Amino acid cycling in plankton and soil microbes studied with radioisotopes: measured amino acids in soil do not reflect bioavailability

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Abstract In radioisotope studies in plankton, bacteria turn over the nanomolar ambient concentrations of dissolved amino acids within a few hours. Uptake follows Michaelis–Menten kinetics. In contrast, within minutes the very abundant bacteria and fungi in soil take up all labeled amino acids added at nanomolar to millimolar final concentrations; uptake kinetics accordingly cannot be measured. This rapid uptake agrees with earlier findings that soil microbes exist in a starving or low-activity state but are able to keep their metabolism poised to take up amino acids as they become available. How can this rapid uptake of added amino acids be reconciled with persistent soil concentrations of 10–500 μM of total dissolved amino acids? Although respiration of added amino acid carbon has been used to deduce uptake kinetics, the data indicate that in both soil and in eutrophic natural waters constant percentages of individual amino acids are respired; this percentage varies from less than 10% of the amount taken up for basic amino acids to more than 50% for acidic amino acids. We conclude that relatively fixed internal metabolic processes control the percent of amino acid respired

and that the μM concentrations of amino acid measured in water extracts from soil are unavailable to microbes. Instead, these relatively high concentrations reflect amino acids in soils that are chemically protected, hidden in pores, or released from fine roots and microbes during sample preparation.

Keywords Amino acids · Cycling · Kinetics · Plankton · Respiration · Soil

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Overview

From the biological standpoint, the breakdown of particulate and dissolved organic nitrogen (N) compounds into amino acids and other N-containing compounds and the subsequent uptake into microbes (considered here as bacteria and fungi) follow identical pathways in aquatic and soil ecosystems. Microbes produce exoenzymes and take up the resulting products of hydrolysis. Given that most non-gaseous nitrogen in ecosystems is stored in organic forms, for example, in the upper layers of the ocean (Capone 2000) and in soils (Brady 1974; Hawkes et al. 2007), this breakdown pathway is very important for the N cycle. Nitrogen cycling by soil microbes is driven by depolymerization of organic N compounds (Schimel and Bennett 2004), since microbes generally require inorganic N, organic monomers, or organic oligomers for uptake. Amino acids support a high fraction of bacterial production in

oceans and lakes (Kirchman 2000). This production by heterotrophic bacteria is responsible for most of the transformation of organic N compounds into inorganic forms taken up by phytoplankton. In addition, amino acids may also be N sources for algae and higher plants (Neff et al. 2003) although their overall importance is uncertain. Because of the ubiquity and importance of amino acids to N dynamics, both aquatic and soil ecologists have sought to understand what controls microbial uptake by adding amino acids to samples.

Nearly all studies of amino acid cycling in both planktonic and soil systems use amino acids labeled with isotopes of carbon and nitrogen; this approach is necessary for measuring small rate changes in very large pools of organic nitrogen. The radioisotopes ^{14}C and ^{3}H are particularly important because they can be detected at very low concentrations compared to stable isotopes such as ^{13}C, ^{15}N, and [2]H. The amount of sample disturbance during measurement, however, varies greatly between planktonic and soil systems. Disturbance is least with homogeneous samples of plankton where measurements are carried out within minutes of sample collection, isotopes are added at low concentrations, and temperatures are carefully controlled during short incubations. In contrast, soil structure is usually destroyed by the sieving, drying, slurrying, mixing, grinding, and centrifuging that is a usual part of the measurement procedure. Every study uses different methods and relating measured rates to in situ rates in undisturbed soils is very difficult.

The relative ease of study in planktonic ecosystems has led to good agreement on methods and 40 years of data on patterns of amino acid cycling. Accordingly, the techniques and conceptual models of the rates and controls of amino acid cycling in plankton are well developed. In this review we summarize current understanding of the plankton model of amino acid cycling and compare the isotopic techniques used and patterns reported in plankton with isotopic data on amino acid cycling in soils. We end with conclusions on how amino acids cycle in soils.

Development of studies of amino acid cycling

Investigations of microbial cycling of simple organic compounds, including amino acids, began in planktonic systems in the 1960s (Parsons and Strickland 1962). These investigations were inspired by the ^{14}C method of measuring primary productivity in which bottles containing water samples and ^{14}C-bicarbonate were incubated for short periods. In this method, the added substrate is always at much lower concentrations than the natural amounts; it is a tracer, which in this review means that the amount added does not alter the rates of the reaction. However, because the importance of tracer-level concentrations with organic substrates was not initially realized, very high concentrations of substrate were added in early experiments. For example, Parsons and Strickland (1962) used a final concentration of glucose of 3.5 micromolar (μM). Studies of the uptake kinetics of LMW (low molecular weight) compounds revealed that in situ concentrations of LMW compounds were very low (Wright and Hobbie 1966); this generalization was confirmed when high performance liquid chromatography analysis became available (Williams 2000). The concentrations of simple sugars, fatty acids, and amino acids actually present in lakes and oceans were at nanomolar (nM) levels (Table 1). Therefore, to maintain the appropriate conditions for tracer studies and to ensure that the added isotope does not affect the rates measured (Blackburn and Knowles 1993), isotopes must be added at very low concentrations. A related problem is that simple organic compounds are also taken up by planktonic algae (Wright and Hobbie 1966). At the natural concentrations (nM), uptake rates are very low but increase linearly at higher concentrations. This uptake by algae is likely first order kinetics at these low concentrations: uptake increases directly with added substrate concentration. One consequence of these kinetics is that at relatively high concentrations of added organic compounds algal uptake may be greater than bacterial uptake. This may lead to the erroneous conclusion that algae outcompete bacteria for organic compounds in nature.

Today, estimates of bacterial production in aquatic systems (Kirchman et al. 1985, 2000) are based upon incorporation experiments in which samples of natural waters are incubated with nM concentrations of ^{3}H-leucine, the incorporation into protein measured, and a conversion factor used to estimate microbial biomass and growth. The final concentrations correspond to the 0.1–50 nM of individual amino acids and monosaccharides in the ocean (Table 1). From the many studies of uptake of [14]C- and ^{3}H-labeled organic compounds at nM concentrations, a general agreement has

Table 1 Concentrations of dissolved amino acids measured in lakes, estuaries, and oceans

Location	Amino acid	
	Concentration (nM)	Notes
Ocean[a]	0.1–50	Individual amino acids
Ocean[b] (New York Bight)	1–15	Individual amino acids
Estuary[c] (North Carolina)	300–500	12 amino acids
Productive lakes[d]	78–377[e]	Total for five sampling days

[a] Williams (2000)

[b] Fuhrman and Ferguson (1986)

[c] Crawford et al. (1974)

[d] Jørgensen (1987)

[e] 1200 and 1500 nM found once

emerged that planktonic bacteria are responsible for most of the rapid cycling of labile compounds (Carlson and Ducklow 1995; Aluwihare and Meador 2008), that bacteria have well-adapted transport systems to grow at these concentrations, and that bacterial activity controls the environmental concentration of labile compounds. One consequence of bacterial adaptation is that the K_m, the Michaelis constant for uptake of compounds such as sugars, acetate, and amino acids, is always close to the ambient substrate concentration (Billen 1984).

Soil measurements of cycling of simple organic compounds are much more difficult than those in plankton systems because of the complex soil structure and high amounts of organic and mineral particles. As a consequence, researchers have applied various concentrations of isotopically labeled compounds and assessed the resulting isotopic enrichment in different compounds. For example, Panikov et al. (1992) added glucose to produce concentrations ranging from 252 μM to 126 mM and measured only the respired $^{14}CO_2$. Jones and Murphy (2007) added 50 mM glucose and 50 mM glycine and also measured only the production of $^{14}CO_2$. Bååth (1994) incubated soil with 775–1550 nM ^{14}C-leucine and then treated the soil with NaOH and tricholoroacetic acid to separate incorporated ^{14}C into DNA and protein fractions.

One major difference between plankton and soils is that concentrations of water-soluble amino acids in soil range from a few to hundreds of μM (Table 2) while in planktonic systems nM concentrations of amino acids are present. This raises a fundamental question: why are concentrations of amino acids in soil so high when bacteria, at least those in the plankton, can take up amino acids at extremely low concentrations? In the following sections we suggest that the free amino acids extracted in soil water are not available to microbes. We first present the plankton model of cycling of amino acids in lakes and oceans and then review and interpret soil studies that added isotopically labeled amino acids to measure cycling. Finally, we discuss our interpretation of soil measurements of amino acid cycling and present the ecological implications of that view.

