**Utilisation and transformation of organic and inorganic nitrogen by soil microorganisms and its regulation by excessive carbon and nitrogen availability**

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**Abstract**

The process of nitrogen (N) transformation after microbial utilisation of organic and inorganic N is unclear. 15N-glycine (Gly), 15NH4+, and 15NO3− were used to investigate the uptake, release, and reutilisation of N by microorganisms over 9 days. In addition, high amounts of unlabelled carbon (C) or N were added to explore how C or N availability affects the cycling of inorganic and organic N by microorganisms. Within 15 min, 67% of the added 15N-Gly was taken up by soil microorganisms; within 1 h, 8% was released as NH4+. The released 15NH4+ was reutilised by the microorganisms within hours. Microorganisms took up 50% of the added 15NH4+ (15 min) and released 13% of the taken up NH4+ (1 h). Microorganisms prefer to take up Gly rather than NH4+ because they can directly acquire C from Gly for maintaining its growth and synthesising more complex compounds. NO3− was taken up by microorganisms within minutes but not released into the soil. NO3− was likely stored in the cytoplasm, to be used as an N source to face future N-deficient environments. When high concentrations of C or N were added, the assimilation of Gly and NH4+ increased, whereas N mineralisation and nitrification rates decreased, and the uptake of NO3− remained stable. Overall, Gly and NH4+ were taken up, released, and re-taken up by microorganisms and were preferentially utilised under excess C or N sources, while NO3− was stored in the microbiome. These findings provide new insights into N uptake by microorganisms in short-term.

**Keywords:** Organic nitrogen uptake, Inorganic nitrogen uptake, C and N bioavailability, Isotopic labelling

**Introduction**

Nitrogen (N) is one of the essential nutrients for all organisms (Kuzyakov and Xu 2013). Soil microorganisms can utilise inorganic forms of N, such as NH4+ and NO3− (Geisseler et al. 2009; Wen et al. 2022), as well as a wide range of organic N compounds with different molecular sizes, including amino acids and peptides (Farrell et al. 2011; Hill and Jones 2019; Ma et al. 2020a). The low-molecular-weight organic N forms, such as peptides, glycine (Gly), glutamate, glutamine, alanine, cysteine, and methionine, can be directly incorporated into the microbiome (Wong et al. 2008; Hu et al. 2018; Ma et al. 2021a), used as substrates in the biosynthesis of new compounds (Wilkinson et al. 2014; Broughton et al. 2015; Hill and Jones 2019), and catabolised to inorganic N (Jones et al. 2005; Ma et al. 2021b). Microorganisms are also the primary mineralisers of organic N (Jones et al. 2009). However, most previous studies have focused on mineralisation rather than the cycling processes of organic N including microbial biomass incorporation, the release of inorganic N, and the re-utilisation of released inorganic N. Thus, a thorough understanding of microbial N utilisation processes over time will greatly improve our understanding of how soil microorganisms control soil N cycling and bioavailability.

The mechanisms underlying microbial uptake and utilisation differ for amino acids, NH4+, and NO3−. NH4+ is considered the preferred N source for soil microorganisms (Geisseler et al. 2010). The uptake of NH4+ by microorganisms primarily depends on the proton gradient inside and outside the membrane (González et al. 2006; Geisseler et al. 2010). When NH4+ is taken up by microorganisms, it is assimilated into biomolecules by two different pathways: 1) catalysed by glutamine synthetase and glutamate synthase and 2) catalysed by glutamate dehydrogenase (Masclaux-Daubresse et al. 2006; Geisseler et al. 2009). NO3− can be utilised by several microorganisms as an N source and transferred to the cytoplasm by specific proteins (González et al. 2006). NO3− must be reduced to NH4+ before being assimilated by microorganisms (González et al. 2006; Galland et al. 2019). The uptake of NO3− is regulated by the availability of peptides, amino acids, and NH4+ (Plett et al. 2018), and an increase in NH4+ and organic N uptake decreases NO3− uptake. The uptake of amino acids by microorganisms is facilitated through various transporters including glutamine transporters and the CycA transporter (Chubukov et al. 2014; Pochini et al., 2014; Hook et al. 2022). Amino acids transported into microorganisms first undergo enzymatic removal of the alpha-amino N through deamination or transamination before they are utilised by microorganisms as a C source (Hill et al. 2011). Owing to the different mechanisms of uptake and assimilation of amino acids, NH4+, and NO3−, understanding the mechanisms by which soil microorganisms take up and assimilate the different N forms is essential.

