



## Nitrogen compounds in soil solutions of agricultural land

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### ABSTRACT

Plants are capable of taking up nitrogen (N) in both organic and inorganic forms, so the concentrations and relative proportions of different N forms in soils are likely to be important determinants of their N nutrition. Therefore, there is a need for greater knowledge of the N profiles of soils. In the study presented here we examined the potential plant-available N in soils from four sites with various agricultural histories (one recently fertilized), using small tension lysimeters to collect free and bound amino acids and inorganic N forms in solution, with minimal soil disturbance and with intact plants present. Subsequent analysis showed that concentrations of free amino acids ranged from 0.1 to 12.7  $\mu\text{M}$ , whereas concentrations of bound amino acids were on average 50 times higher, and higher than ammonium and nitrate concentrations in all three unfertilized soils. In contrast, nitrate strongly dominated in the fertilized soil. Bound amino acids are likely to represent a potential replenishment pool for free amino acids, so the abundance and rate at which amino acid-containing substances are depolymerized might be important determinants of the availability of free amino acids. Our results highlight the need for further research on the liberation of free amino acids from polymers in agricultural soil, and the importance of bound amino acids as N sources for plants.

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### 1. Introduction

With carbon dioxide concentrations increasing in the atmosphere, it is becoming increasingly important to elucidate the connections between the carbon (C) cycle and the nitrogen (N) cycle. It is also important to understand the processes controlling the C:N ratios of photosynthetic organisms (Gruber and Galloway, 2008), and the importance of various available N sources for photosynthetic organisms, since plants take up N not only in inorganic forms, but also in organic forms (Näsholm et al., 2009). Most importantly, perhaps, a wide range of plant species, including those inhabiting both natural and managed ecosystems, have been shown to take up amino acids (Kielland, 1994; Näsholm et al., 1998, 2000, 2001; Persson and Näsholm, 2001; Raab et al., 1996, 1999; Schimel and Chapin, 1996; Schmidt and Stewart, 1999). Some of the transporters involved in amino acid uptake have been identified recently, and some information on the molecular mechanisms involved in the process has been acquired, using the model plant *Arabidopsis thaliana* (Hirner et al., 2006; Lee et al., 2007;

Svennerstam et al., 2007, 2008; Forsum et al., 2008). The ability of plants to acquire N in both organic and inorganic forms suggests that concentrations and relative proportions of these N forms in soils are important determinants of plant N nutrition, and thus plant C:N ratios and interactions. However, while the relative proportions of various N forms in soils of natural ecosystems have been measured in many studies, fewer have investigated corresponding patterns in agricultural soils (but see Owen and Jones, 2001; Jones et al., 2005; Jones and Willett, 2006).

Soil N is dominated by organic forms and approximately 40% of total soil N is present in the form polymers such as proteins and peptides (Schulten and Schnitzer, 1997). The polymers in dissolved organic N (DON) in soils play a key role in soil N fluxes, since depolymerization of these compounds by extracellular enzymes yields short peptides and free amino acids (FAAs), and the amino acid reserves in the soil solution are often higher than those of ammonium and nitrate (Young and Aldag, 1982). Due to their high abundance, proteins may be the largest and most reliable source of FAAs (Lipson and Näsholm, 2001). Further, since proteins are the major inputs of N into soil solution, depolymerization of these organic macromolecules to monomeric DON may be considered rate limiting for the overall N cycle in soils (Schimel and Bennett, 2004). Accordingly, Kielland et al. (2007) found that concentrations of FAA (Werden-Pfisterer et al., 2009) and proteins, as well as protease activity, increased along a forest successional gradient,

*Abbreviations:* BAA, Bound amino acid(s); DON, Dissolved organic nitrogen; FAA, Free amino acid(s); IN, Inorganic nitrogen; ON, Organic nitrogen.

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suggesting that soil FAAs are mainly derived from the depolymerization of soil proteins.