Amino acid cycling in planktonic systems

Planktonic bacteria: abundance and activity

In freshwater and coastal marine planktonic systems, bacterial abundance is around 10^6 ml^{-1} (Hobbie et al. 1977); in oligotrophic ocean water it is around 10^5 ml^{-1}. In the coastal ocean, Karner and Fuhrman (1997) and Fuhrman and Ferguson (1986) estimated bacterial numbers from direct counts with the fluorescent dye DAPI (4′,6-diamidino-2-phenylindole) to be 0.5–6.8 × 10^6 ml^{-1}; ∼50% of these cells were visible after incubating with fluorescent oligonucleotide probes for RNA, and ∼50% had taken up a mixture of radioactive amino acids at a 20 nM final concentration, indicating a metabolically active population. In results from 23 lake and ocean locations, 56% of the cells produced RNA detectable by hybridization while microautoradiography with labeled

Table 2 Total dissolved amino acid concentration measured in soil water or soil water extracts or KCl extract

System studied	Amino acid	
	Concentration (μM)	Notes
40 soils worldwide[a]	23 ± 5	OPA fluorometry
Boreal forest, Sweden[b]	42–106	Upper organic layers OPA fluorometry
	5–20	Lower layers OPA fluorometry
Temperate grassland, Wales[c]	23–58	Total for 15 different amino acids,
		Monthly for 6 mo by HPLC
Pine forest, California[d]	35	Leachate of O horizon, by HPLC
Temperate forest, U.S.[e]	301	Organic horizon
	59.9	Mineral horizon

OPA, *ortho*-phthaldialdehyde

[a] Polar, temperate, tropical, agriculture, non-agriculture, Jones et al. (2009)

[b] van Hees et al. (2008)

[c] Jones et al. (2005c)

[d] Yu et al. (2002)

[e] Maple, ash, oak, beech, hemlock. Soil extracted with KCl immediately after collection, and amino acids assessed by ninhydrin method. For calculation of concentration, 0.3 g water assumed per g soil and published bulk density used. Gallet-Budynek et al. (2009)

amino acids indicated that 5–60% incorporated amino acids (Smith and del Giorgio 2003). Thus, by these methods at least half of the bacteria in plankton are active.

Uptake kinetics of planktonic bacteria and algae

Bacteria have active transport systems that are specific for many simple compounds. Planktonic bacteria, for example, have genes for transport proteins for a wide variety of amino acids, sugars, and carboxylic acids (Poretsky et al. 2010). Accordingly, when bacteria in culture are given labeled glucose at a series of concentrations (S), the uptake velocity (v) follows Michaelis–Menten kinetics (Fig. 1a). As the concentration of S increases, the uptake rate approaches saturation which is also called the maximum velocity of uptake (V_{max}). The velocity of uptake, v, is related to S, to V_{max}, and to K_m, the substrate concentration at half maximum uptake.

$$v = \frac{V_{max}(S)}{K_m + S} \tag{1}$$

Additional information is obtained from a form of the Lineweaver–Burk equation (Fruton and

Simmonds 1958). In this form, Eq. 1 is inverted and both sides multiplied by S.

$$\frac{S}{v} = \frac{K_m}{V_{max}} + \frac{S}{V_{max}} \tag{2}$$

When S/v is plotted against S, the slope of the straight line is $1/V_{max}$ and the intercept with the abscissa is $-K_m$. Thus, in Fig. 1b with the same data as in Fig. 1a, V_{max} equals 1.8×10^{-4} mg l^{-1} h^{-1} and K_m equals 0.005 mg l^{-1} (or 28 nM).

When uptake kinetics are measured in field samples from lakes or oceans (Wright and Hobbie 1966), v is the velocity of uptake, f is the fraction of the added isotope taken up by the microbes, and t is the duration of the experiment. In this case, the substrate concentration S in Eq. 2 is unknown; it is really the unknown plus the added substrate, or (S + A).

$$v = \frac{f(S + A)}{t} \tag{3}$$

Cross multiplication and inversion of Eq. 3 gives

$$\frac{S + A}{v} = \frac{t}{f} \tag{4}$$
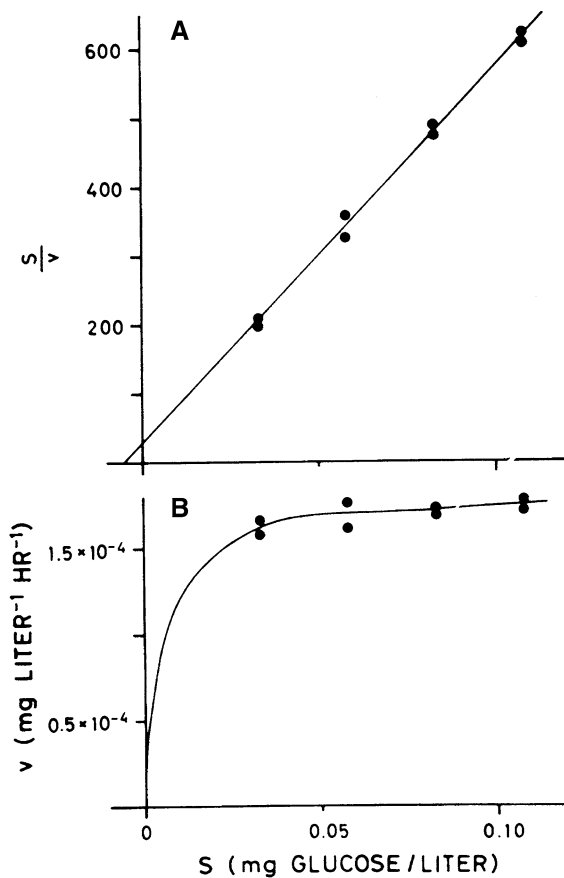
This, substituted into Eq. 2 gives:

Fig. 1 Different concentrations of glucose were added to a bacterial culture at 8°C and the resulting glucose uptake plotted as **a** Lineweaver–Burk and **b** Michaelis–Menten equations. On the abscissa (S) 0.05 mg glucose l^{-1} equals 277 nM. The Michaelis constant (K_m) was 5 μg glucose l^{-1} or 28 nM. From Wright and Hobbie (1966) with permission

$$\frac{t}{f} = \frac{(K_m + S)}{V_{max}} + \frac{A}{V_{max}} \qquad (5)$$

Microbes in field samples take up sugars and amino acids by kinetics that are very similar to those of laboratory cultures. For example, in Fig. 2 leucine uptake kinetics are plotted according to Eq. 5 for a station in the oligotrophic Atlantic Ocean (Zubkov et al. 2008). The uptake was measured at very low concentrations of A, the added leucine (up to 0.8 nM). The V_{max} is the inverse of the slope and the intercept on the abscissa is <0.2 nM; this is $-(K_m + S)$ and gives maximum values for K_m and for S. In Fig. 2b the intercept on the ordinate $[(K_m + S)/V_{max}]$ is the point where the added substrate (A) is zero; therefore, in Eq. 4 this intercept

is S/v (Wright and Hobbie 1966). This value of 5 h is the turnover time (time for complete disappearance of substrate) and takes into consideration both the concentration of substrate and the activity or the velocity of uptake. As M. Zubkov (personal communication) notes, kinetic analysis "allows [scientists] to experimentally "ask" microbial communities about bioavailability of molecules".

The K_m may be estimated when the substrate concentration is separately measured. Fuhrman and Ferguson (1986) measured concentrations of individual dissolved amino acids of 1–15 nM in New York Bight (Table 1) with turnover averaging less than 1 h in summer and around 6 h in winter. The turnover times were measured by kinetic analysis in which ^{3}H-labeled amino acids were added at five concentrations (0.1–3 nM) and incubated for 10–20 min. Half-saturation values (K_m) were 1–10 nM. In a productive estuary sampled over an entire year, Crawford et al. (1974) measured 12 amino acids with a total concentration of 300–500 nM. Turnover times of amino acids ranged from 0.7 to 206 h. In productive lakes, Jørgensen (1987) measured a total of 78–377 nM of amino acid with a mean of 200 nM. We note that studies using ^{3}H-labeled amino acids are preferred for measuring the K_m because the specific activity is higher than with ^{14}C-labeled amino acids (tritium half-life ∼12 years, ^{14}C half-life ∼5700 years); lower experimental concentrations are therefore possible.

In many planktonic studies, measured amino acid concentrations agree with uptake kinetics and estimated K_m values (Billen 1984), suggesting that high affinity active transporters in bacteria take up LMW compounds at low concentrations. Moreover, because bacteria can assimilate LMW compounds at low substrate concentrations yet can grow rapidly when substrate concentrations increase (Morita 1988), bacteria constrain amino acid concentrations to low levels.

Uptake kinetics of algae

Algae have transport systems with much higher K_m values than bacteria. For example, a planktonic *Chlamydomonas* isolate that grew in the dark on glucose (Bennett and Hobbie 1972) showed a K_m for transport of glucose of 27 μM. In contrast, the planktonic bacteria in Fig. 1 had a K_m of 28 nM, one thousand times lower. At low substrate concentrations
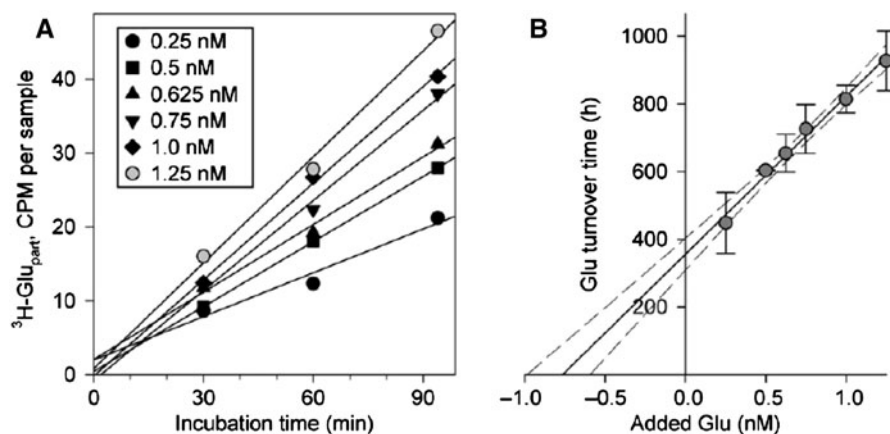
(Fig. 3), algal uptake increased linearly as concentration increased. At concentrations less than 1 μM, the relationship resembled first order equation even though Michaelis–Menten kinetics prevailed over the entire range.

