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Kinetics of soil microbial uptake of free amino acids

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Abstract Amino acids and proteins typically form the biggest input of organic-N into most soils and provide a readily available source of C and N for soil microorganisms. Amino acids can also be taken up directly by plant roots, providing an alternative source of available soil N. However, the degree to which plants can compete against the soil microbial population for amino acids in soil solution remains poorly understood. The aim of this study was to measure the rate of microbial uptake of three contrastingly charged ^{14}C-labelled amino acids (glutamate^{1-}, glycine^{0}, lysine$^{0.9+}$) over a wide concentration range (0.1–5 mM) and in two contrastingly managed soils varying in their degree of erosion, organic-C content and microbial biomass. Amino acid uptake was concentration dependent and conformed to a single Michaelis-Menten equation. The mean maximum amino acid uptake rate (V_{max}) for the non-eroded (control) soil (high organic-C, high biomass) was 0.13 ± 0.02 mmol kg^{-1} h^{-1}, while half maximal uptake occurred at a concentration (K_m) of 2.63 ± 0.07 mM. Typically, V_{max} was fourfold lower and K_m twofold lower in the eroded soil (low available organic-C, low biomass) compared to the non-eroded (control) soil. Amino acid substrate concentration had little effect on the proportion of amino acid utilized in catabolic versus anabolic metabolism and was similar for both. While the results obtained here represent the summation of kinetics for a mixed soil population, they indicate that amino acid uptake is saturated at concentrations within the millimolar range. Because the affinity constants also were similar to those described for plant roots, we hypothesized that competition for amino acids between plants and microbes will be strong in soil but highly dependent upon the spatial distribution of roots and microbes in soil.

Keywords Amino acids · Uptake kinetics · Microorganisms · Root-microbe competition · Dissolved organic nitrogen

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Introduction

Amino acids either in a monomeric or polymeric (protein) state provide the biggest input of organic-N into most soils (Stevenson 1982). In addition, they also provide a readily available source of C and N for the soil microbial biomass (Barraclough 1997; Jones 1999). However, despite many studies on the uptake and use of amino acids by pure microbial cultures, there is very little information available on the concentration-dependent uptake kinetics of amino acids in soil (Anraku 1980). It can be expected that amino acid concentrations in soil will vary spatially and temporally depending on vegetation cover, land management strategy and environmental conditions.

In many cropping situations, the input of below-ground C in the form of root turnover can be significant (Snapp and Shennan 1992). Plant roots are known to be composed of typically 20–40% protein and to possess a free amino acid sap concentration in the region of 1–5 mM (Jones and Darrah 1994). It can therefore be expected that a large input of amino acids into soil may occur upon the lysis of root cells. Upon release, these amino acids can either become sorbed to soil exchange sites, complexed with metals, leached, or taken up by the soil microbial biomass. Measurements of total free amino acids in soil solutions indicate that concentrations are typically in the 1- to 100-μM range with individual amino acids in the 0.01- to 50-μM range (Monreal and McGill 1985; Kielland 1994). The reactions of each individual amino acid will largely depend upon its charge and solubility (Jones et al. 1994). Positively charged amino acids such as lysine can be strong-

ly adsorbed to cation exchange sites in comparison to net neutral and negatively charged amino acids such as glycine and glutamate. This may significantly influence their bioavailability as has been demonstrated for other low molecular weight C substrates (Jones and Edwards 1998).

In addition to microbial uptake of amino acids, the roots of some plant species have also been shown to readily take up a range of intact amino acids directly from the soil solution (Schobert and Komor 1987; Chapin et al. 1993; Jones and Darrah 1993, 1994; Kielland 1994; Raab et al. 1996). This uptake mechanism may be important in recapturing amino acids previously lost in root exudates and also in providing an alternative source of N to plants (Jones and Darrah 1994; Kielland 1994; Raab et al. 1996). However, most of the experiments performed to date have been conducted under sterile conditions where competition by soil microorganisms is not a factor or where root uptake includes a contaminating rhizoplane component. The rhizosphere is an intense zone of microbial activity where competition for amino acids may be great between roots and microbial cells. Although Michaelis-Menten kinetic parameters have been determined for the uptake of amino acids by many plants species, there is still only very limited information available on the amino acid kinetics of the soil microbial biomass (Soldal and Nissen 1978; Jones and Darrah 1993). Without this knowledge it is difficult to determine whether soil microorganisms effectively compete with plant roots for this source of organic-N. Further, it can be expected that microbial and root competition will also be influenced by the isomeric and charge properties of the individual amino acids which are known to significantly influence their interaction with the soil's solid phase (Jones et al. 1994).