Amino acid turnover in soils is very rapid, half-lives may be as long as 20 h in soils with no plants, but they are usually less than 3 h and may be less than 1 h (Jones, 1999; Lipson et al., 2001; Owen and Jones, 2001; Jones and Kielland, 2002; Jones et al., 2005, but see Jones et al., 2009). Consequently, it is important to ensure that the technique used for sampling soil solutions causes minimal disturbance, and hence minimal changes to the concentrations of the measured compounds during sampling and processing. If care is not taken, there is a risk of overestimating or underestimating the soluble N pools due to the production and/or decomposition of N compounds during sampling and handling of the samples. Recent studies suggest that sampling soil solutions using small tension lysimeters may avoid, or at least minimize these problems (Andersson, 2003; Andersson and Berggren, 2005; Roberts and Jones, 2008).

The aim of the present study was to characterize the soil solution N pool in agricultural soils with various use histories and productivity levels. For this purpose we installed small tension lysimeters at four sites with different histories to sample soil solutions with minimal disturbance to the plant–soil systems and with intact plants present. The potentially plant-available forms of N in these soils were then characterized by analyzing, quantitatively and qualitatively, FAAs and BAAs, as well as inorganic N (IN) forms in the soil solution.

## 2. Materials and methods

### 2.1. Study sites

Soil solutions were collected in 2007 and 2008 from four sites with different agricultural histories and current uses. The first site (designated the *thinned birch forest* site) was at Timrå (mid-Sweden), on an east-facing slope (62.532 °N, 17.549 °E, 75 m a.s.l.) with loamy sand soil (Table 1). The sampled area was previously a pasture that had been abandoned ca. 40 years ago and has subsequently been invaded by deciduous trees, mainly *Betula pubescens* Ehrh., with some *Populus tremula* L. and *Salix caprea* L. The tree stand was thinned five years ago. The second sampling site (*old grassland*) was also at Timrå (62.530 °N, 17.552 °E, 80 m a.s.l.), and was an old extensively used grassland on a northeast-facing slope. This site has been harvested for forage once a year, but has not been ploughed during the latest ca. 20 years. Since the yield has been declining to a very low level, the crop has been left in the field for the last ca. 5 years. The third site (*organically grown ley*) was at Ångersjö, Umeå, in northern Sweden (63.631 °N, 19.786 °E, 25 m a.s.l.), where we sampled a 4-year old clover-grassland, now strongly dominated by timothy (*Phleum pratense* L.), with a sandy loam soil (Table 1). The fourth site (*organically grown iceberg lettuce*) was at the same location as the organically grown ley, but the ley had been ploughed two years previously and is now cultivated with iceberg lettuce (*Lactuca sativa* L.) on ridges. The lettuce was planted at the beginning of June and was fertilized with 8 ton ha<sup>-1</sup> of chicken manure (corresponding to 104–132 kg total

N ha<sup>-1</sup> and 57–63 kg ammonium–N ha<sup>-1</sup>), and K and Mg sulfates (Kalimagnesia). At each of the four sampling sites, 15 small tension lysimeters (see below) were installed at 1 m intervals along a transect in the birch forest, at 5 m intervals along a horizontal contour in the old grassland, and at 0.6 m intervals along both a transect in the ley and along a ridge with iceberg lettuce parallel to the sampling line in the ley.

