Microdialysis – a new technology for investigating soil nitrogen fluxes in the rhizosphere

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Abstract:
Extracts of soil are used to provide estimates of plant-available nitrogen sources such as nitrate, ammonium and amino acids (low molecular weight nitrogen, LMW-N). Soil extracts are a blunt tool; they introduce a number of inaccuracies through soil disturbance, and do not indicate the rapidity of N pool turnover. Microdialysis is used predominantly in neuroscience but was recently introduced in soil research. Small *in situ* probes cause minimal disturbance, and passive diffusion of solutes across a semi-permeable membrane allows dialysate to be collected over time allowing study of nutrient flux dynamics. This sampling mode is functionally similar to plant roots, and may provide a good estimate of the N forms available to roots. We used microdialysis to quantify induced diffusive fluxes of LMW-N in a subtropical agricultural soil under three fertiliser regimes. Shifts in LMW-N fluxes were detected over time, suggesting the formation of depletion zones around the probe surface similar to those associated with roots. A pronounced difference was observed between results from microdialysis and soil extracts. In dialysate, amino acids contributed up to 70% of LMW-N in unfertilised soil and 5-20% in fertilised soils. In contrast, amino acids were a minor constituent in soil extracts, highlighting that soil extracts underestimate amino acid availability in soils. Modelling plant N uptake based on soil N fluxes and root uptake kinetics, we show that use of inorganic N in fertilised soil was constrained by the root's uptake. In contrast, fluxes of amino acids and the root's uptake capacity were closely matched.

Keywords: turnover, dialysis, sugarcane, agriculture, amino acids

Introduction

Nitrogen (N) turnover in soils comprises a web of processes which are connected in a dynamic network. A diverse array of organic and inorganic N forms are continuously taken up and released by soil organisms and plants, and metabolised both internally and externally by enzymes. Simultaneously, N moves between the dissolved and soluble pools by being bound to (and released from) soil particles. These characteristics make it problematic to obtain realistic information on the N composition of soil solution and the fate of particular N compounds. Destructive soil sampling followed by sieving and soil extraction using water or a salt solution is the standard technique for measuring both inorganic and organic soil available N fractions, and has been used with only minor modifications for half a century (Keeney and Bremner 1966). Soil extractions have numerous advantages; however, it is evident that extractions introduce artefacts such that the size and composition of the organic N pools considerably deviate from those *in situ* (Hobbie and Hobbie 2012, Inselsbacher 2014). Furthermore, measured concentrations of solutes in soil extracts cannot easily be compared to root and microbial uptake capacities. Thus, soil chemical and biological properties may not share a common basis for characterization, effectively hindering evaluation of their interaction. Microdialysis technology has recently been adopted for minimally-invasive soil sampling, and has revealed large fluxes of high turnover low molecular weight organic N compounds such as amino acids in forest soils (Inselsbacher and Näsholm 2012). In this study microdialysis was used to assess the prevalence and composition of nitrate, ammonium and amino acids in a sugarcane agricultural soil. Results were compared with those obtained by traditional soil extracts. Additionally, changes in N forms over a time course of sampling in a single probe location were determined to quantify compound-specific depletion rates around the probes during sampling.

Methods

A sugarcane farm (27°46'41.79"S, 153°19'37.33"E) at Jacob's Well, Queensland, Australia was used for this study. Samples were taken from three adjacent fields by *in situ* microdialysis, and by two types of soil