Owing to the presence of complex N sources in the soil, the microbial uptake of certain N forms is regulated by the availability of soil C and N (e.g. glucose, amino acid, and NH4+), particularly C bioavailability (Canarini et al. 2017; Chen et al. 2017). The uptake of certain N forms may be inhibited by the presence of other highly available N forms. For example, NH4+ assimilation is repressed by several amino acids, such as Gly and alanine (Geisseler et al. 2010), and the assimilation of NO3− is induced by the lack of NH4+ (González et al. 2006). The uptake of organic N, such as amino acids and peptides, is likely driven by the demand for C rather than for N (Farrell et al. 2014; Zhu et al 2021), and its microbial uptake process may be primarily regulated by excess available C (Jones and Murphy 2007; Creamer et al. 2014; Ma et al. 2020b). Microorganisms prefer NH4+ over organic N when carbohydrates, such as glucose or cellulose, are available (Farrell et al. 2013, 2014). However, at a high availability of C–N-coupled compounds in the soil, such as amino acids, microorganisms may use such organic N molecules to meet their C demand despite the high availability of NH4+ (Geisseler et al. 2010). In addition, applying mineral N to soils reduces microbial respiration and C uptake and can decrease the mineralisation of organic matter, microbial biomass, and respiration (Sinsabaugh 2010; Schleuss et al. 2019; Widdig et al. 2020). Understanding how microbes utilise different forms of N in soil and how these processes are influenced by C and N availability will help to elucidate nutrient transformations.

In this study, two complementary soil incubation experiments were conducted. Firstly, 15N-Gly, 15NH4+, or 15NO3− was added to the soil to assess microbial utilisation of either inorganic N or low-molecular-weight organic N over the culturing time. Then, the same 15N forms were added to the soil, and additional quantities of C (as glucose) or N (as unlabelled NH4+, NO3−, or Gly) were provided to assess the utilisation of contrasting N forms by the soil microorganisms under conditions of excess C or N availability. We aimed to (1) identify the key processes involved in the utilisation of Gly, NH4+, and NO3− by soil microorganisms and (2) determine how the utilisation of one N source is regulated by the high availability of another N form or C. Studying N utilisation by microorganisms, and the impact of additional C or N sources will help to improve our understanding of the mechanisms of microbial N use.

**Materials and methods**

***Soils***

The soils for both incubation experiments, collected from Jinhua City, Zhejiang Province, China (29°19ʹ09ʺ N, 119°43ʹ43ʺ E, 72.8 m a.s.l.), were classified as Haplic Acrisol (Food and Agriculture Organization). All soil samples were thoroughly homogenised, passed through a 5-mm mesh sieve to remove roots and stones, and stored at 4 °C. The physicochemical properties of the soils are summarised in Table S1. The soil pH was measured using soil:H2O suspensions (1:2.5). The moisture content was measured by oven-drying at 105 ℃ for 24 h. Soil organic C was measured by K2Cr2O7 redox titration (Yeomans and Bremner 1988). The total N content was measured using the Kjeldahl method (Hauck and Bremner 1976). Alkali-hydrolysed N was measured using the alkaline diffusion method (Tang et al. 2021). The available P content was tested using the molybdenum blue method (Tang et al. 2022). The NH4+ and NO3− content in the soil extracts were colourimetrically detected using the salicylic acid and vanadate methods, respectively (Mariano et al. 2016). Microbial biomass C (MBC) was determined using the fumigation-extraction method (Vance et al. 1987).