In marine experiments, Billen (1984) concluded that bacteria dominated uptake at low concentrations of amino acids. However, when relatively high concentrations of substrate are used, algae or other eukaryotes may dominate uptake. This is illustrated in Fig. 3 where the same bacteria used in Fig. 1 were added to a culture of a small planktonic alga. Bacterial uptake dominates at low (nM) substrate concentrations while algal uptake dominates at higher concentrations of added substrate because of first order kinetics.

When high concentrations of labeled amino acids are added and subsequently taken up in an experiment,
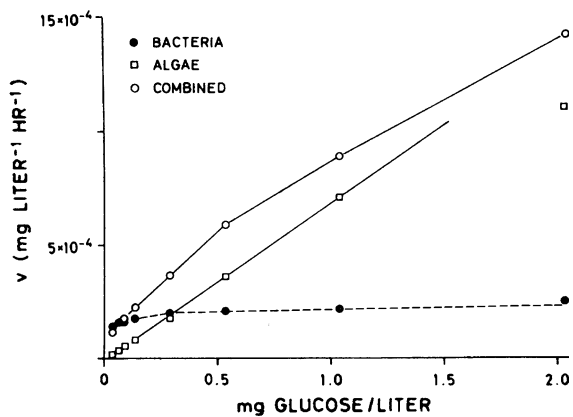

Fig. 3 The velocity of uptake of glucose for a laboratory culture of a small *Chlamydomonas* sp. and the bacterial culture used in Fig. 1. From Wright and Hobbie (1966) with permission

it may appear that an important uptake pathway has been found; however, the experimental results at high substrate concentrations cannot be extrapolated to natural settings. For example, Vonk et al. (2008) added 1–5 μM of ^{15}N-amino acid to a sample of seagrasses and macroalgae and concluded from the resulting ^{15}N labeling patterns that DON uptake gives seagrasses and macroalgae access to additional N resources. In reality, the actual concentration in seawater is in the nM range (not measured by these authors) and the high rate of uptake was very likely caused by first-order kinetics across cell membranes. In a second example, Vincent and Goldman (1980) found that ^{14}C-acetate entered algal cells collected from deep in Lake Tahoe although the levels of applied acetate were considerably higher than the background level. Accordingly, they were uncertain whether this uptake of acetate was important to the algal carbon budget. To assess kinetics, uptake measurements at a number of concentrations, including close to the natural concentrations, must be a part of every study of this type.

Respiration of amino acid carbon in plankton

When ^{14}C-labeled amino acids are added to planktonic samples, some of the C skeleton is respired, releasing $^{14}CO_2$. Detailed experiments with individual amino acids added to mesotrophic and eutrophic waters revealed that the amount of $^{14}CO_2$ produced, expressed as a percentage of the isotope taken up, varied with the type of amino acid used in the experiment. This percentage did not vary with type of water body, incubation time, concentration of the

added substrate, temperature, or season. For example, when ^{14}C-aspartic acid was added to pond water at four different final concentrations in experiments lasting from 0.25 to 4 h (Table 3), respiration varied only between 58 and 63% of the isotope taken up in all cases (Hobbie and Crawford 1969). The turnover time of aspartic acid in the water ranged from 8 to 13 h. In the same pond, the respiration of individual amino acids (Table 4) varied from 12% for lysine to 61% for glutamic acid when labeled amino acids were added at final concentrations of 80 to 2300 nM. Wright (1974) reported similar values for the percentage respired of 14 individual amino acids from an annual study of a mesotrophic lake in Oregon. Respiration ranged from 11 to 45% and averaged 26% (Table 4).

Similar patterns are reported from estuaries and productive oceanic waters. In a year-round study of a eutrophic estuary, Crawford et al. (1974) measured the concentration and cycling rate of 15 amino acids at monthly intervals. The results from the estuarine study (Table 4) resembled those of the pond study, with the average respiration percentage lowest for leucine at 14% and highest for aspartic acid at 51%; glycine averaged 38% with a range of 27–50%. Temperature, which varied from 8 to 30°C, had little effect. In one of the few studies of radiolabeled amino acids carried out in the ocean, respiration averaged 22% of substrate for a mixture of 14 ^{14}C-amino acids (Williams 1970). In a Sargasso Sea study with tritiated compounds, 60–80% of uptake was respired for glutamate, glutamine, alanine, glycine, serine, and glucose. For leucine and ornithine <10% was respired. In this case, respiration was measured from the tritium in water but should be close to the percent respired as carbon.

In offshore ocean waters, percent respiration tends to increase with decreasing nutrient availability and increasing distance from shore. In the northeast Pacific, del Giorgio et al. (in press) measured leucine respiration of less than 10% of total amount taken up in productive coastal waters to >50% in ultraoligotrophic waters some 240 km from shore. Bacterial growth efficiency in the Pacific transect averaged 13% with a range from <5% to >20% in agreement with other oceanic measures (del Giorgio and Cole 2000). In the Atlantic Ocean, Alonso-Sáez et al. (2007) found a range of 40–70% of added leucine-C respired, with the higher values corresponding to the more oligotrophic systems.

Patterns of respiration for specific amino acids reflect the pathways by which those amino acids enter cellular metabolism. When the proportion of amino acids respired relative to the amount taken up is compared for a number of amino acids in plankton (Table 4), acidic amino acids such as aspartic acid and glutamic acid are always highest ($\sim 50\%$), amino acids with hydrophobic side chains such as glycine, isoleucine, and leucine are intermediate, and the basic amino acid lysine is lowest (Hobbie and Crawford 1969; Crawford et al. 1974). As these authors point out, the order follows the length of the catabolic pathway; for example, aspartic acid and glutamic acid readily enter the citric acid cycle after deamination to oxaloacetate and α-ketoglutarate, respectively, while additional transformations are necessary before the carbon skeletons of glycine and leucine enter. We suggest that the similar respiration rates of individual amino acids are a fundamental characteristic of microbes in high-productivity aquatic ecosystems, and therefore must be included in calculations of

Table 3 Kinetics of the total uptake of ^{14}C-aspartic acid over time in a North Carolina pond (calculated from Hobbie and Crawford 1969)

Incubation time (h)	V_{max} (nM h^{-1})	S/v (turnover time, h)	Respiration as % of total uptake	SD of respiration (%)
0.25	4.8	7.7	58.4	1.8
0.50	4.8	11.4	58.7	2.4
1.00	4.8	11.4	61.1	4.6
2.00	6.2	12.8	62.8	1.4
4.00	8.3	12.2	63.1	2.2

For each incubation period the uptake was measured at four substrate concentrations (380, 750, 1139, 1520 nM) as the sum of incorporation as ^{14}C in bacteria and respiration as [14]CO$_2$. Columns 2 and 3 were calculated from a plot of Eq. 2 as shown in Fig. 1b

Table 4 Amino acid carbon respired as either percent of amount taken up by microbes in plankton systems (rows 1, 2, 3) or as percent of amount of amino acid added to the soil (rows 4, 5, 6)

System	Glutamic acid	Aspartic acid	Glycine	Isoleucine	Leucine	Lysine
Pond plankton[a]	61	60	28	27	26	12
Lake plankton[b]	63	62		19	13	14
Estuary plankton[c]	46	51	38	21	14	18
Tundra soil[d]		50				
Grassland soil[e]			25			
Agricultural soil[f]	45		28			20

[a] Pond in North Carolina, USA (Hobbie and Crawford 1969)

[b] Lake in Oregon, USA (Wright 1974)

[c] Estuary in North Carolina (Crawford et al. 1974)

[d] Tussock tundra near Toolik Lake, Alaska, USA (Nordin et al. 2004)

[e] Fertilized grassland, North Wales (Jones et al. 2005c)

[f] Farm soil, Barcelona, Spain (Vinolas et al. 2001)

amino acid cycling and kinetics derived from $^{14}CO_2$ measurements.