Continual erosion of soil by wind and water is known to be a significant limiting factor for agricultural production in many agroecosystems around the world, and as a consequence the fertility level of these degraded soils is progressively declining (Garcia et al. 1997; Bridges and Oldeman 1999). Because of its high turnover rate, the microbial biomass may react quickly to erosion (Syers 1997); however, its ability to re-supply nutrients of intrinsically low availability in eroded soils remains scarcely studied. The aim of this study was therefore to determine the kinetics of the uptake of three contrastingly charged amino acids by a mixed microbial population in two soils differing in erosion status, organic-C content and microbial biomass. The kinetic parameter estimates obtained will then be compared with those previously obtained for plant roots.

Materials and methods

Study site, soils and sampling regime

Soils were taken from experimental field plots located on the Plain of Barcelona at the University of Barcelona, NE Spain (41°22′59″N, 2°6′44″E, at an altitude of 60 m above sea level). The plots were 20 m^2 and located on level ground and have been maintained as fallow for 6 years in all treatments with a mixed herbaceous scrub vegetation. Previous crops included *Medicago sativa* and *Spinacea oleracea*. The parent material consists of Quaternary foothill sediment of several metres depth (Llopis 1942) which has been deposited due to the denudation of the surrounding hill slopes where the dominant lithology is schist with a minor granite component. These Quaternary deposits are underlain by Pliocenic marls. The mean annual precipitation is 614 mm (maximum 180 mm $month^{-1}$, minimum 0 mm $month^{-1}$), and the mean annual temperature is 15.5 °C (maximum 35 °C, minimum 0 °C). The site is characterized by hot dry summers and cool, wetter winters; the site is regularly managed for agriculture (Rovira and Vallejo 1997). Two soils were chosen based on their contrasting organic matter contents. The eroded soil plots possessed an almost identical inorganic nutrient supply to the non-eroded (control) soil plots but possessed a sixfold lower organic-C and organic-N content and a 60% lower soil microbial biomass. The plots had been subjected to two contrasting management regimes namely: (1), non-eroded (control) untreated plots, and (2) eroded plots in which the top 10 cm had been manually removed 4 years previously.

The soil is classified as a Calcic Palexeralf, with loam textured topsoil and clay loam textured subsoil and is typical of "terra-rossa" soils of all the Mediterranean bioclimatic regions of the world (Guerra 1972; Zinke 1973; Oades et al. 1981). The chemistry of the non-eroded (control) untreated plots is dominated by the presence of $CaCO_3$ and the organic matter is well humified, while clays are dominated by 2:1 illites. The moisture content of both soils was 13%, equivalent to a soil matric potential of −0.15 MPa. Samples were collected in March 1998 using a 5-cm auger at a number of random points within each plot from the soil surface (2–7 cm) and passed through a 2-mm mesh followed by field-moist storage at 4 °C. The 0- to 2-cm layer was not sampled due to its extreme dryness and its crust-like structure.

Selected chemical, physical and biological properties of the soils are listed in Table 1. Particle size was determined by the pipette method (Day 1965), soil pH and electrical conductivity in 1:5 (w/w) soil:H_2O extracts and moisture by drying at 105 °C (24 h). Soil microbial biomass was determined by $CHCl_3$ fumigation-extraction (Joergensen 1996), and $CaCO_3$ content by the Van Slyke manometric method (Nelson 1982). Total C and total N were determined with a CHN-2000 analyzer (Leco, St Joseph, Mich.). Exchangeable NH_4^+ and NO_3^- were determined in 1:5 soil:1 M KCl extracts by the methods of Downes (1978) (hydrazine, *N*-1-napthylethylenediame) and Keeney and Nelson (1982) (indophenol blue) while exchangeable cations in 1:5 soil:1 M NH_4Cl extracts were determined by inductively coupled plasma-argon emission Spectroscopy (Jobin Yvon Emission JY138 Ultrace). Soil organic matter size fractionation was undertaken as described by Meijboom et al. (1995) using sieving ranges of Cambardella and Elliot (1994). Soil organic-C <250 μm was taken as the difference between the total soil organic-C and the sum of the 250- to 2000-μm and >2000-μm soil organic-C fractions. All values given are means of two determinations.

