Amino acids comprise a large proportion of soil organic nitrogen (N). Microorganisms can take up amino acids as intact molecules or as ammonium and α-keto acids after enzymatic deamination. The objective of this study was to trace double labeled (13C,15N) glycine and L-leucine from soil solution into microbial biomass using compound specific stable isotope analysis. The two amino acids were utilized rapidly by soil microorganisms, with the half-life of glycine and leucine in soil solution being 2.9 and 5.0 h, respectively. The highest concentration of added glycine and leucine in the microbial biomass was measured after 4 h and corresponded to 10 and 13% of the glycine and leucine added, respectively, showing that a part of the added amino acids was taken up as intact molecules. Based on the results from an accompanying isotope pool dilution experiment, at least 15% of the glycine and 50% of the leucine was taken up as intact molecules. Our results suggest that glycine mainly served as carbon (C) source, while leucine provided C as well as N for the soil microbial community. Labeled keto acids were detected in soil solution and in the microbial biomass; however, their concentration corresponded only to a small fraction of the C added with the amino acids. The fact that 30 and 65% of glycine and leucine N was mineralized within 12 h, while never more than 5% of the amino acid C was recovered in the form of keto acids highlights how quickly keto acids were utilized by soil microorganisms. The combination of compound specific stable isotope analysis and chloroform fumigation extraction proved to be a valuable tool to trace amino acids and keto acids in soil solution and the microbial biomass.
the microbial biomass after an incubation period. A significant linear correlation between the $^{15}$N and $^{13}$C enrichment of the microbial biomass is considered a strong indication for the uptake of intact amino acids (e.g. Clemmensen et al., 2008; Andresen et al., 2011). However, this approach does not distinguish between the uptake of intact amino acids and the separate uptake of NH$_4^+$ and keto acids from the labeled amino acids.

Compound specific isotope analysis is another approach to determine the fate of amino acids in soil. This approach has been used to determine the gross rate of amino acid production in soil (Wanek et al., 2010) and to trace the C and N from added amino acids through various soil amino acid pools (Knowles et al., 2010). We hypothesize that a significant amount of amino acids are taken up as intact molecules by soil microorganisms. To test this hypothesis, we used gas chromatography (GC) coupled with ion-trap mass spectrometry (MS) to perform compound specific stable isotope analysis in combination with chloroform fumigation to determine the fate of added amino acids.

2. Material and methods

2.1. Soil and residue samples

Soil samples were collected in summer 2013 at the UC Davis Russell Ranch Sustainable Agricultural Facility site. Samples were taken from the top 20 cm of the profile from a field planted to corn before the sidedress N application. The soil is mapped as Rincon silty clay loam (fine, montmorillonitic, thermic Haploxeralf; Soil Survey Staff, 1997). The field-moist soil was passed through a 4-mm sieve and stored at 4 °C. The soil had a pH of 7.2 (determined in a 1:2 soil:water solution; Thomas, 1996) and contained 12.3 g C kg$^{-1}$ dry soil and 1.1 g N kg$^{-1}$ dry soil (analyzed by dry combustion on a Costech EA 4010 elemental analyzer; Brenner, 1996; Nelson and Sommers, 1996). Percentages of sand, silt, and clay were 15, 53 and 32%, respectively (pipet method; Gee and Bauder, 1986), and the microbial biomass N was 56.4 mg kg$^{-1}$ dry soil (chloroform fumigation extraction method followed by the alkaline persulfate oxidation method and colorimetric analysis of nitrate; Brookes et al., 1985; Cabrera and Beare, 1993; Doane and Horwath, 2003).

