the biological factors involved is needed. Comparisons of cultivated and non-cultivated soils of the same soil type are useful in shedding light on the abundance, diversity, and biochemical functioning of the soil microbial biomass. This knowledge then directly relates to N mineralization and how dependable management tools such as winter cover crops can be for supplying N to subsequent crop plants. More efficient and accurate methodologies for studying the biochemical functioning of the soil biota are needed to carry this work forward. The creation and continuation of long term study sites will also greatly add to the body of knowledge surrounding N availability in agricultural soils.

BIBLIOGRAPHY

Acea, M. J. and T. Caraballas. 1990. Principal components analysis of the soil microbial population of humid zone of Galicia (Spain). Soil Biol. Biochem. 22:749-759.

Alef, K., T. Beck, L. Zelles, and D. Kleiner. 1988. A comparison of methods to estimate microbial biomass and N-mineralization in agricultural and grassland soils. Soil Biol. Biochem. 20: 561-565.

Ali-Shtayeh, M. S., J. D. Macdonald, and J. Kabashima. 1991. A method for using commercial ELISA tests to detect zoospores of *Phythophthora* and *Pythium* species in irrigation water. Plant Dis. 75:305-311.

Allen, A. L., F. J. Stevenson, and L. T. Kurtz. 1973. Chemical distribution of residual fertilizer nitrogen in soil as revealed by nitrogen-15 studies. J. Environ. Qual. 2: 120-124.

Anderson, E. L. 1986. Maize root response to N in the greenhouse and field. Plant and Soil. 95: 293-296.

Atlas, R. M. 1984. Diversity of microbial communities. pp. 1-47. *In* K. C. Marshall (ed.) Advances in microbial ecology. Plenum Press, New York.

Balabane, M. and J. Balesant. 1992. Input of fertilizer-derived labeled N to soil organic matter during a growing season of maize in the field. Soil Biol. Biochem. 24: 89-96.

Beauchamp, E. G., W. D. Reynolds, D. Brasche-Villeneuve, and K. Kirby. 1986. Nitrogen mineralization kinetics with different soil pretreatments and cropping histories. Soil Sci. Soc. Am. J. 50:1478-1483.

Biederbeck, V. O., C. A. Campbell, and R. P. Zentner. 1984. Effect of crop rotation and fertilization on some biological properties of a loam in southwestern Saskatchewan. Can. J. Soil Sci. 64:355-367.

Blagoveshchenskaya, Z. K. and N. A. Danchenko. 1974. Activity of soil enzymes after prolonged application of fertilizers to a corn monoculture and crops in rotation. Sov. Soil Sci. 5: 569-575.

Bonde, T. A., J. Schnürer, and T. Rosswall. 1988. Microbial biomass as a fraction of potentially mineralizable nitrogen in soils from long-term field experiments. Soil Bio. Biochem. 20: 447-452.

Bossio, D. A. and K. M. Scow. 1995. Impact of C and flooding on the metabolic diversity of microbial communities in soils. Appl. Environ. Microbiol. 61: 4043-4050.

Brandi-Dohrn, F. M., R. P. Dick, M. Hess, S. M. Kauffman, D. D. Hemphill, Jr. and J. S. Selker. 1997. Nitrate leaching under a cereal rye cover crop. J. Environ. Qual. 26: 181-188.

Bremner, J. M. 1965. Inorganic forms of nitrogen. p. 1179-1237. *In* C. A. Black (ed.) Methods of soil analysis, Part 2. American Society of Agronomy, Madison, WI.

Bremner, J. M. and C. S. Mulvaney. 1982. Nitrogen-total. pp. 595-624. *In* A. L. Page, R. H. Miller, and D. R. Keeney (eds.) Methods of soil analysis, Part 2, 2nd edition. American Society of Agronomy, Madison, WI.

Broadbent, F. E. and T. Nakashima. 1967. Reversion of fertilizer nitrogen in soils. Soil Sci. Soc. Am. Pro. 31: 648-652.

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17: 837-842.

Brookes, P. C., J. M. Stark, B.B. Mcinteer, and T. Preston. 1989. Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Sci. Soc. Am. J. 53:1707-1711.

Burket, J. Z. and R. P. Dick. 1997. Long-term vegetation management in relation to accumulation and mineralization of nitrogen in soils. p. 283-296. *In* G. Cadish and K. E. Giller (ed.). Driven by nature: plant litter quality and decomposition. CAB International, Wallingford, U.K.

