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CONTROL OF AMINO ACID CATABOLISM IN SOIL AND DIRECT ASSIMILATION BY PLANTS

BY

DAVID LLOYD BURTON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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University of Alberta
Edmonton

Canada T6G 2E3

Department of Soil Science
Faculty of Agriculture and Forestry

Earth Sciences Building, Telephone (403) 432-3242

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W.B. McGill

Professor
Department of Soil Science
University of Alberta
Edmonton, Alberta.

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David Burton
751 James St.
Delhi, Ont.

(Permanent address)

Date: July 7, 1989

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read , and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled CONTROL OF AMINO ACID CATABOLISM IN SOIL AND DIRECT ASSIMILATION BY PLANTS. submitted by DAVID LLOYD BURTON in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in SOIL MICROBIOLOGY AND BIOCHEMISTRY.

.....
W.B. McGill (Supervisor)

.....
M.J. Dudas

.....
N.G. Juma

.....
W.J. Page

.....
R. Knowles (External)

Date: .....

ABSTRACT

Control mechanisms expressed at the organism-level, such as catabolite repression, could have important consequences to the coordination and control of soil nitrogen mineralization and result in increased nitrogen utilization efficiency in agricultural systems. This thesis examines the applicability of these forms of control to regulation of soil nitrogen-mineralizing enzymes at aggregate and horizon-levels of organization.

Biological control of enzyme content is dependent upon the instability of existing enzymes. As a model system for examining the control of soil nitrogen mineralization, soil histidase was found not to be stable in soil. The decline in soil histidase activity following the addition of a biostatic agent (toluene or azide) followed first order kinetics. Two kinetic components were distinguished having half-lives of 3 and 77 hours. The decline in histidase activity was not attributable to enzyme inactivation by the biostatic agent, substrate limitation, or product (NH_4^+) inhibition.

Soil histidase activity increased 3-4 fold by the addition of 100 $\mu g\ g^{-1}$ of either histidine-C or urocanate-C. Synthesis of soil histidase was sensitive to catabolite repression. The concentrations of glucose required to repress synthesis (4000 $\mu g\ g^{-1}$) call into question the role of glucose catabolite repression in horizon-level control of enzyme synthesis in soil systems. Basal histidase activity persisted in the presence of repressive concentrations of glucose and is likely to be of paramount importance in determining native soil histidase content.

Examination of the interrelationship between histidase activity, protease activity, biomass and net mineral-N production under field conditions revealed that integrative measures such as biomass provided better descriptions of mineral-N production. An inverse relationship between NH_4^+ content and labile soil histidase activity suggested feedback control may be operative. The positive correlation between NH_4^+ content and net mineral-N production did not support negative feedback as a regulatory mechanism in nitrogen-mineralizing reactions in general.

The potential for direct plant uptake of amino acids from the rhizosphere was examined. Although maize was shown to be able to incorporate amino acids directly under axenic conditions, catabolism was the dominant fate of amino acid

added to rhizosphere soil, with only 2% being assimilated by plants. Plant growth increased the rate of amino acid turnover but decreased the amount catabolized. Addition of carbohydrate was unable to mimic the increased retention of amino acid. The catabolism of amino acid in the rhizosphere suggests that conservative control mechanisms do not predominate even in this relatively energy rich zone.

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1. INTRODUCTION

The efficient use of nitrogen in agriculture is of economic and environmental concern. Natural ecosystems generally exhibit higher nitrogen utilization efficiency than do their agricultural counterparts (Power, 1981). This is in part due to a decrease in the concentration of soil inorganic nitrogen, from which pathways of nitrogen loss emanate. Application of chemical fertilizers increases the amount of inorganic nitrogen present in the soil in an attempt to provide optimal nutrient concentrations for plant growth (Halberg, 1987). Strategies which provide for plant nitrogen nutrition while limiting the inorganic nitrogen concentration may result in increased efficiency of nitrogen use without compromising plant yield.

Mineral nitrogen accumulation in soil could be limited if fertilizer application, nitrogen mineralization and plant uptake were synchronized or if direct assimilation of organic nitrogen compounds by plants could be used to supply crop N. Applications of technologies from studies of the former approach have improved nitrogen use efficiency. The latter approach has received relatively little attention.

It is commonly assumed that nitrogen must be mineralized before it becomes available to plants. This view is based primarily on research conducted in the last century and the early part of this century (Brigham, 1917; Vickery, 1941). Similarly most models of the nitrogen cycle equate mineralization-immobilization with turnover, suggesting there is no direct transfer of organic nitrogen between components. Many interpretations of ^{15}N tracer data assume that no organic nitrogen is assimilated directly (Hauck and Bremner, 1976). The microbial literature indicates direct uptake of organic nitrogen compounds such as amino acids is not only possible but is, in some cases, required. Amino acid-dependent microorganisms have been reported in soils (Alexander, 1977). Jansson (1958) discusses the possibility of a direct assimilation of organic nitrogen by microorganisms in soil and its significance to the soil nitrogen cycle.

> "we must bear in mind the possibility that the renewal of the active organic phase [of the soil nitrogen cycle] may start with assimilation of organic decay products, such as amino acids. If this happens to a considerable extent, part of the continuous decay and renewal cycle of the active organic phase will not constitute a mineralization-immobilization turnover and will not be registered in

> experiments of the type used in this investigation [^{15}N tracer studies]... it will cause an underestimation of the true rate of renewal and decay of the active microbial tissue of the soil." p. 325

Microorganisms, as the agents of mineralization, normally require the uptake of simple organics prior to their catabolism, insuring access to the energy produced. The extent to which plants have a similar capacity is not clear. For direct assimilation of organic nitrogen to be of significance to plant nutrition, the transport of simple organics from their source, through a reactive soil matrix to the root surface must occur. This requires mechanisms to suppress their catabolism. Organisms coordinate metabolism by regulating enzyme activity. This occurs by several means; at a molecular-level, by modulating activity of existing enzymes; at a cellular-level by regulating enzyme synthesis[1]; and at higher levels of organization, by compartmentalization of enzyme and substrate. Control mechanisms, such as catabolite repression result in the conservation of biosynthetic molecules (eg. amino acids) in the presence of energetically more favorable catabolites (eg. glucose). Catabolite repression operates at a cellular-level, blocking the synthesis of enzymes catalysing reactions in less energetically favorable catabolic pathways such as the histidine utilization operon (*hut*). Detailed discussions of catabolite repression are provided in the reviews by Magasanik (1961), Ullman (1985) and Saier (1989).

Soils are complex, three dimensional multiphasic systems containing multifarious environments within a small volume which compartmentalizes organisms, enzymes and substrates. The organization or architecture of soil components affect processes within soils and can be examined at several levels of resolution extending from the molecular-level to that of the soil pedon (McGill and Myers, 1987). It is not clear that processes expressed at the organism-level will be similarly expressed at higher levels of organization. The level of resolution at which observation is made is important in determining the applicability of the principles thus derived; extension of such principles to other levels must done with caution (Allen *et al.*, 1984). Architectural constraints at each level may alter the expression of processes operating at lower levels (McGill and Myers, 1987). For further discussion of heirarchical theory the reader is referred to the reviews of

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[1] Synthesis level control refers to mechanisms such as transcription, translation or post translational mechanisms in general without implying any specific mechanism.

Webster (1979) and O'Neill *et al.* (1986). The transition from the organism to the horizon crosses several levels of organization. While organisms are anticipated to respond predictably to their immediate environment, spatial heterogeneity may isolate microbial communities causing them to respond to local conditions in manner not in keeping with bulk determinations of soil conditions. If, however control mechanisms expressed at the organism-level affect system behavior at community-, aggregate- or horizon-levels of organization, such mechanisms could coordinate metabolic activity and facilitate energetically conservative reactions such as the direct assimilation of organic nitrogen compounds. Influences of organism-level controls over the synthesis of enzymes in soil and thus soil enzyme content, has not been demonstrated, however.

This research examines the extension of mechanisms affecting enzyme synthesis, expressed at the organism-level, to the regulation of catalytic activity in soil systems. It focuses on the catabolism of amino acids in soil because this is a major process generating mineral nitrogen in soil. The enzyme L-histidine NH_3-lyase (E.C. 4.3.1.3; histidase), which catalyses the removal of the α-amino group from histidine, was chosen as a model system. Histidase is the first enzyme of the histidine utilization (*hut*) operon, which encodes for histidine catabolism. An enzyme from this operon was chosen in part because the operon has served as a model system for the development of our current understanding of carbon and nitrogen control over enzyme synthesis in microbial systems (Neidhardt and Magasanik, 1957; Lessie and Neidhardt, 1967 and Prival *et al.*, 1971).

Mechanisms of control such as catabolite repression simultaneously provide control over the synthesis of a broad range of enzymes. In so doing entire metabolic pathways can be coordinated (Ingraham *et al.*, 1983). The role of fluctuations in enzyme content in the coordination of reactions contributing to nitrogen mineralization was examined under field conditions. Changes in the size and activity of components ranging from general indices such as soil biomass to specific enzymatic activities such as histidase were monitored in relation to normal fluctuations in mineral nitrogen.

A direct transfer of nitrogen from soil organic pools to growing plants could provide a method by which plant nitrogen needs could be met while restricting the accumulation of mineral nitrogen and thus reducing nitrogen loss. The potential for direct transfer of amino acids from soil solution to growing plants was examined by

following the fate of a mixture of ^{14}C-labelled amino acids introduced into the root zones of three plant species. The relative rates of catabolism and direct uptake were quantified by measuring $^{14}CO_2$ evolution and ^{14}C content of plant parts respectively.

The potential for an organic assimilatory pathway in the soil nitrogen cycle is examined by measuring biochemical activities affecting the fate of amino acids in soil. In particular, the conditions necessary to facilitate such a pathway. The fate of ^{14}C-amino acid introduced into plant rhizosphere provides an empirical evaluation of the validity of the concepts of control developed in earlier experiments.

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2. STABLE ENZYME COMPLEXES IN SOIL: FORMATION AND ROLE IN DETERMINING SOIL ENZYMATIC COMPOSITION

2.1 Microbial vs biochemical characterization of soil

Microbial biomass is an important source of catalyst in soil, mediating transformations involved in processes ranging from nutrient cycling to the formation of soil structure. The extreme physical, chemical and biological diversity of soil complicates characterization of soil biological activity. The first approaches to describing soil populations utilized plating and enumeration techniques of pure microbiological studies. Estimates of microbial numbers in soil provide information relating to the biological potential but do not indicate the extent to which such potential is expressed. Characterization of soil enzymatic activity may be indicative of microbial activity because enzymes mediate the biochemical reactions which constitute microbial activity. Biochemical measures, such as enzymatic activity, have the added advantage of integrating the microbial activity of all species contributing to the process being measured. The integrative nature of biochemical indices is the result of common pathways of metabolism shared by many organisms. The utility of biochemical indices in quantifying the size of soil populations is demonstrated in the chloroform fumigation-incubation technique (Jenkinson and Powlson, 1976).

Common mechanisms of biochemical control also present the possibility of coordination of the biochemical activity of soil populations. Control of metabolic pathways within cells is in response to physical and chemical changes in the external environment. The basic nature of these control mechanisms is similar across a broad range of species. This raises the potential for populations or communities, comprising several species, to respond in a coordinated fashion to the external environment. An understanding of basic control mechanisms, expressed at the horizon-level, would be a useful tool in soil management. A search for such mechanisms requires that enzymatic control[1] within organisms be understood and

[1]Enzyme control refers to the combined effects of the regulation of enzyme synthesis and degradation in determining enzyme content.

expression at community, aggregate and horizon-levels be evaluated (McGill and Myers, 1987).

2.2 The role of stability in determining enzyme content

Synthesis and subsequent inactivation (turnover) of enzymes in soil and the implications of turnover rate for the control of soil enzyme content has not been reported. Soil enzymes are normally categorized in terms of their source or location. In relation to a discussion of the control of enzyme content, classification according to stability may be more appropriate. Soil enzymes or abiontic[1] enzymes are those enzymes which are sufficiently stable to act independently of the soil microbial biomass. Absolute stability cannot be determined and must be expressed relative to the duration of the study. Beyond their impact upon stability, the location of, and processes bringing about the stabilization of soil enzymes do not directly affect the control of enzyme content. Enzyme location is of importance in influencing enzyme activity (Horvath and Engasser, 1974).

Control of enzyme synthesis in microbial cells is achieved through inducer and repressor molecules which convey information about the chemical environment of the cell and dictate protein synthesis. The content of previously synthesized protein, including enzymes, is reduced by gradual dilution in growing cells, or removal by selective degradation in both growing (Bachmair *et al.*, 1986) and non-growing (Willets, 1967) cells. Proteins appear to have characteristic rates of turnover dictated by the N-terminal amino acid residue of the molecule (Bachmair *et al.*, 1986). Proteins catalyzing key metabolic functions and regulatory proteins are degraded rapidly allowing close synchronization between currently favoured protein synthesis and enzymatic composition. Thus the stability or persistence of an enzyme, in combination with selective synthesis, is integral to the regulation of the enzymatic composition of intact, living organisms in soil.

In addition to enzymes mediating intracellular reactions, microorganisms produce a wide array of extracellular enzymes whose functions include the hydrolysis of large potential substrates into smaller, transportable substituents. Increased stability prolongs the functionality of these molecules, reducing the

---

[1] Abiontic refers to enzymes acting independently of their biological source.

amount of energy dedicated to synthesis (Burns, 1982). Release into the soil environment may alter the stability of extracellular enzymes. The stability of enzymes may be altered by denaturation, degradation or inactivation following exposure to the soil environment. Alternatively, stable enzyme complexes may form in soil such as the humic acid-cellulase complexes synthesized by Sarkar (1986) or the peroxidase extracted from soil by Bollag *et al.* (1987). They hypothesized the extracted enzyme (10% of soil peroxidase activity) was either a glycoprotein or adsorbed to a polysaccharide. Thus the stability of an enzyme must be viewed as a function of both inherent chemical stability as well as stability in the environment in which it occurs.

Under conditions of continual inactivation or degradation most enzymes occurring in soil require continued enzyme synthesis for their persistence and thus are under biological control. If enzyme synthesis is not favoured under current environmental conditions activity in the soil environment will diminish. Alternatively the activity of soil stabilized enzymes persists under conditions not favouring synthesis. As a result of their stability, such enzymes are no longer dependent upon continued enzyme synthesis for their persistence and are therefore insensitive to normal mechanisms of biological control. In general the degree of biological control exerted over enzyme content, be it intra- or extra-cellular enzyme, is inversely related to the protein's stability in its environment.

2.3 Stabilization of enzymes in soil

The total enzymatic composition of soil is the result of enzymes occurring in a variety of environments. Burns (1982) grouped soil enzymes into ten categories based upon their location. These groupings are:

1. cytoplasm of proliferating cells
2. periplasm of proliferating Gram-negative bacteria
3. attached to the outer surface of viable cells
4. secreted by living cells
5. associated with non-proliferating cells
6. attached to dead cells and cellular debris
7. released from lysed cells
8. temporarily associated in enzyme-substrate complexes
9. adsorbed to clay minerals
10. associated with humic colloids.

The stability or longevity of enzymes occurring in each of these categories varies. Only the latter two categories are likely to be sufficiently stable for their contents to be considered abiontic or independent of biological control.

Not all enzymes adsorbed to clay minerals or associated with humic colloids will be catalytically active in this form. Binding of the enzyme may alter the conformation of the protein rendering it inactive. Also the geometry of enzyme association may be of importance in determining the potential activity of the complex. Blockage of binding sites for substrate or co-enzyme will result in stable but inactive complexes. Enzyme adsorption has been reported to increase (Aomine and Kobayashi, 1966; Marshall, 1976), decrease (Ambroz, 1970; Burns *et al.*, 1972) or not affect (Harter and Stotsky, 1973; Marshall, 1976) enzymatic activity[1]. Adsorption also influences enzyme kinetics, altering reaction rates and substrate affinities (Horvath and Engasser, 1974; McLaren and Packer, 1970).

While the potential exists for the formation of stable complexes with either intra- or extra-cellular enzymes, the simplicity of reaction, continual release into the environment and inherent longevity of extracellular hydrolytic enzymes favors the formation of stable active complexes. Intracellular enzymes on the other hand, often utilize complex reaction mechanisms involving cofactors or co-enzymes. In the ordered environment of the cell this allows for modulation of enzymatic activity resulting in an additional level of metabolic regulation (eg. glutamine synthase; Tyler, 1978). Thus even if such an enzyme were stabilized in a potentially active conformation, the simultaneous occurrence of enzyme, substrate and co-enzyme in the uncoordinated soil environment is unlikely. Thus enzymes of a relatively simple reaction are more likely to form stable, active complexes in soil.

2.4 The role of stabilized soil enzymes in nitrogen mineralization

Nitrogen mineralization by soil organisms is the result of the degradation of organic nitrogen compounds. The control of the enzymatic potential for nitrogen mineralization in soil systems is achieved through the combined effects of

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[1] In this usage activity refers to the combined effects of physical integrity, functionality and access. Thus, for example, a functional enzyme may be rendered "inactive" by restricted substrate access as a result of physical entrapment.

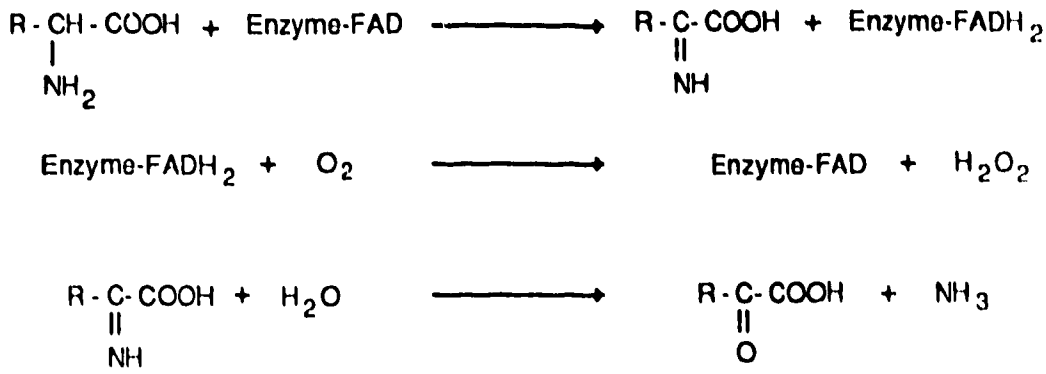
regulation of enzyme content and modulation of enzyme activity. The degree to which enzyme content is regulated will depend upon enzyme stability and synthesis. An accumulation of stable, active nitrogen-mineralizing enzyme would reduce dependence upon continued enzyme synthesis. In the absence of a large amount of stable enzyme, control of enzyme content rests with the biomass and the relative contributions of inducible and constitutive activity. A predominance of constitutive enzyme synthesis reduces the amplitude of response to changes in soil conditions. In situations where constitutive synthesized enzyme accounts for a significant component of total activity the precision of control is restricted. The means by which the content of nitrogen-mineralizing enzymes will be controlled in soil will depend upon both the potential for the formation of stable, active complexes as well as the regulation of enzyme synthesis. The role of the content of mineralizing enzyme in determining the rate of mineralization will relate to activity which is dependent upon substrate availability.

2.4.1 The potential for abiontic deaminase activity in soil

Ladd and Jackson (1982) discuss the reactions by which nitrogen mineralization occurs in soil. In this work the focus is on the deamination of amino acids. Deamination reactions are involved in both catabolic and anabolic pathways. Prior to amino acid catabolism the α–amino group is removed to yield ammonium and the keto acid which may then be catabolized. Alternatively the amino group may be transferred to another organic molecule, often as part of a synthetic pathway, in a transamination reaction. Transamination does not directly result in the mineralization of nitrogen and therefore will not be discussed further.

Deaminase reactions may be oxidative or non-oxidative. In general oxidative deaminases involve more complex reaction mechanism often requiring the participation of nicotinamide or flavin co-enzymes. The participation of interenzymatic co-enzymes, such as nicotinamide, limits the potential for these enzymes to form active, stable complexes in soil. The likelihood of the simultaneous occurrence of substrate, reduced co-enzyme and apo-enzyme would be sufficiently rare to limit the significance of such reactions. Flavin dependent enzymes are more likely to form active stable complexes as the flavin is an intraenzymatic co-enzyme, remaining bound to the apo-enzyme to form a stable holo-enzyme (Walsh, 1979). The likelihood of flavin based reactions forming stable active complexes in soil is further increased by their common use of

molecular oxygen as an electron acceptor (Walsh, 1979). An example of flavin based oxidative deaminase which might form a stable active complex in soil is L-amino acid oxidase found in mammalian liver and in snake venom.

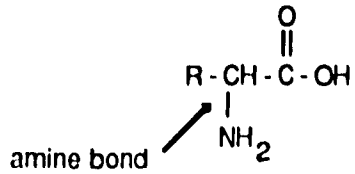


Weetall and Baum (1979) report the preparation of active, immobilized L-amino acid oxidase on silica glass particles.

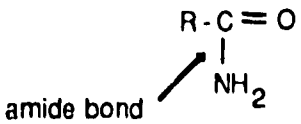
Non-oxidative deaminase reactions include hydrolases and lyases. Hydrolases result in the deamination of amino acids through hydrolysis producing an unstable intermediate which results in formation of ammonium and the α-keto acid (eg. L-serine dehydrase). While these enzymes generally require the participation of pyridoxal phosphate, it remains bound to the apo-enzyme and thus would not prevent the formation of stable active forms of these enzymes. Lyases remove ammonia, generating a double bond (olefin). Two common ammonia lyases are L-histidine NH_3-lyase and L-phenylalanine NH_3-lyase. The simplicity of reaction catalyzed by non-oxidative deaminases may allow for the formation of active stable complexes in soil systems.

2.4.2 Deaminase vs Deamidase

Deaminases catalyze the cleavage of amino bonds while deamidase (also called amidases) cleave amide bonds of amines.



Amino Acid



Amide

Amino acids, which contain an α–amino group, are important structural components of cells, functioning as building blocks for the construction of protein as well as having an important structural role in procaryotic cell walls. Amines are more frequently used as nitrogen storage compounds (Clarke, 1980). They are primarily donors of nitrogen groups in biosynthesis (eg. glutamine and asparagine), for transporting wastes (urea) and in the transport of nitrogen (ureides, allantoin). These differences are reflected in the nature of their respective dissimilatory enzymes. Deaminase reactions are closely regulated and tend to involve complex reactions, involving co-enzymes and energy. Deaminase enzymes appear to be exclusively intracellular. Deamidase reactions involve more simplistic reaction mechanisms and have both intracellular and, in fungi and yeasts, extracellular forms (Wade, 1980).

Evidence for stable deaminase activity in soil is limited. The activities of both phenylalanine NH_3-lyase (Tena *et al.*, 1986) and L-histidine NH_3-lyase (Frankenberger and Johanson, 1982) have been reported in soil. These reports are of short-term studies ($\leq$ 120 h) and are not conclusive evidence of stable activity. The simplicity of reaction for both of these enzymes may explain their activity in soil. There have been numerous reports of stable soil deamidase activity, however. Ladd (1978) reviews these studies which include reports of asparaginase (EC

3.5.1.1), glutaminase (EC 3.5.1.2) and urease (EC 3.5.1.5) activities. There has been extensive research of the origin, persistence, location and kinetics of soil urease activity. Urease has been found to be relatively stable in soil as demonstrated by activity in longer-term (3-12 months) laboratory studies and 9550 year old permafrost soil samples (Ladd, 1978).