### 2.2. Collection of soil solution

Soil solutions were collected during the growing seasons (May–September) in 2007 and 2008 on five occasions from the thinned birch forest and the old grassland sites, and in only 2008 (June–October) from the ley and lettuce sites, using small tension lysimeters (P2.30-1, Rhizon Soil Moisture Samplers; Eijkelkamp, Giesbeek, The Netherlands), 10 cm long, 2.5 mm diameter, with 0.1 µm diameter pores. According to the manufacturer suction of soil solution is achieved between 20 and 50 kPa. The lysimeters were installed at a 45° angle to the soil surface and spanned the soil depth 2–9 cm. At installation, 2 l of water was sprinkled over a circular area of approximately 0.1 m<sup>2</sup> on the soil surface above each lysimeter to promote close contact between it and the soil. All lysimeters remained in the soil throughout the experiment. At each soil solution collection occasion the lysimeter cap was replaced by a sterile needle and a pre-evacuated sterile 10 ml glass tube (BD Vacutainer™ Z) fitted with a rubber membrane stopper. Liquid was collected over a 12 h period in the daytime (at the birch forest and old grassland sites) or overnight (at the ley and lettuce sites). Tubes were covered with aluminum foil or shaded by vegetation during collection. At the end of collection the lysimeters were immediately closed with sterile caps. The temperature at the tubes was measured at the start and end of each collection (5–17 °C). Tubes with the collected liquid were kept in a cool box during transport (ca. 1 h) and then immediately frozen at –20 °C awaiting analysis.

### 2.3. Soils

Soil samples were taken from the soil depth spanned by the lysimeters. Five samples of ca. 0.5 l, taken along the lines of lysimeters, were pooled into a single sample from each of the sampling areas. Samples were collected in early September (from the birch forest and old grassland sites) and early October (from the ley and lettuce sites). The soil samples were kept cool during transportation and then stored in a cold-room, about 5 °C, for four weeks (birch forest and old grassland soil samples) or overnight (ley and lettuce site soils) before being analyzed. Each soil sample was sieved through a 2 mm mesh and a representative subsample of ca. 0.5 l was sent to Agrilab AB, Uppsala, Sweden, for analysis of its soil texture, pH, and contents of humus and macro nutrients. Texturally, the soils were all dominated by sand, which accounted for more than 75% of the soil from the ley and iceberg lettuce sites (Table 1). The grassland soil contained nearly two times more carbon, humus and N than the other soils. The pH was similar (5.7–6.0) in all soils. The nutrient analyses showed that soluble K,

**Table 1**  
Properties of the four soil types (2–9 cm depth) studied. Soil texture is given as the proportion of dry weight and nutrients are given in mg g<sup>-1</sup> dry soil. AL, soil extracted in ammonium lactate; HCl, soil extracted in HCl. Data are from five pooled soil samples from each vegetation type.

Vegetation type	Humus (%)	Clay (%)	Silt (%)	Sand (%)	pH	N	C	P–AL	K–AL	Mg–AL	Ca–AL	P–HCl	K–HCl
Thinned birch forest	7.6	9.0	31.0	52.4	5.7	3.7	38.9	0.15	1.03	1.41	11.8	4.2	1.1
Old grassland	13.3	5.5	26.5	54.7	5.8	5.6	80.4	0.45	2.23	2.48	17.1	5.1	8.2
Ley	6.9	3.5	12.5	77.1	6.0	3.3	41.2	0.98	0.35	0.41	10.4	5.5	1.3
Iceberg lettuce	8.2	4.5	11.5	75.8	6.0	3.1	47.6	1.23	1.25	0.57	12.6	6.3	2.7

Mg and Ca were more abundant in the grassland soil and P was most abundant in the soil from the cultivated iceberg lettuce site.

## 2.4. Analyses

### 2.4.1. Free amino acids and ammonium

Frozen samples from the lysimeters were thawed and immediately prepared for FAA and ammonium analysis. The individual analytes were separated using an AccQ-Tag™ Ultra column and a mixture of (A) 99.9% formic acid with 0.1% ultrapure water and (B) acetonitrile in 90% ultrapure water as the mobile phase at a flow rate of 0.6 ml h<sup>-1</sup> and the following gradients: 0–5.74 min isocratic 99.9% formic acid, declining to 90.9% formic acid from 5.74 to 7.74 min, to 78.8% formic acid at 8.24 min then to 40.4% formic acid at 8.74 min, before re-equilibration with 99.9% formic acid from 8.74 to 9.50 min.