Burket , J. Z., D. D. Hemphill, and R. P. Dick. 1997. Winter cover crops and nitrogen management in sweet corn and broccoli rotations. HortSci. 32:664-668.

Burns, R. G. 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biol. Biochem. 14: 423-427.

Cabrera, M. L. and M. H. Beare. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci. Soc. Am. J. 57: 1007-1012.

Campbell, C. A., V. O. Biederbeck, R. P. Zentner, and G. P. LaFond. 1991. Effect of crop rotations and cultural practices on soil organic matter, microbial biomass and respiration in a thin Black Chernozem. Can. J. Soil Sci. 71: 363-376.

Campbell, C. A., K. E. Bowren, M. Schnitzer, R. P. Zentner, and L. Townley-Smith . 1991. Effect of crop rotations and fertilization on soil organic matter and some biochemical properties of a thin Black Chernozem. Can. J. Soil Sci. 71: 377-387.

Campbell, C. A., G. P. LaFond, A. J. Letshon, R. P. Zentner, and H. H. Janzen. 1991. Effect of cropping practices on the initial potential rate of N mineralization in a thin Black Chernozem. Can. J. Soil Sci. 71: 43-53.

Campbell, C. A., M. Schnitzer, G. P. Lafond, R. P. Zentner, and J. E. Knipfel. 1991. Thirty-year crop rotations and management practices effects on soil and amino nitrogen. Soil Sci. Soc. Am. J. 55: 739-745.

Campbell, C. A. and R. P. Zentner. 1993. Soil organic matter as influenced by crop rotations and fertilization. Soil Sci. Soc. Am. J. 57:1034-1040.

Carter, J. N., O. L. Bennet, and R. W. Pearson. 1967. Recovery of fertilizer nitrogen under field conditions using nitrogen-15. Soil Sci. Soc. Am. Pro. 31: 50-56.

Castellanos, J. Z. and P. F. Pratt. 1981. Mineralization of manure nitrogen-correlation with laboratory indexes. Soil Sci. Soc. Am. J. 45: 354-357.

Cawse, P. A. 1967. The determination of nitrate in soil solutions by ultraviolet spectrophotometry. Analyst 92:311-315.

Chang, C. W. 1949. Effect of long-time cropping on soil properties in northwestern New Mexico. New Mexico Agric. Exp. Sta. No. 57. pp. 359-368.

Cheng, H. H. and L. T. Kurtz. 1963. Chemical distribution of added nitrogen in soils. Soil Sci. Soc. Am. Pro. 27: 312-316.

Christ, R. A. 1993. Effect of long-term residue management and nitrogen fertilization on availability and profile distribution of nitrogen, phosphorus, and sulfur. M.S. Thesis, Oregon State University, Corvallis OR, USA

Collins, H.P., P. E. Rasmussen, and C. L. Douglas, Jr. 1992. Crop rotation and residue management effects on soil carbon and microbial dynamics. Soil Sci. Soc. Am. J. 56: 783-788.

Copeland, R. D. 1965. The evaluation of cereal grains, legumes, and selected winter annuals as cover crops for the Willamette Valley. MS Thesis, Oregon State University, Corvallis, OR.

de Ruiter, P. C., A. M. Neutel, and J. C. Moore. 1995. Energetics, patterns of interaction strengths, and stability in real ecosystems. Science 269:1257-1260.

Decker, A. M., J. F. Holderbaum, R. F. Mulford, J. J. Meisinger, and L. R. Vough. 1987. Fall seeded legume nitrogen contributions to no-till corn. *In* The role of legumes in conservation tillage systems. (J. F. Powers, editor) Soil Conservation Society of America. pp. 21-22.

Dick, R. P. 1992. A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. Agric. Ecosys. Environ. 40:25-36.

Dick, R. P. 1994. Soil enzyme activities as indicators of soil quality. *In* p. 107-124. J. W. Doran, D. C. Coleman, D. F. Bezdicek, and B. A. Stewart (ed.) Defining soil quality for a sustainable environment. Soil Sci. Soc. Amer. Special Pub. 15, Madison, WI.

Dick, R. P. and R. A. Christ. 1995. Effects of Long-term residue management and nitrogen fertilization on availability and profile distribution of nitrogen. Soil Sci. 159: 402-408.