2.2. Microcosm experiment

Samples for the different analyses were prepared by weighing field-moist soil, equivalent to 6 g oven dry soil, into 40 mL glass vials. For the different analyses, 0.64 mL of solutions containing NH$_4^+$, glycine and l-leucine dissolved in DI water were added (see following paragraphs for amounts and treatments). With this addition, the gravimetric moisture content was brought to 28 g g$^{-1}$ dry soil, which corresponded to 50% water filled pore space. The vials were then placed into a 12-L plastic container with a lid, lined with moist paper towels to minimize evaporation, and incubated at room temperature (22 °C). After 0, 0.5, 1, 2, 4, 12, and 24 h, four random replicates per treatment were destructively sampled. Ammonium and amino acids were extracted by adding 30 mL of 0.5 M potassium sulfate (K$_2$SO$_4$) to the soil samples (Mulvaney, 1996). Samples were shaken for 1 h on a reciprocal shaker and the suspension filtered (Fisherbrand, Q5).

2.2.1. Amino acid-N mineralization analysis

The following treatment solutions containing N were added to samples at the beginning of the incubation to analyze amino acid-N mineralization by pool dilution: (a) $(^{15}$NH$_4$)$_2$SO$_4$ (40 atom%); (b) $(^{15}$NH$_4$)$_2$SO$_4$ (40 atom%) + unlabeled glycine and unlabeled l-leucine; (c) $(^{15}$NH$_4$)$_2$SO$_4$ + $^{13}$C$^{15}$N-glycine and unlabeled l-leucine; (d) $(^{15}$NH$_4$)$_2$SO$_4$ + unlabeled glycine and $^{13}$C$^{15}$N-l-leucine. 10 mg N kg$^{-1}$ dry soil were added in the form of NH$_4^+$ and 5 mg N kg$^{-1}$ dry soil in the form of each glycine and l-leucine. The atom% $^{15}$N of both amino acids was >95% and the atom% $^{13}$C at the C2 position was 99%. The treatment solutions were applied uniformly to 4 replicates (n = 4) using a syringe with needle. Glycine and l-leucine were chosen because they have the lowest and highest C to N ratios of all the aliphatic protein-forming amino acids. The samples of treatment (a) were extracted immediately with (K$_2$SO$_4$) as described below. The remaining treatments (b–d) were extracted after the predetermined incubation time.

2.2.2. GC–MS amino acid analysis

For the GC–MS analysis, two samples were prepared for each incubation time and replicate (n = 4). The solutions added with the DI water contained (NH$_4$)$_2$SO$_4$ (10 mg N kg$^{-1}$ dry soil), $^{13}$C$_2$$^{15}$N-glycine, and $^{13}$C$_2$$^{15}$N-l-leucine (5 mg N kg$^{-1}$ dry soil each). The addition of each amino acid corresponded to 0.33 mmol kg$^{-1}$ dry soil. One sample was extracted immediately at the end of the incubation as described above; the other was chloroform fumigated first for 24 h. A standard chloroform fumigation extraction method (Brookes et al., 1985) was used with one adjustment: Preliminary analyses showed that the concentration of double-labeled amino acids in the fumigated samples was lower than in the corresponding control samples, while the concentration of single-labeled amino acids was increased. A series of tests indicated that aminotransferases released during fumigation were responsible for this reaction (Geisseler and Horwath, submitted for publication). In order to inactivate these enzymes, 0.6 mL of a 5% sodium dodecyl sulfate (SDS) solution were added to the samples prior to fumigation. With this addition, the gravimetric soil moisture content was raised to 0.38 g g$^{-1}$. After the fumigation, the samples were extracted as described above.

All extracts from the above experiments were kept in the fridge at 4 °C and analyzed within two days. Preliminary test showed that the amino acid concentration in the samples did not change during this time.

2.3. Nitrogen mineralization from amino acids

Nitrogen mineralized from amino acids was determined at all sampling dates using a mirror image pool dilution approach (Equations (1) and (2)) described by Watkins and Barraclough (1996).