Amino acid uptake kinetics

Three contrastingly charged amino acids were used for the kinetic studies: glycine, glutamate and lysine. Using GEOCHEM-PC (Parker et al. 1995) it was predicted that for the non-eroded (control) soil (pH 7.86) the net charges on the amino acids were as follows: lysine, +0.95; glycine, 0; glutamate, −1, whereas for the eroded soil (pH 8.29), the net charges were: lysine, +0.86; glycine, 0; glutamate, −1. The uniformly ^{14}C-labelled, isomeric, amino acids were added to soil at concentrations ranging from 0.1 to 5 mM.

Fifty microlitres of an amino acid solution (0.1, 0.25, 0.5, 1.0, 2.5 and 5 mM) was briefly mixed with 0.3 g soil in a 15-ml polypropylene tube to give a final concentration ranging from 15 to

Table 1 Physical, chemical and biological properties of the two soils

Property	Non-eroded (control)	Eroded
Sand (%)	48	35
Silt (%)	31	44
Clay (%)	21	21
Bulk density (g cm^{-3})	1.21	1.51
$CaCO_3$ (%)	5.4	2.9
Total soil organic-C (%)	1.8	0.3
Soil organic-C (>2000 μm) (%)	0.13	0.15
Soil organic-C (250–2000 μm) (%)	0.096	0.006
Soil organic-C (<250 μm) (%)	1.57	0.14
Total soil N (%)	0.12	0.02
C:N ratio	15	15
Microbial biomass (mg N kg^{-1} soil)	285	115
Electrical conductivity (μS cm^{-1})	1030	226
pH (H_2O)	7.86	8.29
NO_3^- ($mmol_c$ kg^{-1})	26	9
NH_4^+ ($mmol_c$ kg^{-1})	12	12
K ($mmol_c$ kg^{-1})	333	322
Na ($mmol_c$ kg^{-1})	0.04	0.04
Ca ($mmol_c$ kg^{-1})	34	30
Mg ($mmol_c$ kg^{-1})	0.2	0.1
Al ($mmol_c$ kg^{-1})	<0.01	<0.01
P ($mmol_c$ kg^{-1})	1.04	0.58

900 nmol amino acid g^{-1} soil and a final moisture content of 31%. To each sample tube, a 5-ml polypropylene vial containing 1 ml of 1 M NaOH was added to capture respired $^{14}CO_2$ and the sample tubes sealed with a gas-tight rubber stopper and incubated at 20 °C. The efficiency of the static $^{14}CO_2$ traps was estimated to be >98% by comparison with a continuous $^{14}CO_2$ flow method. To quantify $^{14}CO_2$ production, the NaOH traps were removed after 3, 6 and 9 h for ^{14}C determination. Subsequently, the soils were extracted with 5 ml of 0.5 M K_2SO_4 to remove unutilized amino acid (15 min, 200 revolutions min^{-1}) followed by centrifugation (15,000*g*, 5 min) and counting the ^{14}C label remaining in the supernatant solution (Jones 1999). All radioactivity was determined using a Wallac 1409 liquid scintillation counter, and NaOH/K_2SO_4 compatible scintillation fluid (Wallac Optiphase 3; EG and G, Milton Keynes, UK). Any ^{14}C label not recovered in either the NaOH traps or K_2SO_4 extracts was assumed to be in the microbial biomass (biomass-^{14}C; Jones 1999). Microbial biomass yields (Y) from the amino acid substrate were determined using the following equation: Y = biomass-^{14}C/(biomass-^{14}C + $^{14}CO_2$).

Effect of microbial inhibitors

To determine whether amino acid mineralization is predominantly a biological process, incubations on the non-eroded (control) soil were carried out in the presence of microbial toxins or after sterilization treatments. Essentially the method is identical to that described above except either glycine, glutamate or lysine was added to the soil at a final concentration of 5 mM and $^{14}CO_2$ evolution measured over a 24-h period. Incubations were carried out on untreated (control), autoclaved (30 min, 121 °C) or freeze-thawed (one cycle, −5 °C, 16 h) soil. Alternatively, $HgCl_2$ (12 mM) and sodium azide (10 mM) were added to the amino acid solution prior to addition to the control soil.

