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Short communication

Interference by amino acids during the determination of ^{15}N ammonium in soil

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Abstract

In the study of terrestrial N cycling, NH_4^+ concentration and ^{15}N enrichment are routinely determined by colorimetric continuous flow analysis and microdiffusion methods. Amino acids can interfere in these determinations; consequently the aim of the present study was to evaluate the significance of the interference. Glycine and glutamine are key amino acids in soil and were therefore used as 'models'. Both glycine and glutamine interfered during continuous flow analysis, whereas interference during microdiffusion was of little importance. The effects of interference can be significant, e.g. estimates of gross mineralisation rate were reduced up to 33%, where we allowed for amino acid interference during determination of NH_4^+ concentration. The potential influence of amino acid interference emphasises that development of continuous flow analysis to increase NH_4^+ specificity is needed.

Keywords: Amino acids; Dissolved organic nitrogen; Glycine; Glutamine; Interference; Method; ^{15}N; Gross mineralization

The stable isotope of nitrogen (^{15}N) represents an important tool in the study of N cycling processes in soils (Powlson and Barraclough, 1992). Microdiffusion is commonly used as a method to prepare soil solutions and extracts for the determination of the ^{15}N enrichment of NH_4^+ and other soluble N pools (Brooks et al., 1989). However, complete recovery of N from the diffusion disks during isotope ratio mass spectrometry is difficult to achieve, and colorimetric continuous flow methods are used routinely for the quantitative determination of NH_4^+ concentrations. The data giving total NH_4^+–N concentration and ^{15}N enrichment are then combined to calculate the amount of NH_4^+–^{15}N in soil extracts. Interference of alkali-labile organic N during microdiffusion is of minor importance when diffusions are carried out at room temperature using small sample volumes (Mulvaney and Khan, 1999). In contrast, the Berthelot reaction, which forms the basis of determination of NH_4^+–N during colorimetric continuous flow analysis, is not entirely specific to NH_4^+–N (Searle, 1984) and interference from organic N compounds, such as amino acids, is known to occur (Rhine et al., 1998; Husted et al., 2000).

In a preliminary study, we dissolved 17 L-isomeric amino acids (2.5 mg N l^{-1}) individually in deionised water, and observed that different amounts of amino acid-N were measured as 'NH_4^+–N' (Table 1) by the salicylate–nitroprusside modified Berthelot method (Mulvaney, 1996) during continuous flow analysis. The degree of interference was not related to the chemical structures of the amino acids. Although free amino acids usually occur at low concentration in soil solution, it has been suggested that they have an important role in the soil N cycle (Appel et al., 1999); both glycine and glutamine make up a major proportion of the amino acid pool (Chapin et al., 1993; Friedel and Scheller, 2002; Jones et al., 2005). These findings have particularly relevance to N-limiting soil environments, where soluble N concentrations are low (e.g. arctic tundra) and where the relative balance of amino acids and NH_4^+ may be important

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Table 1
Interference by 17 amino acids (2.5 mg N l^{-1}) during the colorimetric determination of NH_4^+ in deionised water

	% Amino acid measured as NH_4^+–N
Threonine	100 ± 2
Serine	89 ± 1
Glycine	73 ± 1
Histidine	45 ± 1
Methionine	44 ± 1
Alanine	43 ± 1
Phenylalanine	39 ± 2
Leucine	37 ± 1
Valine	28 ± 2
Isoleucine	24 ± 1
Glutamine	12 ± 2
Tyrosine	12 ± 1
Arginine	11 ± 1
Glutamic acid	5 ± 2
Asparagine	4 ± 1
Proline	1 ± 3
Asparatate	−2 ± 2

Interference is expressed as the percentage of the added amino acid that is measured as if it is NH_4^+. Values represent means ± SD (n = 3).

in regulating the competitive ability of individual plant species (Chapin et al., 1993). In these circumstances, it is vital that interference-free measurements of soluble N are made. The present study, therefore, used glycine and glutamine as model amino acids, as they had shown high and low interference, respectively, (Table 1) on the determination of NH_4^+ concentration and ^{15}N enrichment by both continuous flow and microdiffusion methods. We then evaluated the effect of amino acid interference on the calculation of gross mineralisation rates.