Dick, R. P., P. E. Rasmussen, and E. A. Kerle. 1988. Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. Biol. Fertil. Soils 6:159-164.

Dick, W. A. 1984. Influence of long-term tillage and crop rotation combinations on soil enzyme activities. Soil Sci. Soc. Am. J. 48:569-574.

Ditsch, D. C. and M. M. Alley. 1991. Nonleguminous cover crop management for residual N recovery and subsequent crop yields. J. Fert. Issues 8:6-13.

Ebelhar, S. A., W. W. Frye, and R. L. Blevins. 1984. Nitrogen from legume cover crops for no-tillage corn. Agron. J. 76:51-55.

Eivazi, F. and M. A. Tabatabai. 1990. Factors affecting glucosidase and galactosidase activities in soils. Soil Biol. Biochem. 22: 891-897.

El-Harris, M. K., V. L. Cochran, L. F. Elliott, and D. F. Bezdicek. 1983. Effect of tillage. cropping, and fertilizer management on soil mineralization potential. Soil Sci. Soc. Am. J. 47: 1157-1161.

Fauci, M. F. and R. P. Dick. 1994. Plant response to organic amendments and decreasing inorganic nitrogen rates in soils from a long-term experiment. Soil Sci. Soc. Am. J. 58: 134-138.

Fauci, M. F. and R. P. Dick. 1994. Soil microbial dynamics, short- and long-term effects of inorganic and organic nitrogen. Soil Sci. Soc. Am. J. 58: 801-806.

Ferguson, W. S. and B. J. Gorby. 1971. Effect of various periods of seed-down to alfalfa and bromegrass on soil nitrogen. Can. J. Soil Sci. 51: 65-73.

Fisk, M. C. and S. K. Schmidt. 1995. Nitrogen mineralization and microbial biomass nitrogen dynamics in three alpine tundra communities. Soil Sci. Soc. Am. J. 59: 1036-1043.

Follet, R. F. and D. S. Schimel. 1989. Effect of tillage practices on microbial biomass dynamics. Soil Sci. Soc. Am. J. 53:1091-1096.

Fox, R. H. and W. P. Piekielek. 1978. A rapid method for estimating the nitrogen-supplying capacity of a soil. Soil Sci. Soc. Am. J. 42: 751-753.

Frankenberger, W. T. and W. A. Dick. 1983. Relationships between enzyme activities and microbial growth and activity indices in soils. Soil Sci. Soc. Am. J. 47: 945-951.

Franzluebbers, A. J., F. M. Hons, and D. A. Zuberer. 1995. Soil organic carbon, microbial biomass, and mineralizable carbon and nitrogen in sorghum. Soil Sci. Soc. Am. J. 59: 460-466.

Freund, R. S. and R. C. Little. 1986. SAS system for regression. SAS Institute, Cary, NC.

Gaikawad, S. T., S. K. Goel, and N. G. Raghumohan. 1977. Numerical classification of some Indian soils. J. Indian Soc. Soil Sci. 25: 288-296.

Garland, J. L. 1996. Patterns of potential C source utilization by rhizosphere communities. Soil Biol. Biochem. 28:223-230.

Garland, J. L. and A. L. Mills. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57:2351-2359.

Garland, J. L., and A. L. Mills. 1994. A community-level physiological approach for studying microbial communities. British Soc. Soil Sci. Wiley-Sayce Pub. pp. 77-83.

Gass, W. B., G. A. Peterson, R. D. Hauck, and R. A. Olson. 1971. Recovery of residual nitrogen by corn (*Zea mays* L.) from various soil depths as measured by ^{15}N tracer techniques. Soil Sci. Soc. Am. Pro. 35: 290-294.

Gasser, J. K. R. and S. J. Kalembasa. 1976. The effects of leys and organic manures on the available N in clay and sandy soils. J. Soil Sci. 27: 237-249.

Goh, K. M. 1972. Amino acid levels as indicators of paleosols in New Zealand soil profiles. Geoderma 7: 33-47.

Goyal, S., M. M. Mishra, I. S. Hooda, and R. Singh. 1992. Organic matter-microbial biomass relationships in field experiments under tropical conditions: effects of inorganic fertilization and organic amendments. Soil Biol. Biochem. 24: 1081-1084.