Prior to analysis, samples were derivatized with a Waters AccQ-Tag Ultra Derivatization kit for amino acid analysis, as follows. 100 µl of each soil solution sample was evaporated until dry and then dissolved in 70 µl of 1 M borate solution, then 10 µl of norvaline (100 µM), as an internal standard, and 20 µl of AccQ-Tag Reagent were added. Samples were heated for 10 min at 55 °C and then analyzed. Total FAA concentrations were calculated as the sum of the 21 amino acids measured.

### 2.4.2. Bound amino acids

BAAs (e.g. those in proteins and peptides) were analyzed after hydrolyzing the soil solution samples, as follows. 100 µl of soil solution was evaporated until dry, dissolved in 1 ml of 6 M HCl and kept at 105 °C for 16 h (Knicker, 2004). 100 µl of the HCl solution was then evaporated and the dry sample was derivatized and analyzed as above. The BAA concentration was calculated as the total amino acids in hydrolyzates minus the FAAs.

### 2.4.3. Nitrate

Nitrate concentrations were analyzed by suppressed ion chromatography (Metrohm, Herisau, Switzerland) and a Metrosep A Supp 5-150 column, with an aqueous solution of 3.2 mM Na<sub>2</sub>CO<sub>3</sub>

and 1.0 mM NaHCO<sub>3</sub> as the mobile phase at a flow rate of 0.7 ml min<sup>-1</sup>.

## 2.5. Data handling

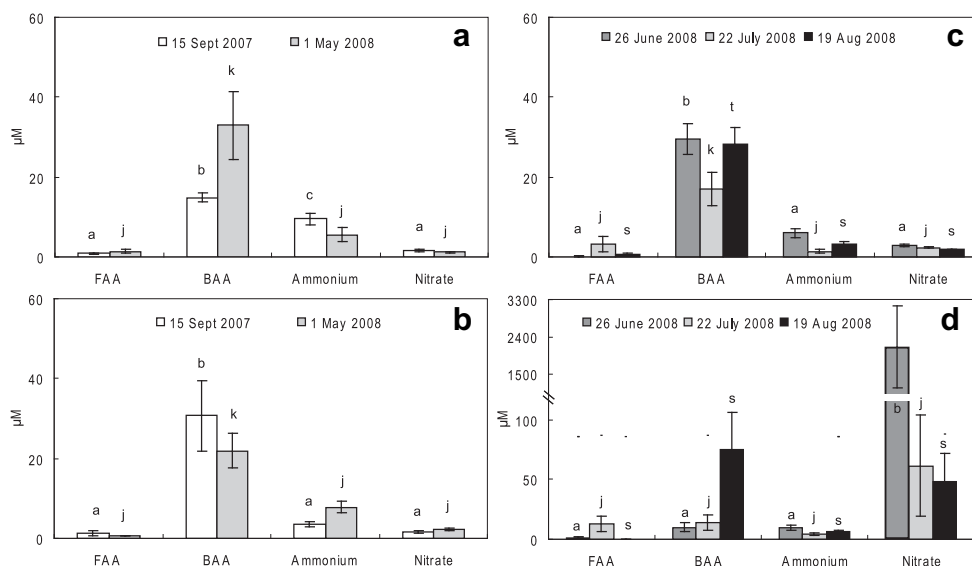
The volumes of the soil solutions collected from the lysimeters varied both among the lysimeters and among sampling occasions, thus it was not possible to obtain repeated measurements of the samples collected from single lysimeters, and the presented results are based on the lysimeter samples for which the volume collected was sufficient (≥4 ml) for analysis of all studied compounds (FAAs, BAAs, ammonium and nitrate). To avoid pseudo-replication, samples were randomly chosen from different (independent) lysimeters on different sampling occasions. The resulting data are from soil solutions sampled in September 2007 and May 2008 from the birch forest and old grassland sites, and in June, July and August 2008 from the ley and lettuce sites. There was no correlation between sampled soil solution volumes and the concentrations we found (data not shown).