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3. ROLE OF ENZYME STABILITY IN CONTROLLING HISTIDINE DEAMINATING ACTIVITY IN SOIL[1]

3.1 Introduction

Amino acids commonly account for up to 50% of organic N in soil and thus their deamination is an important source of ammonium in soil. Microbial amino acid deamination occurs intracellularly and is subject to carbon and nitrogen catabolite control (Lessie and Neidhardt, 1967). In soil however there is the potential for immobilization and stabilization of enzymes by soil particles to yield abiontic (stabilized in association with abiotic components) enzyme activity. Thus total soil enzyme activity comprises labile enzymatic activity of recent biological origin as well as stabilized activity.

Total Enzymatic Activity = Biological Activity + Abiontic Activity

The stability of abiontic components circumvents cellular control of enzyme content by mechanisms such as catabolite repression (Chapter 2; Burns, 1986). Therefore the existence of abiontic amino acid deamination in soil could modify carbon and nitrogen control of nitrogen mineralization and thus there is a need to consider the relative magnitude of microbial and abiontic enzymatic activities contributing to nitrogen mineralization in soil.

The mechanism of enzymatic reaction influences the potential for abiontic activity (Chapter 2). Active abiontic forms of cellular enzymes requiring the participation of an interenzymatic redox cofactor (eg. NAD; as defined by Walsh, 1979) are unlikely to occur. The simultaneous occurrence of substrate, interenzymatic cofactor and a functional enzyme would be sufficiently unlikely in a non-coordinated system to limit the extent of reaction. Most deaminating enzymes are oxidoreductases requiring the participation of the interenzymatic cofactors (eg. L-amino acid dehydrogenase) and thus are unlikely to have active abiontic forms.

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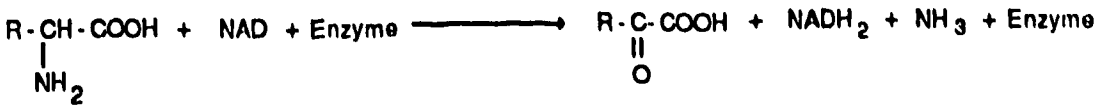
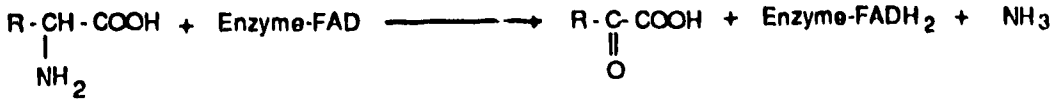
[1] A version of this chapter has been accepted for publication. D.L. Burton and W.B. McGill (Soil Biology and Biochemistry)



Other deaminating oxidoreductases utilize intraenzymatic redox carriers such as flavoproteins in which FAD is alternately oxidized and reduced, using O_2 as the electron acceptor (eg. L-amino acid oxidase; Walsh, 1979) and thus may facilitate abiontic deaminase activity in soil.



Carbon-nitrogen lyases eliminate NH_3 through hydrolysis and form a double bond, thereby avoiding the necessity of a redox carrier. L-histidine NH_3-lyase (E.C. 4.3.1.3; histidase) is an example. Thus there is potential for abiontic deaminase activity in soil but catalyzed by only a subset of the range of enzymes capable of deaminating.

The contribution of abiontic deaminase activity to soil nitrogen mineralization remains unresolved (Ladd and Jackson, 1982). Based on evidence presented by Greenwood and Lees (1960) and Voets and Dedeken (1965), Skujins (1978) questioned Subrahmanyan's report of abiontic glycine deamination in soil. Greenwood and Lees (1960) did not observe alanine degradation products in leachates of alanine-amended soils. Voets and Dedeken (1964) noted amino acids and not ammonium accumulated following the proteolysis of gelatin in toluene-treated soils. Similarly, Kiss *et al.* (1975) concluded, based on the work of Ambroz that the deamination of amino acids in soil is due exclusively to the enzymes of proliferating microorganisms.

Ladd and Jackson (1982) cite unpublished work by Ladd and Amato in which NH_4^+ slowly accumulated in soils exposed to chloroform vapor. This was taken as evidence for at least transient deaminase activity in the absence of microbial growth. Subsequent studies have reported the deamination of L-histidine in soils treated with toluene (Frankenberger and Johanson, 1982) and the deamination of L-phenylalanine in azide-treated soils (Tena *et al.*, 1986). Enzymes not requiring the participation of an interenzymatic redox carrier have been reported for both of these deaminations; L-histidine NH_3-lyase for the deamination of histidine and a number

of enzymes for phenylalanine including L-phenylalanine NH_3-lyase (Walsh, 1979) and phenylalanine oxidase (Koyama, 1982).

Our objective was to employ a range of assay durations together with kinetic analysis (Shipley and Clark, 1972) to examine the kinetic components of L-histidine NH_3-lyase in soil, their stability, and the implications for the control of this enzyme.

3.2 Materials and Methods

3.2.1 Soil description

Soil samples used were from the Ap horizon of a black chernozem (Typic Cryoboroll) under *Festuca rubra* at the Ellerslie Research Station, Edmonton, Alberta. The soil had a SiCl texture, organic carbon content of 6.0%, pH of 5.4 ($CaCl_2$) and retains 38% H_20 ($w.w^{-1}$) at field capacity (Maulé and Chanasyk, 1987). Morphological and chemical characteristics of this soil have been published by Pawluk (1986).

3.2.2 Soil preparation

Soil samples were stored field moist (25 % $w.w^{-1}$) at 4 °C until use. With each experiment the soil was sieved (< 2 mm) and held at 12 °C for at least a week before being assayed. An incubation temperature of 12 °C was felt to be representative of local conditions. For air-dry treatments the soil was air-dried following sampling and stored at room temperature (22 °C) until onset of the assay. Soils treated with a carbon source, glucose-C or urocanate-C (100 $\mu g\ g^{-1}$), were aerobically incubated for 3 days at 12 °C following the addition of 1 ml of solution containing the carbon source to 5 g soil at 25% ($w.w^{-1}$) H_2O.

3.2.3 Assay procedure

Changes in potential histidase activity were used as a measure of soil histidase content. In enzyme systems which exhibit saturable reaction kinetics the velocity of reaction (activity) approaches the maximum velocity (V_{max}) and the system approaches zero order as substrate concentration increases well beyond K_m. The V_{max} of reaction is a function of the amount of enzyme present and the rate of turnover of the enzyme-substrate complex. If for a given set of conditions, the rate

of enzyme-substrate turnover (specific activity of the enzyme) is constant, then potential enzyme activity (velocity under conditions where substrate concentration is an order of magnitude greater than K_m) is directly related to total active enzyme content.

The assay of L-histidine NH_3-lyase described by Frankenberger and Johanson (1982) was modified as follows: 5 g portions of field moist, soil were weighed into round 30 ml screw-top vials. One ml of toluene was added to each vial which was capped and equilibrated at room temperature (22 °C) for 5 min before the assay. At the time of initiation (t=0) 9 ml of 0.1 M Tris (hydroxymethyl) aminomethane buffer (pH 9.0) were added to each vial, followed by 1.0 ml of a 0.5 M histidine solution. The vials were capped, mixed and shaken at 120 oscillations min^{-1} (reciprocal shaker) or 200 revolutions min^{-1} (rotary shaker) at 37 °C for the duration of the assay. All reactions were terminated with 5 mM uranyl acetate - 2.5 M KCl solution. Control samples received histidine solution after the assay. After the assay mixtures were stored at 4 °C until distillation. Treatments were replicated 3 times with 2 control samples for each treatment.

Ammonia[1] content was determined by steam distillation with MgO (Bremner, 1965). The entire assay mixture and rinse water was transferred to a 500 ml round-bottomed flask for distillation. Ammonia was collected in 5 ml of 2% boric acid solution and titrated with 5 mM H_2SO_4 to pH 4.5 using a memo titrator (Mettler, Model# DL 40 RC). Gravimetric soil water content was determined on the bulk soil and reaction rates expressed on a per g dry soil basis.

3.2.4 Influence of assay conditions

Effect of amount of toluene - Toluene additions of 0, 0.2, 1 or 2 ml were added to 5 g of moist soil 5 min before the assay.

Denaturation by toluene - The effects of toluene were evaluated by comparison with azide, a biostatic agent differing in mode of action. One ml of 1% w/v azide (Tena *et al.*, 1986) replaced the toluene normally added to the soil before the assay.

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[1] The term ammonium is used here to indicate the protonated form NH_4^+ and ammonia is used to specify the total of the species NH_4^+, NH_4OH and NH_3 present in a given system.

Adsorption induced substrate limitation - The possibility of adsorption induced substrate limitation was examined by altering the substrate additions. Samples receiving all substrate at time=0 were compared to those receiving 50% of the substrate at time=0 and the remainder at time=24 h of the 48 h assay.
Ammonium inhibition - The potential for end product inhibition of histidase was considered by treating soils 48 hours before assay with 20 $\mu g\ g^{-1}$ as $(NH_4)_2SO_4$-N and held at 12 °C.

3.2.5 Influence of soil history

The decay of histidase was directly examined in a series of four experiments. The first examined field moist (25% w w^{-1}) and air-dry soils. Seven assay durations were used (0, 2, 4, 8, 16, 24 and 48 h). The second experiment concerned the effect of glucose and urocanate treatment (C at 100 $\mu g\ g^{-1}$) on enzymatic components. Soils were treated with 1.0 ml of solution to the 5 g soil samples (C at 500 $\mu g\ ml^{-1}$). Samples were then held at 12 °C for 3 days. The final two experiments, in combination with the previous experiments, documented the temporal variation in component size in field moist soil (25% w w^{-1}). The final three experiments all used 12 assay durations (0, 1, 2, 3, 4, 6, 10, 16, 24, 48, 72 and 96 h). In each case assay durations were replicated three times with two control samples. In the first and third experiments samples were shaken on a reciprocal shaker the remaining two on a rotary shaker.

3.2.6 Mathematical analysis

Histidase activity was measured by monitoring ammonia accumulation. Average activity (calculated by dividing the cumulative product by the period of accumulation) can be used to estimate an instantaneous rate only under conditions of constant enzyme activity over the assay period. A non-linear least square fitting procedure (Systat NONLIN, Systat Inc., Evanston, Il.) was used to fit a linear model as well as integrated forms of both single and double component first-order decay models to the data. The integrated first-order expressions can be derived from the first-order expression for decay in enzymatic activity. The instantaneous activity of an enzyme decaying according to first-order kinetics can be expressed as,

$$A_t = A_o e^{-kt} \qquad [3.1]$$

where: A_t = Initial component size ($\mu g\ g^{-1}\ h^{-1}$) at time t(h)
A_o = Initial component size ($\mu g\ g^{-1}\ h^{-1}$) at time t=0
k = decay constant (h^{-1}).

Equation [3.1] can be expressed in terms of cumulative product at time t by integrating over time, under conditions of non-limiting substrate and no enzyme synthesis.

$$\text{Cum. } NH_4^+ \text{ Prod.}_t = (A_o/k)\ (1 - e^{-kt}) \qquad [3.2]$$

Activity may be the result of two components, in such cases component B may be stabilized by a different mechanism or in a different location than component A, and decay at a different rate. The corresponding expressions for a two component model are,

$$A_t = A_o e^{-k_1 t} + B_o e^{-k_2 t} \qquad [3.3]$$

where: B_o = Initial size of component B ($\mu g\ g^{-1}\ h^{-1}$) at time t=0.

$$\text{Cum. Prod.}_t = (A_o/k_1)\ (1 - e^{-k_1 t}) + (B_o/k_2)\ (1 - e^{-k_2 t}) \qquad [3.4]$$

The adequacy of linear, single-component first-order and two-component first-order models in describing ammonium accumulation curves was examined by testing the significance of the reduction in the residual sum of squares (RSS) relative to the complexity of each model. Significance was determined by computing the F-statistic (Robinson, 1985). The statistic is calculated by dividing the difference between the RSS of the two models by the residual mean square (RMS) of the more complex model. The result was compared to an F value at $p \leq 0.05$ with 1 and n-p degrees of freedom, where n = the number of data points and p = the number of parameters.

3.3 Results

3.3.1 Effect of assay conditions

The necessity for and an optimum quantity of toluene required to ensure biostasis was examined. Toluene addition reduced NH_4^+ accumulation during 48 h to about 25% of non-treated soil regardless of the quantity added (Table 3.1). All toluene additions examined were sufficient to prevent enzyme synthesis-induced increases in soil histidase. In keeping with additions recommended by Skujins (1967), 1.0 ml of toluene was added to 5 g of moist soil (0.25 ml g^{-1} dry soil) in all subsequent work.

Table 3.1 Evaluation of the effect of the amount of toluene added to 5 g (moist) soil samples on soil histidase.

Toluene (ml)	Cum. NH_4^+-N Prod. ($\mu g\ g^{-1} 48\ h^{-1}$)
0	388 a*
0.2	88 b
1.0	101 b
2.0	94 b

* values followed by different letters are significantly different at p=0.01

Toluene addition had no effect on the rate of ammonium accumulation in assays lasting 6 h or less (Fig. 3.1). Lower soil histidase was observed in toluene-treated soils in the 8 h assay. Accumulation of product in the absence of toluene was linear whereas in the presence of toluene accumulation was curvilinear (Fig. 3.1). Average activities (determined by dividing the product accumulated by the assay duration) were plotted against assay duration (data not shown). In soils not receiving toluene the slope was not significantly different from zero whereas in toluene-treated soils a highly significant ($p \leq 0.001$) decline in soil histidase was observed.

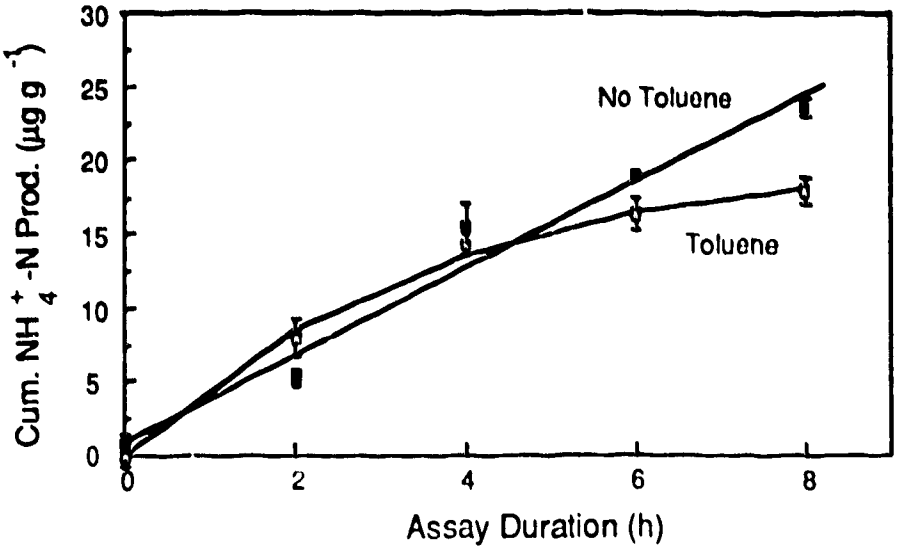

Fig. 3.1 Short-term accumulation of ammonium as a result of histidine addition in the presence and absence of toluene. Curves are simple linear regression of untreated samples and non-linear least squares first-order fit of treated samples (points are means of three replications, bars indicate standard error).

Toluene and azide were compared as biostatic agents in assay durations ranging from 0 to 96 h (Fig. 3.2). Rate of ammonium accumulation declined with time in both toluene and azide treatments. This can be seen more explicitly in the decline in average activity with increasing assay duration (Fig. 3.2b). This decline in average activity with assay duration was highly significant ($p \leq 0.001$). Soil histidase in the presence of toluene, was not significantly different for the common assay times in this and the preceding experiment (Fig. 3.1).

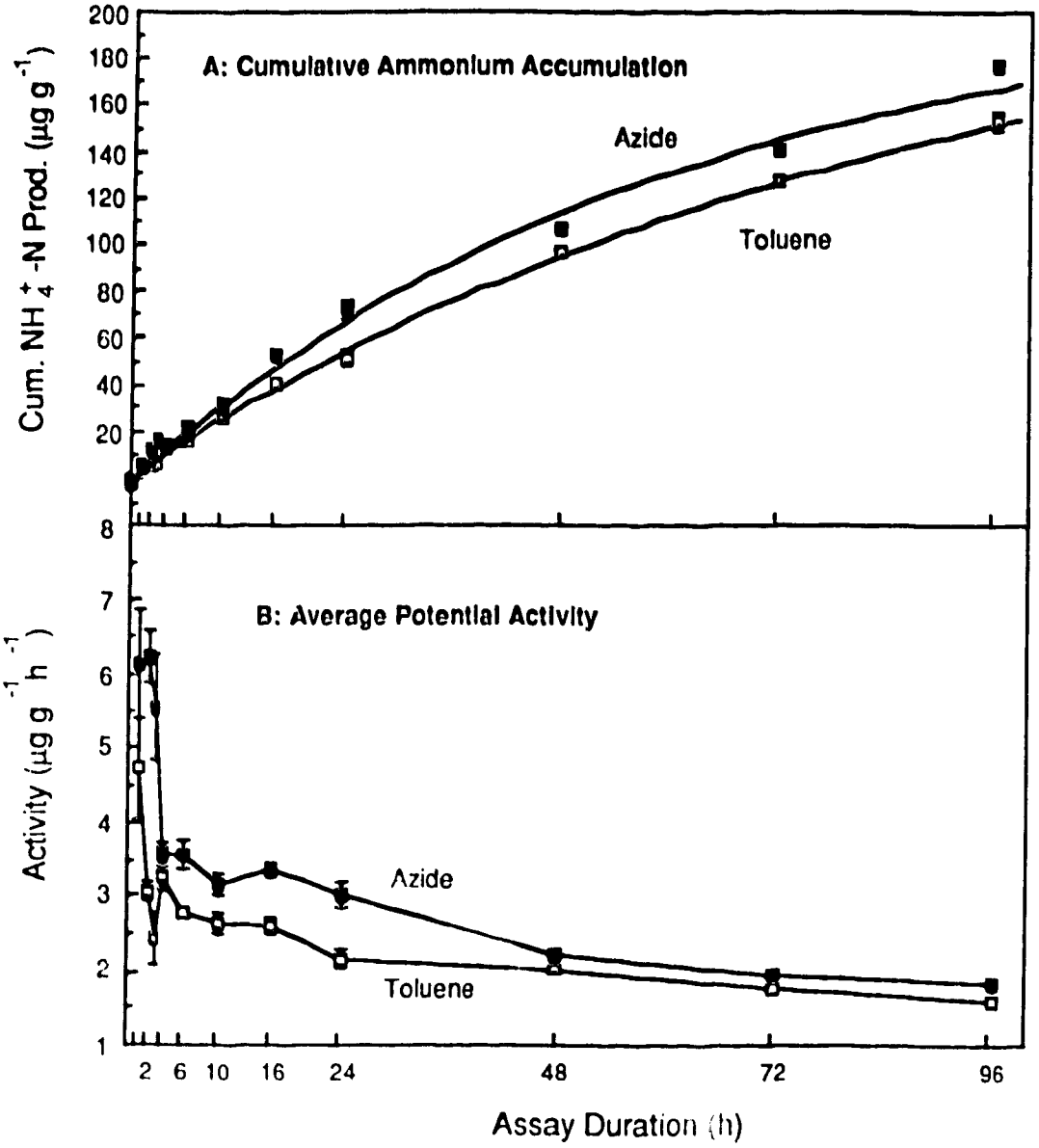

Fig. 3.2 Accumulation of ammonium (A) and average histidase activity (B) resulting from histidine addition in soil treated with toluene or azide. Curves are A) equation predicted from a two-component, first-order fit of the data and B) interpolation of data points. Points are the mean of three replications, bars indicate standard error.

Soil histidase was not significantly affected by either single or split substrate addition. Mean NH_4^+-N accumulation during 48 h was 81.9 ± 2.4 µg g^{-1} soil when the substrate was added in a single dose at t=0, and 81.2 ± 4.8 µg g^{-1} soil in treatments receiving substrate in split doses at t=0 and 24 h. Soil treatment with NH_4^+-N (20 µg g^{-1}) did not significantly affect soil histidase either (data not shown).

3.3.2 Kinetic analysis of ammonium accumulation curves

Components contributing to soil histidase were examined to determine whether there are one or more components of activity and their size and stability. Examination of the residual sum of squares (RSS) indicated single component first-order models provided a significantly better fit of the data than do linear models (Table 3.2). Further, two-component first-order models produce a RSS which is significantly smaller than those of single-component models. Table 3.2 contains a representative analysis for glucose and urocanate treated soils as well as non-amended soil samples. The residual values for the two-component model are uniformly grouped around zero (Fig. 3.3). Linear approximations of the data over estimate initial rates of ammonium accumulation and underestimate accumulation in assays lasting 12-48 h. The single-component, first-order model reverses the trend found in the residuals of the linear model; under estimating initial accumulation, while over estimating ammonium accumulation in assays of intermediate duration. The two-component model identifies a labile component with a half-life of less than 5 h and a more stable component with a half-life ranging from 30 to 140 h (Table 3.2).

Moist, non-amended treatments were included in each of the four experiments to allow an examination of the variation in parameters between experiments. The coefficient of variation of estimated size of the labile component in non-amended soil was 32% between experiments (4.28 ± 1.36), whereas it was only 8% for the stable component (2.12 ± 0.17; Table 3.3). The decay rate of labile and stable components of non-amended samples also varied between experiments (k_1 = 0.272 ± 0.048; k_2= 0.011 ± 0.003). Air drying or soil amendment increased k_1 (0.441 ± 0.020) which is interpreted as a decrease in the stability of the labile component. The decay rates of the stable component were less affected by amendment (0.018 ± 0.002, in amended; 0.011 ± 0.003, in non-amended). The half-lives of the stable components (non-amended = 77.35 ± 21.47; amended = 39.37 ± 3.41) are similar to the assay duration (48 h), contributing to the uncertainty in the estimates.

Air drying soil reduced the size of the labile pool of soil histidase (Fig. 3.4) in addition to increasing its turnover rate. The size of the stable component was unaffected by air-drying although its stability decreased (Table 3.3). Glucose treatment did not significantly affect the size of labile or stable components. Soil treatment with the inducer of histidase, urocanate, resulted in significant increases in the size of both labile and stable components of histidase (Fig. 3.5).

Table 3.2: Evaluation of models to describe accumulation of ammonium (Y) with time (t) in three soil treatments. The F statistic was calculated as the difference in residual sum of squares (RSS) of the two models divided by the residual mean square (RMS) of the more complex model described by Robinson (1985).