Griffen, T. S. and O. B. Hesterman. 1991. Potato response to legume and fertilizer nitrogen sources. Agron. J. 83:1004-1012.

Griffin, G. F. and A. F. Laine. 1983. Nitrogen mineralization in soils previously amended with organic wastes. Agron. J. 75: 124-129.

Gupta, V. V. S. R. and J. J. Germida. 1988. Distribution of microbial biomass and activity in different soil aggregate size classes as affected by cultivation. Soil Biol. Biochem. 20: 777-786.

Gupta, U. C. and H. W. Reuszer. 1967. Effect of plant species on the amino acid content and nitrification of soil organic matter. Soil Sci. 104: 395-400.

Haack, S. K., H. Garchow, M. J. Klug, and L. J. Forney. 1995. Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Appl. Environ. Microbiol. 61:1458-1468.

Haider, K., J. P. Martins, and Z. Filip. 1975. Humus biochemistry. p. 195-244. *In* E. A. Paul and A. D. McLaren (eds). Soil biochemistry, vol. 4. Marcel Dekker Inc. New York, NY.

Hargrove, W. L. 1986. Winter legumes as a nitrogen source for no-till grain sorghum. Agron. J. 78:70-74.

Harlan, C. 1899. Farming with green manures. Alfred J. Ferris, Printer, Philadelphia.

Harris, G. H., O. B. Hesterman, E. A. Paul, S. E. Peters, and R. R. Janke. 1994. Fate of legume and fertilizer nitrogen-15 in a long-term cropping systems experiment. Agron. J. 86: 910-915.

Hauck, R. D. and J. M. Bremner 1976. Use of tracers for soil and fertilizer nitrogen research. Adv. Agron. 28: 219-266.

Hesterman, O. B., C. C. Scheaffer, D. K. Barnes, W. E. Lueschen, and J. H. Ford. 1986. Alfalfa dry matter and nitrogen production and fertilizer nitrogen response in legume-corn rotations. Agron. J. 78:19-23.

Holmes, W. E. and D. R. Zak. 1994. Soil microbial biomass dynamics and net nitrogen mineralization in northern hardwood forests. Soil Sci. Soc. Am. J. 58: 238-243.

Horwath, W. R., L.E. Elliot, J. J. Steiner, J. H. Davis, and S. M. Griffith. 1998. Denitrification in cultivated and non-cultivated riparian areas of grass cropping systems. J. Environ. Qual. 27: 225-231.

Horwath, W. R. and E. A. Paul. 1994. Microbial biomass. p. 753-773. *In* R. W. Weaver, J. S. Angl, and P. S. Bottomley (ed.) Methods of Soil Analysis. Part 2 Microbiological and Biochemical Properties. Soil Sci. Soc. Am. Book Series : 5. Madison, Wi.

Janzen, H. H. 1987. Soil organic matter characteristics after long-term cropping to various spring wheat rotations. Can. J. Soil Sci. 67: 845-856.

Janzen, H. H. and G. D. Radder. 1989. Nitrogen mineralization in a green manure-amended soil as influenced by cropping history and subsequent crop. Plant and Soil 120: 125-131.

Jenkinson, D. S., and D. S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol. Biochem. 8:209-213.

Jenny, H. 1980. The soil resource. Ecological studies, vol. 37. Springer-Verlag, New York.

Jha, D. K., G. D. Sharma, and R. R. Mishra. 1992. Soil microbial population numbers and enzyme activities in relation to altitude and forest degradation. Soil Biol. Biochem. 24:61-767.

Kai, H., Z. Ahmad, and T. Harada. 1973. Factors affecting immobilization and release of nitrogen in soil and chemical characteristics of the nitrogen newly immobilized. Soil Sci. Plant Nutr. 19: 275-286.

Katyal, J. C., S. P. Doshi, and P. K. Malhotra. 1985. Use of cluster analysis for classification of benchmark soil samples from India in different micronutrient availability groups. J. Agric. Sci., Camb. 104:421-424.

Keeney, D. R. and J. M. Bremner. 1966. Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. Agron. J. 58: 498-503.

Keeney, D. R. and J. M. Bremner. 1966. Characterization of mineralizable nitrogen in soil. Soil Sci. Soc. Am. Proc. 30: 714-719.