Kinetic analysis

Experimental data points were used to obtain parameter estimates for the Michaelis-Menten kinetic equation, where the rate of uptake (V; mmol kg^{-1} soil h^{-1}) can be defined as: $V = V_{max} \times (C/(C + K_m)$, where C denotes added substrate concentration (mM), V_{max} is the maximum uptake rate (mmol kg^{-1} soil h^{-1}) and K_m is the concentration (mM) at which half maximal uptake occurs. Kinetic parameter estimates were determined using the computer-based equation-fitting package Sigmaplot 4.01 (SPSS, Chicago, Ill.) using a least sum of squares optimization routine. Linear regression analysis was carried out with the computer package Sigmaplot 4.01 (SPSS).

Results and discussion

Inhibitor studies

Evolution of $^{14}CO_2$ was significantly reduced by treatments designed to modulate the size and activity of the soil's microbial biomass (Fig. 1). Autoclaving and enzyme inhibitors such as $HgCl_2$ and sodium azide were the most effective inhibitor treatments. The results also confirmed that the mineralization assay used throughout this study was sensitive to changes in biological activity. Autoclaving almost completely inhibited microbial activity whereas $HgCl_2$ and, to a lesser extent, sodium azide only partially suppressed mineralization. A single freeze-thaw cycle affected the mineralization rate to a different extent depending on the individual amino acid, with lysine degradation strongly inhibited, gluta-

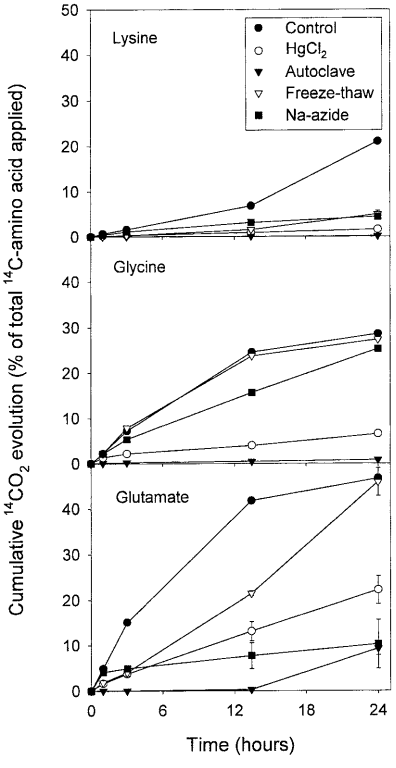

Fig. 1 The effect of toxins ($HgCl_2$, 12 mM; sodium azide, 10 mM), soil sterilization (autoclaving, 30 min) and freeze-thaw (1 cycle, −5 °C, 16 h), on the time-dependent mineralization of three ^{14}C-labelled amino acids (lysine, glycine and glutamate; 5 mM) to $^{14}CO_2$ in the non-eroded (*control*) soil. Values represent means ± SE (n = 2)

mate mineralization partially inhibited and glycine decomposition unaffected (Fig. 1).

Amino acid uptake kinetics

The concentration-dependent uptake of amino acids from the two soils is shown in Fig. 2. Longer incubation periods (6–9 h) gave similar trends (data not shown) to those observed in Fig. 2. Generally, the rates of substrate utilization had a saturating tendency and were well described by a single Michaelis-Menten kinetic equation with r^2 values ranging from 0.944 to 0.999. The rates of amino acid uptake tended to be approximately twofold lower in the eroded soil compared to the non-eroded (control) soil when the amino acid uptake rates in the non-eroded soil were <0.040 mmol kg^{-1} soil h^{-1} ($r^2=0.87$; Fig. 3). This is consistent with the eroded soil having an approximately twofold lower microbial biomass (Table 1, Fig. 3). While this relationship was consistent for amino acid uptake rates <0.035 mmol kg^{-1} soil h^{-1}, above this point the relationship failed to hold as uptake by the microbes in the eroded soil appeared to become saturated. This is consistent with a measured mean V_{max} for the three amino acids in the eroded soil of 0.039 mmol kg^{-1} soil h^{-1} (Table 2). On average the maximum uptake rate (V_{max}) was fourfold higher in the non-eroded (control) soil compared to the eroded soil, while the affinity constant (K_m) was twofold higher. Comparison of amino acid uptake rates per unit of microbial biomass indicated that the eroded soil only had a 33% lower uptake rate that of the non-eroded control soil (Table 2).

The concentration-dependent use of amino acid-C in respiration indicates that, as for substrate uptake, CO_2 production is much greater in the non-eroded (control) soil compared to the eroded soil (Fig. 4). Generally, the rate of CO_2 production in the non-eroded (control) soil was closely correlated with that of the eroded soil ($r^2=0.93$); however, the rates were approximately fourfold higher in the non-eroded (control) soils than in the eroded soil. In most cases, the concentration-dependent respiration profiles showed a saturating tendency similar to that observed for substrate uptake (Fig. 2).