	Model	RSS	RMS	F-Ratio	%TSS§
	Nonamended				
Linear	$Y = 2.0\ t + 19.1$	6094	197		98.0
Single	$Y = \frac{4.5}{0.021}(1 - e^{-0.021t})$	4458	144	11.38*	98.5
Double	$Y = \frac{5.1}{0.147}(1 - e^{-0.147t}) + \frac{2.2}{0.005}(1 - e^{-0.005t})$	3021	104	13.80*	99.0
	Glucose-C (100 μg .g $^{-1}$)				
Linear	$Y = 1.7\ t + 16.3$	6320	204		97.0
Single	$Y = \frac{4.0}{0.023}(1 - e^{-0.023t})$	3619	117	23.19*	98.3
Double	$Y = \frac{4.6}{0.463}(1 - e^{-0.463t}) + \frac{3.1}{0.016}(1 - e^{-0.016t})$	3161	109	4.20*	98.5
	Urocanate-C (100 μg .g $^{-1}$)				
Linear	$Y = 2.7\ t + 33.2$	9566	309		98.0
Single	$Y = \frac{7.2}{0.025}(1 - e^{-0.025t})$	2856	92	72.85*	99.5
Double	$Y = \frac{8.4}{0.401}(1 - e^{-0.401t}) + \frac{5.1}{0.016}(1 - e^{-0.016t})$	1163	40	42.25*	99.8

§ Percent of total sum of squares accounted for by the model
* Significantly different at $p \leq 0.05$ ($F_{0.05(1,29)} = 4.18$)

Table 3.3: Values for parameters of double exponential model describing the decay in soil histidase calculated from experimental data using non-linear least-squares fitting techniques

Treatment	labile			stable		
	$A^{\dagger}$ ($\mu g\ g^{-1}\ h^{-1}$)	k_1 (h^{-1})	$t_{1/2}$ (h)	B ($\mu g\ g^{-1}\ h^{-1}$)	k_2 (h^{-1})	$t_{1/2}$ (h)
	----------Effect of Air Drying*----------					
Moist, Non-amended	3.58	0.358	1.9	2.29	0.011	63.0
Air Dry	1.67	0.460	1.5	2.48	0.022	31.5
	----------Effect of Soil Treatment----------					
Moist, Non-amended	5.12	0.147	4.7	2.17	0.005	138.6
Glucose	4.59	0.463	1.5	3.08	0.016	43.3
Urocanate	8.40	0.401	1.7	5.09	0.016	43.3
Moist, Non-amended*	7.46	0.338	2.1	1.63	0.018	38.5
Moist, Non-amended•	0.97	0.245	2.8	2.39	0.010	69.3

* reciprocal shaker
• rotary shaker
† initial soil histidase (t=0)

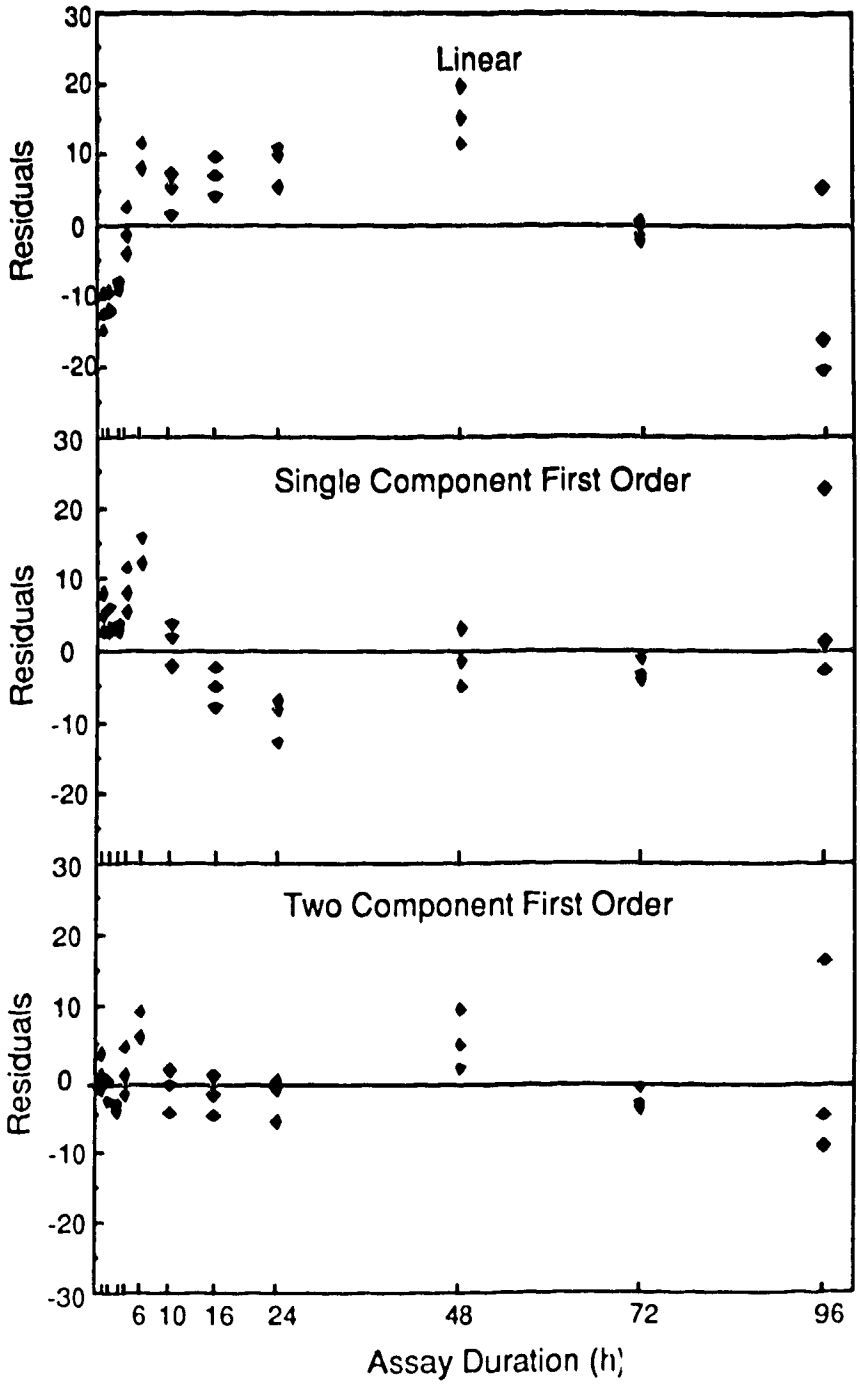

Fig. 3.3 Distribution with time for residuals of non-linear least squares analysis of the data using linear, single component and two-component first-order models.

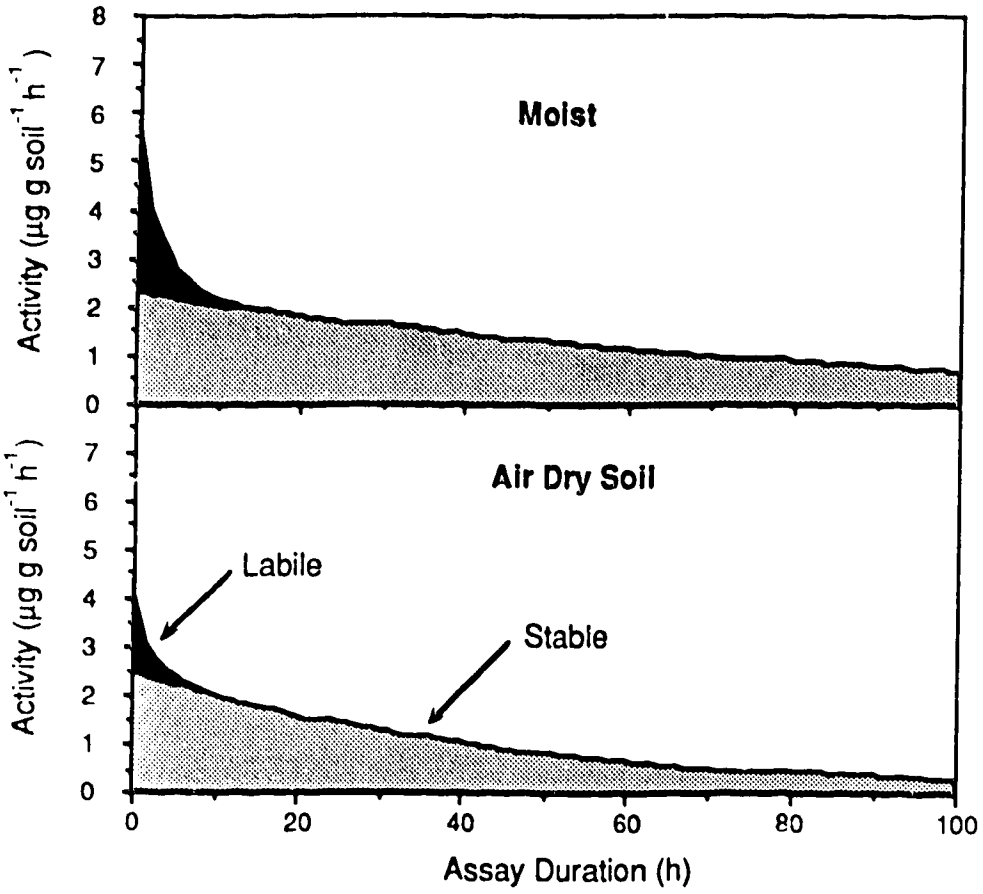

Fig. 3.4 Contribution of labile and stable components (Eq. 3.3) to soil histidase as predicted by kinetic analysis of data from moist and air dried samples. Decay rate constants and pool sizes from Table 3.3.

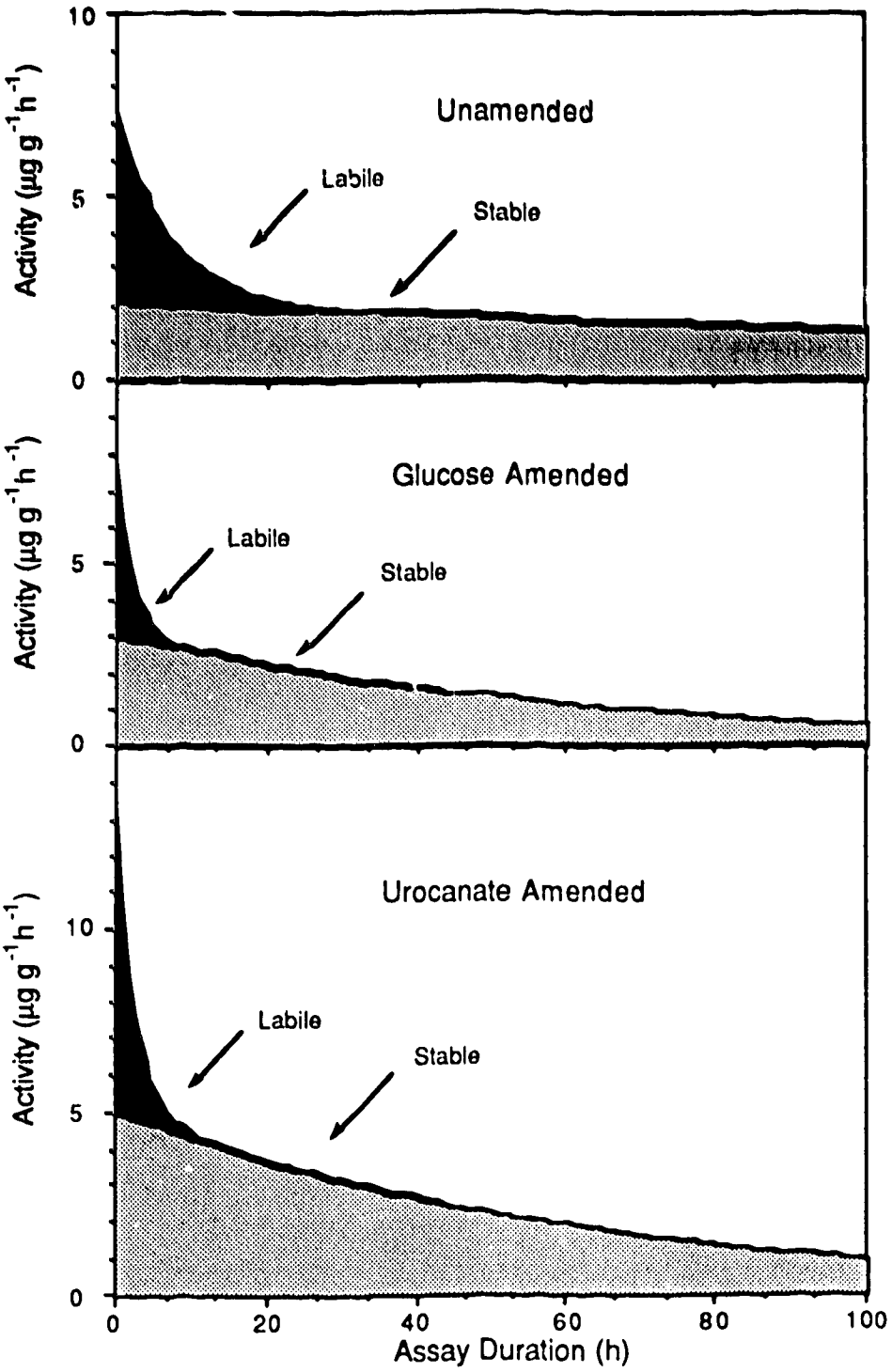

Fig. 3.5 Effect of soil treatment with urocanate or glucose on labile and stable components (Eq. 3.3) of soil histidase in moist soil. Decay rate constants and pool sizes from Table 3.3.

3.4 Discussion

3.4.1 Decline of soil histidase

Residual soil histidase persisted in soils treated with sufficient toluene to inhibit microbial growth completely. Frankenberger and Johanson (1982) observed persistent histidase activity in soils treated with 1.3% toluene. While this concentration of toluene inhibited induction of enzyme synthesis, it is below the concentration of toluene recommended to ensure complete inhibition of microbial activity. Beck and Poschenrieder (1963) found soil treatment influenced the quantity of toluene required for biostasis. At least 20% by volume toluene was required to inhibit microbial activity completely in moist soils while 5 - 10% was sufficient for soil suspensions (Skujins, 1976). At concentrations of 0.05 - 0.1%, toluene serves as a substrate for microorganisms (Kaplan and Hartenstein, 1979); and at 1%, it increases the ratio of bacteria to fungi colony forming units (Waksman and Starkey, 1923). In our study increasing toluene concentration from 1.3 to 13.3 % ($v.w^{-1}$) had no effect on histidase activity (Table 3.1).

The persistence of enzyme activity in toluene-treated soils has been taken as evidence for abiontic enzyme activity (Burns, 1986). This question can be examined more directly by determining the degree of persistence, or stability, of enzymatic activity over the duration of the assay. This allows a differentiation of activity associated with unstable, remnant microbial enzyme, from catalysis by stabilized enzyme.

If histidase activity in soil is a result of stable abiontic enzyme, the rate of catalysis should not be affected by the addition of a biostatic agent. Stable activity would thus result in linear product (NH_4^+) accumulation during prolonged assays. Curvilinear accumulation of NH_4^+ was observed, however (Fig. 3.2a) which suggests declining enzymatic activity with increasing assay duration. Histidase activity may decline over time in the presence of a biostat due to one, or a combination, of four processes: i) substrate immobilization; ii) end product inhibition of enzyme activity; iii) enzyme destruction by the biostatic agent; or iv) gradual loss of enzyme activity due to degradation or inactivation of the enzyme in the absence of enzyme synthesis or both.

The amount of substrate added was in excess of that required to saturate enzymatic activity allowing the evaluation of potential activity and enzyme content (>> K_m; Frankenberger and Johanson, 1982). In soil systems the extensive charged surface area associated with soil particles provides the potential for binding of organic molecules such as histidine, reducing their concentration in solution and thus restricting the opportunity for participation in enzyme catalysis. If this were the case, split applications would alter the rate of substrate immobilization increasing substrate availability and thus histidase activity. Estimates of histidase activity were similar in assays in which substrate was added in single or split applications. These results also preclude the possibility of allosteric modulation of enzyme activity, as a result of reduced substrate concentration, being the causal agent (Frankenberger, 1983). Product (NH_4^+) accumulation was also found not to contribute to declining activity. Therefore substrate limitation and end-product inhibition were not the causal agents for declining activity. *In situ* degradation of enzymatic activity is considered the reasonable and probable alternative.

Toluene may alter instantaneous histidase activity as a result of cell rupture, increased membrane permeability or direct inhibition of enzyme activity (Frankenberger and Johanson, 1986). The extent of this alteration was evaluated in short-term assays of histidase activity in the presence and absence of toluene. Short-term assays (< 4 h) provide insufficient time for induction, synthesis and expression of additional microbial enzymes and thus exclude increased biological response to substrate addition. Histidase activity was similar in the presence and absence of toluene in short-term (< 4 h) assays (Fig. 3.1). If histidase activity in the presence of toluene were enhanced by changes in cell wall permeability or cell lysis, lower rates of activity would be anticipated in intact cells where access to the enzyme had not been altered. Such lowering was not observed in untreated samples. Alternatively direct inhibition of histidase by toluene, which would reduce short-term activity relative to the non-treated soil, was also not observed. The decline in histidase activity in assay durations greater than 6 h is likely the result of enzyme turnover. The consistency of histidase activity in soils not receiving toluene may result from continued basal rates of enzyme synthesis equalling the rate of enzyme degradation. Toluene therefore does not appear to affect substrate access to this enzyme nor to inhibit histidase directly in the short-term.

While toluene and azide are both biostatic agents their mode of action differs. Azide acts as an uncoupling agent, inhibiting cytochrome oxidase, an enzyme important in cellular energy generation (Harold, 1972). Toluene inhibits activity by increasing the permeability of the cell membrane, allowing the dissipation of the proton motive force required for ATP generation. If the long-term decline in histidase activity were an artifact of toluene addition rather than its biostatic role, azide would not be expected to have a similar effect. Either azide or toluene treatment resulted in a declining rate of ammonium accumulation with time. Thus declining activity can not be considered an artifact of toluene addition. Normal soil denaturation processes are left as possible causes. The magnitude of the decline was, however, different for the two biostats (Fig. 3.2). Therefore, while the decline in rate of accumulation with time is due to soil processes, the magnitude of its expression is specific to the nature of the biostat-soil interaction.

At least a portion of the observed histidase activity in soil is due to a labile enzyme. Losses of activity associated with the cessation of enzyme synthesis and continued enzyme degradation are consistent with the observed decline in average activity during long-term assays (Fig. 3.2b), and with the fit of first-order decay models to such declines (Table 3.2).

3.4.2 Components of soil histidase and their stability

In heterogeneous systems the rate of enzymatic catalysis is dependent upon the amount of enzyme, concentration of substrate, and the rate of transport of substrate to the enzyme (McLaren and Packer, 1970). This is especially true of soil histidase activity which may be the result of enzymes associated with the biomass; enzymes associated with dormant cells or cell fragments; extracellular histidase; or abiontic histidase. Microbial control over enzyme content is facilitated by controlling relative rates of enzyme synthesis in growing cells; enzyme degradation in growing (Bachmair, *et al.*, 1986) and non-growing (Willetts, 1967) cells; or the denaturation or inactivation of the enzyme upon lysis (Burns, 1982). Soil stabilization can free enzymes from such microbial mechanisms of control (Burns, 1986).

For large immobile substrates, enzyme location is critical to the likelihood of reaction occurring. While the precise location of enzymic activity in soil is of interest, for an enzyme acting on a soluble molecule such as histidine, control of

enzyme content is the more relevant issue. The content of nitrogen catabolic enzymes in soil is key to the extent of mineralization relative to re-utilization of biosynthetic molecules such as amino acids. The balance of these two processes determines the relative importance of mineral and active organic forms of N in N-cycling (Jansson and Persson, 1982).

Kinetic analysis allows theoretical separation, according to the assumed model, of processes occurring concurrently (Shipley and Clark, 1972). It is useful in systems such as soil where physical separation is either impractical or impossible. Results of kinetic analysis of ammonium accumulation in the soils used in our study are consistent with the hypothesis that histidase activity in soil is a function of two diminishing components (Table 3.2; Fig. 3.3). The labile component ($t_{1/2}$ ~ 2-4 h) is highly dependent upon recent microbial metabolism for its activity and is thus expected to be sensitive to mechanisms of microbial control. The more stable second fraction ($t_{1/2}$ ~ 30 - 140 h) may be indicative of a second protein (Bachmair *et al.*, 1986) or result from the compartmentalization or adsorption of previously-labile protein by soil colloids or cellular debris. Both components of soil histidase appear to have sufficiently rapid turnover rates that neither should be considered truly abiontic. Thus it would appear that there are two microbially dependent components to histidase, differing in their stability and perhaps in their function.

The origins of the two enzymatic components is not clear. Each may be produced by separate taxa of the soil population, with the contrasting stabilities of the two components reflecting the different metabolic strategies of each. Alternatively each component may be a functionally different enzyme from the same organism. Dunlop *et al.* (1978) present evidence for two forms of asparaginase in *Saccharomyces cerevisiae*, one being an internal constitutive enzyme and the other being an inducible exoenzyme. Kroening and Kendrick (1987) concluded, based on the discontinuity of an Arrhenuis plot, that two forms of histidase existed in crude extracts of *Saccharomyces griseus*. There are ecological benefits to production of basal amounts of enzymes, such as histidase, in which the product serves as inducer for the operon (Burns, 1986). Further, it would be energetically advantageous for such enzymes to be relatively stable, maximizing catalytic capacity without compromising metabolic control.

Based upon our current understanding of the control of histidase synthesis in microbial systems, the following responses to treatment are anticipated: addition of urocanate will induce histidase synthesis and increase the amount of biologically dependent activity, glucose addition will increase the quantity of biomass but not cause histidase induction, addition of sufficient glucose (in the presence of ammonium) will repress histidase induction. Constitutive activity is anticipated to follow a trend similar to that of the biomass. Abiontic activity is not dependent upon biological activity and thus should not be affected by treatment. The response of kinetic components to treatments known to effect biological activity was used to examine the relationship between dynamics of enzyme activity and biological constituents. The contribution of the labile component varied considerably between experiments in response to differences in antecedent microbial environments including physical conditions and substrate quality. The stabilized component is more consistent (Fig. 3.4 and 3.5), both in pool size and in decay constant (Table 3.3). The insensitivity of the stable component to perturbations of metabolism in soil indicates greater independence from direct biological control and may result in part from short-term soil stabilization. It may also reflect the activity of a constitutive enzyme whose role is the production of inducer, urocanate in the presence of histidine.

Treating the soil with urocanate increased the size of both components. This suggests that both components are of relatively recent biological origin and are derived, in part, from operons sensitive to induction. Frankenberger (1983) also argued that because of its sensitivity to allosteric control, soil histidase activity was of microbial origin. Glucose treatment did not significantly affect the quantity of either component of enzymatic activity. Glucose did not repress activity indicating that either activity was not repressible or that the amount of glucose addition was insufficient to repress activity. Carbon and nitrogen control of the components of soil histidase is the subject of further study.

Both glucose and urocanate treatment increased the turnover rates of the labile component. This is consistent with the suggestion that this component is associated with the biomass and the increased rates of turnover are a reflection of an increase in the growth rate or turnover rate of the population. The turnover rate of the stable component was increased slightly. If the more stable component is a conglomeration of abiontic, extracellular and other more stable enzymatic

components, added carbon sources may simply increase the contribution of less stable components to the mix and decrease the half-life of the association. An increased growth rate of a second component of the population can not be eliminated. This underscores the inability of kinetic methods to identify physical enzymatic components but emphasizes its strength in describing groupings which are important to dynamics of soil systems.