Keeney, D. R. and R. F. Follet. 1991. Managing nitrogen for groundwater quality and farm profitability: overview and introduction. Soil Sci. Soc. Amer., Madison WI.

Kelley, K. R. and F. J. Stevenson. 1985. Characterization and extractability of immobilized ^{15}N in soil microbial biomass. Soil Biol. Biochem. 17: 517-523.

Killham, K. and R. A. Rashid. 1986. Assay of activity of a soil deaminase. Plant Soil 92: 15-21.

Klein, T. M. and J. S. Koths. 1980. Urease, protease, and acid phosphatase in soil continuously cropped to corn by conventional and no-till methods. Soil Biol. Biochem. 12: 293-294.

Kowalenko, C. G. 1989. The fate of applied nitrogen in a Fraser Valley soil using ^{15}N in field microplots. Can J. Soil Sci. 69: 825-833.

Kowalenko, C. G. and J. W. Hall. 1987. Effects of nitrogen applications on direct-seeded broccoli from a single harvest adjusted for maturity. J. Amer. Soc. Hort. Sci. 112:9-13.

Kuo, M. H. and W. V. Bartholomew. 1966. On the genesis of organic nitrogen in decomposed plant residue. p. 329-335 *In* The Use of Isotopes in Soil Organic Matter Studies. Pergamon Press, London.

Ladd, J. N. and M. Amato. 1986. The fate of nitrogen from legume and fertilizer sources in soils successively cropped with wheat under field conditions. Soil Biol. Biochem. 18: 417-425.

Ladd, J. N., M. Amato, Zhou Li-Kai, and J. E. Schultz. 1994. Differential effects of rotation, plant residue and nitrogen fertilizer on microbial biomass and organic matter in an Austrsilian Alfisol. Soil Biol. Biochem. 26: 821-831.

Ladd, J. N. and J. H. A. Butler. 1972. Short-term assays of soil proteolytic enzyme activities using protein and dipeptide derivatives as substrates. Soil Biol. Biochem. 4:19-30.

Ladd, J. N., J. M. Oades, and M. Amato. 1981. Distribution and recovery of nitrogen from legume residues decomposing in soils sown to wheat in the field. Soil Biol. Biochem. 13: 251-256.

Larson, W. E., C. E. Clapp, W.H. Pierre, and Y.B. Morachan. 1972. Effects of increasing amounts of organic residues on continuous corn, II. Organic carbon, nitrogen, phosphorus, and sulphur. Agron. J. 64: 204-208.

Legg, J. O. and J. J. Meisinger. 1982. Soil nitrogen budgets. P. 503-566. *In* F. J. Stevenson, (ed.) Nitrogen in Agricultural Soils. Amer. Soc. Agron. Madison Wis.

Lethbridge, G. and M. S. Davidson. 1983. Microbial biomass as a source of nitrogen for cereals. Soil Bio. Biochem. 15: 375-376.

Magurran, A. E. 1988. Ecological diversity and its measurement. Princeton University Press. Princeton, NJ. 179 p.

McCarty, G. W., D. R. Shogren, and J. M. Bremner. 1992. Regulation of urease production in soil by microbial assimilation of nitrogen. Biol. Fertil. Soils 12: 261-264.

McCracken, D. V., S. J. Corak, M. S. Smith, W. W. Frye, and R. L. Blevins. 1989. Residual effects of nitrogen fertilization and winter cover cropping on nitrogen availability. Soil Sci. Soc. Am. J. 53:1459-1464.

McGill, W. B., K. R. Cannon, J. A. Robertson, and F. D. Cook. 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. Can. J. Soil Sci. 66:1-19.

Meints, V. W. and G. A. Peterson. 1977. The influence of cultivation on the distribution of nitrogen in soils of the Ustoll suborder. Soil Sci. 124:334-342.

Mitchell, W. H. and M. R. Teel. 1977. Winter-annual cover crops for no-tillage corn production. Agron. J. 69:569-573.

Muller, M. M. and V. Sundman. 1988. The fate of nitrogen (^{15}N) released from different plant materials during decomposition under field conditions. Plant and Soil 105: 133-139.

Myrold, D. D. 1987. Relationship between microbial biomass nitrogen and a nitrogen availability index. Soil Sci. Soc. Am. J. 51: 1047-1049.