Multiphasic uptake kinetics of bacteria

In a detailed examination of the different transport kinetics present in a plankton community, Azam and Hodson (1981) collected a single water sample from Scripps pier in the Pacific Ocean and added radiolabeled glucose to subsamples in six short-term uptake experiments. Each experiment covered a 10-fold range of added glucose concentrations (A) with the total range from 1 nM to 1 mM. They discovered a different value of $K_m + S$ for each experiment, with values varying from 2 to 5 nM at the lowest concentration to 600 μM at the highest (Table 5). Because

Table 5 Details of the experiments designed to measure multiphase kinetics in a single water sample collected from Scripps pier, California (Azam and Hodson 1981)

Glucose concentrations (moles)	$(K_m + S)$ (moles)	V_{max} (nM l^{-1} h^{-1})
2.5×10^{-9}–10^{-8}	2.3×10^{-9}	0.64
2.5×10^{-9}–10^{-8}	3.2×10^{-8}	1.86
2.5×10^{-9}–10^{-8}	1.5×10^{-7}	3.60
2.5×10^{-9}–10^{-8}	2.4×10^{-6}	11.5
2.5×10^{-5}–10^{-48}	5.8×10^{-5}	66.0
2.5×10^{-4}–10^{-3}	5.9×10^{-4}	300

The concentrations in the subsamples are those after ^{3}H-glucose is added. Uptake kinetics, calculated as $(K_m + S)$ and V_{max}, were measured with a 40–60 min incubation

the S was constant in every subsample, it is the K_m that changed in each experiment. If all the data were plotted on a single graph it would resemble Fig. 4. In this single planktonic sample, the different transport systems could have been on different bacterial species. However, a single species may possess multiphasic transport systems, as found for a culture of planktonic bacteria from the same site by Nissen et al. (1984). K_m values for uptake covered six orders of magnitude (20 nM to 16 mM). These findings underscore the importance of making measurements of amino acid cycling at ecologically relevant concentrations to avoid errors.
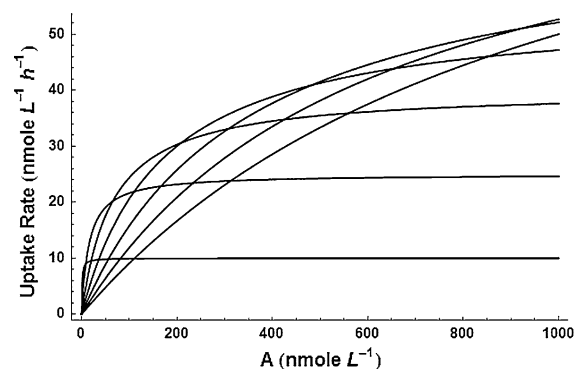
Fig. 4 Theoretical construct of kinetics of uptake of a simple organic compound such as a carbohydrate or amino acid into a plankton sample when multiple transport systems are present with a variety of half saturation constants (K_m) and V_{max} values. The kinetics could be the result of uptake of many species of bacteria in the water sample or of multiphasic kinetics in a single species of bacteria. Figure courtesy of J. Vallino

Summary of the model of amino acid cycling in planktonic systems

In the plankton model, microbial enzymes release amino acids from dissolved organic N compounds in the water. These amino acids are removed from solution by bacteria with transport systems effective at nM concentrations. Half-saturation values (K_m) are in the nM range. The bacterial activity limits the concentration of amino acids to a few nM, similar to the K_m values; at these low concentrations all amino acids are completely removed in a few hours or a few days. Because the bacteria are strongly limited by energy and carbon, the carbon skeletons of most of the amino acids immediately enter the cell's active metabolic pathways, where they are partially respired. The percentage respired depends upon the type of amino acid. To study amino acid cycling in plankton, low concentrations of isotopically labeled amino acids are added to samples and incubated for short periods. At high concentrations, labeled amino acids enter algae or other eukaryotes by a diffusion-like process; this can mask the dominant pathway in nature of bacterial uptake by active transport. In addition, at high concentrations of added substrate, bacteria can switch to transport systems with high K_m and high V_{max} leading to high estimated uptake rates (Table 5; Fig. 4). Finally, in the plankton model of amino acid cycling, bacteria have a remarkable capacity to withstand extreme substrate limitation and to scavenge for small traces of amino acids (del Giorgio and Cole 2000).

This view of how amino acids cycle in plankton is widely accepted and forms the conceptual basis for measuring bacterial productivity. The known physiological abilities of bacteria fit well with the measurements of very low concentrations of labile substrate, uptake kinetics of amino acids into bacteria, and growth rates of microbes. We suggest that this plankton model reflects bacterial capabilities in nature and may be applied to understanding microbial functioning in soils.

Amino acid cycling in terrestrial soils

Soil microbes: abundance and activity

In soils, fungi and bacteria dominate the microbial fauna. Estimates of bacterial numbers by direct counts vary widely; Bittman et al. (2005) reported values of 5–11×10^8 per gram of soil in the upper 30 cm of grassland soil whereas Whitman et al. (1998) reported 2×10^9 bacteria per gram of soil in the top meter of soils, about 1000 times higher than numbers per milliliter in oceans and lakes. Microbial biomass correlates strongly with soil organic carbon (in a literature review Fierer et al. 2009 calculated an r^2 of 0.91), so much of the reported variability in bacterial numbers may simply reflect underlying differences in the organic carbon available for bacterial production.

In contrast to planktonic systems, where fungi are minor actors, fungi are often dominant components of the soil microbial community. According to Bardgett (2005), fungal biomass, both saprotrophic and mycorrhizal, may exceed that of other microbes, plants, and animals. For example, using direct microscope counts in a temperate hardwood forest, Frey et al. (2004) found 501 and 53 μg C g^{-1} soil for fungi and bacteria, respectively. In an extensive review of the fungal contribution to microbial biomass and respiration, Joergensen and Wichern (2008) surveyed microscopic methods, selective inhibition, specific cell membrane components, and specific cell wall components in soils of arable lands, grasslands, and forests. The authors concluded that the various biomass methods agreed and that fungi usually dominated the biomass of soil microbes. However, the widely used method of selective inhibition of respiration (Bååth and Anderson 2003) has recently been shown to overestimate fungal respiration, since respiration of many bacteria is also limited by supposedly fungal-specific inhibitors (Rousk et al. 2009).

Estimating bacterial and fungal activity is difficult in soils. In one of the few reports for fungi, Ingham and Klein (1984) found that about one-third of the fungal hyphae were active based on staining with fluorescein diacetate. In another measure of activity, 40–50% of the fungal cells in a pine rhizosphere could reduce the electron acceptor INT (iodonitro-tetrazolium chloride) to deposits of INT formazan (Norton and Firestone 1991). For bacteria, around half of the cells in soil are active. Thus Zarda et al. (1997), Chatzinotas et al. (1998), Barra Caracciolo et al. (2005), and Bertaux et al. (2007) found that 40–58% of bacteria were positive for fluorescent in situ hybridization (FISH).
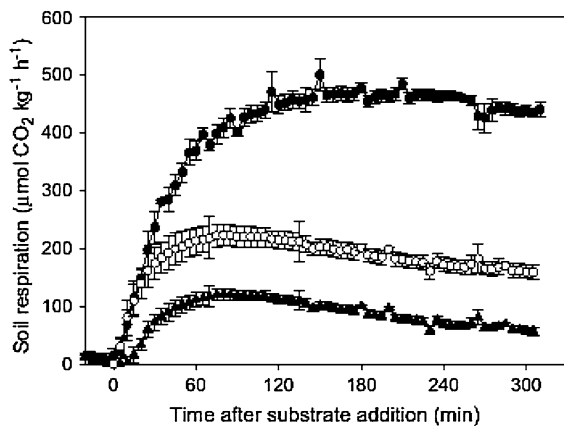
Fig. 5 Soil respiration following the addition of 50 mM glucose (*top*), 50 mM glycine (*middle*), or rainwater (*bottom*) to a sample of grassland soil at Time 0. The basal respiration value of 209 μmol CO_2 kg^{-1} h^{-1} has been subtracted from all treatments. From Jones and Murphy (2007) with permission

The response of soil microbes to added LMW substrates, such as glucose and amino acids, has also been used to estimate the active biomass of the soil microbes (Bailey et al. 2002). This method, substrate-induced respiration (Kandeler 2007), measures the initial respiration after substrates are added to soil and then uses a calibration factor to estimate the amount of carbon in living heterotrophic microorganisms. The rapid response of respiration is shown in Fig. 5 where the total soil respiration doubled in minutes after adding 50 mM glycine and tripled after adding 50 mM glucose. Microbes in this particular soil even responded to added water (other examples from Xu et al. 2004). As explained in detail in the "Microbes remove amino acid molecules from soil solution as soon as they appear" section, most of the abundant soil microbes are dormant in situ yet are poised for activity (White 1995; Morita 1997; De Nobili et al. 2001).

Amino acid concentrations and cycling in soil

Dissolved amino acids in soil are present at total concentrations of a few to hundreds of μM (Table 2). These are usually measured in soil water after roots are removed and the soil mixed. Extraction procedures may include centrifugation and the use of 2 M KCl. The resulting measure of amino acid concentration is for bulk soil which gives an average across microhabitats that include both hotspots of amino

acid concentration and regions where microbes have removed most of the amino acid. Such hotspots are important because of the patchy distribution of resources and the low diffusivity of many compounds in soil. Jones et al. (2005a) state that bulk soil techniques may underestimate DON concentrations especially where root activity is maximal. For example, they calculate that a bursting of a root cell releases enough amino acids to increase the concentrations in a 1 mm rhizosphere around the root by three orders of magnitude. Various sources of organic matter that may create a hot spot include dead animals, root exudates, worm tubes, and animal excreta. Rather than being unimportant in the total picture of nutrient cycling, hot spots are regarded by some as the main drivers of the cycles of most elements, especially of C and N (Kuzyakov et al. 2009).