***First experiment: Utilisation of amino acids, NH4+, and NO3− by soil microorganisms***

To trace the utilisation of amino acids, NH4+, and NO3− by microorganisms over time, 0.5 mL of 100 μM 99.8% atom 15N-labelled Gly (0.15 μg N g-1 soil), 50 μM 99.8% atom(15NH4)2SO4 (0.15 μg N g-1 soil), or 100 μM Na15NO3 (0.15 μg N g-1 soil) was added to 5 g field-moist soil samples. Each treatment was prepared in four replicates at six time points (24 tubes per treatment). The Gly concentration used in this study reflects the typical free amino acid content after microbial cell lysis in rhizosphere soils (Jones et al. 2005). After incubation for 15 min, 1 h, 6 h, 1 d, 3 d, and 9 d at 20 °C, the soil sample was extracted by 30 mL 0.5 M K2SO4 (Soil:H2O was 1:6) and centrifuged at 18,000 × *g* for 1.5 min. Then the soil was extracted by 0.5 M K2SO4 thrice until 120 mL was extracted (Cao et al. 2013). A part of the soil extract (20 mL) was used to measure NH4+ and NO3− contents using the salicylic acid and vanadate methods, respectively, and the content of total organic matter (OM) produced by the microorganisms was determined via semi-automatic Kjeldahl digestion at each time point. The remaining soil extract (100 mL) was used to measure the concentrations of 15NH4+, 15NO3−, and 15N-OM.

The concentration of 15NH4+, 15NO3−, and 15N-OM at each time point was determined using the 15NH3 release method (Wanek et al. 2010). In brief, MgO (0.4 g) was added to a bottle containing 100 mL of the 0.5 M K2SO4 soil extract, and a 7-mm filter disc acidified with 2.5 M KHSO4 was suspended below the cap before sealing the bottle. The bottles were carefully shaken daily, and the filter discs were removed after 7 d and used to measure 15NH4+ abundance. Next, MgO (0.4 g) and Devarda’s alloy (0.8 g) were added to each bottle again to measure 15NO3− abundance in the soil extraction (Wanek et al. 2010). Acidified filter discs were then suspended below the cap, and the bottles were sealed and shaken daily. The filter discs were removed after 7 d and used to measure 15NO3− abundance. After collecting 15NO3-, the extract was digested using 5 mL of H2SO4 at 380 °C to determine the abundance of 15N-OM in the soil extracts. After digestion, all solutions were transferred to a bottle, and 20 mL of 1 M NaOH was added to induce the release of 15NH3, which was produced from 15N-OM. The released 15NH3 was captured using acidified filter discs, as described above, and used to measure 15N-OM abundance. All filter discs were freeze-dried, and their 15N was analysed using elemental analyser-stable isotope mass spectrometry (IsoPrime100; Isoprime Ltd., Cheadle Hulme, UK). The difference between the total 15N in the soil and the 15NH4+, 15NO3–, and 15N-OM amounts was used to calculate the 15N immobilised in the microbiome (15N-MB = 15Ntotal – 15NH4+ – 15NO3– – 15N-OM) (Jones et al. 2013). The 15N was extracted four times by 0.5 M K2SO4 at a soil:H2O ratio of 1:6; most of the 15N was extracted, and a limited amount of 15N was adsorbed by the soil particles.

***Second experiment: Effect of C and N availability on the utilisation of amino acids, NH4+, and NO3− by soil microorganisms***

To assess whether the soil microbial uptake of amino acids, NH4+, and NO3− was affected by C or N availability, 5 g of field-moist soil was placed in 50 mL centrifuge tubes, and 0.5 mL of tracer solution with highly bioavailable C or N was evenly added to the soil samples (Spohn and Kuzyakov 2013; Ma et al. 2020b). Nine solution combinations were used in the treatments: 15N-Gly+1 M NO3−, 15N-Gly+1 M NH4+, 15N-Gly+1 M Glucose; 15NH4++1 M NO3−, 15NH4++1 M Gly, 15NH4++1 M Glucose; 15NO3−+1 M NH4+, 15NO3−+1 M Gly, 15NO3−+1 M Glucose. The amount of 15N added was 0.15 μg N g-1 soil and the amounts of N and C added were 1.4 mg N g-1 soil and 7.2 mg C g-1 soil, respectively. The amounts of 15N assimilated by the microorganisms and remaining in the soil as 15NH4+, 15NO3−, and 15N-OM were determined at 15 min and 3 d after the labelled N forms were added, as stated above, and the 15N was analysed using elemental analyser-stable isotope mass spectrometry.

