RESEARCH SUB-PROGRAM

DEVELOPMENT OF STANDARD METHODOLOGIES: BIO-INDICATORS AND METHOD0LOGIES TO QUANTIFY SOIL QUALITY

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FORWARD

This report is one of a series of **COESA** (Canada-Ontario Environmental Sustainability Accord) reports from the Research Sub-Program of the Canada-Ontario Green Plan. The **GREEN PLAN** agreement, signed Sept. 21, 1992, is an equally-shared Canada-Ontario program totalling $64.2 M, to be delivered over a five-year period starting April 1, 1992 and ending March 31, 1997. It is designed to encourage and assist farmers with the implementation of appropriate farm management practices within the framework of environmentally sustainable agriculture. The Federal component will be delivered by Agriculture and Agri-Food Canada and the Ontario component will be delivered by the Ontario Ministry of Agriculture and Food and Rural Assistance.

From the 30 recommendations crafted at the Kempenfelt Stakeholders conference (Barrie, October 1991), the Agreement Management Committee (AMC) identified nine program areas for Green Plan activities of which the three comprising research activities are (with Team Leaders):

1. **Manure/Nutrient Management and Utilization of Biodegradable Organic Wastes** through land application, with emphasis on water quality implications
   - **A.** Animal Manure Management (nutrients and bacteria)
   - **B.** Biodegradable organic urban waste application on agricultural lands (closed loop recycling) (Dr. Bruce T. Bowman, Pest Management Research Centre, London, ONT)

2. **On-Farm Research**: Tillage and crop management in a sustainable agriculture system. (Dr. Al Hamill, Harrow Research Station, Harrow, ONT)

3. **Development of an integrated monitoring capability** to track and diagnose aspects of resource quality and sustainability. (Dr. Bruce MacDonald, Centre for Land and Biological Resource Research, Guelph, ONT)

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This Research Sub-Program is being managed by the Pest Management Research Centre, Agriculture and Agri-Food Canada, 1391 Sandford St., London, ONT. N5V 4T3.

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DEVELOPMENT OF STANDARD METHODOLOGIES: BIO-INDICATORS AND METHOD0LOGIES TO QUANTIFY SOIL QUALITY

Final Report Canada-Ontario Green Plan

January 1998

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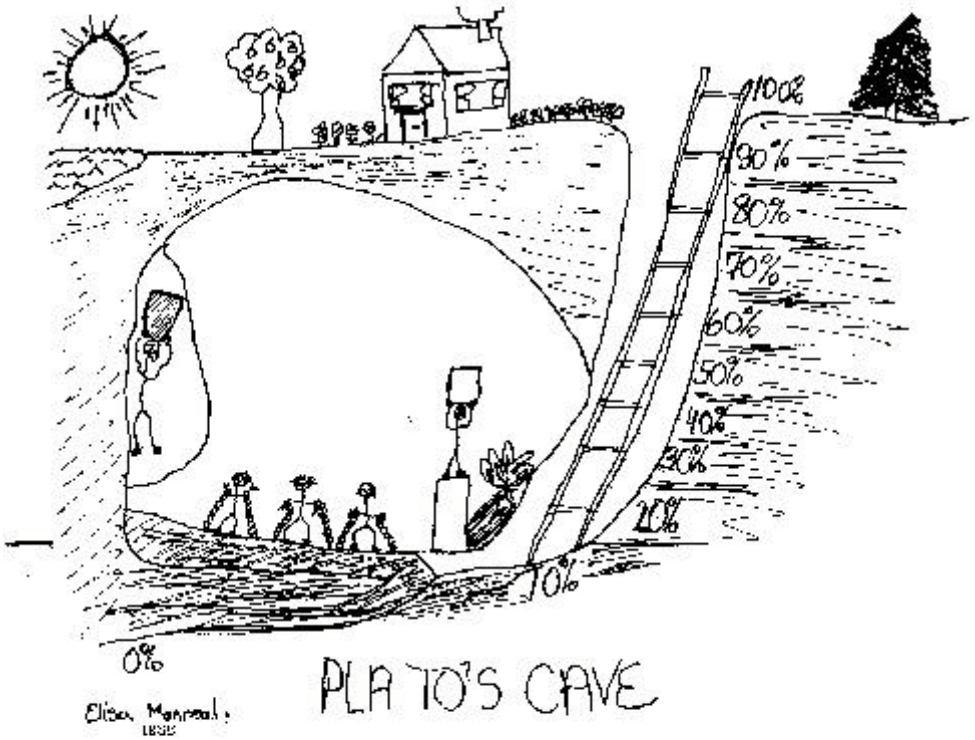
To those who have taken care of the land,
since the beginning...



Building Soil Health at Plato's Cave

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EXECUTIVE SUMMARY

The objectives of this research were to:

1) Develop methodologies for assessing soil health using indicators of soil organic matter (SOM) quality, and to inform the public on the impacts of farming practices on soils.
2) Characterize the variabilities of soil health indicators, and to determine the influence of management practices on their random variability.
3) Establish quantitative relationships between soil indicators with soil attributes of soil qality in soil agro-ecosystems. The tested indicators were soil enzymes, lipids and light fraction of SOM. This report emphasizes information on soil enzymes and lipids as indicators of soil health relative to that of the light fraction of soil organic matter (SOM).

Soil mineralizing enzymes transform soil organic matter and provide essential nutrients for the growth of crops and soil organisms. Soil lipids help form stable aggregates and influence the structural support for rooth growth. Establishing quantitative methodologies to use in assessment of soil health requires knowledge on how soil enzymes and lipids are influenced by farming practices in soil landscapes. Methodologies presented in this report will assist farmers in defining the state of soil nutrient mineralization power and the structural stability of soils; and will indicate when to implement changes in farming practices once changes in nutrient mineralization power are detected. Assessment of soil health also informs policy makers and the public on the current state of soils and the sustainability of crop production systems.

The 3 yr research was conducted on four farms with differing soils, climate and crop production systems. At two of these sites (Rockwood and Clinton) there has been significant amount of other information collected. Soil lipids and six nutrient mineralizing enzymes ($-glucosidase, dehydrogenase, L-glutaminase, urease, alkaline phosphatase and arylsulfatase) were sensitive to the effects of land use, tillage system, manure and fertilizer application. Some soil enzymes (i.e. dehydrogenase) behaved as early indicators of SOM accumulation by expressing changes before soil organic carbon (OC) content showed any similar change. Other soil enzymes (i.e. urease, alkaline phosphatase and arylsulfatase) correlated with OC

content and behaved as surrogate measurements of SOM. Crop growth and slope position influenced soil enzyme activities and lipid content. These factors need to be considered when assessing the functions of soil nutrient mineralization and structural stability.

In earlier studies conducted *in vitro*, pure cultures of organisms showed that the six tested soil enzymes control key processes of mineralization in the C, N, P and S cycles. This project tested specifically for the ecological function of enzymes and N mineralization. Soil L-glutaminase, dehydrogenase, and to a lesser extent urease, were associated with the power of soils to mineralize N. Simple linear models predicted the N mineralization power of soils based on enzyme activity level. Seven enzyme activity levels defined for each of the six enzymes were the same in all soils and ranged from very low to very high. Overall, very high levels of enzyme activity, lipid content and soil structural stability were found in undisturbed soils under forest, sod and zero-tillage. Soils cropped with conventional tillage always presented medium to low enzyme activity levels and low soil structural stability.

This report presents two methods for monitoring and evaluating the fuctions of soil nutrient mineralization and structural stability. Quality control charts (QCC) were used succesfully for monitoring large and small shifts in the activities of enzymes asociated with N mineralization power. A method using principal component analysis (PCA) integrated soil chemical and physical properties into a vectorial scale defining the relative status of soil structure stability. Both methods consistently represented the effects of different management systems on nutrient mineralization and structural stability in landscapes of soils with different pedogenesis. We predicate that high enzyme activity level and soil structural stability represent a high state of soil health. Conversely, low soil health is associated with soils having both low soil enzyme activity level and structural stability.

Sommaire

La recherche visait les objectifs suivants :

1) Élaborer des méthodes d'évaluation de la santé des sols à partir d'indicateurs de la qualité des matières organiques des sols (MOS) et informer le public des effets des pratiques agricoles sur les sols.
2) Caractériser la variabilité des indicateurs de santé des sols et déterminer l'influence des pratiques de gestion sur leur variabilité aléatoire.
3) Établir des relations quantitatives entre les indicateurs de santé des sols et les attributs servant à la détermination de la qualité des sols dans les agro-écosystèmes. Les indicateurs étudiés étaient les enzymes et les lipides des sols et la fraction légère des MOS.

Ce rapport fournit de l'information principalement sur les enzymes et les lipides des sols en tant qu'indicateurs de la santé des sols par rapport à celle de la fraction légère des MOS.

Les enzymes minéralisantes transforment les matières organiques des sols et fournissent les substances nutritives essentielles à la croissance des végétaux et des organismes vivant dans le sol. Les lipides des sols contribuent à former des agrégats stables et jouent un rôle dans le soutien structural nécessaire au développement racinaire. Pour élaborer des méthodes devant servir à évaluer la santé des sols, il faut savoir de quelle façon les pratiques agricoles influent sur les enzymes et les lipides dans les paysages pédologiques. Les méthodes présentées dans ce rapport aideront les agriculteurs à déterminer la puissance de minéralisation des substances nutritives du sol et la stabilité structurale du sol. De plus, elles indiqueront quand modifier les pratiques agricoles une fois que des modifications de cette puissance de minéralisation seront décelées. L'évaluation de la santé des sols permet également d'informer les décideurs et le public sur l'état des sols et le caractère durable des systèmes culturaux.

Les recherches, qui ont duré trois ans, ont été menées dans quatre exploitations agricoles caractérisées par des sols, un climat et des systèmes culturaux différents. À deux des quatre endroits (Rockwood et Clinton), une grande quantité d'autres données ont été recueillies. Les lipides des sols et six enzymes minéralisantes des substances nutritives ($-glucosidase, déshydrogénase, L-glutaminase, uréase, phosphatase alcaline et arylsulfatase) étaient sensibles aux effets de l'utilisation des terres et au système de travail du sol ainsi qu'à l'épandage du fumier et des engrais. Certaines enzymes, comme la déshydrogénase, signalaient l'accumulation des MOS en indiquant les changements avant que le carbone organique du sol n'en fasse autant. On a établi une corrélation entre la teneur en carbone organique et d'autres enzymes (c.-à-d. l'uréase, la phosphatase

alcaline et l'arylsulfatase), et ces dernières constituaient des indicateurs de substitution des MOS. La croissance des cultures et la position des pentes influaient sur les activités des enzymes du sol et la teneur en lipides. Il faut tenir compte de ces facteurs quand on évalue les fonctions de minéralisation des substances nutritives et de stabilité structurale des sols.

Lors d'études in vitro antérieures, des cultures pures d'organismes ont montré que les six enzymes des sols étudiées contrôlent les processus clés de minéralisation dans les cycles du carbone, de l'azote, du phosphore et du soufre. Les travaux qui nous occupent visaient spécialement à examiner la fonction écologique des enzymes et la minéralisation de l'azote. La L-glutaminase, la déshydrogénase et, dans une moindre mesure, l'uréase influaient sur la puissance de minéralisation de l'azote par les sols. On a utilisé des modèles linéaires simples pour prévoir cette puissance en fonction du niveau d'activité enzymatique. Les sept niveaux d'activité établis pour chacune des six enzymes étaient les mêmes dans tous les sols et allaient de très faible à très élevé. En général, les niveaux très élevés d'activité enzymatique ont été mesurés dans les sols forestiers non perturbés, les sols engazonnés et les sols cultivés sans labour. Les sols cultivés par des méthodes de labour traditionnelles présentaient toujours des niveaux d'activité enzymatique moyens à faibles et une faible stabilité structurale.

Ce rapport présente deux méthodes de surveillance et d'évaluation des fonctions de minéralisation des substances nutritives et de stabilité structurale des sols. Des tables de contrôle de qualité se sont révélées efficaces pour la surveillance des petites et des grandes variations de l'activité enzymatique liées à la puissance de minéralisation de l'azote. Une méthode des composantes principales intégrait les propriétés chimiques et physiques des sols à une échelle vectorielle définissant l'état relatif de la stabilité structurale des sols. Les deux méthodes représentaient constamment les effets de différents systèmes de gestion sur la minéralisation des substances nutritives et la stabilité structurale dans des paysages pédologiques caractérisés par différentes pédogenèses. Il ressort que l'état de santé des sols est directement proportionnel à l'activité enzymatique et à leur stabilité structurale. Inversement, les sols présentant un piètre état de santé sont caractérisés par une activité enzymatique et une stabilité structurale faibles.

Chapter 1

GENERAL INTRODUCTION

1.1 Rationale and objectives

Soil quality may be defined as "the fitness of a specific kind of soil, to function within its capacity and within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Karlen et al., 1997). At present there are, however, no quantitative methods to determine soil fitness (quality) at the farm or regional level. The absence of methodologies to evaluate soil health is in part associated with a lack of understanding the soil ecological processes vital in sustaining plant growth and environmental quality (Hatfield and Stewart, 1994).

Soil components may be used as indicators of land health if they control agroecological processes important for plant growth and respond rapidly to anthropogenic disturbances. Soil enzymes, which are components of SOM, influence the release of nutrients necessary for plant and microbial growth, the gas exchange between soils and the atmosphere, and other properties of soils (Skujins, 1978; Conrad et al., 1978; Martens et al., 1992). Soil enzymes controlling processes of nutrient mineralization, and soil lipids involved in the stabilization of soil structure may be useful indicators to evaluate the effects of soil management on soil health (Monreal et al., 1998).

Soil enzymes playing a central role in the mineralization of organic C, N, S and P, and energy flow through microorganisms and terrestrial ecosystems may be usefull indicators of health. Measurement of soil dehydrogenase activity provides information of *in-situ* respiration or oxidative activity of soil organisms (Schaefer, 1963; Casida et al., 1968). The enzyme $-glucosidase is important to the C cycle. $-glucosidase provides important C and energy sources for the growth of soil microbes by hydrolyzing polymers (i.e. cellobiose and maltose) existing in plant residues (Hayano and Katami, 1977; Hayano and Tubaki, 1985). Alkaline phosphatase control the mineralization of soil esters of P (Speir and Ross, 1978), and arylsulfatase that of organic esters of S (Castellano and Dyck, 1991), to produce plant available inorganic phosphate and sulfate, respectively. The enzymes L-glutaminase and urease are central regulators of the N cycle (Prusiner, 1973; Rotini, 1935). These two enzymes produce available ammonium for plant growth during the microbial

decomposition of aliphatic and aromatic N compounds in soil organic matter (SOM). L-glutaminase produces ammonium through deamination of L-glutamine (Prusiner, 1973). Urease hydrolyzes urea to NH_3 and CO_2 (Gorin, 1959). Molecular regulatory mechanisms for the latter two and other enzymes have been established (i.e. Prusiner and Stadtman, 1973; Gorin, 1959; Blakeley et al., 1969; Mandesltam and McQuillen). Methods of enzyme activity measurements and potential interferences are discussed in detail by Tabatabai (1982).

Soil lipids in SOM are known to influence the stability of soil aggregates (Capriel et al., 1990; Dinel et al., 1992; Monreal et al., 1995). Soil lipid fractions extractable with diethyl ether (DEE) and chloroform ($CHCl_3$) together with water stable aggregates have been used to asess the structural stability of soils (Dinel et al., 1997). The status of soil structure may be indicated by measuring aggregate stability and the non-limiting water range (Gregorich et al., 1994; Topp et al., 1995). The use of soil enzymes and lipids as functional indicators of health require characterizing their variabilities in soil pedons and landscapes (Monreal et al., 1998).

Within this context, this project had the following objectives:

1. Develop, test and adapt methodologies to examine the use of soil enzymes, lipids and light fraction of SOM as indicators of soil fitness, current agro-ecological status and to inform the public on the impacts of management on the soil resource.

2. Characterize the normal or random variation of soil enzymes, lipids and light fraction and separate such random variation from that associated with management practices.

3. Establish quantitative relationships between soil enzyme properties, lipids and light fraction with other soil attributes that relate to quality in soil agro-ecosystems.

1.2 Addressing the Project's Objectives

The field component of this research was carried out on four farm sites in Ontario. Soil samples were also collected at a farm near Stayner, ON, and subsequently used in a laboratory incubation experiment. Summaries of field studies are presented in two chapters, based on objectives outlined in the General Introduction. Chapter 2 (sections 2.1-2.3) deals with effects of

management on soil enzyme activities, and addresses objective 1. The results from the Clinton and Rockwood sites were combined to provide a wider scope for evaluation of results. Chapter 3 (sections 3.1 and 3.2) deals with measurement of temporal and spatial variation of soil enzyme activities, and addresses objective 2. Chapters 4 and 5 address that part of objective 1 concerning future soil health assessments. Together they present methodologies for evaluating basic functions of nutrient mineralization and stability of soil structure. Objective 3, that of establishing quantitative relationships between soil enzyme activities and other soil attributes, is addressed in the interpretation of results of individual studies in Chapters 2 and 3, and in the methodologies presented in Chapters 4 and 5. The light fraction did not associate with any other measured soil property and thus was mentioned very briefly in Chapter 3. Chapter 6 is a brief synthesis of the main findings of individual studies, and identification of future research needs in assessing the health of N cycle. All measurements made during the study are presented in the Appendix.

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Chapter 2

EFFECTS OF MANAGEMENT ON SOIL ENZYME ACTIVITIES

2.1 Sensitivity of Soil Enzyme Activities To Conservation Practices[1]

D.W. Bergstrom, C.M. Monreal, D.J. King, A. Tomlin and J. Miller

2.1.1 ABSTRACT

There is a need to assess change in soil quality resulting from introduction of conservation practices. This study tested for an effect of tillage practice and crop rotation on activity of six soil enzymes (dehydrogenase, urease, glutaminase, phosphatase, arylsulfatase, and $-glucosidase). Samples of the Ap horizon were collected from adjacent no-till and tilled fields. At one site, fields were located on a simple, single slope averaging 4%, and differed in previous cropping history. The second site included coarse- and fine-textured soils at lower and upper slope-positions, respectively. Enzyme activities of field-moist samples were measured over two growing seasons, and values of V_{max} were estimated for three enzymes on a subset of air-dry samples. At the first site implementation of no-till and previous cropping to forages increased activity of all enzymes. At the second site, there was no consistent response of enzyme activities to tillage practice in the coarse-textured soils which had relatively large organic matter contents. In the fine-textured soil, activity of phosphatase and arylsulfatase, and dehydrogenase in the surface (0-8 cm) layer, was greater in the no-till field. At this location, these enzyme activities may have been more sensitive than total C content to tillage practice. Alternatively, they may have been indices of some property other than soil organic matter content. Slope-position, and time and depth of sampling influenced enzyme activities, and affected management comparisons.

2.1.2 INTRODUCTION

There is a need to assess soil quality in development of sustainable agricultural systems. The soil attribute measured

[1]A version of this Chapter has been accepted for publication by the Soil Science Society of America Journal (1998).

depends upon the soil function of interest. Warkentin (1995) suggested that soil quality be defined in terms of the unique ecosystem functions performed by soil, one of which is the cycling of nutrients and C. He indicated that soil enzyme activities can be measured to assess this function. Likewise, Kennedy and Papendick (1995) considered soil enzyme activities as one of numerous measurements of soil microbial quality. Although crude in comparison with molecular methods, soil enzyme activities are easily measured, with reaction rate indicating the amount of enzyme present (Galstian, 1974). Moreover, soil enzyme activities are sensitive indicators of management-induced changes in soil properties, although in most instances their use has been limited to sites at which comparisons can be made between management practices, or within a chrono- or topo-sequence (Dick, 1992). At the same time, in most field studies the ecological meaning of soil enzyme activities has not been clearly defined. Rather, they have been used as a surrogate measure of soil organic matter (SOM) content, or interpreted based on reactions at the scale of the microbial cell.

Tillage is one management practice in crop production that affects soil quality. Decrease in SOM content resulting from excessive tillage reduces fitness of soil for crop production. Hence, farmers are shifting to management practices with reduced or no tillage, and there is a need to assess the change in soil quality that results from this change in management.

There is a strong relationship between SOM content and enzyme activities (e.g. Garcia et al., 1994). Both parameters are influenced by cultivation (Kennedy and Smith, 1995; Farrell et al., 1994). We hypothesized that enzyme activities would respond to decreased tillage in a manner similar to SOM content (Karlen et al., 1994; Alvarez et al., 1995). At sites where no-till is imposed following years of conventional tillage, we expected enzyme activities of the surface layer of the Ap horizon to increase. Conversely, we expected enzyme activities of the lower layer of the Ap horizon to either remain the same or decrease. On farms, soil enzyme activities are also affected by other agronomic practices such as crop rotation, and show spatial and temporal variability. The magnitude of tillage-induced changes in enzyme activities, relative to variation due to these other factors, needs to be assessed.

The objective of this study was to determine whether conservation practices (no-till and crop rotation) affected six different soil enzyme activities at two sites. Effects of management on enzyme activities were measured against other sources of variation (time and depth of sampling, and slope).

2.1.3 MATERIALS AND METHODS

2.1.3.1 Site Description and Soil Sampling Procedures

The first site near Rockwood, Ontario (hereafter referred to as Rockwood) was a loam (Gray Brown Luvisol) on a simple, single slope averaging 4%. Samples were collected from two fields: a no-till field uncultivated since 1987, and previously cropped to forages including alfalfa (*Medicago sativa* L.) and to corn (*Zea mays* L.); and a conventionally tilled field previously cropped to corn for 12 y. The fields had the same rotation of corn-soybeans (*Glycine max* L.)-winter wheat (*Triticum aestivum* L.) since 1991. This site provided a comparison of tillage and cropping history together, and the effect of one could not be assessed independent of the other.

Samples of the Ap horizon were collected from strips (25 by 220 m) in each field planted to soybeans in 1994 and winter wheat in 1995. Each field was sampled at 3 slope positions: shoulder, mid-slope and foot-slope. For each slope position, a composite sample of 4 cores was collected at each of 4 locations across the slope. Sampling locations were separated by a distance of 4 m; cores for each composite sample were collected within 1 m. Soil samples were collected on April 28, and August 4 and 7 in 1994. In 1995 sampling dates (May 2, June 6 and 28, and August 7) were chosen to coincide with phenological stages of the wheat crop (early growth, tillering, grain-filling and post-harvest, respectively).

The second site was located on a farm near Clinton, Ontario (hereafter referred to as Clinton). The field was divided into areas of no-till (uncultivated since 1981) and conventional tillage along a slope with soils of different textural classes. Fine-textured soils occurred on the upper part of the slope and coarse-textured soils occurred on the lower part of the slope. The site had been cropped to corn and soybeans since 1981, with one year of winter wheat and of oats (*Avena sativa* L.). It was cropped to soybeans in 1994 and oats in 1995.

Soil samples were collected at 4 positions in the field along the textural gradient--2 positions on fine-textured soil, and 2 positions on coarse-textured soils (Table 2.1). At each position, 4 soil cores were collected from each of the no-till and conventionally tilled areas. Soil samples were collected on May 13, June 13, July 11 and August 12 in 1994. In 1995 sampling dates (April 11, June 1, July 5 and August 18) were chosen to coincide with phenological stages of the oat crop (shortly after seeding, tillering, grain-filling and post-harvest, respectively).

At both sites, soil samples were collected at 2 depths (0-8 cm, and 8 cm to the bottom of the Ap horizon) using Oakfield split-tube samplers (id 2 cm). Samples were shipped to the laboratory in a cooler with cold-packs, and held at approx. 5EC until analysis.

2.1.3.2 Soil Analyses

Soil samples were broken-up by hand and thoroughly mixed before analysis. Water content was determined gravimetrically after drying at 105EC for 24 h. Dehydrogenase, urease (urea amidohydrolase, EC 3.5.1.5), alkaline (pH 11) phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1), $-glucosidase (EC 3.2.1.21) and arylsulfatase (arylsulfate sulfohydrolase, EC 3.1.6.1) activities were determined as described by Tabatabai (1982), with minor modifications as follows.

Dehydrogenase activity was measured for 1.5 g moist soil. Approximately 0.02 g $CaCO_3$ was added to each sample. Sufficient water (1.5 mL) was added to cover the soil in the centrifuge tubes used for the assay, and thereby provide a relatively uniform barrier against diffusion of O_2 among samples. In order to maintain the same concentration of substrate as in the standard method, 0.6 mL 2,3,5-triphenyltetrazolium chloride (3% w/v) was added to the tubes. The reaction was stopped by addition of 10 mL methanol. Tubes were subsequently centrifuged, and the supernatant was decanted for colorimetric measurement of triphenyl formazan (TPF). Reaction rates were adjusted for adsorption of TPF by soil.

For the phosphatase, arylsulfatase and $-glucosidase assays, 0.1 *M* *tris*(hydroxymethyl)aminomethane (TRIS, pH 12) was added to stop the reaction and extract -nitrophenol. Toluene was not added for the phosphatase, arylsulfatase and $-glucosidase assays because initial tests showed that it did not affect activity. Because there was substantial phosphatase activity in all samples, 0.5 g soil was used for the phosphatase assay. For samples with very high phosphatase activity, the incubation time was reduced to 30 min.

Glutaminase (L-glutamine amidohydrolase, EC 3.5.1.2) activity was determined by the method of Frankenberger and Tabatabai (1991). For both the urease and glutaminase assays, the reaction was stopped by addition of 20 mL KCl(3 *M*)-Ag_2SO_4 (100 ppm) solution (final volume 30 mL). The initial NH_4^+ content of each soil sample was determined and subtracted from the amount of NH_4^+ produced during the urease and glutaminase assays. Ammonium was extracted from moist soil (5 g) with 30 mL

2 *M* KCl. The 6:1 (solution:soil) ratio was comparable to the extraction ratio of the urease and glutaminase assays. Extract and assay solutions were filtered, then frozen until analysis of NH_4^+ content.

Kinetic analyses for determination of V_{max} were carried out with air-dried (<2 mm) soil samples from one date at Rockwood (1 replicate, n = 12), and two dates (combined) at Clinton (n = 16). Urease, phosphatase and $-glucosidase activities were measured in duplicate over an appropriate range of substrate concentrations, and kinetic parameters were estimated by fitting the Michaelis-Menten equation

$$v = (V_{max} * S)/(K_m + S) \qquad (1)$$

to data, where v is initial reaction velocity, S is initial substrate concentration, V_{max} is maximum reaction velocity and K_m is the Michaelis-Menten constant. For soils, V_{max} is an indirect measure of enzyme content. Total C content was also determined on the samples used for kinetic analyses (duplicate analyses per sample). Particle size was determined on a separate set of air-dry (<2 mm) soil samples by the pipette method (McKeague, 1978).