It is not clear how stable an enzyme must be before the term abiontic applies. Any decay in activity imparts a requirement for resynthesis over time and therefore a dependence on biological activity. Bachmair *et al.* (1986) report turnover times ranging from 2 min to >20 h, depending on terminal amino acid residue, for a protein of *Saccharomyces cerevisiae*. The longevity of the two components observed in our study only slightly exceed the stability reported for the completely biological system of Bachmair and co-workers. Reporting the turnover rate of the enzyme, if measurable, in categorizing an enzyme as labile or abiontic is necessary to clarify in the context in which these relative terms are being used.

3.5 Conclusions

It is concluded that soil histidase declines in the absence of enzyme synthesis and thus has limited abiontic character. Normal mechanisms of enzyme turnover are the most probable agents of declining activity. The decay in soil histidase was best described by a two-component first-order model. The response of both components of soil histidase to addition of inducer (urocanate) further indicates both components are composed of enzyme of recent biological origin.

Enzyme inactivation and resulting dependence upon biological enzyme synthesis should be considered when evaluating the abiontic character of soil enzymes. Enzyme assays over a wide range of durations, coupled with kinetic analysis of product accumulation allow a separation of the components contributing to enzymatic activity and provide insight into enzyme longevity.

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4. CARBON AND NITROGEN CONTROL OF HISTIDASE ACTIVITY IN A BLACK CHERNOZEMIC SOIL[1]

4.1 Introduction

In natural ecosystems regulation of nitrogen mineralization is important in optimizing quantities of inorganic nitrogen and minimizing potential losses. Regulation of nitrogen mineralization involves variation in the content and activity of degradative enzymes and their proximity to substrate molecules. The enzymes of decomposition in soil are produced by microorganisms in association with catabolic pathways. Microbial synthesis of catabolic enzymes is regulated at the cellular-level primarily by induction and repression. Enzyme synthesis can be induced by exposure to a substrate of the pathway, increasing the amount of enzyme present in the cell. The induction of catabolic pathways, and thus synthesis of constituent enzymes, is sensitive to carbon and nitrogen availability in pure cultures of bacteria in liquid media (Neidhardt and Magasanik, 1957). In the presence of sufficient concentrations of a superior catabolite such as glucose, synthesis of enzymes degrading less favorable catabolites is repressed. This phenomenon has been termed "catabolite repression" (Magasanik, 1961; Ullman, 1985).

Regulation of L-histidine NH_3-lyase (E.C. 4.3.1.3; histidase) in pure cultures in liquid media is well documented (Lessie and Neidhardt, 1967; Brill and Magasanik, 1969; Kroening and Kendrick, 1987) and a soil assay for this enzyme has previously been described (Frankenberger and Johanson, 1982). Studies examining the expression of the *hut* operon in *Aerobacter aerogenes* (Schlesinger *et al.*, 1965) and in *Pseudomonas aeruginosa* (Lessie and Neidhardt, 1967) indicate urocanate induces histidase activity. It was concluded in Chapter 3 that soil histidase activity comprised at least two enzymatic components: a labile component of recent biological origin and a more stable component. Instability renders each component dependent on continued enzyme synthesis and thus potentially sensitive to synthesis-level control. Responsiveness to synthesis-level control is inversely related to enzyme stability (Chapter 2).

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[1]A version of this chapter will be submitted for publication. D.L. Burton and W.B. McGill (Soil Biology and Biochemistry)

The architecture associated with soil pores and the surrounding solid matrix creates unique habitats for soil organisms. Such spatial relations yield environmental diversity and substrate variability over distances of a few μm. Similarly, decomposition of plant residues provides a unique situation when the substrate becomes the habitat (Juma and McGill, 1986; McGill and Myers, 1987). Organisms in soil can be expected to respond predictably to defined environmental conditions in their immediate vicinity. Processes expressed at the organism-level, such as the control of enzyme synthesis, may not be observed at higher levels of organization such as aggregate- or horizon-levels (McGill and Myers, 1987). Microsite spatial variability, together with constraints on substrate accessibility and microbial movement may prevent aggregate or horizon-level observation of many genetically controlled microbial responses (McGill and Myers, 1987). Architectural constraints may limit the opportunity for control mechanisms observed in homogeneous liquid cultures to coordinate enzyme activity in heterogeneous soil environments.

The expression of cellular control mechanisms such as induction and catabolite repression on the synthesis of enzymes in soil has received little attention. Macura and Kubátová (1973) found the synthesis of enzymes of carbohydrate metabolism in soil not to be sensitive to glucose-C additions of 2400 μg g^{-1}. Macura (1975) reported that glucose and ammonium repressed glutamic acid degradation in microorganisms isolated from soil. The role of these control mechanisms in regulating the content of enzymes catalysing nitrogen-mineralizing reactions in soil has not been explicitly examined.

This research examines the extension of control mechanisms expressed at a cellular-level to the regulation of soil histidase content at the aggregate-level and expression under normal soil conditions. The overall objective of this study was to discover the degree to which mechanisms controlling enzyme synthesis in liquid culture are expressed in soil in reference to their role in regulating nitrogen-mineralizing activity in soil. The specific objective was to determine the role of induction and repression in regulating soil histidase activity. This was achieved by examining the response of each component of soil histidase to amendment with compounds known to induce histidase in liquid culture, in the presence and absence of repressive amounts of glucose.

4.2 Materials and Methods

4.2.1 Soil Description

Soil samples used were from the Ap horizon of a black chernozemic soil (Typic Cryoboroll) under grass (predominantly ***Festuca rubra***) at the Ellerslie Research Station, Edmonton, Alberta. The soil had a SiCL texture, organic carbon content of 6.0%, pH of 5.4 ($CaCl_2$) and retains 38% H_20 (w/w) at field capacity (Maulé and Chanasyk, 1987). Morphological and chemical characteristics of this soil have been published by Pawluk (1986). Soil samples were stored field moist at 4 °C until use. Prior to each experiment the soil was sieved through a 2 mm mesh sieve and incubated at 12 °C until assay initiation to establish a constant environment similar to that encountered under natural conditions.

4.2.2 Assay Procedure

Changes in potential histidase activity were used as a measure of soil histidase content. In enzyme systems which exhibit saturable reaction kinetics the velocity of reaction (activity) approaches the maximum velocity (V_{max}) and the system approaches zero order as substrate concentration increases well beyond K_m. The V_{max} of reaction is a function of the amount of enzyme present and the rate of turnover of the enzyme-substrate complex. If for a given set of conditions, the rate of enzyme-substrate turnover (specific activity of the enzyme) is constant, then potential enzyme activity (velocity under conditions where substrate concentrations is an order of magnitude greater than K_m) is directly related to total active enzyme content.

The assay procedure followed that of Frankenberger and Johanson (1982) with modification as follows. Following incubation at 12 °C, 5 g portions of field moist soil were weighed into round 30 mL screw top vials. One mL toluene was added to each vessel which was then stoppered and equilibrated at 22 °C for 5 minutes prior to initiation. At the time of initiation (t=0) 9 mL of 0.1 M Tris (hydroxymethyl) aminomethane buffer (pH 9.0) were added to each vessel, followed by 1.0 mL of a 0.5 M histidine solution. Following additions the vials were capped, mixed and placed in a rotary shaker-incubator. The samples were maintained at 37 °C and shaken at 200 rpm for the duration of the assay. Reactions were terminated with 5 mM uranyl acetate - 2.5 M KCl solution, at which time

control samples received histidine solution. Following termination, solutions were stored at 4 °C until distillation. Treatments were replicated 3 times with 2 control samples for each treatment.

Ammonium content was determined by steam distillation with MgO (Bremner, 1965). The entire assay mixture and rinse water was transferred to a 500 mL distillation flask for distillation. Ammonia was collected in 5 mL of 2% boric acid solution and titrated with 0.005 M H_2SO_4 to pH 4.5 using a memo titrator (Mettler, Model# DL 40 RC). Gravimetric soil water content was determined (105 °C, 48 h) on the bulk soil and reaction rates expressed on a per g oven dry soil basis (ODB).

4.2.3 Induction and Catabolite Control Experiments

Induction is normally measured as the increase in enzyme content per unit cell mass. Due to difficulties in accurately measuring cell mass in soil systems, induction was defined as the increase in enzyme content, as measured by potential enzyme activity, per unit of soil mass resulting from the addition of the inducer compound. The effect of increased cell mass as a result of carbon addition was evaluated by adding non-inducing carbon sources (eg. glucose).

The effect of histidine and urocanate addition on soil histidase was examined by incubating 5 g field moist soil with urocanate-C or histidine-C at the rate of 100 $\mu g\ g^{-1}$ soil. Soils were amended with 1 mL of 6.9 mM histidine or urocanate solution, non-amended samples received 1 mL of distilled water. The soils were incubated for periods of 0, 1, 2, 3, and 4 days at 12 °C. Following incubation histidase was determined in 4 and 48 hour assays.

The effect of glucose and ammonium addition on histidase response to urocanate addition was examined. Ammonium was added with glucose to prevent nitrogen immobilization-induced enzyme synthesis; thereby allowing catabolite supply, rather than nitrogen availability to affect histidase synthesis (Neidhardt and Magansanik, 1957). Soils were incubated with solutions containing glucose-C and $(NH_4)_2SO_4$-N at a C:N ratio of 10:1. Glucose-C concentrations of 50, 100, 250, 500, 1000, 2000, and 4000 $\mu g\ g^{-1}$ were used. One mL of solution containing the prescribed amount of glucose and ammonium plus urocanate (C at 100 $\mu g\ g^{-1}$) was

added to 5 g of soil and incubated for 3 days at 12 °C. Following incubation histidase was determined in 4 hour assays.

Carbon and nitrogen control over native soil histidase was examined by incubating soil with glucose-C (4000 $\mu g\ g^{-1}$), ammonium-N (400 $\mu g\ g^{-1}$), casamino acids (N at 400 $\mu g\ g^{-1}$) or both glucose and ammonium. Amendments were added to 5 g of soil in a 1.0 mL aliquot; non-amended soils received distilled water. The soils were incubated for 3 days at 12 °C. Following incubation histidase was determined in 4 and 48 hour assays to determine labile and stable enzyme components (Chapter 3).

Ammonium feedback control of native (non-induced) histidase synthesis was examined. Soils were incubated (12 °C) with a 0.5 mL solution containing glucose-C at 400 or 4000 $\mu g\ g\ soil^{-1}$ to immobilize native mineral nitrogen. Following the 7 day immobilization period an additional 0.5 mL of solution containing NH_4^+-N equivalent to 0, 5, 10, 20, 40 $\mu g\ g\ soil^{-1}$ was added to each soil. After an additional 3 day incubation soil histidase was determined in 4 and 48 hours assays. Measured activity was compared to soil receiving 1.0 mL of distilled water and incubated at 12 °C for 10 days.

4.2.4 Compartment Analysis

The decline in soil histidase following the addition of a biostatic agent can be described by a two component first order model (Chapter 3). The two components describe a labile component having a half life of about 3 hours and a more stable component having a half life of about 77 hours in non-amended soils. Soil amendment with a carbon source decreased the stability of each component to 2 and 43 hours for labile and stable components respectively (Chapter 3). Histidase assays of 4 and 48 hours were used to examine the response of each component to carbon and nitrogen control. The initial size of each component (A_0 and B_0) of non-amended samples was determined from [4.1] written as two simultaneous equations.

$$\text{Cum. } NH_4^+ \text{ Prod.}_t = \frac{A_0}{0.272}(1 - e^{-0.272\,t}) + \frac{B_0}{0.011}(1 - e^{-0.011\,t}) \qquad [4.1]$$

Similarly for carbon (substrate) amended samples [4.2] was written as two simultaneous equations which were solved for A_0 and B_0.

$$\text{Cum. } NH_4^+ \text{ Prod.}_t = \frac{A_o}{0.441}(1 - e^{-0.441\,t}) + \frac{B_o}{0.018}(1 - e^{-0.018\,t}) \quad [4.2]$$

where: Cum. NH_4^+ Prod.$_t$ = Cumulative NH_4^+ produced in t hours

Ao = initial size of labile component ($\mu g\ g^{-1}\ h^{-1}$)

Bo = initial size of stable component ($\mu g\ g^{-1}\ h^{-1}$)

t = time (hours)

4.3 Results

Soil amendment with histidine or urocanate increased histidase by 3-4 fold after 3 days of incubation at 12 °C (Fig. 4.1). In two replicate experiments, estimates of the initial size of stable histidase (B_o) increased as a result of the addition of histidine or urocanate (Table 4.1). The increases in mean values for labile histidase (A_o) as a result of histidase or urocanate amendment were not statistically significant ($p \leq 0.05$).

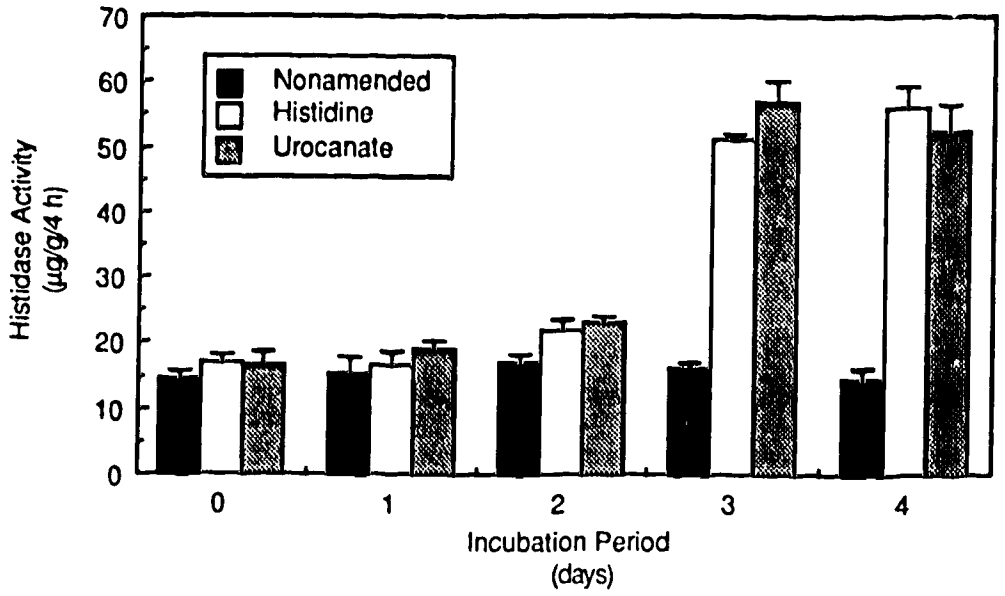

Figure 4.1 Potential soil histidase activity over a 4 day incubation at 12 °C following histidine-C or urocanate-C addition (100 $\mu g\ g^{-1}$). Histidase activity expressed as ammonium-N accumulated ($\mu g\ g^{-1}$) during a 4 hour assay period.

Table 4.1 Ammonium accumulation during 4 and 48 hour assays, labile (A_0) and stable (B_0) components of histidase in control, urocanate or histidine treated soil after 3 days. Estimated initial sizes of components were calculated using Eq. [4.1] or Eq. [4.2] and ammonium accumulation in 4 and 48 hour assays. Values are the mean of three replications, results from two separate but identical experiments are shown.

Treatment	Cumulative NH_4^+-N Production ($\mu g\ g^{-1}$ soil)		Histidase Components ($\mu g\ g^{-1}$ soil h^{-1})	
	4 hour assay	48 hour assay	Labile (A_0)	Stable (B_0)
	---------- Experiment 1 ----------			
No Amendment	14.94 a	98.50 a	2.22 a §	2.43 a
Histidine	30.44 b	179.25 b	4.99 a	5.80 b
Urocanate	29.30 b	183.98 b	6.03 a	5.56 b
	---------- Experiment 2 ----------			
No Amendment	14.76 a	90.95 a	2.52 a	2.19 a
Histidine	25.47 b	146.40 b	3.49 a	7.50 b
Urocanate	32.46 b	232.39 c	5.30 a	4.51 c

§ values within columns and followed by different letters are significantly different at $p \leq 0.05$

A urocanate-C amendment of 100 μg g^{-1} was required to elicit increased soil histidase content (Table 4.2). Amendment with glucose-C (100 μg g^{-1}) resulted in soil histidase in excess of non-amended soil, but significantly lower than in soils receiving the same amount (on a C basis) in the form of urocanate. Similarly the addition of 4000 μg glucose-C in combination with 400 μg ammonium sulfate-N increased histidase relative to non-amended soil but not to the degree observed in histidine or urocanate amended soil (Table 4.3).

Table 4.2 Potential soil histidase activity in control, urocanate and glucose amended soil following 3 days incubation at 12 °C.

Amendment	Level (μg g^{-1}) as C	Histidase (Cum. NH_4^+ Prod.) (μg g^{-1} 4 h^{-1})
Non-amended	-	13.99 a§
Urocanate	5	9.25 a
	10	14.38 a
	25	15.92 ab
	50	18.44 ab
	100	35.91 c
Glucose	100	19.66 b

§ means followed by different letters indicate are significantly different at p = 0.05

Glucose-C additions of 1000 and 2000 μg g^{-1} caused a 30% reduction in soil histidase in urocanate amended soils (Fig. 4.2). With 4000 μg g^{-1} of glucose-C, histidase in urocanate amended soils was not significantly different from soils not receiving urocanate. Histidase persisted even at the highest rates of glucose addition.

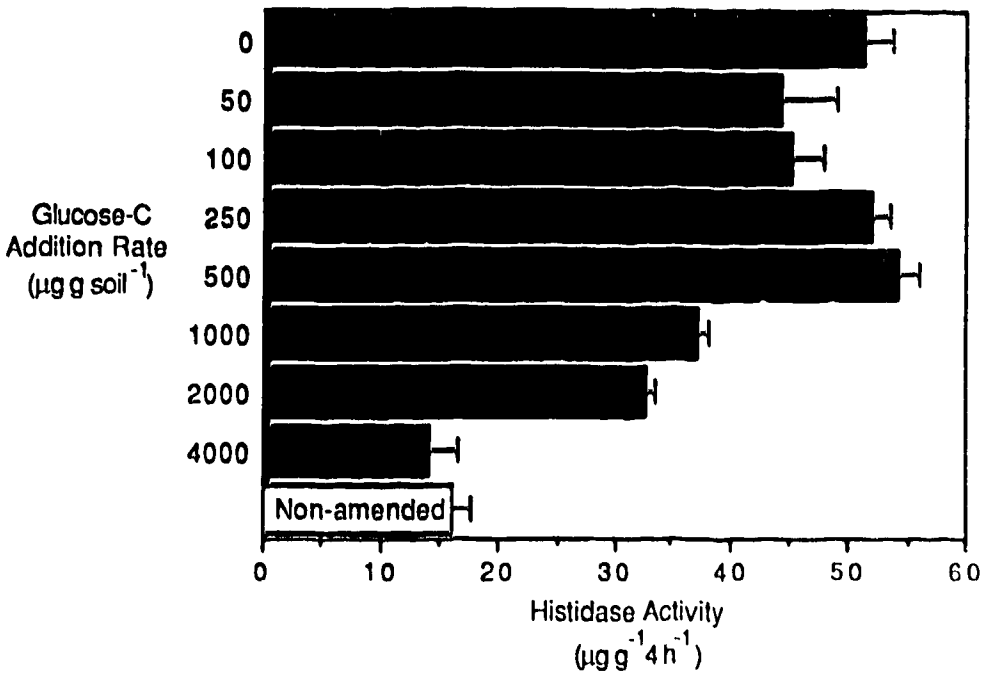

Figure 4.2 Potential soil histidase activity after 3 days incubation at 12 °C in soils receiving glucose-C (0-4000 µg g^{-1}) and ammonium-N (0-400 µg g^{-1}; C:N = 10:1). All samples received urocanate-C (100 µg g^{-1}) with the exception of the non-amended control.

Organic carbon addition in the absence of histidine or urocanate either had no effect or increased soil histidase (Table 4.3). Ammonium addition had no effect on 4 hour measures of potential histidase activity. The effect of soil amendment upon estimates of the initial size of components identified by equations [4.1] and [4.2] was determined. Addition of carbon alone resulted in increased ($p \leq 0.10$) estimates of the size of the labile (A_0) component of soil histidase (Table 4.3). The size of the stable component of histidase (B_0) increased ($p \leq 0.05$) as a result of the addition of either casamino acids or glucose with ammonium or casamino acids but did not respond to glucose or ammonium alone (Table 4.3). Casamino acid addition resulted in a statistically significant increase in histidase activity as measured in 4 and 48 hour assays. The increased histidase was primarily associated with stable histidase (B_0).

Table 4.3 Ammonium accumulation during 4 and 48 hour assays, labile (A_0) and stable (B_0) components of histidase activity in soils amended with glucose-C (4000 μg.g soil^{-1}), ammonium-N (400 μg.g soil^{-1}), glucose and ammonium or casamino acid (N at 400 μg.g soil^{-1}). Component size (A_0 and B_0) calculated using Eq. [4.1] or Eq. [4.2] and ammonium accumulation in 4 and 48 hour assays.

Treatment	Level (μg g^{-1})	Cum. NH_4^+-N Production (μg g^{-1} soil) 4 hour	48 hour	Histidase Components (μg g^{-1} soil h^{-1}) Labile (A_0)	Stable (B_0)
Non-amended	0	14.94 a†	102.0 a	2.06 a	2.53 a
C + N	4000 + 400	19.07 ab	148.1 b	0.79 a	4.55 b
C only	4000	22.77 b	106.3 a	6.23 a	2.87 a
N only	400	15.62 a	110.0 a	1.99 a	2.75 ab
Casamino Acid N at 400		41.15 c	322.0 c	1.55 a	9.91 c

† means followed by different letters indicate significance at $p \leq 0.05$

To evaluate the role of ammonium concentration in affecting soil histidase, soils were treated with glucose to immobilize soil ammonium. A significant increase in stable histidase (B_0) was observed following a 10 day incubation of soils with either 400 or 400 μg g soil^{-1} of glucose-C (Table 4.4). This increase was offset by the addition of as little as 5 μg g soil^{-1} of NH_4^+-N. Estimates of labile histidase activity (A_0) were not significantly different.

Table 4.4 Response of labile (A_0) and stable (B_0) components of native soil histidase to ammonium addition in glucose treated soils. Soils were treated for 7 days at 12°C with glucose-C at 400 or 4000 μg.g soil^{-1} to immobilize native mineral nitrogen. Ammonium-N was then added at indicated rates and soils were incubated for an additional 3 days at 12 °C. Component sizes calculated using Eq. [4.1] or Eq. [4.2] and ammonium accumulation in 4 and 48 hour assays and are expressed as μg NH_4^+-N produced g soil^{-1} h^{-1}.