First, the gross rate of N mineralization ($m$) was calculated using Equation (1).

$$A_t = \frac{A_t}{1 + \frac{t}{m}}$$

(1)

Where $A_0$ and $A_t$ are the NH$_4^+$ pool at time 0 and $t$, respectively. The $^{15}$N excess of the NH$_4^+$ pools is indicated by *. The rate at which the pool size changes ($\theta$) is given by ($A_t - A_0)/t$. Values for $A_0$ and $A_0^*$ were derived from the samples which were immediately extracted (treatment a), while $A_t$ and $A_t^*$ were determined on the samples to which unlabeled amino acids and $^{15}$NH$_4^+$ were applied (treatment b). The value for $m$ from Equation (1) is substituted into Equation (2) together with the size and $^{15}$N excess of the NH$_4^+$ pool in the samples to which labeled amino acids and NH$_4^+$ were applied (treatments c and d).
\begin{align}
A_i' &= a_P' + \frac{(A_0' - a_P')}{\left(1 + \frac{\sigma_i}{\sigma_0}ight)^{0.5}} \\
\end{align}

Where $P$ is the $^{15}$N excess of the amino acids. The equation is solved for $a$, which is the proportion of the mineralization flux resulting from the amino acid. Multiplying $a$ with $m$ gives the amount of N mineralized from the amino acids (Barraclough, 1997).

The NH$_4^+$ concentration in soil extracts was determined using the salicylate method (Verdouw et al., 1978; Foster, 1995). Amino acids can interfere with the analysis, leading to an overestimation of the NH$_4^+$ concentration (Ros et al., 2011). To correct for this interference, 1.5 mL of the extracts were added to 40 mL glass vials and a small quantity of MgO was added to raise the pH. The samples were swirled briefly and placed into the plastic container together with moist towels for 24 h at room temperature (22 °C). Preliminary tests with solutions containing NH$_4^+$, amino acids (L-leucine and glycine), or both showed that under these conditions, NH$_4^+$ volatilizes completely as ammonia (NH$_3$) while the amino acid concentration is not decreased significantly. The samples were analyzed for NH$_4^+$ again after the 24 h diffusion period. The NH$_4^+$ concentration determined with this second measurement was assumed to be entirely due to amino groups reacting with the reagents and was subtracted from the initial measurement for a corrected NH$_4^+$ concentration.

To determine the $^{15}$N signature of the NH$_4^+$ pool, the solutions were transferred into glass vials with screw caps followed by adding about 0.5 g of MgO to raise the solution pH and convert NH$_4^+$ to NH$_3$ (Brooks et al., 1989). The vials were immediately covered with PTFE tape, a 7-mm filter paper disc to which 10 mL of KHSO$_4$ (2.5 M) had been added was placed on the tape and covered with another layer of PTFE tape before the vials were capped. After one week, the paper discs were removed, dried at 40 °C and placed into tin capsules for atom%$^{15}$N analysis at the UC Davis Stable Isotopes Facility. To calculate the mineralization rate of glycine, treatments a, b and c were used, while treatments a, b and d allowed calculating the mineralization rate of L-leucine.

### 2.4. Sample derivatization

As a GC can only separate volatile compounds, amino acids need to be derivatized before analysis. A methyl chloroformate derivatization procedure described by Smart et al. (2010) was used. Briefly, 180 μL of soil extract were added to salinized borosilicate test tubes and 20 μL of a d-2,3,3,3-alanine solution (10 mg N L$^{-1}$) was added as internal standard. Under the fume hood, 167 μL of methanol, 32 μL of pyridine, and 20 μL of methyl chloroformate were added to the samples in sequential order. The samples were vortex-mixed for 30 s and additional 20 μL of methyl chloroformate were added and the samples vortex-mixed again for 30 s. In a next step, 400 μL of chloroform were added and the samples vortex-mixed for 10 s. These steps were carried out in short succession to prevent volatilization of the reagents. Finally, 400 μL of a NaHCO$_3$ solution (50 mM) were added and the samples vortex-mixed one last time for 10 s. After this step a double meniscus formed and the aqueous phase on top was removed with a Pasteur pipet. To bind any water left in the remaining chloroform solution, a few crystals of Na$_2$SO$_4$ were added. Approximately 200 μL were then transferred to 2 mL amber glass vials with a 300 μL insert and screw caps using a glass syringe.