***Data processing and statistical analysis***

The amount of 15N in the 15NH4+, 15NO3−, and 15N-OM in the soil after adding the tracers was obtained by subtracting the amount of 15N determined in the control from the corresponding amount determined in the labelled sample. This calculation was conducted as previously described by Pan et al. (2022):

PS = CS– C (As – Ac)/CT × 100% (1)

where PS is the percentage of 15N remaining in the soil as 15NH4+, 15NO3−, or 15N-OM; CS-C is the total N of the NH4+, NO3−, or OM in the soil extraction; AS and AC are the 15N atom% in the test and control samples, respectively; CT is the total 15N tracer added.

The amount of 15N uptake by the microorganisms was calculated according to the difference between the total 15N and 15N extracted from the soil solutions (Jones et al. 2013; Hill and Jones 2019; Ma et al. 2021):

where is the percentage of 15N taken up by soil microorganisms; is the total percentage of 15N added to the soil; , , and are the percentages of 15NH4+, 15NO3−, and 15N-OM in the soil extracts, respectively.

Data are presented as mean ± standard error. After testing for normality and homogeneity of the residuals, one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test (*p* < 0.05) was performed to calculate statistical differences among treatments using SAS v. 8.2 (SAS Institute Inc., Cary, NC, USA). The figures were produced using Origin v. 8.1 (OriginLab, Northampton, MA, USA).

**Results**

***Utilisation of 15N-Gly, 15NH4+, and 15NO3− by microorganisms in the soil***

*Mineral N and MBC after the addition of contrasting N forms*

After adding 15NO3−, 15NH4+, or 15N-Gly to the soils, the MBC increased by 50, 63, and 51%, respectively, within 9 days (Fig. 1). The concentrations of NH4+ and NO3− in the soil extractable pool over time are shown in Figure S1.

*Incorporation and utilisation of 15N-glycine, 15NH4+ and 15NO3− by soil microbiome*

Within 15 min, 66% of the 15N-Gly was taken up by the microorganisms (Fig. 2A); 33% of 15N-Gly mineralised to NH4+ (Fig. 2B), whereas half of NH4+ was oxidised to NO3− (Fig. 2C), and almost all 15N-Gly was consumed. One hour after its addition to soil, 8% of Gly after incorporation continued to mineralise to NH4+, and NH4+ production from Gly mineralisation was increased to 27%. The released NH4+ was then reutilised by microorganisms. The content of NO3− produced by nitrification was maintained at 14% (Fig. 2C).

The utilisation of 15NH4+ significantly differed from that of 15N-Gly over time. Within 15 min, 15NH4+ (50%) uptake was 13% lower than that of 15N-Gly. From the added 15NH4+, 13% of the N was released by microorganisms after 1 h, and the released NH4+ was re-incorporated into the microbiome after 6 h. The utilisation of 15NH4+ increased with time, and 81% of 15NH4+ was incorporated into the microbiome at 9 days (Fig. 2A). In addition, 24% of NH4+ was oxidised to NO3− within 15 min and subsequently remained stable at 20% (Fig. 2C).

The incorporation and utilisation of 15NO3− differed from those of 15NH4+ and 15N-Gly. 15NO3− was not released from the microbiome and was continuously immobilised by the microbiome (up to 70% of the 15N applied; Fig. 2A). Almost no 15NO3− was denitrified to NH4+ or synthesised to OM (Fig 2D).

***Effect of C and N availability on the utilisation of Gly, NH4+, and NO3− by soil microorganisms***

Within 15 min, the addition of excess C or N led to a 34 and 37% increase in Gly uptake into the microbiome, respectively (Fig. 3A). Furthermore, the mineralisation of Gly by microorganisms was decreased (Fig. 3B, 3C). Moreover, when 1 M NO3−, NH4+, and glucose were added to the soil, 12, 13, and 13% of NH4+ were produced, while only 0.5, 2.6, or 6.7% of NH4+ were oxidised to NO3−, respectively (Fig. 3B, 3C). After 3 days, the Gly utilisation from 1 M NO3− and 1 M glucose increased to 97 and 99%, respectively (Fig. 3A), but Gly utilisation from 1 M NH4+ remained at 85%. In addition, almost all Gly was consumed (Fig. 3D).

