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The potential of microdialysis to monitor organic and inorganic nitrogen compounds in soil

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ABSTRACT

Plant nitrogen (N) acquisition is strongly controlled by the concentration of available inorganic and organic N in the soil solution and by biogeochemical processes in the rhizosphere. However, until now it was hardly possible to reliably estimate plant-available N in soil microsites. Here, a novel microdialysis approach based on passive diffusion sampling is presented and compared qualitatively and quantitatively with lysimeter and soil extraction techniques when analyzing two contrasting boreal soils. Further, preliminary dialysis membrane calibration issues for sampling plant-available N compounds are discussed. Due to its miniaturized design microdialysis was shown to be a suitable tool for continuous sampling of ammonium, nitrate and free amino acids from the soil solution with only minimal disturbance of the soil structure. Microdialysis proved to be outstanding regarding the possible spatial (<0.5 mm) and temporal (<30 min) resolution of soil solution N chemistry. The different methods for soil N sampling resulted in significantly different results. In lysimeter and soil extraction samples, nitrate and ammonium were found at the highest concentrations, while results from microdialysis revealed that the pool of plant-available amino acids was contributing most to the total N pool tested. Application of a standard N solution to the tested soils led to an immediate peak of recovery via the microdialysis probes followed by a rapid decrease due to the formation of a depletion zone at the probe surfaces. Therefore, this relatively new technique will not only provide essential data on diffusion rates of a variety of N compounds in the soil but might be used for monitoring quantitative and qualitative changes in plant-available N in soil microsites such as the rhizosphere.

1. Introduction

The availability of soil nitrogen (N) largely regulates plant biomass production as well as species composition in terrestrial ecosystems and directly influences a wide range of ecological processes, both above and below ground. Especially the importance of organic N, comprising more than 80% of the total soil N pool (e.g. Schulten and Schnitzer, 1997), was increasingly recognized during the last decade (cf. Näsholm et al., 2009). A major part of this N consists of proteinaceous substances which are mostly not directly accessible for plant uptake. These polymeric N forms are, however, sources for the production of amino acids mediated by extracellular proteases (Schimel and Bennett, 2004). Gross protein depolymerization of plant litter can substantially exceed gross mineralization rates indicating that only a minor fraction of amino acids released by extracellular enzymes is actually mineralized to ammonium (Chapin et al., 1988; Fisk et al., 1998; Lipson and Näsholm, 2001; Raab et al., 1999; Wanek et al., 2010). The resulting pool of free amino acids can be taken up by plant roots and often comprises an important direct source of N for a variety of plant species in a broad range of ecosystems (Gärdenäs et al., 2010; Näsholm et al., 2009). However, plant N acquisition is strongly controlled by the concentration of available inorganic and organic N (especially amino acids) in the soil solution and by soil processes in the rhizosphere. Therefore, in order to examine the importance of individual N compounds for plant nutrition it is crucial to reliably estimate their concentrations and also their rates of replenishment in soil. Unfortunately, due to the heterogeneity and the highly dynamic nature of soils, *in situ* monitoring of plant-available N remains a difficult task and depending on the method used

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results might differ substantially (Chen and Xu, 2008). The most common techniques for investigating organic and inorganic N concentrations in soils are soil extractions with water or salt solutions, soil centrifugation or collection of soil water with tension lysimeters (e.g. Andersson, 2003; Kielland et al., 2007; Rousk and Jones, 2010). While soil extraction and centrifugation methods might be convenient in many respects, they inevitably involve disruption of the *in situ* soil structure during the sample preparation and extraction processes. There are also considerable time lags between sampling the soil and obtaining and analyzing the extracts, which could cause chemical alterations prior to analyses. Given the rapidity of N turnover in soils, in the case of amino acids with measured half-lives of usually only a few hours (Jones, 1999; Jones et al., 2005; Jones and Kielland, 2002; Kielland et al., 2007; Lipson et al., 2001; Owen and Jones, 2001; Rousk and Jones, 2010) results from soil extractions may differ significantly from actual concentrations in the soil solution. Further, depending on the extractant of choice different results may be obtained. Compared to soil extraction with water, strong salt solutions (e.g. 0.5 M K_2SO_4, 1 M KCl) will yield higher concentrations of ammonium and the less mobile, positively charged amino acids such as arginine and lysine, which are easily adsorbed to negatively charged surfaces of soil particles and soil organic matter (e.g. Lipson and Näsholm, 2001; Murphy et al., 2000; Zhong and Makeschin, 2003). However, to which extent N forms extracted with water or strong salt solutions are actually available for plant uptake remains unknown.

To obtain representative samples for analysis of organic and inorganic N concentrations in soil solution, ideally both disturbance of the plant–soil system and the risks of over- or under-estimating soluble N pools due to the production or decomposition of N compounds during sampling and handling of soil samples should be minimized. Several recent studies suggested that sampling of soil solutions using small tension lysimeters may fulfill these requirements (Andersson, 2003; Andersson and Berggren, 2005; Jämtgård et al., 2010; Roberts and Jones, 2008). This technique reduces the risk of degradation of N compounds before analysis since the likelihood of microbial decomposition of organic compounds is minimized when the solution is sampled by percolation through the small pores (0.1 μm diameter) of the lysimeter (Andersson, 2003). Still, some disturbance of the soil can be expected when the lysimeters are inserted into the soil, but these are minor in comparison with soil extraction techniques. The main obstacles of tension lysimeter sampling are the dependency on rather high soil moisture contents and the disruption of the natural soil conditions due to extraction of pore soil water. Further, similar to soil extraction techniques, results obtained from tension lysimeter sampling represent average bulk concentrations and are probably biased towards the concentrations in the largest water-filled soil pores, since pores tend to empty in order of their size, starting with the largest soil pores, in accordance with associated differences in water potential (Miro et al., 2010). Because of this, and because of the size of tension lysimeters (5–10 cm length, 2.5 mm diameter), this technique does not allow estimating N concentrations in soil microsites, e.g. in the rhizosphere.

All together, this indicates that the methods most commonly used until now are still lacking the possibility of specifically analyzing N concentrations in soil microsites without seriously disturbing the soil structure. Recently, a novel *in situ* sampling technique based on the microdialysis principle has been introduced for environmental research (Miro et al., 2010; Miro and Frenzel, 2005; Miro and Hansen, 2006; Miro et al., 2005; Sulyok et al., 2005). Microdialysis is a well established and extensively studied membrane-based technique in neurosciences and pharmacokinetic studies (e.g., Kehr, 1993; Davies, 1999), but surprisingly its use in other areas has only been recognized in the last few years. Passive microdialysis is a membrane-based sampling technique where the sole driving force for sampling the target compounds is the concentration gradient across a semi-permeable barrier (Seethapathy et al., 2008; Torto et al., 2001; Ungerstedt, 1991). Since microdialysis is hardly invasive and only dissolved compounds but no soil solution *per se* is withdrawn, this technique has been recognized as appropriate for direct application in environmental studies (Miro and Frenzel, 2004, 2005). Implantation of microdialysis probes into soils clearly has the potential to open new research directions in soil science, especially in rhizosphere studies, since relevant information about plant-available nutrient concentrations, mobility, and turnover rates may be obtained on site and in nearly real time. While the principle of microdialysis has been previously evaluated for the impact and availability of trace metals (Miro et al., 2010; Miro and Hansen, 2006), chloride (Miro and Frenzel, 2003) and low-molecular weight organic anions (Sulyok et al., 2005) in soil samples, so far there is no available information about the use of microdialysis for studying plant-available N compounds in soils.

This study therefore aimed at (1) evaluating the potential of microdialysis to estimate plant-available organic and inorganic N, (2) evaluating the possible spatial and temporal resolution achieved by microdialysis and (3) comparing the results from microdialysis sampling with soil extractions and lysimeter applications.

2. Materials and methods

2.1. Setup of the microdialysis system and calibration of microdialysis probes

The microdialysis system consisted of two syringe infusion pumps (CMA 100 and CMA 400) equipped with a total of 7 microsyringes (5 ml) that were used to provide the perfusate solution. Each syringe was connected to a microdialysis probe (CMA 20) with a polyarylethersulphone membrane (10 mm long, 500 μm outer and 400 μm inner diameter) with a 20 kDa molecular weight cut-off. Samples were collected with two microfraction collectors (CMA 140 and CMA 470) in 300 μl glass vials. All equipment is commercially available and was purchased from CMA Microdialysis AB, Solna, Sweden. A detailed scheme of the microdialysis system setup and the CMA 20 probe is given in Fig. 1.