Glucose-C	NH_4^+-N	Cum. NH_4^+-N Production (μg g^{-1} soil)		Histidase Components (μg g^{-1} soil h^{-1})	
(μg.g soil^{-1})		4 hour	48 hour	Labile (A_0)	Stable (B_0)
0	0	10.50 a	88.89 a	0.92 a†	2.10 a
400	0	16.82 a	97.55 a	3.24 a	2.77 b
	5	14.54 a	87.57 a	2.57 a	2.51 ab
	10	12.15 a	83.44 a	1.38 a	2.47 ab
	20	15.97 a	86.41 a	3.54 a	2.41 ab
	40	11.13 a	94.36 a	-0.06 a	2.91 b
4000	0	11.21 a	96.93 a	-0.20 a	3.00 b
	5	11.98 a	88.60 a	1.27 a	2.48 ab
	10	9.51 a	79.74 a	-0.01 a	2.45 ab
	20	12.45 a	87.10 a	1.30 a	2.59 ab
	40	9.89 a	83.79 a	-0.05 a	2.58 ab

† means followed by different letters indicate significance at p = 0.05

4.4 Discussion

4.4.1 Induction

Histidase content, as measured by potential soil histidase activity, increased after 3 days exposure to either histidine or urocanate (Fig. 4.1). Increased content results from either increased enzyme production per unit of cell mass (induction) or from an increase in cell mass (growth). The increase in histidase observed here is consistent with induction of enzyme synthesis and not microbial growth because the addition of glucose or glucose with ammonium sulfate did not result in increases of similar magnitude (Tables 4.2 and 4.3).

Estimates of stable histidase (B_0) increased in response to both histidine and urocanate addition (Table 4.1). Variation in estimates of the size of the labile

component (A_0) masked response to soil amendment. This is the result of variability in short term (4 h) assay data resulting from small differences between proportionally large NH_4^+ contents in control samples and those receiving substrate. Estimates of stable histidase are more dependent upon ammonium accumulation in longer assay durations where variability is small relative to background ammonium.

In heterogeneous systems such as soil it is often difficult to determine with certainty the enzyme responsible for measured activity. Frankenberger and Johanson (1982) observed ammonia production following the addition of histidine to soil, and attributed this activity to histidase. This was supported by the similarity of pH optima of the measured soil activity and literature reports of histidase. Increased histidase activity in response to urocanate addition provides further evidence to support histidase, and not a broad spectrum lyase, as the enzyme catalysing the production of ammonium in response to histidine addition to soil. The activity of a broad spectrum lyase would not be positively affected by addition of a specific reaction product such as urocanate.

Expression of increased histidase required 3 days at 12°C, much longer than response time of a few hours commonly observed in liquid culture systems (Wiseman, 1975). The lag period prior to the expression of increased potential activity is related to growth rate (Wiseman, 1975). Soil substrate availability and incubation temperature (12°C) were likely limiting growth rate; conditions more typical of field soils than liquid culture systems. Lags in response may be advantageous in substrate limited environments, where substrate concentrations sufficient to justify synthesis of elevated amounts of enzyme of a particular pathway seldom persist. Basal activity may, for the most part, be sufficient for competitive access to available substrate without the additional energetic investment of enzyme synthesis.

4.4.2 Repression

The induction of many catabolic enzymes may be repressed by addition of a superior catabolite such as glucose (catabolite repression). The stimulatory effect of urocanate addition on potential histidase was reduced by the addition of 2000 $\mu g\ g^{-1}$ glucose-C (with NH_4^+) and completely offset by the addition of 4000 $\mu g\ g^{-1}$ glucose-C (Fig. 4.2). This is consistent with catabolite repression of enzyme

synthesis and provides further support for increased potential activity being the result of induction and not microbial growth.

The relationship between catabolite supply and induction by urocanate is consistent with the control of the synthesis of histidase in liquid culture systems (Lessie and Neidhardt, 1967). At 25% gravimetric water content, 4000 μg g^{-1} glucose-C corresponds to a solution concentration of 0.4% glucose (w/v). In liquid culture, repression of histidase synthesis is commonly observed in broths containing 0.2% glucose (Magasanik *et al.*, 1965). The higher amounts of glucose required in soil systems may reflect non-uniformity of substrate distribution or the adsorption/entrapment of substrate by soil particles. This points to the role of soil surfaces and spatial discontinuity in the regulation of processes occurring in soil relative to homogeneous systems.

4.4.3 Native (Non-induced) Soil Histidase

Addition of glucose in amounts shown to repress urocanate-induced increases in soil histidase (Fig. 4.2) did not repress native soil histidase. Thus histidase synthesis as a result of induction was not contributing significantly to native histidase. This observation is consistent with basal, non-induced synthesis as the origin of native histidase. Increased histidase in response to the addition of glucose with ammonium, conditions which favour an increase in biomass, is also consistent with basal synthesis of native histidase. Substrate-induced increases in native histidase were small relative to urocanate-induced changes in histidase.

Native histidase was not associated with a single kinetic component; equal amounts of labile and stable activity were identified in non-amended soil (Table 4.3). Constitutive synthesis contributing to each component of histidase is not consistent with these components representing inducible and constitutive enzymes, as has been observed for asparaginase activity in *Saccharomyces cerevisiae* (Dunlop *et al.*, 1978).

The failure of NH_4^+ addition to suppress native soil histidase (Table 4.3) indicates that either NH_4^+ feedback control does not affect histidase content or that normal NH_4^+ amounts in this soil (3 μg g $soil^{-1}$) are repressive. Increased labile histidase (A_0) in response to glucose addition ($p \leq 0.10$), in the absence of NH_4^+ (Table 4.3), is consistent with immobilization-induced reduction in NH_4^+ content

and a subsequent release from feedback control. While variation in estimates of labile histidase (A_0) failed to detect differences, increased stable histidase (B_0) as a result of 10 day incubation with glucose (Table 4.4) provides further support for a limited role of NH_4^+ feedback control of native soil histidase. The increase in histidase resulting from glucose treatment was offset by the addition of as little as 5 $\mu g\ g\ soil^{-1}$ NH_4^+-N. While NH_4^+ addition did not affect a large portion of native histidase, NH_4^+ feedback control may be of importance in the regulation of soil histidase as the NH_4^+ concentration range over which control was expressed (0 - 5 $\mu g\ g\ soil^{-1}$) was similar to values anticipated in soil.

4.4.4 Soil as an Oligotrophic Environment

The observation that soil histidase can be induced by histidine or urocanate addition or that induction is sensitive to catabolite repression is not unexpected. However amounts of inducer and/or catabolite required to evoke these responses in soil systems have not previously been reported. The concentrations of both inducer and catabolite required suggest these mechanisms of control, characteristic of diauxic growth, are likely to be active only at microsites of organic matter deposition/accumulation. The frequency of occurrence and longevity of such microsites will determine the significance of induction and repression in the determination of the enzyme content of soil systems.

While the availability of substrate varies in soil, the majority of the soil matrix is likely sufficiently depleted of available substrate to be considered an oligotrophic environment (Morgan and Crawford, 1986). If synthesis-level control mechanisms observed under conditions of energetic abundance (eg. catabolite repression) are not expressed under such conditions how is the enzymatic composition of these systems regulated? In such environments constitutive enzyme synthesis, characteristic of mixed substrate utilization, will be the dominant source of enzyme activity (Harder and Dijkhuizen, 1982). This is also consistent with the observations on protease and cellulase activity which suggest these enzymatic reactions in soil are limited by substrate and not enzyme content (Tateno, 1988). In such a system there remains the need to maintain basal amounts of enzymatic activity to allow for utilization of the small but consistent supply of histidine entering the free amino acid pool. From these observations the role of the regulatory mechanisms operative in energetically rich environments in controlling

the enzymatic composition of nutrient limited environments such as soil, may be questioned.

4.5 Conclusions

The synthesis of soil histidase was sensitive to both induction and catabolite repression. Variability in the estimates of labile histidase (A_0) masked response to addition of histidine or urocanate. Stable histidase (B_0) was affected by both induction and repression. The amount of amendment required for each in bulk soil exceeded those anticipated under normal soil conditions, bringing into question the role of these control mechanisms in determining soil histidase content at the horizon level. Basal enzyme synthesis appears to be the dominant source of native soil histidase.

While broad based control does not appear to dominate soil histidase activity, a small portion of native soil histidase was sensitive to feedback control by NH_4^+-N. While comprising only a small component of total histidase, the concentration ranges over which control was expressed (0 - 5 μg g soil^{-1}) suggest this form of control may be operative under normal soil conditions.

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5. NITROGEN MINERALIZATION, PROTEASE AND HISTIDASE CONTENTS IN RELATION TO AMMONIUM IN A FIELD SOIL CROPPED TO BARLEY[1]

5.1 Introduction

The mineralization of organic nitrogen is an important source of plant available inorganic nitrogen in both agricultural and natural ecosystems. Inorganic nitrogen forms, particularly nitrate, are susceptible to loss through volatilization and leaching. Management strategies which better coordinate mineralization with plant nitrogen demand would minimize nitrate accumulation and reduce nitrogen loss. The development of such approaches require an understanding of the mechanisms controlling nitrogen mineralization.

Nitrogen mineralization is a cascade of enzymatic reactions resulting in the breakdown of soil organic matter into smaller organic molecules, ultimately producing inorganic nitrogen (Fig. 5.1). Broad based control mechanisms having the potential to coordinate the synthesis of enzymes catalysing nitrogen-mineralizing reactions have been demonstrated in liquid culture systems. Catabolite repression which regulates the synthesis of many catabolic enzymes is an example of such a control mechanism (Magasanik, 1961). The concentration of catabolite (glucose-C) required for repression in soil systems (2000 - 4000 $\mu g\ g\ soil^{-1}$) brings into question its role in regulating soil enzyme content (Chapter 4). Feedback inhibition is another mechanism of enzyme control in which enzymatic activity is suppressed by end-product accumulation (Brock, 1979). Smith and Paul (1986) observed that the addition of large amounts of ammonium-N (18 $mg\ g^{-1}$) to soil repressed the mineralization of organic nitrogen. The ammonium content of the medium may also affect enzyme synthesis as demonstrated by the synthesis of glutamine synthetase under conditions of ammonium starvation (Tyler, 1978). Rice and Paul (1987) found enhanced rates of amino acid degradation in soils in which inorganic nitrogen had been depressed by carbon amendment. The potential exists for expression of these mechanisms in soil, resulting in the coordination of nitrogen-mineralizing

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[1]A version of this chapter will be submitted for publication. D.L. Burton and W.B. McGill (Canadian Journal of Soil Science)

reactions (Chapter 4). The generality of such control mechanisms and impact upon nitrogen mineralization under field conditions have yet to be determined.

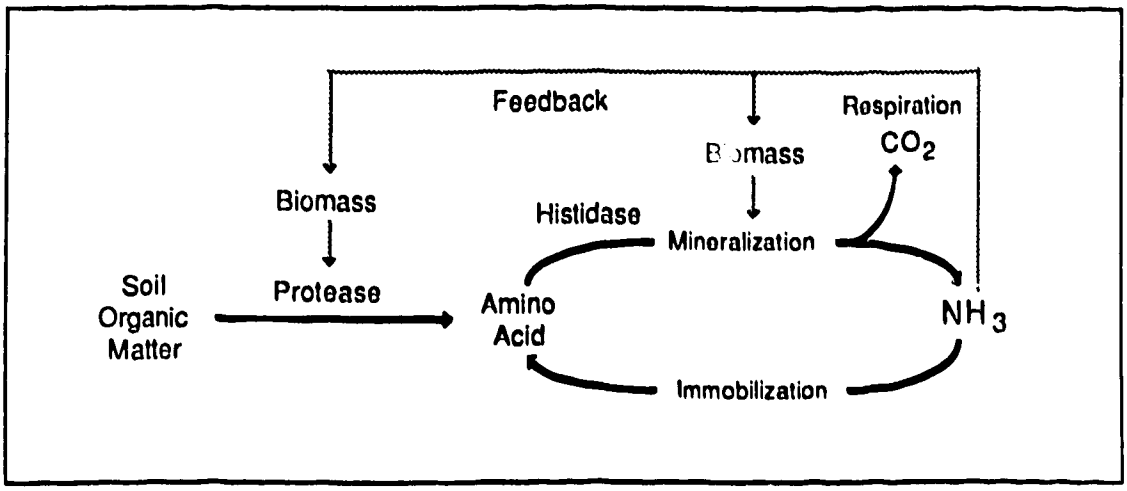

Figure 5.1 Schematic representation of components and processes involved in the mineralization of organic nitrogen in soil. Solid lines indicate mass transfer, dotted lines indicate the transfer of information.

Do changes in the content of nitrogen-mineralizing enzymes in soil reflect synthesis-level coordination? Synthesis-level coordination would be manifest through simultaneous changes in content in response to changes in the soil environment. Seasonal patterns in specific measures of nitrogen-mineralizing enzyme content, such as histidase or protease, were examined in relation to changes in soil biomass, nutrient content and net mineral-N production to address this question. To be effective, control mechanisms must respond to the combined effects of mineralization and immobilization (net mineral-N production) and not simply gross mineralization. Changes in enzyme content in relation to fluctuations in soil ammonium concentration were examined to assess the role of ammonium ion in feedback control[1] of mineralization. Spatial and temporal variation in specific measures of the content of nitrogen-mineralizing enzymes were compared to variation in measures integrating a wide range of mineralizing reactions or potential such as biomass-C.

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[1] Here feedback control refers to a positive or negative change in enzyme content in response to product accumulation without infering mechanism.

5.2 Materials and Methods

5.2.1 Site Description and Sampling

The experiment was conducted at the Ellerslie Research Station, Edmonton (53° 25' N, 113° 33' W). The soil was a black chernozem (Typic Cryoboroll) under conventional tillage cropped to barley (*Hordeum vulgare L.*). The barley crop was planted on May 25, 1987 at a rate of 255 seeds m^{-2} in a 22.5 cm row spacing and harvested on September 3, 1987. Nitrogen fertilizer (urea-N) was applied at a rate of 77 kg ha^{-1}. Detailed cropping history of this site, which was part of a project examining the effect of tillage and cropping sequence on soil communities, is described by Izaurralde *et al.* (submitted). The soil had a SiCl texture, organic carbon content of 6.0%, pH of 5.4 (0.1 M $CaCl_2$) and retains 38% H_2O (w/w) at field capacity (Maulé and Chanasyk, 1987). Morphological and chemical characteristics of this soil were reported by Pawluk (1986).

Samples were collected on six dates during the summer and fall of 1987. On each occasion 5 cores (2 cm diameter) were collected to a depth of 15 cm and combined. Two locations (within the barley rows and between barley rows) were sampled on each of four replicate field plots within the experiment. Samples were returned to the laboratory and analyzed on the same day.

5.2.2 Soil Analyses

Biomass - Biomass carbon of freshly sampled, non-sieved soils, was determined by chloroform-fumigation and incubation (Jenkinson and Powlson, 1976). Plant roots and the associated microflora were not removed from soil samples because they represent a significant microbial habitat. Incubation temperature was 25°C and soil water content was brought to 55% of water holding capacity prior to the ten day incubation. Carbon dioxide evolved was collected in 0.25 M NaOH and quantified by titration with 0.5 M HCl to pH 8.1 following the addition of $BaCl_2$.

Controversy exists about whether subtracting the CO_2 evolution from a non-fumigated control is appropriate, especially in disturbed, amended soils. Two approaches were used to convert the flush of CO_2 evolution over the ten day incubation following fumigation to biomass-C. The first approach involved the subtraction of CO_2 respired by a non-fumigated control (Jenkinson and Powlson, 1976), with biomass calculated as:

$$\text{Biomass-C1} = (\text{Fumigated } CO_2\text{-C} - \text{Non-fumigated } CO_2\text{-C})/ k_{c1} \quad [5.1]$$

where $k_{c1} = 0.45$ (Jenkinson *et al.*, 1979)

The second approach directly converted CO_2 evolution from fumigated samples to biomass (Paul and Voroney, 1980) as given by:

$$\text{Biomass-C2} = \text{Fumigated } CO_2\text{-C} / k_{c2} \quad [5.2]$$

where $k_{c2} = 0.41$ (Paul and Voroney, 1980).

Biomass nitrogen was simultaneously determined as the flush of mineral nitrogen ($NH_4^+ + NO_3^-$) following $CHCl_3$ fumigation (Jenkinson and Powlson, 1976). Biomass-N was calculated as:

$$\text{Biomass-N} = (\text{Fumigated-N} - \text{Non-fumigated-N}) / k_n \quad [5.3]$$

where $k_n = 0.68$ (Shen *et al.*, 1984).

An index of soil respiration was calculated as the amount of CO_2-C evolved from non-fumigated samples divided by the duration of incubation (10 days) and expressed per gram oven dry soil. The amount of mineral nitrogen per g of soil produced (mineral nitrogen $_{t0}$ - final mineral nitrogen $_{t10}$) during incubation of non-fumigated samples divided by the duration of incubation (10 days) was used as an index of nitrogen mineralization (net mineral-N production) and expressed per gram of oven dry soil.

Protease - Casein-hydrolysing activity was determined by the method of Ladd and Butler (1972) modified as follows: moist soil (4 g) was weighed into a 50 mL centrifuge tube and 10 mL of sodium caseinate (10 mg/mL) in 0.1 M TRIS buffer (pH = 8.1) added. The mixture was shaken at 120 oscillations/minute on a reciprocating shaker bath at 50 °C. After 1 hour the reaction was stopped by adding 4 mL of 17.5% trichloroacetic acid (TCA). The tubes were then centrifuged at 2000 g (4500 rpm) for 20 minutes. 2 mL of supernatent was removed and added to 3.0 mL of 1.4 M Na_2CO_3 and 1 mL of threefold diluted Folin's reagent. Absorbance at 700 nm was determined after 10 minutes and compared to tyrosine standards. Results are expressed as µmoles of tyrosine equivalent/g oven dry soil/h.

Histidase - The assay of L-histidine NH_3-lyase activity followed Frankenberger and Johanson (1982) as modified in chapter 3, using 0.5 M histidine as substrate. Potential histidase activity was measured as the production of NH_4^+ by toluene treated field moist soil (5 g) shaken at 200 rpm on a rotary shaker at 37°C. Following termination with 5 mM uranyl acetate - 2.5 M KCl solution, assay mixtures were stored at 4 °C until distillation. Control samples received histidine solution after assay termination. A response (+ histidine) and control (- histidine) assay were conducted for each soil and assay duration. Ammonium content of assay mixtures was determined by steam distillation with MgO (Bremner, 1965). The entire assay mixture and rinse water was transferred to a 500 mL round-bottomed flask for distillation. Ammonia was collected in 5 mL of 2% boric acid solution and titrated with 0.005 M H_2SO_4 to pH 4.5 using a memo titrator (Mettler, Model# DL 40 RC).

Histidase activity over 4 and 48 hour assay durations was measured to allow determination of the initial size of labile and stable components of histidase activity (Chapter 3). The initial size of labile and stable components were algebraically determined from equation [5.4] written as two simultaneous equations.

$$\text{Cum. } NH_4^+ \text{ Prod.}_t = \frac{A_o}{0.272}(1 - e^{-0.272\,t}) + \frac{B_o}{0.011}(1 - e^{-0.011\,t}) \qquad [5.4]$$

where: Cum. NH_4^+ Prod.$_t$ - Cumulative NH_4^+ produced in t hours[1]

A_o - initial size of labile component ($\mu g\ g^{-1}$)

B_o - initial size of stable component ($\mu g\ g^{-1}$)

t - time (hours)

Numerical constants were derived from a non-linear least-squares fit of a two component first order model [3.4] to ammonium accumulation in histidase assays ranging in duration from 1 to 96 hours (Chapter 3).

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[1] The term ammonium is used here to indicate the protonated form NH_4^+ and ammonia is used to specify the total of the species NH_4^+, NH_4OH and NH_3 present in a given system.

Mineral Nitrogen - Soil mineral nitrogen content was measured by extracting 25 g of moist soil in 125 mL of 2 M KCl. Ammonium and nitrate concentrations of the extract were determined by the automated indophenol procedure (Technicon Industrial Method# 98-70W, 1973), and Cd-reduction followed by colorimetric determination of NO_2^- (Technicon Industrial Method# 497-77A, 1977), respectively.

Water Content - Gravimetric soil water content was determined (105 °C, 48 h) on a sample of the bulk soil and reaction rates and nutrient contents are expressed on a per g oven dry soil basis.

5.3 Results

5.3.1 Comparison of estimates of biomass-C

The two methods used to calculate biomass-C provided significantly different estimates, biomass-C1 averaged 501 ± 187 μg g^{-1} over the season as compared to 1065 ± 253 μg g^{-1} for biomass-C2. The large difference between estimates is due to high non-fumigated respiration. This is likely the result of additional substrate associated with roots and root exudates. Paul and Voroney (1980) recommended the use of direct calculation when disturbed substrate amended soils are being used. Biomass-C1, as calculated by the method of Jenkinson and Powlson (1976), was more variable within treatments and did not exhibit the seasonal trends observed in biomass-C2 estimates (Table 5.1). Biomass-C did not vary between sampling locations, regardless of method of calculation. The amount of biomass-N in the soils did not differ significantly between sampling locations or over the season.

Estimates of the C:N ratio of the biomass are dependent upon the method used to calculate biomass-C. Based upon biomass-C1 the C:N ratio is estimated to be 8.4 ± 3.7, use of biomass-C2 estimates yields a biomass C:N ratio of 17.7 ± 4.9. The ratio of carbon respired to nitrogen mineralized in non-fumigated soils was highly variable averaging 27.7 ± 45.6, largely the result of soils immobilizing N or mineralizing only small amounts of N over the 10 day incubation.

5.3.2 Spatial and temporal variation

For most variables measured, soils taken from within the barley row did not significantly differ from those obtained between rows (Table 5.1). Respiration varied significantly, both between locations ($p \leq 0.05$) and over time ($p \leq 0.01$). Respiration was consistently higher in samples taken from within the barley row. Soils sampled from within barley rows had a higher ammonium content ($p \leq 0.05$) than samples taken from between rows. The labile component of histidase (A_0) was significantly ($p \leq 0.01$) higher between rows than within the row prior to crop harvest, after which location had no significant effect (Table 5.1).

Significant temporal variations were observed in water content, respiration, net mineral-N production and protease activity (Table 5.1). Mineral nitrogen declined over the growing season but increased after crop harvest (Sept. 3). Fluctuation in mineral nitrogen was primarily the result of changes in NO_3^-. Soil NH_4^+ was low except for a period of higher concentration on the June 24 and July 8 sample dates. Estimates of biomass-C in which the control was subtracted (biomass-C1) did not indicate significant temporal variation, whereas the CO_2 flush following fumigation (biomass-C2) varied over time ($p \leq 0.05$). No seasonal patterns in biomass-N were detected. Graphically, seasonal trends in the average labile (A_0) histidase activity seem apparent (Fig. 5.2), but statistical significance of this variation is limited by variation within dates (Table 5.1). Estimates of stable (B_0) activity were more consistent than labile activity both within dates and over the course of the season; no statistically significant differences were observed.

Table 5.1: Mean values and analysis of variance for four replicate measurements of parameters measured in soil sampled from within (In Row) and between (Between) barley rows for 6 dates at the Ellerslie Research Station, Edmonton.