Colorimetric measurements were made with a Perkin-Elmer Lambda 3 UV/VIS Spectrophotometer. Ammonium content of extracts of enzyme assay mixtures was determined by steam distillation or NH_4^+-electrode (Keeney and Nelson, 1982). These methods were used because buffers added to the assay mixture apparently interfered with automated measurement using the indophenol blue method. Ammonium content of 2 *M* KCl extracts was determined with a TRAACS 800 autoanalyzer, using Technicon Industrial Method No. 780-86T. Total C content was determined by a LECO dry combustion method (Nelson and Sommers, 1982). All data were expressed based on the oven-dry weight of soil.

2.1.3.3 Statistical Analysis and Data Presentation

At both sites, there were two fields--one with and one without tillage. Within each field, we sampled at replicated locations that represented slope-positions (Rockwood) or soil textural classes (Clinton). As is common for soils experiments located on farms, the design did not group experimental units into blocks, and lacked randomization. Constraints on analysis of data from such a sampling scheme are similar to those discussed by Wardle (1994) and Reganold (1994) for comparison of soil quality of two types of farm. As well, at Clinton soil texture was confounded with slope. Preliminary analysis of data from Clinton indicated large differences in some enzyme activities between soil textural classes, resulting in skewed frequency

distributions. For this reason, the two textural classes were analyzed separately. Data were analyzed using the Multivariate General Linear Hypothesis (MGLH) module of SYSTAT (Wilkinson, 1988a), with sampling dates as repeated measures (Hall, 1994). We combined data from both years at each site. Differences in crop between the two years was one, but not the only, source of temporal variation. Because our sampling dates were restricted to two growing seasons with different crops, our experimental design was not suitable for assessing variation over yearly intervals. When necessary, data were transformed for homogeneity of variance.

Kinetic constants were estimated using the Nonlinear Estimation (NONLIN) module of SYSTAT. Values of V_{max} for the three enzyme activities, and total C contents for management treatments were compared at the same depth and slope-position at Rockwood, and the same depth and position along the soil texture gradient at Clinton, using 95% confidence limits based on the *t* distribution.

Notched box plots (SYGRAPH, Wilkinson, 1988b) provided a visual comparison of enzyme activities between management treatments at the two sites. These plots indicated both the central tendency and distribution of data. The upper and lower edges of the central box define the upper and lower limits of the interquartile range (midrange). The vertical lines above and below the box (inner fences) extend 1.5 times the midrange above and below the edges of the box. Values lying outside this inner fence are plotted with asterisks; far outside values are plotted with circles. The boxes are notched at the median, and return to full width at the upper and lower confidence interval values. When the confidence intervals around two medians do not overlap, the two population medians are different at approximately the 95% level. The mean values of untransformed data were plotted as triangles.

2.1.4 RESULTS

Data analysis primarily tested for differences in enzyme activities between management practices, and identified interactions in which other factors influenced this comparison. The comparison of management practices was affected by some but not all of the interactions which involved management. The interactions and other sources of variation in enzyme activities (slope, and sampling depth and date) were not discussed in detail.

2.1.4.1 Effect of Tillage and Cropping History at Rockwood

At Rockwood, differences in enzyme activities between the two fields were attributed to management as inclusive of both tillage practice and cropping history. Management accounted for most of the variation in all six enzyme activities (25-48% of total sum of squares [%SS]; Table 2.2). On average, all enzyme activities were greater in the no-till field than in the tilled field (Fig. 2.1). Slope affected activity of four enzymes--dehydrogenase, urease, phosphatase and arylsulfatase. There was temporal variation in all six enzyme activities (Table 2.2), with dehydrogenase and phosphatase most strongly affected (Fig. 2.2a).

The interaction between sampling date and management for urease, glutaminase, phosphatase and arylsulfatase (Table 2.2) indicated that the temporal patterns of activity in each field were not exactly alike. Thus, there were larger differences in activity between the two fields on some dates than on others. For dehydrogenase and $-glucosidase, both sampling depth and date influenced the management comparison. For example, on the fifth sampling date, dehydrogenase activity was unexpectedly greater in the surface layer of the tilled field, relative to the no-till field. It is noteworthy that on this sampling date, the no-till field was considerably wetter than the tilled field (30 and 20% gravimetric water content, respectively). Slope affected the management comparison on some dates for arylsulfatase activity. For example, on the third sampling date, activity was greater in the no-till field than in the tilled field at the foot-slope and mid-slope positions, but not at the shoulder.

Values of V_{max} and total C content for the two management treatments were compared at the same depth and slope-position (Table 2.3). For urease, V_{max} was greater for the no-till field than for the tilled field, with larger differences at the shoulder and foot-slope positions. Values of V_{max} of phosphatase were greater for the no-till field than for the tilled field at the shoulder and foot-slope positions, but not at the mid-slope. For $-glucosidase, V_{max} was greater for the no-till field than for the tilled field at the shoulder position, and in the lower depth at the mid-slope and foot-slope positions. Total C content was greater in the no-till field than in the tilled field at the shoulder position, and in the surface layer at the foot-slope position.

2.1.4.2 Effect of Tillage on Fine-textured Soil at Clinton

The fine-textured soil at Clinton had lower total C contents in the Ap horizon than the coarse-textured soils (Table 2.4). Phosphatase and arylsulfatase activities were greater in no-till soil (Table 2.5; Fig. 2.1d,e). Tillage practice accounted for 27 and 22% of the variation in activity of phosphatase and arylsulfatase, respectively. Dehydrogenase activity of the surface layer was greater for the no-till treatment; activity in the lower layer was greater for the conventional tillage treatment. Activity of four enzymes (dehydrogenase, urease, glutaminase and $-glucosidase) was greater in the surface than in the lower layer of the Ap horizon. There was temporal variation in activity of all enzymes (Table 2.5; Fig. 2.2b). The influence of tillage on urease and $-glucosidase activities varied with sampling date. On most dates, activity of both enzymes was either greater in no-till soil, or similar for tillage treatments. Both sampling depth and date influenced the tillage comparison for glutaminase. On most sampling dates, glutaminase activity of the surface layer was either greater in the no-till soil or similar for tillage treatments.

Values of V_{max} and total C content for the two tillage treatments were compared at the same depth and position along the soil texture gradient (Table 2.4). For urease and $-glucosidase, V_{max} was greater for conventional tillage compared to no-till in the lower layer of the Ap horizon at both positions in the field. As well, V_{max} for $-glucosidase was greater in the surface layer of no-till soil, relative to tilled soil, at position 2. For phosphatase, V_{max} was greater in the lower layer of the no-till treatment, relative to tilled soil, at position 1. It was greater in the lower layer in tilled soil, relative to no-till soil, at position 2. At both positions, there was no difference in total C content between tillage treatments.

2.1.4.3 Effect of Tillage on Coarse-textured Soils at Clinton

The coarse-textured soils at Clinton occurred at the lower part of the slope. The subgroup classification indicated poor drainage (gleyed characteristics; Table 2.1), and they had relatively large total C contents (Table 2.4). Tillage did not influence any of the enzyme activities independently of sampling depth and date (Table 2.6; Fig. 2.1). The difference in arylsulfatase activity between tillage treatments apparent in Fig. 2.1e was not significant in analysis of variance because of the large amount of variation associated with the error terms (15 and 30%SS). There was temporal variation in activity of all enzymes except arylsulfatase (Table 2.6; Fig. 2.2c), and

$-glucosidase activity was greater in the surface layer of the Ap horizon. The influence of tillage on phosphatase activity varied with sampling date. On most dates it was either greater in no-till soil, or there was little difference in activity between the tillage treatments. The effect of tillage on urease and $-glucosidase activities was influenced by both sampling depth and date. In the surface layer, activity of both enzymes was most often greater in no-till soil than in tilled soil. In the lower layer of the Ap horizon, on most sampling dates activity of both enzymes was either similar for both tillage treatments or greater in the tilled soil.

Values of V_{max} and total C content for the two tillage treatments were compared at the same depth and position along the soil texture gradient (Table 2.4). For urease, V_{max} was greater in the surface layer of no-till soil compared with tilled soil at position 4. For phosphatase, V_{max} was greater in the surface layer of no-till soil compared with tilled soil at position 3. It was greater at both depths in tilled soil compared to no-till soil at position 4. For $-glucosidase, V_{max} was greater in the surface layer of no-till soil compared with tilled soil at position 3. It was greater in the lower layer of tilled soil compared to no-till soil at position 4. Total C content was greater in tilled soil compared to no-till soil at position 4.

2.1.5 DISCUSSION

2.1.5.1 Sensitivity of Enzyme Activities to Conservation Practices

At Rockwood, all enzyme activities were strongly affected by management, and on average, greater in the no-till field for the entire depth of the Ap horizon. Differences in enzyme activities between the two fields derived from both tillage practice and cropping history. The influence of previous cropping of the no-till field to forages was evident in the greater enzyme activity in the lower layer of the Ap horizon of the no-till field, compared to the lower layer of the tilled field (data not presented). Miller and Dick (1995) found that green manures and crop rotation strongly influenced soil enzyme activities. Hence, the larger differences in enzyme activities between fields at Rockwood compared to Clinton (Fig. 2.1) may be due primarily to cropping history. At Rockwood the strong response of enzyme activities to management practice was indicative of differences in total C content between the two fields. In this instance, enzyme activities were behaving as a surrogate measure of SOM content. Lovell et al. (1995) compared

the sensitivity of various soil biochemical and microbial indices to short- and long-term fertilizer N treatments on long-term grassland. They found that microbial biomass C and N contents, and urease and phosphatase activities responded similarly to long-term treatments, and were indicative of SOM content.

At Clinton, topography and its effects on drainage and SOM content influenced results. As well, the six enzymes differed in sensitivity to tillage practices. Because soil texture was confounded with slope-position, effects of soil texture alone on enzyme activities could not be assessed. The coarse-textured soils were located in a poorly drained location at the bottom of the slope and had relatively large total C contents (Table 2.4). Activity of glutaminase, phosphatase, arylsulfatase and $-glucosidase also tended to be greater in the coarse-textured soils than in the fine-textured soil (Fig. 2.1c-f). At position 4 on coarse-textured soils, total C content was unexpectedly greater in conventionally tilled soil than in no-till soil. These site characteristics may account for the fact that in the coarse-textured soils no enzyme activity was consistently responsive to tillage practice alone. Topography rather than tillage had a strong effect on soil biochemical properties in this part of the field. Similarly, Ross et al. (1995a) found that changes in enzyme activities in response to pasture management were small relative to background activity deriving from large amounts of stabilized enzymes.

The fine-textured soil at Clinton had lower total C contents than the coarse-textured soils, with no differences in total C content between tillage treatments (Table 2.4). Yet, activity of three enzymes (dehydrogenase in the surface layer, and phosphatase and arylsulfatase in both layers) was greater in the no-till field than in the tilled field. These enzyme activities, like soil microbial biomass (Powlson et al., 1987), may have been more sensitive than total C content to management practice. Such sensitivity would make soil enzyme activities effective indicators of changes in soil quality (Kennedy and Papendick, 1995; Park and Seaton, 1996). Verification of this explanation requires comparable replication of both measurements, which the present study lacked. As well, enzyme activities should be compared to organic rather than total C content. An alternative explanation was that these enzyme activities were indices of change in some soil property other than SOM content.

We hypothesized that soil enzyme activities respond to implementation of no-till in a manner similar to SOM content. Results for fine-textured soil at Clinton refute this

hypothesis, notwithstanding the fact that dehydrogenase activity of the Ap horizon showed the stratified response observed by other workers for SOM content. Tillage practice strongly influences vertical distribution of crop residues and SOM in the soil profile; residues and SOM accumulate at the surface with reduced or no tillage. In light of this fact, the sampling depth of the surface layer (8 cm) may have been too large. As a result, enzyme activities and total C content of residue and SOM at and immediately beneath the soil surface in no-till plots would have been under-estimated.

Temporal changes in enzyme activities were sometimes large, accounting for up to 69%SS. Moreover, at Clinton sampling date was the single most important source of variation in enzyme activities--more important than either tillage practice or sampling depth. Other workers have reported seasonal fluctuations in enzyme activities (e.g. Burton and McGill, 1992; Ross et al., 1995b). Seasonal variation in some enzyme activities may be related to crop phenology and rhizodeposition of C. Swinnen et al. (1995) reported maximum below-ground C fluxes in winter wheat and barley (*Hordeum vulgare* L.) at tillering. Moreover, they found greater rhizodeposition of C by barley under conventional versus integrated management. At Rockwood, dehydrogenase activity of the surface layer was unexpectedly greater in the tilled field compared with the no-till field on the fifth sampling date. The difference in activity probably derived from the relative water contents of the two fields at that time. The no-till field was much wetter (30% vs. 20% gravimetric water content). Dehydrogenase activity is a respiratory measurement which would be expected to decrease in very wet soil. Whatever its source, temporal variation in enzyme activities is large relative to tillage effects, and can influence soil quality comparisons based on these measurements. As well, if soil enzyme activities such as dehydrogenase and $-glucosidase are indicative of biological oxidation processes, then they may be expected to increase temporarily in conventional tillage systems, over that of no-till systems, in the period following incorporation of crop residues (cf. Reicosky et al., 1995). Franzluebbers et al. (1995) observed large seasonal changes in active soil C and N pools, which were affected by tillage practice and related to inputs of organic C from rhizodeposition during the growing season and from crop residues after harvest. Temporal variation in some enzyme activities would be expected to accompany such seasonal changes in labile C and N pools.

2.1.5.2 Comparison of Enzyme Activity vs. V_{max}

For three enzymes, activities of field-moist soil and V_{max} values of air-dry soil were compared as indices of soil quality. The two measurements may differ, depending on the relative amounts of enzyme associated with live microbial cells vs. extracellular, stabilized enzyme in the moist and air-dry soil samples (Burns, 1982). As well, in our study the limited number of samples used for kinetic analysis did not allow for replication across the slope at Rockwood. Therefore, V_{max} estimates may have been more affected by small-scale spatial variability at this site than were enzyme activities of field-moist samples. Finally, our study did not account for temporal variation in V_{max} at either site. At Rockwood and for fine-textured soil at Clinton, enzyme activities of field-moist samples were more useful than V_{max} estimates in distinguishing between management treatments. Management comparisons based on V_{max} sometimes varied between slope-positions or along the texture gradient at Rockwood and Clinton, respectively. For coarse-textured soils at Clinton, both activities of field-moist samples and V_{max} estimates gave inconsistent results.

Within each field at Rockwood there were similar trends in total C content and V_{max} estimates along the slope in the surface layer of the Ap horizon (Table 2.3). In the no-till field, total C content and V_{max} estimates were greater at the shoulder and foot-slope positions than at the mid-slope. In the conventionally tilled field, total C content and V_{max} of urease and phosphatase were greater at the mid-slope and foot-slope positions than at the shoulder. Likewise, differences in V_{max} of phosphatase and $-glucosidase of coarse-textured soils at Clinton were related to differences in total C content (Table 2.4). These observations are consistent with the strong relationship between V_{max} of some enzymes and SOM content observed in other studies (e.g. Farrell et al., 1994). For extracellular enzymes, V_{max} estimates from kinetic analysis of air-dry samples may primarily represent amounts of stabilized enzyme. As such, they may be less affected by seasonal variation, and more suited to comparisons over a longer time-scale. Activity measurements on field-moist samples include a transient component resulting from microbial activity over the growing season (Fig. 2.2), and may represent intracellular enzyme production. On such a temporal scale, enzyme activities of field-moist samples may be primarily an intracellular as opposed to extracellular measurement.

2.1.5.3 Use of Enzyme Activities in Management Comparisons, and Perspectives in Further Research

Because of their sensitivity and ease of measurement, soil enzyme activities may be key variables for soil assessments related to sustainability (Park and Seaton, 1996). They may be especially useful for validation of changes in soil quality predicted by simulation models from existing databases (Acton, 1994), or to verify effectiveness of conservation practices (Kennedy and Papendick, 1995). The present study provided ground for limited comment about how enzyme activities might be used. Slope, and time and depth of sampling influenced management comparisons. Thus, comparisons should be made at the same slope-position(s), and for a layer of the solum that adequately represents effects of management. Sampling depth may be particularly important for dehydrogenase, urease and $-glucosidase activities. Very wet or very dry soil, and crop phenology may be sources of temporal variation in enzyme activities that affects comparisons. Thus, comparisons restricted to one sampling date within the growing season should be avoided. Finally, activity measurements of field-moist samples and V_{max} values from kinetic analysis of air-dry samples may not always provide the same information.

Further research is required to link enzyme activities to fundamental soil processes. Enzyme activities may be sensitive indicators of changes in SOM content, but at present their measurement rarely informs us about the underlying soil processes driving the changes. Moreover, in some instances enzyme activities may be primarily affected by soil properties other than SOM content, and cannot be used in the conventional sense as a surrogate measure of the latter. Information about the ecological meaning of these measurements may also enable us to use enzyme activities in evaluations that do not rely upon treatment comparisons.

2.1.6 CONCLUSIONS

Soil enzyme activities are sensitive to changes in soil quality resulting from conservation practices. Implementation of no-till following a crop rotation that included forages increased SOM content and six enzyme activities. Enzyme activities differed in their sensitivity to tillage alone. Implementation of no-till following years of conventional tillage may increase some enzyme activities at some sites. Conversely, in soils in poorly drained locations with relatively large SOM contents, SOM content and enzyme activities may not be strongly affected by tillage practice. Slope-position, and time and depth of

sampling also influence enzyme activities, and affect management comparisons. Enzyme activities behave in most but not all instances as surrogate measures of SOM content.

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Table 2.1. Soil characteristics at Clinton.

Position[†]	Tillage	Subgroup[‡]	Depth[§]	Sand	Silt	Clay
				------ percent -----		
1.	No-till	Orthic Gray Brown	1	16	43	41
		Luvisol	2	17	49	34
	Conventional	Orthic Gray Brown	1	18	47	35
		Luvisol	2	18	45	37
2.	No-till	Orthic Gray Brown	1	21	43	36
		Luvisol	2	17	57	26
	Conventional	Orthic Gray Brown	1	8	52	40
		Luvisol	2	12	52	36
3.	No-till	Orthic Humic Gleysol	1	62	26	12
			2	62	26	12
	Conventional	Gleyed Brunisolic	1	64	24	12
		Gray Brown Luvisol	2	65	23	12
4.	No-till	Orthic Humic Gleysol	1	71	19	10
			2	70	19	11
	Conventional	Gleyed Brunisolic	1	70	20	10
		Gray Brown Luvisol	2	70	20	10

[†] position along soil textural gradient in field.
[‡] Canadian system of soil classification.
[§] 1. 0-8 cm; 2. 8 cm to the bottom of the Ap.

Table 2.2. Summary analysis of variance for enzyme activities of the Ap horizon at Rockwood (significant effects only, *P* 0.05).

Variables	df	Dehydrogenase†	Urease†	Glutaminase	Phosphatase†	Arylsulfatase‡	$-glucosidase‡
		——————————percent of total sum of squares——————————					
Management (M)	1	29	47	48	48	42	25
Slope (S)	2	4	3		7	4	
Layer (L)	1						
Date (D)	6	7	20	7	27	17	17
Interactions		M*D L*D M*L*D	M*D L*D	M*D L*D M*L*D	M*S M*D S*D M*S*D M*L*D	M*S M*D M*S*D M*L*D	M*D L*D M*L*D S*L*D

† data transformed by square-root.
‡ data log-transformed.

Table 2.3. Total C content and maximum velocity (V_{max}) for three enzymes of the Ap horizon at Rockwood.

Slope-position	Depth†	Total C content (%)	Urease (g N g^{-1} h^{-1})	Phosphatase (mole -nitrophenol g^{-1} h^{-1})	$-glucosidase
		no-till following forage			
Shoulder	1	3.4*	141 (3)‡*	5.0 (0.2)*	1.5 (0.06)*
	2	2.7*	63 (3)*	2.7 (0.4)*	0.77 (0.08)*
Mid-slope	1	2.0	59 (1)*	1.7 (0.1)	0.87 (0.05)
	2	1.7	62 (1)*	1.2 (0.1)	0.60 (0.03)*
Foot-slope	1	2.8*	105 (3)*	3.1 (0.4)*	1.0 (0.04)
	2	2.4	64 (3)*	3.7 (0.2)*	0.65 (0.01)*
		conventional tillage following corn			
Shoulder	1	1.7	17 (1)	O.74 (0.03)	0.74 (0.05)
	2	1.5	29 (2)	0.54 (0.04)	0.31 (0.01)
Mid-slope	1	2.3	27 (2)	1.5 (0.09)	0.70 (0.05)
	2	2.0	14 (2)	0.91 (0.05)	0.22 (0.02)
Foot-slope	1	2.3	41 (2)	0.91 (0.04)	0.83 (0.04)
	2	1.9	12 (1)	0.60 (0.03)	0.44 (0.02)

† 1. 0-8 cm; 2. 8 cm to the bottom of the Ap horizon.
‡ standard error in parentheses.
* significant difference between fields at the indicated slope-position and depth at *P* 0.05, based on confidence limits calculated from the *t* distribution (larger values marked).

Table 2.4. Total C content and maximum velocity (V_{max}) for three enzymes of the Ap horizon at Clinton.

Position†	Tillage	Depth‡	Total C content (%)	Urease (g N g^{-1} h^{-1})	Phosphatase (mole -nitrophenol g^{-1} h^{-1})	$-glucosidase
			fine texture			
1.	No-till	1	2.2	53 (2.8)	1.7 (0.09)	0.87 (0.01)
		2	2.0	24 (1.6)	1.8 (0.06)§*	0.43 (0.01)
	Conventional	1	2.3	67 (3.3)	1.7 (0.11)	0.78 (0.03)
		2	2.1	39 (2.7)*	1.3 (0.06)	0.54 (0.01)*
2.	No-till	1	2.0	44 (4.8)	3.0 (0.12)	1.0 (0.02)*
		2	1.7	26 (0.8)	1.6 (0.11)	0.33 (0.02)
	Conventional	1	1.9	46 (4.4)	2.5 (0.11)	0.81 (0.03)
		2	1.9	41 (2.0)*	2.3 (0.07)*	0.81 (0.01)*
			coarse texture			
3.	No-till	1	3.8	39 (1.4)	3.3 (0.06)*	1.5 (0.03)*
		2	3.4	27 (0.8)	3.1 (0.38)	0.70 (0.02)
	Conventional	1	3.5	35 (0.5)	2.6 (0.08)	0.66 (0.03)
		2	3.5	28 (0.7)	2.1 (0.06)	0.78 (0.04)
4.	No-till	1	2.9	25 (0.8)*	2.5 (0.08)	1.2 (0.06)
		2	3.0	19 (1.3)	2.2 (0.09)	0.5 (0.01)
	Conventional	1	3.7*	12 (1.4)	3.0 (0.13)*	1.3 (0.04)
		2	3.5*	23 (1.0)	3.3 (0.11)*	1.5 (0.03)*

† position along textural gradient in field.
‡ 1. 0-8 cm; 2. 8 cm to the bottom of the Ap horizon.
§ standard error in parentheses.
* significant difference between fields at the indicated depth at *P* 0.05, based on confidence limits calculated from the *t* distribution (larger value marked).

Table 2.5. Summary analysis of variance for enzyme activities of the Ap horizon of fine-textured soil at Clinton (significant effects only, *P* 0.05).

Variables	df	Dehydrogenase†	Urease‡	Glutaminase‡	Phosphatase‡	Arylsulfatase	$-glucosidase
			percent of total sum of squares				
Tillage (T)	1				27	22	
Layer (L)	1	30	17	8			22
Date (D)	7	41	26	47	44	24	36
Interactions		T*L	T*D	L*D T*L*D		L*D	T*D

† data transformed by square-root.
‡ data log-transformed.

Table 2.6. Summary analysis of variance for enzyme activities of the Ap horizon of coarse-textured soils at Clinton (significant effects only, *P* 0.05).

Variables	df	Dehydrogenase†	Urease†	Glutaminase†	Phosphatase†	Arylsulfatase	$-glucosidase†
			percent of total sum of squares				
Tillage (T)	1						
Layer (L)	1						11
Date (D)	7	13	31	69	35		55
Interactions			T*D L*D T*L*D		T*D		T*L T*D L*D T*L*D

† data log-transformed.

2.2 Tillage Practice and Preceding Crop Influence Soil Enzyme Activities

D.W. Bergstrom and C.M. Monreal

2.2.1 INTRODUCTION

This study tested for an effect of tillage practice on soil enzyme activities at a site in eastern Ontario. At one farm a chisel-plowed field was cropped continuously to corn (*Zea mays* L.). At a second farm, a ridge-tilled field was strip-cropped to corn and soybeans (*Glycine max* L.). Ridge tillage was practiced for two reasons. Firstly, the ridges provided a warmer and drier seedbed in the spring. Prior to implementation of ridge tillage, seepage of water to the soil surface resulted in a cool, wet seedbed in the spring. Secondly, ridge tillage was used to conserve crop residues by keeping them for the most part on the soil surface where they decompose slowly. The differences in rotation between the two fields (continuous corn vs. corn alternating with soybeans) may have confounded the comparison of tillage practices. For this reason, our study also tested for an effect of the preceding crop within the strip-cropped field.

The objectives of this study were to test for influence of (1) tillage practice (chisel plow vs. ridge tillage) and (2) preceding crop on six soil enzyme activities.

2.2.2 MATERIALS AND METHODS

2.2.2.1 Site Description and Sampling Procedure

Soil samples were collected in 1994 and 1995 from two fields on separate farms near Bainsville in eastern Ontario, one of which was chisel-plowed and the other ridge-tilled. Soil characteristics of the two sites are presented in Table 2.7. The chisel-plowed field was planted to corn for the sampling period and in the previous 4 y. The ridge-tilled field was strip-cropped to corn and soybeans, with alternate crops on each strip in successive years. For this study, one strip in the ridge-tilled field was planted to corn following corn in each of 1994 and 1995. In each year, two strips in this field were

sampled--one planted to corn following corn, and the other planted to corn following soybeans. Thus, in the two fields the sampling scheme compared three management treatments (combinations of tillage practice and preceding crop): (1) chisel plow and corn; (2) ridge tillage and corn; and (3) ridge tillage and soybeans. Both fields received starter fertilizer with the seed at planting. Anhydrous NH_3 was applied to the chisel-plowed field prior to seeding, while a solution of urea-NH_4NO_3 was knifed-in beside corn rows in late June in the ridge-tilled field.