After the addition of 1 M NO3−, Gly, and glucose, the microbial utilisation of 15NH4+ increased to 89, 69, and 71%, respectively, while the utilisation of 15NH4+ without C or N substrates was 52% within 15 min (Fig. 4A). After the addition of 1 M NO3−, Gly, and glucose for 3 days, microbial uptake of NH4+ increased to 96, 89, and 99.9%, respectively (Fig. 4A). With the increase in microbial biomass, the produced NO3− was also taken up by microorganisms (Fig. 4C). Furthermore, 1.5% of the total 15N addition in OM produced by MB was released into the soil after 9 days (15NH4+ + 1 M Gly; Fig. 4D).

Within 15 min, the uptake of NO3− by microorganisms was not affected by the addition of 1 M Gly and glucose, of which 63 and 66% were incorporated into the microbiome, respectively (Fig. 5A). However, 94% of 15NO3− was taken up by the microorganisms following the addition of 1 M NH4+. After 3 d, 94, 90, and 99% of NO3− were taken up by the soil microbiome from 1 M NH4+, Gly, and glucose, respectively (Fig. 5A). Furthermore, the content of NO3− remaining in the soil from 1 M NH4+, 1 M Gly, and 1 M glucose decreased to 4.0, 7.9, and 0.6%, respectively (Fig. 5C). In addition, 0.4 and 1.1% of the total 15N addition were immobilised as OM (Fig. 5D).

**Discussion**

***Mechanisms underlying microbial utilisation of Gly, NH4+, and NO3− over time***

The cycling of Gly in the soil occurred in three steps in the short-term culture (Fig. 2): (1) The added Gly was rapidly depleted from the soil (Fig. 2A) and was rapidly taken up by the microbiome (67% within 15 min). Due to all Gly was consumed within 15 min, the rapid use of Gly may occur in a shorter period of time [within 1–2 min (Hill and Jones 2019)]. (2) Once taken up by the microbiome, a high ratio of the N caused the release of NH4+. Microorganisms strip N from Gly to produce pyruvate, which can be used in the tricarboxylic acid (TCA) cycle for energy and growth (Kuzyakov and Xu 2013; Zhang et al. 2022). The NH4+ was released in the form of other metabolites to ensure the stoichiometric balance of microorganisms (Pan et al. 2022). Ma et al. (2021a) showed that the utilisation and mineralisation of different amino acids by microorganisms are rapid processes; methionine, cysteine, and glutamate were rapidly mineralised and excreted by the microbial cells, resulting in 28, 34, and 50% of 15NH4+ being rapidly returned to the soil within 15 min, respectively. (3) The released NH4+ was subsequently reutilised by the microbial community to support growth (Fig. 1). The increase in microbial biomass is the main reason for the reutilisation of released NH4+ by microorganisms. Microorganisms need sufficient N to make complex compounds, such as extracellular enzymes, for digging up soil N or breaking down SOM (Zhang et al. 2022).

The transfer of NH4+ in microorganisms also occurred in three steps: (1) 50% of the added 15NH4+ was rapidly taken up by the microorganisms (within 15 min). Then, NH4+ utilisation decreased from 50 to 37% within 1 h, while the NH4+ in the soil increased to 42% (Fig. 2). (2) Excess NH4+ was initially taken up by microorganisms and subsequently released back into the soil. Microorganisms likely released N from 15NH4+ to avoid reductions in microbial activity caused by excess NH4+ absorption (Ma et al. 2021b). (3) The increase in microbial biomass with time forces the microbe to require more N to meet its N requirement, thereby promoting the reuse of the released NH4+. The N from 15NH4+ utilised by microorganisms reached 81% by the end of the incubation period. Gly rather than NH4+ was more readily available to microorganisms during the entire incubation period (Fig. 2). The uptake of NH4+ must be accompanied by a supply of C sources from the soil to maintain microbial cellular activity (Wilkinson et al. 2014); the uptake of C and N from Gly is an efficient process reducing the need for additional C sources.