Statistically significant differences between different soil N pools were tested with one-way ANOVA (one-way ANOVA,  $p < 0.05$  and Tukey's test,  $p < 0.02$ ) for each soil type and date. For seasonal mean values statistically significant differences between each soil N pool was tested for each soil type (one-way ANOVA,  $p < 0.05$  and Tukey's test,  $p < 0.02$ ). Samples were sampled from a single site of each vegetation type and therefore no analyses of differences between vegetation types and soil N pools were carried out.

## 3. Results

FAA concentrations were generally low (individual values ranged from 0 to 29 µM) in the soil solutions from all four sites and from all sampling dates; mean values are shown in Fig. 1. Furthermore, the FAA concentration within each soil type did not differ significantly ( $p > 0.05$ ) between sampling dates (Fig. 1).

Among the N forms, BAA concentrations were by far the highest, with individual values ranging from 2 to 167 µM, and were on average 50 times higher than the FAA concentrations, in soil from



**Fig. 1.** Concentrations of free amino acids, bound amino acids, ammonium and nitrate in soil solutions repeatedly sampled at four sites with different histories and vegetation types: a) thinned birch forest; b) old grassland; c) organically cultivated ley; and d) organically cultivated lettuce. Mean  $\pm$  SE,  $n = 4$ . Note broken scale on the Y-axis (d). Statistically significant differences between soil N pools were tested for each soil type and date (one-way ANOVA,  $p < 0.05$  and Tukey's test,  $p < 0.02$ ).

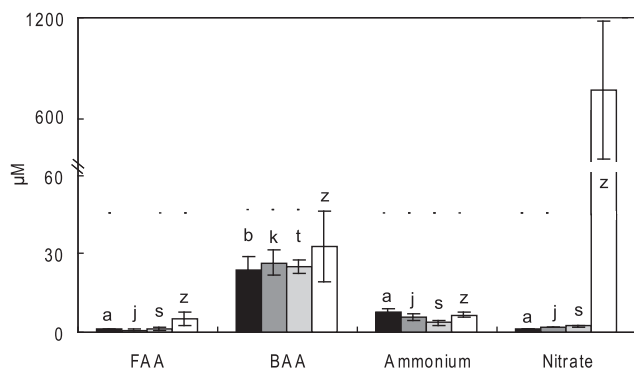
all four sites and on all occasions (Fig. 1a–c), except for nitrate at the lettuce site in June and July (Fig. 1d).

Ammonium concentrations were low (0.6–14.9  $\mu\text{M}$ ), although they were slightly higher than the FAA concentrations in the soil solution from all four sites (Fig. 1). The difference between ammonium and FAA concentrations was only significant in September in the birch forest (Fig. 1a). Ammonium was the second largest N pool in all sampled soils, except the soil from the lettuce site.

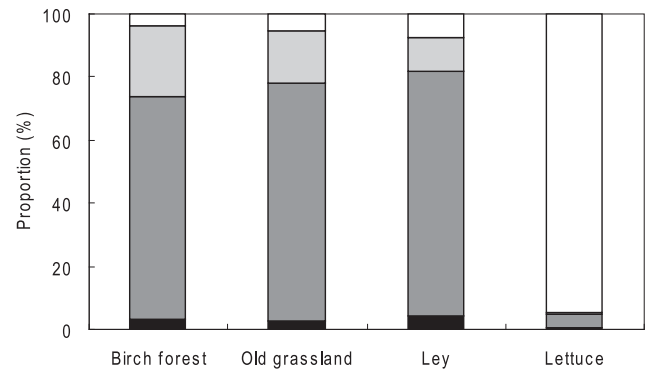
Concentrations of nitrate (0.9–3.4  $\mu\text{M}$ ) were slightly lower than ammonium concentrations, but did not differ significantly from them, except in the birch forest soil in September (Fig. 1a–c). In contrast, the lettuce soil solution had a very high nitrate concentration (mean value 2200  $\mu\text{M}$ ) in June, shortly after fertilization, but the concentration successively decreased during the season (Fig. 1d). At the last sampling date, in August, the BAA concentration was higher than the nitrate concentration at the lettuce site, as well as at the sites with the other histories and vegetation. No significant differences were found between the concentrations of BAA and the other N compounds on this occasion, probably due to the large variance and the limited number of replicates. At the lettuce site, BAA concentrations increased considerably from July to August, while nitrate concentrations were lowest in August (Fig. 1d).