Soil samples were collected from the Ap horizon on 4 dates in 1994 (May 19, July 12, August 12 and September 14) and 3 dates in 1995 (May 16, June 28 and July 31) with an Oakfield split-tube sampler (id = 2 cm). One each date, samples were collected from the plant row at two depths: 0-8 cm, and 8 cm to the bottom of the Ap horizon. In both fields, samples were collected along transects marked off into four 100-m lengths. A composite sample of 4 cores was collected for each length of the transect, providing 4 replicate samples per treatment. Samples were transported to the laboratory in a cooler with cold-packs, and thereafter held at 5EC until analysis.

2.2.2.2 Laboratory Analyses

In the laboratory, soil samples were thoroughly mixed before analysis. Water content was determined gravimetrically with 10 g moist soil. Ammonium and NO_3^- plus NO_2^- were extracted from 5 g moist soil with 30 mL 2 *M* KCl. Extracts were filtered through Whatman No. 2 filter paper, then frozen until analysis by TRAACS 800 autoanalyzer using Technicon Industrial Methods No. 780-86T and No. 782-86T for NH_4^+ and NO_3^- plus NO_2^-, respectively. Dehydrogenase, urease, phosphatase, arylsulfatase and $-glucosidase activities were determined as described by Tabatabai (1982), and glutaminase activity was determined by the method of Frankenberger and Tabatabai (1991), with modifications outlined in section 2.1.3.2. Total C content of finely ground (<100 mesh) soil was determined by a LECO dry combustion method (Nelson and Sommers 1982). All data were expressed based on the oven-dry weight of soil.

2.2.2.3 Statistical Analysis

Data were analyzed using the Multivariate General Linear Hypothesis (MGLH) module of SYSTAT (Wilkinson, 1988a), with sampling dates as repeated measures (Hall, 1994). Data were combined for both years. Because sampling dates were restricted to two growing seasons, the scope of this study was not large enough for assessing variation over yearly intervals. When necessary, data were transformed for homogeneity of variance prior to ANOVA.

Enzyme activities of the three management treatments (tillage practice and preceding crop) were compared graphically using notched box plots prepared with SYGRAPH (Wilkinson, 1988b). These plots provided a comparison of both the central tendency and distribution of data. The upper and lower edges of the central box define the upper and lower limits of the interquartile range (midrange). The vertical lines above and below the box (inner fences) extend 1.5 times the midrange above and below the edges of the box. Values lying outside this inner fence are plotted with asterisks; far outside values are plotted with circles. The boxes are notched at the median, and return to full width at the upper and lower confidence interval values. When the confidence intervals around two medians do not overlap, the two population medians are different at approximately the 95% level. The mean values of untransformed data are plotted as triangles.

2.2.3 RESULTS

On average, there were differences in activities of all enzymes except glutaminase among the three management treatments (Table 2.8). Orthogonal contrasts for effects of management summed over sampling dates indicated that urease, phosphatase, arylsulfatase and $-glucosidase activities differed between tillage practices, with greater activity in the chisel-plowed field (Table 2.9; Fig 2.3). These four enzyme activities were not influenced by preceding crop in the ridge-tilled field. Dehydrogenase activity was affected by both tillage and preceding crop. It was greater in the chisel-plowed field, and following corn (Table 2.9; Fig. 2.3a). Of the five enzymes that

were responsive to management, phosphatase was most affected (27% total sum of squares[%SS]), and $-glucosidase was least affected (3%SS). The interaction between management and sampling date for dehydrogenase, urease, phosphatase and $-glucosidase indicated that there were no differences among treatments on some dates (Fig. 2.4). Sampling date accounted for most of the variation in glutaminase activity (53%SS).

Dehydrogenase, urease and $-glucosidase activities were also strongly affected by sampling depth, with greater activity in the surface (0-8 cm) layer. Among the six enzymes, activity of glutaminase, arylsulfatase and $-glucosidase varied most over sampling dates. For all enzymes except phosphatase sampling date and/or depth accounted for more variation in enzyme activities than management (Table 2.8).

2.2.4 DISCUSSION

Greater mean activity of five enzymes in the Ap horizon of the chisel-plowed field relative to the ridge-tilled field was consistent with the greater total C content (standard deviation) of the chisel-plowed field, relative to the ridge-tilled field (3.0 (0.14) vs. 2.6 (0.15) % in the A1 (0-8 cm) layer, and 2.4 (0.21) vs. 2.1 (0.06)% in the A2 (8 cm to bottom of Ap) layer. Soil enzyme assays indicate relative enzyme contents (Galstian, 1974), rather than activity *in situ*. While greater enzyme content may be associated with greater activity *in situ*, conventional soil enzyme assays do not directly assess the latter. Two questions were posed by the results of this study. Firstly, were the greater enzyme activities and total C content of the chisel-plowed field, relative to the ridge-tilled field, a consequence of site characteristics or management (tillage and rotation)? Secondly, how might the difference in enzyme activities between the two fields be interpreted?

Differences in the five enzyme activities and total C content between the two fields can be attributed to both site characteristics and management practice. The chisel-plowed and ridge-tilled fields differed in soil texture (loam and very fine sandy loam, respectively; Table 2.7). As well, the Ap horizon of the chisel-plowed field had a lower bulk density (1.33 Mg m^{-3}) than that of the ridge-tilled field (1.44 Mg m^{-3}). Both

properties influence soil aeration and porosity, and indicated a more favorable environment for soil microorganisms in the chisel-plowed field. Porosity also influences distribution and retention of water in the soil matrix. Water content was greater in the chisel-plowed field on all but one sampling date (data not presented). The soil physical environment influences the soil biota and its activity (Richards, 1974; Smiles, 1988; Coleman and Crossley, 1996). Greater activity of five enzymes in soil of the chisel-plowed field may reflect, in part, its more favorable microbial environment, if soil enzyme activities were behaving as indices of general microbial activity (Kennedy and Papendick, 1995).

The chisel-plowed field was repeatedly cropped to corn, while strips in the ridge-tilled field were planted to corn and soybeans in alternate years. Thus, over successive growing seasons more plant biomass and crop residue was produced on the chisel-plowed field. This difference in rotation may account, in part, for the greater total C content of the Ap horizon. While crop residue was incorporated by chisel-plowing in the one field, in the ridge-tilled field crop residue was left for the most part on the soil surface. Only some of the residue was mixed with surface soil during the ridging operation in June. Incorporation of crop residues may also account, in part, for the greater total C content in the chisel-plowed field. It is impossble to determine the excat cause because the initial C content for both sites is unknown.

Greater activity of five enzymes in the chisel-plowed field may have been a consequence of the greater soil organic matter (SOM) content, if enzyme activities behave as surrogate measures of the latter. Alternatively, the greater activity of five enzymes in the chisel-plowed field may have derived from increased microbial activity during biological oxidation of incorporated residue. Greater dehydrogenase activity in the strip-cropped field when the preceding crop was corn rather than soybeans supports the second hypothesis. There was little or no difference in total C content between the two strips in soil samples from 1995 (2.7 vs. 2.6% in the surface layer of the Ap horizon, and 2.1 vs. 2.2% in the lower layer, following corn and soybeans, respectively). Increase in activity of enzymes that mediate microbial decomposition processes would be consistent

with the influence of cultural practices on seasonal changes in size of labile soil C and N pools (Franzluebbers et al., 1995). Increased enzyme activity would also be expected during substantial mineralization of SOM following incorporation of crop residue by tillage (Reicosky et al., 1995). If such was the case, the enzyme activities were behaving an indices of nutrient and C cycling, as postulated by Warkentin (1995).

For four enzyme activities, sampling date influenced comparison of management treatments. Sampling depth was also an important source of variation in activity of dehydrogenase, urease and $-glucosidase. Both factors need to be accounted for in sampling protocols of soil quality assessments.

On average, glutaminase activity was not responsive to tillage practice. The fact that soil enzymes in some instances behave differently is plausible, given the differences in the reactions they catalyze and in their functions in microbial metabolism. Behavior of glutaminase activity in soil may be affected by the role of glutamine as an intermediate in microbial assimilation of NH_3 (Stanier et al., 1986). Moreover, McCarty (1995) postulated a role for glutamine synthetase in regulation of N metabolism within the soil microbial community. Behavior of glutaminase activity in soil may be explained by its role in such a paradigm. In a study of the influence of crop rotation on activity of soil enzymes, Miller and Dick (1995) hi-lighted contrasts in behavior between amidase and L-asparaginase, and suggested that L-asparaginase in soil is confined to cells and cell debris. Activity of glutaminase and asparaginase in soils may be similar in these and other respects, given that asparagine, like glutamine, is an intermediate in microbial assimilation of NH_3. The fact that both glutaminase and asparaginase activities of soil samples are increased by toluene (Frankenberger and Tabatabai, 1991; Miller and Dick, 1995) also points to a predominantly intracellular identity for both enzymes, if toluene increases permeability of the microbial cell-membrane.

The effect of the preceding crop on dehydrogenase activity of the Ap horizon of the ridge-tilled field, and appreciable temporal variation of activity of some enzymes prompted a more detailed study of the influence of the crop on soil enzyme

activities in the ridge-tilled field at Bainsville in 1995 (reported in section 3.2).

2.2.5 CONCLUSIONS

On average, five enzyme activities of the Ap horizon were greater in a chisel-plowed field than in a ridge-tilled field. Site characteristics as well as tillage practice and rotation may have influenced enzyme activities. Greater activity of five enzymes in the chisel-plowed field had three plausible explanations, which affect interpretation of these measurements. It may have resulted from a more favorable microbial environment, with enzyme activities behaving as indices of general microbial activity. It may have derived from the greater SOM content of the chisel-plowed field, if enzyme activities behave as surrogate measures of SOM content. It may have resulted from greater microbial activity during biological oxidation of incorporated crop residue, with enzyme activities behaving as indices of nutrient and C cycling.

2.2.6 ACKNOWLEDGMENTS

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Table 2.7. Soil and site characteristics at Bainsville.

Characteristics	Chisel-plowed field	Ridge-tilled field
Rotation	corn	strip-cropped to corn and soybeans
Parent material	fluvial sand	fluvial sand over lacustrine clay
Drainage	poor	poor
Classification	Orthic Humic Gleysol	Rego Humic Gleysol
Soil Series	Bainsville	Bainsville
Texture of Ap	loam	very fine sandy loam
Bulk density of Ap (Mg m^{-3})	1.33	1.44
Total C content (%) Ap1[†]	3.0	2.6
Ap2[‡]	2.4	2.1

[†] 0 - 8 cm.
[‡] 8 cm to bottom of the Ap.

Table 2.8. Summary analysis of variance for enzyme activities of the Ap horizon at Bainsville (significant effects only, *P* 0.05).

	df	Dehydrogenase	Urease†	Glutaminase	Phosphatase	Arylsulfatase	$-glucosidase
Variable		———————---------——— percent of total sum of squares ———————————————————					
Management (M)	2	10	23		27	10	3
Layer (L)	1	52	41	17		7	46
Date (D)	6	7	7	53	5	29	26
Interactions		M*D	M*D	M*D	M*D		M*D L*D

† data transformed by square-root.

Table 2.9. Orthogonal contrasts for effect of management (tillage practice and preceding crop) summed over sampling dates on soil enzyme activities of the Ap horizon at Bainsville.

Variable	df	Dehydrogenase‡		Urease‡		Phosphatase		Arysulfatase		$-glucosidase	
		SS	*P*	SS	*P*	SS	*P*	SS	*P*	SS	*P*
Management	2	68.96	0	53.69	0	43.54	0.009	0.641	0.013	0.920	0.027
1 vs. 2 and 3†	1	54.92	0	53.44	0	40.89	0.003	0.539	0.007	0.905	0.009
2 vs. 3	1	14.04	0.02	0.25	0.476	2.65	0.397	0.102	0.199	0.015	0.71

† 1. chisel plow and corn.
2. ridge-tillage and corn.
3. ridge-tillage and soybeans.

‡ data transformed by square root.

2.3 Temporal Variation in Soil Enzyme Activities Resulting From Application of Inorganic N Fertilizer and Manure

D.W. Bergstrom, C.M. Monreal and P. Gasser

2.3.1 INTRODUCTION

Use of soil enzyme activities as indicators of soil quality assumes that they are site characteristics. Temporal variation in soil enzyme activities, if substantial, may make this assumption untenable, and require explicit attention in sampling protocols for soil quality assessments. For example, in a study of effects of inorganic and organic N inputs on soil microbial activity, Fauci and Dick (1994) found that over the long-term microbial biomass and enzyme activities were correlated with amounts of added C. At the same time, recent organic inputs had a large effect on these soil biological properties, regardless of long-term management.

On farms agronomic inputs such as fertilizer and manure may be sources of temporal variation in soil enzyme activities. For example, Kandeler et al. (1994) reported increased N mineralization and activity of protease, deaminase and urease at 0-50 cm below grassland that had received cattle slurry at 480 kg N ha^{-1} y^{-1} for 14 y, compared to control plots.

The objective of this study was to test for temporal variation in six soil enzyme activities resulting from application of inorganic N fertilizer and poultry manure.

2.3.2 MATERIALS AND METHODS

2.3.2.1 Site Description and Soil Sampling Procedure

Soil samples were collected in 1994 and 1995 from a field experiment conducted by Pierre Gasser (Ag-Knowledge) on a farm near Ste-Isidore in eastern Ontario. The soil was a clay loam and the field was strip-cropped to corn (*Zea mays* L.) and soybeans (*Glycine max* L.), using ridge tillage. The experimental design was a randomized complete block, with 4 replicates. In each block, 8 fertilizer treatments were applied to strips (4.5 m wide) of corn. Soil samples were collected from 3 treatments: (1) an unfertilized check, (2) N added at 150 kg N ha^{-1} as 28% urea-NH_4NO_3 (UAN) solution, and (3) 150 kg N ha^{-1} added as poultry manure (6,400 gallons manure ha^{-1}, assumed that 80% of the manure N becomes available for following crop).

In 1994, corn was planted on May 11 (Julian day 131). Fertilizer (UAN, 160 kg N ha^{-1}) was applied in split applications: 25% at planting, and the remaining 75% on July 9

(d 190) onto the soil surface. Poultry manure (160 kg N ha^{-1}) was applied to the soil surface between the plant rows and incorporated by disks on June 21 (d 172). Wet weather in June prevented the ridging operation. Samples of the Ap horizon were collected on 4 dates during the growing season in 1994 (May 10, June 9, July 27 and August 26; d 130, 160, 208 and 238, respectively), using Oakfield split-tube samplers. On the first 2 dates samples were collected from the ridge; on the last 2 dates samples were collected from the furrow where the manure was applied. Within each block, for each treatment a composite sample of 4 cores was collected along a 70 m transect. Samples were collected at 2 depths: 0-8 cm, and 8 cm to the bottom of the Ap horizon. Soil samples were transported from the field to the laboratory in a cooler with cold-packs, and thereafter held at 5°C until analysis.

In 1995 the size of the field experiment was reduced, leaving 2 of the original 4 blocks. Each of the 2 remaining blocks was divided into halves to provide 4 replicates. The individual treatments had been randomized within the original 2 blocks. For each treatment, a composite sample of 4 cores was collected along a 35 m transect. In 1995 corn was planted in May. Poultry manure was applied on June 7 (d 158). The post-planting application of UAN (75% of total dose, 25 % of dose was added at seeding) occurred on June 20 (d 171), after soil sampling. Soil samples were collected on 4 dates in 1995: May 3, June 9, June 20 and July 20 (d 123, 160, 171 and 201, respectively). On the first three dates, samples were collected from the furrow; on the last date which followed the ridging operation, samples were collected from the plant row. There was only one sampling date following the post-planting application of UAN--July 20 (d 201), with an elapse of 30 d between application and sampling. Hence, analysis of data in 1995 was limited to a comparison of the check and manure treatments.

2.3.2.2 Laboratory Analyses

In the laboratory, soil samples were thoroughly mixed before analysis. Water content was determined gravimetrically with 10 g moist soil. Ammonium and NO_3^- plus NO_2^- were extracted from 5 g moist soil with 30 mL 2 *M* KCl. Extracts were filtered through Whatman No. 2 filter paper, then frozen until analysis by TRAACS 800 autoanalyzer using Technicon Industrial Methods No. 780-86T and No. 782-86T for NH_4^+ and NO_3^- plus NO_2^-, respectively. Dehydrogenase, urease, phosphatase, arylsulfatase and $-glucosidase activities were determined as described by Tabatabai (1982), and glutaminase activity was determined by the

method of Frankenberger and Tabatabai (1991), with modifications outlined in section 2.1.3.2. All data were expressed based on the oven-dry weight of soil.

2.3.2.3 Statistical Analysis

Because UAN and manure were added to the surface soil, data for each layer of the Ap horizon were analyzed separately. The Multivariate General Linear Hypothesis (MGLH) module of SYSTAT (Wilkinson, 1988) was used to analyze data, with sampling dates as repeated measures (Hall, 1994). An effect of fertilizer treatment on soil enzyme activities was indicated by a fertilizer*date interaction, with differences between treatments following UAN or manure application. Because this statistical approach tested for differences among fertilizer treatments amidst temporal variation in enzyme activities over the growing season, it was more rigorous than separate analyses of data for each sampling date. When the effect of fertilizer was significant, treatments were compared on individual sampling dates by LSD (P = 0.05), using the mean-square-error and df for comparisons within subjects unless otherwise noted. When necessary, data were transformed for homogeneity of variance prior to ANOVA.

2.3.3 RESULTS

2.3.3.1 Effects of UAN and Manure Application in 1994

In 1994, fertilizer and manure application increased mineral N contents of the Ap horizon (Fig. 2.5 and 2.6). There was relatively little NH_4^+ and NO_3^- in unfertilized soil, with <1 g N g^{-1} by the fourth sampling date. Application of UAN at seeding (day 131, following soil sampling on day 130) and later in the season (day 190) increased NH_4^+ content in the surface layer over that of the other two treatments. Manure application on day 172 did not increase NH_4^+ content of the surface layer above that of the check treatment on the last two sampling dates. Ammonium contents for all three fertilizer treatments were relatively small (<2 g N g^{-1}) in the lower layer of the Ap horizon throughout the sampling period. Nitrate content was increased in both layers of the Ap horizon by UAN application. Manure application also increased NO_3^- content of the surface layer. Increase in NO_3^- content for the check and manure plots on the second sampling date, relative to the first, presumably resulted from mineralization and subsequent nitrification of soil N.

In the surface layer of the Ap horizon, urease activity averaged over sampling dates differed between fertilizer treatments (Table 2.10). The fertilizer*date interaction

indicated that differences among treatments occurred on the third and fourth sampling dates, following UAN and manure applications (Fig. 2.7a). Comparison of treatment means (LSD = 9.2) indicated the following differences: manure>check>UAN on the third sampling date, and manure>UAN on the fourth sampling date.

In the lower layer of the Ap horizon, phosphatase and arylsulfatase activities averaged over sampling dates differed between fertilizer treatments (Table 2.10). Differences in phosphatase activity among treatments were greatest on the third sampling date, following UAN and manure application (Fig. 2.7b). Analysis of data for that sampling date alone indicated greater activity for the manured treatment compared to the check (LSD = 0.73). While there was an interaction between fertilizer treatment and sampling date for arylsulfatase activity, no single date accounted for much of the difference among treatments (Fig. 2.7c). Differences occurred on the last three sampling dates as follows (LSD = 0.047): UAN>check and manure on the second date; UAN and manure>check on the third date; and UAN>check and manure on the fourth date. Hence, most of the difference in arylsulfatase activity among treatments resulted from greater activity for the UAN treatment.

Of the three enzyme activities, urease was more strongly affected by fertilizer treatment than were phosphatase and arylsulfatase (Table 2.10). There was substantial temporal variation in all six enzyme activities over the growing season. Moreover, sampling date accounted for more variation in enzyme activity than did fertilizer treatment for phosphatase and arylsulfatase (Table 2.10; Fig. 2.7b,c). Water content of the surface layer of the Ap horizon increased over the growing season (Fig. 2.5a), and may have influenced temporal variation of enzyme activities.

2.3.3.2 Effect of Manure Application in 1995

In 1995, analysis of data was restricted to the check and manure treatments. Manure application increased the NH_4^+ content of the surface layer of the Ap horizon over that of the check treatment (Fig. 2.8). It increased NO_3^- content of the surface layer on the second and third sampling dates. Application of manure increased NH_4^+ content of the lower layer of the Ap horizon on the fourth sampling date, over that of the check treatment (Fig. 2.9). It increased NO_3^- content of the lower layer on the second, third and fourth sampling dates.

In the surface layer, dehydrogenase, urease, glutaminase and phosphatase activities differed on some sampling dates, as indicated by the fertilizer*date interaction (Table 2.11; Fig.

10). Manure application increased dehydrogenase activity over that of the check treatment on the second and fourth sampling dates (LSD = 19.9). For urease and phosphatase, the manured treatment had greater enzyme activity than the check treatment on the second sampling date, 2 d after application (LSD = 0.30 and 0.30 for log-transformed urease, and phosphatase activities, respectively). The increase in activity was limited in duration for both enzymes, with no difference between treatments on the third sampling date, 13 d after manure application. Glutaminase activity differed between treatments on the first sampling date, prior to manure application. This difference was be attributed to variation within the sampling area, and confounded comparison of treatments on subsequent dates.

Manure application had no effect on enzyme activities of the lower layer of the Ap horizon (Table 2.11). All enzymes varied temporally over the sampling period (Table 2.11). Moreover, sampling date accounted for most of the variation in enzyme activities. Water content of the Ap horizon tended to decrease over the sampling period (Fig. 2.8a).

2.3.4 DISCUSSION

2.3.4.1 Implications of Temporal Variation of Enzyme Activities in Fertilized Soils for Soil Quality Assessments

Temporal variation in soil enzyme activities, if substantial and unaccounted for, may confound soil quality assessments that assume these measurements to be site characteristics. Cultural practices influence seasonal variation of labile pools of soil C and N (Franzluebbers et al., 1995), and may be expected to influence some soil enzyme activities. The present study indicated that fertilizer practices were one source of temporal variation of some but not all enzyme activities. Application of UAN markedly decreased activity of urease, and increased activity of arylsulfatase. Application of poultry manure increased activity of dehydrogenase, urease and phosphatase. In 4 of 6 cases, the difference in enzyme activity between the fertilizer treatment and the check was transient and did not persist over the sampling period. This behavior was consistent with a study of Nannipieri et al. (1983) where microbial biomass and enzyme activities of soil tended to decrease to indigenous levels following organic amendments. In 2 cases (arylsulfatase in 1994 and dehydrogenase in 1995), differences in activity among treatments persisted over successive sampling dates. In 1995, activity of dehydrogenase increased on the second and fourth sampling dates, but not on the third. This observation may be explained by the fact that dehydrogenase is a measure of

microbial respiratory activity. As such, it is influenced by C substrate supply (hence manure application), as well as other soil properties such as water content that influence microbial growth. Like denitrification, an alternate respiratory pathway in some microorganisms, dehydrogenase activity may also be influenced by antecedent events during the growing season (Bergstrom and Beauchamp, 1993). The absence of an increase in dehydrogenase activity for the manured treatment in 1994 may have resulted, in part, from the elapse of time (36 d) between application and subsequent sampling. Except for urease activity in 1994, fertilizer practice was not the major source of variation in enzyme activities.

Explanations of increased and decreased soil enzyme activity are most often conjectural, based on indirect evidence. There are several mechanisms by which enzyme activity can increase in soils amended with organic materials. Increased enzyme activity (i.e., enzyme content, Galstian, 1974) may result from increased numbers of microorganisms, or induction of enzyme production by a relatively stable population. For example, increased urease activity within the surface layer (0-10 cm) of grassland which received cattle slurry at 480 kg N ha^{-1} y^{-1} for 14 y was attributed to both a larger microbial biomass and increased enzyme production by it (Kandeler and Eder, 1993). Stabilization of enzymes such as urease, phosphatase and arylsulfatase by adsorption to humic material or clay may also increase amounts of extracellular enzymes. Finally, organic amendments themselves may contain considerable amounts of enzymes (Martens et al., 1992; Dick and Tabatabai, 1984).

Explanation of effects of inorganic N fertilizer on enzymes of the soil N cycle is less straightforward, with possible effects on both activity and amount of enzyme. For soil samples collected after fertilizer application, activity of enzymes in the assay mixture may be inhibited by a high concentration of NH_4^+. Alternatively, high concentrations of NH_4^+ may repress enzyme synthesis *in situ*. Thus, Burton and McGill (1992) interpreted an inverse relationship between labile histidase activity and soil NH_4^+ content as indirect evidence for control by NH_4^+ of soil histidase content. Other explanations may emerge from schemes such as that of McCarty (1995), which focuses on the role of glutamine synthetase in regulation of nitrogen metabolism within the soil microbial community. In the present study, increase in arylsulfatase activity from UAN application was unexplained.

Implications of this study for soil quality assessments can be summarized as follows. Additions of organic materials such as manure may temporarily increase activity of enzymes such as dehydrogenase, urease and phosphatase which are involved in mineralization of organic compounds. The duration of the effect on dehydrogenase activity may be influenced by wetting and drying of soil through the growing season (cf. Loro et al.). Application of inorganic N fertilizer may temporarily decrease activity of enzymes such as urease which mediate reactions that produce NH_4^+. Soil quality assessments should avoid sample collection from the surface horizon following manure or fertilizer application. This conclusion assumes that over the longer term stabilization and synthesis of enzymes in soil are controlled by the soil microbial environment (Burton and McGill, 1992), and are indicative of C inputs and soil organic matter content (Fauci and Dick, 1994; Martens et al., 1992).

2.3.4.2 Significance of Temporal Variation in Enzyme Activities for Soil Fertility Analyses

The observation of increased enzyme activities following additions of organic materials to soil raises the question of whether soil enzyme activities indicate rates of mineralization of organic materials. In particular, are dehydrogenase, urease and phosphatase activities indicative of mineralization rates of organic C, N and P, respectively?

Where soil enzyme activities increase following organic inputs, they may be indicative of rates of nutrient cycling (Warkentin, 1995). Kandeler et al. (1994) related increase N mineralization at 0-50 cm beneath slurry-amended grassland to increased activities of enzymes of the N cycle (protease, deaminase and urease). Asmar et al. (1994) reported that increased activity of extracellular proteases in glucose-amended soils had a priming effect on solubilization of ^{15}N-labeled active and non-labeled passive fractions of soil organic N. Sinsabaugh (1994) described other instances where enzyme activities have been used to monitor macronutrient transformations in soils. Yet, relationships between enzyme activities and rates of soil processes have for the most part not been well defined. Thus, Burton and McGill (1992) concluded that biomass-C, an integrative measure of substrate supply, potential biological activity and enzymatic activity, describes net mineral-N production better than do indices of any single step of the mineralization process.