### 2.5. Gas chromatography—mass spectrometry

The samples were analyzed within 12 h of sample derivatization on a Varian 3800 GC connected to a Varian Saturn 2000 ion-trap MS equipped with a 1177 injector and a 8410 autoinjector. The GC was equipped with an Agilent VF-1701s capillary column (length 30 m plus 5 m EZ-guard column, ID 0.25 mm, film thickness 0.25 μm). The GC program was adapted from Smart et al. (2010). Briefly, a 1-μL sample was injected using the splitless mode. After 0.7 min the mode was switched to split with a ratio of 100. Helium was used as carrier gas at a flow rate of 1 mL min$^{-1}$. The inlet temperature was 290 °C. The GC temperature was initially held at 40 °C for 2 min, then raised to 180 °C (9 °C min$^{-1}$), and held for 5 min. The temperature was then raised to 220 °C (40 °C min$^{-1}$), held for 5 min, raised at the same rate to 240 °C and held for 11.5 min. Finally, the temperature was raised to 280 °C (40 °C min$^{-1}$) where it was held for 2 min. The interface temperature was held at 250 °C and the ion trap temperature was 150 °C. Samples were ionized by electron impact at 70 eV. Mass spectra were obtained in full-scan mode (38–650 m/z) starting at 5.5 min. The retention times and the detected ions of the molecules analyzed for this study are listed in Table 1. Glyoxylate and 4-methyl-2-oxovaleric acid are the α-keto acids formed when glyoxylate and leucine, respectively, are deaminated. In the following section, 4-methyl-2-oxovaleric acid will be referred to as oxovaleric acid.

### 2.6. Data analysis

All results are expressed on a moisture-free basis. Moisture content was determined by drying the soil samples at 105 °C for 24 h.

Agilent MS workstation software version 7.0.1 was used to integrate the area under the peaks. To quantify the concentration of the amino- and keto acids in the samples, standard curves for each amino- and keto acid were prepared by analyzing 5 samples of different concentrations in the expected recovery range.

Statistical analyses were conducted with the SAS program (SAS Institute, 2010), using the general linear model (GLM) procedure for analysis of variance and the REG procedure for regression analyses. The assumptions of the statistical models were tested for every data set. Normality of the residuals was evaluated graphically and with the Shapiro–Wilks test. Homogeneity of variances was tested by plotting the residuals vs. the predicted values and with Levene’s test. When necessary, the data were transformed. Mean comparisons were performed using the Tukey test, which controls the experimentwise type I error rate $\alpha$ (SAS Institute, 2010). Effects were considered significant for $P < 0.05$.

### 3. Results

#### 3.1. Glycine

After the immediate extraction of samples following the treatment additions, 78% of the double-labeled glycine was recovered from solution (Table 2, Fig. 1). During the initial 4 h of the

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
<th>Integration range (min)</th>
<th>Detected ion (m/z)$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>13C,15N Glycine</td>
<td>13.08</td>
<td>13.05–13.50</td>
<td>90</td>
</tr>
<tr>
<td>13C,15N L-Leucine</td>
<td>15.69</td>
<td>15.65–15.74</td>
<td>146</td>
</tr>
<tr>
<td>d4-2,3,3,3 alanine</td>
<td>12.63</td>
<td>12.55–12.90</td>
<td>106</td>
</tr>
<tr>
<td>13C Glyoxylate</td>
<td>12.80</td>
<td>12.76–13.00</td>
<td>76</td>
</tr>
<tr>
<td>13C 4-methyl-2-oxovaleric acid</td>
<td>9.53</td>
<td>9.40–9.70</td>
<td>86</td>
</tr>
</tbody>
</table>