The relationship between the use of amino acid-C for either biomass production or respiration can be described by Y. Typically, 90% of each of the amino acids was used for biomass production with the remaining 10% used for respiration after a 9-h incubation period (Figs. 5, 6). A comparison of the microbial yields between the non-eroded (control) and eroded soils indicates that the use of amino acid-C for biomass purposes was marginally higher in the eroded soils in >80% ($n=18$) of the experiments (Fig. 5). Further, microbial yield also appeared to be largely independent of amino acid concentration in both soil types (Figs. 5, 6).

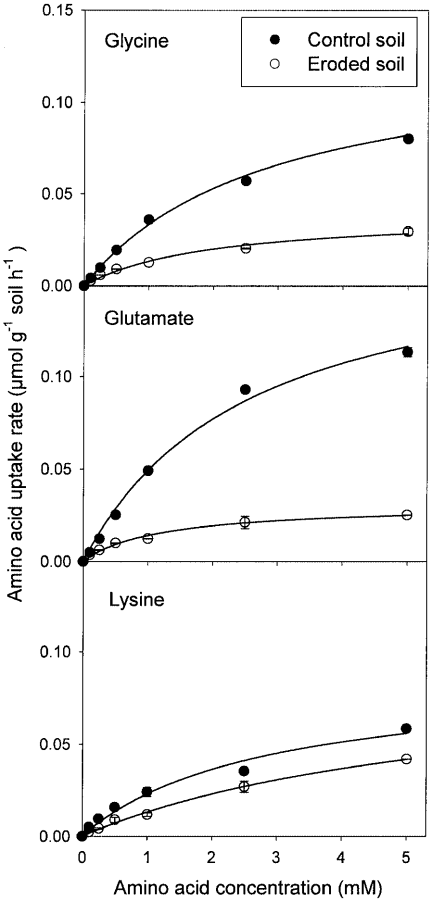

Fig. 2 Concentration-dependent microbial uptake kinetics of three amino acids (glycine, glutamate and lysine) measured after a 3-h substrate incubation in soils with either a high (*non-eroded, control soil*) or low (*eroded soil*) organic-C content. *Symbols* represent experimentally determined data points (means ± SE), while *lines* represent curves fitted to a single Michaelis-Menten equation using a least-squares optimization procedure

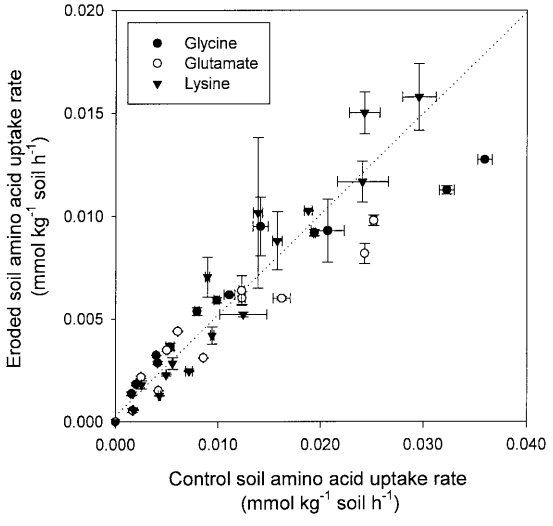

Fig. 3 The relationship between the uptake rate of glycine, glutamate and lysine in the non-eroded (*control*) and eroded soils. *Symbols* represent experimentally determined data points at 3, 6 and 9 h (means ± SE), while the *line* is a linear regression fitted to the data [$r^2=0.87$; eroded $=(0.49\times$ non-eroded$)+2.8\times10^{-4}$].

Table 2 Kinetic parameters describing the microbial uptake of three contrastingly charged amino acids (glycine, glutamate and glycine) after an incubation time of 3 h in either non-eroded (control) or eroded soil. Parameters were derived by a least-squares optimization procedure. Values describe the curves illustrated in Fig. 3