In order to perform microdialysis and to interpret the results correctly, it is necessary to calibrate the microdialysis probes for their recovery of the target compounds prior to the experiments. The probes were positioned in a standard solution containing 100 μmol N l^{-1} of each of the following compounds: NH_4^+, NO_3^- and 12 amino acids (alanine, arginine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, leucine, lysine, proline, serine, valine). The solution was kept at a constant temperature of 20 °C and stirred with a magnetic stirrer throughout the calibration period. Probes were perfused with high-purity deionized (MilliQ) water at flow rates of 1.0, 3.0, 5.0, 7.0 and 10.0 μl min^{-1} and dialysate samples were collected at 26 min intervals for each flow rate during a period of 182 min. The relative recovery (RR), given as percent of concentrations of the standard solution recovered in the dialysate, was then calculated according to the microdialysis probe calibration (Bungay et al., 1990; Nandi and Lunte, 2009; Torto et al., 2001), as shown in equation (1):

$$RR\ (\%) = 100 \times C_{dial}/C_{std} \quad (1)$$

where C_{dial} is the concentration of the measured N compound in the dialysate, i.e., that has diffused through the microdialysis membrane, and C_{std} is the concentration of the compound in the standard solution.

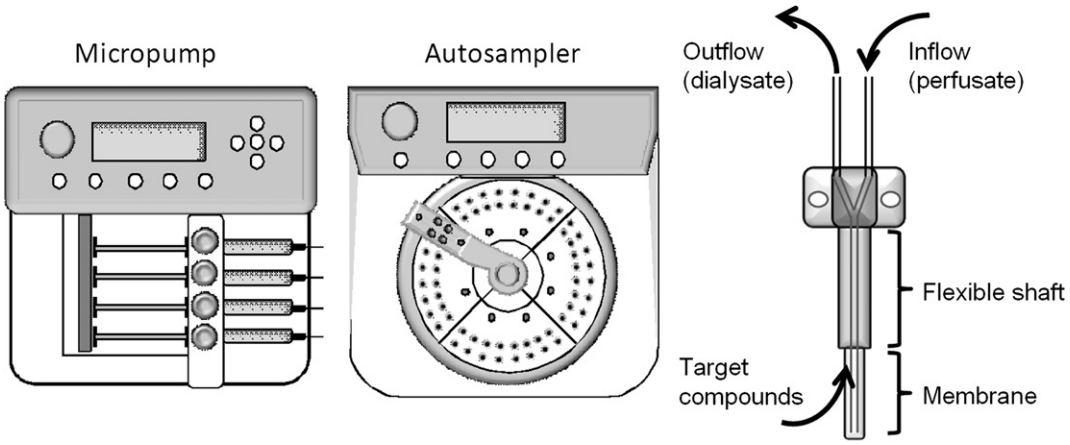

Fig. 1. Schematic experimental setup of the microdialysis system consisting of a high-precision infusion pump (CMA 400), a microfraction collector (CMA 470) and microdialysis probes (CMA 20) with a polyarylethersulphone membrane (10 mm long, 500 μm outer and 400 μm inner diameter) with a 20 kDa molecular weight cut-off. In the present study high-purity deionized water was used as perfusate.

To verify these results a second calibration method was performed, namely retrodialysis or reverse dialysis, which measures the loss of analyte used as perfusate through the microdialysis probe. Thereby, each sample probe was placed into a beaker containing MilliQ water and the same N standard solution as given above (100 μmol N l^{-1} for each compound) was perfused through the probes at flow rates of 1.0, 3.0, 5.0, 7.0 and 10.0 μl min^{-1}. Dialysate samples were collected at 26 min intervals over 182 min and the relative recovery (RR) due to the loss of N through the probe was calculated as shown in equation (2):

$$RR\ (\%) = 100 \times ((C_{std} - C_{dial})/C_{std}) \quad (2)$$

The absolute recovery (AR; i.e. the flow rate of compounds over the probe surface) was determined in the same way and was calculated as amount of N diffusing over the membrane surface during a given sampling time and is given as nmol N cm^{-2} h^{-1}.

2.2. Soil sampling

Soil was collected in October 2009 from an agricultural field and a poor Scots pine heath forest in the vicinity of Umeå, Sweden (63°49′ N, 20°17′ E). The agricultural soil is a silty loam Dystric Cambisol (FAO, 1998) formed by marine sediments as seen by a layer of iron sulfide at 2 m depth (4% clay, 58% silt, 38% fine sand). The C/N ratio was 10.5 and soil pH (H_2O) 6.2. The forest soil is a sandy glacial till Haplic Podzol (FAO, 1998) with 2% silt, 97% sand and 1% gravel, a C/N ratio of 38.7 and a pH (H_2O) of 5.2. Average annual precipitation at both sites is 587 mm and the annual mean air temperature 1.9 °C. Soil samples were collected from the uppermost soil layer (0–10 cm) from both sites and immediately stored at 4 °C until further analysis. Prior to the start of the experiments each of the two soils was thoroughly mixed, homogenized and sieved (<2 mm).

2.3. Determination of soil N pools

2.3.1. Soil extractions

Aliquots of homogenized soil (4 g FW, corresponding to 2.9 and 2.2 g DW, of agricultural and forest soil, respectively) were extracted with 30 ml of either MilliQ water, 0.5 M K_2SO_4 or 1 M KCl, shaken for 90 min, and filtered through ashless Whatman filter paper. Samples were immediately processed for analyses of NH_4^+, NO_3^- and amino acids as described below.

2.3.2. Lysimeter sampling

Aliquots of homogenized agricultural and forest soil samples (300 g DW) were filled into 500 ml polyethylene (PE) beakers and slightly compressed by tapping the beakers until a final volume of 350 ml was achieved, resulting in a bulk density of 0.86. Rhizon tension lysimeters (Rhizon SMS, Rhizosphere Research Products, Wageningen, The Netherlands) were installed in these jars at an angle of approximately 15° to the soil surface. These lysimeters (10 cm length, 2.5 mm diameter) consist of a porous polymer with a mean pore size of 0.1 μm. At each soil solution collection the lysimeter cap was replaced by a sterile needle and a pre-evacuated 10 ml sterile sampling tube (Beliver Industrial Estate, Plymouth, UK) fitted with a rubber membrane stopper. Sampling was done at room temperature (20 °C) and sampling time was set to 26 min for each set of samples. Samples were immediately processed for analyses of NH_4^+, NO_3^- and amino acids as described below.

2.3.3. Microdialysis

A series of microdialysis experiments were conducted measuring soil N pools at a high spatial and temporal resolution. Aliquots of homogenized soil (350 and 230 g DW of agricultural and forest soil, respectively) were weighed into rectangular PE jars (15 × 9 × 4 cm) and slightly compressed by tapping the jars until a final volume of 350 ml was achieved, resulting in bulk densities of 1.0 and 0.66 of the agricultural and the forest soil, respectively. Microdialysis probes were inserted vertically to approximately 1.5 cm beneath the soil surface at 91 positions along a 1 cm grid pattern. MilliQ water was used as perfusate, the flow was set to 5.0 μl min^{-1} and sampling time was set to 26 min. At each position three replicates were taken during a total sampling time of 78 min. Thereafter, samples were immediately processed for chemical analysis as described below. The resulting data were used to illustrate the variability of soil N pools depending on the individual site taken as well as for calculating average soil N pool sizes.