$^*$ The m/z for unlabeled leucine and glycine is two units lower, for unlabeled keto acids 1 unit lower.
incubation, the concentration of added glycine decreased nearly linearly from 4 to about 1 mg N kg\(^{-1}\) soil. After 12 h, the concentration of added glycine in solution had decreased to less than 1% of the amount added. In the microbial biomass, the concentration of double-labeled glycine reached its highest concentration of 0.5 mg N kg\(^{-1}\) soil after 4 h, which corresponds to 10% of the added glycine. After 12 h, added glycine was no longer detectable in the microbial biomass. While the concentration of unlabeled glycine in solution decreased almost linearly during the initial 12 h from 3.9 to 0.3 mg N kg\(^{-1}\) soil (Table 2, Fig. 1). The extraction efficiency of glycine was 78%. As was the case with glycine, added leucine was no longer detectable in solution after 24 h. The highest concentration of added leucine in the microbial biomass, 0.65 mg N kg\(^{-1}\) soil, was measured after 4 h and corresponds to 13% of the added leucine. The concentration of unlabeled leucine was very low in solution, but reached 0.8 mg N kg\(^{-1}\) soil in the microbial biomass in the samples where no labeled leucine was applied (data not shown).

The oxovaleric acid concentration in soil solution reached a maximum after 2 h, which corresponded to about 1.6% of the added leucine. In the microbial biomass, oxovaleric acid was highest after one hour, representing 4.6% of the added leucine (Fig. 2). After 12 h, the oxovaleric acid concentration had decreased to a very low level, which was no longer significantly different from the unamended control. Little oxovaleric acid was present in soil solution when the samples were extracted immediately after the addition of leucine. However, oxovaleric acid was found in the microbial biomass of samples that were fumigated directly after the leucine addition (Fig. 2). This suggests that leucine was either taken up and deaminated by microorganisms that survived for some time before the chloroform treatment took full effect, or that leucine was deaminated by deaminases that were released during fumigation.

About 25% and 20% of the added leucine-N was in found in soil solution in the form of \(^{15}\)NH\(_4\) after 12 and 24 h, respectively. The proportion of leucine mineralized reached 30% after 12 h and remained constant. Subtracting the 20% of leucine that could not be extracted immediately after its addition, this suggests that at least 50% of the leucine was taken up as intact molecules.

### 4. Discussion

The combination of compound specific stable isotope analysis and chloroform fumigation extraction proved to be a valuable tool to trace the fate of added amino acids in soil solution and microbial biomass. However, the complex and dynamic nature of the microbial N transformations make it difficult to quantify some of the processes.

The two amino acids were utilized rapidly by soil microorganisms. The glycine concentration decreased rapidly between 2 and 4 h, corresponding to 0.91 mmol g\(^{-1}\) microbial biomass N h\(^{-1}\). Measuring the CO\(_2\) evolution after the addition of glycine, Vinolas et al. (2001) determined a \(V_{\text{max}}\) of 0.42 mmol g\(^{-1}\) microbial biomass N h\(^{-1}\). The higher value found in our study may be due to the fact that only part of the C from amino acids taken up is respired, while the other is used in anabolism. In addition, the higher estimate could be a result of extracellular deamination. In a study with a large number of agricultural soils under a variety of managements, Jones et al. (2005) found that about 25% of the C of an amino acid mix was respired, while 75% was incorporated into

### Table 2

Concentration (mg N kg\(^{-1}\) oven dry soil) of double-labeled glycine and L-leucine in soil solution and the microbial biomass. 5 mg N kg\(^{-1}\) oven dry soil were added with each amino acid, which corresponds to 0.33 mmol kg\(^{-1}\) dry soil.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>In soil solution</th>
<th>In the microbial biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glycine</td>
<td>L-leucine</td>
</tr>
<tr>
<td>0</td>
<td>3.89 (0.022) a</td>
<td>3.90 (0.072) a</td>
</tr>
<tr>
<td>0.5</td>
<td>3.69 (0.136) ab</td>
<td>3.68 (0.086) a</td>
</tr>
<tr>
<td>1</td>
<td>3.35 (0.104) b</td>
<td>3.50 (0.135) ab</td>
</tr>
<tr>
<td>2</td>
<td>2.63 (0.076) c</td>
<td>3.05 (0.057) b</td>
</tr>
<tr>
<td>4</td>
<td>1.09 (0.039) d</td>
<td>2.40 (0.096) c</td>
</tr>
<tr>
<td>12</td>
<td>0.00 (0.000) e</td>
<td>0.29 (0.011) d</td>
</tr>
<tr>
<td>24</td>
<td>0.00 (0.000) e</td>
<td>0.00 (0.000) e</td>
</tr>
</tbody>
</table>