Enzyme activities may be indicative of relative rates of reactions in soil, at small spatial and temporal scales, yet not strongly related to pool sizes of substrates or products. For

example, measurements of NO_3^- and NH_4^+ contents of soil have often been poorly related to rates of denitrification and nitrification, respectively, in part because microsite rather than bulk soil concentrations influence reactions rates (Bergstrom and Beauchamp, 1993; Davidson and Hackler, 1994). Enzyme activities may be more strongly related to measurements of rates of related processes in soils, rather than pool sizes.

In Ontario there is current interest in a soil N test that accounts for potentially mineralizable N, and hot chemical extractants are being used as indices of the latter. In a European study, activities of deaminase and urease were more sensitive than hot-water soluble N as indices of increased N mineralization under slurry-amended grassland (Kandeler et al. 1994). In the present study, inputs of inorganic and organic N resulted in temporal variation in soil enzyme activities (including urease). Further research on use of soil enzyme activities as indices of soil N status is warranted.

2.3.5 CONCLUSIONS

Additions of organic materials such as manure temporarily increased activity of soil enzymes such as dehydrogenase, urease and phosphatase which are involved in mineralization of organic compounds. Application of inorganic N fertilizer temporarily decreased activity of enzymes such as urease which mediate reactions that produce NH_4^+. Soil quality assessments should avoid sample collection from the surface horizon following application of manure or fertilizer. Enzyme activities provide one approach for study of regulation of mineralization of N in soil, in support of more efficient fertilization practices.

2.3.6 ACKNOWLEDGMENTS

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Table 2.10. Summary analysis of variance for enzyme activities of the Ap horizon at Ste-Isidore, 1994 (significant effects only, *P* 0.05).

Variable	df	Dehydrogenase†	Urease	Glutaminase	Phosphatase	Arylsulfatase	$-glucosidase
		percent of total sum of squares					
		layer 1‡					
Fertilizer (F)	2		20				
Date (D)	3		18	42	51	64	62
F*D	6		17				
		layer 2§					
Fertilizer (F)	2				11	6	
Date (D)	3	45	75	44	40	79	21
F*D	6					2	

† data log-transformed.
‡ 0 - 8 cm.
§ 8 cm to the bottom of the Ap horizon.

Table 2.11 Summary analysis of variance for enzyme activities of the Ap horizon at Ste-Isidore, 1995 (significant effects only, *P* 0.05).

Variable	df	Dehydrogenase	Urease†	Glutaminase	Phosphatase	Arylsulfatase	$-glucosidase
			percent of total sum of squares				
			layer 1‡				
Fertilizer (F)	1						
Date (D)	3	56	52	61	41	24	
F*D	3	13	13	8	15		
			layer 2§				
Fertilizer (F)	1						
Date (D)	3	87	68	67		61	30
F*D	3						

† data log-transformed.
‡ 0 - 8 cm.
§ 8 cm to the bottom of the Ap horizon.

Chapter 3

MEASUREMENT OF SPATIAL AND TEMPORAL VARIATION OF SOIL ENZYME ACTIVITIES

3.1 The spatial dependence of soil enzyme activities along a slope[2]

D.W. Bergstrom*, C.M. Monreal, J.A. Millette and D.J. King

3.1.1 ABSTRACT

This study measured the spatial dependence of soil enzyme activities and other properties of the Ap horizon of a Gray Brown Luvisol. Soil samples were collected at 74 positions along a slope following harvest of soybeans (*Glycine max* L.) and fall tillage. Parameters measured were activity of dehydrogenase, urease, glutaminase, phosphatase, arylsulfatase and $-glucosidase; water, organic C, mineral N and inorganic P contents; the light fraction of soil organic matter, and depth of the Ap horizon. Rank correlation indicated strong relationships between water and organic C contents, and urease, dehydrogenase, phosphatase and arylsulfatase activities. Depth of the Ap horizon, water content and arylsulfatase activity were strongly spatially dependent; organic C and inorganic P contents, and phosphatase activity were moderately spatially dependent. Other properties showed little or no spatial dependence. The ranges of spatial dependence were similar for depth of the Ap horizon, inorganic P content and phosphatase activity (approx. 20 m). The range for arylsulfatase activity was 16 m, while that of organic C content was 32 m. The relatively long range estimate for water content (98 m) was influenced by a trend along the slope. Maps of water and organic C contents, and phosphatase and arylsulfatase activities indicated approximately similar spatial patterns along the slope. The magnitude of these soil properties was minimal in the middle or upper portion of the slope, and maximal at the foot-slope. Similarity in spatial patterns along the slope was interpreted as evidence for influence of water and/or organic C content on amounts of phosphatase and arylsulfatase at that scale.

3.1.2 INTRODUCTION

Soil enzyme activities are sensitive indicators of management-induced changes in soil quality (Dick, 1992; Kennedy and

[2]A version of this Chapter has been accepted for publication by the Soil Science Society of America Journal (1998).

Papendick, 1995). Soil enzyme assays have also been used to monitor microbial activity related to specific macronutrient transformations (Sinsabaugh, 1994). In both assessments of soil quality and studies of macronutrient cycling, the spatial variability of measured soil properties must be accounted for at a scale that adequately represents sites or treatments (Groffman and Tiedje, 1991; Parkin, 1993). Geostatistical methods have been used to assess spatial dependence in ecology (Rossi et al., 1992). They are one of several techniques for analyzing spatial and temporal heterogeneity of field soils (Nielsen and Alemi, 1989). For example, Cambardella et al. (1994) used semivariograms to compare the range and degree of spatial dependence of soil properties in two fields. Gonzalez and Zak (1994) measured the spatial autocorrelation of soil properties in a secondary tropical dry forest, then compared maps of soil properties derived by kriging to assess relationships between properties. To date, few studies have measured and compared the spatial variability of soil enzyme activities.

There were two principal reasons for this study. An initial study which compared the effect of management practices on soil enzyme activities in two adjacent fields indicated an influence of slope on enzyme activities and on their sensitivity to management (Bergstrom et al., 1998). To further examine the effect of slope, geostatistical methods were used to measure the spatial dependence of enzyme activities in one field. A second interest lay in understanding the ecological meaning of soil enzyme activities at the field scale. Conventional statistical approaches (e.g. Burton and McGill, 1992; Bonmati et al., 1991) have yielded little information of this kind. Studies in landscape ecology have recently focused on the relationship between spatial pattern and ecological processes (Pickett and Cadenasso, 1995; Allen and Hoekstra, 1992). Spatial patterns of enzyme activities and other soil properties along the slope were compared to identify hypothetical causal relationships at this scale.

The objective of this study was to measure the range and degree of spatial dependence of six soil enzyme activities and related properties along a slope. As well, maps of selected soil properties obtained by block kriging were used to compare spatial patterns along the slope.

3.1.3 MATERIALS AND METHODS

3.1.3.1 Site Description and Sampling Procedure

Soil samples were collected from the Ap horizon of a conventionally tilled field at a site near Rockwood, ON on October 25, 1994, following harvest of soybeans and subsequent tillage prior to seeding winter wheat (*Triticum aestivum* L.). The soil was a Guelph loam (Gray Brown Luvisol), on a simple, single slope averaging 4% over its entire length. The parent material was loamy calcareous till, and the landform was

rolling. The field had been cropped in a rotation of corn (*Zea mays* L.)-soybeans-winter wheat since 1991. In the previous 12 years it had been cropped to continuous corn. Soil samples were collected with an Oakfield split-tube sampler (id 2 cm) at 74 locations along or between two east-west transects running along the slope from the shoulder to the foot-slope (Fig. 3.1). Sampling depth was equal to that of the Ap horizon and was measured at each sampling location. Soil samples were placed into plastic bags, shipped to Ottawa in a cooler with cold-packs, and thereafter stored field-moist at 5EC until analysis.

3.1.3.2 Laboratory Analyses

In the laboratory, soil samples were broken-up by hand and thoroughly mixed before analysis. Water content was determined gravimetrically with 10 g moist soil. Ammonium and NO_3^- plus NO_2^- were extracted from 5 g moist soil with 30 mL 2 *M* KCl. Extracts were filtered through Whatman No. 2 filter paper, then frozen until analysis by TRAACS 800 autoanalyzer, using Technicon Industrial Methods No. 780-86T and No. 782-86T for NH_4^+ and NO_3^- plus NO_2^-, respectively. Dehydrogenase, urease, phosphatase, arylsulfatase and $-glucosidase activities were determined as described by Tabatabai (1982), and L-glutaminase activity was determined by the method of Frankenberger and Tabatabai (1991), with modifications outlined in Bergstrom et al. (submitted). The light fraction of soil organic matter (SOM) was determined by the method of Janzen et al. (1992). A portion of each sample was air-dried, then finely ground (<100 mesh). Finely ground soil was used for determination of total C (combustion by Leco CR-12 Carbon Determinator) and inorganic C (Tiessen et al., 1983) contents. Organic C content was taken as the difference between total and inorganic C contents. Inorganic P was extracted with 0.5 *M* $NaHCO_3$ (pH 8.5), using 1 g soil and 20 mL solution, and measured by the ammonium-molybdate ascorbic acid method (McKeague, 1978). All data were reported based on the oven-dry weight of soil.

3.1.3.3 Statistical Analysis

Spearman rank correlation (SYSTAT, Wilkinson, 1988), a non-parametric test which does not require that data follow any known distribution, was used to identify the strongest relationships among the measured soil properties. GS+ (Version 2.3, Gamma Design Software, Plainwell, MI) was used for geostatistical calculations. Application of geostatistical methods in soils' studies has been described by Trangmar et al. (1985) and Nielsen and Alemi (1989), and for ecological studies by Rossi et al. (1992). The semivariance ( ) was estimated by:

$$\frac{1}{N(h)}$$

$$(h) = \frac{}{2N(h)} \sum_{i=1} [Z(x_i)-Z(x_i + h)]^2 \quad (1)$$

where Z is the measured soil property, x is the sample location, and N(h) is the number of pairs of locations separated by a lag distance h. The semivariogram expresses the relationship between the semivariance ( ) and the lag distance (h). It typically rises from a value at $h = 0$ (identified as the nugget) to a maximum value (identified as the sill). The distance at which the sill occurs indicates the average maximum distance over which samples are related, and is called the range of spatial dependence. For comparisons among soil properties, semivariograms were standardized by dividing each semivariance estimate by the overall sample variance.

Calculation of the semivariance assumes strict stationarity--that there is no trend (change in local means and variance) over the entire sampling space (Clark, 1979; Rossi et al., 1992). Water and organic C contents, and dehydrogenase, urease, phosphatase and arylsulfatase activities followed a trend along the slope, with increasing values towards the foot-slope. Moreover, initial semivariograms of water and organic C contents, and phosphatase activity showed a continual increase in the semivariance with increasing lag distance, to values >1 (Fig. 3.2), which indicated a trend in the data (Clark, 1979; Rossi et al., 1992). Therefore, these variables were de-trended by a procedure similar to that of Berndtsson and Bahri (1995), in which a second-order polynomial was fitted to the data by least squares and then subtracted. Residuals were subsequently used in semivariance analysis for organic C content and dehydrogenase, urease, phosphatase and arylsulfatase activities. Because the semivariogram of residuals of water content showed a pattern similar to that for the original data, the original data were used in calculating the semivariance. For parameters that did not show trends, the fit of data to a normal distribution was tested using the Kolmogorov-Smirnov one-sample test (SYSTAT). For variables with a skewed, approx. log-normal frequency distribution, data were log-transformed before analysis. Outliers can strongly influence semivariance calculations (Rossi et al., 1992). Two outliers were removed from the data-set for inorganic P content and dehydrogenase activity; and one datum was removed for phosphatase, arylsulfatase and $-glucosidase activities. Because most of the 74 samples were collected along two east-west transects spaced 10 m apart, with only 15 samples collected between transects in a north-south direction over short distances (1, 2 or 5 m) (Fig. 3.1), isotropic models were used and it was assumed that there was no anisotropy.

Fitting of semivariograms to data using GS+ has a degree of subjectivity, and it seemed necessary to briefly describe the rationale. The approach used was based on the premise of Clark

(1979) that the larger the number of pairs used in calculating the semivariance at a particular lag, the more reliable the estimate. Size of both the active step and maximum lag influenced the number of pairs used in calculating the semivariance with GS+. Increasing the active step increased the interval over which an average semivariance value was calculated and consequently increased the number of pairs used in the calculation. Thus larger active steps smoothed the semivariogram and reduced noise. At the same time, increasing the active step reduced the number of interval classes and hence the number of points with which to define the curve. All data were initially fit with a maximum lag equal to approx. half the distance along the slope (110-120 m). This restriction assured that all intervals were truly representative of the sampling space, because lags greater than half the maximum distance compare only points on the edge of the sampling region (Rossi et al., 1992). For those properties with a relatively short range of spatial dependence, models were fit with shorter maximum lags (40-60 m) to improve precision of parameter estimates. The active step and maximum lag were varied among soil properties to optimize fit of semivariogram models to data, based on visual inspection and r^2 values. Jack-knife (cross-validation) analysis was also used to test fit of the semivariogram models.

The nugget represents all unaccounted-for spatial variability at distances smaller than the smallest lag, while the semivariogram models the structural spatial dependence (Rossi et al., 1992). Hence, the ratio of the nugget to the sill was used to compare the spatial dependence among soil properties (Cambardella et al., 1994). The smaller the ratio, the stronger the spatial dependence. For selected soil properties, maps were constructed from the semivariograms by block kriging at an interval of 5 m.

3.1.4 RESULTS

3.1.4.1 Descriptive Statistics and Rank Correlation

There was little or no NH_4^+ in the soil samples. Hence this parameter was excluded from statistical analysis. The measured soil properties differed in their variability (Table 3.1). Nitrate and organic C contents, the light fraction, and dehydrogenase, phosphatase and arylsulfatase activities had skewed (approx. log-normal) frequency distributions. Geometric means, standard deviations and CV's were calculated by the method of Rogowski (1972) to characterize these data.

Rank correlation of soil properties was used to identify the strongest relationships (P = 0.001; r 0.375) between the variables (Table 3.2). Water content was strongly correlated with organic C content and all enzyme activities except $-glucosidase. Organic C content was strongly correlated with dehydrogenase, urease, phosphatase and arylsulfatase activities.

Dehydrogenase, phosphatase and arylsulfatase were strongly correlated with each of the other five enzymes.

3.1.4.2 Geostatistical Analysis

Nitrate content, the light fraction, and dehydrogenase, urease, glutaminase and $-glucosidase activities showed little or no spatial dependence at the scale of our sampling grid (data not presented). The other soil properties differed in spatial dependence (Fig. 3.3 and 3.4; Table 3.3). All semivariograms except water content had sill values of approx. 1 (Table 3.3; Fig. 3.3 and 3.4), which is typical for standardized semivariograms. The larger sill for water content (1.35) resulted from a trend along the slope, as previously discussed. Semivariograms of water and organic C contents, and arylsulfatase activity showed moderate fluctuation about the sill (Fig. 3.3). The semivariogram of inorganic P content showed cyclical variation at a period of approx. 40 m (Fig. 3.4a). Semivariograms of depth of the Ap horizon and phosphatase activity also showed approx. cyclical fluctuation at a similar period. This periodicity was attributed to clustering of sampling locations at intervals of 40 m along the slope. It was diminished in semivariograms for subsets of data which excluded some of the clustered sampling locations.

All of the variation in water content was spatially dependent (nugget = 0). Depth of the Ap horizon and arylsulfatase activity were also strongly spatially dependent (nugget/sill = 0.19 and 0.13, respectively). Inorganic P and organic C contents, and phosphatase activity were moderately spatially dependent (nugget/sill = 0.38, 0.52 and 0.46, respectively). Depth of the Ap horizon, inorganic P content and phosphatase activity had similar ranges (approx. 20 m). The range of arylsulfatase activity was 16 m, while that of organic C content was 32 m. The relatively long range estimate for water content (98 m) was influenced by the trend along the slope.

Comparison of maps of depth of the Ap horizon, and water and organic C contents indicated that the surface horizon was thinnest, and water and organic C content were least in the middle of the slope--approx. 110-150 m east (Fig. 3.5a,b,c). Maps of water and organic C contents, and phosphatase and arylsulfatase activities showed approximately similar patterns along the slope, with more definition for water and organic C contents (Fig. 3.5b-e). A common trend was also evident in the semivariograms with original data for water and organic C contents, and phosphatase activity (Fig. 3.2). Water and organic C contents and the two enzyme activities were greatest at the foot-slope position. Phosphatase and arylsulfatase activities were minimal in the middle and upper portions of the slope, at distances of approx. 110-190 m east.

3.1.5 DISCUSSION

3.1.5.1 Influence of Methodology

Results of this study were influenced to some extent by methodology. Analysis of spatial dependence is affected by sample support--in the present study, diameter and depth of the soil cores (Clark, 1979; Bramley and White, 1991). All of the Ap horizon was sampled to provide enough soil for analysis from one core at each location in the field. In this conventionally tilled field the mean depth of the Ap horizon was 21 cm. Under these conditions, variation of most of the measured properties with depth was expected to be small relative to that between sampling locations. In no-till soils, and for enzyme activities such as dehydrogenase and $-glucosidase which decrease with depth in the surface horizon, sample support (depth of sampling) may influence measurements of spatial dependence. The scale of our sampling grid also influenced results. For some of the soil properties which were not spatially dependent, the scale of our sampling grid may have been too large. A grid at spacings of 1 m rather than 10 m may have been more appropriate. As well, a finer sampling grid with more intervals of 1-5 m would have improved definition of the semivariograms at lags shorter than the range for depth of the Ap horizon, inorganic P content and phosphatase and arylsulfatase activities. Location of sampling sites also influenced results. Clustering of sampling locations resulted in periodicity in the semivariograms for inorganic P content, depth of the Ap horizon and phosphatase activity.

Our sampling site was located on a simple, single slope and some soil properties showed a trend along the slope. Because semivariance analysis assumes strict stationarity, we de-trended organic C content and four enzyme activities prior to calculation of semivariance with GS+. An alternative approach would be to calculate the "non-ergodic" correlogram as described by Rossi et al. (1992), to model the lag-to-lag changes without the effect of changes in local means and variances across the sampling space. Their approach would also have removed the periodicity in some semivariograms. A trend will increase the sill and range estimates (Clark, 1972; Rossi et al., 1992). Thus, the larger sill and range estimates of water content compared to organic C content in this study probably resulted in part from the trend in water content along the slope.

3.1.5.2 Interpretation of spatial dependence of soil enzyme activities

Knowledge of the spatial dependence of soil enzyme activities has practical value in two respects: it informs measurement of these soil properties, and it aids interpretation of their ecological meaning at a particular scale. Inasmuch as the range is the average maximum distance over which samples are related,

it estimates the scale (i.e. m, 10's of m or longer) at which a measurement is representative. This information is particularly relevant in assessments of soil quality that use these properties as site characteristics. In the present study, range estimates were similar for phosphatase and arylsulfatase--approx. 15-20 m, and less than that for organic C content (32 m). Other studies have measured the range of spatial dependence for soil biochemical properties and selected enzyme activities (Table 3.4). Ranges for phosphatase and arylsulfatase were comparable to those of N mineralization and nitrification potential in a secondary tropical dry forest (Gonzalez and Zak, 1994), and NO_3^- content in a ryegrass-clover pasture (Bramley and White, 1991). They were larger than the range of nitrification potential in the same ryegrass-clover pasture, and shorter than the ranges reported for biochemical properties of Iowa farmland by Cambardella et al. (1994). As previously discussed, trends in data inflate range estimates and could account for differences in comparisons between sites with different landforms. Finally, comparison of range values indicated that a more intensive sampling scheme is required in field measurements of soil enzyme activities, than for organic C content.

Knowledge of the spatial dependence of soil enzyme activities can also aid interpretation of their ecological meaning. In many studies soil enzyme activities behave as surrogate measures of SOM content. Comparison of the spatial dependence of organic C content and the six enzyme activities tested the fore-going hypothesis. The ranges of phosphatase and arylsulfatase were shorter than that of organic C content, yet of the same order of magnitude (10's of m). Both phosphatase activity and organic C content were moderately spatially dependent, while arylsulfatase activity was strongly spatially dependent. The other four enzymes were not spatially dependent at the scale of our sampling grid. Hence, at this site there was a stronger spatial relationship between organic C content and phosphatase and arylsulfatase, than between organic C content and the other four enzymes. At the same time, because phosphatase and arylsulfatase activities did not behave exactly as organic C content, the three soil properties did not have the same ecological identity. It is noteworthy that rank correlation indicated that arylsulfatase was more strongly related to organic C content than were the other enzymes (Table 3.2). Farrell et al. (1994) also found a strong relationship between arylsulfatase activity and organic C content for soils with differing cultivation histories. The ranges of both phosphatase and arylsulfatase were similar to that of the depth of the Ap horizon (19 m), indicating a possible influence of the latter on these enzyme activities. The similarity in range between phosphatase and inorganic P content also warrants attention, because of regulation of activity and microbial

synthesis of phosphatase by inorganic P content (Spiers and McGill, 1979).

3.1.5.3 Comparison of spatial patterns of soil properties

Maps of water and organic C contents, and phosphatase and arylsulfatase activities showed similar patterns along the slope, notwithstanding the differences in spatial dependence already noted. The spatial pattern of these two enzyme activities was less similar to that of depth of the Ap horizon. Thus, along the slope these two enzyme activities behaved in varying degree as surrogate measures of SOM content. Furthermore, similarity in spatial pattern along the slope indicated influence of water and/or organic C content on amounts of phosphatase and arylsulfatase at that scale. While dehydrogenase and urease activity were not spatially dependent in this study, both properties showed a trend along the slope (for a second order polynomial, r^2 = 0.38 and 0.36, respectively). Water and organic C contents were greater at the foot-slope position because of drainage and erosion. Enzyme activity may increase with organic C content because of the dependence of microbial activity (hence enzyme production) on supply of substrate C. In such cases, organic C content and enzyme activities would be related to each other via microbial biomass. Zelles et al. (1992) found that both organic C content and soil enzyme activities were strongly correlated with microbial biomass as measured by phospholipid fatty acid content in eight soils under different cropping and management practices. Urease, phosphatase and arylsulfatase exist in part as extracellular enzymes in soil. Hence, activity of these three enzymes may increase with organic C content because of greater stabilization of existing enzymes by adsorption to SOM. Water content may influence enzyme activity indirectly by its effect on plant growth and/or microbial activity, although such an effect may be temporal. These three hypotheses remain to be tested in further research. While our study focused upon slope-position, surface geometry at particular slope-positions is also important (Pennock et al., 1994).

Influence of topography on the four enzyme activities may be relevant to precision-farming studies. In fields where variation in SOM content with topography influences crop yield, soil enzyme activities may provide proxy measures of SOM content. Whether topographic variation in specific enzyme activities such as urease and phosphatase is indicative of changes in N mineralization rate or available P content, respectively, remains undetermined. Soil nutrient availability has been shown to vary predictably within catenas in natural ecosystems (e.g. Silver et al., 1994).

Maps also indicated a spatial context in which to test for relationships among soil properties. For example, a relationship between phosphatase activity and inorganic P

content (Spiers and McGill, 1979) may exist within areas of relatively uniform organic C content, as opposed to the entire strip. Cross-correlation could be used to test for relationships at defined lags (Smith et al., 1994), and factorial kriging analysis could be used to characterize sources of variation at different scales (Dobermann et al., 1995). Water content was strongly spatially dependent, and strongly correlated with all enzyme activities except $-glucosidase. Thus, in future studies water content might be used in a cokriging procedure to improve the precision of estimates of the enzyme activities (Zhang et al., 1995).

3.1.6 CONCLUSIONS

Six soil enzyme activities differed in spatial dependence at this site. Arylsulfatase was strongly spatially dependent, phosphatase was moderately spatially dependent, and dehydrogenase, urease, glutaminase and $-glucosidase showed no spatial dependence. There was a stronger spatial relationship between organic C content and phosphatase and arylsulfatase, than between organic C content and the other four enzymes. Similarity in spatial pattern along the slope indicated influence of water and/or organic C content on amounts of phosphatase and arylsulfatase at that scale. Trends in data strongly influenced estimates of spatial dependence of soil properties. Geostatistical methods can be used to compare soil enzyme activities between heterogeneous sites, and to study relationships between soil enzyme activities and ecological processes.

3.1.7 ACKNOWLEDGMENTS

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Table 3.1. Summary statistics for samples of the Ap horizon of a conventionally tilled field collected in the fall (n = 74).

Variable	Mean	Standard deviation	CV (%)
Depth of Ap (cm)	21	4	18
Water content (g g-1)	0.18	0.02	13
Nitrate content (mg N kg^{-1})†	5.3	2.5	28
Inorganic P content (mg P kg^{-1})	12.4	6.1	49
Organic C content (%)†	2.0	0.3	24
Light fraction (%)†	0.46	0.19	35
Dehydrogenase (g triphenyl formazan g^{-1} $24h^{-1}$)†	30	12	12
Urease (g N g^{-1} h^{-1})	30	6	18
Glutaminase (g N g^{-1} h^{-1})	176	22	12
Phosphatase (mole -nitrophenol g^{-1} h^{-1})†	2.3	0.7	30
Arylsulfatase (mole -nitrophenol g^{-1} h^{-1})†	0.54	0.11	17
$-glucosidase (mole -nitrophenol g^{-1} h^{-1})	0.72	0.11	16

† skewed (approx. log-normal) frequency distribution; mean, standard deviation and CV calculated by method of Rogowski (1972).

Table 3.2. Spearman rank correlation coefficients for properties of the Ap horizon of a conventionally tilled field (significant relationships only at $P = 0.001$, n = 74).

Variable	W	C	L	D	U	G	P	S
Depth of Ap								
Water content (W)								
NO_3^- content								
Inorganic P content								
Organic C content (C)	0.62							
Light fraction (L)								
Dehydrogenase (D)	0.67	0.44						
Urease (U)	0.54	0.43		0.49				
Glutaminase (G)	0.44			0.58				
Phosphatase (P)	0.63	0.45		0.59	0.48	0.43		
Arylsulfatase (S)	0.74	0.59		0.65	0.41	0.39	0.65	
$-glucosidase			0.42	0.43			0.53	0.40

Table 3.3. Spatial dependence of properties of the Ap horizon of a conventionally tilled field, as described by a spherical model with standardized semivariograms.

Variable	r^2	Nugget	Sill	Nugget/sill	Range (m)
Depth of Ap	0.86	0.19	0.99	0.19	19
Water content	0.97	0	1.35	0	98
Inorganic P content	0.90	0.45	1.17	0.38	21
Organic C content†	0.83	0.54	1.03	0.52	32
Phosphatase†	0.96	0.49	1.06	0.46	19
Arylsulfatase†	0.78	0.13	1.00	0.13	16

† data de-trended before analysis.