	Non-eroded (control) soil				Eroded soil			
	V_{max}		K_m (mM)	r^2	V_{max}		K_m (mM)	r^2
	(mmol kg^{-1} h^{-1})	(mmol g^{-1} biomass N h^{-1})			(mmol kg^{-1} h^{-1})	(mmol g^{-1} biomass N h^{-1})		
Glycine	0.119 ±0.005	0.417 ± 0.017	2.55 ± 0.23	0.998	0.039 ± 0.003	0.339 ± 0.026	1.95 ± 0.41	0.986
Glutamate	0.176 ± 0.013	0.617 ± 0.045	2.58 ± 0.41	0.994	0.030 ± 0.002	0.260 ± 0.017	1.21 ± 0.19	0.989
Lysine	0.086 ± 0.013	0.302 ± 0.045	2.78 ± 0.85	0.977	0.049 ± 0.009	0.426 ± 0.078	1.78 ± 0.83	0.944
Mean ± SE	0.127 ± 0.023	0.445 ± 0.092	2.63 ± 0.07		0.039 ± 0.005	0.341 ± 0.047	1.64 ± 0.22	

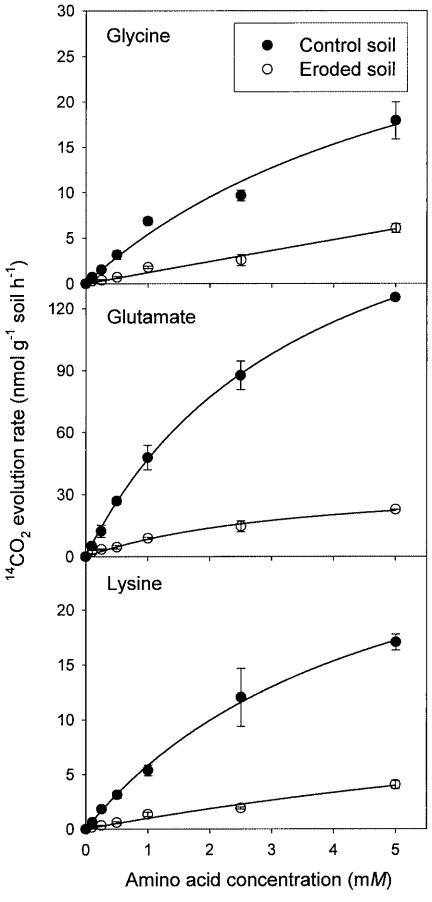

Fig. 4 Concentration-dependent production of $^{14}CO_2$ after the addition of three ^{14}C-labelled amino acids (glycine, glutamate and lysine) to soils possessing either a high (*non-eroded, control*) or low (*eroded soil*) organic-C content. $^{14}CO_2$ evolution rates were calculated after a substrate incubation time of 9 h. *Symbols* represent experimentally determined data points (means ± SE), while *lines* represent curves fitted to a single Michaelis-Menten equation using a least-squares optimization procedure

Biotic and abiotic reactions of amino acids in soil

It has been shown that amino acid mineralization in soil can occur by non-biological oxidation (Wang and Huang 1991; Wang and Lin 1993). Treatments designed to modulate the size and the activity of the soil's microbial biomass used here, however, strongly suggested that in our soils amino acid uptake was caused predominantly by soil microorganisms. This supports evidence presented by Jones (1999) who showed that amino acid depletion from the soil was closely correlated, and intrinsically linked, with $^{14}CO_2$ production. This is also supported by Fitzgerald and Andrew (1985) who showed that methionine utilization was inhibited by autoclaving and sodium azide treatment.

Amino acid uptake kinetics

It can be expected that most soil microorganisms will possess the capability of taking up and utilizing L-amino acids. Amino acid uptake by microorganisms is typically an energy-dependent process whereby amino acids are transported into the cell by amino acid group (e.g. neutral, basic, acidic) and isomer-specific membrane transporters (Anraku 1980). Although our results represent the summation of many microbial uptake systems operating simultaneously, they indicate that amino acid uptake can be adequately described by a single Michaelis-Menten equation for amino acid concentrations ≤ 5 mM. Further, our results indicate that high substrate concentrations do not saturate the potential for microbial uptake.