In a review of the microscale heterogeneity in the distribution of soil microbes, Frey (2007) describes intact soil as a mosaic of microbial habitats containing various mixes of mineral particles, organic materials, pore spaces, and organisms. The size and water content of soil pores are extremely important in determining microbial abundance and activity. Peak aerobic activity of the microbial community occurs when 60% of the total pore space is filled with water and microbes have adequate moisture and oxygen. Both bacteria and fungal hyphae are encased in mucilage which protects against predation and desiccation, helps bind particles into aggregates, and retains extracellular enzymes near to substrates (e.g., Lindahl et al. 2005).

Sampling and preparation of soil for amino acid measurement may release amino acids from hot spots in soil pores; amino acids may also be released from the biota. Jones et al. (2005a) state that it is difficult to extract soil waters without disrupting fine roots and root hairs that generally contain 10 mM amino acids. Amino acids may also be released when fungal hyphae are disrupted or when the KCl used in extraction affects the charges on clay particles.

Microbes remove amino acid molecules from soil solution as soon as they appear

When nM amounts (final concentration) of labeled amino acids are added to samples of plankton, only a small amount of the total is taken up during the few

hours of incubation. For example, in the detailed study of an estuary by Crawford et al. (1974), incubation times were adjusted so that less than 5% of the added isotope was taken up by bacteria during the incubation. In contrast, in soil when μM concentrations are added 100% of the amino acid addition is taken up in a matter of minutes (Figs. 6, 7).

From papers such as Jones et al. (2005c), we conclude that amino acid molecules in soils are taken up by microbes as fast as they are produced. This uptake and induced respiration occur because large numbers of soil microbes with low activity are poised to respond quickly to added substrate (White 1995). Morita (1988) calls this state metabolic arrest while Kuzyakov et al. (2009) go even further and state that this concept, called the excessive pool principle, is one of the two main principles of soil microbial ecology. Zvyagintsev (2001) explains that soils have a large and diverse pool of microorganisms only a small number of which are active; this sustains homeostasis in soil. Often the pool is excessive, that is, it is out of balance with the supply rate of organic matter and other nutrients. How can this pool of microbes persist? Morita (1988, 1997) states that bacteria can live for several years without added nutrients then can respond quickly when resources do become available. The rapid response of some of the community is achieved by maintaining their adenylate energy charge and ATP content at high levels (De Nobili et al. 2001).
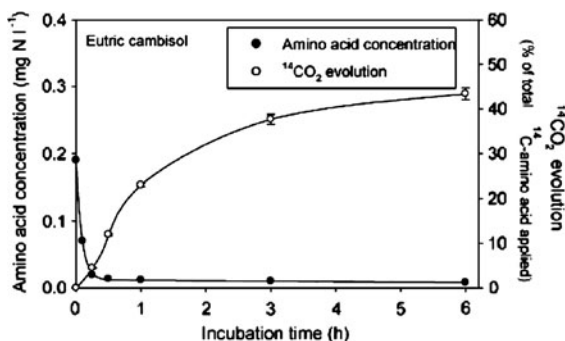

Fig. 6 A mixture of 15 ^{14}C-labeled amino acids (total of 11 μM) was added to a soil in Wales and the concentration of free amino acids remaining in soil was sampled over time. Also shown is the percent of added amino acid carbon transformed to $^{14}CO_2$. The amount of ^{14}C-labeled amino acids remaining was determined by shaking the soil with 1 M KCl and analyzing the ^{14}C in the supernatant. Values represent means ± SEM (n = 4). From Jones et al. (2004) with permission
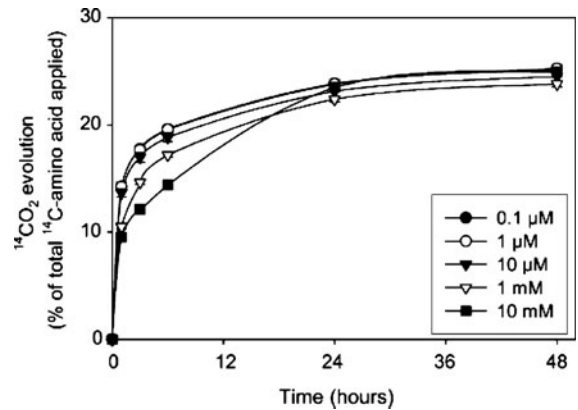

Fig. 7 The percent of ^{14}C-labeled glycine added to a sample of soil that is transformed to $^{14}CO_2$ over time. From (Jones et al. 2005c) with permission

The uptake and induced respiration also occurs at concentrations of added substrate orders of magnitude above the μM level. When Jones and Murphy (2007) extended the concentrations added into the mM range, the rate of $^{14}CO_2$ production increased linearly as the concentration increased (Fig. 8) but finally leveled off at the mM concentration level. We suggest that the capacity of microbes to take up and respire added substrate finally became saturated; however, the data should not be fit to Michaelis–Menten kinetics as all the substrate was immediately taken up.

We conclude that soil microbes have a tremendous capacity for uptake. Some of the soil bacteria and fungi can quickly ramp up their activity to respond to local addition or release of amino acids.

Bacterial growth measurement using leucine as a tracer is independent of uptake

Measuring the rate of incorporation of labeled amino acids into soil microbes is difficult because microbes are so abundant, their distribution is heterogeneous, and most bacteria and fungi are attached to particles. The one case where the addition of radioactive amino acids may give usable information is the use of labeled leucine to calculate bacterial production (Bååth 1994). The key to this method is that incorporated isotope is measured in protein; making protein requires a number of amino acids and energy which is very different than measuring the uptake of a single labeled amino acid into cells. Thus, the
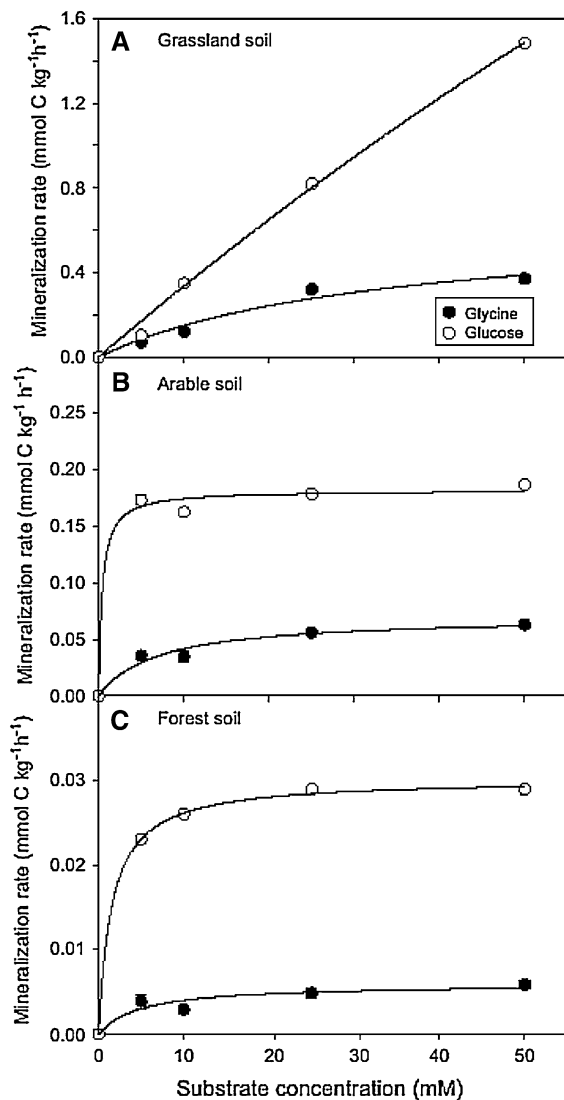
Fig. 8 Effect of high concentrations of glycine or glucose substrate on carbon respiration rate in samples from grassland, arable, and forest soils. From Jones and Murphy (2007) with permission

problems of rapid uptake, high biomass of soil microbes, and respiration of the label are avoided. From what is known of the process, the production of labeled protein is independent of the concentration of added substrate and of the amount of substrate taken up. In nature, it is the rate of provision of the substrates and energy needed for protein synthesis that determines the microbial growth rate. However, in the various steps of this method the microbes are highly disturbed. First a soil sample (<10 g) is

diluted with 200 ml of distilled water and centrifuged to form a pellet of the microbes. Then microbes in the pellet are resuspended in distilled water containing 775 nM of labeled leucine and incubated, trichloro-acetic acid is added and the sample centrifuged again, the protein in the precipitate is dissolved in NaOH, and the ^{14}C or ^{3}H in the protein is measured. Because leucine makes up a constant percentage of the total bacterial protein, bacterial growth may be calculated from incorporation. This method assumes that the rate of the internal process of growth of the cell in nature is unchanged by the multiple manipulations required to measure leucine incorporation. Bååth (1994) did show that after the start of the incubation with isotope, protein formation increased linearly over time. This is certainly a piece of the information needed to test the method but whether this protein incorporation method reflects microbial growth rates in the undisturbed soil is still uncertain.