Table 3.4. Estimates of the range of spatial dependence for soil biochemical properties.

Reference	Site description	Property	Range (m)
Bramley and White (1991)	ryegrass-clover pasture, New Zealand	nitrification potential	3.3
		NO_3^- content	12.0
Cambardella et al. (1994)	conventional tillage, Iowa, sampled prior to planting corn	total organic C content	104
		microbial biomass C	46
		respiration rate	68
		NO_3^- content	201
	no-till, Iowa, sampled after harvest of soybeans	total organic C content	129
		microbial biomass C	190
		NO_3^- content	201
		dehydrogenase activity	51
Gonzalez and Zak (1994)	secondary tropical dry forest, St. Lucia	organic C content	71.2
		N mineralization	13.7
		nitrification potential	13.4

3.2 Increased Soil Enzyme Activities Under Two Row Crops[3]

D.W. Bergstrom and C.M. Monreal

3.2.1 ABSTRACT

This study examined influence of the crop on the spatial pattern of soil enzyme activities in a very fine sandy loam strip-cropped to corn (*Zea mays* L.) and soybeans (*Glycine max* L.). Soil samples (0-23 cm) were collected at 20-cm spacings along transects across adjacent plant rows. Activities of dehydrogenase, urease, glutaminase, phosphatase, arylsulfatase and $-glucosidase were compared for soil samples collected from within vs. between crop rows. Spectral analysis was used to test for a cyclical pattern of soil enzyme activities along transects. The spatial pattern of all six soil enzyme activities was sometimes influenced by the crop. Greater enzyme activity in the row than in the furrow was observed in approx. one-third of the cases examined. In approx. two-thirds of the instances where the class comparison indicated greater enzyme activity in the row than in the furrow, enzyme activity appeared to cycle at a period equal to the row-spacing. Periodograms also indicated cycling at other periods, and there was considerable random variation in enzyme activities along the transects. Dehydrogenase and $-glucosidase activities differed between strips of corn and soybeans on some sampling dates. The spatial pattern of soil enzyme activities indicated that they behaved similarly as general indices of microbial activity at the scale of measurement. Comparisons of soil enzyme activities between sites within the growing season should account for influence of the crop.

3.2.2 INTRODUCTION

In studies of the effect of management on soil quality, enzyme activities have been used as general indices of microbial activity for site or treatment comparisons (Dick, 1992; Kennedy and Papendick, 1995). If soil enzyme activities are used as site characteristics, influence of the crop on temporal and spatial variation of soil enzyme activities must be accounted for in the sampling protocol for comparisons made within the growing season. Moreover, in cases where soil biochemical indices are intended to account for rates of macronutrient transformations, it may be argued that the soil volume immediately around the plant roots, as opposed to bulk soil, provides a relevant sample.

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[3]A version of this Chapter has been accepted for publication by the Soil Science Society of America Journal (1998).

In natural ecosystems plants significantly influence soil properties (e.g. Smith et al., 1994; Vogt et al., 1995). Soils have even been considered as biotic constructs resulting from activities of plants and decomposers (Van Breemen, 1993). Influence of the plant on soil microorganisms has been recognized from studies of the rhizosphere. Soil microbial biomass is influenced by plants. For example, Carter and White (1986) found less biomass C and N in the row, relative to the near or mid-row position, under direct-planted silage corn on a sampling date prior to harvest. Conversely, Winter and Beese (1995) found greater microbial biomass C in the row than between the rows on two soils of different textures under a permanent crop of hops (*Humulus lupulus* L.). Production of enzymes such as phosphatase by roots of some plants would be expected to increase activity in the rhizosphere (Ozawa et al., 1995). Yet, there have been relatively few measurements of the effect of plants on soil enzyme activities in the field. Neal (1973) reported that potential phosphatase activity of soil can be altered by growth of certain grasses and forbs. Burton and McGill (1992) found no difference in histidase and protease activities in soil samples from within vs. between rows of barley (*Hordeum vulgare* L.), for six sampling dates. Because labile histidase activity was greater between rows than within the row on the first four sampling dates, growth stage of the crop may have influenced the comparison. Hojberg et al. (1996) showed that the rhizosphere of young barley plants contained greater potential activities (enzyme contents) of NH_4^+-oxidizing, NO_2^--oxidizing, NO_3^--reducing and denitrifying bacteria. The influence of the plant on the spatial pattern of soil physical properties such as water content (Van Wesenbeeck and Kachanoski, 1988) may also indirectly affect microbial ecology and enzyme activities.

Within a field, the crop affects the spatial pattern of soil properties. The study of spatial pattern in landscape ecology provides information about the mechanisms of ecological processes (Pickett and Cadenasso, 1995). Likewise, the spatial pattern of soil enzyme activities within a field may provide information about controls on enzyme activities, hence their ecological meaning, at this scale.

The objective of this study was to test for influence of the crop on the spatial pattern of six soil enzyme activities by (1) comparing activities of soil samples from within vs. between crop rows, and (2) testing for a cyclical pattern of soil enzyme activities along transects across adjacent crop rows. The magnitude of soil enzyme activities under two different crops--corn and soybeans--was also examined.

3.2.3 MATERIALS AND METHODS

3.2.3.1 Site Description and Soil Sampling Procedure

The sampling site was a ridge-tilled field near Bainsville, ON, located on a poorly drained very fine sandy loam (Rego Humic Gleysol). The field was strip-cropped, with alternating strips of corn and soybeans each 6 plant rows wide. Soil samples were collected on three dates in 1995--May 16, June 28 and July 31. On May 16, samples were collected from a strip that had been planted to corn, following soybeans in 1994. Three transects of 5 m length were marked out across the strip, spaced 10 m apart. Twenty-five soil samples were collected at intervals of 20 cm along each transect, using an Oakfield split-tube sampler (2 cm id, 23 cm deep). With this scheme, sample no. 3 and every fourth sample thereafter (no. 7, 11, 15, 19 and 23) were collected from the plant row.

On June 28 and July 31, samples were collected from the corn strip following the same scheme but with transects located 5 and 2.5 m, respectively, north of transects of the first sampling date to avoid disturbed soil. On these two dates, soil samples were also collected from transects located on the adjacent strip of soybeans, which followed corn in 1994. Transects on the soybean strip were contiguous with those on the corn strip. Soil samples were transported to the laboratory in a cooler with cold-packs, and thereafter held at 5EC until analysis.

3.2.3.2 Laboratory Analyses

In the laboratory, soil samples were thoroughly mixed prior to analysis. Water content was determined gravimetrically with 10 g moist soil. Ammonium was extracted from 5 g moist soil with 30 mL 2 *M* KCl. Extracts were filtered through Whatman No. 2 filter paper, then frozen until analysis by TRAACS 800 autoanalyzer using Technicon Industrial Method No. 780-86T. Dehydrogenase, urease, phosphatase, arylsulfatase and $-glucosidase activities were determined as described by Tabatabai (1982), and L-glutaminase activity was determined by the method of Frankenberger and Tabatabai (1991), with modifications outlined in section 2.1.3.2. All data were expressed based on the oven-dry weight of soil.

3.2.3.3 Statistical Analysis

For the purpose of this study, the effect of the ridge itself on soil enzyme activities was assumed to be small relative to that of the plant row and sampling date. For each sampling date, measurements of soil water content and enzyme activities were analyzed as follows. Spearman rank correlation was used to compare the relative strength of relationships between soil properties along each transect (n = 25) using SYSTAT (Wilkinson, 1988). For each transect and enzyme activity, a t-test (small samples with unknown variances assumed equal; Bailey, 1981) was

used to compare two classes--samples in the plant rows, and samples between the plant rows. This class comparison assumed that the influence of the plant was restricted to samples collected from the row, and ignored the position of samples in the furrow relative to the row.

For transects which showed a difference in enzyme activity between samples from the row and furrow, spectral analysis was used to test for cycling of enzyme activity along the transect. The hypothesis tested was that influence of the plant results in a cyclical pattern, with maximum enzyme activity in soil samples from the plant row and a period approximately equal to the distance between plant rows (0.8 m). A spectral analysis was carried out as described by Kachanoski et al. (1985a). For soil enzyme activity E_j [j = 1, 2, ..., N] measured at N discrete equally spaced intervals over a finite distance L, the variance (power) spectrum $S^2(f_K)$ can be estimated by:

$$S^2(f_K) = (2m + 1)^{-1} N^{-1} \sum_{l=-m}^{m} \left(\left[\sum_{j=1}^{N} E_j \cos(2 f_{K+l} j) \right]^2 + \left[\sum_{j=1}^{N} E_j \sin(2 f_{K+l} j) \right]^2 \right) \qquad (1)$$

where f_K = K/N, K = 0, 1, 2, ..., N/2, and m = smoothing coefficient determining the degree of averaging of adjacent independent frequencies and the degrees of freedom for the individual spectral variance estimates. Peaks of the power spectrum indicate the frequencies of the dominant cycles in the series (Nielsen and Alemi, 1989). Unsmoothed periodograms (m = 0) were more useful for detecting peaks at specific frequencies than were power spectra (smoothed periodograms). Periodograms were plotted as $\log(S^2)$ vs. spatial period (m), and used to test for a peak (a local maximum greater than neighboring maxima) at a period equal to the row spacing (0.8 m). Representative periodograms for the last sampling date were included as figures.

Data for the corn and soybean strips of the second and third sampling dates were analyzed separately. When required, enzyme activities were transformed by square root or logarithm for homogeneity of variance before statistical analysis. When strong trends were evident along a transect, the series was de-trended by fitting a second-order polynomial to data by least

squares and then subtracting (cf. Grundmann et al., 1988). Residuals were subsequently used for spectral analysis.

3.2.4 RESULTS

On all three sampling dates, there was no difference in soil water content between samples from the plant row and samples from the furrow (data not presented). The effect of the plant on soil enzyme activities depended in part on growth stage, hence sampling date. Moreover, farming operations influenced soil conditions on the second sampling date, June 28. Therefore, results for each sampling date were presented separately.

3.2.4.1 Transects on the Corn Strip, May 16

On May 16, shortly after planting, corn seedlings had not yet emerged. Along transect 1, there was a strong relationship (P = 0.001) between arylsulfatase and each of urease and glutaminase (r = 0.62 and 0.65); along transect 3, there was a strong relationship between dehydrogenase and phosphatase (r = 0.67). Arylsulfatase activity was greater in the row than in the furrow for all three transects (Table 3.5). For the other five enzymes, activity was greater in the row than in the furrow for only one transect of the three. A peak in the periodogram occurred at a spatial period of 0.8 m for dehydrogenase, urease, phosphatase, arylsulfatase (transect 1) and $-glucosidase.

3.2.4.2 Transects on the Corn Strip, June 28

On June 28, most corn plants had 11 leaves. Fertilizer had been recently knifed-in alongside the corn rows, resulting in a wide range of NH_4^+ contents (0-295 g N g^{-1}). In the urease and glutaminase assays, soil NH_4^+ content was measured on a separate subsample of soil and subtracted from the NH_4^+ content of the assay mixture, to estimate NH_4^+ produced by enzymatic hydrolysis of substrate. Large soil NH_4^+ contents resulted in numerous negative urease values. The variation in soil NH_4^+ content also influenced glutaminase activities. For this reasons, urease and glutaminase activities of the corn strip were excluded from statistical analysis on this date.

There were no strong relationships (P = 0.001) between soil properties along the transects. Dehydrogenase activity was greater in the row than in the furrow for two of three transects, while $-glucosidase activity was greater in the row than in the furrow for only one transect (Table 3.5). A peak in the periodogram occurred at a period of 0.8 m for dehydrogenase activity along one transect.

3.2.4.3 Transects on the Soybean Strip, June 28

Soybean plants had two simple leaves opposite each other on the stem above the cotyledons, then 4 or 5 compound leaves above those. Plants were nodulated and fixing N. The surface soil between the soybean rows had been recently tilled, and was fairly dry.

Strong relationships (P = 0.001) occurred along transect 1 between glutaminase and arylsulfatase (r = 0.65), along transect 2 between phosphatase and each of water content and urease (r = 0.69 and 0.67), and along transect 3 between phosphatase and each of arylsulfatase and urease (r = 0.75 and 0.70). Activity of glutaminase, phosphatase, arylsulfatase and $-glucosidase was greater in the row than in the furrow along transect 1 (Table 3.5). The periodogram of de-trended phosphatase activity was dominated by a peak at a period of 0.8 m, and peaks also occurred at 0.8 m for glutaminase and $-glucosidase.

3.2.4.4 Transects on the Corn Strip, July 31

On July 31, most of the silk on the corn cobs was still green, and anthers were conspicuous on some tassels. Glutaminase activities varied widely between transects. Because this variation may have been due to analytical error, glutaminase activities of the corn strip on this sampling date were omitted from statistical analysis.

Phosphatase was strongly related (P = 0.001) to urease along transect 1 (r = 0.74), and to arylsulfatase along transect 2 (r = 0.75). Arylsulfatase and dehydrogenase activities were greater in the row than in the furrow along three and two transects, respectively (Table 3.5). Urease, phosphatase and $-glucosidase activities were greater in the row than in the furrow along one transect. For dehydrogenase activity along transect 1, de-trended phosphatase activity, de-trended arylsulfatase activity along transects 2 and 3, and $-glucosidase activity, a relatively large peak in the periodogram occurred at a period of 0.8 m.

3.2.4.5 Transects on the Soybean Strip, July 31

On July 31, pods had begun to fill on some soybean plants. Phosphatase was strongly related (P = 0.001) to urease along all three transects (r = 0.70, 0.67 and 0.80), and to arylsulfatase along transects 1 and 2 (r = 0.80 and 0.62). Arylsulfatase was strongly related to urease along transect 1 (r = 0.72), and to $-glucosidase along transect 3 (r = 0.63). Water content was strongly related to phosphatase and urease along transect 1 (r = 0.69 and 0.66), and transect 2 (r = 0.78 and 0.84). Urease, phosphatase and arylsulfatase activities were greater in the row than in the furrow along transect 2 (Table 3.5; Fig. 3.6). For each of these three enzymes, the largest peak of the periodogram occurred at a period of 0.8 m (Fig. 3.7).

3.2.4.6 Differences in Enzyme Activities Under Corn vs. Soybeans

Comparison of soil properties between the corn and soybean strips indicated an influence of the crop on water content, and dehydrogenase and $-glucosidase activities (Fig. 3.8). On both June 28 and July 31 water content was greater under soybeans, while dehydrogenase activity was greater under corn. Activity of $-glucosidase was greater under soybeans than corn on June 28. Phosphatase and arylsulfatase did not differ between crops on either sampling date (data not presented). Because of missing values, urease and glutaminase were omitted from the comparison.

3.2.5 DISCUSSION

3.2.5 1 Influence of the Crop on Soil Enzyme Activities

The crop influenced the spatial pattern of soil enzyme activities in some but not all instances tested. Greater enzyme activity in the row than in the furrow was observed in approx. one-third of the cases examined. In approx. two-thirds of the instances where a class comparison indicated greater enzyme activity in the row than in the furrow, enzyme activity appeared to cycle at a spatial period equal to the row-spacing. Periodograms also indicated cycling at other periods, and there was considerable random variation in enzyme activities along the transects. Finally, for some transects a strong correlation was found between enzyme activities that appeared to cycle along the transect (e.g. urease, phosphatase and arylsulfatase for transect 2 on the soybean strip, July 31).

On the first sampling date, corn had only been recently planted. Thus, greater enzyme activity in the plant row relative to the furrow, when it occurred, probably resulted from influence of the previous soybean crop through residual dead roots and a lingering rhizosphere. Alternatively, greater enzyme activity in the row relative to the furrow may have been due to fertilizer applied with the seed, or physical effects of the ridge on soil biological processes. The latter is unlikely because the ridges were very shallow on this sampling date. Moreover, there was no difference in water content between the plant row and furrow. On the second and third sampling dates greater enzyme activities in the plant row, when they occurred, could be attributed to influence of the growing crop. Greater influence of the crop as the growing season progressed was indicated by larger differences in enzyme activities between row and furrow (Table 3.5), and stronger cycling at the period of the row spacing for the third sampling date, relative to the first and second (data not presented).

Because the field was ridge-tilled, enzyme activities of the soil samples may have been affected by position of the ridge as well as the plant row. The ridges were relatively shallow on

all three sampling dates, and especially on the first date before ridging in mid-June. Samples from the plant row, and in some but not all cases those from locations adjacent to the plant row, were on the ridge; intervening samples were from the furrow between the ridges. Movement of soil from the furrow onto the ridge during ridging would result in a deeper surface horizon on the ridge than in the furrow. The ridges may have been a source of variation at frequencies other than that of the row-spacing in the periodograms.

Differences in $-glucosidase activity between the corn and soybean strips on the second sampling date, and in dehydrogenase activity on the second and third sampling dates also indicated influence of the crop on soil enzyme activities. Greater dehydrogenase activity under corn than soybeans is consistent with greater root production, hence input of organic C, under corn (Coleman and Crossley, 1996). On the last two dates when samples were collected from both the corn and soybean strips, there were more instances of greater enzyme activity in the row compared to the furrow for corn than soybeans (11 vs. 7), which indicated a greater influence of corn than soybeans on soil enzyme activity in general. Greater $-glucosidase activity under soybeans than corn on June 28 but not on July 31 was less easy to account for. It may have resulted from decomposition of corn residues from the previous year on the soybean strip.

Influence of the crop on the spatial variation and magnitude of soil enzyme activities has implications for soil quality assessments based on these measurements. At the very least, for site comparisons within the growing season the position of the soil sample relative to the plant row will sometimes influence the amount of enzyme activity measured. In the present study, mean enzyme activities for samples from the row were larger than those of samples from the furrow by as much as 30%. Variation of this magnitude could affect soil quality comparisons based on these measurements (section 2.1). A sampling scheme that accounts for influence of the crop is preferable to random sampling (cf. Kachanoski et al., 1985b). For example, soil samples could be collected in all cases from either the row or the furrow. For comparisons between different sites, soil samples should be collected at the same time in terms of crop phenology--especially if from the plant row. As well, differences in crop between sites may confound assessments which measure activity of dehydrogenase and $-glucosidase within the growing season.

3.2.5.2 Comparison and Interpretation of Enzyme Activities Along Transects

Influence of the crop on the spatial pattern of soil enzyme activities provided information about their ecological meaning at the scale of measurement. All six enzyme activities were sometimes increased by the crop, albeit on different sampling

dates and on different transects. This fact suggests that, in part, the spatial pattern derived from an effect of the plant on microbial activity in general, rather than on regulation of any specific enzyme at the scale of the microorganism, or production of specific enzymes by roots. Similarly, Hojberg et al. (1996) measured greater potential activities (enzyme contents) of four distinct groups of bacteria (NH_4^+-oxidizing, NO_2^--oxidizing, NO_3^--reducing, and denitrifying bacteria) in the rhizosphere of barley vs. bulk soil. Over short distances between plant rows, soil enzyme activities were behaving as indices of microbial activity. They were also behaving as indices of microbial activity over the growing season, when manifesting temporal variation. Over both scales, spatial and temporal, they were influenced by the plant. This interpretation is consistent with the strong relationships found between the amount of phospholipid fatty acid (a measure of microbial biomass) and enzyme activities for differing soils by Zelles et al. (1992), and behavior of soil enzyme activities in microcosm studies of degradation of xenobiotic compounds added to soil (Felsot and Dzantor, 1995; Pozo et al., 1995) and during composting (Herrmann and Shann, 1993). At other scales, soil enzyme activities may behave more as biochemical indices of soil organic matter content (section 3.1).

Other studies have shown the potential for an effect of the plant on specific enzyme activities, such as increased secretion of phosphatase by plant roots in phosphorus-deficient media (e.g. Ozawa et al., 1995). In the present study, activity of arylsulfatase increased in the plant row relative to the furrow more often than did activity of the other enzymes. This result was unexpected, inasmuch as enzymes related to substrate C supply (dehydrogenase and $-glucosidase), or produced by the plant root (phosphatase) were expected to be most affected by the crop. Whether the crops in our study influenced arylsulfatase activity in some particular manner remains to be determined. Our study was not designed to study regulation of soil enzyme activities in the rhizosphere. In future research coherency analysis could be used to test for relationships between enzyme activity and specific regulators in the vicinity of the plant (cf. Grundmann et al., 1988).

A spatial pattern in enzyme activity deriving from position of the crop in the field also implies that enzymes are produced and degraded in appreciable amounts over the growing season. Burton and McGill (1992) differentiated between labile and stable fractions of soil histidase in a study of controls on enzyme activity and mineralization of organic N. Such an approach may be useful in future studies of soil quality based on enzyme activities.

3.2.6 CONCLUSIONS

The spatial pattern of six soil enzyme activities was in some but not all cases influenced by the crop, with greater activity in the plant row than in the furrow. Dehydrogenase and $-glucosidase activities differed between strips of corn and soybeans on some sampling dates. The spatial pattern of soil enzyme activities indicated that they behaved similarly as general indices of microbial activity at the scale of measurement. Comparisons of soil enzyme activities between sites within the growing season should account for influence of the crop.

3.2.7 ACKNOWLEDGMENTS

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Table 3.5. Comparison by *t*-test of soil enzyme activities measured along a transect across six adjacent plant rows for samples from the plant row vs. samples from the furrow.

Crop/transect		Dehydrogenase	Urease	Glutaminase	Phosphatase	Arylsulfatase	$-glucosidase
		calculated values of *t*					
				May 16			
corn	1	1.60	1.41	2.76*	0.82	3.91***	0.87
	2	0.85	0.95	0.26	0.75	2.68*	2.16*
	3	2.21*	2.79*	1.73	3.99***	2.75*	0.98
				June 28			
corn	1	4.30***	nd†	nd	0.44	1.52	1.35
	2	2.84**	nd	nd	1.26	1.80	2.92**
	3	2.04	nd	nd	1.64	1.05	1.48
soybeans	1	1.63	0.59	3.97***	2.89**	4.02***	2.23*
	2	0.82	1.29	0.60	1.53	1.08	1.53
	3	0.18	0.65	0.84	1.22	1.22	0.22
				July 31			
corn	1	3.82***	1.45	nd	0.51	2.82**	2.99**
	2	3.02**	0.83	nd	1.97	4.90***	0.82
	3	1.97	2.16*	nd	4.16***	3.14**	0.63
soybeans	1	0.33	0.66	0.37	0.55	1.45	0.86
	2	0.92	3.96***	1.86	3.51**	5.02***	0.53
	3	1.14	0.45	1.27	1.26	1.63	0.35

*,**,*** significant at *P* 0.05. 0.01 and 0.001, respectively (df = 23).
† not determined.

Chapter 4

ASSESSING SOIL NUTRIENT MINERALIZATION

4.1 A Method for Monitoring Soil Nutrient Mineralizing Enzymes: N Mineralization Power[4]

C.M. Monreal[1], D. Bergstrom[1], K. Henderson-Dube[2]

4.1.1 ABSTRACT

There is a need to establish methodologies assessing soil health on the basis of agroecological functions. We developed a quantitative method to monitor the impact of crop management on the activity of soil nutrient mineralizing enzymes. The activities of $-glucosidase, urease, L-glutaminase, dehydrogenase, alkaline phosphatase, and arylsulfatase were measured in soil samples taken from A and B horizons at different slope positions in Gleysolic, Luvisolic and Podzolic soils. Soil samples were collected on four different dates during the growing season of corn, soybean and wheat under conventional tillage (CT) and zero-tillage (ZT).

For each soil enzyme, cluster analysis of field data helped define seven levels of activity ranging from very low to very high. Land use and tillage system influenced the activity level of nutrient mineralizing enzymes. Overall, forested soils and conservation tillage systems maintained higher levels of soil enzyme activity than soils cropped with conventional tillage. We found that soil N mineralization power was highly associated with the activities of soil glutaminase and dehydrogenase (r^2= 0.85 $p<0.001$, $n=24$). Quality control charts (QCC) were used succesfully for monitoring large and small shifts in the activities of enzymes associated with N mineralization power and caused by crop growth and tillage system at different slope positions in the field.

[4]A version of this manuscript is being prepared for submision to the Journal of Environmental Quality.

4.1.2 INTRODUCTION

Excessive tillage induces the degradation of agricultural land (Sparrow, 1984). Changes in soil health needs to be diagnosed timely to avoid long-term productivity losses and environmental degradation. The present absence of methodologies to evaluate soil health is, in part, associated with a lack of understanding the soil ecological processes vital in sustaining plant growth and environmental quality (Hatfield and Stewart, 1994).

Soil components may be used as indicators of land health if they control agroecological processes important for plant growth and respond rapidly to anthropogenic disturbances. Soil enzymes play a central role in the transformations of organic C, N, S and P, microbial growth and energy flows through terrestrial ecosystems (Hayamo and Katami, 1985; Casida et. al., 1968; Speir and Ross, 1978; Castellano and Dick, 1991; Prusiner, 1973; Gorin, 1959; Rotini, 1973). The agroecological functions for the six measured soil enzymes are well known and were clearly defined in the General Introduction section. Chapters 2 and 3 demonstrated that soil nutrient mineralizing enzymes were sensitive to tillage system, crop sequence, fertilizer and manure additions. Soil enzymes varied spatially and such variability needs to be taken into account when evaluating the effects of agricultural practices on soil health. Quality control models designed to improve processes of industrial production (Montgomery, 1991) may be used to evaluate the state of soil health by monitoring changes in the activity level of nutrient mineralizing enzymes. Monreal et al., (1998) proposed that soil enzymes involved in nitrogen (N) mineralization may be used as indicators of sustainable agriculture.

Specific objectives of this study were to: a) Define activity levels for soil nutrient mineralizing enzymes in relation to land use and management system; b) Identify sensitive biochemical indicators of nitrogen (N) mineralization as they relate to management practices; and c) Use quality control charts to monitor changes of indices assessing the N mineralization power of cultivated land.

4.1.3 MATERIALS AND METHODS

4.1.3.1 General Approach

The general approach used to develop a method for assessing the status of nutrient cycling involved the following steps: a) Measurement of enzyme activities in soil samples taken from cultivated fields; b) Identification of soil nutrient mineralizing enzymes sensitive to land use and farming practices; c) Application of cluster analysis to define levels of enzyme activity; d) Determine soil enzymes associated with N mineralization power; e) Design and use quality control charts to determine the health state of N mineralization power.