To investigate the potential of microdialysis sampling at a high temporal resolution, aliquots of homogenized soil samples were filled into 100 ml glass beakers (75 and 60 g DW, 0.93 and 0.75 bulk density of agricultural and forest soil, respectively). The microdialysis probes were installed vertically at 7 positions to approximately 1.5 cm beneath the soil surface. MilliQ water was used as perfusate at a constant flow rate of 5.0 μl min^{-1}. After equilibration for 52 min 12.5 and 30 ml of an N standard solution (100 μmol N l^{-1} of each of NH_4^+, NO_3^- and 12 amino acids; see above) were applied to the agricultural and the forest soil, respectively. Inserting a 7 cm

long side-hole needle to the bottom of the soil cores in 12 positions and slowly injecting the solution while withdrawing the needle ensured homogenous distribution of applied N. This technique has already been shown to yield uniform distributions in earlier studies (Inselsbacher et al., 2009; Pörtl et al., 2007). After N application, samples were taken every 26 min over a total monitoring time of 130 min. Samples were immediately processed for chemical analysis as described below. In parallel, a second experiment was conducted using the same setup but with soils being sterilized (autoclaved) prior to the start of the experiment.

2.4. Chemical analyses

2.4.1. Amino acids and ammonium

Samples from soil extractions, lysimeter and microdialysis sampling were immediately prepared for analysis by reversed-phase liquid chromatography using a Waters Ultra High Performance (UPLC) system with a Waters Tunable UV (TUV) detector. Samples were derivatized with a Waters AccQ-Tag Ultra Derivatization kit for amino acid analysis. Briefly, 70 µl borate buffer (1 M) and 10 µl of internal standard (nor-valin, 100 µmol N l^{-1}) were added to aliquots (10 µl) of samples or standards. Thereafter 20 µl of AccQ-Tag derivatisation reagent were added and thoroughly mixed immediately and subsequently heated to 55 °C for 10 min. After cooling down to room temperature, an aliquot of the reaction mixture was used for UPLC injection. The individual amino acids and NH_4^+ were determined after separation on an AccQ-Tag™ Ultra column by elution with a mixture of 99.9% formic acid and 10% acetonitrile at the following gradient: 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 and then to 40.4% formic acid at 8.74 min, before re-equilibration with 99.9% formic acid from 8.74 to 9.54 min. The flow rate was 0.6 ml min^{-1} and column temperature was 55 °C. Amino acid and NH_4^+ standards were prepared fresh daily in the range of 100–0.1 µmol N l^{-1} by serial 1:2 dilution in MilliQ water. The limit of quantification was 26 pmol amino acid-N in each dialysis sample (130 µl).

2.4.2. Nitrate

Nitrate in soil extracts, lysimeter samples and microdialysis samples was analyzed by the vanadium (III) chloride (VCl_3) and Griess method as described by Hood-Nowotny et al. (2010) based on

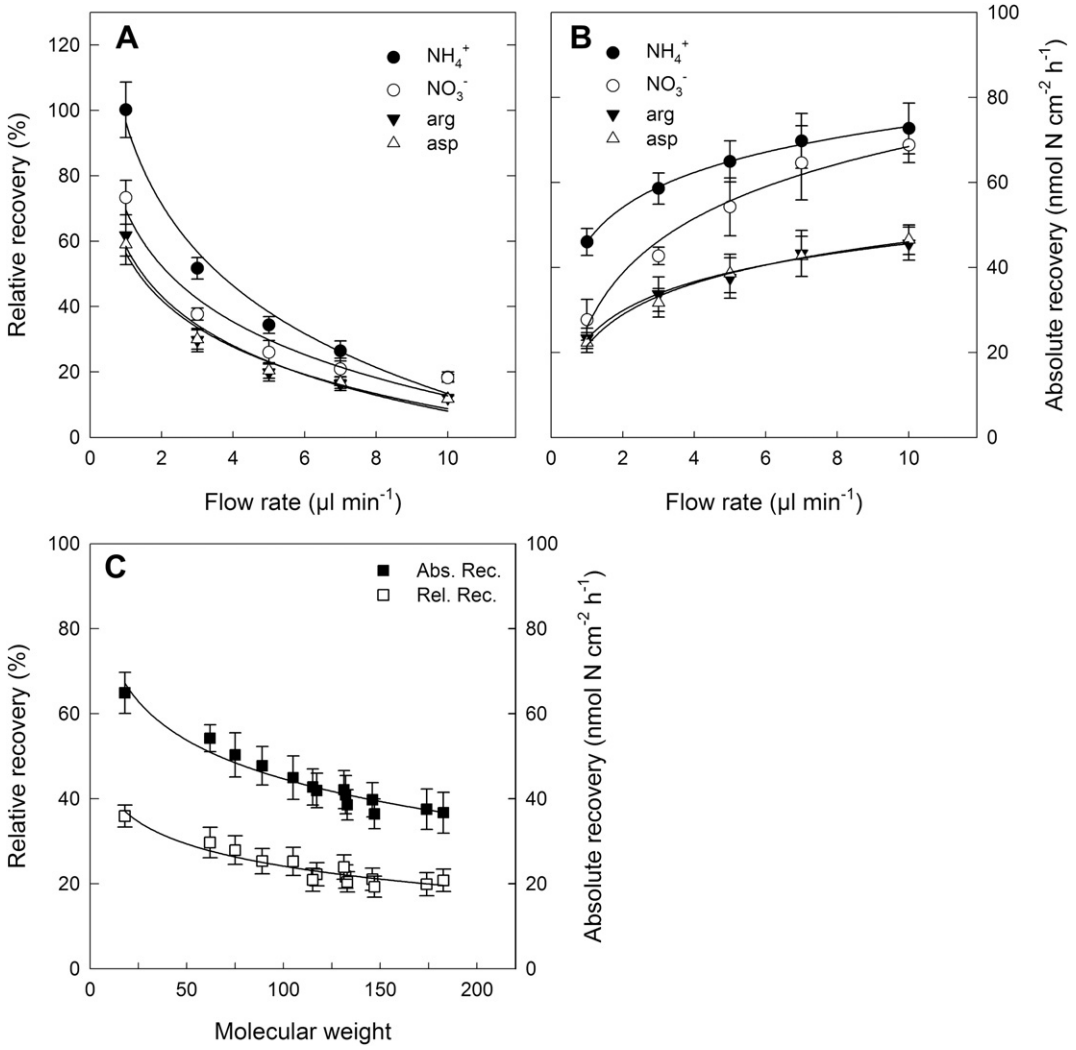

Fig. 2. The effect of perfusion fluid flow rate on (A) the relative recovery of NH_4^+, NO_3^-, and two representative amino acids, arginine and aspartic acid (mean of 7 concentrations (0.05–4 mmol N l^{-1}) and 7 replicates at each concentration) and (B) the absolute recovery of NH_4^+, NO_3^-, arginine and aspartic acid (each 100 µmol N l^{-1}, n = 7) and (C) the effect of molecular weight on the relative and absolute recovery of NH_4^+, NO_3^- and 12 amino acids (each 100 µmol N l^{-1}) at a constant perfusion fluid flow rate of 5 µl min^{-1}. Symbols and error bars represent means ± SE (n = 7). Equations for regression curves of relative and absolute recoveries of all analyzed N compounds are given in Table 1. For additional details on relative and absolute recoveries see Section 2.5.

Table 1
Exponential functions of relative recoveries (RR) and absolute recoveries (AR) of NH_4^+, NO_3^- and 12 amino acids by CMA 20 dialysis probes inserted into a standard solution as functions of perfusion flow rate (FR) and exponential functions of RR and AR as functions of molecular weight (MW) of each N compound analyzed. Functions were calculated from data shown in Fig. 2. Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Gln, glutamine; Glu, glutamic acid; Gly, glycine; Leu, leucine; Lys, lysine; Pro, proline; Ser, serine; Val, valine.