	Moisture	Mineral Nitrogen			Biomass Carbon		Biomass	Respiration	Net Mineral-N	Histidase Activity		Protease
		Ammonium	Nitrate	Total	C1[1]	C2[2]	Nitrogen	CO_2-C	Production	A_o	B_o	
	(% w w^{-1})	($\mu g\ g^{-1}$)	($\mu g\ g^{-1}$)	($\mu g\ g^{-1}$)	($\mu g\ g^{-1}$)	($\mu g\ g^{-1}$)	($\mu g\ g^{-1}$)	($\mu g\ g^{-1}day^{-1}$)	($\mu g\ g^{-1}day^{-1}$)	($\mu g\ g^{-1}h^{-1}$)/	($\mu g\ g^{-1}h^{-1}$)/	($\mu mole\ g^{-1}h^{-1}$)
	June 8											
Between	31.6	0.6 A[†]	22	23	485	1090	68	23 A	1.3	3.7	2.7	0.41
In Row	31.8	0.7 A	11	12	469	1002	77	25 A	0.8	0.9	3.0	0.27
	a[£]	a	ab	a		ac		a	ac			ac
	June 24											
Between	27.4	11.4 A	33	44	510	1082	57	21 A	2.4	2.9	2.8	0.26
In Row	26.6	12.8 A	17	30	562	1247	67	25 A	1.7	1.4	2.8	0.29
	b	b	b	b		ab		a	ab			a
	July 8											
Between	24.6	2.8 A	8	11	582	1269	77	26 A	3.0	6.3	2.3	0.38
In Row	23.4	10.6 B	14	25	541	1351	64	31 A	4.4	1.5	2.9	0.43
	c	c	ac	a		b		a	bd			c
	July 22											
Between	25.9	2.1 A	4	5	324	817	70	19 A	0.8	4.2	2.8	0.4
In Row	26.9	4.1 A	4	8	347	898	69	27 B	0.5	0.1	3.1	0.25
	b	ac	c	a		c		a	c			b
	September 15											
Between	34.3	0.4 A	8	8	641	1004	48	24 A	0.7	0.0	3.3	0.11
In Row	33.7	1.0 B	7	8	381	1202	61	30 A	0.6	3.8	3.2	0.19
	d	a	ac	a		ab		a	c			b
	October 14											
Between	31.0	1.4 A	12	13	578	796	50	7 A	2.1	0.1	3.1	0.27
In Row	30.8	2.4 B	13	16	592	937	50	14 B	2.6	0.6	3.3	0.28
	a	a	ac	a		c		b	ad			ab
	Analysis of Variance[§]											
Date	***	***	**	***	NS	***	NS	**	*	NS	NS	**
Location	NS	*	NS	NS	NS	NS	NS	*	NS	NS	NS	NS
Date x Location	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] Biomass-C calculated by subtracting CO_2 respired from unfumigated soil (Jenkinson and Powlson, 1976)

[2] Biomass-C calculated without subtracting basal respiration (Paul and Voroney, 1980)

[§] significant difference at * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and NS = not significantly different (or sig. different at $p > 0.05$)

[†] values followed by different capital letters indicate significant ($\alpha \leq 0.05$) differences between locations.

[£] values followed by different lower case letters indicate significant ($\alpha \leq 0.05$) differences between dates.

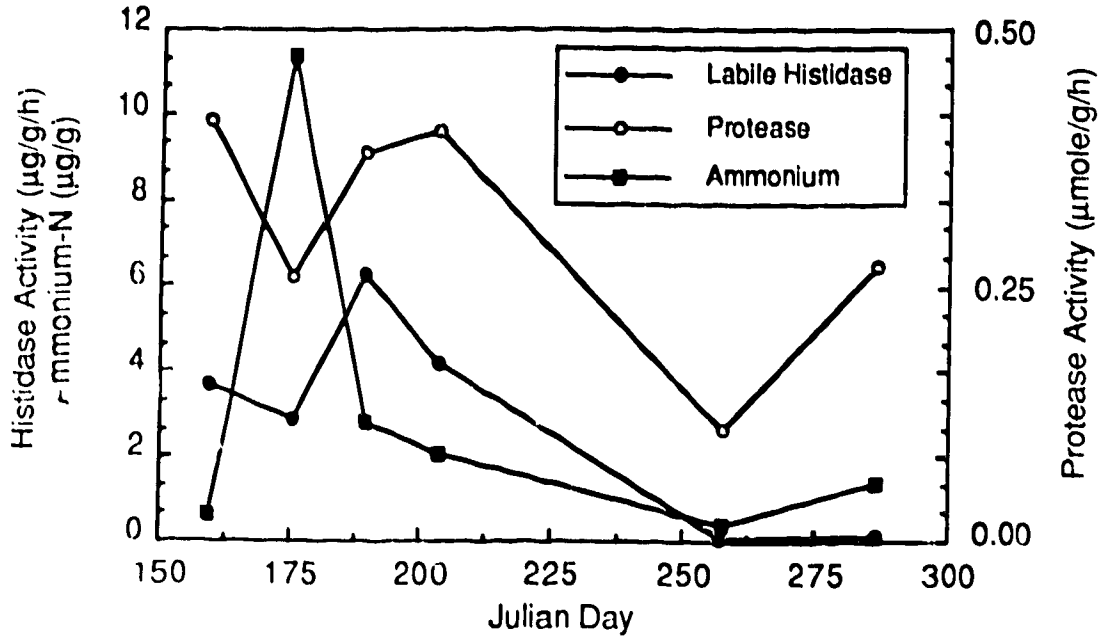

Figure 5.2 Relationship between labile histidase activity, protease activity and ammonium-N in soils collected from the inter-row of soils cropped to barley at the Ellerslie Research Station, Edmonton, Alberta during the 1987 growing season. (Solid symbols refer to left-hand axis, hollow symbols refer to right-hand axis.)

5.3.3 Correlation between variables

Net mineral-N production was positively correlated with biomass-C1 ($p \leq 0.05$), biomass-C2 ($p \leq 0.01$), protease activity ($p \leq 0.05$) and soil ammonium content ($p \leq 0.01$). Individually these variables accounted for 13, 17, 8 and 16% of the variation in net mineral-N production respectively. Net mineral-N production was not correlated ($p > 0.05$) with biomass-N, respiration or either component of histidase activity (A_o and B_o). (Table 5.2)

Table 5.2 Correlation (r) between mineralizing reactions and variables in the schematic diagram of nitrogen mineralization (Fig. 5.1).

Variable		Net Min.-N Prod.	Protease	Histidase A_o	Histidase B_o
Biomass-C1		0.36 **§	0.29 *	- 0.06	0.11
Biomass-C2		0.41 ***	0.45 ***	0.09	0.03
Biomass-N		0.15	0.52 ***	0.09	0.03
Respiration		0.09	0.09	0.06	- 0.13
Protease		0.28 **	---	0.20	- 0.22
Histidase	A_o	0.08	0.20	---	- 0.64 ***
	B_o	- 0.22	- 0.22	- 0.64 ***	---
Ammonium		0.40 ***	0.17	- 0.06	0.04
Nitrate		0.09	0.04	- 0.09	- 0.02
Resp.:Min.†		---	- 0.22	- 0.21	0.07

§ correlations significant at * $p \leq 0.10$, ** $p \leq 0.05$, *** $p \leq 0.01$

† The ratio of respiration to net mineral-N production

Comparison of labile histidase (A_o) activity and soil ammonium content across all treatments reveals two groups of data (Fig. 5.3). One group of data is typified by low ammonium content (< 3 $\mu g.g\ soil^{-1}$) and a range of labile histidase activities. It is dominated by soils sampled in the spring (June 8, day 159) and fall (Sept. 15 and Oct. 14, days 258 and 287). The second group of data, sampled in mid-June and July, spans a larger range of ammonium contents (0 - 12 $\mu g.g\ soil^{-1}$) and an inverse linear relationship between ammonium content and labile histidase (A_o) activity is evident (Fig. 5.3). Labile histidase activity (Ao) in this group will

be referred to as ammonium-dependent. Soils not exhibiting ammonium-dependent histidase activity had a significantly ($p \leq 0.001$) higher water content (30.5 %) than did the ammonium-dependent samples (26.5 %). Labile histidase activity (A_o) in the low ammonium samples correlated positively ($r = 0.58$; $p \leq 0.001$) with soil nitrate content.

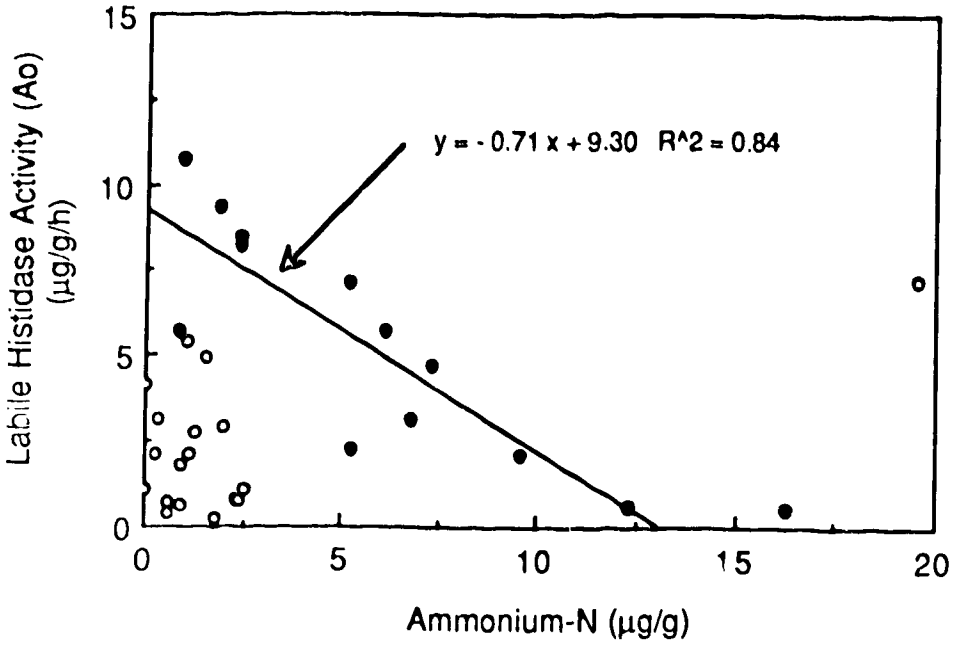

Figure 5.3 Relationship between labile histidase activity (A_o) and soil ammonium-N content. Solid symbols represent the population of samples exhibiting ammonium-dependent labile histidase activity.

Despite the inverse relationship in Fig. 5.2, casein hydrolysing (protease) activity did not relate significantly to ammonium content (Table 5.2). Protease activity however correlated positively with both biomass-C2 ($p \leq 0.01$) and biomass-N ($p \leq 0.01$), individually accounting for 20 and 27% of the variation in protease activity respectively. Relationships between protease activity and biomass-C1, biomass-C2 and respiration were stronger in soils exhibiting ammonium-dependent labile histidase activity (Table 5.3). Protease activity was significantly ($p \leq 0.05$) related to net mineral-N production ($r = .28$). This relationship accounted for a greater amount of the variation in net mineral-N production (18 %) in soils

exhibiting ammonium-dependent histidase activity although only significant at $p \leq 0.10$ in this subset of all observations. Protease did not correlate with biomass-C1 or respiration ($p > 0.5$).

Table 5.3 Correlation (r) between mineralizing reactions of soils exhibiting ammonium-dependent labile histidase activity (A_0) and components identified in the conceptual model of nitrogen mineralization (Fig. 5.1).

Parameter		Net Min.-N Prod.	Protease	Histidase A_0	Histidase B_0
Biomass-C1		0.39	0.62 ***§	- 0.11	- 0.08
Biomass-C2		0.51 **	0.75 ***	- 0.19	0.02
Biomass-N		0.32	0.46 *	- 0.07	0.10
Respiration		0.29	0.38	- 0.16	0.14
Protease		0.43 *	---	0.02	- 0.31
Histidase	A_0	- 0.05	0.02	---	- 0.66 ***
	B_0	- 0.20	- 0.31	- 0.66 ***	---
Ammonium		0.42 *	- 0.06	- 0.92 ***	0.56 **
Nitrate		- 0.02	- 0.31	- 0.43 *	0.19
Resp.:Min.†		---	- 0.33	- 0.26	0.32

§ correlations significant at * $p \leq 0.10$, ** $p \leq 0.05$, *** $p \leq 0.01$
† The ratio of respiration to net mineral-N production

5.4 Discussion

5.4.1 Spatial dependence of measured variables

Field-scale spatial variability was not examined in this study; rather measures were made of plant-induced variability in soil biological and biochemical activities in samples taken from within or between rows of barley seeded at 22.5 cm spacings. Response of soil variability to row position is dependent upon the range

of values such variables may exhibit under field conditions and their rate of change during crop growth.

Zhai *et al.* (1988) reported increased microbial biomass and higher respiration rates within rows of soil cropped to maize than in either quarter-row or mid-row positions. Similarly, Carter and White (1986) found soil ammonium contents were spatially distributed in maize crops according to row position. Spatial distribution of soil biotic variables in response to row position of close seeded crops such as used here has not been reported.

Integrated variables such as ammonium-N and respiration were spatially distributed according to row position; both were higher within than between rows. The ratio of nitrate-N/ammonium-N was also lower within rows than between rows at all sampling dates. In general, detailed measures of biomass or enzyme potential were not affected by row position. For sampling dates up to and including July 22 labile histidase potential (A_0) was lower within rows than between rows. The spatial distribution of labile histidase appears be related to crop growth disappearing after harvest (September 3).

This study extends results from earlier work with maize to crops with narrower (22.5 cm) rows, even where root exploration of the entire soil volume would be expected to be complete, spatial variability is observed in integrated measures of soil biological activity (e.g ammonium-N), but not in high resolution variables (e.g. enzyme activity).

5.4.2 Correlation of variables with net mineral-N production

Mineral-N production is the result of the opposing processes of nitrogen mineralization and immobilization. To be effective, mechanisms controlling mineral-N content of soil must compensate for concurrent mineralization and immobilization and thus affect net mineral-N production. To determine whether broad based control mechanisms are involved in the regulation and coordination of nitrogen mineralization, correlation of components contributing to nitrogen mineralization on several levels were related to net mineral-N production (Fig. 5.1).

The soil biomass is an active component of soil organic nitrogen, a potential source of mineral-N and as a result provides a sensitive index of mineralizable nitrogen (Voroney and Paul, 1984; Carter, 1986). Biomass-C, as measured by

both approaches, correlated with net mineral-N production (Table 5.2). Biomass provides an indirect measure of biological activity, potential for enzyme production in addition to a measure of the mass of microbial material. The correlation with biomass-C was not simply a reflection of its role as substrate as indicated by the poor correlation with biomass-N. Biomass-N is a more direct evaluation of the nitrogen content of the biomass yet accounted for much less of the variation in net mineral-N production. Neither was the correlation with biomass-C solely related to its metabolic activity as reflected by the poor correlation with *in vivo* respiration. The correlation with biomass-C may also be derived from its role as an indicator of soil enzymatic or catalytic potential. Measures of potential activity of specific enzymes contributing to mineralization (protease and histidase) were not as well correlated with net mineral-N production as was biomass-C. Biomass-C as an integrative measure of substrate supply, potential biological activity and enzymatic activity, was more strongly related to net mineral-N production than were indices of any single aspect. As an integrative measure, biomass-C reflects both biological potential and chemical composition and thus provides information at a higher level of organization than specific indices.

Much higher levels of correlation between soil biomass and measures of nitrogen mineralization or mineralizing activity have been reported (Alef *et al.*, 1988; Frankenberger and Dick, 1983). These studies are generally those examining activities across a range of soils. Studies where relationships are examined within a soil generally have found much lower correlation between net mineral-N production and the activity of mineralizing enzymes (Ross *et al.*, 1984). In widely differing soils it is difficult to determine whether observed correlation is indicative of causal relationships or result of fundamental pedogenic differences. For example high correlations between enzymatic parameters and total organic carbon have been reported in studies examining a range of soils (Alef *et al.*, 1988; Frankenberger and Dick, 1983) but is seldom observed within a soil (Ross *et al.*, 1984). The current work examines a single soil and thus correlations are not confounded by pedogenic differences.

Potential casein hydrolysing (protease) activity is a specific measure of protein breakdown, presumably leading to mineralization. Protease catalyses only one of a number of reactions which ultimately result in mineral N production. Poor correlations between protease and mineral-N production have been noted (Ross and

McNeilly, 1975; Ross, 1977; Haynes and Swift, 1988). Similarly in this study protease accounted for only 8% of the variation in net mineral-N production. This poor correlation may stem from one, or a combination of factors: i) The protease assay is a measure of potential, not actual, activity. The supply of substrate *in situ* is not evaluated and may well limit the rate of reaction (Tateno, 1988). A more direct correlation might be anticipated if substrate supply were also considered. Amato and Ladd (1988) found ninhydrin reactive material accounted for 68% of the NH_4^+ released in fumigated-incubated soils, suggesting substrate supply is limiting nitrogen mineralization. ii) The time frame of the two measures is different, potential protease activity was measured over a period of hours while net mineral-N production is measured over days. Thus the net mineral-N production is a measure of both the amount and persistence of enzymatic activities such as protease. iii) Net mineral-N production is the result of a wide range of enzymatic reactions. Fluctuation in casein hydrolysing activity may not reflect changes in the entire suite of mineralizing enzymes, and thus may not be representative. iv) Net mineral-N production reflects the balance of mineralizing reactions, including proteolysis, and immobilizing reactions. Protease activity may be more closely related to total mineralization.

The deamination of histidine, catalyzed by histidase, is a nitrogen-mineralizing reaction. Due to the small amounts of histidine occurring in soil this reaction is not itself a significant component of nitrogen mineralization. Despite its relatively small contribution, soil histidase would be expected to respond to any broad-based biochemical coordination of nitrogen mineralization. In general, both components (labile and stable) of soil histidase were poorly correlated with net mineral-N production, accounting for less than 5% of the variation in net mineral-N production. The poor correlation with net mineral-N production may result from; i) deaminase activity, represented by histidase, not being the rate limiting step in mineralization and/or ii) histidase not being representative of general deaminating reactions in soil. Both of these explanations are plausible in a soils context. The rapid turnover of amino acids in soil (Monreal and McGill, submitted) suggest substrate and not catalytic potential is limiting the rate of deamination in soil. This is also supported by previous work (chapter 4) which indicates the majority of soil histidase activity is the result of constitutively synthesized enzymes.

The inability of specific measures of the content of nitrogen-mineralizing enzymes to correlate with net mineral-N production reflect both the diversity of reactions involved and the lack of a common, broad-based control of enzyme content. Specific measures of enzyme content (potential activity) fail to account for substrate supply and its role in regulating enzyme activity in first order systems. Poor correlation between enzyme content and net mineral-N production is consistent with substrate-limited enzyme activity observed for soil protease and cellulase activity (Tateno, 1988). Thus while synthesis-level control mechanisms have the potential to regulate enzyme content of soil systems (Chapter 4), limited substrate availability effects both the expression and efficacy of these mechanisms. In substrate limited systems (first order) enzyme activity is not simply a function of content. In addition many of the synthesis-level mechanisms discussed earlier are operative under conditions of energy abundance and are not expressed under conditions of substrate limitation. Measures which integrate enzymatic potential and substrate supply, such as biomass, provided better descriptors of net mineral-N production.

In a nitrifying soil transitory ammonium concentration may be indicative of the magnitude of mineralization. This is supported by the correlation of the ammonium content of freshly sampled soils and net mineral-N production over a ten day period. The initial soil ammonium content was able to account for 16% of the total variation in net mineral-N production. The positive correlation between ammonium and net mineral-N production also suggests that feedback inhibition did not impact upon the majority of reactions contributing to net mineral-N production in this soil. Ammonium content is an integrative measure of nitrogen-mineralizing reactions, further supporting the observation that integrative measures are better descriptors of diverse processes such as nitrogen mineralization.

5.4.3 Nitrogen control of histidase activity

Seasonal trends in protease and histidase (A_0) were inversely related in 150-200 d but positively related in 200-275 d to soil ammonium content (Fig. 5.2). Examination of labile histidase activity in relation to soil ammonium content reveal two separate responses (Fig. 5.3). A strong inverse relationship with ammonium was evident in soils with high ammonium content, the majority of which were associated with late June and July sampling dates. Low histidase activity is not unique to soils of high ammonium content. Conditions limiting biological activity,

lack of available substrate and the presence of an inhibitor are among other potential limiting factors. The relatively high rates of respiration during this period do not support inhibition of biological activity as the causal agent (Table 4.1). Low ammonium content is necessary, but not sufficient, for increased amounts of labile histidase activity.

The inverse relationship between ammonium content and labile histidase (A_0) provides indirect evidence for ammonium control and is consistent with feedback inhibition. Ammonium content was not the only factor regulating histidase however, a range of histidase contents were observed in samples low in ammonium (Fig. 5.3). Since labile histidase is not a unique function of ammonium content it is difficult to detect such relationships using correlation analysis. Poor correlations between component enzymatic activities and net mineral-N production does not eliminate the possibility of broad based control but suggests this control, if operative, was not expressed in the majority of cases. Rice and Paul (1987) noted ammonium addition decreased the metabolism of ^{14}C-glutamate and ^{14}C-leucine relative to immobilization-induced N-deficient soils. In earlier work (Chapter 4) ammonium addition resulted in a marginal reduction in stable histidase (B_0) but did not significantly affect on labile histidase (A_0). The failure to detect significant differences as a result of ammonium addition in laboratory studies (Chapter 4) may reflect the presence of other limiting factors (open circles in Fig. 5.3) or may have been obscured by the high variability in the size of the labile component. The control of reactions contributing to mineralization is likely the result of several factors operating simultaneously. Individual elements will only become apparent when other limitations are removed.

Labile histidase (A_0) was positively related to nitrate content in soils low in ammonium. The positive relationship with nitrate may reflect the role of nitrification in removing ammonium from the system, derepressing histidase. These soils also had significantly higher water contents than did soils exhibiting ammonium-dependent histidase. Aeration may have limiting biological activity, favouring nitrate respiring (denitrifying) organisms. Increased histidase may be associated with increased activity of nitrate respiring organisms in moist soils having a high NO_3^- content. This observation is not consistent with increased assimilatory utilization of amino acid observed in nitrate respiring *Pseudomonas stutzeri* however (Patkai, 1972).

Casein hydrolysing (protease) activity was not related to soil ammonium content (Table 5.2). Protease activity was correlated with biomass-C, particularly in the soils identified as having an ammonium-dependent labile histidase activity (Table 5.3). This is consistent with the predominantly microbial origin of soil protease activity (Vágnerová and Macura, 1974). In general, as in this study, soil protease activity has been found to be only slightly effected by ammonium content (Ross, 1977; Coy, 1984). Vágnerová and Macura (1974) found microbial protease activity on the surface of roots to be related to solution content of amino nitrogen and reducing compounds. Soil protease activity responds primarily to fluctuations in soil biomass and thus its content is related to biological potential and not soil ammonium content. This observation does not support broad based coordination of nitrogen mineralization reactions.

5.4 Conclusions

Variables examined relate to mineralization in a very specific sense, in that they are constituent reactions, as well as more general integrative measures. Integrative measures such as biomass were better correlated with net mineral-N production than were the more specific enzymatic activities. For a specific enzymatic activity to be predictive of a cascade of reactions such as mineralization it must either be rate limiting or responsive to common control mechanisms. The poor correlation between potential soil protease (casein-hydrolysing activity), soil histidase (potential histidase activity) and net mineral-N production suggest that enzyme content was not rate limiting nor sensitive to common control mechanisms. The correlation with estimates of biomass-C reflect the integrative nature of this variable, combining metabolic, synthetic and chemical aspects of the soil biomass.