Respiration of added amino acid is a questionable estimate of turnover or half-life in soils

Because all of the added labeled amino acid is immediately taken up by microbes, the rate of evolution of respired $^{14}CO_2$ or $^{13}CO_2$ from the addition is often used as a proxy for amino acid cycling (Jones et al. 2005b; Kielland et al. 2007). In the literature this rate is called mineralization time, turnover time, removal time, or transformation time. It is calculated as a half-life of the labeled substrate that was added. In contrast to the hours to days for aquatic bacteria to completely remove amino acids at natural concentrations, 100% of the labeled amino acid was very quickly taken up into microbes in soil as is shown in Fig. 6 where the amount of a mixture of amino acids that remained in solution was followed. In this experiment, Jones et al. (2004) found a half-life of the mixture of 4 min (Fig. 5). In this and similar experiments (Figs. 5, 6, 7) respiration begins immediately and the time course of CO_2 evolution was similar at concentrations between 0.1 and 10000 μM (Jones et al. 2005c). In studies of Boddy et al. (2007), CO_2 production reached half maximal rates in 10–40 min.

In the experiment shown in Fig. 7, about 25% of the labeled glycine was recovered as $^{14}CO_2$ no matter what concentration was added. By measuring the released $^{14}CO_2$, the authors determined that the half-life of glycine in soil ranged from 0.75 to 3.5 h

depending upon glycine concentration. In boreal soils, Kielland et al. (2007) estimated a half-life of 3–6 h for a mix of ten ^{13}C-labeled amino acids added to 5 g of soil (final concentration of 333 μM). We emphasize that in all these soil experiments, the estimated half-life reflects metabolic cycling after uptake, and not a turnover time calculated from uptake kinetics (see "Uptake kinetics of planktonic bacteria and algae" section).

Remarkably, the percentages of individual amino acids respired in the planktonic system and in the soil are quite similar (Table 4). In the plankton system these values are calculated as a percent of the amount taken up but in the soil the calculation was as a percent of the total substrate added. Note that soil samples are usually moistened to field capacity and that all of the added substrate is quickly taken up by the microbes in the soil; thus, plankton and soil experiments both measure the percentage of substrate taken up that is respired. For glycine, for example, from 25 to 38% was respired. The percent respired is presumably related to the biochemical pathway leading from the specific amino acid to deamination and the subsequent respiration of the resulting citric acid cycle intermediates such as oxaloacetate and α-ketoglutarate. This link between amino acid type and respiration applies to short incubations in both productive marine plankton (Crawford et al. 1974) and soil (Vinolas et al. 2001). This fixed percent of respired CO_2 is independent of the concentration of amino acid added, at least at ecologically reasonable concentrations (Fig. 6). We conclude that bacteria in soil and water follow the same biochemical pathways for processing amino acids; these pathways are characteristic for bacteria in natural ecosystems. However, based on data from leucine incorporation experiments, bacteria in ultraoligotrophic ocean waters may respire higher percentages of amino acids than given in Table 4 (see "Respiration of amino acid carbon in plankton" section).

Under a wide range of conditions, respiration percentages for specific amino acids remain quite similar. In one study, the percent respiration of added ^{14}C-glycine was similar across agricultural plots where the pH ranged from 3.8 to 6.4 (Jones et al. 2005b). These authors concluded (p. 1273) "Surprisingly, the gross partitioning of amino acid carbon into microbial catabolic and anabolic processes was similar between a range of geographically disparate

and contrastingly managed soils." In another experiment with a mixture of ten labeled amino acids, half lives for amino acids, 3–6 h, were similar for five types of taiga forest soils (Kielland et al. 2007). Finally, Jones et al. (2009) measured the capacity of 40 soils worldwide to process a mixture of amino acids under standard conditions; all soils were at 10°C and at field moisture capacity. There was a remarkable constancy of respiration of amino acid carbon and of concentrations. Some $30 \pm 1\%$ of the amino acid mix was respired and the total amino acid concentration measured was 23 ± 5 μM (SEM). These findings reinforce the conclusion that each type of amino acid has a typical respired percentage. It is likely that this percentage is the same for all soil bacteria.

What is the difference between turnover times measured by uptake kinetics in plankton and the half-life for amino acids measured by the release of labeled CO_2 respired from added substrate in the soil? First, in plankton the turnover time is calculated as $(K_m + S)/V_{max}$ (Eq. 5) which is the same as S/v. For example, in Fig. 2b the estimated turnover in the plankton habitat is 5 h; this value is affected both by the concentration of amino acid and by the actual activity of the microbes. Second, in the soil 1) all the added labeled substrate is immediately taken up and 2) the time course of the subsequent respiration of the label is used to calculate a half-life by fitting a first-order exponential decay equation to the amino acid respiration and dividing the natural log of 2 by the exponent (Jones et al. 2009). Therefore, the key difference between a turnover time in the plankton and a half-life value for the soil amino acid is that the added labeled substrate is thoroughly mixed with in situ substrate in the plankton and is not mixed with the in situ substrate in the soil. Instead the labeled substrate added to the soil is immediately taken up by microbes. Thus, complete mixing, a basic requirement for estimating turnover, is not met. The rate of evolution of the CO_2 is related to how fast dormant or semi-starved microbes transform this newly added amino acid carbon into CO_2 after the rapid uptake of amino acids added to the soil. The relationship between this rate and the actual turnover time in the undisturbed soil is unknown.

According to this interpretation, the results of Jones et al. (2009) that the average half-life of amino acids in 40 soils from around the world is similar

(1.8 ± 0.1 h SEM) suggest only that the metabolic activity inside microbial cells acting on the labeled amino acids they have taken up is similar in many or all soils. The half-life of the added amino acids is probably unrelated to the removal rate of amino acids by microbes in intact soils.

The amino acids measured in soil solution are not necessarily available to microbes

If microbes take up amino acids as soon as they become available, how then can appreciable concentrations of amino acid build up in the soil? As noted earlier, microbes in soils remove and respire amino acids at concentrations ranging from hundreds of nM to mM (Figs. 6, 7, 8). Given this ability of microbes, bacteria in soil should immediately grow and remove the μM concentrations of amino acids that are consistently measured. An additional possibility is that small pores inaccessible to microbes may sequester amino acids. If so, then the inverse relationship between soil water potential and pore diameter (Ghazanfari et al. 2007) suggests that water at different matric potentials may differ in the concentration of amino acids.

Very rapid uptake of added, isotopically labeled amino acids despite background μM concentrations of amino acids suggests that most amino acids naturally present in soil are protected from microbial attack. They may be hidden in microaggregates (Frey 2007), located in pores inaccessible to microbes, or chemically protected. For example, Kleber et al. (2007) and Sollins et al. (2009) described a complex model for the retention and protection of organic compounds in soil. In this model, the soil organic matter (SOM) sorbs to mineral surface in a zonal or layered fashion. A key concept is that many SOM molecules are amphiphilic, that is, contain distinct hydrophilic and hydrophobic regions that promote the formation of micelles or colloidal-size aggregates in water. These structures can sequester amino acids from microbes; the rate of release from these structures is unknown. Marine sediments also contain relatively high concentrations of dissolved free amino acids that appear to be protected from microbes. In an anaerobic incubation of pore waters extracted from a coastal sediment and then inoculated with sediment bacteria, dissolved free amino acids decreased by 150 μM over 14 days (Guldberg et al. 2002).

However, there was no evidence that the free amino acids were bioavailable prior to extraction and reinoculation.

The preceding discussion assumes that microbes behave similarly in planktonic and in soil systems and that all microbes are able to employ high affinity transport systems to take up very low levels of substrates such as carbohydrates and amino acids. This assumption is valid for microbes in the planktonic system where the half-saturation constant (K_m) closely matches the substrate concentration; that is, both are at the nM concentration. In kinetic measurements Wright and Hobbie (1966) measured a value of $K_m + S$, where S is the natural substrate concentration. If S is separately measured then K_m may be estimated and if S is not measured the value of ($K_m + S$) is still a useful indicator of the maximum values for K_m and for S. Fuhrman and Ferguson (1986) estimated K_m values of a few nM for amino acids in the ocean and Zubkov et al. (2008) found that the ($K_m + S$) for the system in Fig. 2 was less than 1 nM for leucine. However, if multiple bacterial species with differing K_m values are present, then the assemblage of microbes may display multiphasic kinetics (Azam and Hodson 1981) as explained earlier in the "Multiphasic uptake kinetics of bacteria" section. Based on these experiments, the value of K_m measured in a plankton sample may depend on the concentrations of added substrate employed in the experiment; kinetics should accordingly be estimated for added substrate concentrations close to the natural level of substrate, S. Finally, the K_m of bacteria in culture may also change. The bacterium in Fig. 1 had a K_m of 28 nM for glucose when freshly isolated but the K_m increased after later subculturing on rich media (Hobbie and Wright 1965). The K_m for a *Vibrio* isolate decreased from 7 to 1 μM when the culture was starved for glucose (Davis and Robb 1985).

High affinity and low affinity values of K_m are often estimated from bacterial cultures. In the summary by Jones and Hodge (1999) the high affinity values for different amino acids range from 1 to 41 μM while the low affinity values range from 500 to 18,000 μM in *Bacillus subtilis* and *Thiobacillus novellus.* In some species the high affinity values of K_m are even lower; *Pseudomonas aeruginosa* had a K_m of 400–500 nM for phenylalanine and tyrosine (Kay and Gronlund 1971) and *E. coli* had a high

affinity K_m of 26 nM for arginine and 150 nM for glutamine (Anraku 1980). One possible problem with these studies is that the high affinity measurements were carried out only at μM concentrations; as discussed earlier, multiphasic kinetics may exist with the results that the very high affinity kinetics are found only when very low concentrations of substrate are added (e.g., Azam and Hodson 1981).