	Relative recovery	R^2	Absolute recovery	R^2
NH_4^+	$RR = 122.2 \times \exp(-0.24 \times FR)$	0.95	$AR = 66.8 \times (1-\exp(-1.07 \times FR))$	0.82
NO_3^-	$RR = 86.1 \times \exp(-0.22 \times FR)$	0.90	$AR = 68.8 \times (1-\exp(-0.36 \times FR))$	0.92
Ala	$RR = 122.2 \times \exp(-0.24 \times FR)$	0.92	$AR = 54.9 \times (1-\exp(-0.61 \times FR))$	0.89
Arg	$RR = 75.5 \times \exp(-0.25 \times FR)$	0.93	$AR = 42.8 \times (1-\exp(-0.63 \times FR))$	0.83
Asn	$RR = 79.2 \times \exp(-0.22 \times FR)$	0.93	$AR = 52.3 \times (1-\exp(-0.53 \times FR))$	0.80
Asp	$RR = 70.2 \times \exp(-0.23 \times FR)$	0.93	$AR = 43.3 \times (1-\exp(-0.60 \times FR))$	0.91
Gln	$RR = 76.4 \times \exp(-0.22 \times FR)$	0.94	$AR = 49.2 \times (1-\exp(-0.56 \times FR))$	0.83
Glu	$RR = 71.6 \times \exp(-0.23 \times FR)$	0.92	$AR = 46.5 \times (1-\exp(-0.52 \times FR))$	0.79
Gly	$RR = 109.6 \times \exp(-0.29 \times FR)$	0.91	$AR = 63.2 \times (1-\exp(-0.39 \times FR))$	0.96
Leu	$RR = 79.4 \times \exp(-0.24 \times FR)$	0.94	$AR = 45.4 \times (1-\exp(-0.66 \times FR))$	0.85
Lys	$RR = 78.2 \times \exp(-0.22 \times FR)$	0.94	$AR = 51.6 \times (1-\exp(-0.58 \times FR))$	0.81
Pro	$RR = 82.5 \times \exp(-0.22 \times FR)$	0.94	$AR = 54.1 \times (1-\exp(-0.56 \times FR))$	0.88
Ser	$RR = 98.9 \times \exp(-0.26 \times FR)$	0.95	$AR = 50.3 \times (1-\exp(-0.83 \times FR))$	0.88
Val	$RR = 77.3 \times \exp(-0.22 \times FR)$	0.94	$AR = 49.4 \times (1-\exp(-0.57 \times FR))$	0.89
MW	$RR = 37.5 \times \exp(-0.004 \times MW)$	0.89	$AR = 67.9 \times \exp(-0.004 \times MW)$	0.95

the technique described by Miranda et al. (2001). Briefly, a saturated vanadium reagent solution was prepared by dissolving 400 mg VCl_3 in 50 ml 1 M HCl, and excess solids were removed by filtration through ashless Whatman filter paper. Griess reagent 1 was prepared by dissolving 50 mg of N-naphthylethylenediamine dihydrochloride in 250 ml of MilliQ water. Griess reagent 2 was prepared by dissolving 5 g of sulphanilamide in 500 ml 3 M HCl. For the colour reaction, each 50 µl sample, standard or blank were pipetted into a 96-well, flat-bottomed polystyrene microtiter plate and 50 µl VCl_3 were added, rapidly followed by 25 µl each of Griess reagents 1 and 2 to give a total volume of 150 µl. The Griess reagents were mixed in equal volumes immediately before being added to the assay mixtures. The plates were incubated at 37 °C for 60 min and the absorbance of the reaction product in the wells was measured by a microtiter plate reader (Spectramax 190, Molecular Devices, Sunnyvale, CA, USA) at 540 nm. Nitrate standards were prepared fresh daily from a stock solution of KNO_3 (1000 mg N l^{-1}) in the range of 5–0.02 mg N l^{-1} by serial 1:2 dilution in MilliQ water.

2.5. Calculations and statistical analysis

Concentrations of N compounds in dialysates were used to calculate soil solution concentrations using the relative recovery rates of individual compounds established in the probe-recovery calibration described above. Calculated values were then expressed as µmol N l^{-1}. Absolute recoveries were used to calculate flux rates (diffusion rates) of N compounds over the membrane surface (15.9 mm^2) during each experimental time period (26 min). Diffusion rates were expressed as nmol N cm^{-2} h^{-1}. Data were analyzed using one-way and multi-factorial ANOVA followed by Tukey's HSD post-hoc test using Statistica 6.0 (StatSoft Inc., Tulsa, OK, USA) and Statgraphics 5.0 (Statistical Graphics Inc., Rockville, MD, USA). When necessary, data were either square root- or $\log_{10}$-transformed prior to analysis to meet the assumptions of ANOVA after testing normality using Kolmogorov–Smirnov test and homogeneity of variances using Bartlett's test.

3. Results

3.1. Relative and absolute recovery of microdialysis probes

The microdialysis probes were calibrated for their relative and absolute recoveries of NH_4^+, NO_3^- and 12 amino acids prior the experiments. No significant differences in relative recoveries were found between the two calibration techniques, direct dialysis and reverse dialysis, for any of the N compounds tested (one-way

Table 2
Concentrations of NH_4^+, NO_3^- and 12 amino acids in water and salt extracts of an agricultural and a forest soil (values are given as µmol N kg^{-1} soil DW and represent means (±SE), n = 5). Different letters indicate significant differences between different extractants within each soil (one-way ANOVA, Tukey's post-hoc test, $P < 0.95$). Not detectable indicated as "–".

	Agricultural soil			Forest soil		
	MilliQ	KCl (1 M)	K_2SO_4 (0.5 M)	MilliQ	KCl (1 M)	K_2SO_4 (0.5 M)
NH_4^+	52 (4)^a	86 (3)^b	84 (3)^b	111 (14)^A	290 (39)^B	232 (13)^B
NO_3^-	1646 (65)^a	1718 (47)^a	1699 (64)^a	74 (17)^A	50 (15)^A	47 (7)^A
Asn	–	–	–	–	25.6 (0.6)	–
Arg	–	–	–	–	22.3 (1.0)	–
Ser	1.8 (0.4)^a	4.5 (0.9)^b	1.8 (0.2)^a	4.8 (0.6)^A	16.1 (0.6)^B	4.6 (0.2)^A
Gln	–	–	–	–	73.0 (2.4)	–
Gly	2.2 (0.2)^a	4.1 (0.6)^b	2.0 (0.1)^a	5.4 (0.4)^A	25.0 (1.1)^B	4.9 (0.2)^A
Asp	–	2.0 (0.0)	–	–	11.2 (0.4)	–
Glu	–	–	–	–	22.3 (0.7)	–
Ala	0.3 (0.1)^a	1.7 (0.3)^b	0.3 (0.1)^a	1.6 (0.3)^A	28.1 (0.8)^B	1.1 (0.2)^A
Lys	–	1.5 (0.0)	–	–	15.5 (0.3)	–
Pro	–	–	–	–	–	–
Val	–	–	–	–	5.1 (0.5)	–
Leu	–	–	–	–	3.8 (0.1)	–
AA_{tot}	4.2 (1.0)^a	13.7 (1.4)^b	4.2 (0.5)^a	11.8 (1.5)^A	248 (11)^B	10.6 (0.8)^A

Table 3
Concentrations of NH_4^+, NO_3^- and 12 amino acids in soil solutions of two homogenized, field moist soils sampled by tension lysimeters (μmol N l^{-1}; means (±SE), n = 9) and by microdialysis (sampled along a 13 × 9 cm grid pattern, μmol N l^{-1}; means (±SE), n = 91). Significant differences between soils within each sampling method are indicated as $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (one-way ANOVA, Tukey's post-hoc test). Not detectable indicated as "–".

	Lysimeter		Dialysis	
	Agricultural soil	Forest soil	Agricultural soil	Forest soil
NH_4^+	25.32 (2.07)	2.44 (0.36)***	2.69 (0.18)	7.17 (0.15)***
NO_3^-	110.2 (5.88)	89.8 (6.87)	3.34 (0.10)	3.12 (0.02)*
Asn	0.34 (0.06)	0.12 (0.01)*	–	–
Arg	0.52 (0.08)	0.29 (0.02)*	–	–
Ser	0.96 (0.20)	0.46 (0.06)	0.63 (0.06)	0.40 (0.04)**
Gln	0.69 (0.11)	0.97 (0.11)	1.04 (0.03)	3.14 (0.04)***
Gly	2.13 (0.24)	0.28 (0.06)***	3.04 (0.04)	0.85 (0.06)***
Asp	0.43 (0.07)	0.30 (0.06)	0.27 (0.06)	–
Glu	0.68 (0.12)	0.55 (0.11)	0.09 (0.01)	0.98 (0.04)***
Ala	0.62 (0.10)	0.54 (0.05)	0.69 (0.05)	1.20 (0.07)***
Pro	0.16 (0.05)	0.11 (0.01)*	0.12 (0.03)	–
Lys	0.24 (0.04)	0.09 (0.01)*	–	–
Val	0.49 (0.10)	0.15 (0.02)*	0.97 (0.07)	1.38 (0.08)***
Leu	0.40 (0.04)	0.13 (0.01)***	–	–
AA_{tot}	7.65 (1.32)	3.99 (0.49)***	6.84 (0.16)	7.95 (0.17)***