We dissolved ^{15}N-labelled $(NH_4)_2SO_4$ (6.0 at.% ^{15}N), glycine (Gly), glutamine (Gln) and a mixture of glycine/glutamine (1:1 Gly/Gln) separately in 2 M KCl to give either a 'low' (3.6 mg N l^{-1}) or 'high' (12.5 mg N l^{-1}) concentration of each solute. These amino acid concentrations are typical of those in soil solutions (low concentration) and plant tissues (high concentration) (Jones and Darrah, 1994). The NH_4^+ solution was mixed with each of the three amino acid solutions in combination to give the following NH_4^+-amino acid treatments: (i) low–low, (ii) low–high, (iii) high–low, and (iv) high–high. Each treatment had four replicates. The amount of NH_4^+–N in the KCl solutions was determined colorimetrically by the salicylate-nitroprusside method of Mulvaney (1996) on a Skalar autoanalyser (Skalar UK Ltd, New York, UK). The KCl extracts were prepared for determination of at.% ^{15}N using a modified diffusion technique (Goerges and Dittert, 1998; Herrmann et al., 2004) and ^{15}N enrichment was determined after conversion of NH_4^+–N to molecular N_2 using an isotope-ratio mass spectrometer (Europa Tracermass, Europa Scientific Ltd, Crewe, UK). An at.% ^{15}N recovery of 99% was measured for this process. Total N recovery was 86 and 94% on average for low and high NH_4^+ concentrations, respectively.

The interference of amino acids in the determination of NH_4^+ by continuous flow and microdiffusion methods is expressed: (i) as the apparent amount of ^{15}N recovered as NH_4^+ (Fig. 1); and, (ii) as the percentage of the added amino acid that is measured as if it is NH_4^+ (Tables 1 and 2). The measured NH_4^+ concentrations and ^{15}N enrichments were normally distributed; consequently differences between

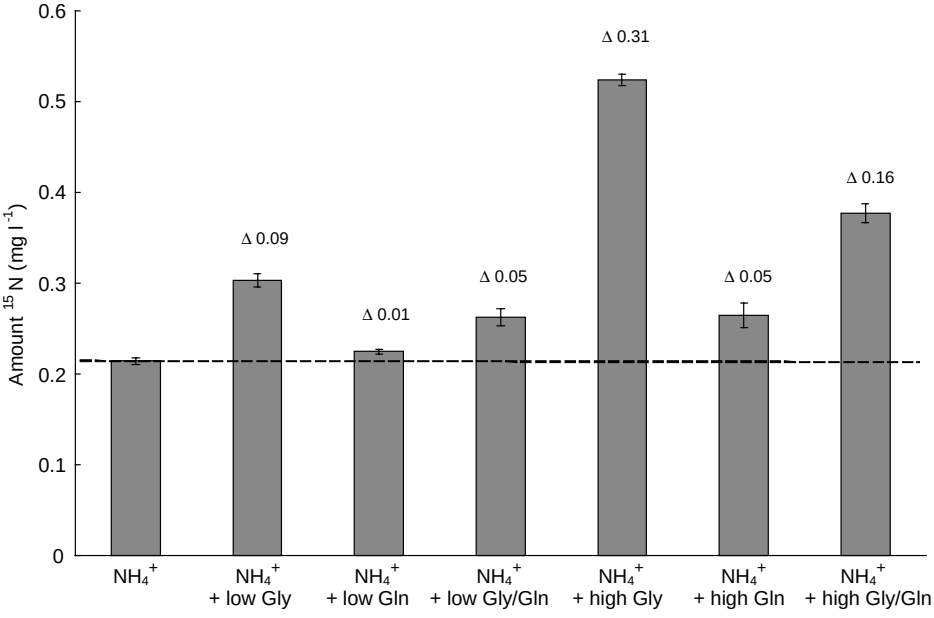

Fig. 1. Apparent amount of ^{15}N as NH_4^+–N (mg N l^{-1}) calculated from the NH_4^+ content measured by continuous flow analysis and the ^{15}N enrichment measured after microdiffusion for solutions with low NH_4^+ concentration in the presence and absence of either high or low amounts of the amino acids glycine (Gly) and glutamine (Gln). Gly/Gln indicates a 1:1 mixture of the two amino acids. The solutions containing high NH_4^+ concentrations showed a similar pattern and are not shown here. Δ gives the differences between apparent and true amount of ^{15}N as NH_4^+–N (mg N l^{-1}). Values represent mean ± SD (n = 4).