The role of efficiency control mechanisms commonly observed in microbial systems, such as catabolite repression or nitrogen metabolite control, is limited in nutrient limited or stressed environments (Poindexter, 1987). The apparent absence of unifying control mechanisms, coordinating the reactions contributing to nitrogen mineralization, is consistent with an oligotrophic description of the soil. Metabolic diversity, rather than diauxic growth is a more appropriate characterization of biochemical activity in soil systems.

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6. ASSIMILATION OF AMINO ACIDS BY PLANTS IN SOIL AND EFFECT OF PLANT GROWTH ON AMINO ACID METABOLISM[1]

6.1 Introduction

Inorganic nitrogen has long been considered to be the sole source of plant available nitrogen in soil (Vickery, 1941). This view continues to dominate research in soil fertility despite evidence supporting direct assimilation of organic nitrogen sources such as amino acids (Bringham, 1917; Virtanen, 1946; Gosh and Burris, 1950; Miettinen, 1959; Mori and Nishimura, 1979). Direct assimilation of organic nitrogen sources may contribute to efficient utilization of soil nitrogen. Soil management strategies which provide for plant nitrogen nutrition while limiting the quantity of nitrate-N, from which most losses emanate, would result in increased nitrogen utilization efficiency. Such strategies would have favorable economic and environmental consequences. Knowledge about the fate of amino acids in the rhizosphere, and extent of amino acid uptake from soil by plants is necessary background for studies of associative plant-bacterial nitrogen fixing biotechnologies under field conditions.

Soil biomass is a source of nitrogen for rice (Maudinas *et al.*, 1981), wheat and maize (Lethbridge and Davidson, 1983). Results from mycorrhizal systems suggest amino acids are candidates as vectors of this transfer (Bledsoe and Sangwaint, 1986). While plants can assimilate amino acids directly from sterile solutions (Virtanen and Linkola, 1946; Ghosh and Burris, 1950; Mori and Nishizawa, 1979) direct utilization of amino acids from soil by plants has not been examined. Virtanen and Linkola (1946) hypothesized that direct amino acid uptake is unlikely in agricultural soils due to the rapid conversion of organic compounds to ammonium and nitrate, this hypothesis was not tested however. Monreal and McGill (1989) reported turnover times of a few seconds for soluble cystine in several Chernozemic and Luvisolic soils under laboratory conditions. For a plant to be able to assimilate amino acid from soil solution it must be able to compete

[1]A version of this chapter will be submitted for publication. D.L. Burton and W.B. McGill (Plant and Soil)

favorably with the combined effects of transport through the heterogeneous soil matrix and concurrent microbial utilization.

The ability of maize to assimilate amino acids, relative to inorganic nitrogen sources, was evaluated in sterile solution culture studies. ^{14}C-amino acids were introduced into the rhizosphere of soil-plant systems (barley, canola and maize) to allow an evaluation of the extent of direct assimilation of amino acids and the effects of plant growth on amino acid metabolism.

In addition to being a potential sink for free amino acids, plant growth may influence the metabolism of amino acids in soil. Plant roots are a major source of substrate in soil. Their impact on soil processes is widely accepted and define the rhizosphere, a volume in which physical and biological components of the soil are altered by the presence of plant roots. The availability of energy rich compounds, in sufficient amounts, has the potential to affect catabolic processes in soil (Chapter 4). The impact of plant growth on the metabolism of amino acids introduced into the rhizosphere were examined.

6.2 Materials and Methods

6.2.1 Solution culture

Maize (*Zea mays* L.) seeds were surface sterilized using 0.5% NaOCl and transferred to sterile germination pouches ("DiSPo" growth pouch, Canlab #B1220). Germinated seedlings were aseptically transferred to 1 L Kerr jars containing 800 mL of sterile nutrient solution. Seedings were suspended from sterile cotton plugs fit in holes in the jar lids. The nutrient solution was that of Taylor and Foy 1985) with the nitrogen source modified to meet experimental objectives. Nitrogen sources were: i) inorganic nitrogen (NH_4NO_3); ii) casamino acids; iii) two equimolar solutions of (a) alanine, aspartate, glycine and glutamate (AAGG) and (b) alanine, arginine, proline and glycine (AAPG); and iv) a 1:1 mixture of NH_4NO_3 and amino acid solution (AAGG). Final concentrations of all nutrient solutions contained 0.5 mM nitrogen. Kerr jars were covered with aluminum foil to exclude light. After 60 days plants were harvested, shoots and roots separated, dried and dry matter determined. Solution sterility at harvest was checked by plating 1 mL of solution on nutrient agar and incubating for 7 days at 25 °C. Ammonium content of amino acid solutions was determined by steam

distillation (Bremner, 1965) because amino acids interfere with the automated indophenol (Technicon Industrial Method #98-70W) method (Appendix). All treatments were replicated five times; only those remaining sterile throughout the course of the experiment were used in statistical analysis.

6.2.2 Soil description

Soils were from the Ap horizon of a black chernozem (Malmo SiCl) under grass (predominantly *Festuca rubra* L.) at the Ellerslie Research Station, Edmonton, Alberta. The samples had a silt loam texture, moderate granular structure, friable consistence when moist, an organic carbon content of 6.0%, pH of 5.4 ($CaCl_2$) (Pawluk, 1986) and retained 38% H_20 (w/w) at field capacity (Maulé and Chanasyk, 1987). Additional morphological and chemical characteristics of this soil were published by Pawluk (1986). Prior to use soil was stored at 4 °C; one week prior to planting the soil was passed through a 2 mm mesh sieve.

6.2.3 ^{14}C-amino acid metabolism in soil and uptake by plants

Plants were grown in modified Tinus containers (Spencer Lamar Industries, Edmonton, Alberta) containing approximately 120 g of soil. After 2 weeks of growth 2 mL of a 0.3 μM solution containing equimolar amounts of uniformly labelled ^{14}C-alanine (5.6 x 10^3 GBq.mole^{-1}), ^{14}C-aspartic acid (6.7 x 10^3 GBq.mole^{-1}), ^{14}C-glycine (3.3 x 10^3 GBq.mole^{-1}) and ^{14}C-glutamic acid (8.3 x 10^3 GBq.mole^{-1}; ICN Biomedicals) was injected into the rhizosphere of each plant by injecting approximately 0.2 mL into 10 different locations. The amount of ^{14}C-amino acid added was small (7 ng.g^{-1}) relative to free amino acid pools in soil (> 300 ng.g^{-1}; Monreal and McGill, 1985) to minimize perturbation. Following injection, containers were placed in 2 L Kerr jars with the shoot protruding from a hole in the jar lid. The soil atmosphere was sealed by fitting a modified one-hole rubber stopper around the shoot and sealing with parafin wax. Growth room temperatures were 25 °C day and 20 °C night. The plants were grown in this fashion for 14 days with a 16 hour day length. Carbon dioxide evolved from the soil was trapped in a microcentrifuge tube containing 1 mL of 1 M NaOH. A second hole in the jar lid allowed periodic changing of CO_2 traps. The ^{14}C content of each CO_2 trap was determined by transferring 0.2 mL of trapping solution to 4 mL of Hionic scintillation cocktail (Packard) for liquid scintillation counting (Tri-

Carb 2000 CA, Packard). All ^{14}C contents are expressed as percent of the initial dose.

At the time of plant harvest, shoots were cut at the soil surface and dried at 60 °C. The soil and roots were immediately frozen in liquid N_2, to stop further amino acid metabolism. The frozen soil-root samples were subsequently freeze dried and roots separated from soil by hand sorting. Soils were then sub-divided into three portions. The first and second portions were used to determine biomass ^{14}C by the chloroform fumigation-incubation method (Jenkinson and Powlson, 1976); the first was fumigated, and the second was a non-fumigated control. The final portion of soil was used for total soil ^{14}C determination. The ^{14}C content of the shoots, roots and soil was determined by dry oxidation (Biological Oxidizer OX 300, Harvey) followed by liquid scintillation counting. Total soil ^{14}C was determined on 5 samples (100 mg) taken from a finely ground (ball mill) and homogenized sub-sample (25 g) of soil. To ensure complete oxidation, cellulose was added to soil samples at a rate of 7-10 mg of cellulose in each 100 mg sample. Initial ^{14}C recovery was measured by immediately harvesting plants following the injection of ^{14}C-amino acid into soils planted to maize, barley or canola as well as a non-planted soil.

Barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), canola (*Brassica napus* L.) as well as non-planted soil were the treatments. All treatments were replicated five times; with a 23 day growth period. This experiment was replicated, but over a 14 day interval using only maize and canola with a non-planted control soil to confirm observations made in the first experiment. The species were selected to include a variety of rooting habits.

0.2.4 Effect of soil amendment on amino acid metabolism

Field moist soil (5 g at 25 % water $w.w^{-1}$) was weighed into 30 mL screw top vials and incubated with 0.5 mL of either distilled water or glucose, succinate or casamino acids solutions at a C addition rate of 400 $\mu g\ g^{-1}$ of soil for 12 hours. Following incubation, a 0.5 mL of a 0.3 $\mu\underline{M}$ solution containing equimolar amounts of uniformly labelled ^{14}C-alanine, ^{14}C-aspartic acid, ^{14}C-glycine and ^{14}C-glutamic acid (specific activity as noted above) was added to each vessel. The $^{14}CO_2$ evolved was collected in 1 mL of 1 $\underline{M}$ NaOH contained in microcentrifuge

tubes. The CO_2 traps were periodically changed. The ^{14}C content of each trap was determined as above.

The experiment was repeated using a 72 hour incubation period prior to amino acid addition to insure sufficient time for substrate addition to affect soil metabolism. CO_2 evolution was monitored for an additional 500 hours in the second experiment.

6.2.5 Mathematical analysis of $^{14}CO_2$ evolution

The turnover of soil metabolic pools in situ can be estimated by the addition of a pulse of radioactive tracer. The rate of $^{14}CO_2$ evolution follows first-order kinetics if the quantity of ^{14}C added to the soil is low relative to the potential for metabolism. Under steady state conditions added ^{14}C is diluted by ^{12}C and the specific respiration rate of ^{14}C can be used to calculate the rate of pool turnover (Shipley and Clark, 1972). Kinetic analysis of the rate of $^{14}CO_2$ evolution can be obtained by analysis of incremental data (Ellert and Bettany, 1988) or by summing the $^{14}CO_2$ collected in each time interval, for each vessel, over the course of the incubation period to give the cumulative $^{14}CO_2$ evolution. Turnover time (k^{-1}) of the ^{14}C-amino acid mixtures were calculated by fitting single- or double-component first-order models to the data for incremental and cumulative evolution of $^{14}CO_2$.

A non-linear least square fitting procedure (Systat NONLIN, Systat Inc., Evanston, Il.) was used to fit single- or double-component first-order incremental or cumulative models. The amount of ^{14}C remaining in solution (S) is described by the equation,

$$S_t = S_o e^{-kt} \qquad [6.1]$$

where: S_t = Amount of ^{14}C in solution at time t (h) (% ^{14}C g $soil^{-1}$)
S_o = Amount of ^{14}C in solution at time t=0 (% ^{14}C g $soil^{-1}$)
k = rate constant for amino acid pool turnover (h^{-1})

The rate of $^{14}CO_2$ evolution (A) follows first order kinetics and is given by,

$$A = -k S \qquad [6.2]$$

Substituting equation [6.2] into [6.1] and dividing by -k gives a first-order expression describing the instantaneous rate of $^{14}CO_2$ evolution (A_t),

$$A_t = A_o e^{-kt} \qquad [6.3]$$

where: A_t = Rate of $^{14}CO_2$ evolution at time t (h) (% ^{14}C g soil^{-1} h^{-1})
A_o = Rate of $^{14}CO_2$ evolution at time t=0 (% ^{14}C g soil^{-1} h^{-1})
k = rate constant for amino acid pool turnover (h^{-1})

The rate of $^{14}CO_2$ evolution (A_t) reflects the combined effects of the rate of amino acid oxidation and diffusion in soil. Equation [6.3] can be integrated over time to yield cumulative $^{14}CO_2$ evolved by time t.

$$\text{Cum. } ^{14}CO_{2\,t} = \frac{A_o}{k}(1 - e^{-kt}) \qquad [6.4]$$

Ellert and Bettany (1988) derive an expression to describe the incremental quantity of $^{14}CO_2$ evolved:

$$\text{Inc. } ^{14}CO_{2\,it} = \frac{A_o}{k}\left(e^{-k(t-i)} - e^{-kt}\right) \qquad [6.5]$$

where: Inc. $^{14}CO_{2\,it}$ = the $^{14}CO_2$ evolved during the increment i preceding time t
t = time from the start of the incubation (h)
i = interval of CO_2 collection (h)

While equation [6.3] can be further simplified, the current form avoids the subtraction of large numbers and thus minimizes roundoff error.

Amino acid metabolism may be the result of the activity of two or more components or populations, each having a characteristic evolution rate, and thus the added tracer is diluted at different rates. The corresponding expressions for a two-component model are,

$$A_t = A_o e^{-k_1 t} + B_o e^{-k_2 t} \qquad [6.6]$$

where: B_o = loss of $^{14}CO_2$ from component B at time t=0;
with all other terms as previously defined.

The integrated form of the two-component model is,

$$\text{Cum. } {}^{14}CO_{2\,t} = \frac{A_o}{k_1}(1 - e^{-k_1 t}) + \frac{B_o}{k_2}(1 - e^{-k_2 t}) \qquad [6.7]$$

and the incremental model is,

$$\text{Inc. } {}^{14}CO_{2\,it} = \frac{A_o}{k_1}\left(e^{-k_1(t-i)} - e^{-k_1 t}\right) + \frac{B_o}{k_2}\left(e^{-k_2(t-i)} - e^{-k_2 t}\right) \qquad [6.8]$$

The adequacy of one and two-component first order models to describe $^{14}CO_2$ evolved was examined by testing the significance of the reduction in the residual sum of squares (RSS) relative to the complexity of each model. Significance of the reduction in RSS was determined by computing the F-statistic described by Robinson (1985). The statistic is calculated by dividing the difference between the RSS of the two models by the residual mean square (RMS) of the more complex model. The result was compared to an F value at $p = 0.05$ with 1 and n-p degrees of freedom, where n = the number of data points and p = the number of parameters.

6.3 Results

6.3.1 Sterile solution culture

All N sources significantly ($p \leq 0.05$) increased total dry matter and nitrogen content of maize over that in the control (Table 6.1). Plants receiving organic nitrogen produced dry matter equal to or greater than did those growing on inorganic nitrogen sources. The combination of amino acid and NH_4NO_3 significantly increased ($p \leq 0.05$) dry matter production over NH_4NO_3 alone. Nitrogen uptake followed similar trends as dry matter accumulation. Casamino acids resulted in significantly lower dry matter accumulation than did the two amino acid mixtures. There was greater nitrogen uptake by plants grown on amino acid solutions containing aspartate and glycine than on those containing arginine and proline.

Table 6.1 Nitrogen content and dry matter yield of maize grown in sterile solution culture containing diverse nitrogen sources

Nitrogen Source†	Shoots		Roots		Total	
	Dry Weight (g)	Nitrogen (mg)	Dry Weight (g)	Nitrogen (mg)	Dry Weight (g)	Nitrogen (mg)
Amino Acid (AAGG)	1.26 a§	11 a	0.37 ab	4 ab	1.63 a	15 a
Amino Acid (AAPG)	1.01 ab	7 b	0.39 ab	3 a	1.40 ab	10 b
Casamino Acids	0.85 b	7 b	0.26 a	3 a	1.12 c	10 b
Amino Acid (AAGG) + NH_4NO_3	1.09 ab	9 c	0.40 b	5 b	1.49 a	14 a
NH_4NO_3	0.90 b	7 b	0.29 a	3 a	1.19 bc	10 b
No Nitrogen	0.40 c	3 d	0.21 c	2 c	0.58 d	5 c

§ nitrogen treatments followed by different letters are significantly different at $p \leq 0.05$

† AAGG - Alanine, aspartate, glutamate and glycine; AAPG - alanine, arginine, proline and glutamate.

Differences in dry matter accumulation and nitrogen uptake were primarily the result of shoot growth. Root growth was more consistent across treatments, but the control treatment yielded significantly smaller root mass than any other treatment (Table 6.1). Roots growing in solutions containing amino acids were smaller in diameter and more extensively branched than those growing in solutions containing inorganic nitrogen.

Sterility of nutrient solution was determined at the time of harvest. Statistical analyses of dry matter yield used only the data from solutions which remained sterile during the course of the experiment, although inclusion of all samples did not alter interpretation.

6.3.2 Plant uptake of ^{14}C-amino acid in soil

A small but statistically significant ($p \leq 0.05$) proportion of the ^{14}C-amino acid injected into the rhizosphere was found in plant tissues (Table 6.2). Total uptake was in all cases less than 2% of the added amino acid. The majority of plant ^{14}C was associated with shoot tissue. The amount of ^{14}C associated with the soil

biomass was not affected by plant growth. In soils in which plants were growing, more ^{14}C was associated with non-biomass soil components ($p \leq 0.10$).

Table 6.2 Recovery after 14 or 23 days of ^{14}C from a mixture of alanine, aspartate, glycine and glutamate introduced into the rhizosphere of barley, canola and maize.

Plant Species	Plant		Soil		
	Shoot (%)	Root (%)	Biomass (%)	Non-Biomass (%)	Respiration (%)
	14 days				
Barley	0.4 a§	0.1 ab	14.6 a	34.1 ab	35.3 a
Canola	0.4 a	0.3 a	14.8 a	37.0 ab	34.2 a
Corn	0.4 a	0.2 a	11.3 a	42.2 a	25.6 b
Soil Only	0 b	0 b	12.5 a	32.9 b	39.8 c
	23 days				
Canola	0.4 a	0.0 a	12.2 a	30.0 a	31.8 a
Corn	1.1 b	0.5 b	16.5 a	25.1 ab	26.1 b
Soil Only	0 c	0 a	19.4 a	21.2 b	33.9 a

§ mean within an experiment, means followed by different letters are significantly different at $p \leq 0.05$

The amount of added amino acid evolved as $^{14}CO_2$ was significantly ($p \leq 0.05$) reduced in soils in which plants were growing (Table 6.2). Soils planted to maize evolved the smallest amount of $^{14}CO_2$. Soil in which barley and canola were growing evolved similar amounts of $^{14}CO_2$, intermediate between the amounts observed in maize soils and those of non-planted controls. The amount of $^{14}CO_2$ evolved and the effect of plant growth on $^{14}CO_2$ evolution was similar in both the 23 and 14 day experiments.

Total recovery of ^{14}C was relatively low, averaging 79% and ranging from 70-87% of added ^{14}C-amino acid. There were no significant differences in ^{14}C recovery between treatments. Recovery of ^{14}C in time 0 controls averaged 89% ranging from 79-97% of added ^{14}C-amino acid. Recovery may have been effected by one or a combination of factors including: 1) Soil handling (freezing, freeze

drying and grinding) may have resulted in the volatilization of ^{14}C as CO_2 originating from organics or in equilibrium with carbonates in soil solution. The presence of a NaOH trap in the headspace would be anticipated to minimize but not eliminate ^{14}C-carbonates in the soil solution. 2) Water absorption by NaOH traps was not accounted for in calculation of $^{14}CO_2$ content. 3) estimates of total ^{14}C were based on a small proportion of the total soil volume. Although multiple injections (10) of ^{14}C-amino acids were used, introduction would still result in a relatively non-homogeneous distribution of label. Bulk soil analysis of a non-homogeneous point source is on average likely to underestimate total ^{14}C.

6.3.3 Analysis of $^{14}CO_2$ evolution

Non-linear least squares analysis of $^{14}CO_2$ evolution using either incremental or cumulative $^{14}CO_2$ evolution data (Table 6.4; Fig. 6.1) indicated two-component first-order models were able to account for a significantly ($p \leq 0.05$) greater proportion of the total sum of squares relative to single-component first-order models for all samples (Table 6.3). A physiological basis for this separation cannot be inferred. The derived constants suggest a majority (60%) of amino acid catabolism was associated with the labile pool ($t_{1/2}$ = 3-8 hours). The remaining $^{14}CO_2$ emanated from a pool of more gradual metabolism ($t_{1/2}$ = 35 - 63 hours). The partitioning of amino acid between the two pools was not affected by the presence of growing plants (Table 6.4).

Table 6.3: Representitive analysis of varience to evaluation the statistical appropriateness of one and two component first order models to describe $^{14}CO_2$ evolution (Y) with time (t) in soils in which maize or canola is growing relative to an unplanted soil. The F statistic was calculated as the difference in residual sum of squares (RSS) of the two models divided by the residual mean square (RMS) of the more complex model described by Robinson (1985).

	Model	RSS	RMS	F-Ratio
	Maize			
Single	$Y = \frac{2.25}{0.093}(1 - e^{-0.093t})$	47.85	2.28	
Double	$Y = \frac{2.84}{0.174}(1 - e^{-0.174t}) + \frac{0.17}{0.017}(1 - e^{-0.017t})$	3.34	0.18	252.9*
	Canola			
Single	$Y = \frac{1.88}{0.061}(1 - e^{-0.061t})$	21.08	1.05	
Double	$Y = \frac{2.04}{0.088}(1 - e^{-0.088t}) + \frac{0.15}{0.017}(1 - e^{-0.017t})$	4.17	0.23	73.1*
	Soil Only			
Single	$Y = \frac{1.39}{0.043}(1 - e^{-0.043t})$	35.61	1.78	
Double	$Y = \frac{1.64}{0.077}(1 - e^{-0.077t}) + \frac{0.16}{0.012}(1 - e^{-0.012t})$	1.75	.10	348.5*

* Two component model accounts for significantly greater deviation at $p \leq 0.05$ ($F_{0.05(.,18)} = 4.41$)

Table 6.4: Values for parameters of double exponential model calculated from incremental and cumulative $^{14}CO_2$ evolved from soil in the presence and absence of plant growth.