ANOVA, $P > 0.05$; data not shown). Further, no significantly different relative recoveries were found between different N concentrations in the standard solution within the tested range of 0.05–4 mmol N l^{-1} (one-way ANOVA, $P > 0.05$). Both relative and absolute recoveries of all N compounds tested followed exponential functions of perfusate flow rate (NH_4^+, NO_3^-, arginine and aspartic acid are shown as representatives in Fig. 2, details are given in Table 1). Relative recoveries were strongly dependent on perfusate flow rate reaching between 60% and 100% recovery at 1 μl min^{-1} flow rate and only between 10 and 20% recovery at a flow rate of 10 μl min^{-1} (Fig. 2A). The opposite pattern was found for absolute recoveries which were lowest (between 25 and 45 nmoln N cm^{-2} h^{-1}) at the lowest flow rate (1 μl min^{-1}) and significantly higher at 10 μl min^{-1} (between 45 and 70 nmol N cm^{-2} h^{-1}; Fig. 2B). Additionally to the perfusate flow rate relative and absolute recoveries were dependent on the molecular weight of the N compounds tested (Fig. 2C, Table 1). For the main experiments a perfusion flow rate of 5 μl min^{-1} was chosen. This resulted in reduced, but for the purposes of this study satisfactory recovery rates. A decrease in flow rates would have led to insufficient amounts of sample at the same sampling times or to a loss of temporal sampling resolution, another aim of this study.

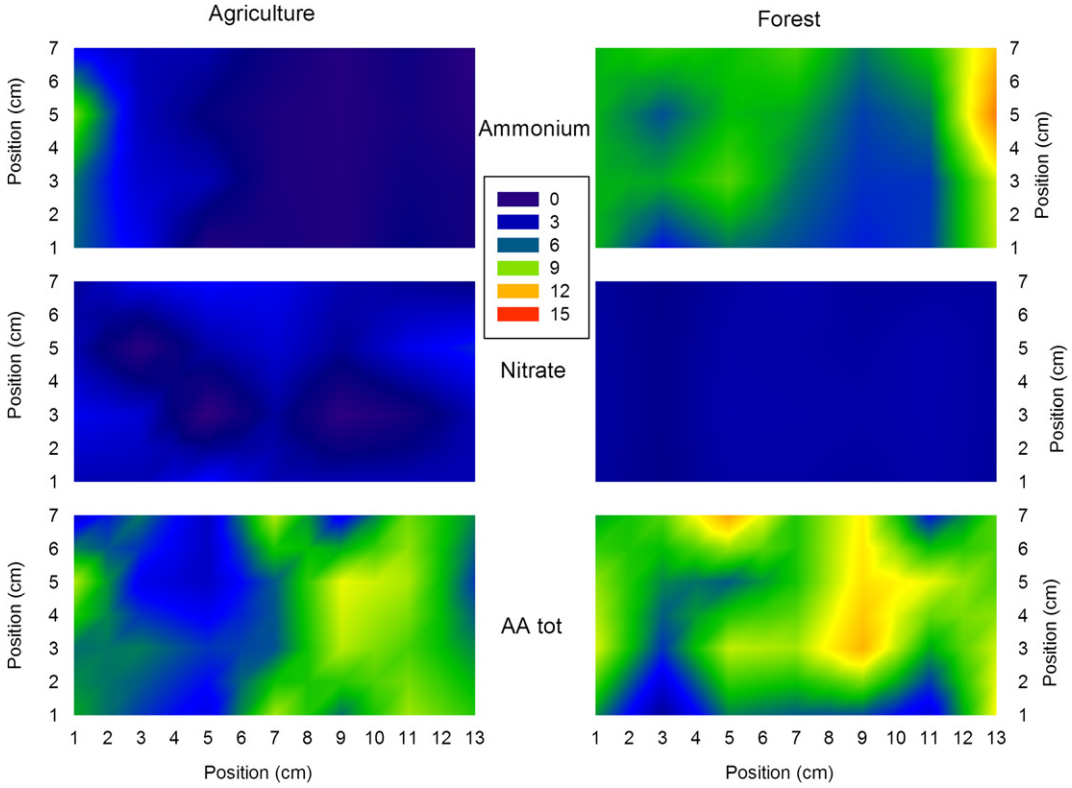

Fig. 3. Concentration of NH_4^+–N, NO_3^-–N and total measured amino acid N (AA tot) in an agricultural and a forest soil at 91 positions in a 13 × 7 cm grid pattern (μmol N l^{-1}). Each data point within the area represents the mean of 3 technical replicates.

3.2. Comparison of different methods to estimate soil N pools

3.2.1. Soil extraction

Within each of the two studied soils the extraction with high-purity distilled (MilliQ) water and with K_2SO_4 (0.5 M) resulted in similar amounts of N compounds (one-way ANOVA, $P > 0.05$; Table 2, Supplementary Table 2). The only exception were NH_4^+ concentrations that were 1.6 and 2.1 times as high in the K_2SO_4 extracts as in water extracts in the agricultural and the forest soil, respectively. When using K_2SO_4 or water as extractant only three out of 12 analyzed amino acids were found in both soils, namely serine, glycine and alanine. Using KCl (1 M) as extractant resulted in significantly higher concentrations of all analyzed N compounds (one-way ANOVA, $P < 0.05$) except NO_3^- which was found at similar concentrations in all three extracts ($P > 0.05$). Additionally, more amino acid species could be extracted from both soils with KCl compared to water or K_2SO_4 (Table 2). In the agricultural soil two additional amino acids (aspartic acid and lysine) and in the forest soil all amino acids analyzed (except proline) were detected in the KCl extracts. This resulted in three and 22 times higher total amino acid N concentrations found in the KCl extracts compared to water or K_2SO_4 extracts in the agricultural and forest soil, respectively. In the agricultural soil the dominant N form clearly was NO_3^- with concentrations being approximately 20–30 times as high as NH_4^+, the second highest N pool (Table 2). Further, irrespective of soil extraction method the sum of all amino acids analyzed was significantly lower than the NH_4^+ pool in the agricultural soil. In the forest soil, on the other hand, NH_4^+ was the dominant N pool in all three types of extractions. In the water and K_2SO_4 extracts NO_3^-–N was higher than total amino acid N, but extracting this soil with KCl resulted in significantly higher total amino acid N concentrations compared to NO_3^-–N ($P < 0.05$).