Note: All values are means (n = 4) with SE given in parenthesis. Means followed by a different letter within each column are significantly different at \(P < 0.05\) according to Tukey’s test.

The two amino acids were utilized rapidly by soil microorganisms. The glycine concentration decreased rapidly between 2 and 4 h, corresponding to 0.91 mmol g\(^{-1}\) microbial biomass N h\(^{-1}\). Measuring the CO\(_2\) evolution after the addition of glycine, Vinolas et al. (2001) determined a \(V_{\text{max}}\) of 0.42 mmol g\(^{-1}\) microbial biomass N h\(^{-1}\). The higher value found in our study may be due to the fact that only part of the C from amino acids taken up is respired, while the other is used in anabolism. In addition, the higher estimate could be a result of extracellular deamination. In a study with a large number of agricultural soils under a variety of managements, Jones et al. (2005) found that about 25% of the C of an amino acid mix was respired, while 75% was incorporated into

<fig src="image.jpg" alt="Fig. 1. Recovery of N from added glycine (left panel) or L-leucine (right panel)."> Fig. 1. Recovery of N from added glycine (left panel) or L-leucine (right panel).


the microbial biomass (Jones et al., 2005). Based on the decrease in concentration of labeled amino acids in solution, the half-life of glycine and leucine in our study was 2.9 and 5.0 h, respectively. This compares well with an average half-life of mixed amino acids of 2.3 h reported by Jones et al. (2005). Shorter half-lives have been reported in the literature. Across a global latitudinal transect of soils under different land uses, the average half-life of amino acids was 1.8 h (Jones et al., 2009), while Boddy et al. (2007) reported values ranging from 0.37 to 0.61 h in a grassland soil.

The highest concentration of added glycine and leucine in the microbial biomass was measured after 4 h and corresponded to 10 and 13% of the glycine and leucine added, respectively. The occurrence of double labeled amino acids in the microbial biomass clearly shows that at least a proportion of the added amino acids was taken up as intact molecules. The concentration of glycine and leucine measured in the microbial biomass most likely underestimated the total amount taken up, as amino acids are incorporated into proteins or deaminated in the cytoplasm.

Another approach to determine direct uptake is measuring the N mineralization rate of added amino acids. The approach relies on the assumption that the difference between amino acids utilized and mineralized is due to uptake of intact amino acids. The estimate needs to be adjusted for the amino acids immobilized abiotically. Only about 80% of the added amino acids could be recovered from solution or the microbial biomass directly after their addition. This suggests that some leucine and glycine were immobilized abiotically, which seems likely. Hedges and Hare (1987) found that glycine and leucine can be immobilized on montmorillonite, a major clay mineral present in the soil used for the present study. However, the possibility of some microbial uptake during extraction with 0.5 M K₂SO₄ cannot be excluded. Assuming that abiotic immobilization was dominant, the pool dilution approach suggests that at least 15% of the glycine and 50% of the leucine was taken up as intact molecules. Similar to the compound specific isotope results, the pool dilution approach may underestimate direct uptake, as ¹⁵N in amino acids that were taken up as intact molecules may have been released into solution after intracellular deamination. Under the conditions of our experiment, this was a likely pathway. Therefore, the measured mineralization rate may be due to extracellular deamination.