Generally, the concentrations of each type of N compound in the soil solutions from each site were similar, and the concentrations of BAAs were high, as shown by the seasonal means (Fig. 2). The N sources were ranked, in order of concentration, as follows: BAA  $\gg$  ammonium  $>$  nitrate and FAAs, except for nitrate under the lettuce at the beginning of the season. Although the FAA concentrations were the lowest of all the N sources, they were still in the same order of magnitude as ammonium and nitrate concentrations in the birch forest, old grassland and organically cultivated ley sites.

The proportions of FAAs, BAAs, ammonium and nitrate, based on seasonal mean concentrations (expressed as  $\mu\text{M}$  of each compound) found in each soil, are shown in Fig. 2. Deamination of bound glutamine and asparagine during hydrolyzation prevents the calculation of BAA–N, but the proportion of BAA–N is probably equal to or larger than that shown in Fig. 3. In the soil solution under the thinned birch forest, old grassland and organically cultivated ley, BAAs constituted approximately 75% of the total N compounds (Fig. 3).



**Fig. 2.** Seasonal concentrations of free amino acids, bound amino acids, ammonium and nitrate in soil solutions collected under thinned birch forest (black bars), old grassland (dark grey bars), organically cultivated ley (light grey bars) and organically cultivated lettuce (white bars). Mean  $\pm$  SE,  $n = 8$  (thinned birch forest, old grassland),  $n = 12$  (lettuce, ley). Note broken scale on the Y-axis. Statistically significant differences between each soil N pool were tested for each soil type (one-way ANOVA,  $p < 0.05$  and Tukey's test,  $p < 0.02$ ).

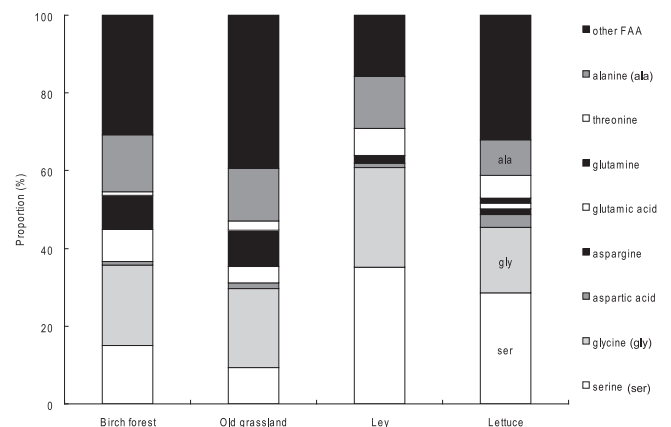


**Fig. 3.** Proportions of free amino acids, bound amino acids, ammonium and nitrate in soil solutions, based on seasonal means (in  $\mu\text{M}$ ) for free amino acids (black bars), bound amino acids (dark grey bars), ammonium (light grey bars) and nitrate (white bars).  $n = 8$  (thinned birch forest, old grassland),  $n = 12$  (lettuce, ley).

The relative proportions of individual amino acids in the FAAs (Fig. 4) showed similarities, but also some differences, to the proportions in the total BAA pool (Fig. 5). The most abundant FAAs were serine, glycine and alanine, which collectively accounted for approximately 56% of the total FAAs. The most abundant BAAs were glycine, serine, alanine, aspartic acid + asparagine and glutamic acid + glutamine, which accounted for approximately 60% of the BAAs. The FAA composition in the soil solution from the birch forest and the old grassland were similar, as were the FAAs in the ley and lettuce soil solutions. The most obvious difference between these two groups was that the samples from the birch forest and old grassland all contained free gamma-amino butyric acid (gaba), but this was not found in the soil solutions from the ley or lettuce sites (data not shown). The proportions of individual amino acids in the BAAs were similar in soil solutions from all four sites (Fig. 5).