Interestingly, 15NO3− was rapidly taken up by microorganisms (within 15 min), with a utilisation rate comparable to that of 15N-Gly (Fig. 2A). However, the utilised 15NO3− was not released back into the soil. Based on the limited production of 15N-OM (Fig. 2D), we hypothesise that most of the NO3− was stored in the cytoplasm rather than being assimilated by microorganisms. Storage of NO3− was brought about by the following: 1) alternative sources of highly bioavailable N, such as Gly and NH4+, may be preferentially utilised to fulfil the N demand (Geisseler et al. 2010); 2) (González et al. 2006); 3) microorganisms must store an N source to cope with the effect of potential nutrient changes in the soil (Mooshammer et al. 2014). Consequently, the uptake processes of Gly and NH4+ are similar; however, the absorption process of NO3− is largely different, and NO3−is not released back into the soil (Fig. 6).

Owing to the differences in the molecular structure and transport of Gly, NH4+, and NO3−, the metabolic pathways involved may explain the relative difference in the utilisation rates of these three N sources. When substrates are assimilated into microorganisms, their original molecular structures determine the derived biomolecules (Xu et al. 2014). Gly is a C-containing organic substrate, and its decomposition could supply energy to support microbial activity, whereas the assimilation of NH4+ and NO3− requires energy. Gly induces C and N assimilation in microorganisms. Gly, a building block of proteins, serves as a precursor to complex organic molecules such as purines (Pedley and Benkovic 2017) and is involved in the synthesis of important substances (Hong et al. 2020). After Gly is ingested by microorganisms, it is converted to serine by serine hydroxymethyltransferase (SHMT), followed by deamination to pyruvate and release of NH4+, producing a major central carbon metabolite (Cheng et al. 2019; Hong et al. 2020). Wu et al. (2015) found that the enhancement of Gly-operated metabolism promotes the biosynthesis of fatty acids and upregulates TCA cycle, which can promote bacterial growth (Peng et al. 2015). By comparison, NH4+ is required to convert N-containing compounds via the glutamine synthetase/glutamate synthase pathway and NO3− must be reduced to NH4+ before being utilised by microorganisms (Patriarca et al. 2002). Overall, both Gly and NH4+ can be released after being taken up by microorganisms. Subsequently, the released N is reutilised by the microorganisms. NO3− remained in the microbiome as reserve N.

During the 9-day experiments, < 0.2% of the 15N-OM was produced from 15N-Gly, 15NH4+, and 15NO3− and released by microorganisms (Fig. 2D). Further long-term experiments should be conducted to explore in detail the organic N produced by the addition of 15N-Gly, 15NH4+, and 15NO3− and subsequent release by microorganisms. In addition, the results of the present study were based only on the current analysis; further studies involving isotopic labelling combined with metabolomics are warranted to advance our understanding of the transformation pathways of Gly, NH4+, and NO3− during microbial metabolism and the effects of these substrates on soil N cycling (Fig. 6).

***Effects of excess C or N source on the utilisation of Gly, NH4+, and NO3− by microorganisms***

Organic N uptake by microorganisms is subject to N and C regulation (Li et al. 2021). Our results showed that microorganisms preferentially utilise organic N to meet their C demand even when the concentrations of NH4+ or NO3− in the soils was high (Fig. 3A). When C is available mainly in the form of molecules that also contain N, such as amino acids and peptides, microorganisms can take up the organic molecules directly even when NH4+ is available at high concentrations; this process allows for the fulfilment of C demand from organic N sources (Yang et al. 2016). However, when C is readily available in the form of carbohydrates, the C of organic N sources is not required; in these cases, NH4+ becomes the preferred source of N, and extracellular deamination takes precedence (Pinggera et al. 2015). After the addition of 1 M glucose, microorganisms could still rapidly take up Gly, and the N utilisation rate increased within 15 min. Our results indicated that soil microorganisms can quickly assimilate amino acids regardless of the presence of readily available C sources. Consistent with these findings, Ma et al. (2021a) reported that microbes can rapidly assimilate cysteine and methionine even in the presence of high amount of C or S. Owing to the addition of high amounts of bioavailable C to the soil, the shift from C to N limitation can also promote the efficient assimilation of N (Mooshammer et al. 2014). Moreover, the addition of excess amounts of N and C sources can increase microbial demand owing to an increase in MBC (Fig. 1) (Blagodatskaya and Kuzyakov 2008).