Measures of K_m in fungal cultures are generally similar to those in bacteria, in the μM range. Chalot and Brun (1998) reported K_m value for glutamate of 180 μM for *Aspergillus*, 24 μM for *Penicillium*, 27 μM for the ectomycorrhizal fungus *Paxillus*, and 30 to 1999 μM for that of the yeast *Saccharomyces cerevisiae*. The yeast *Cryptococcus albidis* had a K_m values for glutamate of 12 μM. Jennings (1995) summarized earlier work and reported K_m values for glutamate of 40 μM in *Neurospora*, 16 μM in N-starved *Neurospora*, and 25 μM in *Achlya*.

Because of unknown effects of long-term culture on the K_m of microbes and of possible effects of estimating the K_m with μM concentrations of added substrate, whether soil microbes and planktonic microbes differ in K_m values is unknown. Measurements of kinetics are needed at low concentrations of added substrate in freshly isolated microbial cultures or in natural microbial communities in soils.

If the K_m values for amino acid transport in the uM range for microbes are correct, then 1 μM might be close to the lower limit to which soil microbes can remove substrate from solution. The concentrations of amino acid present are all greater than 1 μM because microbial transport systems cannot operate effectively at lower concentrations. Raven et al. (1992) suggested that the much slower diffusion of ammonium in soils compared to oceans contributed to the higher concentrations of ammonium in soils relative to marine systems; similar arguments could be made for the behavior of amino acids in soils relative to marine systems. If diffusion greatly limits amino acid movement to the uptake surface, then very low K_m values and low soil concentrations would result in insufficient uptake of amino acids to fulfill microbial requirements.

Finally, the concentrations of amino acids reported (for example, in Table 2) have been used to guide the amount of labeled amino acids added back into soils and cultures as tracer levels in experiments. For example, hundreds of experiments have measured the rate of movement of amino acids into roots by adding labeled amino acids at concentrations of 100–1000 μM. The effect of the concentrations on uptake rates is almost never considered but Warren (2009) demonstrates that substrate concentration greatly affects plant preference for N forms.

In summary, we conclude that much of the amino acid measured in soils is not microbially available. This conclusion is based on the ability of microbes to take up amino acids at extremely low concentrations, on the immediate and complete removal from solution of labeled amino acids by microbes, and on the many mechanisms for amino acid sequestration in biologically unavailable forms that are possible in the complex soil media. From this conclusion it is, however, difficult to explain the constancy of concentration of free amino acids. Do all soils have a similar capacity to sequester free amino acids in pores? Another possibility is that the release of amino acids from decomposition and the efflux of amino acids from microbes combines with the rate of influx of amino acids into microbes to determine the amino acid concentration in the soil solution. The K_m values for transport of amino acids into soil microbes would therefore influence the upper and lower limits of concentration of the amino acids, but these values would be modified by other effluxes and influxes.

Role of fungi in cycling of amino acids in soil

Reconciling the simultaneous existence of fungi with high K_m values in culture studies with bacteria having low K_m values in cultures is possible. Many fungi can enzymatically degrade protein or chitin, thereby creating a concentrated nutrient solution composed of small peptides, amino acids, and amino sugars at concentrations higher than in the free soil solution (Hobbie and Hobbie 2008). This enzymatic activity may take place within a matrix of mucopolysaccharides secreted by fungi to limit diffusion by enzymes and their hydrolysis products (Lindahl et al. 2005). Uptake of N-containing compounds by fungi from this diffusion-limited environment may therefore overcome the bacterial advantages for nutrient acquisition at low ambient concentrations. In addition, fungi secrete numerous compounds that inhibit bacterial activity (de Boer et al. 2005). The presumably low diffusion of these compounds in the soil environment and the high concentrations of biologically

active fungi may further favor fungal over bacterial uptake. Because proteolytic enzymes generally create oligopeptides from protein before amino acids are released, microbes capable of degrading protein and then taking up the oligopeptides produced may avoid competition for amino acids. Many fungi possess proteolytic capabilities, can grow on oligopeptides, and possess specific oligopeptide transporters (Benjdia et al. 2006; Hobbie and Wallander 2006). Thus, these fungi may compete against bacteria for uptake of oligopeptides rather than for amino acids.

Apparent half-saturation constants (K_m) measured in soils are not related to cycling in nature

In enzyme kinetic studies with bacteria in aquatic systems or in liquid culture (Hobbie and Wright 1965; Litchman et al. 2007), the uptake of substrate by the microbial cell was measured at a series of increasing substrate concentrations and followed Michaelis–Menten (saturating) kinetics. As described earlier, the half-saturation concentration or K_m is usually close to the actual substrate concentration in plankton.

In laboratory cultures, measuring K_m values for soil microbes or microbial assemblages is possible. However, soil microbes in the field have excess uptake capacity and therefore take up amino acids as soon as they are produced. Kuzyakov et al. (2009) state that the large pool of dormant microbes have a broad spectrum of potential metabolic activities and respond to the input of any available substrate. This uptake happens at all reasonable concentrations of added substrate; therefore, substrate concentration does not affect uptake and kinetics cannot be calculated. Thus, we conclude that measuring the uptake kinetics of intact soil communities is not possible using current techniques.

Despite the above conclusion, the literature contains several papers that estimate K_m from the amount of substrate respired at different substrate concentrations. At high levels of added substrate the rate of CO_2 production becomes constant; this leveling off was used to estimate K_m values in the mM range (e.g., Fig. 7). However, estimated K_m values in the mM range do not agree with concurrent measures of amino acid concentrations in the 1–20 μM range. For example, in Fig. 8 the K_m calculated for glycine in grassland soils was 7 mM while the concentration of glycine measured for grassland soils in the same study was 3–5 μM. As described earlier, the simplest explanation is that the abundant low-activity bacteria and fungi present in the sample have a large capacity to take up and respire added amino acid. When the concentration of added amino acid reaches the mM level, as in Fig. 8, total microbial uptake eventually becomes saturated, that is, a V_{max} is reached, and the rate of CO_2 production becomes constant. However, this saturation is unrelated to uptake patterns in nature where amino acid molecules are taken up as soon they become available. We conclude that the K_m values calculated at very high concentrations of added substrate reflect a saturation of the potential for soil microbes to assimilate amino acids.

What happens when labeled amino acids are experimentally injected into soil?

The studies of the uptake of labeled amino acids discussed so far for soil have all been carried out in laboratory flasks on small samples, for example, 1–10 g soil, and with the water content optimized. These studies are open to the criticism that, in these unusually wet soils, added amino acids will diffuse rapidly and respiration will be enhanced. Another anomaly is the observation that respiration of the labeled amino acid carbon may be respired in hours in the laboratory flasks rather than in days measured in field additions.

These observations raise the question, how does uptake of amino acids into microbes and subsequent metabolism differ between the lab-based and field-based situations? A related question, for which we have no answer, is how do amino acid concentrations and turnover differ between soils where rhizodeposition is lacking and soils where there is significant rhizodeposition and hot spots of microbial activity. Hot spots are discussed in the "Amino acid concentration and cycling in the soil" section.

There are a number of studies in which high concentrations of ^{15}N- or ^{13}C-labeled amino acids or inorganic nitrogen were added to intact soils; time-course experiments suggest that the isotopes moved quickly into microbial biomass and the percent respired of the amino acids was similar to that found in laboratory incubations (e.g., Table 5). For example, Nordin et al. (2004) measured the fate of four

types of ^{15}N-labeled inorganic and organic nitrogen injected into tundra soil; injections included a total of 2.4 mM in glycine and aspartic acid. After 4 h 49% of the N appeared in microbial biomass and less than 1% in plant roots. At the same time, 50% of the ^{13}C-aspartic acid was present in microbes and 50% of the C was lost in microbial respiration, in agreement with the 50% respiration of aspartic acid found in other soils (Table 5). Sorensen et al. (2008) injected ^{15}N-glycine into an Arctic soil and found up to 80% in microbes and 2% in tree roots after 1 day. This rapid uptake into microbes agrees with the concept of high levels of microbial biomass capable of rapid switching to an active state. The percent in the tree roots was unchanged a year later. In a boreal forest soil, McFarland et al. (2002) injected ^{13}C-glycine and recovered close to 50% of the isotope in bulk soil carbon after 45 min. The $^{13}CO_2$ was not collected. This agrees with the prediction that uptake was rapid and that much of the ^{13}C was lost as $^{13}CO_2$. It is likely that the isotope recovery in the bulk soil would have been closer to the predicted amount of 60% if the experimental period had been even shorter so less was lost as $^{13}CO_2$. This double-label experiment also included adding ^{15}N-glycine and the recovery of 31% and 53% of the added isotope in microbes after 45 and 120 min, respectively. Less than 1% of the ^{15}N was recovered in the fine roots during this period but the authors suggested that a large amount could have been quickly transported aboveground and outside the experiment. In the field injection experiments described above, the microbes likely take up almost all of the added amino acid very rapidly as expected.