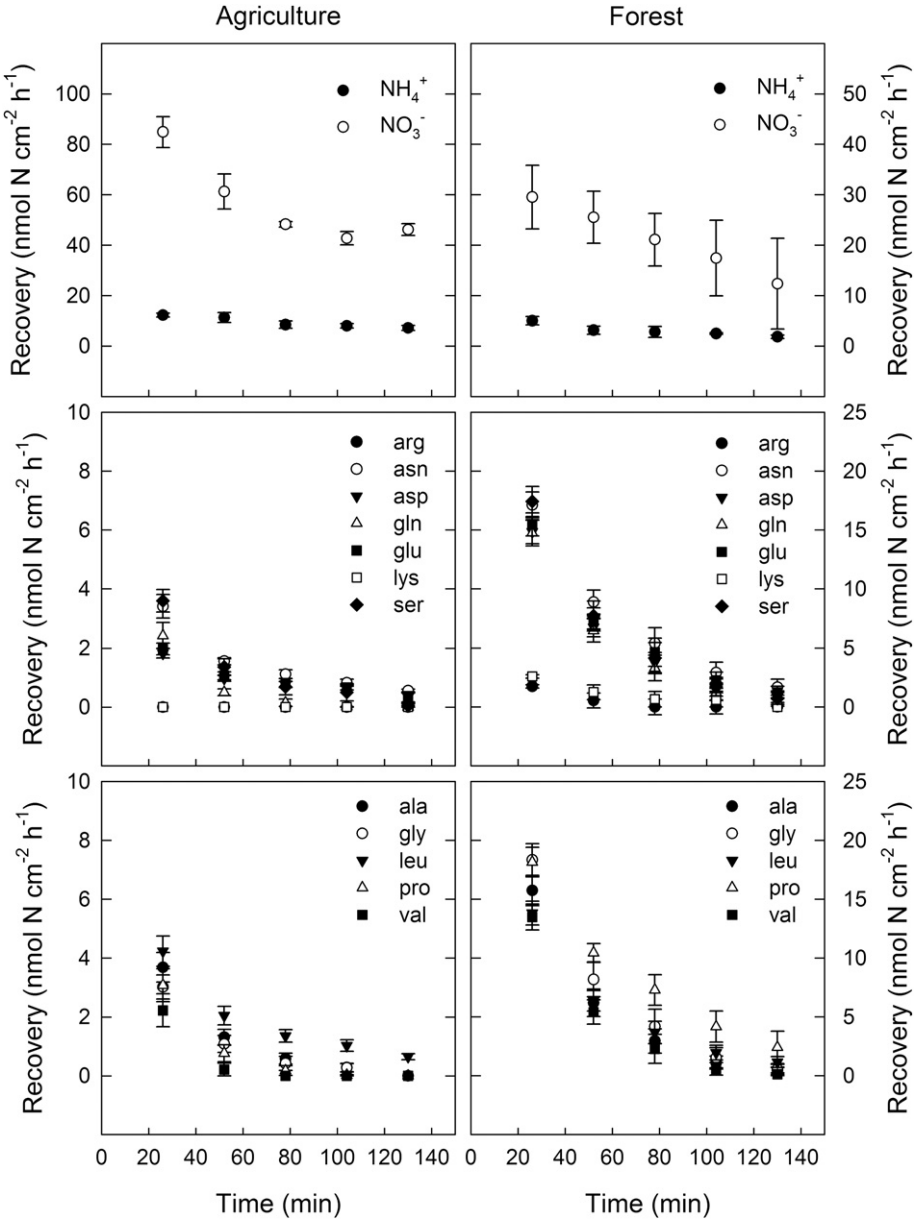

Fig. 4. Flow rates of NH_4^+, NO_3^-, 7 polar and 5 unpolar amino acids over the microdialysis membrane surface (expressed as absolute recoveries) after application of a standard solution (100 μmol N l^{-1} of each compound) to an agricultural and a forest soil. Symbols represent means ± SE (n = 4). In the agricultural soil symbols for arginine and lysine are overlapping. Exponential decay functions of all analyzed N compounds are given in Table 5.

3.2.2. Lysimeter sampling

In both soils all target N compounds (NH_4^+, NO_3^- and 12 amino acids) were detected in the samples obtained by tension lysimeters (Table 3). NO_3^- was the dominant N form in both soils and was found at similar concentrations in both soils (110.2 and 89.8 μmol N l^{-1} in the agricultural and forest soil, respectively). In the agricultural soil NO_3^- concentrations were 4.3 times as high as NH_4^+ concentrations which, in turn, were 3.3 times as high as total amino acid N concentrations (one-way ANOVA, $P < 0.05$). On the contrary, in the forest soil the total amino acid N pool was 1.6 times as high as NH_4^+–N which on the other hand was 37 times lower than NO_3^-–N. Surprisingly, not only the sum of analyzed amino acid N was higher in the agricultural compared to the forest soil but also individual amino acid N concentrations. Only glutamine was found at higher concentrations in the forest soil, although this difference was not significant (one-way ANOVA, $P > 0.05$; Table 3). Further, the dominant amino acid in the agricultural soil was glycine ($P < 0.001$) and glutamine in the forest soil ($P < 0.001$), accounting for 28% and 24% of total analyzed amino acid N, respectively.

3.2.3. Microdialysis sampling

In order to compare the results from microdialysis sampling with soil extractions and lysimeter sampling N concentrations of soils were calculated as the average values obtained from 91 sampling points in a 13 × 7 cm grid pattern (Table 3, Fig. 3). The most striking result found by diffusion-based microdialysis sampling was that, in clear opposition to lysimeter sampling and soil extractions, not inorganic N but the total amino acid N pool comprised the biggest N pool in both soils (one-way ANOVA, $P < 0.001$). However, the individual amino acid composition within each soil differed significantly (Table 3). In the forest soil, six of the 12 amino acids tested were found while in the agricultural soil additionally aspartic acid and proline were detected. Similar to lysimeter sampling glutamine was the dominant amino acid in the forest soil and glycine in the agricultural soil, accounting for 40 and 44% of the total analyzed amino acid N pool. Regarding the inorganic N pools NO_3^- was found at slightly, but still significantly higher concentrations than NH_4^+ in the agricultural soil ($P < 0.05$) while in the forest soil the opposite was the case with NH_4^+ being more than twice as high as NO_3^- ($P < 0.001$).

3.3. Spatial and temporal resolution of microdialysis sampling

An important issue of microdialysis performance is the possibility to work at high spatial and temporal resolutions. Analysis of homogenized soil within a 7 × 13 cm jar at a 1 cm resolution revealed that the homogeneity of N concentrations was strongly dependent on the form of N (Fig. 3). Concentrations of NO_3^- were similar at all sampling positions in the forest soil and were in the range of 2.71 and 3.42 μmol N l^{-1}. In the agricultural soil, NO_3^- concentrations were slightly more variable, ranging from 0.58 to 5.14 μmol N l^{-1}. On the contrary, NH_4^+ concentrations differed strongly in both soils ranging between 0.82 and 8.63 μmol N l^{-1} in the agricultural soil and between 5.20 and 12.58 μmol N l^{-1} in the forest soil. The variation of NH_4^+ concentrations in the agricultural soil was caused by only one specific area with significantly higher NH_4^+ concentrations compared to the rest of the sampling area while in the forest soil NH_4^+ concentrations varied over the whole analyzed area (Fig. 3). However, in both soils the strongest variation of N concentrations within the sampling area was found for amino acids. Concentrations of total analyzed amino acids ranged between 3.81 and 9.77 in the agricultural soil and between 2.99 and 11.72 μmol N l^{-1} in the forest soil. Although the variation of amino acid N was quantitatively in approximately the same range as the variation of NH_4^+ in both soils, the amino acids clearly occurred more patchy over the analyzed area (Fig. 3).

To investigate the possibility of temporal resolution of microdialysis sampling, a standard solution containing NH_4^+, NO_3^- and 12 amino acids was applied to the soils and samples were subsequently taken at 26 min intervals (Fig. 4). The absolute recovery (i.e. the flux rates) of all N forms was highest directly after N application and decreased significantly ($P < 0.05$) within the subsequent 130 min (Table 4). The only exceptions were NH_4^+ in both soils, which also decreased but not significantly ($P > 0.05$), and the amino acids arginine and lysine, which were only recovered during one hour after their application in the forest soil and not recovered at all in the agricultural soil (Fig. 4). The highest flux rate in the agricultural soil was found for NO_3^- with an initial rate of 96 nmol N cm^{-2} h^{-1} (Table 5). Recovery rates of NH_4^+ and amino acids were similar in the agricultural soil with initial rates of 2.6 (aspartic acid) to 14.3 (NH_4^+) nmol N cm^{-2} h^{-1}. The high initial recovery of valine (23.4 nmol N cm^{-2} h^{-1}) calculated by exponential regression was the result of the strong decrease of recovery during the first 52 min, after which no valine was recovered any more. In the forest soil, on the other hand, the lowest initial flux rates were found for NH_4^+ (6.1 nmol N cm^{-2} h^{-1}) while flux rates of NO_3^- and amino acids were similar and ranged between 27.1 nmol N cm^{-2} h^{-1} (leucine) and 40.3 nmol N cm^{-2} h^{-1} (glycine). Further, the initial flux rate of all amino acids was higher in the forest than in the agricultural soil ($P < 0.001$). More importantly, in general the gradual decrease in flux rates was similar for all amino acids (except

Table 4
F-statistics from two-way ANOVA of the effects of sampling time (26, 52, 78, 104 and 130 min after N application) and treatment (untreated or sterilized soil) on the absolute recovery of NH_4^+, NO_3^- and 12 amino acids in an agricultural and a forest soil. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, *na*, not applicable.