The non-eroded (control) soil, which had a greater microbial biomass, had significantly greater maximal rates of amino acid uptake (V_{max}) in comparison to the eroded soil. The kinetic parameter K_m reported in Table 2 also indicates that the microbial community in the eroded soil possesses a higher affinity than the non-eroded (control) soil microbial community. This may reflect a greater limitation of available substrates in the eroded soil which possesses a lower total organic-C pool; however, measurements of organic-C fractions indicate that there is a similar supply of newly added organic-C (>2000 μm in size) in both soils but that organic-C is significantly less processed in the eroded soil (<250 μm; Table 1). In addition, the mean K_m values of the two soils ($K_m = 2.2 \pm 0.2$ mM) are approximately 3 orders of magnitude greater than reported previously

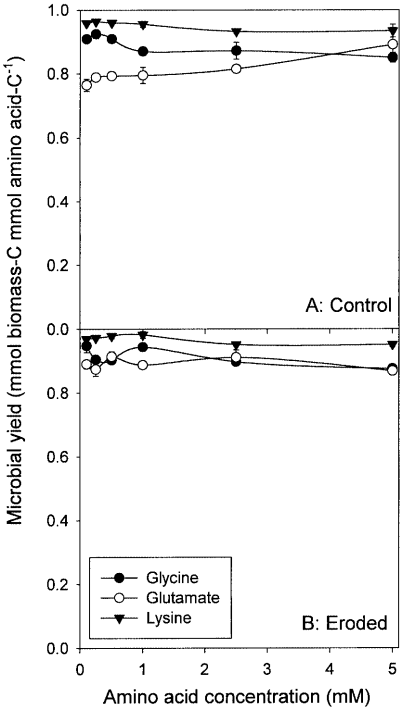

Fig. 5 Microbial biomass yield as a function of amino acid concentration in soils with either **A** high (*non-eroded, control soil*) or **B** low (*eroded soil*) organic-C content. Values represent means ± SE

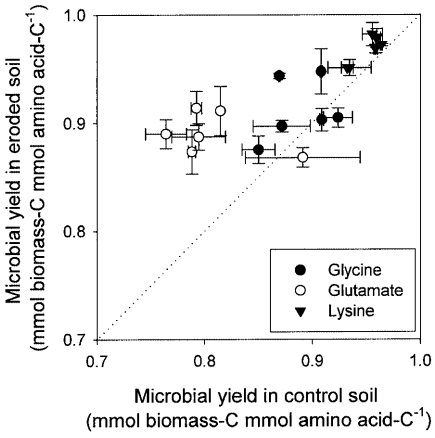

Fig. 6 The relationship between microbial biomass yield in the non-eroded (*control*) and eroded soil after 9 h incubation with varying concentrations of amino acid substrate (0–5 mM). Values represent means ± SE. The *line* represents a theoretical 1:1 relationship

for bacteria and yeasts in pure culture (1–10 μM) (Kay and Gronlund 1971; Miller and Rodwell 1971; Anraku 1980). The K_m values reported for the two soils in this study are typical of those for low-affinity amino acid uptake systems (K_m in the low millimolar range); whereas those reported for pure cultures are typical of high-affinity uptake systems (K_m in the low micromolar range).

From previous studies with low molecular weight C substrates (e.g. malate) it has been demonstrated that an increase in low affinity uptake systems occurs when substrates are in abundance, and that this typically involves a de novo synthesis of transport proteins (Jones et al. 1995). Due to the short time over which we measured uptake (3 h), the de novo synthesis of transport enzymes is unlikely; therefore, values reflect transporter affinity before substrate addition (i.e. $t=0$). Although soil solution amino acid concentrations were not measured here, it can be expected from previous studies and the management of this soil (no organic amendments) that free amino acids will be present at the low micromolar level. These low levels are due in part to the removal of amino acids from the solution as a result of sorption to soil exchange sites, indicating that the reserves of amino acids on exchange sites in soil may be much higher. The microbial K_m values measured here, however, represent the sum of amino acids held both in the bulk soil solution and on the exchange surfaces. The amount of amino acid sorption in these soils, as defined by the dimensionless buffer power (total soil amino acid concentration/solution amino acid concentration; Barber 1995), was 14.8 for lysine, 1.0 for glycine and 0.4 for glutamate. This indicates that the rate of uptake is likely to be influenced to different degrees by sorption of substrate to the soil particles. Taking this into account, the measured K_m values can be adjusted to reflect equilibrium soil solution concentrations giving approximate mean (±SE)K_m values for the two soils of 34 ± 16 μM for lysine, 504 ± 18 μM for glycine and 1035 ± 175 μM for glutamate. These "soil solution" K_m values indicate that there may be little difference between the K_m values of the two soils relative to the differences between the individual amino acids.

Although small differences were observed between amino acid-C use by the two soils, typically 90% of the amino acid-C was partitioned into structural C, probably reflecting the ease of amino acid incorporation into proteins and other microbial structural compounds. This is in contrast to other low molecular weight solutes found in abundance in plant cells (e.g. organic acids and sugars) which are used by the microbial biomass preferentially for energy production leading to a loss of soil C as CO_2 (Coody et al. 1986; Jones et al. 1995).