extraction (water and 1.5 M KCl). The three fields had received different N supply treatments – urea, organic fertiliser and no fertiliser. The 'urea fertilised' field received 135 kg N ha^{-1} as urea 10 days prior to sampling, with ~2.3mm of rain and irrigation occurring before sampling. The organic field had residual plant litter from the previous rotation of soybeans, harvested six months prior to sampling, and had received approximately 330 kg N ha^{-1} of organic N through an application of mill mud (a sugar mill waste). A full site description is included in Brackin *et al*., (2015). We sampled six sub-sites on each field. Eight independent samples were collected at each of the 6 sites, resulting in 48 samples per field. The six sites were separated by ~10 m and formed an approximate rectangular shape (10 × 30 m). The microdialysis system consisted of two syringe pumps (CMA 400, CMA Microdialysis AB, Kista, Sweden) equipped with eight syringes (2.5 ml, Hamilton, Bonaduz, Switzerland) and two refrigerated fraction collectors (CMA 470). Microdialysis probes (CMA 20) had a polyarylethersulphone membrane (10 mm long, 500 µm outer diameter) with a 20 kDa cut-off. Diffusive fluxes from the soil across the probe membrane to the microdialysis sampler were estimated by calculating the total amount of each N compound diffusing across the membrane surface and expressed as nmol N cm^{-2} h^{-1}. Probes were inserted to 15 mm depth. Water (~2 ml) was added to the soil surrounding each probe site for ease of probe insertion. Microdialysis sampling occurred for 1 h using a perfusate flow rate of 5 µl min^{-1} of MilliQ water, except for the first site on each field, where five consecutive samples of 20 minutes duration (totalling 100 minutes) were collected from each probe location to determine if fluxes change over time. After microdialysis sample collection, soil was sampled to ~2 cm depth in the immediate ~3cm vicinity around the probe, and subsequently extracted using deionised water or 1.5 M KCl within 24 h as per Holst *et al.*,(2012). Effects of probe N depletion on KCl values are likely to be negligible due to the difference in relative volumes of the probe and the extracted soil. Nitrate, NH_4^+ and amino acids were analysed as per Richards *et al.* (2012). A model was used to estimate plant uptake of soil N using compound-specific sugarcane root N uptake capacity and soil fluxes, as per Brackin *et al.* (2015). Data were analysed using one-way ANOVA with Tukey's Post-hoc test (Statistica, StatSoft Inc, Tulsa, USA).

Results & Discussion

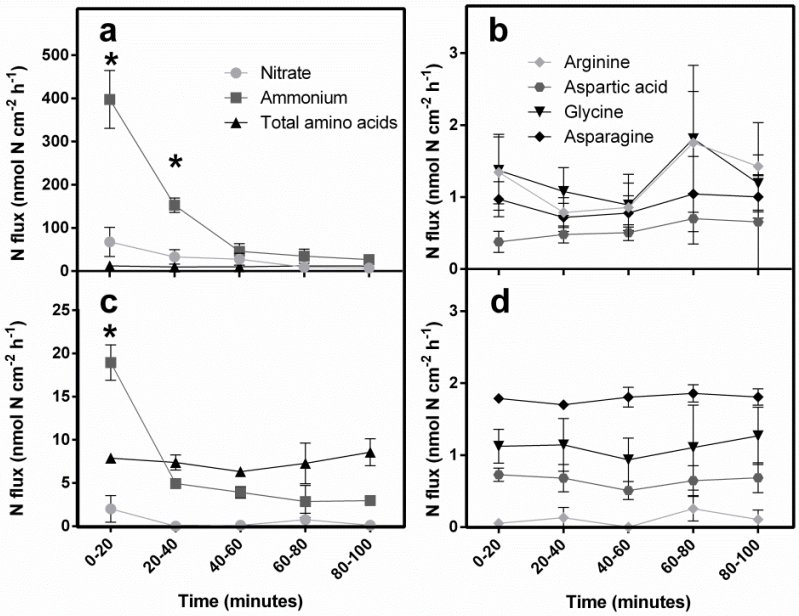

Figure 1: Time sequence of N compound fluxes in a single location in 20 minute time intervals. Urea-fertilised (a, b) and unfertilised (c, d) soils are shown. Nitrate, NH_4^+ and total amino acids are shown in the left panels, and amino acids with contrasting chemical properties in the right panels. Error bars are SEM, n=8, asterisks represent significant differences compared to the 80-100 minute sample fraction.