	Agricultural soil			Forest soil		
	Time	Treatment	Time × treatment	Time	Treatment	Time × treatment
NH_4^+	2.87	526.1***	2.34	0.83	201.7***	0.74
NO_3^-	5.16**	21.3***	2.29	4.56*	65.22***	0.01
Ala	12.01***	153.3***	0.2	5.3**	14.87**	0.6
Arg	*na*	*na*	*na*	*na*	*na*	*na*
Asn	27.31***	21.53***	1.01	6.66**	0.01	1.15
Asp	13.33***	218.2***	2.68	2.97*	47.78***	0.01
Gln	17.42***	3.81	1.47	7.01**	0.05	1.59
Glu	10.25***	13.37**	0.54	5.68**	0.21	0.81
Gly	6.28**	263.6***	1.28	6.06**	8.6**	0.79
Leu	18.61***	6.63*	1.92	6.7**	0.16	0.99
Lys	*na*	*na*	*na*	*na*	*na*	*na*
Pro	17.96***	99.63***	0.54	6.6**	0.12	1.34
Ser	5.64**	1.69	0.32	7.1***	1.1	1.46
Val	15.71***	5.72	0.11	6.16**	0.45	1.19

Table 5
Exponential functions of absolute recoveries (AR) of NH_4^+, NO_3^- and 12 amino acids as functions of time (t, in minutes) after application of a 100 μmol N l^{-1} standard solution to an agricultural and a forest soil. For details see Fig. 4. *na*, Not applicable.

	Agricultural soil	R^2	Forest soil	R^2
NH_4^+	$AR = 14.3 \times \exp(-0.006 \times t)$	0.93	$AR = 6.1 \times \exp(-0.01 \times t)$	0.90
NO_3^-	$AR = 96.0 \times \exp(-0.007 \times t)$	0.81	$AR = 36.8 \times \exp(-0.008 \times t)$	0.97
Ala	$AR = 9.8 \times \exp(-0.04 \times t)$	0.99	$AR = 38.2 \times \exp(-0.03 \times t)$	0.99
Arg	*na*		*na*	
Asn	$AR = 5.6 \times \exp(-0.02 \times t)$	0.95	$AR = 30.8 \times \exp(-0.02 \times t)$	0.99
Asp	$AR = 2.6 \times \exp(-0.02 \times t)$	0.93	$AR = 30.3 \times \exp(-0.03 \times t)$	0.99
Gln	$AR = 11.4 \times \exp(-0.06 \times t)$	0.99	$AR = 32.7 \times \exp(-0.03 \times t)$	0.99
Glu	$AR = 3.0 \times \exp(-0.02 \times t)$	0.95	$AR = 29.2 \times \exp(-0.03 \times t)$	0.99
Gly	$AR = 8.1 \times \exp(-0.04 \times t)$	0.99	$AR = 40.3 \times \exp(-0.03 \times t)$	0.99
Leu	$AR = 7.0 \times \exp(-0.02 \times t)$	0.97	$AR = 27.1 \times \exp(-0.03 \times t)$	0.99
Lys	*na*		*na*	
Pro	$AR = 12.5 \times \exp(-0.05 \times t)$	0.99	$AR = 29.4 \times \exp(-0.02 \times t)$	0.99
Ser	$AR = 8.4 \times \exp(-0.03 \times t)$	0.99	$AR = 37.4 \times \exp(-0.03 \times t)$	0.99
Val	$AR = 23.4 \times \exp(-0.09 \times t)$	0.99	$AR = 34.0 \times \exp(-0.04 \times t)$	0.99

arginine and lysine) and could be well described as exponential functions of time after N application (Table 5). Accordingly, at the end of the experimental period, i.e. after 130 min, flux rates of amino acids were approaching initial levels before N application again (Fig. 4).

Sterilizing soils (autoclavation) prior to the microdialysis experiments resulted in significantly higher initial flux rates of NH_4^+ being 77.6 in the agricultural and 120 nmol N cm^{-2} h^{-1} in the forest soil ($P < 0.001$, Table 4; for details see Supplementary Fig. 1 and Supplementary Table 1). Nitrate flux rates, on the other hand, increased in the agricultural soil but decreased in the forest soil (300 and 20.9 nmol N cm^{-2} h^{-1}, respectively). In the forest soil, only two amino acids, namely alanine and glycine, were recovered at different rates in the sterilized soil compared to the unsterilized soil, while in the agricultural soil significant differences were found for all amino acids except glutamine, serine and valine (Table 4). As was the case in the unsterilized soils, arginine and lysine could not be recovered in the sterilized soil, not even directly after application of the standard solution containing 100 μM of a range of N forms including these two basic amino acids. However, although these differences in flux rates were found after sterilizing the soil, there was no significant difference in the observed pattern of time dependent decrease of all N forms (two-way ANOVA, $P > 0.05$, Table 4). While the gradual decreases of flux rates were slightly less than in the unsterilized soils, they still were exponential functions of time after N applications.

4. Discussion

Soil solution content and composition of N is a critical determinant of plant N acquisition. However, the turnover of N compounds in soils is rapid and the dynamics of root–soil interactions are large. These factors restrict our possibilities to gain a correct representation of plant-available N in soils and hence there is a strong need for non-invasive techniques for sampling soil solution. Moreover, plant acquisition of N is, in many soils, dependent on diffusion of N compounds from the surrounding soil towards root surfaces while techniques (e.g. lysimeters) for sampling soil solution do not normally mimic this process well. In this context, microdialysis offers specific advantages compared to standard techniques used in soil science so far.

Our results show that the use of microdialysis probes can provide valuable information about soil N concentrations relevant for plant nutrition causing minimal disturbance of the natural soil system at an unrivaled spatial and temporal resolution.

There are, however, several important issues that need to be considered prior to application of microdialysis. One such issue concerns probe calibration in solution and should consider a wide range of possible interfering factors (such as membrane, soil and target compound properties, temperature, convection effects and membrane fouling) and is necessary before the microdialysis probes can be used in soils (Kehr, 1993; Stenken, 1999). In our study initial calibration of the probes in solution showed that the relative and absolute recoveries of individual N forms were strongly dependent on their molecular weight and on perfusate flow rates (Fig. 2). Relative recoveries (i.e. concentrations in the dialysate) were increasing exponentially with decreasing flow rates and decreasing target compound molecular weight, as has been shown previously (Kehr, 1993; Stenken, 1999; Torto et al., 2001). On the other hand, different concentrations and different physical or chemical properties of individual amino acids did not affect diffusion rates over the microdialysis membrane. Calibration of the microdialysis probes used in our study also showed a satisfying compromise of relative recoveries (between 19 and 36%) and amount of sample (100–150 μl) at a perfusion flow rate of 5.0 μl min^{-1} and a sampling time of 26 min.

Sampling soil with microdialysis is strictly passive and therefore the N concentrations in the dialysates reflect how much of each N form was diffusing through the soil towards, and eventually over the membrane surface. The rate of diffusion is proportional to the concentration difference over the membrane and depends thus both on the external concentration of the soil solution and the internal concentration of the probe. The latter would approach that of the former when rates of perfusate flow approach zero and thus maximum relative recovery would be achieved. At high rates of perfusate flow, however, concentration differences between soil and probe solutions would be maximal and hence lead to maximal diffusion rates and rates of absolute recovery. Comparing lysimeter and microdialysis sampling revealed that several amino acids that were detected with the former technique were not found with the latter (Table 3). The most likely reason for this might have been the recovery-related dilution of some amino acids in the samples to undetectable levels (below 0.2 μmol N l^{-1}). As indicated above, this dilution effect could be minimized by a perfusion flow rate close to 0 μl min^{-1}, leading to relative recoveries around 100%. However, this would require significantly longer sampling intervals in order to gain sufficient sample amounts for chemical analysis. Besides sample dilution another difficulty when using microdialysis is the comparison with the results gained by other techniques, due to the different underlying principles. In our study for example, compared to lysimeter sampling or soil extractions microdialysis sampling not only gave different results in quality but also in relative quantity of each N form. Especially NO_3^- was found at much lower relative concentrations in the dialysates while the sum of amino acids

analyzed constituted the dominant part of N in dialysates (Table 3). These differences can be attributed to the fact that lysimeter sampling is based on applying a vacuum, while microdialysis is solely based on diffusion. The application of a vacuum when using lysimeters causes a mass flow of soil water and solutes, another important pathway of plant nutrient acquisition. This mass flow might explain the high concentrations of comparably mobile NO_3^- and the fact that also less strongly bound amino acids were detected in lysimeter samples in both soils. One drawback of lysimeter sampling is the dependency on quite high soil moisture contents and that the resulting bulk concentrations could be biased towards the concentrations in the largest water-filled soil pores (Miro et al., 2010). Microdialysis, on the other hand has been proven to give reliable results even at low soil water contents (Miro et al., 2010) and sample soluble N compounds from the surrounding soil independent of soil pore sizes. Thus, the two techniques may, at least partly, reflect different fractions of the soil and the soil solution.