4.1.3.2 Soils and Crop Rotations

The first site near Rockwood, Ontario, consisted of a Gray Brown Luvisolic soil (Loam) with a single slope averaging 4% (Table 4.1). Prior to 1987, the site had been cropped to corn (*Zea mays*) and hay forage including alfalfa (*Medicago sativa*). In 1987, treatments of conventional tillage (CT) and zero-tillage (ZT), herein called the conservation tillage system (CTS), were established on two adjacent plots. The two plots had the same crop rotation of corn-soybean (*Glycine max*)-winter wheat (*Triticum aestivum*). Each field was sampled at three slope positions: shoulder, mid-slope, and foot-slope. Representative soil samples were collected from the Ap horizon (0-8 cm and 8 cm to bottom of Ap) within strips (25 by 220m) in each tillage plot. Soil samples were taken during phenological stages of crops (i.e. early growth, tillering, grain filling, and post-harvest.

The second site near Clinton, Ontario consisted of a complex of Gray Brown Luvisol, Humic Gleysol and Humic Brunisol (Table 4.1). In 1981, the cropped field was divided into a ZT and CT plots (560 by 9 m) along a slope that included two soil Catenas. Catena 1 with fine textured soils (SiCL) occurred in the upper and mid parts of the slope. Catena 2 with coarse textured soil (Fine Sandy Loam) occurred at the lower part of the slope. The site was cropped to corn, soybean and oat (*Avena sativa*) since 1981. Representative soil samples were collected from the Ap horizon (0-8 cm and 8 cm to bottom of Ap) at four slope positions (2 positions in each Catena) during phenological stages of crops and from each tillage plot.

The third site near Bainsville, Ontario located on poorly drained land consisted of a Rego Humic Gleysol (Very Fine Sandy Loam) with a single slope averaging 1 % (Table 4.1). The field was stripped cropped continuously to corn and soybean with ridge tillage. Alternating strips of corn and soybean were 6 plants row wide. A second adjacent field was cropped continuously to corn with chisel plow. Soil samples were taken from the Ap horizon during the spring and growing season of both crops.

The fourth site near Stayner, Ontario consisted of a Grey Brown Podzolic soil (Alliston series)(Table 4.1). Soil samples were taken from the top 15 cm of adjacent forested and cultivated fields. Field 1 (Hay) was cropped for ten years to a mixture of timothy (*Phleum pratense*) and alfalfa (*Medicago sativa*) between 1985 and 1995. Field 2 (Grain) was cropped annually to a mixture of oats and barley for feed during the same period. Field 3 was under forest (Whitebark Pine, *Pinus albicaulis*) located between Fields 1 and 2, and had not been disturbed for the last 50 years. Fields 1 and 2 were amended frequently with cattle manure and fertilized with COOP Triple 15 according to soil test made every 5 yr.

4.1.3.3 Soil Enzyme Assays and Other Analysis

The methods of Tabatabai (1982) with modifications described by Bergstrom et al., (1998a) were used to measure the activity of soil dehydrogenase and urease. The activity of soil L-glutaminase was determined according to Frankenberger and Tabatabai (1991). Ammonium-N was determined by steam distillation or NH_4^+ electrode (Keeney and Nelson, 1982). Organic carbon was measured by dry combustion (Nelson and Sommers, 1982). Carbon dioxide evolved from each soil sample during the experiment was collected in 10 ml 1 M NaOH, and measured according to the method of Anderson (1982). All data is expressed on the oven dry weight of soil. Data were subject to analysis of variance and a Pearson correlation matrix analysis (Wilkinson, 1996).

4.1.3.4 Cluster Analysis and Soil Enzyme Activity Level

We used cluster analysis to detect natural groupings (similarities) of all cases (Romesburg, 1985). All collected data was organized in a matrix consisting of ca. 750 (cases) x 6 (soil enzymes) (Appendix). Distances between cases were

computed using the normalized euclidean distance in Systat (Wilkinson, 1996). Before cluster analysis, raw data for soil ureases and dehydrogenase were square-root transformed for normal distribution. Data for L-glutaminase were normally distributed and not transformed.

Data for each enzyme were grouped into clusters to satisfy two criteria:

i) Maximize the sum of squares (variation) between clusters and minimize the sum of squares within clusters. This was achieved using the algorithms of K-means in Systat.

ii) Obtain a number of adjacent and non-overlapping clusters.

4.1.3.5 Designing Quality Control Charts

We used concepts of statistical process control to develop a methodology for evaluating the power of soils to mineralize N in agricultural land. The quality control chart (QCC) was developed for industrial use by W.A. Shewhart in the 1920's (Montgomery, 1991). Figure 4.1 shows a Shewhart control chart representing the value of a soil quality characteristic (i.e. dehydrogenase activity). Two horizontal lines called the upper control limit (UCL) and lower control limit (LCL) define the in- and out-of control state. Sample points of a process in-control will plot between these limits in a randomized manner. Control limits for each enzyme activity level were established at distances from the center line equal to 3 times the cluster's standard deviation (sigma):

UCL = CL + 3 sigma
LCL = CL - 3 sigma

Upper and lower warning limits (UWL and LCL, respectively) were established at: CL 2 sigma. Points plotting between the control and warning limits indicate that the process may not be operating properly. Concepts and principles of statistical quality control, and patterns indicating an out-of-control state in control charts are discussed extensively by Montgomery (1991). Shewhart control charts were used to detect and provide warning signals of large shifts (3 sigma) in soil enzyme activity. Small changes in soil enzyme activity (< 1 sigma) were

detected with either the cumulative sum (CUSUM), moving average (MA), or exponentially weighted moving average (EWMA) charts (Barnard, 1959; Roberts, 1959; Wilkinson, 1996). Control charts were prepared using the Graphics module of Systat (Wilkinson, 1996), and designed by setting the probability limits at 0.99865 for the UCL, and at 0.00135 for the LCL.

4.1.3.6 Associating Enzyme Activity With N Mineralization

Soil samples (35 g air-dried) sieved to 2 mm were moistened to 80% of water holding capacity and pre-incubated for 10 d at room temperature ca. 18 C. The same unamended samples were subsequently incubated at constant moisture and temperature (20 C) for 28 days in closed 1 liter plastic containers. Quadruplicated samples representing soils for each site were removed destructively every forth day. Enzyme activities, together with ammonium and nitrate (mineral-N)content, and the amount of CO_2 evolved were determined at each sampling time.

4.1.4 RESULTS AND DISCUSSION

4.1.4.1 Defining Levels of Soil Enzyme Activity

Cluster analysis maximized the variability between groups of enzyme activity levels, and defined non-overlapping contiguous clusters. Grouping data into seven clusters satisfied the latter two criteria. Analyses defining lower number of clusters always produced overlapping between the lower and upper boundary values of adjacent clusters. The seven clusters were arbitrarily classified into different soil enzyme activity levels: low (very-low, low), medium (medium-low, medium, medium-high), and high (high, very-high).

Table 4.2 shows the boundaries for the seven activity levels of all six enzymes. For example, a net mineralization of 133 g of NH4-N $g^{-1}h^{-1}$ represents the mean value for the cluster with very low L-glutaminase activity. On the other hand, a net mineralization value of 531.2 g of NH_4-N $g^{-1}h^{-1}$ represents the mean value for the cluster with very high L-glutaminase activity. The standard deviation in each cluster was < 15% of the mean. Table 4.3 shows that the mean value for each enzyme activity level for individual sites were the same as those calculated when data for all sites were pooled together. The latter strongly indicates that data in Table 2 represent common levels of enzyme activity in soils cultivated with different

management systems and developed under different pedogenetic histories.

Clusters 1 to 6 were always made up of cases represented by enzyme activities measured in samples from the Clinton, Rockwood, and Bainsville sites. A seventh cluster (very high activity) was purposely not defined for the Bainsville site due to lower enzyme activities consistently measured at the site over 3 y. The lack of very high enzyme activity at Bainsville may be associated with the site's redox potential. A shallow aquifer underlies the field trials at Bainsville and creates a high water table and frequent periods of moisture saturation, especially in undisturbed forested areas. Under low redox potential and slow flow of groundwater, the rate of microbial growth and associated metabolic processes are reduced (Bohn et al., 1979).

We tested the null hypothesis that cultivated soils and conservation tillage systems (CTS) do not influence soil enzyme activities. Table 4.4 shows the relative distribution of cases for all seven clusters in relation to land use and tillage system. In general, the proportion of soil samples (cases) with high enzyme activity was greater in the forested than in adjacent cultivated soil. Most forested soil samples (>92 % of all cases) had medium to very high enzyme activities. Less than 8% of all cases for all soil enzymes from the forested site had low activitiy. Most soil samples (> 85 % of all cases) taken from the conventionally tilled fields (CT) had low to medium activity for all enzymes. Only a very low proportion (<4 % of all cases) of samples from the soil under CT had high enzyme activities (mostly in soil depositional areas). Greater than 75% of all cases obtained from fields with CTS and ZT consisted of samples with medium to very high enzyme activities. Chapters 2 and 3 showed that higher enzyme activity in soils is associated with higher amounts of active enzyme. Table 4.4 also shows that CT decreased the activity of intracellular L-glutaminase and of dehydrogenase to a larger extent than for urease. The colloidal stabilization of extracellular urease, exudated by plant roots (Juma and Tabatabai, 1978), may help explain the lower reduction in urease activity observed under CT. Data in Tables 4.2 and 4.4 help reject our hypothesis, and show that forested land and conservation tillage systems (CTS) including zero-tillage (ZT) maintained higher enzyme activity levels than CT for all enzymes

at all sites. We further hypothesized that higher enzyme activity relates to greater substrate availabilty.

4.1.4.2 Testing An Agroecological Function for Soil Enzymes

4.1.4.2.1 Sensitivity of Soil Enzymes to Cropping System

Chapters 2 and 3 indicated that soil enzymes were more or as sensitive as organic carbon in reflecting the effects of management on soil health. The ensuing section briefly discusses the relative sensitivity of biochemical and chemical properties to farming practices in the Podzolic soil near Stayner. The properties of soil samples taken from the field cropped to cereals (Grain field) with CT appear more deteriorated than in those taken from the Forest and Hay fields (Table 4.5).

The activities of L-glutaminase, dehydrogenase and urease were more sensitive than organic-C (OC) or pH to indicate changes in soil health. For example, the forest-to-grain ratio for each soil property shows that L-glutaminase activity was 12 times more sensitive than OC in expressing cropping effects (Table 4.5). These results also complement and support those reported on the sensitivity of soil enzymes to tillage system in soil landscapes of Ontario (Chapters 2 and 3). In comparison, Kachanoski and von Bertoldi (1993) reported that tillage system did not affect OC concentration in a soil of Ontario. Kachanoski (1996) reported that ZT significantly decreased the amount of light fraction in the soil landscape of our Clinton site.

4.1.4.2.2 Soil Enzyme Activities and N Mineralization

During the incubation, the activity of soil L-glutaminase was highest in samples of the Forest field, lower in the Hay field, and lowest in the Grain field (Figure 4.2). The same was observed for dehydrogenase and urease (data not shown). Michaelis-Menten kinetics showed that higher levels of activity in samples of the Forest and Hay fields were associated with increased synthesis and preservation of active soil enzyme as indicated by Vmax values (Henderson, 1986). We hypothesized that the combination of tillage and cropping to cereals reduced the pool size of enzymes through increased oxidation of easily metabolizable C and deteriorated living habitats.

Indicators of soil health need to be associated with an agroecological function important to plant growth like N

mineralization (Monreal et al., 1998). We tested the hypothesis that the activities of $-glucosidase, L-glutaminase, urease and dehydrogenase are associated with the power of soil to mineralize N. Table 4.6 shows that N mineralization power was highly correlated with activities of soil dehydrogenase (g TPF $g^{-1}d^{-1}$) and L-glutaminase (g N $g^{-1}h^{-1}$), to a lesser extent with urease (g N $g^{-1}h^{-1}$) (r^2=0.56, p=0.065), or not at all with $-glucosidase (mole -NP $g^{-1}h^{-1}$)(r^2=0.2).

The linear models

$Y = 13.85 + 0.096\ X,$ [n=24] (1)
$Y = 11.68 + 1.084\ X_{2,}$ [n=24] (2)
$Y = 11.64 + 0.786\ X_2 + 0.031\ X,$ [r^2=0.85, n=24] (3)

described the change in soil mineral N (Y, g N g^{-1}) in relation to the activities of L-glutaminase [(X), Figure 4.3], and dehydrogenase (X_2) during the incubation period.

The cycling of N in soils depends on the interactions between active microorganisms, their biochemical processes, their living habitats and abiotic factors. Figure 4.4 is an example of such intricacies in the Grey Brown Podzolic soil. The type of soil habitat (i.e. Forest vs. Hay vs. Grain) regulates the net flux of N from soil organic matter to mineral forms by influencing the respiratory activity of soil microbes and their deamination capacity. The highest mineral-N flux was measured in the forested samples with the highest activities of dehydrogenase and L-glutaminase. Tillage and cropping to cereals reduced the power to mineralize N by decreasing the soil's microbial respiratory and deaminating capacity. Our results support the hypothesis associating enzyme activities with the capacity of soils to mineralize N. We attribute the high N flows to greater availability of metabolizable substrates in samples of the Forest and Hay fields than in samples of the Grain field.
Higher supply of C increases the synthesis of energy sources for microbial growth and thus NH_3 excretion in soils. Our results are consistent with and support those obtained *in vitro* and pure culture of microorganisms (i.e. Prusiner, 1973; Hartman, 1973). The activities of cell glutaminases are controlled by nitrogenous metabolites, presence of energy precursors, divalent cations and carboxylic acids (Prusiner, 1973). Our data obtained with soils and showing the association of L-glutaminase and dehydrogenase with N mineralization power, are consistent with

the pure culture studies conducted in the lab; and strongly support the use of these enzymes as indicators to evaluate a specific ecological function as proposed by Monreal et al., (1998). Thus, L-glutaminase and dehydrogenase appear as useful indicators for assessing the state of soil N mineralization power. Future research studies need to focus on L-glutaminase and dehydrogenase as important model enzymes for further characterizing N mineralization from SOM at the pedon and field level. The potential association of these two enzymes with functions of nitrate leaching, denitrification and N crop uptake also needs to be determined. Noteworthy, the ecological functions of nitrate leaching and denitrification are different from the N mineralization power of soils. Information to determine the effects of C and N inputs in different tillage system, the quantity and quality of soil organic substrates, and the architecture of soil particles on the activities of these two soil enzymes is also needed.

Section 4.1.4.1 defined seven levels of enzyme activity and showed that these levels were the same for soils with different parent material, climate and management. Based on the latter, we used equation (1) to estimate the power of unamended soils to mineralize N on the basis of enzyme activity level. Table 7 shows the range for soil mineralizable N ranged between 27 g N g^{-1} for soils with very low L-glutaminase activity, and 64 g N g^{-1} for soils with very high L-glutaminase activity. We propose Table 4.7 as a simple model to estimate the power of any unamended soil to mineralize N. Future research is warranted on the relations between crop N uptake and the activities of glutaminase and dehydrogenase.

4.1.4.3 Quality Control Charts and Enzyme Activity Level

The most important use of a control chart (QCC) is to improve a given process. High activity level of soil glutaminase and dehydrogenase, and to a lesser extent urease were associated with the power of soil to mineralize N. Thus, building and using QCC is highly desirable to monitor and improve the health of N mineralization in degraded soils, and to prevent health decline of fertile soils. The ensuing sections discusses the use of QCC to monitor enzyme activity in soil landscapes.

4.1.4.3.1 Control Limits for Quality Control Charts

We tested the hypothesis that the establishment of control limits for QCC are influenced by the number of soil samples and the date when samples are taken. Table 4.8 shows that the variability for control limits and sigma values tended to be lower when taking samples in the absence of growing crops. Table 4.8 indicates that control limits in QCC for L-glutaminase may be established by taking as few as 4 soil samples from the 0-8 cm depth. The variability for control limits increased quite significantly when enzyme activities measured during the growing season were considered (n=28). We observed similar trends of the effects of the number of samples on the variability of control limits for other soil enzymes and sites (data not shown).

The contribution of crop growth to L-glutaminase is shown in Figure 4.5. The control limits were defined using 28 enzyme activity measurements made in samples taken in the spring and growing season. The warning signals showed large shifts (> 3 sigma) in glutaminase activity during the growing season of soybean grown with CT in 1994, and of winter wheat under ZT in 1995. The more sensitive EWMA chart of Figure 4.6 statistically confirmed temporal "out-of-control" states defined for soil glutaminase activity during the growing season. Large "upward shifts" in soil dehydrogenase activity were also detected during the grain filling stages of soybean and oat at Clinton (Figure 4.10). The effect of crop growth on soil dehydrogenase was greater than that observed for L-glutaminase (Figures 4.5, 4.6 vs 4.10). The contribution of crop rows on increased soil enzyme activity was well documented for crops growing in the same fields (Chapter 3). The exact mechanisms controlling the shifts in soil glutaminase activity during the growing season are unknown, but may be associated with substrate availability and productiuon of intra- and extra-cellular enzymes in the rhizosphere. Therefore, and in order to remove the contributions made by active crops on enzyme activities, assessments of soil health require testing for enzyme activity in soil alone.

Our data strongly indicate that control limits for QCC may be established by measuring enzyme activity in four soil samples taken in the absence of growing crops either in early spring or late fall (Table 4.8 and Figures 4.5, 4.6 and 4.10). Further, and considering information from Chapters 2 and 3, we suggest that soil sampling for assessing soil health needs to be made in

landscape units having similar characteristics of texture, topography, and hydrology. Present soil test methods for fertilizer use also require taking soil samples during the fall or spring to remove the effects of crop growth on nutrient availability.

4.1.4.4 Monitoring Nitrogen Supply Power in Soil Landscapes

4.1.4.4.1 Monitoring enzyme activity and topography

Quality control charts may be also used for evaluating the effects of tillage system on L-glutaminase activity at different topographic positions. The center line (CL) in Figures 4.5 and 4.7 show that conservation tillage system (CTS) with ZT maintained significantly higher enzyme activity than CT at the shoulder and mid-slope positions, respectively. These results were confirmed by a t-test (data not shown). Noteworthy, the QCC charts show that enzyme activity levels measured in soil samples taken in the spring and before seeding were reproducible from year to year. Figures 4.5, 4.7, 4.8, 4.9 and 4.10 suggest that different steady-state condition for L-glutaminase, urease and dehydrogenase activity level has been attained under both tillage systems as indicated by samples taken in the spring. These temporary steady-state levels consistently expressed in the spring appear to be disrupted by crop growth in an annual cyclic fashion.

Relative to CT, CTS with ZT also reduced the variability in activity between the shoulder and mid-slope position for activities measured in the spring of 1994 and 1995. The mean glutaminase activity under ZT was 332 (g N g^{-1} h^{-1}) for the shoulder slope, and 329 (g N g^{-1} h^{-1}) for the mid-slope position. The mean glutaminase activity under CT was 250 (g N g^{-1} h^{-1}) for the shoulder, and 216 (g N g^{-1} h^{-1}) for the mid-slope position. Greater carbon supply and energy flow together with better aggregate architecture under CTS may help increase the synthesis and preservation of L-glutaminase or other soil enzymes. This hypothesis is supported by greater amounts of enzyme found under CTS than under CT (Chapters 2 and 3). Excesive tillage reduces carbon storage and destroys living habitats for soil organisms (Monreal and Kodama, 1997). Consequently, CT appears to decrease the rate of enzyme synthesis and increase the rate of enzyme catabolism. According to Tables 4.2 and 4.7, higher soil glutaminase activity under ZT results in higher power to

mineralize N. Thus, implementation of CTS and 12 y of ZT resulted in a more fit soil for supplying N to crops.

4.1.4.4.2 Monitoring enzyme activity in soil catenas

The QCC were also used to monitor the activities of dehydrogenase and urease in two soil catenas. Catena 1 consists of fine textured soils located in upper slope positions, and Catena 2 consists of coarse textured soils located in lower slope positions (Table 5.1). The QCC for both soil enzymes were also designed to remove the contributions made by crop growth (Figures 4.8 and 4.9). The QCC of Figure 4.9 and a t-test showed that higher urease activity was maintained under ZT than under CT only in Catena 2. These results are attributed to the presence of a larger free urease pool in coarse than in fine textured soil, and higher protein turnover in soils under CT than under ZT. In comparisson, a t-test, showed that the activity of dehydrogenase was greater in soil with ZT than with CT in Catena 1, but not in Catena 2 (data not shown). Stabilization of soil enzymes by clay colloids in fine textured soils of Catena 1 may also help explain these results. Kinetic studies showed that the amount of urease in soil of Catena 1 was higher under ZT than under CT (Chapters 2 and 3). Higher dehydrogenase activity indicates more active and healthier soil microbial populations under ZT than under CT. Lack of soil disturbance by tillage creates better living habitats and enhances the population size of soil microbes (Monreal and Kodama, 1977).

Our results indicate that QCC may be used to quantify the temporal and spatial variability caused by management on enzyme activities associated with N mineralization. Because crop growth influences soil enzyme activity, control limits in QCC need to be established with data obtained from uncropped soils in early spring or fall. Noteworthy, control limits in QCC charts to monitor enzyme activities may be defined on the basis of economic and environmental goals, and not just the 3 sigma principle (Montgomery, 1991). The establishment of control limits based on economic and environmental targets require further research.

We also predicate that differences in the level of soil enzyme activity reflect differences in the health status of cultivated soils. We provide two reasons supporting the latter statement.

First, a large number of studies (molecular, cell culture and soils) have shown that our measured soil enzymes regulate nutrient and microbial activities in terrestrial ecosystems (see General Introduction). Second, low enzyme activity level were paralleled by poor structural stability in the same soils (Chapter 5). Highest enzyme activity level and microbial population size, and soil structural stability are always found in undisturbed soils under forest, sod or zero-tillage. Therefore, high soil enzyme activity level reflect land with a high capacity to mineralize nutrients for plant growth, and healthier soil microbial populations. High enzyme activity level and soil structural stability as defined in Chapter 5 represent high states of soil health. Therefore, low enzyme activity and structural stability appear to represent low state of soil health.

4.1.5 CONCLUSIONS

This study permits the following conclusions:

1. Land use and tillage system influenced the activity of nutrient mineralizing enzymes. Forested soils and CTS maintained higher levels of soil enzyme activity than soils cropped with conventional tillage.

2. The activities of L-glutaminase and dehydrogenase were highly associated with the power of soil to mineralize N, and are useful to asess the function of N mineralization.

3. Quality control charts are useful techniques to evaluate and monitor large and small changes in activity level of enzymes associated with nutrient cycling processes in soil landscapes.

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Table 4.1. Soil physical and chemical characteristics of the 0-8 cm depth at four sites in Ontario.

Slope position	Sampling station[1]	Soil	pH ($CaCl_2$)	TC[2] (%)	Soil texture (g kg^{-1}) Sand	Silt	Clay
		Site 1, Rockwood					
Shoulder	NT	Gray Brown Luvisol	7.0	3.1	419	424	157
	CT	Gray Brown Luvisol	7.2	1.57	451	404	145
Mid-slope	NT	Gray Brown Luvisol	-	1.84	-	-	-
	CT	Gray Brown Luvisol	-	2.14	-	-	-
Foot-slope	NT	Gray Brown Luvisol	-	2.63	-	-	-
	CT	Gray Brown Luvisol	-	2.10	-	-	-
		Site 2 - Clinton, Catena 1					
Mid-upper	CT-107	Orthic Gr. Br. Luvisol	7.4	1.87	210	450	340
Upper	CT-108	Orthic Gr. Br. Luvisol	7.3	2.27	170	460	370
Mid-upper	NT-207	Orthic Gr. Br. Luvisol	7.4	1.97	180	450	380
Upper	NT-208	Orthic Gr. Br. Luvisol	7.2	2.25	130	500	370
		Site 2 - Clinton, Catena 2					
Lower	CT-102	Gleyed Brunisol	7.2	3.72	670	240	90
Mid-lower	CT-103	Gray Brown Brunisol	7.3	3.51	620	250	130
Lower	NT-202	Orthic Humic Gleysol	7.1	2.91	720	210	80
Mid-lower	NT-203	Orthic Humic Gleysol	7.2	3.82	590	270	140
		Site 3 - Bainsville					
1-2 %	RT	Rego Humic Gleysol	6.4	2.12	616	282	102
		Site 4 - Stayner					
<1 % slope	Forest	Grey Brown Podzolic	5.5[3]	3.21	sandy loam[4]		
	Hay	Grey Brown Podzolic	6.4	1.64			
	Grain	Grey Brown Podzolic	5.5	1.39			

1. CT= conventional tillage, NT= zero-tillage, RT= ridge tillage. 2. TC= total carbon. 3. soil pH at Stayner was measured in water (2:1 water-to-soil ratio). 4. Soil texture was determined by hand.

Table 4.2. Seven activity levels determined for soil enzymes in the A1 and A2 layers[1] of the A horizon at all sites.

		Enzyme activity in cluster number						
		1	2	3	4	5	6	7
Soil enzyme	Statistics	Very low	Low	Medium low	Medium	Medium high	High	Very High
L-glutaminase	Mean	133.33	198.48	252.27	298.9	350.13	423.23	531.19
(g NH_4-N g^{-1} h^{-1})	St. dev.	25.4	16.8	14.1	14.1	16.4	26.0	47.5
	n[4]	58	152	160	153	108	54	16
Dehydrogenase	Mean	7.80	17.66	30.27	47.29	68.44	103.85	180.77
(g TPFg^{-1} d^{-1})[2]	St. dev.	2.82	3.26	4.08	5.89	7.82	13.48	28.34
	n	63	144	138	144	144	95	30
$-glucosidase	Mean	0.34	0.62	0.86	1.13	1.47	1.88	2.39
(mole p-NP g^{-1} h^{-1})[3]	St. dev.	0.09	0.07	0.07	0.09	0.11	0.12	0.24
	n	53	156	177	177	95	67	35
Alkaline phoshatase	Mean	1.48	2.37	3.37	4.47	5.63	7.62	10.48
(mole p-NP g^{-1} h^{-1})	St. dev.	0.29	0.27	0.29	0.33	0.40	0.67	1.35
	n	81	166	189	118	100	81	26
Arylsulphatase	Mean	0.16	0.34	0.51	0.71	0.95	1.34	2.07
(mole p-NP g^{-1} h^{-1})	St. dev.	0.06	0.05	0.05	0.06	0.08	0.15	0.32
	n							
Urease	Mean	10.31	19.10	29.82	42.40	61.70	90.97	151.30
(g NH_4-N g^{-1} h^{-1})	St. dev.	2.73	2.71	3.18	4.31	7.12	11.43	19.13
	n	47	101	200	203	132	69	8

1. A1=0-8 cm, A2=8 cm to bottom of Ap or Ah.
2. TPF=triphenylformanzan
3. p-NP=p-nitrophenol
4. n=number of cases

Table 4.3. Mean L-glutaminase activity measured during 1994 and 1995 in the A horizon (A1 and A2 layers)[1] at all sites.