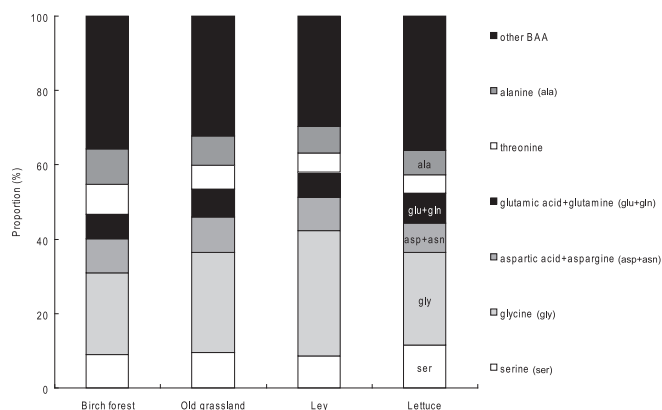
#### 4. Discussion

The aim of the presented study was to characterize the N fractions in soil solutions from four different agricultural land types, using small tension lysimeters to collect soil solutions with minimal soil disturbance. Earlier studies using this technique (e.g. Andersson, 2003; Andersson and Berggren, 2005; Roberts and Jones, 2008) have shown that this method causes very little



**Fig. 4.** Proportions of individual free amino acids in solutions from each soil type. Other free amino acids are the sum of histidine, arginine, gaba, proline, ornithine, lysine, tyrosine, methionine, valine, isoleucine, phenylalanine and tryptophane. Values are seasonal means, where  $n = 8$  (thinned birch forest, old grassland) and  $n = 12$  (organically cultivated ley, lettuce).





**Fig. 5.** Proportions of individual bound amino acids in solutions from each soil type. Other bound amino acids are the sum of histidine, arginine, gaba, proline, ornithine, lysine, tyrosine, methionine, valine, isoleucine, phenylalanine and tryptophane. Values are seasonal means, where  $n = 8$  (thinned birch forest, old grassland) and  $n = 12$  (organically cultivated ley and lettuce).

alteration of the chemical composition of collected samples, allowing *in situ* levels of organic nitrogen (ON) and IN compounds to be conveniently investigated. However, the soil must have a sufficiently high moisture content for the lysimeters to collect sufficient volumes of solution for analysis. We found that of the four N fractions studied, soil solutions of all the studied ecosystems were dominated by BAAs, while FAAs accounted for only a minor fraction (Fig. 1). The fraction of IN in the studied soil solutions was generally lower than the ON fraction, but fertilization gave high and variable nitrate concentrations, occasionally as high or even higher than the organic fraction of the soil solution N pool.

It is likely that BAAs represent a potential replenishment pool for FAAs and the rate at which BAAs are depolymerized should therefore be an important determinant of the availability of FAAs over the long term. Moreover, plants have been shown to take up both peptides and amino acids (Komarova et al., 2008; Paungfoo-Lonhienne et al., 2008; Näsholm et al., 2009), suggesting that BAAs may play important roles as both direct and indirect N sources for plants. The concentration of BAAs was similar in all soils studied, irrespective of vegetation type (Fig. 2) and their total N and humus contents (Table 1). In three of the four studied soils, BAA concentrations were higher than IN concentrations (Fig. 2), in accordance with the summary by Young and Aldag (1982), who also found amino acid reserves to be higher than those of ammonium and nitrate in several types of soil. We applied acid hydrolysis to soil solutions collected with lysimeters (pore size 0.1  $\mu\text{m}$ ), thus our BAA analysis was limited to soluble N compounds, which would generally be largely peptides and polypeptides. In contrast, most studies of BAAs have not targeted compounds present in soil solutions, but instead have focused on the total amount of BAAs in soils, i.e. they have included the BAAs in the solid soil fraction (Sowden et al., 1977; Stevenson, 1982; Schulten and Schnitzer, 1997; Senwo and Tabatabai, 1998). Such analyses will also inevitably include living microbial biomass as well as fractions of fine roots, and thus are likely to overestimate soluble BAA concentrations (Roberts and Jones, 2008).