Myrold, D. D. 1988. Denitrification in ryegrass and winter wheat cropping systems of western Oregon. Soil Sci. Soc. Am. J. 52: 412-416.

Nannipieri, P. F., L. Muccini, and C. Ciardi. 1983. Microbial biomass and enzyme activities; production and persistence. Soil Biol. Biochem. 15: 679-685.

National Research Council. 1989. Alternative Agriculture. National Academy Press. Washington, D.C.

Norman, R. J., J. C. Edberg, and J. W. Stucki. 1985. Determination of nitrate in soil extracts by dual-wavelength ultraviolet spectrophotometry. Soil Sci. Soc. Am. J. 49: 1182-1185.

Pancholy, S. K. and E. L. Rice. 1973. Soil enzymes in relation to old field succession: amylase, cellulase, invertase, dehydrogenase, and urease. Soil Sci. Soc. Am. Proc. 37:47-50.

Paul, E. A. and F. E. Clark. 1989. Soil microbiology and biochemistry. Academic Press, Inc., San Diego, CA.

Petit, G. 1988. Assessment of Oregon's groundwater for agricultural chemicals. Report to the State of Oregon, Department of Environmental Quality, Portland, Oregon.

Pieters, A. J. 1927. Green Manuring Principles and Practices. John Wiley and Sons, New York.

Pscheidt, J. W., J. Z. Burket, S. L. Fischer, and P. B. Ham. 1992. Sensitivity and clinical use of *Phytophthora*- specific immunoassay kits. Plant Dis. 76: 928-932.

Raimbault, B. A., T. J. Vyn, and M. Tollenaar. 1991. Corn response to rye cover crop, tillage methods, and planter options. Agron. J. 83:287-290.

Rassmussen, P. E. and H. P. Collins. 1991. Long-term impacts of tillage, fertilizer, and crop residue on soil organic matter in temperate semiarid regions. Adv. Agron. 45:93-134.

Romesburg, C. A. 1984. Cluster Analysis for Researchers. Lifetime Learning Publications, Belmont, CA.

Rosswall, T. and K. Paustian. 1984. Cycling of nitrogen in modern agricultural systems. Plant and Soil 76: 3-21.

SAS Institute, Inc. 1985. SAS user's guide: Statistics. SAS Institute, Inc., Cary, NC.

SAS Institute, Inc. 1990. SAS user's guide: Statistics. SAS Institute, Inc., Cary NC.

Schnitzer, M. 1977. Recent findings on the characterization of humic substances extracted from soils from widely differing climatic zones. *In* Organic Matter Studies, vol. II. Proceedings of a symposium. International Atomic Energy Agency, Vienna. pp. 117-132.

Schnürer, J., M. Clarholm, and T. Rosswall. 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents. Soil Bio. Biochem. 17: 611-618.

Schnürer, J. and T. Rosswall. 1982. Flourescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl. Environ. Micro. 43:1256-1261.

Segal, W. and R. L. Mancinelli. 1987. Extent of regeneration of the microbial community in reclaimed spent oil shale land. J. Environ. Qual. 16: 44-48.

Sheaffer, C. C., M. P. Russelle, G. H. Heichel, M. M. Hall, and F. F. Thicke. 1991. Non-harvested forage legumes: nitrogen and dry matter yields and effects on a subsequent corn crop. J. Prod. Agric. 4:520-525.

Shen, S. M., G. Pruden, and D. S. Jenkinson. 1984. Mineralization and immobilization of nitrogen in fumigated soil and the measurement of microbial biomass nitrogen. Soil Biol. Biochem. 16: 437-444.

Singh, H. 1995. Nitrogen mineralization, microbial biomass and crop yield as affected by wheat residue placement and fertilizer in a semi-arid tropical soil with minimum tillage. J. of Appl. Ecol. 32: 588-595.

Sisworo, W. H., M. M. Mitrosuhardjo, H. Rasjid, and R. J. K. Myers. 1990. The relative roles of N fixation, fertilizer, crop residues and soil in supplying N in multiple cropping systems in a humid, tropical upland cropping system. Plant and Soil 121: 73-82.

Smith, S. J. and L. B. Young. 1975. Distribution of nitrogen forms in virgin and cultivated soils. Soil Sci. 120: 354-360.

Smith, S. J., F. W. Chichester, and D. E. Kissel. 1978. Residual forms of fertilizer nitrogen in field soils. Soil Sci. 125: 165-169.