Cluster	Activity level	L-glutaminase activity (g NH_4-N g^{-1} h^{-1})			
		All sites	Rockwood	Clinton	Bainsville
1	Very low	133	134	132	132
2	Low	198	200	197	195
3	Medium low	252	260	256	244
4	Medium	299	308	321	295
5	Medium high	350	363	428	346
6	High	423	438	511	414
7	Very high	530	516	564	a.n.d[2]

1. A1=0-8 cm, A2=8 cm to bottom of Ap or Ah horizon.
2. a.n.d = activity not detected

Table 4.4. The relative distribution of enzyme activity levels as influenced by land use and tillage system in the A1 and A2 layers[1] of the A horizon at all sites.

Activity level[2]	Relative distribution as a proportion of the total number of cases (%)								
	Dehydrogenase			$-glucosidase			Arylsulfatase		
	CT[3]	CTS[4]	Forest	CT	CTS	Forest	CT	CTS	Forest
Low	44	21	8	48	21	0	24	7	4
Medium	41	39	17	48	49	28	64	48	4
Med. high	11	24	17	3	18	15	9	33	7
High	4	16	58	1	12	57	3	12	85
No. of cases	256	419	83	254	425	81	255	423	81
	L-glutaminase			Urease			Alkaline phosphatase		
Low	58	19	3	21	21	6	51	26	3
Medium	40	51	27	70	48	31	41	48	43
Med. high	0	22	28	9	21	23	7	17	12
High	2	8	42	0	10	40	1	9	42
No. of cases	230	396	75	228	448	84	256	421	84

1. A1=0-8 cm; A2=8 cm to bottom of A horizon.
2. Low activity level includes all cases with very low and low activity; Medium level includes all cases with medium-low and medium activity; and High level includes all cases with high and very high enzyme activity.
3. CT=conventional tillage.
4. CTS= conservation tillage system includes zero-tillage.

Table 4.5. Chemical and biochemical properties in the A horizon (0-15 cm) of a Podzolic soil at three adjacent sites near Stayner, Ontario.

Soil property	Site			
	Forested	Hay	Grain	Forest/Grain
pH (H_20)	5.5	6.4	5.5	1.0
Organic-C (%)	3.2	1.6	1.4	2.3
Dehydrogenase (g TPF $g^{-1}d^{-1}$)	29.4	14.2	18.5	1.6
L-Glutaminase (g NH_4-N $g^{-1}h^{-1}$)	340.6	176.4	13.4	25.4
Urease (g NH_4-N $g^{-1}h^{-1}$)	24.2	5.4	4.7	5.1
$-glucosidase (mole p-PNP $g^{-1}h^{-1}$)	0.5	0.5	0.4	1.3

Table 4.6. Pearson correlation matrix for biochemical properties measured during a 28 d incubation of A horizon (0-15 cm) samples of a Podzolic soil.

	Time[v]	NH_4-N	NO_3-N	Min.-N	CO_2	Ure[z]	Gluta[y]	Dehy[x]
Time	1.00							
NH4-N	-0.52	1.00						
NO3-N	0.56	-0.24	1.00					
Min.-N	0.37	0.15	0.92**	1.00				
CO_2	0.58	-0.29	0.84**	0.74**	1.00			
Ure[z]	0.39	-0.27	0.66*[w]	0.56	0.56	1.00		
Glut[y]	0.16	0.08	0.83**	0.87**	0.55	0.56	1.00	
Dehy[x]	0.11	0.28	0.79**	0.91**	0.63*	0.43	0.89**	1.00

z. Ure= urease
y. Gluta= L-glutaminase
x. Deh= dehydrogenase
w. *= 0.01 p 0.05; ** = p 0.01; n=24
v. Time=days of incubation

Table 4.7. The estimated amount of mineralizable N in the A horizon for the seven levels of enzyme activities defined in Table 4.2.

		Enzyme activity and N mineralization power		
Activity level[1]		Minimim	Mean	Maximum
Very low (1)	Glutaminase[2]	64	133	166
	Mineral-N[3]	20	27	30
Low (2)	Glutaminase	167	198	225
	Mineral-N	30	33	35
Medium-low (3)	Glutaminase	226	252	275
	Mineral-N	36	38	40
Medium (4)	Glutaminase	276	299	324
	Mineral-N	40	43	45
Medium high (5)	Glutaminase	325	350	386
	Mineral-N	45	47	51
High (6)	Glutaminase	387	423	475
	Mineral-N	51	54	59
Very high (7)	Glutaminase	484	531	669
	Mineral-N	60	64	78

1. Number in brackets for activity levels correspond to cluster number in Table 4.2.
2. Glutaminase units are expressed in g NH_4-N $g^{-1}h^{-1}$
3. Mineral-N units are expressed in g N g^{-1} soil. Mineral N was estimated from the equation: 13.853 + 0.096 X, where X is glutaminase activity

Table 4.8. The infuence of the number of soil samples and date of sampling on the control limits determined for a quality control chart of L-glutaminase. Soil samples were taken from the 0-8 cm of the Ap horizon at the upper slope position with zero-tillage during the spring and growing season (with crop) and in the spring or fall (no crop) at Rockwood.

Control	Number of samples (with crop)				Number of samples (no crop)		
Limits[1]	4	6	8	28	4	6	8
CL	338.12	325.08	318.24	326.71	338.12	339.44	347.28
Sigma	30.05	31.18	29.36	52.90	30.05	48.88	45.26
LCL	247.97	231.54	230.16	168.02	247.98	192.80	211.51
LWL	278.02	262.72	259.52	220.92	278.02	241.68	256.76
UWL	398.22	387.44	376.96	432.50	398.22	437.20	437.80
UCL	428.27	418.62	406.32	485.40	428.27	486.08	483.05

1. CL=center line; Sigma=standard deviation; LCL=lower control limit; LWL=lower warning limit; UWL=upper warning limit; upper control limit.

4.2 Guidelines for Implementing Quality Control Charts (QCC)

Section 4.1 showed that QCC may monitor changes in nutrient mineralization capacity of soils. Programs monitoring soil health at the farm need to consider QCC not just for process surveillance, but also as a tool decision making to improve soil processes and productivity.

4.2.1 General comments on using QCC for assessing nutrient mineralizing enzymes

We recommend the use of control charts for individual cases (i.e. soil samples). Control charts for individual cases are appropriate for monitoring the health of agricultural soils because data become available more slowly (spring or fall of each year); are cost effective ($3/sample, only four soil samples per representative area in the field are needed). Corrective action to a problem can be taken rapidly before waiting to collect a large number of soil samples, thus preventing further soil or environmental degradation.
In addition, because soil nutrient mineralizing enzymes are spatially and temporally dependent (Chapters 2 and 3), we recommend the preparation of separate control charts for individual areas within fields. Noteworthy, the set of control limits values in Appendix Tables A1, A2, and A3 may be used for monitoring purposes if the mean enzyme activity measured for a representative area plot within 0.5 sigma units from the cluster's CL. We recommend the use of standard deviation values of the appendix for designing control charts assessing the state of soil enzyme activities in soils or areas where available data is limited or non existant.

QCC enable farm producers to rapidly implement changes in management to stop out-of-control processess. The QCC techniques may be easily adopted by farm producers as an integral part in

the production of agricultural commodities, and by policy makers designing programs securing the health of agricultural land.

4.2.2 Specific comments for using QCC in soil health assesments

We propose the following general guidelines for implementing QCC at the farm level:

1. Select spatially representative areas within fields on the basis of topography, soil texture, parent material, and hydrology. For fields with heterogeneous soil properties choose areas represented by soil catenas with similar management.

2. Use Shewhart control charts for individual cases to detect large shifts (3 sigma) in soilenzyme activity together with a moving-range chart like EWMA, CUSUM, or MA to detect small shifts (< 1 sigma) in soil enzyme activity.

3. Measure the activity of the nutrient mineralizing enzyme (i.e. glutaminase) in 4 soil samples taken from the 0-8 cm or the whole Ap horizon in each representative area. Soil samples must be taken in the spring or early summer before active crop growth, or in the fall after harvest.

4. Set the control and warning limits in the QCC for the soil nutrient mineralizing enzyme at the farm.

5. Plot the enzyme activity data in the chart and analyse temporal trends. This may be a cooperative effort between the farmer and a professional. Professional advice need to be provided by trained agricultural extensionists, private consultants, service labs or soil scientists. Preparation and analysis of control charts may be done manually or with the help of computer software.

7. Take management action to improve the desired nutrient mineralizing function (i.e. N mineralization) if needed. For example, reduce the frequency of tillage or implement zero-tillage to increase enzyme activity improving nutrient mineralization. Introducing a hay crop in the rotation, manure and fertilizer addition all tend to enhance enzyme activities, as inicated by Chapters 2, 3, and 4.

Chapter 5

ASSESSING SOIL STRUCTURAL STABILITY

5.1 A Method for Evaluating the Status of Soil Structure Stability[5]

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5.2 ABSTRACT

There is a lack of methodologies for assessing the health of agricultural land. A quantitative method was developed to evaluate structure stability in soils cropped to corn and soybean, and in undisturbed soils under forest and sod. Soil samples were taken from the A and B horizons at two farms, one near Clinton and the other near Wodslee, Ontario. Soil structure stability was evaluated against slacking, and the dispersive and dissolution forces of water by measuring water stable aggregates and soil lipids extracted with chloroform ($CHCl_3$) and diethyl ether (DEE). A method using principal component analysis (PCA) integrated soil chemical and physical properties into a vectorial scale defining the relative status of soil structure stability. Results indicate that soil extractable lipids were sensitive indicators evaluating the effects of management on soil structure stability. Soils under forest and sod had the highest relative structure stability (83-100%), and soils cropped with CT had the lowest relative soil structural stability (<20 %). Cropping with no-till (NT) helped reduced soil structure deterioration by preserving soil lipids and protecting aggregate stability. The scale defining the relative status of soil structure stability appears is a reliable method to assess the impact of management on the stability of soil structure in soil landscapes.

[5]A version of this Chapter has been submitted to Geoderma for publication (1998).

5.3 INTRODUCTION

There is a lack of methodologies for assessing the health of agricultural land. This limits our abilities to secure the long-term sustainability of agriculture. The absence of methodologies is due in part to a lack of understanding soil ecological processes vital in sustaining plant growth and environmental quality (Hatfield and Stewart, 1994). Timely diagnosis of soil degradation is essential in preventing further resource and environmental deterioration. Soil organic matter (SOM) together with physical properties have been proposed as indicators of soil quality (Larson and Pierce, 1991). A key ecological process influencing crop growth is the stabilization of soil structure (Monreal et al., 1998). Soil structure refers to the size, shape and arrangement of aggregates and pore size. Soil structure influences plant growth, the movement and storage of air, nutrient cycling, and living habitats of soil organisms. The condition of soil structure may be indicated through measurements of aggregate stability and the non-limiting water range (Gregorich et al., 1993; Topp et al., 1995). Aggregagte stability, however, does not always correlate with the total content of SOM (Malik et al., 1965), but more often relates to specific classes of soil organic molecules like long-chain aliphatics and inorganic constituents (Dinel et al., 1991, 1997; Monreal et al., 1995a; Nwadialo and Mbagwu, 1991). The quantity and quality of organic matter in agricultural land is influenced by land use, crop rotation and tillage system among other factors (Monreal et al., 1995a,b; Schlesinger, 1986). It was hypothesized that tillage affects the content of long-chain aliphatics, and that change in their content influences the stability of soil structure. The objectives of this study were to: a) test the effects of land use and tillage system on the content of soil extractable lipids, and b) develop an integrated method assessing the relative status of soil structure on the basis of soil lipid content and aggregate stability.

5.4 MATERIALS AND METHODS

5.4.1 Soils and Cropping Systems

The study site was located near Clinton, Ontario (lot 18 concession XV), and consisted of a Gray Brown Luvisol and Humic

Gleysol complex. Based on settlement history, the site was broken in 1860's and cropped to forage and annual crops with conventional tillage until 1980. No-till (NT) and conventional tilled (CT) treatments were established in two plots (561 by 9 m each) along two soil catenas in 1981. The plots were oriented in a NW to SE direction. Each tillage plot was cropped to a three year rotation, including maize (*Zea mays*), soybean (*Glycine max*), and winter wheat (*Triticum cereale*). Winter wheat was sporadically replaced by oat (*Avena sativa*).

Table 1 shows the properties for two soil catenas at Clinton. Catena 1 consisted of fine-textured soils (SiCL) found in the upper slope positions of the landscape. Catena 2 consisted of coarse-textured soils (FSL) found the lower slope positions of the landscape. The field was divided into NT and CT along the gradient of soil texture. At Clinton, soil samples were taken from the field cropped to corn at four slope positions along the textural gradient in the fall of 1994 (Table 1). At each position, four soil cores (2.5 cm dia.) were taken, pooled and mixed thoroughly to obtain one representative sample. Soil cores were divided according to their genetic horizon into 0-8 cm (A1), 8 cm to the bottom of the A horizon (A2) (approx. 25 cm deep), and the top 10 cm of the B horizon (B). Soil samples were air-dried, ground to pass a 2 mm sieve and stored before chemical analysis.

The field trials at Woodslee were established in 1946 on a fine textured Orthic Humic Gleysol (Brookston series). Management treatments consisted of fertilized (F.M) and unfertilized (UF.M) maize grown with CT; fertilized (F.S) and unfertilized (UF.S) sod; and a wooded plot (W). Maize residues were ploughed under in the fall of each year whereas bluegrass residues were left on the soil surface. Four replicate soil samples were collected from the Ap1 and Ap2 layers of each plot, air-dried and passed through a 2-mm sieve (Dinel and Gregorich, 1995).

5.4.2 Soil Lipids and Soil Structure Stability

A methodology was designed to determine the relative status of structure stability in soils of Ontario. The approach included collection of soil samples from farm fields, measuring the

content of extractable soil lipids and the stability of soil aggregates in the lab. Principal component analysis (PCA) assisted in the integration of chemical and physical data to produce a relative scale of structure stability in relation to tillage and topographic properties of the landscape.

5.4.3 Soil Lipid Extraction

Soil lipids were extracted according to Dinel et al., (1996b), and fractionated into easily biodegradable (diethyl ether extractable) and more bioresistant (chloroform extractable) fractions. Briefly, 30 g of air-dried soil sample was sequentially extracted for 20 h with 150 mL diethyl ether (DEE), followed by an 18 hr overnight extraction with 150 ml of $CHCl_3$ in a Soxhlet extractor. Each extract was dried in a rotary evaporator at 45° C to remove most of the solvent, and then in a vacuum oven at 30° C for 5 days. All data was expressed on oven dry weight of soil samples.

5.4.4 Aggregate Stability and Structural Properties

Moist field soil samples were air-dried at room temperature. Air-dried soil samples were forced through a 2-mm sieve, and sieved to retain aggregates > 150 µm. Each aggregate sample was subject to the stability test of Dinel et al., (1991a). Briefly, the proportion (Rw) of soil aggregates stable to implosion (slacking) was determined by wet sieving after sudden immersion in water for 5 minutes. A separate aggregate sample was pretreated with ethanol for 30 min to minimize effects of slacking on dried aggregates, and immediately wet sieved to determine the proportion (Re) of stable aggregates resistant to the forces of dispersion and dissolution of water.

The same aggregate stability tests were carried out after unbounds lipids (u) were extracted with chloroform. A schematic diagram of the aggregate stability test is described by Dinel et al., (1997). From these stability tests, the aggregated materials were distributed into five fractions corresponding to the destructive forces (i.e. slacking, dissolution and dispersion), and to the unbound lipids and bound organic materials (Dinel and Gregorich, 1995). The proportion of stable

aggregates remaining after wet sieving was expressed on oven-dry weight basis. In this article, UAt1 and UAt2 represent the proportion of unstable aggregates due to the dispersive and dissolution action of water, and slaking, respectively.

The aggregate stability tests conducted after lipid extraction with $CHCl_3$ were used to evaluate the effects of unbound lipids on the proportion of water stable aggregates (Sat) resistant to the dispersive and dissolution action of water (SAu1), slacking (Sau2) and SAb representing the remaining stable aggregates associated with bound organic and inorganic materials.

5.4.5 Stastitical Analysis

Principal-component analysis (PCA) was utilized to find the most significant descriptors for structural properties: these analyses were performed with PRINCOMP (SAS Institute Inc., Cary, NC), and the conceptual approach of Gabriel (1971) and Jolicoeur and Moismann (1960).Input data for PCA analysis was obtained from fine textured soils at Clinton and Woodslee. Data from undisturbed wooded site were used as a reference to describe the relative stability of structure in cultivated soil landscapes. In order to keep consistency of the input data, we ommitted data for the coarse soil in Catena 2 due to a lack of available information on other coarse textured soils similar to that of Catena 2.

5.5 RESULTS AND DISCUSSION

5.5.1 Tillage Effect on Soil Extractable Lipids

In general, the amounts of total soil extractable lipids (TEL=DEE+$CHCl_3$) in conventional (CT) and no-till (NT) systems tended to decrease with soil depth (Figure 5.1). In Catena 2, TEL increased with depth by about 30 % in samples from the NT system, and either increased or decreased in samples from the CT system. In Catena 1, TEL decreased with depth in soil with CT and NT systems. Some of the observed differences in TEL content with depth may be associated with the amounts of organic residues added to the surface layer of the A horizon and frequency of soil tillage, rate of lipid decomposition, and physico-chemical reactions stabilizing lipids in soils.

For both tillage systems, amounts of DEE-extractable soil lipids are generally lower in Catena 2 than in Catena 1, especially that present in the top 10 cm of the B horizon. DEE-extractable lipids were slightly higher in samples of the no-till (NT) than in samples of the CT plot in Catena 1. No clear trend was observed for the vertical distribution of DEE-extractable lipids in horizons from both catenas (Figure 5.1). The coefficient of variation for DEE-extractable soil lipids was about 32.5 % in samples of Catena 1, and 21 % for samples in Catena 2.

Amounts of $CHCl_3$-extractable lipids tended to decrease with depth. This decrease was more pronounced in Catena 2 than in Catena 1 (Figure 5.1). These results may be associated with lower physico-chemical stabilization and greater biochemical decomposition of lipids in coarse textured than in fine textured soil. Slower turnover of SOM in aggregate fractions of fine textured soils are associated with the type of clay minerals that favor the formation of organo-mineral complexes (Monreal et al., 1997; Dinel et al., 1997). The coefficient of variation for $CHCl_3$ content was about 74 % for samples in Catena 2, and 29 % for samples in Catena 1.

The sequential solvent extraction of soil lipids may help to differentiate between easily biodegradable and bioresistant lipids. The DEE-to-CHCl3 and $CHCl_3$-to-TEL ratios indicate degree of biodegradability and stability of organic materials, respectively (Dinel et al., 1996a,b). Based on the latter, it was hypothesized that a high DEE-to-$CHCl_3$ ratio in soils indicates the presence of less decomposed and easily metabolizable substrates for soil organisms. A high CHCl3-to-TEL ratio would indicate that SOM is highly stabilized. Data for the A1 soil layer (0-8 cm) shows that the DEE-to-CHCl3 ratio is higher in samples from the NT than in those from the CT system (Table 2), indicating that NT increases the content and preservation of easily metabolizable substrates for soil organisms. The $CHCl_3$-to-TEL ratio tended to be slightly higher in soil under CT than under NT, suggesting greater SOM stabilization under CT than under NT system.

Principal component analysis was used to separate the individual contributions of the descriptors and to graphically represent

the relative quality of SOM for each layer and sampling station. The inset of Figure 5.2 shows the vectors representing soil organic-C and nitrogen, the ratio TEL-to-C, DEE- and $CHCl_3$-fatty acids, DEE- and $CHCl_3$-n-alkyl benzenes and DEE-unsaturated n-alcohols. The latter variables contributed significantly to the first two principal components (PRIN1 and PRIN2). These two principal components explained 72.3 % of the total variance. Figure 5.2 shows that the quality of SOM in each layer and landscape position was affected by tillage system and soil texture. The relative short distances between the three soil layers indicate that conventional tillage tended to homogeneize and reduce the diversity of SOM in coarse textured soil located at the lower slope position (CT-103). In comparisson, the relatively long distances and orientations between soil layers and sampling stations in Figure 5.2 indicate that NT increased the molecular diversity of soil lipids in the coarse and fine textured soils in lower (NT-203) and mid-lower (NT-208) slope positions. Conventional tillage maintained SOM quality in fine textured soil at the mid-lower slope position (CT-108). These results are consistent with the tested hypothesis. The DEE-to-$CHCl_3$ and $CHCl_3$-to-TEL ratios, and lipid constituents are useful indicators of SOM quality.

5.5.2 Soil Aggregate Stability

Figure 5.3 shows that cropping with NT increased the amount of water stable aggregates (SAt=SAu1+SAu2+SAb) in both soil catenas. In Catena 2, the increase was associated with a 15 % reduction in aggregates unstable to slaking forces (UAt_2). In Catena 1, the increase in aggregate stability was associated with a reduction of aggregates unstable to dissolution and dispersive actions of water (UAt1). In both soil catenas, the increased aggregate stability under NT was also associated with bound organic and inorganic materials (SAb). Differences in SAb between Catenas 1 and 2 may be due, in part, to differences in soil texture (Table 1). A high sand content in Catena 2 resulted in a high proportion of coarse individual unaggregated soil particles.

In Catena 2, the removal of extractable unbound lipids from soil under NT decreased the proportion of stable aggregates

associated with the dissolution and dispersive actions of water (SAu1) by 22 %, and of those associated with slaking (SAu2) by 37 %. In contrast and for Catena 1, the removal of extractable unbound lipids from soil under NT decreased SAu1 by 10 %, and slightly increased SAu2 by 9 %. In both soil catenas, the increase of water-stable aggregates appeared to be mainly associated with an increase in the amount of bound aggregated materials (SAb), and a decrease in unstable aggregated materials (UAt1 and UAt2).

The ability of soil aggregates to resist breakdown produced by any disruptive force is a major attribute of soil quality. Increased breakdown of aggregates reduces the infiltration and drainage of water and solutes, and increases the risk of erosion, compaction and crusting (Dinel et al., 1991a,b). Studies have reported positive, negative and/or negligible influences of total soil organic matter content on soil aggregation and aggregate stability (Capriel et al., 1990, Dinel et al., 1991b). Specific pools of SOM and their interactions with phyllosilicates, however, influenced the resistance of stable aggregates to various disruptive forces (Dinel et al., 1992 and 1997). For instance, unbound lipids were responsible for a 72 % increase of the relative strength of intra-aggregate bonds against the dispersive and dissolution action of water, and for a 10 % increase in the resistance of aggregates to the disruption by slaking. Soil minerals also increased soil aggregate stability (Nwadialo and Mbagwu, 1991; Dinel et al., 1997).

5.5.3 Relative Status of Soil Structure Stability

A relative index for assesing the relative status of soil structure stability was developed by integrating soil structural and textural characteristics with soil management practices using PCA techniques. The relative soil structure stability axis of Figure 5.4 was obtained by making the vectorial sum of unit-normalized eigenvectors representing each variable (Dinel et al., 1991). The distance between the projection of each observation on the axis was used to prepare the scale of soil structure stability. Figure 5.4 shows the orthogonal distribution of the first two principal components, and they

were influenced by soil structural property (i.e., UAt1), textural parameter (i.e., SA, SI), and crop management (i.e. F.M.CT). The three principal components explained 84.9 % of the total variation in the data set. Cropping of soils to maize at Woodslee and soybean at Clinton with conventional tillage increased the susceptibility of soil aggregates to be disrupted (all observations made in the field with CT are located on the left side of the resulting vector). Figure 5.4 also shows that soils cropped to soybean with NT prevented further deterioration of soil structure (NT observations plotted immediately to the right of CT observations). Forested and sod samples showed the highest stability of soil structure.

Figure 5.5 clearly shows that soil under forest (W) and sod (S), which was fertilized (F.S) and unfertilized (UF.S), have a relatively high (80%) structural stability. Soils cropped to maize (M) that were fertilized (F.M) and unfertilized (UF.M) with CT are located towards the lower part of the scale, indicating low structure stability and a high susceptibility for soil aggregates to be disrupted. In comparison, soil cropping with maize and NT minimized the physical deterioration of fine textured soils. These results support the findings of Karlen et al., (1994), and indicate that NT helps maintain and improve the structural characteristics of fine textured soils. The integrated approach presented above allowed the establishment of a relative index to assess structure stability in cultivated soils. The present index for the status of soil structure stability requires data on soil texture, and extractable lipids in relation to aggregate stability. Additional information on soil mineralogy also helps explaining mechanisms of interaction between inorganic and organic soil constituents.

5.6 CONCLUSIONS

The content of DEE and CHCL3 extractable lipids and the DEE-to-CHCl3 and CHCl3-to-TEL ratios may be used as indicators of SOM quality. Extractable soil lipids were sensitive to changes induced by tillage system, and influenced the stability of soil aggregates in cultivated soil landscapes. The scale for soil structural stability status shows that relative to conventional tillage, NT helps reduce the physical deterioration of fine

textured soils. The relative scale designed using PCA analysis is a reliable and objective method to assess the status of soil structural stability.

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Table 5.1. Physical and chemical characteristics of the A1 (0-8 cm) layer in two soil Catenas at Clinton.

Slope postion	Tillage/ station	Soil	pH ($CaCl_2$)	Total-C (%)	Texture (g kg^{-1}) Sand	Silt	Clay
		Catena 1					
Upper	[1]CT-108	Orthic Gray Brown Luvisol	7.3	2.27	170	460	370
Mid-upper	CT-107	Orthic Gray Brown Luvisol	7.4	1.87	210	450	340
Upper	[2]NT-208	Orthic Gray Brown Luvisol	7.2	2.25	130	500	370
Mid-upper	NT-207	Orthic Gray Brown Luvisol	7.4	1.97	180	450	380
		Catena 2					
Mid-lower	CT-103	Gleyed Brunisolic/ Gray Brown Brunisolic	7.3	3.51	620	250	130
Lower	CT-102	Gleyed Brunisol/ Gray Brown Luvisol	7.2	3.72	670	240	90
Mid-lower	NT-203	Orthic Humic Gleysol	7.2	3.82	590	270	140
Lower	NT-202	Orthic Humic Gleysol	7.1	2.91	720	210	80

1. CT=conventional tillage.
2. NT=no-till; numbers 107, 108 etc.. indicate soil sampling stations.

Table 5.2. DEE-to-$CHCl_3$ and $CHCl_3$-to-TEL ratios in the 0-8 cm depth of two soil catenas cropped with no-till (NT) and conventional tillage (CT) .