Competition between roots and microbes for amino acids

It has been shown recently that in addition to inorganic-N, plant roots can also absorb all proteinaceous, but not all non-proteinaceous amino acids, intact from their surroundings (Jones and Darrah 1994). This had led to speculation that root amino acid uptake may be important in supplementing the plant's N requirements, especially when NO_3^- and NH_4^+ supplies become limiting (Schobert et al. 1988; Chapin et al. 1993; Jones and Darrah 1994). Although plant roots will always exude

small amounts of amino acids into the soil in response to the large diffusion gradient across the plasma membrane, it has been shown that they can also simultaneously take up amino acids (Jones and Darrah 1993). From these observations it has been hypothesized that amino acid uptake by roots is a mechanism to recapture root exudates, reducing C availability in the rhizosphere and therefore reducing microbial proliferation (Jones and Darrah 1994). It has been estimated that the influx-efflux equilibrium, at which point net uptake equals zero, occurs at external amino acid concentrations of approximately 1–10 μM in maize roots (Jones and Darrah 1994). Thus, it can be speculated that soil solution concentrations must be higher than this equilibrium point in order to initiate a significant supplementation of the plant's N requirement. From the limited data available, it is known that the individual amino acid concentrations of the bulk soil solution are typically in the region of 0.01–10 μM, but rarely exceed 100 μM (Monreal and McGill 1985). Therefore, it could be postulated that plant uptake of amino acids may be insignificant compared to the rate of efflux. However, soil is thought to be composed of hotspots of biological activity and therefore concentrations of amino acids may vary greatly over short distances. Whether amino acid concentrations may be high in these hotspots and whether the plant can benefit from these remains unknown.

To date there have been few in vivo studies comparing the ability of plants to compete with the soil microbial biomass for amino acids. Estimates of the uptake kinetics for cereal roots grown in hydroponic culture indicate that their affinity for amino acids (K_m) is similar to that for soil microorganisms ($K_m = 10$–500 μM; Soldal and Nissen 1978; Jones and Darrah 1993). One in vivo study where a large volume and high concentration of ^{15}N-, ^{13}C-labeled amino acid was added (1.2 mM) to soil indicated that plants absorbed amino acids in an intact form, but uptake by soil microorganisms was much greater (Näsholm et al. 1998). Similar results have also been obtained for *Ricinus communis* grown in soil microcosms (Schobert et al. 1988). In addition, the uptake of amino acids by the plant could have been almost entirely due to mycorrhizae. In addition, no account was made for a loss of unlabelled amino acids in root exudates providing no estimate of net uptake. Further experimentation is therefore required to determine the relative importance of both root and mycorrhizal amino acid uptake systems in the plant's N budget.

Soil microorganisms possess the capacity to release large amounts of protease and peptidase into soil to facilitate the breakdown of complex organic-N into assimilatable forms (Watanabe and Hayano 1996). These enzymes have been shown to be regulated by the availability of N and are thought to be inactivated by sorption to soil particles (Rao et al. 1996; Watanabe and Hayano 1996). Therefore, in the presence of clays, most protease enzymes will be active only within a localized sphere (a few micrometres) of the microbial cell. Further, the hydrolysis products of these enzymes also are frequently adsorbed on the exchange complex, limiting their outward diffusion. Although plant roots are capable of releasing many enzymes into the rhizosphere (e.g. phosphatases) it is uncertain whether they release proteases. Therefore, roots may have to be close to the production sites of the microbial proteases in order to benefit from the released amino acids. More experimentation is therefore required to determine the importance of soil spatial variability on the potential for root capture of amino acids (i.e. proximity of roots to the amino acid production sites).

In conclusion, the use of free amino acids by soil microorganisms is rapid and the K_m values normalized for amino acid sorption ($K_m = 20$–1000 μM) are an order of magnitude higher than most reported soil solution concentrations (1 μM). Our results also show that the processing of amino acids in eroded soil appears to be limited by a low microbial population; the uptake kinetics were similar for both eroded and non-eroded soils when normalized to microbial biomass. The results suggest that soil microorganisms will be effective competitors with plant roots for low molecular weight free amino acids in soils of high and low organic-N status. The results also provide data for the mechanistic modelling of organic-N cycling in soil which may provide a basis for understanding the concentration-dependent competition between plant roots and soil microorganisms for amino acids.

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