The utilisation of 15NH4+ increased by ~30% within 15 min after the addition of 1 M of Gly, glucose, and NO3− (Fig. 4). The microbial biomass increased with the addition of C and N, which reflects the need for a high rate of microbial assimilation of NH4+. The input of these organic substances may trigger soil microorganisms from dormancy to activity (Brant et al. 2006). Once soil microbes are activated, nutrient requirements, such as NH4+, will increase with microbial growth and proliferation (Brant et al. 2006). Ding et al. (2021) also found that organic C input into soils influences the amount of inorganic N immobilisation. By contrast, the utilisation of 15NO3− did not increase at 15 min after the addition of 1 M Gly and glucose (Fig. 5). As stated above, microorganisms may preferentially take up amino acids and NH4+ rather than NO3− since NO3− must be converted into NH4+ by enzymes before being utilised (González et al. 2006). Therefore, the overall uptake of 15NO3− did not increase; however, the assimilation of NH4+ and Gly increased in the presence of readily available C and N.

***N use efficiency of different N forms over time***

In previous studies, microbial N use efficiency (NUE) is typically determined at a single time point (Mooshammer et al. 2014; Li et al. 2021), which may lead to over- or underestimation of NUE. The results of this study provide insights into how microbial NUE changes over time (Fig. 2A). The NUE of microorganisms from 15N-Gly initially increased sharply, followed by a small decrease and subsequent continuous increase for the remaining period (Fig. 2A). When N is limited, microorganisms retain most of the fixed organic N (high NUE), and N mineralisation is low (Mooshammer et al. 2014). By contrast, when the metabolic control of microbial decomposers switches from being N- to C-limited (Li et al. 2021), high amounts of N are released as NH4+ (low NUE), which was a suitable short-term mechanism. In the 15NH4+ treatments, the released NH4+ could be reused by microorganisms, and the utilisation of 15NH4+ reached 81% at the end of incubation. Throughout the incubation period, the utilisation rate of 15NH4+ was lower than that of 15N-Gly. Compared with NH4+, Gly can be used more efficiently by microbes because it is a small-molecule C–N coupling compound, and microorganisms do not need to take up other C sources or only require less C to support their growth and activities (Farrell et al. 2014; Ma et al. 2020a). Thus, microbial NUE changes with time, and different N sources could affect the NUE of microbes.

**Conclusions**

Microorganisms take up Gly, NH4+, and NO3− from the soil within minutes; intracellular mineralisation of Gly, NH4+ release, and reutilisation occur within hours. The utilisation of Gly was higher than that of NH4+ since microorganisms prefer to utilise Gly. NO3− was not released after being taken up by microorganisms; only a small amount of 15NH4+ was released into the soil, and no 15N-OM was produced, indicating that NO3− was used as an N storage source by microbes. The NUE of microorganisms from Gly and NH4+ changed over time. When the availability of C and N was high, the utilisation of Gly and NH4+ increased by 30 and 20%, respectively, whereas Gly mineralisation and nitrification decreased. The utilisation of NO3− was not affected by the addition of 1 M Gly and glucose within 15 min, but it increased within 3 days. Hence, the short-term utilisation of organic N by soil microorganisms was higher than that of inorganic N. The process of NO3− utilisation differed from that of Gly and NH4+, and the excess C or N availability in the soil can promote the uptake of Gly and NH4+.

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**Declaration of competing interest:** The authors declare no competing interests.