	Labile				Stable			
	A_o/k_1 (%)§	k_1 (h^{-1})	$t_{1/2}$ (h)	Contribution (%)¥	B_o/k_2 (%)	k_2 (h^{-1})	$t_{1/2}$ (h)	Contribution (%)
	Analysis of Cumulative $^{14}CO_2$ Evolution							
Canola	19.5 a†	0.102 a	6.8	61.7	12.1 a	0.018 a	38.5	38.3
Maize	16.2 ab	0.180 b	3.9	62.5	9.7 a	0.018 a	38.5	37.5
Soil	21.1 b	0.086 c	8.1	62.2	12.8 a	0.011 b	63.0	37.8
	Analysis of Incremental $^{14}CO_2$ Evolution							
Canola	15.6 a†	0.126 a	5.5	56.7	11.9 a	0.020 a	34.7	43.3
Maize	15.8 a	0.201 b	3.4	65.0	8.5 a	0.017 b	40.8	35.0
Soil	19.4 c	0.090 c	7.7	61.2	12.3 a	0.011 c	63.0	38.8

† numbers followed by different letters significantly different at $p \leq 0.05$
§ percent of added amino acid evolved
¥ percent of $^{14}CO_2$ evolved

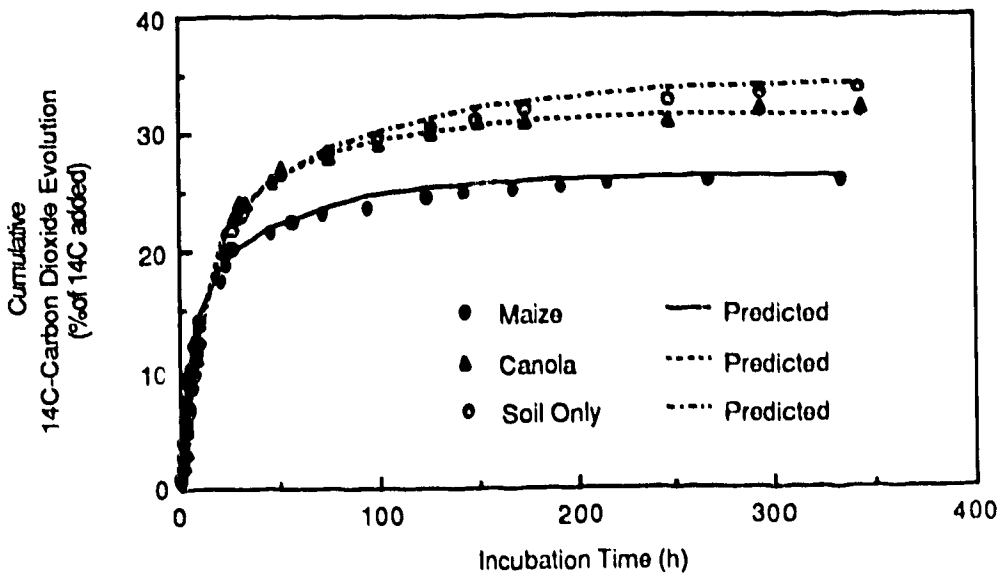

Figure 6.1 Observed and predicted cumulative $^{14}CO_2$ evolution from planted and non-planted soil.

The rate of turnover of the amino acid pools from which $^{14}CO_2$ was oxidized was significantly ($p \leq 0.05$) increased by the growth of plants as indicated by an increase in the rate constants (k) (Table 6.4). The magnitude of the increase in turnover was species specific, with maize resulting in the greatest increase. Despite the increased rate of catabolism a smaller proportion of the added ^{14}C-amino acid was catabolized to $^{14}CO_2$ in soils in which plants were grown (Fig. 6.1).

Analysis of incremental $^{14}CO_2$ evolution data tended to produce higher estimates of turnover and smaller pool sizes (Table 6.4; Fig. 6.2). The method of calculation had little effect on conclusions about relative differences between treatments or partioning between the two pools.

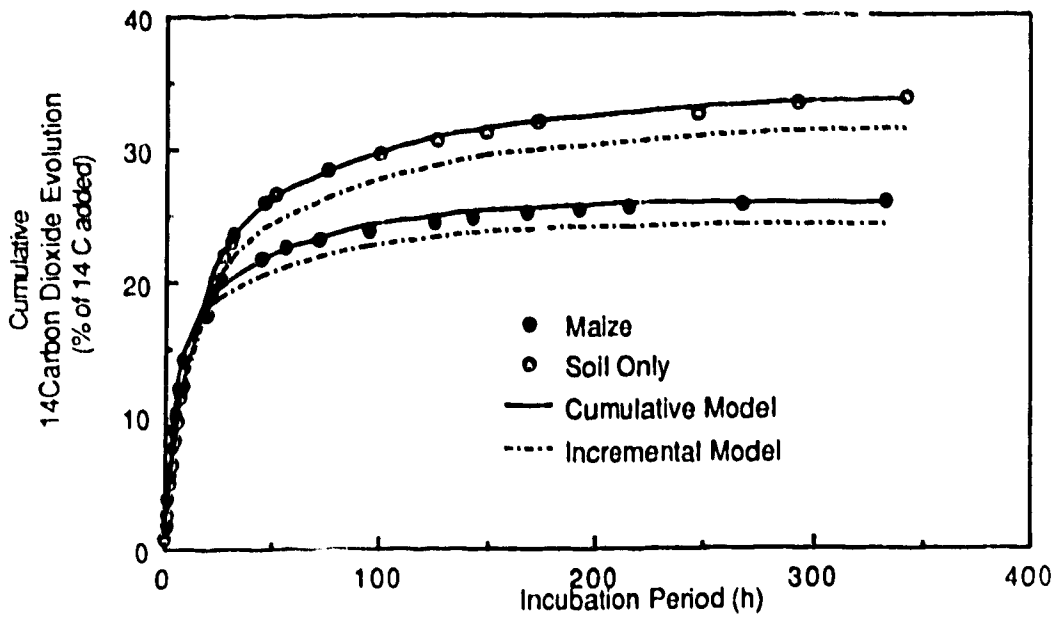

Figure 6.2 Comparison of the ability of models using parameters estimated using cumulative vs incremental data to predict cumulative $^{14}CO_2$ evolution.

6.3.4 Effect of substrate addition on amino acid metabolism

The addition of carbohydrate (glucose or succinate) to soil either had no effect on, or increased ($p \leq 0.05$) amino acid oxidation (Fig. 6.3; Table 6.5). Despite an overall increase in the extent of oxidation the turnover of soil free amino acid pools from which oxidation was occurring was either not affected or decreased following carbohydrate addition. Soil treatment with casamino acids resulted in a short-term (12 h) increase in both the turnover of free amino acids and the extent of amino acid oxidation.

Table 6.5: Values for parameters of double exponential model calculated from cumulative $^{14}CO_2$ evolved from amended soil.

	Labile				Stable			
	A_o/k_1 (%)§	k_1 (h^{-1})	$t_{1/2}$ (h)	Contribution (%)¥	B_o/k_2 (%)	k_2 (h^{-1})	$t_{1/2}$ (h)	Contribution (%)
	12 Hour Preincubation							
Glucose	27.6 a†	2.28 a	0.30	59.1	19.1 a	0.003 a	201.5	40.8
Succinate	23.8 b	2.05 b	0.34	59.2	16.4 b	0.029 b	24.3	40.7
Casamino Acids	26.6 a	2.97 c	0.23	56.8	20.2 a	0.005 ac	131.8	43.2
Soil	24.2 b	2.36z a	0.29	58.9	16.9 b	0.011 c	64.1	41.1
	72 Hour Preincubation							
Glucose	24.1 a†	2.96 ab	0.23	66.9	11.9 a	0.036 a	19.3	33.1
Succinate	21.6 ab	2.68 b	0.26	63.1	12.6 a	0.035 a	19.8	36.9
Casamino Acids	17.6 b	3.30 ac	0.21	60.1	11.4 a	0.036 a	19.3	39.9
Soil	19.7 b	3.48 c	0.20	63.5	11.3 a	0.029 a	23.9	36.5

† numbers followed by different letters significantly differnt at $p \leq 0.0$
§ percent of added amino acid evolved
¥ percent of $^{14}CO_2$ evolved

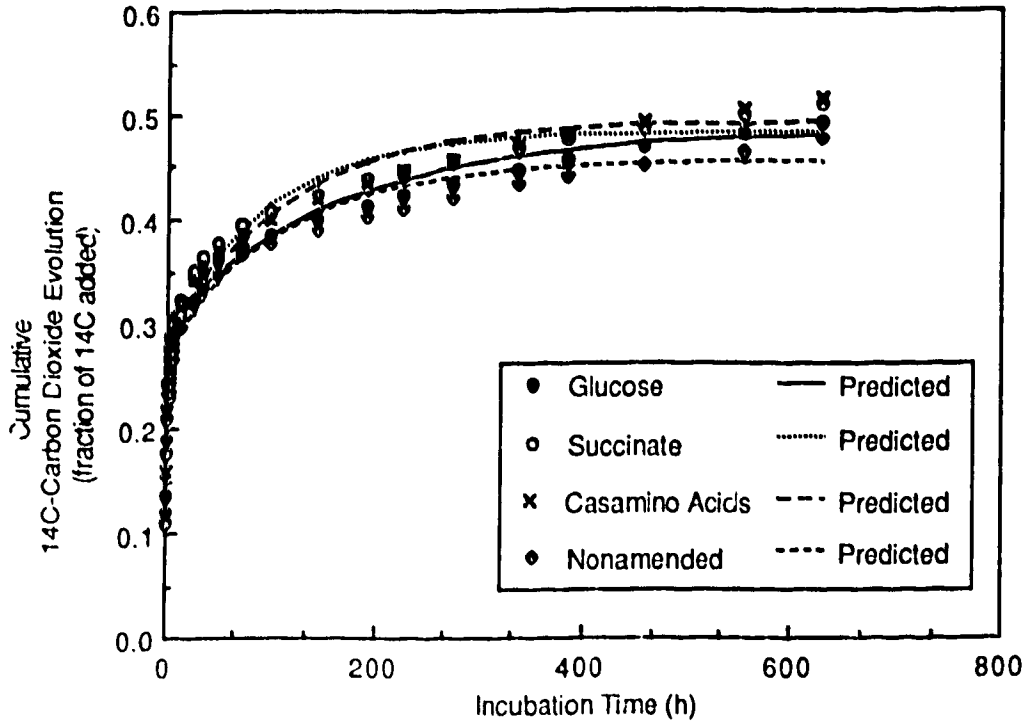

Figure 6.3 Observed and predicted cumulative $^{14}CO_2$ evolution from glucose, succinate, casamino acid or non-amended soils. Predicted response calculated using Eq. [6.7] and the values given in Table 6.5.

The effects of substrate amendment were primarily expressed in changes in the rapid component of oxidation. Succinate addition resulted in an increase in the turnover of soil pool from which the in the slower component of oxidation was emanating. The contribution of each component to overall activity was relatively constant across all experiments with 60 % activity being derived from rapid catabolism and 40 % from more gradual oxidation (Table 6.5).

6.4 Discussion

6.4.1 Amino acid uptake in sterile solution

Increased plant growth and nitrogen uptake in response to nitrogen addition, whether organic or inorganic, confirms that both forms of nitrogen are available to maize. Maize growth was as high or higher when nitrogen was supplied from organic sources (Table 6.1). Direct amino acid uptake is consistent with reports of amino acid utilization by plants in sterile systems (Virtanen and Linkola, 1946; Ghosh and Burris, 1950; Miettinen, 1959; Schobert and Komor, 1987; Schobert *et al.*, 1988). Amino acids have been found to be superior (Mori et al, 1979), inferior (Ghosh and Burris, 1950; Miller and Schmidt, 1965) or equivalent (Virtanen and Lionkola, 1946) relative to inorganic nitrogen sources across a variety of plant species.

6.4.2 Uptake in soil systems

Based on evidence collected from sterile systems, Schobert and Komor (1987) suggest amino acid uptake could potentially contribute 15 - 25% of the nitrogen requirements of the plant. Soil is not however a sterile system and thus plants must be able to effectively compete with soil microflora for amino acid uptake to be of significance to plant nutrition. Transport through a heterogeneous, reactive soil matrix is a factor in determining the potential for amino acid uptake. Transport and plant uptake must be sufficiently rapid to compete with microbial metabolism of amino acids.

Despite the ability to assimilate amino acid, in all three species studied (barley, canola and maize) less than 2% of the added ^{14}C added was recovered in plant tissue. Microbial metabolism was the dominant fate and 25 and 40% of the added amino acid was respired as CO_2 (Table 6.2). This is consistent with Schobert *et al.* (1988), in which less than 3% of the added label was detected in castor bean seedlings exposed to ^{14}C-proline under "natural" conditions. The rate of microbial metabolism of amino acid limits direct plant utilization of amino acids occurring in bulk and rhizosphere soil.

A large proportion of added ^{14}C-amino acid was retained in the soil, both in biomass and non-biomass forms. The large amount of non-biomass ^{14}C may be

the result of physical and chemical stabilization of added amino acids (Monreal and McGill, 1989). This material may be available to the plant over the long-term.

The relative roles of transport and uptake affinity in favouring microbial metabolism cannot be separated in this study. While an attempt was made to introduce the amino acid solution near the root mass of the plant, presumably dominated by rhizosphere soil, it is likely that the amount introduced into the rhizoplane or root surface would be quite small. Thus further research is needed to examine the fate of amino acids occurring at the rhizoplane. Re-adsorption of amino acids occurring at the root surface as discussed by Schobert and Komor (1987) may be of greater significance than indicated by these data.

Mycorrhizal infection enhanced amino acid uptake in Douglas-fir and western hemlock seedlings (Bledsoe and Sangwaint, 1986). Abuzinadah *et al.* (1986) found mycorrhizal infection of aseptic *Pinus contorta* allowed superior plant utilization of proteinaceous nitrogen sources. Although the predominant effect was likely the result of fungal protease activity, enhanced amino acid uptake would also benefit growth. Mycorrhizal plants access a much larger volume of soil allowing assimilation of non-mobile nutrients. Mycorrhizal plants may therefore possess a superior ability to utilize free amino acids by reducing the distance over which transport must occur and thus limit opportunities for microbial metabolism. Further research examining amino acid uptake by mycorrhizal and non-mycorrhizal plants under non-sterile soil conditions is needed.

6.4.3 Effect of plant growth and substrate addition on amino acid metabolism

The turnover ($k_1 + k_2$) of free amino acid pools undergoing oxidation was increased in soils in which plants were growing, yet a smaller portion of the added amino acid ($A_o + B_o$) was being oxidized to CO_2. Reduced catabolism may be indicative of greater biosynthetic utilization. The consistency in the amount of ^{14}C associated with the soil biomass does not support this assertion however. Plant growth has been observed to reduce $^{14}CO_2$ evolution from labelled organic matter (Jenkinson, 1977; Reid and Goss, 1982; Sparling *et al.*, 1982). This effect was attributed to plant-induced drying of the soil (Jenkinson, 1977) but this interpretation was later questioned (Reid and Goss, 1982; Sparling *et al.*, 1982). Reid and Goss (1983) noted maize growth resulted in a significant reduction in water soluble ^{14}C in soil. Decreased oxidation of added amino acid in planted soils

and reports of plant suppression of soil organic matter catabolism are consistent with the hypothesis that plant growth affects soil metabolism through favouring biosynthesis as a result of the exudation of high C/N ratio compounds. The exuded material, primarily carbohydrate, would provide a readily available substrate for the microflora. The presence of superior catabolites would increase the overall rate of metabolism, favor anabolic use of biosynthetic precursors such as amino acid and suppress the synthesis of enzymes catabolizing these compounds. This hypothesis can be tested by examining the catabolism of amino acid in carbohydrate amended soils.

Carbohydrate amendment did not mimic the increased amino acid conservation observed in plant systems. the rate of free amino acid turnover was decreased and the extent of oxidation was increased. Soil amendment with casamino acid also resulted in increased amino acid oxidation over the short-term. This is consistent with substrate induced synthesis of catabolic enzymes.

The turnover of free amino acids associated with rapid component of CO_2 production was an order of magnitude greater in the substrate amendment experiment (Table 6.5) than observed in the plant uptake study (Table 6.4). Turnover of pools associated with more gradual metabolism were of similar magnitude in the two experiments. While the reason for differences in parameter estimates is not clear, differences may relate to the amount of soil and geometry of the two systems. $^{14}CO_2$ evolution is a function of both the rate of $^{14}CO_2$ production (amino acid oxidation) as well as its diffusion from the soil. In the plant uptake experiment the larger volume of soil (200 g) likely provided a greater impediment to $^{14}CO_2$ diffusion from the system than did the smaller volume (5 g) used in the substrate amendment study. Diffusion resistance to the release of $^{14}CO_2$ would be expressed over the short-term, primarily affecting estimates of the rate of dilution of pools of more rapid metabolism.

The opportunity for transport to the root surface and subsequent utilization of rapidly turning over pools ($t_{1/2} < 10$ h and $t_{1/2} < 70$ h) is limited. The combined effects of rapid catabolism of amino acid and transport through the soil are consistent with the small amounts of ^{14}C found in plant tissues (Table 6.2). While plants have the physiological capacity to assimilate amino acids, soil architectural constraints restrict the volume of soil from which amino acid can be drawn intact and thus limit the significance of direct uptake in soil. Mycorrhizal infection may

act to increase uptake affinity as well as reduce the distance over which transport must occur.

Increased catabolism as a result of substrate amendment is not consistent with root exudate driven biosynthetic utilization of ^{14}C-amino acid. Plant induced changes in soil water content and subsequent effects on diffusion and rates of biological activity may account for observed differences in catabolism.

The half-lives determined in this work are those for the catabolism of primary metabolites such as amino acid. Slower rates of turnover, expressed in terms of years, have been reported for the utilization of more complex secondary metabolites such as microbial cells or soil organic matter (Jenkinson, 1966; Juma and McGill, 1986). Monreal and McGill (submitted) described the catabolism of ^{14}C-cystine by two kinetic components, which they attributed to cytoplasmic and protein pools. The half-life of cystine was affected by substrate amendment and ranged between 0.07 hours and 12 hours for cytoplasmic and protein pools, respectively. The half-lives observed in the current study reflect rates of metabolism slightly slower than those reported by Monreal and McGill (submitted). The differences in estimated rates of metabolism may also reflect differences in diffusion restraints to CO_2 evolution in each of the systems.

6.5 Conclusions

Evidence from this work as well as that of others strongly suggest that plants have the physiological capacity for direct assimilation of amino acid as a nitrogen source in sterile systems. Despite this ability the three plant species studied in this work failed to assimilate more than 2% of amino acid introduced into the rhizosphere. The predominant fate of was microbial metabolism as indicated by the evolution of 25-40% of the added amino acid as $^{14}CO_2$. In view of the small proportion of free amino acid available to the plant the significance of amino acid to plant nitrogen nutrition in soil systems would appear to be limited. Longer duration studies are required to fully assess the role of direct amino acid uptake in plant nitrogen nutrition.

Plant growth significantly affected amino acid metabolism. Less ^{14}C-amino acid was oxidized in soil in which plants were growing. Plant species affected the extent of amino acid retention, with soil planted to maize evolving the smallest

amounts of $^{14}CO_2$, barley and canola intermediate, and non-planted soil evolving the greatest amount. The turnover rate of amino acids pools was seen to increase in planted soil. Amendment of soils with carbohydrate did not reduce the catabolism of added amino acids suggesting reduced oxidation was not the result of substrate induced anabolic utilization of amino acid.

6.6 References

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7. SYNTHESIS

The potential for direct assimilation by plants of simple organics such as amino acids exists in soil. The extent of direct assimilation depends upon the degree to which catabolism of such molecules is suppressed so that they may be transported in soil. While such mechanisms are expressed within organisms, their expression at higher levels of organization in complex environments such as soil has not been demonstrated.

Synthesis-level controls over the content of nitrogen mineralization reactions are pertinent if these reactions are dependent upon continued enzyme synthesis and therefore are under biological control. Although all enzymes in soil are of biological origin, the possibility of soil stabilization may free these enzymes from a continued dependence upon synthesis. The presence of stabilized enzymes catabolizing amino acids could prevent expression of conservative mechanisms such as catabolite control and thus limit the extent of transport in soil and in turn the significance of direct assimilation of amino acid. L-histidine NH_3-lyase, the model system used in this study, was unstable in soil and thus is dependent upon continued enzyme synthesis and potentially sensitive to synthesis-level control (Chapter 3). Histidase activity declined following the addition of a biostatic agent and the rate of this decline was best described by a two component first order equation. The two components correspond to one of rapid turnover ($t_{1/2}$ = 2 h) and one of more gradual decay ($t_{1/2}$ = 77 h). After examining several possible causes, instability was attributed to normal mechanisms of enzyme turnover.

The expression of synthesis-level control of enzyme content has not been explicitly examined in soil systems. Soil histidase was found to be sensitive to induction by histidine or urocanate and this induction could be repressed by the addition of glucose (Chapter 4). The concentrations of both inducer (histidine or urocanate) and catabolite (glucose) required to elicit a response were well in excess of concentrations expected to normally occur in bulk soil however. Thus while the synthesis-level control can be elicited in amended systems their significance under normal soil conditions may be restricted to microsites of substrate accumulation. The majority of soil histidase activity in non-amended soils is the result of constitutive enzyme synthesis rather than induction. Extrapolating from

From the response of histidase to induction and repression it would appear that while synthesis-level controls are operative in soil, they are likely to be of significance only within energy rich microsites of the soil environment. From this perspective the bulk of the soil environment may be more appropriately described as an oligotrophic environment (Morgan and Crawford, 1986). In such environments the constitutive synthesis of a wide range of catabolic enzymes for the utilization of mixed substrates is favoured over the production of a few very specific enzymes characteristic of diauxic growth. The enzymatic composition of soil under such conditions is more closely related to the size and composition of the entire microbial population rather than the activity of a few metabolic specialists. Further enzyme content will exhibit less temporal variation under such conditions and be less responsive to short-term changes in chemical environment.

Laboratory studies are conducted to standardize or normalize soil conditions. In so doing there is the potential to mask field responses. Evidence for the coordination of reactions contributing to mineralization with net mineral-N production was sought under field conditions. Feedback inhibition of labile histidase activity by ammonium was apparent in soils collected from the field during mid-summer sampling dates (Chapter 5). Despite apparent control of histidase activity, the majority of nitrogen-mineralizing reactions were apparently not sensitive to feedback regulation as indicated by the positive correlation between ammonium content and net mineral-N production.

Protease activity and net mineral-N activity were best related to soil biomass content. The correlation between the size of the biomass and net mineral-N production is likely the result of both its catalytic activity and its role as a source of readily metabolizable substrate. In this regard soil biomass is an integrative parameter, simultaneously characterizing a spectrum of parameters impacting on nitrogen mineralization. The correlation between biomass and protease and net mineral-N production is also consistent with the description of soil as an energy limited, oligotrophic environment.

As demonstrated with maize in sterile culture, plants have the capacity to directly assimilate simple organics such as amino acids. Despite this ability,

microbial metabolism was the predominant fate of amino acids added to the rhizosphere of planted soils and less than 2% was directly assimilated by plants (Chapter 6). High microbial demand for energy rich compounds such as amino acids and the inability of amino acid to be transported over even small distances in soil without being catabolized are consistent with these observations.

While plant uptake was not a major sink for added amino acids, plant growth reduced the catabolism of added amino acids (Chapter 6). The suppression of catabolism was not the result of exudate-driven conservative reactions (biosynthesis) as indicated by the failure of carbohydrate or casamino acid additions to mimic the plant effect. Reduced catabolism in the presence of plants is likely the result of physical changes in the soil matrix as a result of soil wetting and drying in response to plant transpiration. Increased physical adsorption would decrease the availability of amino acid to microorganisms and is consistent the observed trends.

The quantity and spatial distribution of substrate (energy) in soil combine to restrict the expression of synthesis-level control of soil enzyme content such as catabolite repression. Thus while these soil organisms are sensitive to such control mechanisms, architecture constraints prevent the coordination of reactions at higher levels of resolution (aggregate and horizon-levels). Direct assimilation of organic nitrogen (eg. amino acid) will be limited by the ability of these compounds to be transported from their source to the plant root intact. From this standpoint, soil may be best described as a series of nutritionally isolated micro-environments linked by changes in the physical environment and the movement of more stable chemical species. The predominance of an inorganic phase in the soil nitrogen cycle, as described by mineralization-immobilization turnover (MIT) is realized as a result of the impact of soil architecture on biochemical activity. The transport of inorganic nitrogen forms is favoured by their stability resulting from lower energy content relative to organic forms.

7.2 References

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