Deamination of amino acids results in the production of NH₃ and α-keto acids. Therefore, insight into microbial utilization of amino acids can also be gained by tracking keto acids. Keto acids derived from the added amino acids were present in soil solution. The solution with ammonium and amino acids added before the incubation contained 1 mol of N per 2 mol of C. It is therefore unlikely that the microorganisms took up intact amino acids and released the keto acids into solution after intracellular deamination. This pathway has been found to occur mainly under N-limited conditions (Linton and Musgrave, 1983; Ahmed et al., 1984). Therefore, the keto acids in soil solution were most likely derived from the added amino acids without prior deamination. However, the low concentration of keto acids in solution, corresponding to less than 2% at any sampling time, suggests that they were either taken up rapidly or that extracellular deamination was insignificant. Direct uptake of amino acids without prior deamination would be in line with several other studies (Barak et al., 1990; Drury et al., 1991; Barraclough, 1997).

Labeled keto acids were detected in very small amounts in the microbial biomass and represented a small fraction of the C added as amino acids. The fact that 30 and 65% of glycine and leucine N was mineralized within 12 h, while never more than 5% of the C was recovered in the form of keto acids highlights how quickly keto acids are utilized by soil microorganisms. The keto acids detected in the microbial biomass may have been the product of extracellular deamination by enzymes that were excreted actively by soil microorganisms or released by decaying cells. However, the low concentration of keto acids in solution, corresponding to less than 2% at any sampling time, suggests that they were either taken up rapidly or that extracellular deamination was insignificant. Direct uptake of amino acids without prior deamination would be in line with several other studies (Barak et al., 1990; Drury et al., 1991; Barraclough, 1997).

Keto acids in the cytoplasm may have served different purposes. As mentioned earlier, the release into soil solution seems unlikely under the conditions of our experiment. However, they may have been used for the synthesis of other molecules. Knowles et al. (2010) showed that over a period of 8 days, 8% of the N and 2.8% of the C added to a soil sample in the form of glycine were used to synthesize other amino acids. Furthermore, keto acids may be mineralized and released as CO₂. How fast amino acid-C is respired is evident from the studies that determined amino acid half-life by measuring CO₂ evolution (Vinolas et al., 2001; Jones et al., 2005). This discussion highlights the dynamic and complex pathways of
keto acid utilization by soil microorganisms. The concentration of keto acids in soil solution and the microbial biomass does not provide a reliable estimate of the pathway of amino acid utilization.

Our results suggest that glycine and leucine served different functions. Both were taken up and utilized in the microbial biomass rapidly. A large proportion of the glycine-N was recovered in soil solution in the form of NH₄⁺ after only 12 h, suggesting that glycine mainly served as C source, with the C either incorporated into other molecules or respired as carbon dioxide, as discussed above. In contrast, a much smaller proportion of the added leucine-N was found in soil solution as NH₄⁺ after 24 h, suggesting that leucine provided C as well as N for the soil microbial community.

4.1. Conclusions

Our study showed that compound specific stable isotope analysis in combination with the chloroform fumigation extraction method could distinguish between free amino acids and keto acids in soil solution and in the microbial biomass.

While the N mineralization rate of added amino acids suggested that direct uptake was an important process, compound specific stable isotope analysis provided clear evidence that amino acids are taken up as intact molecules by microorganisms in soil. Compound specific stable isotope analysis could also be used to track keto acids, the products of deamination.

The results of this study highlight the complex and dynamic nature of microbial amino acid utilization. Amino acids were deaminated rapidly either in soil solution or in the cytoplasm of microbial cells. While amino acid N accumulated in soil solution in the form of NH₄⁺, the keto acids were utilized quickly. Only a small fraction of the added C was detectable in the form of keto acids at any one time after the addition of amino acids.

Compound specific stable isotope analysis proved to be a valuable tool to trace the microbial utilization of amino acids and their degradation products.

Acknowledgments

We would like to thank the editor and two anonymous reviewers for their valuable comments and suggestions. Funding for this study was provided by California Department of Food and Agriculture Fertilizer Research and Education Program (CDFA-FREP, Grant number 11-04855A) and by the J. G. Boswell Endowed Chair in Soil Science.

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