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**Figure captions:**

**Fig. 1** Microbial biomass C (MBC) in the soil over time. Symbols and error bars represent the mean ± standard error of the mean (SEM; *n* = 4); results are expressed on a dry soil weight (DW) basis. Gly, glycine.

**Fig. 2** Transformation of N from added 15N-Gly, 15NH4+, and 15NO3− in the soil. N assimilation into microbial biomass (A), remaining NH4+ in the soil extractable pool (B), remaining NO3− in the soil extractable pool (C), and the production of organic matter (OM) by microbiome (D) were determined for 15N-Gly, 15NH4+, and 15NO3− during the incubation period (15 min, 1 h, 6 h, 24 h, 3 d, and 9 d) after the addition of tracers. Symbols and error bars represent the mean ± standard error of the mean (SEM; *n* = 4). Capital letters indicate significant differences in the abundance of 15N-Gly, 15NH4+, and 15NO3− at the indicated time points, while lowercase letters indicate significant differences in each treatment over time.

**Fig. 3** Transformation of N from 100 μM 15N-Gly with 1 M NO3− or NH4+ or glucose in the soil. N assimilation into microbial biomass (A), remaining NH4+ in the soil (B), remaining NO3− (C), and the production of organic matter (OM) by the microbiome (D) were determined for 15N-labelled Gly, NH4+, and NO3− during the incubation period (15 min and 3 days) after the addition of tracers. Symbols and error bars represent ± standard error of the mean (SEM; *n* = 4). Capital letters indicate significant differences in the 15N-Gly + 1 M NO3−, 15N-Gly + 1 M NH4+, 15N-Gly + 1 M glucose, and 15N-Gly at the indicated time points, while lowercase letters indicate significant differences in each treatment over time.

**Fig. 4** Transformation of N from 100 μM 15NH4+ with 1 M NO3− or Gly or glucose in the soil. N assimilation into microbial biomass (A), remaining NH4+ in the soil (B), remaining NO3− (C), and the production of organic matter (OM) by the microbiome (D) were determined for 15N-labelled Gly or NH4+ or NO3− during the incubation period (15 min and 3 days) after the addition of tracers. Symbols and error bars represent the mean ± standard error of the mean (SEM; *n* = 4). Capital letters indicate significant differences in the concentration of 15NH4+ + 1 M NO3−, 15NH4+ + 1 M Gly, 15NH4+ + 1 M glucose, and 15NH4+ at the indicated time points, while lowercase letters indicate significant differences in each treatment over time.

**Fig. 5** Transformation of N from 100 μM 15NO3− with 1 M NH4+,or Gly, or glucose in soil. N assimilation into microbial biomass (A), remaining NH4+ in the soil (B), remaining NO3− (C), and production of organic matter (OM) by the microbiome (D) were determined for 15N-labelled Gly or NH4+ or NO3− during the incubation period (15 min and 3 days) after the addition of tracers. Symbols and error bars represent the mean ± standard error of the mean (SEM; *n* = 4). Capital letters indicate significant differences in the abundance of 15NO3−+ 1 M NH4+, 15NO3−+ 1 M Gly, 15NO3− + 1 M glucose, and 15NO3− at the indicated time points, while lowercase letters indicate significant differences in each treatment over time.

**Fig. 6** Simplified mechanism of N cycling of Gly, NH4+, and NO3− in the soil. Gly, NH4+, and NO3− are rapidly taken up through different metabolic processes. In the soil, Gly (67%) and NH4+ (50%) were rapidly immobilised into the microbial biomass (within 15 min). Concurrently, 33% of Gly was mineralised to NH4+, whereas 16% of 15NH4+ was oxidised to NO3−. Within 1 h, 8 and 13% of Gly and NH4+, respectively, were released. The released NH4+ was reutilised by microorganisms. NH4+ production decreased over time, while NH4+ was partly oxidised to NO3−. Approximately 60% of NO3− was rapidly taken up by microorganisms and stored in the cytoplasm without subsequent release. Gly was taken up by microorganisms and converted into serine (by serine hydroxymethyltransferase, SHMT), releasing NH4+ after deamination; serine was converted into pyruvate and entered the tricarboxylic acid cycle (TCA) cycle.