Results in other studies applying labeled amino acids have sometimes contradicted the above patterns; we believe that examining the likely metabolic pathways of the amino acid applied may be instructive. For example, Hofmockel et al. (2007) sprayed ^{13}C- and ^{15}N-labeled alanine on intact soils of the Duke Forest at a concentration of 125 μM; after 3 h, 1 day, and 1 week the soil organic matter (SOM) contained 50%, 84%, and 58% of the ^{13}C added in alanine while microbial biomass contained 7%, 5%, and 3% for the same sampling times. After 1 month 8% and 2% of the added ^{13}C was still present in the SOM and microbial biomass, respectively. No $^{13}CO_2$ was collected. At the same four time points the $^{13}C:^{15}N$ ratio of SOM was 3.2, 6.6, 4.3, and 0.66 and

of microbes was 0.93, 1.07, 0.65, and 0.64. These patterns indicate that for the first week of the study, microbes had a strong preference for retaining only the ^{15}N of labeled alanine, whereas the ^{13}C of labeled alanine preferentially accumulated in SOM. We suggest that alanine dehydrogenase was probably important in the movement of ^{13}C and ^{15}N among different pools, as it breaks down alanine into ammonia and pyruvate. The released ammonia was then assimilated by microbes into amino acids while the pyruvate was excreted into the SOM or metabolized. The low $^{13}C:^{15}N$ ratio after 1 month of both SOM and microbes (0.66 and 0.64) indicates that most of the pyruvate would have been metabolized, with perhaps about 20% assimilation of labeled alanine ($\sim 0.65/3$) into protein. Thus, the high amounts of ^{13}C from alanine remaining in the SOM for as long as a week and the low percentages in the microbial biomass can still be compatible with immediate uptake by microbes of all added amino acids.

In all of the studies of labeled amino acids added to intact soil, a small amount of the label does end up in tree roots in accordance with the concept that amino acids will cross biological membranes, especially when high concentrations of substrate are present. The kinetics of this uptake likely resemble those described for algae in the "Uptake kinetics of algae" section and for Fig. 3. If these same kinetics apply, then the uptake should be proportional to the concentration of labeled substrate. This has been found by Warren (2009) for an evergreen tree (*Eucalyptus regnans*) and the herb basil (*Ocimum basilicum*); the author cautioned researchers to pay more attention to the concentrations used in experiments. Dual labeling of carbon and nitrogen also show that intact amino acid molecules enter tree roots (Näsholm et al. 1998; McFarland et al. 2002). Studies are needed at a range of concentrations of the time course of transfers of labeled amino acids from soil into microbes, soils into tree roots, and microbes into soils and then into tree roots. If the μM concentrations of amino acid measured in forest soils are found to be largely unavailable for plant and microbial uptake, a shift to low concentrations of experimental additions could dramatically alter current assumptions that plants can take up significant amounts of amino acids from soil (Neff et al. 2003; Näsholm et al. 2009).

Other methods of investigating amino acid cycling

The rapid turnover of amino acids relative to the temporal frequency with which soils are sampled means that quantifying the cycling of free amino acids in the soil by measuring their concentration is usually not possible. Accordingly, other methods have been developed. In one approach, amino acids labeled with the stable isotopes ^{13}C and ^{15}N are used as tracers. The ^{13}C should move into microbes and be respired and retained in exactly the same way as ^{14}C. However, the ^{15}N provides information about the fate of the N added in the amino acid molecule and has the advantage that it is not lost by respiration. McFarland et al. (2002) added ^{15}N and ^{13}C in glycine to an intact deciduous forest soil and recovered 31% and 53% of the N in microbial biomass after 45 and 120 min, respectively. Around 50% of the ^{13}C added in glycine was recovered for the same time intervals. The authors suggested that glycine is being used for energy by soil microbes. One interesting finding was a peak of movement of ^{15}N into fine roots at the beginning of the measurement when 187 μM ^{15}N-glycine were added. Only about 1.5% of the added ^{15}N ended up in the fine roots after 2 weeks of incubation but this percentage continued to increase after 2 weeks. Apparently the plants take up higher amounts in the initial stages of the experiment when concentrations of labeled glycine and ammonium are higher. The authors also made the same conclusion as Johnson (1994) that plants are long-lived compared with microbes and will sequester N resulting from the continuous turnover of substrates. This experiment with stable isotopes still does not give information on the cycling rate of in situ amino acids because of the high concentrations used and the large pool of microbes ready to quickly take up most of the added substrate.

The presence of enzymes is routinely measured by measuring the fluorescence produced when an enzyme cleaves a fluorescent molecule from a substrate. Wallenstein and Weintraub (2008) have critically reviewed the approach and concluded that the resulting rates are not the natural activity level but a potential rate. The substrate is added at high concentrations and the enzyme could be attached to soil organic particles or clays (Kandeler 2007).

The only method (Lipson et al. 1999) designed to be a chemical measure of production is the toluene method (toluene is added to soil samples to destroy microbial membranes so the enzymes in the soil will continue to release amino acids but in the absence of live microbes the concentration of amino acids builds up). The change in concentrations of amino acids is the production rate. In one test of the method, for the production of carbohydrates, Boschker et al. (1995) found that the continued increase of sugars could not be the result of leaching from killed microbes. However, whether the increase in amino acids could come from the killed microbes remains an open question. The toluene method may eventually be confirmed or refuted by isotope dilution, a very promising method for measuring the production rate of amino acids (Wanek et al. 2010). In this method, a mixture of amino acids, labeled with ^{15}N, is added to a sample and the change in the isotopic composition of individual amino acids followed over time. By appropriate samplings and treatments, such as chloroform extractions for ^{15}N in microbial biomass, a number of rates and pools were measured including rates of protein depolymerization, N mineralization, nitrification, and microbial immobilization of amino acids, ammonium, and nitrate. In this research, the substrate was finely chopped beech litter. Adding 1% toluene did not reduce the microbial respiration, amino acid concentration, or protease activity (i.e., fluorometric analysis), which contradicts the assumptions of the toluene method that adding toluene will reduce microbial respiration and increase amino acid concentration. However, adding toluene reduced amino acid production (protein depolymerization) by 71% and reduced amino acid immobilization by 74%. We would argue that the "excessive microbial pool" principle of Kuzyakov et al. (2009) can explain these results, with those authors suggesting that the limitation on soil CO_2 production is not microbial biomass, which is still present in excess even after fumigation, but the supply rate of amino acids and other simple organic molecules. In the same way, in the isotope dilution experiment of Wanek et al. (2010) we suggest that enough microbes survived the toluene treatment so that respiration was not reduced.

Conclusions

When labeled amino acids are added to plankton and to soils, the microbes in both systems respond

similarly. The amino acids are rapidly taken up and a percentage of the amino acid carbon taken up is respired; for a given amino acid, similar percentages are respired in both plankton and in soil assemblages. The crucial differences between the two habitats is the thousand-fold higher abundance of microbes in the soil than in aqueous environments, the heterogeneity of the soil environment which allows hot spots of activity and substrate concentration to exist, and the prevalence of diffusional constraints. The relatively low abundance of microbes in aqueous environments means that the kinetics of uptake of amino acids into planktonic microbes can be measured using dilution experiments. In contrast, the high concentration of microbes in the soil leads to rapid uptake of added amino acids, so that the kinetics of amino acid uptake cannot be studied using added radioisotopes.

This immediate uptake of added amino acids is very rapid in soils studied in vitro and in in situ incubations of amino acids injected into intact soils. In addition, soil microbes can take up and respire amino acids over an enormous range of amino acid concentrations. In spite of these abilities, measurements always show µM concentrations of free amino acids in soil water. Therefore, we conclude that these µM concentrations cannot be available to the soil microbes. Instead, in the undisturbed soil these amino acids are hidden in aggregates and micropores, affected by surface charges of clay particles, chemically bound to mineral particles, or dissolved inside plant roots. While this conclusion is robust for a variety of soils, it must be noted that experiments are often carried out after the soil is thoroughly mixed and water added. Not only is microbial activity optimized but it is impossible to investigate effects of hot spots of rhizodeposition.

Immediate uptake of all of the labeled amino acid added to soil precludes kinetic analysis; however respiration of the carbon skeleton of the amino acid can still be measured. The percentage respiration is a function of metabolic turnover of amino acids in microbes; the percentage is a constant for a given amino acid in both productive waters and in all soils. The rate of production of CO_2 appears to reflect the processing of freshly added amino acids by semi-starved or dormant soil microbes. Because the microbes in most soils are likely in this low-activity condition, the half-life of the added labeled amino acids is nearly the same in every soil, worldwide. Thus,

measuring microbial respiration of labeled amino acids added to soil does not give useable half-life rates.

We conclude that cycling of amino acids in soils is very rapid and cannot be measured by experiments with labeled amino acids. Heterotrophic microbes can take up amino acid molecules as quickly as they become available. As a consequence, competition for the immediate precursors of amino acids, protein and oligopeptides, may be equally important in structuring soil microbial communities. For heterotrophic bacteria in soil that are faced with strong competition for free amino acids by a tremendous number of microbes, the strategy for survival is to maintain low activity yet maintain the ability to take up every amino acid molecule that becomes available. Soil fungi may follow the same strategy but their activity and uptake ability have been little studied.

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