In all of the soils we studied, despite the differences in vegetation types, the amino acid composition of the BAA fraction was dominated by six amino acids – serine, glycine, aspartic acid/asparagine, glutamic acid/glutamine, threonine and alanine (Fig. 5) – which accounted for nearly 70% of the total BAA pool in all soils. The similarity of the composition of BAAs suggests that this N pool has common origins, irrespective of soil type. In contrast, variations

in BAA compositions have been found in hydrolyzates of different soil types (Sowden et al., 1977; Senwo and Tabatabai, 1998; Friedel and Scheller, 2002). Increases in abundance of glycine, alanine and threonine have been found during organic matter decomposition (Rovira et al., 2008) and are thought to be linked to their abundance in resistant structures, including the cell walls of bacteria, fungi and plants. Although we cannot explain the high concentrations of glycine in our studied soil solutions, it is interesting to note that glycine is the most abundant amino acid in peptidoglycan, which is found in the cell wall of some bacteria and is also a constituent of glycine-rich proteins associated with plant cell walls (Stevenson, 1982; Rovira et al., 2008). A high fraction of bacterial proteins in soil is also supported by a previous proteomic study of plant debris decomposition, which showed that the amount of microbial protein can increase over time (Schulze, 2005).

As found for BAAs, serine, glycine and alanine were relatively abundant FAAs in all of the soils we studied. However, FAA concentrations in the soil samples from all of the sites were consistently low (Figs. 1 and 2), and in a similar range to those found in our previous studies of soil solutions in agricultural fields cropped with barley (Jämtgård et al., 2008), and other studies of agricultural soils (Jones et al., 2005). Comparisons with FAAs in natural ecosystems are complicated by the small number of studies on undisturbed soil, and by results being generally expressed in relation to soil dry matter. However, higher concentrations of FAAs (13–158  $\mu\text{M}$ ) have been extracted from alpine soils using lysimeters (Raab et al., 1999), and FAA concentrations have been found to increase with forest succession in boreal ecosystems, possibly because rates of protein degradation increase more rapidly than rates of amino acid breakdown as succession proceeds (Kielland et al., 2007). The gradient spanned by Kielland et al. (2007) was most likely larger than the one encompassed by our study. In contrast, Hofmockel et al. (2010) suggested that the activity of ammonification is more decisive than proteolysis for plant amino acid availability. However, this suggestion was based on incubated soils and therefore needs field validation. Protein breakdown in grasslands has been found to be slower than ammonification (Jan et al., 2009), which might at least partly explain the low FAA concentrations of agricultural soils.

The predominance of BAAs in the examined soils, under all vegetation types (Fig. 5), and their correlations with FAAs (Fig. 4) found in our study are comparable to findings of analyses of soil solutions from conifer forests in regions with Mediterranean climates (Yu et al., 2002) and from a fertilized boreal spruce forest (Andersson and Berggren, 2005). In our study, the size and proportion of each N pool, including ammonium, was very similar in soils from all of the examined sites, except the lettuce site (Figs. 2 and 3). The high concentration of nitrate under lettuce, especially in June, probably reflects the application of fertilizer at this time, at this site, but not any of the others (Fig. 1d).

## 5. Conclusions

By using small tension lysimeters we have repeatedly, and with minimal disturbance of the soil and with intact plants still present, characterized both ON and IN in soil solutions from different types of agricultural soil. Similar, high concentration of BAAs was found in all soil solutions, corroborating the potential importance of BAAs as N sources for plants (Komarova et al., 2008; Paungfoo-Lonhienne et al., 2008). From our results we cannot draw any conclusions about flux rates or processes involved in the N cycle, but the high BAA concentrations highlight the need for further studies of depolymerization of BAAs and consequent liberation FAAs as a potential N source for plants in agricultural soils.

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