	[1]DEE/$CHCl_3$		[2]$CHCL_3$/TEL	
Catena	NT[3]	CT[4]	NT	CT
1	0.8	0.6	0.5	0.6
2	1.0	0.7	0.5	0.6

1. DEE/$CHCl_3$= diethyl ether-to-chloroform ratio.
2. $CHCL_3$/TEL= chloroform-to-total extractable lipids.

Chapter 6

SYNTHESIS

6.1 Sensitivity of Soil Enzyme Activities and Lipid Content to Land Use and Management Practices

A central objective of this project was to identify sensitive biological and chemical indicators to asess the health state of cultivated soils in Ontario. In general, undisturbed forested soils, and agricultural land cropped with conservation management systems including zero-tillage (ZT), ridge tillage (RT) and chisel plow (CP) maintained higher levels of biochemical and chemical properties than soils cropped with conventional tillage (CT). Our results indicate that soil enzymes and lipids were always sensitive to management practices and tillage system. The tested soil enzymes control aspects of C, N, P and S mineralization. Soil lipids are associated with the stability of soil aggregates. Therefore these indicators may be used to evaluate the agroecological functions of soil nutrient mineralization and structure stability. In comparisson, the content of soil organic carbon (OC) was not always sensitive to reflect management effects. For example, fifteen years of zero-tillage (ZT) did not influence the content of OC in two soil catenas at Clinton. A soil incubation study showed that soil enzymes were up to 12 times more sensitive than OC in reflecting the effects of land use, and management system on soil health. Furthermore, there was no association between light fraction and other soil properties.

Measurement of activities and kinetic properties of soil enzymes indicated that the effect of conservation management on soil health differed between farms, depending on agronomic practices and site characteristics. For example, at Rockwood implementation of ZT following decades of crop rotations that included forage maintained higher OC content and six enzyme activities. At Bainsville, a CP system maintained higher enzyme activities and concentration of total C than RT in the Ap horizon after 10 y of implementing the tillage systems. The

exact reasons for higher activity under CP are unknown but may be associated with the initial levels of enzyme activities or the rate of enzyme synthesis under the two tillage systems. At Bainsville and Clinton, soils with poor drainage had lower enzyme activities than well drained soils managed with the same tillage system. Kinetic studies showed that level of enzyme activity in cultivated soils were associated with the amount of active soil enzyme.

Time and depth of sampling also influenced enzyme activities, and affected management comparisons. Fertilizer inputs were a minor source of temporal variation in some soil enzyme activities. Soil amendment with manure temporarily increased the activity of soil dehydrogenase, urease and alkaline phosphatase which are involved in mineralization of organic compounds. Application of inorganic N fertilizer may temporarily decrease the activity of soil urease which mediate reactions producing NH_4^+. Temporal variation in soil enzyme activities over the growing season was also influenced by crop growth.

Conservation tillage systems also influenced the amount and composition of lipids in two soil catenas. Diethyl ether (DEE)- and chloroform ($CHCl_{3)}$-extractable lipids were lower in Catena 2 than in Catena 1 at Clinton. Differences in soil lipid content between soil catenas was attributed to differences in physico-chemical stabilization and biochemical decomposition of soil lipids. Soils cropped to ZT increased the preservation of the easily biodegradable (DEE) fraction, and soils cropped with CT appeared to increase the humification and physico-chemical stabilization of soil lipids. The increase in lipid content under ZT was paralleled by an increase in the amount of water stable aggregates in the Gleysolic soil at Clinton. Higher amounts of water stable aggregates was associated with a reduction in the negative effects caused by slaking and the dissolution and dispersive action of water.

6.2 Monitoring Nutrient Mineralization and Structural Stability

Another important project objective was to develop methods to assess soil health. Assessment of soil quality needs to be made based on unique ecosystem functions performed by soil components (Warkentin, 1995; Monreal et al., 1997). Working hypotheses of the project proposal were that soil enzymes are components controlling nutrient cycling and lipids control soil structure stability. Ordinarily the soil attribute measured depends upon the soil function of interest. The present study began with specific attributes--soil enzyme activities--and hypotheses about their sensitivity to management practices that influence soil quality.

Our results indicated that some soil enzyme activities responded to management differently than OC content. In fine-textured soils at Clinton, three enzyme activities were influenced by tillage practice whereas total C content was not. This difference in response may be interpreted in two ways. Soil enzyme activities are more sensitive than OC content to management, in a manner similar to microbial biomass (Powlson et al., 1987). As such, they have diagnostic value, indicating early change in SOM content that is not yet measurable at a site. Alternatively, they are indicators of change in some soil property other than OC content. Thus, at specific spatial and temporal scales soil enzyme activities may be indices of macronutrient cycling (Warkentin, 1995; Sinsabaugh, 1994).

Some soil enzyme activities provided similar information on soil health as that given by SOM content. This was especially true for enzymes such as urease, alkaline phosphatase and arylsulfatase. These enzymes exist in part as extracellular enzymes stabilized onto humic materials and clays. As indices of SOM content, the latter extracellular enzyme activities may be related to those soil functions within agro-ecosystems which are affected by SOM content and influence crop production.

Data obtained from an incubation study of a Podzolic soil tested for and showed the ecological association of the soil's N mineralization power with the activities of soil dehydrogenase, L-glutaminase and urease. These results may have important implications for sustainable crop production systems. For instance, predicting N mineralization from measurements of soil enzyme activities improves fertilizer-N recommendations for different crops by including amounts of N derived from soil organic matter during the growing season. Thus, fertilizer recommendations may not only consider production and economic benefits, but also benefits to the environment by maintaining a desired level of enzyme activities and SOM. These results and concepts need further testing in the field, especially to better define the links between soil enzyme activities and crop N uptake in the field. Future research on soil enzymes may also improve understanding the mechanisms governing synchronization between soil N mineralization and crop N uptake to minimize environmental degradation (i.e. NO_3 leaching, denitrification).

We developed novel quantitative methodologies to evaluate soil health. Concepts of statistical control and quality control charts (QCC) used by industry were applied to monitor shifts in soil enzyme activities influencing nutrient mineralization. Control limits for control charts were defined using the 3-sigma limits. The latter approach has given "good practical" results to industry in North America (Montgomery, 1991). The QCC approach was tested with data for soil L-glutaminase, dehydrogenase and urease obtained form different slope positions at Rockwood and two soil catenas at Clinton. The QCC described the temporal variability of enzyme activities induced by tillage and crop growth. Shewhart in conjunction with the EWMA, CUSUM or MA control charts were very effective in recording large (> 3-sigma) and small (< 1-sigma) shifts in enzyme activities induced by land use, tillage or cropping system. The method is simple, cost effective and may be established at any farm to monitor and evaluate the state of soil health. Nutrient (i.e. N) mineralization power of soils may now be assessed in relation to

the soil's natural fertility rather than relative to "undisturbed" or virgin sites. A control chart is a decision making tool warning producers of changes in the nutrient mineralization power of soil, and assists them to rapidly implement changes in farm practices if required.

Vectorial and principal component analysis were used as the basis to develop a method to assess the relative status of soil structure stability. The method is effective in integrating data on chemical and physical properties of soils, and was sensitive to detect effects of tillage and cropping systems in fine textured soils. The best soil structural stability was observed in samples from undisturbed sites under forest and sod, followed by that in samples taken from soils cropped with ZT. Soils cropped to corn and soybean with CT had the lowest structural stability.

6.3 Linking Soil Enzyme Activities and Structural Stability with the State of Soil Health.

Results in Chapters 2, 3, 4 and 5 gave complementary information on critical biochemical, chemical and physical properties indicating the state of nutrient mineralization power and structural stability. This information allows to relate soil health state in relation to level of biochemical activity and state of soil chemical and physical properties. For example, soils having high enzyme activity levels presented high lipid content and structural stability, mostly in undisturbed sites under forest and sod or cropped with ZT. Soils with high enzyme activity level provide soils with a high power to mineralize nutrients (i.e. helps lower fertilizer inputs). High aggregate stability provide soils with greater resistance to erosion, and improved water and air regimes of soils, thus better soil conditions for root growth. We propose that high enzyme activity level and soil structural stability represent a high state of soil health. Conversely, low soil health is associated with soils having low enzyme activity level and structural stability. We predicate that global assessment of soil health need to

intregrate functions of nutrient cycling and the physical conditions affecting soil productivity and crop growth. Finally, considering the ecological functions in crop production systems, the information on soil enzymes and lipids appear as essential complementary components to taxonomic information contained in soil databases.

6.4 References

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APPENDICES

Table A1. **Control limits defining boundary conditions for soil dehydrogenase and $-glucosidase in the A horizon[1] of all studied soils of Ontario.**

Control limits[2]	Enzyme activity in cluster number						
	1	2	3	4	5	6	7
	Dehydrogenase (g TPF $g^{-1}d^{-1}$)						
CL	7.8	17.7	30.3	47.3	68.4	103.9	180.8
LCL	0	7.9	18.1	29.6	45.0	63.4	95.8
LWL	2.2	11.1	22.1	35.5	52.8	76.9	124.2
UWL	13.4	24.3	38.5	59.1	84.0	130.9	237.4
UCL	16.3	27.4	42.5	65.0	91.9	144.3	256.8
	$-glucosidase (mole p-NP $g^{-1}h^{-1}$)						
CL	10.3	19.1	29.8	42.4	61.7	91.0	151.3
LCL	2.1	11.0	20.3	29.5	40.4	56.7	93.9
LWL	4.9	13.7	23.4	33.8	47.5	68.2	113.1
UWL	15.7	24.5	36.2	51.0	75.9	113.8	189.5
UCL	18.5	27.2	39.4	55.3	83.1	125.7	208.7

1. A horizon includes layers A1=0-8 cm and A2=8 cm to bottom of A.
2. CL = center line; LCL = lower control limit; LWL = lower warning limit; UWL = upper warning limit; UCL = upper control limit.

Table A2. Control limits defining boundary conditions for L-glutaminase and urease in the A horizon[1] of all studied soils of Ontario.

Control limits[2]	Enzyme activity in cluster number						
	1	2	3	4	5	6	7
	L-glutaminase (g NH_4-N $g^{-1}h^{-1}$)						
CL	133.3	198.5	252.3	298.9	350.1	432.2	531.2
LCL	57.3	148.1	210.0	256.6	300.9	345.4	388.6
LWL	82.6	164.9	224.1	270.7	317.3	371.2	436.2
UWL	184.0	232.1	280.5	327.1	382.9	475.2	626.2
UCL	209.4	248.9	294.6	341.2	399.4	501.2	673.8
	Urease (g NH_4-N $g^{-1}h^{-1}$)						
CL	10.3	19.1	29.8	42.4	61.7	91.0	151.3
LCL	2.1	11.0	20.3	29.5	40.4	56.7	93.9
LWL	4.9	13.7	23.4	33.8	47.5	68.2	113.1
UWL	15.7	24.5	36.2	51.0	75.9	113.8	189.5
UCL	18.5	27.2	39.4	55.3	83.1	125.7	208.7

1. A horizon includes layers A1=0-8 cm and A2=8 cm to bottom of A.
2. CL = center line; LCL = lower control limit; LWL = lower warning limit; UWL = upper warning limit; UCL = upper control limit.

Table A3. Control limits defining boundary conditions for alkaline phosphatase and arylsulfatase in the A horizon[1] of all studied soils of Ontario.

	Enzyme activity in cluster number						
Limits[1]	1	2	3	4	5	6	7
	Alkaline phosphatase (mole p-NP $g^{-1}h^{-1}$)						
CL	1.48	2.37	3.37	4.47	5.63	7.62	10.48
LCL	0.61	1.56	2.50	3.48	4.42	5.60	6.42
LWL	0.90	1.83	2.79	3.81	4.83	6.28	7.78
UWL	2.06	2.91	3.95	5.13	6.43	8.96	13.18
UCL	2.35	3.18	4.24	5.46	6.84	9.64	14.54
	Arylsulfatase (mole p-NP $g^{-1}h^{-1}$)						
CL	0.16	0.34	0.51	0.71	0.95	1.34	2.07
LCL	0.0	0.19	0.36	0.53	0.71	0.89	1.08
LWL	0.04	0.24	0.41	0.65	0.79	1.04	1.43
UWL	0.28	0.34	0.61	0.83	1.11	1.64	2.71
UCL	0.35	0.49	0.66	0.89	1.20	1.78	3.06

1. A horizon includes layers A1=0-8 cm and A2=8 cm to bottom of A.
2. CL = center line; LCL = lower control limit; LWL = lower warning limit; UWL = upper warning limit; UCL = upper control limit.

Table A4. Kinetic parameters for selected soil enzymes at the Clinton site.

Means of 4 field replicates

ASE=asymptotic standard error; A1=0- 8 cm, A2=8 cm to bottom of Ap, B=top 10 cm of B horizon

CT=conventional tillage; NT=no-till

$-glucosidase (Vmax = umole p-nitrophenol/g/h; Km = mM)

Station	Texture	Tillage	Depth	Vmax	ASE	Km	ASE
102	coarse	CT	A1	1.26	0.04	5.54	0.49
102	coarse	CT	A2	1.54	0.03	3.75	0.23
102	coarse	CT	B	0.10	0.004	3.33	0.47
202	coarse	NT	A1	1.21	0.06	0.88	0.14
202	coarse	NT	A2	0.49	0.01	0.68	0.06
202	coarse	NT	B	*0.03	0.01	4.13	1.90
103	coarse	CT	A1	0.66	0.03	0.60	0.12
103	coarse	CT	A2	0.78	0.04	0.61	0.11
103	coarse	CT	B	*0.07	0.01	1.44	0.64
203	coarse	NT	A1	1.50	0.03	2.47	0.27
203	coarse	NT	A2	0.70	0.02	1.64	0.28
203	coarse	NT	B	0.20	0.002	1.29	0.08
107	fine	CT	A1	0.81	0.03	0.91	0.10
107	fine	CT	A2	0.81	0.01	1.09	0.05
107	fine	CT	B	0.10	0.01	1.75	0.31
207	fine	NT	A1	1.02	0.02	1.14	0.08
207	fine	NT	A2	0.33	0.02	1.26	0.19
207	fine	NT	B	0.12	0.02	5.45	1.90
108	fine	CT	A1	0.78	0.03	2.63	0.42
108	fine	CT	A2	0.54	0.009	1.95	0.14
108	fine	CT	B	*0.03	0.002	6.25	1.26
208	fine	NT	A1	0.87	0.009	1.26	0.07
208	fine	NT	A2	0.43	0.008	2.01	0.17
208	fine	NT	B	0.19	0.005	2.01	0.22

Alkaline phosphatase (Vmax = umole p-nitrophenol/g/h; Km = mM)

Station	Texture	Tillage	Depth	Vmax	ASE	Km	ASE
102	coarse	CT	A1	3.04	0.13	2.24	0.51
102	coarse	CT	A2	3.28	0.11	3.61	0.61
102	coarse	CT	B	0.74	0.18	12.40	7.40
203	coarse	NT	A1	3.30	0.06	4.79	0.39
203	coarse	NT	A2	3.11	0.38	5.16	2.52
203	coarse	NT	B	1.24	0.15	14.80	4.90
108	fine	CT	A1	1.67	0.11	3.72	0.95
108	fine	CT	A2	1.34	0.06	3.06	0.58
108	fine	CT	B	0.68	0.17	9.40	5.50
208	fine	NT	A1	1.71	0.09	2.60	0.62
208	fine	NT	A2	1.76	0.06	3.44	0.49
208	fine	NT	B	1.15	0.03	2.62	0.34

* very low activity ^ high variability of the replicates

(continued...)

Urease (Vmax = ug N/g/h; Km = mM)

Station	Texture	Tillage	Depth	Vmax	ASE	Km	ASE
102	coarse	CT	A1	12.13	1.43	1.73	0.63
102	coarse	CT	A2	22.85	1.04	1.90	0.27
102	coarse	CT	B	very low activity, not measurable			
202	coarse	NT	A1	24.77	0.84	1.38	0.16
202	coarse	NT	A2	18.61	1.34	3.07	0.60
103	coarse	CT	A1	^35.67	8.05	4.48	2.37
103	coarse	CT	A2	^34.86	3.70	3.95	0.96
203	coarse	NT	A1	^58.22	5.64	3.55	0.84
203	coarse	NT	A2	27.25	0.77	1.17	0.12
203	coarse	NT	B	*9.67	0.12	0.41	0.03
107	fine	CT	A1	45.74	4.42	6.23	1.19
107	fine	CT	A2	^52.01	6.68	12.26	2.48
107	fine	CT	B	13.96	2.14	3.27	1.24
207	fine	NT	A1	44.43	4.85	11.10	1.93
207	fine	NT	A2	^32.365	4.53	5.79	1.71
207	fine	NT	B	very low activity, not measurable			
108	fine	CT	A1	66.65	3.34	2.67	0.35
108	fine	CT	A2	38.74	2.67	3.19	0.55
108	fine	CT	B	15.61	1.61	8.91	1.58
208	fine	NT	A1	52.85	2.84	3.44	0.45
208	fine	NT	A2	24.21	1.55	2.49	0.45
208	fine	NT	B	9.98	0.67	0.86	0.24

* very low activity ^ high variability of the replicates

Table A5. Kinetic parameters for selected soil enzymes at the Rockwood site.

Means of 4 field replicates
ASE=asymptotic standard error; A1=0-8 cm, A2=8 cm to bottom of Ap; B=top 10 cm of B horizon.

Alkaline phosphatase (Vmax = umole p-nitrophenol/g/h; Km = mM)

Management	Slope	Depth	Vmax	ASE	Km	ASE
No-Till	Shoulder	A1	5.02	0.19	2.13	0.35
	Shoulder	A2	2.72	0.39	5.63	2.51
	Shoulder	B	0.43	0.02	0.71	0.17
	Mid-slope	A1	1.74	0.13	1.22	0.54
	Mid-slope	A2	1.20	0.10	0.63	0.45
	Mid-slope	B	0.72	0.03	0.42	0.09
	Foot-slope	A1	3.11	0.42	5.61	2.43
	Foot-slope	A2	3.75	0.17	1.42	0.34
	Foot-slope	B	1.21	0.06	0.77	0.12
Conventional tillage	Shoulder	A1	0.74	0.03	0.53	0.21
	Shoulder	A2	0.54	0.04	0.70	0.37
	Shoulder	B	0.85	0.11	1.61	0.49
	Mid-slope	A1	1.47	0.09	2.94	0.72
	Mid-slope	A2	0.91	0.05	3.62	0.81
	Mid-slope	B	0.18	0.03	1.43	0.57
	Foot-slope	A1	0.91	0.04	0.49	0.19
	Foot-slope	A2	0.60	0.03	0.53	0.27
	Foot-slope	B	0.19	0.02	1.04	0.27
Woods	Shoulder	A1	5.80	0.32	2.75	0.72
	Shoulder	A2	5.27	0.10	1.14	0.11
	Shoulder	B	1.46	0.16	6.16	2.13
	Mid-slope	A1	6.23	0.45	3.59	1.09
	Mid-slope	A2	3.81	0.10	2.02	0.27
	Mid-slope	B	1.78	0.06	1.92	0.32
	Foot-slope	A1	6.29	0.35	2.56	7.16
	Foot-slope	A2	5.06	0.23	1.67	0.39
	Foot-slope	B	1.57	0.04	1.04	0.15

$-glucosidase (Vmax = umole p-nitrophenol/g/h; Km = mM)

Management	Slope	Depth	Vmax	ASE	Km	ASE
No-Till	Shoulder	A1	1.487	0.062	1.28	0.185
	Shoulder	A2	0.774	0.08	2.459	0.654
	Shoulder	B	0.384	0.061	6.031	1.787
	Mid-slope	A1	0.871	0.054	1.261	0.277
	Mid-slope	A2	0.596	0.034	1.121	0.238
	Mid-slope	B	0.124	0.01	1.634	0.0393
	Foot-slope	A1	1	0.039	0.961	0.151
	Foot-slope	A2	0.647	0.004	0.828	0.024
	Foot-slope	B	0.155	0.004	0.68	0.092
Conventional	Shoulder	A1	0.742	0.045	1.176	0.259
tillage	Shoulder	A2	0.313	0.013	0.832	0.152
	Shoulder	B	0.095	0.008	1.347	0.354
	Mid-slope	A1	0.695	0.05	1.083	0.295
	Mid-slope	A2	0.224	0.022	4.585	0.939
	Mid-slope	B	very low activity			
	Foot-slope	A1	0.827	0.039	1.109	0.196
	Foot-slope	A2	0.435	0.018	1.136	0.177
	Foot-slope	B	very low activity			
Woods	Shoulder	A1	1.8	0.079	1.14	0.185
	Shoulder	A2	1.008	0.047	0.718	0.129
	Shoulder	B	0.216	0.01	0.711	0.155
	Mid-slope	A1	2.019	0.072	0.976	0.119
	Mid-slope	A2	0.663	0.018	0.577	0.063
	Mid-slope	B	0.339	0.013	1.075	0.157
	Foot-slope	A1	1.785	0.07	0.951	0.128
	Foot-slope	A2	0.981	0.029	0.758	0.083
	Foot-slope	B	0.202	0.01	0.89	0.149

Urease (Vmax = ug N/g/h; Km = mM)

Management	Slope	Depth	Vmax	ASE	Km	ASE
No-Till	Shoulder	A1	155.315	2.928	2.708	0.239
	Shoulder	A2	83.748	2.605	1.987	0.35
	Shoulder	B	40.241	1.283	0.779	0.221
	Mid-slope	A1	69.37	2.089	1.433	0.285
	Mid-slope	A2	69.839	1.234	2.073	0.203
	Mid-slope	B	34.027	1.354	1.682	0.414
	Foot-slope	A1	129.08	2.443	3.103	0.276
	Foot-slope	A2	77.917	3.175	2.345	0.469
	Foot-slope	B	38.466	2.732	1.867	0.802
Conventional	Shoulder	A1	28.586	1.056	0.187	0.08
tillage	Shoulder	A2	43.458	1.589	1.564	0.357
	Shoulder	B	22.72	0.837	0.995	0.293
	Mid-slope	A1	42.683	1.488	1.671	0.373
	Mid-slope	A2	26.35	0.492	0.306	0.067
	Mid-slope	B	22.615	1.109	1.22	0.438
	Foot-slope	A1	50.821	2.132	1.9	0.459
	Foot-slope	A2	31.683	0.613	0.312	0.05
	Foot-slope	B	24.629	0.789	0.798	0.235
Woods	Shoulder	A1	86.596	4.108	2.693	0.441
	Shoulder	A2	46.426	1.773	2.157	0.354
	Shoulder	B	-	-	-	-
	Mid-slope	A1	105.46	9.6	8.47	2.1
	Mid-slope	A2	-	-	-	-
	Mid-slope	B				
	Foot-slope	A1	73.849	4.065	1.5	0.395
	Foot-slope	A2	47.819	1.85	0.941	0.217
	Foot-slope	B	-	-	-	------

Table A6. Kinetic parameters for selected soil enzymes at the Bainsville site.

Means of 4 field replicates
ASE=asymptotic standard error; A1=0-8 cm; A2=8 cm to bottom of Ap; B=top 10 cm of B horizon

$-glucosidase (Vmax= umole p-nitrophenol/g/h; Km=mM)

Tillage	Crop	Depth	Vmax	ASE	Km	ASE
Chisel plow	Corn	A1	1.649	0.015	0.652	0.023
Chisel plow	Corn	A2	0.678	0.016	0.571	0.051
Chisel plow	Corn	B	very low activity, not measurable			
Ridge tillage	Corn	A1	1.316	0.038	0.675	0.075
Ridge tillage	Corn	A2	0.589	0.008	0.519	0.029
Ridge tillage	Corn	B	0.029	0.007	1.642	1.084
Ridge tillage	Soybean	A1	1.322	0.019	0.538	0.033
Ridge tillage	Soybean	A2	0.842	0.011	0.501	0.03
Ridge tillage	Soybean	B	0.023	0.003	0.431	0.266

Alkaline phosphatase (Vmax= umole p-nitrophenol/g/h; Km=mM)

Tillage	Crop	Depth	Vmax	ASE	Km	ASE
Chisel plow	Corn	A1	1.806	0.082	2.523	0.3
Chisel plow	Corn	A2	1.319	0.065	2.023	0.312
Chisel plow	Corn	B	0.3	0.019	2.477	0.457
Ridge tillage	Corn	A1	1.477	0.062	2.359	0.267
Ridge tillage	Corn	A2	0.948	0.055	1.034	0.206
Ridge tillage	Corn	B	0.414	0.022	4.693	0.567
Ridge tillage	Soybean	A1	2.253	0.11	1.364	0.214
Ridge tillage	Soybean	A2	1.212	0.028	1.179	0.093
Ridge tillage	Soybean	B	0.307	0.038	1.063	0.641

Urease (Vmax=ug N/g/h; Km=mM)

Tillage	Crop	Depth	Vmax	ASE	Km	ASE
Chisel plow	Corn	A1	37.892	2.188	1.117	0.228
Chisel plow	Corn	A2	23.167	1.258	1.223	0.239
Chisel plow	Corn	B	very low activity, not measurable			
Ridge tillage	Corn	A1	24.242	1.579	2.015	0.37
Ridge tillage	Corn	A2	11.728	0.743	1.264	0.278
Ridge tillage	Corn	B	very low activity, not measurable			
Ridge tillage	Soybean	A1	17.621	1.235	1.12	0.281
Ridge tillage	Soybean	A2	13.255	0.76	0.489	0.134
Ridge tillage	Soybean	B	very low activity, not measurable			

Glutaminase (Vmax=ug N/g/h; Km=mM)

Tillage	Crop	Depth	Vmax	ASE	Km	ASE
Chisel plow[1]	Corn	A1	563.13	11.82	66.9	3.38
Chisel plow[2]	Corn	A1	626.45	28.98	81.17	6.78

1. Sample taken on June 28, 1995; 2. Sample taken on July 8, 1996

Table A7. Kinetic parameters for selected soil enzymes at the Ste. Isidore (Embrun) site.

Means of 4 field replicates in the control plot (0 kg N/ha)

ASE=asymptotic standard error; A1=0-8 cm; A2=8 cm to bottom of Ap; B=top 10 cm of B horizon

$-glucosidase (Vmax= umole p-nitrophenol/g/h; Km=mM)

Depth	Vmax	ASE	Km	ASE
A1	0.918	0.021	0.616	0.057
A2	0.525	0.05	0.466	0.021
B	0.052	0.005	0.766	0.253

Alkaline phosphatase (Vmax= umole p-nitrophenol/g/h; Km=mM)

Depth	Vmax	ASE	Km	ASE
A1	0.642	0.021	2.686	0.162
A2	0.301	0.014	0.892	0.123
B	0.265	0.012	1.554	0.168

Urease (Vmax=ug N/g/h); Km=mM)

Depth	Vmax	ASE	Km	ASE
A1	36.421	4.92	2.093	0.776
A2	25.581	2.523	2.164	0.638
B	very low activity, not measurable			