Soil extractions with MilliQ water or 0.5 M K_2SO_4, on the other hand, resulted in similar concentrations of N pools with a strong dominance of NH_4^+ and NO_3^- but low concentrations of amino acids in both extracts (Table 2). In comparison, extracting soils with 1 M KCl generally resulted in higher concentrations of all N forms and more species of amino acids were detected, which is in agreement with previous studies (e.g. Chen et al., 2005; Hannam and Prescott, 2003; Jones and Willett, 2006; Murphy et al., 2000). Especially in the forest soil a significantly higher number of amino acids and substantially higher concentrations were found in the KCl extract compared to water or K_2SO_4 extraction (Table 2). These differences were smaller in the agricultural soil, which shows that a greater proportion of amino acids was bound to the solid phase in the forest soil, which was rich in organic compounds. These findings indicate the difficulty, if not invalidity, to directly compare soil N pools qualitatively and quantitatively estimated with different methods. Soil extractions are not only seriously disrupting the natural soil structure, but also lead to inevitable time lags between sampling and soil extractions, which can cause chemical alterations prior to analysis. For example, it has been shown that the pool of amino acids rapidly (within 15 min) turned over when extracting soil with distilled water or 0.5 M K_2SO_4 (Rousk and Jones, 2010). Therefore, the concentrations of amino acids, NH_4^+ and NO_3^- found in soil extracts may not reflect actual natural conditions in the field.

Studies of soil solution N availability should seek a representation of the system with as high relevance as possible for plant N uptake. Theoretically, root N uptake depends on interception, mass flow and diffusion processes. While soil extractions may provide information on the total amount of N available for uptake, lysimeter sampling mimics mass flow processes in the soil. Dialysis methods, such as the here described microdialysis technique, preferentially sample soil through diffusion. These differences give rise to different fractions of the soil solution and hence to different N sources for plant uptake. Additionally, it has to be pointed out that plant nutrient uptake is also dependent on ion exchange processes. For example, organic acids secreted by root tips into the soil or protons (H^+) carried across the cell membrane change the chemical state of nutrients improving their uptake into the root tissues. However, reliably simulating and investigating the effects of these exchange processes *in situ* remains a challenging task for future studies.

In our study microdialysis allowed the detection of even small differences in concentrations of amino acids and NH_4^+ at distances of only 1 cm (Fig. 3). By choosing smaller dimensions for the probes (e.g. 1 mm instead of 10 mm length), this resolution could have been further improved easily. Still, smaller microdialysis probes would inevitably have led to decreased recovery rates due to the decreased surface area resulting in lower N concentrations in the samples and therefore would have severely limited chemical analyzes. On the other hand, for estimating NO_3^- alone, a lower spatial resolution than chosen in the present study would have been sufficient due to its relatively homogenous distribution in both soils (Fig. 3). This homogeneity of NO_3^- in contrast to NH_4^+ or amino acids was not surprising as it reflects the comparably free movement of NO_3^- through the soil. Still, the patchy distribution of amino acids and NH_4^+ was unexpected as soils were homogenized before the experiment. This might have been caused by a heterogeneous initial distribution of amino acids or increased turnover rates in hotspots of high microbial activity. While we can only speculate about the causes, our results show that even thorough mixing and sieving (<2 mm) was not sufficient to get a completely homogenized soil regarding soil N concentrations.

Our results show that the optimal choice of probe dimensions and spatial resolution strongly depends on the physico-chemical nature of the target compounds and the soil. However, when a study aims at investigating plant-available N concentrations and diffusion rates in specific soil microsites, our results show that this can be achieved with microdialysis. Due to this excellent potential spatial resolution (<0.5 mm), microdialysis has the capability to monitor the turnover of individual amino acids in hotspots such as the rhizosphere, as it has already been proposed for low-molecular weight organic anions and trace metals (Miro et al., 2010; Miro and Hansen, 2006; Sulyok et al., 2005).

Still, in order to monitor concentration changes of free amino acids, NH_4^+ and NO_3^- in soil, not only an ability to sample at a high spatial but also at a high temporal resolution is required. In our study, shortly (<30 min) after spiking the soils with a standard N solution the recovery of all N forms tested (except arginine and lysine) was rising to a maximum followed by a significant decrease within the subsequent 2 h (Fig. 4). These findings indicate that a short pulse of N, as can occur in the rhizosphere or in soil microsites with high microbial activity, can be detected by microdialysis, as has been suggested previously for organic anions (Sulyok et al., 2005). The subsequent decrease in recovery, however, could have been caused by several factors, e.g. microbial degradation, adsorption to soil particles, formation of a so-called diffusion shell around the microdialysis probes or a combination of these processes. Adsorption to soil particles was probably the major cause in our study, at least for arginine and lysine, which were not recovered at higher-than-background rates even directly after N application (Fig. 4). The fact that we found similar temporal patterns of decreased recoveries in both unsterilized and sterilized soils suggests that microbial degradation during the experimental period of 2 h was probably only of minor influence (Supplementary Fig. 1, Supplementary Table 1, Table 4). Thus, diffusional depletion around the probe was probably the main cause for the temporal decrease of N flow rates over the dialysis membrane. Therefore, microdialysis sampling could be compared with nutrient uptake of roots, as, to some extent similar to plant roots, sampling with microdialysis probes can be considered as a sink of free moving compounds. In a recent study, Leitner et al. (2010) modeled the formation of nutrient depletion zones around plant roots showing that many factors together determine the dynamic patterns of these zones. One important factor was diffusion of the target nutrient through the soil matrix to the root surface as in many soils diffusion is a key factor for nutrient supply of plants. Still, these findings were based on mathematical models and need to be verified with data from actual experiments. Until now it was practically impossible to measure diffusion rates of nutrients, such as inorganic and organic N compounds, in the heterogeneous soil matrix in the field. Our results show that microdialysis could indeed be a suitable tool meeting the requirements for diffusion-based studies. Therefore,

systematic implantation of microdialysis probes into the soil could help elucidating the relative importance of diffusion to the supply of specific N compounds for plant uptake.

5. Conclusions

This study has highlighted the potential of microdialysis for estimating plant-available inorganic and organic N pools as well as diffusion rates in two contrasting soils. Due to its miniaturized design and the diffusion-controlled passive sampling approach, microdialysis probes proved to be suitable tools for continuous sampling of NH_4^+, NO_3^- and amino acids from the soil solution with only minimal disturbance of the soil structure. Compared to commonly used soil extraction techniques or lysimeter applications microdialysis proved to be superior regarding the possible spatial and temporal resolution. Differences in N concentrations could be well detected on a small spatial scale (<1 cm) and within short time intervals (<30 min). Further, in contrast to the other techniques, diffusion-based microdialysis revealed that the pool of plant-available amino acids was contributing most to the total N pool analyzed. Application of N led to an immediate peak of recovery via the microdialysis probes followed by a rapid decrease due to the formation of a depletion zone at the probe surface. Therefore, this relatively new technique will not only provide essential data on diffusion rates of a variety of N compounds in the soil but might be used for monitoring quantitative and qualitative changes of N pools in soil microsites such as the rhizosphere. However, our results are still based on laboratory experiments and the challenge now is to apply this new technique in a field setting. Additional field studies including contrasting soils as well as different environmental conditions will therefore be necessary to thoroughly test the reliability and applicability of microdialysis sampling. We believe that, after establishment in the field, this approach will provide valuable information about the dynamics of plant-available N compounds in soils, such as diffusion rates, adsorption to soil particles and turnover rates in soil microsites.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2011.03.003.

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