Soderstrom, B., E. Baath, and B. Lundgren. 1983. Decrease in soil microbial activity and biomasses owing to nitrogen amendments. Can. J. Micro. 29: 1500-1506.

Sorensen, P. and E. S. Jensen. 1991. Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene trap for ^{15}N determination. Anal. Chim. Acta 252: 201-203.

Sparling, G. P. and P. L. Searle. 1993. Dimethyl sulphoxide reduction as a sensitive indicator of microbial activity in soil: the relationship with microbial biomass and mineralization of nitrogen and sulfur. Soil Biol. Biochem. 25: 251-256.

Stanford, G. 1982. Assessment of soil nitrogen availability. p. 651-688. *In* F. J. Stevenson (ed.) Nitrogen in agricultural soils. No. 22. Amer. Soc. Agron., Madison, WI.

Stanford, G. and S. J. Smith. 1972. Nitrogen mineralization potentials of soils. Soil Sci. Soc. Am. J. 36: 465-472.

Staver, K. W. and R. B. Brinsfield. 1990. Patterns of soil nitrate availability in corn production systems: Implications for reducing groundwater contamination. J. Soil and Water Cons. 45:318-32.

Stevenson, F. J. 1956. Effect of some long-time rotations on the amino acid composition of the soil. Soil Sci. Soc. Proc. 20: 204-208.

Stevenson, F. J. 1994. Humus Chemistry. John Wiley & Sons, New York.

Stewart, B. A., D. D. Johnson, and L. K. Porter. 1963. The availability of fertilizer nitrogen immobilized during decomposition of straw. Soil Sci. Soc. Am. Pro. 27: 656-659.

Tabatabai, M. A. 1994. Soil enzymes. p. 775-833. *In* R. W. Weaver, J. S. Angle, and P. J. Bottomley (ed.) Methods of soil analysis, Part 2. Microbiological and biochemical properties. Soil Sci. Soc. Amer. book series, no 5. Madison, WI.

Torsvik, V., K. Salte, R. Sorheim, and J. Goksoyr. 1990. Comparison of phenotypic diversity and DNA heterogeneity in population of soil bacteria. Appl. Environ. Microbiol. 56: 776-781.

Touchton, J. T., W. A. Gardner, W. L. Hargrove, and R. R. Duncan. 1982. Reseeding crimson clover a N source for no-tillage grain sorghum production. Agron. J. 74: 283-287.

Van Veen, J. A., J. N. Ladd, and M. J. Frissel. 1984. Modelling C and N turnover through the microbial biomass in soil. Plant and Soil 76: 257-274.

Voroney, R. P. and E. A. Paul. 1984. Determination of k_c and k_n *in situ* for calibration of the chloroform fumigation incubation method. Soil Biol. Biochem. 16: 9-14.

Wagger, M. G., D. E. Kissel, and S. J. Smith. 1985. Mineralization of nitrogen from nitrogen-15 labeled crop residues under field conditions. Soil Sci. Soc. Am. J. 49: 1220-1226.

Wassel, R. A. and A. L. Mills. 1983. Changes in water and sediment bacterial community structure in a lake receiving acid mine drainage. Microb. Ecol. 9:155-169.

Westerman, R. L. and L. T. Kurtz. 1972. Residual effects of ^{15}N fertilizers in a field study. Soil Sci. Soc. Am. Pro. 36: 91-94.

Winding, A. 1994. Fingerprinting bacterial soil communities using Biolog microtitre plates. *In* K. Ritz, J. Dighton, and K. E. Giller (eds.) Beyond the biomass. British Society of Soil Science, Wiley-Sayce Pub. pp. 85-94.

Wyland, L. J., L. E. Jackson, and K. F. Schulbach. 1995. Soil-plant dynamics following incorporation of a mature rye cover crop in a lettuce production system. J. Agric. Sci. 124:17-25.

Zak, J. C., M. R. Willig, D. L. Moorhead, and H. G. Wildman. 1994. Functional diversity of microbial communities: a quantitative approach. Soil Biol Biochem 26: 1101-1108.

Zielke, R. C. and D. R. Christenson. 1986. Organic carbon and nitrogen changes in soil under selected cropping systems. Soil Sci. Soc. Am. J. 50: 363-367.