Sequential microdialysis fractions collected at 20 minute intervals revealed a significant decrease in NH_4^+ fluxes over the initial 60 minutes in the fertilised soils (Fig. 1a), and over 40 minutes in the unfertilised soil (Fig. 1c).. Nitrate flux rates were unaltered over time (the decline in the unfertilised soil was not statistically significant). Amino acid flux rates remained constant over the 100 minute sampling period in urea- and non-fertilised soils (Fig. 1). Flux rates of individual amino acids with contrasting chemical properties did not vary significantly over the 100 minute sampling period in either soil (Fig. 1 b, d). Plant roots have been shown to deplete NH_4^+ levels in a zone of <10 mm around the root, however this occurs to a lesser extent with NO_3^-

(Jungk 2001). Diffusion into a plant root (or microdialysis probe) is driven by a concentration gradient and depletion rates are determined by uptake rates and by the mobility of each N compound in soil. As NO_3^- is more mobile in soil than NH_4^+ by a factor of 5-10 times (Abaas et al. 2012), it is reasonable to expect that the microdialysis probe draws NO_3^- from a greater volume of soil around the probe, resulting in less pronounced depletion. In general, amino acids have a comparatively low mobility in soils similar to NH_4^+ (Abaas et al. 2012), however soil flux rates of amino acids did not significantly decrease over the sampling period. This may be a result of relatively smaller concentration gradients between soil and perfusate, or an indicator of high amino acid turnover and production, allowing the amino acid pool size to stay reasonably similar despite continual withdrawal from the pool by the microdialysis probe over 100 minutes. While free amino acids are rapidly consumed by microbes in soil, having half-lives estimated as 0.75 to 3.5 h (Jones et al. 2009) or minutes (Fischer et al. 2010), studies worldwide have shown surprisingly consistent levels of amino acids in KCl extracts of soils and in soil solution (Jones et al. 2009, Holst et al. 2012). These findings suggest that amino acid production rates in soil are similar in magnitude to consumption rates, indicating that large quantities of N move rapidly through amino acid pools over time (Holst et al. 2012).

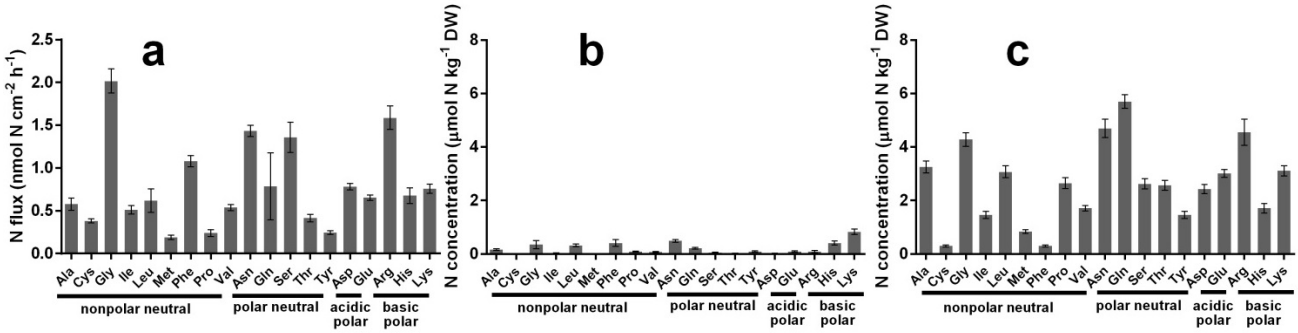

Figure 2: Diffusion rates of individual amino acids estimated by (a) microdialysis, (b) free soil amino acid N concentrations estimated by water extraction and (c) exchangeable amino acid N estimated by KCl (1.5 M) extraction averaged across all soil treatments. Bars represent means (± standard errors), n=120.

Amino acid profiles differed between N sampling methods: the largest N fluxes were in the forms of glycine, arginine, asparagine, serine, and phenylalanine (Fig. 2a), the free N pool had very low concentrations of most amino acids, and the most prominent compounds were lysine, asparagine, leucine, phenylalanine and histidine (Fig. 2b); and the exchangeable N contained glutamine, asparagine, arginine, glycine, and alanine as the main amino acids (Fig. 2c). Prominent in diffusive flux and free N pools, phenylalanine was a small contributor to exchangeable N pools (Fig. 2c). No clear trends were observed between amino acid polarity class and prevalence in each sampling method, although polarity class has previously resulted in differences in depletion of amino acid standard solution added to soil (Inselsbacher et al. 2011).

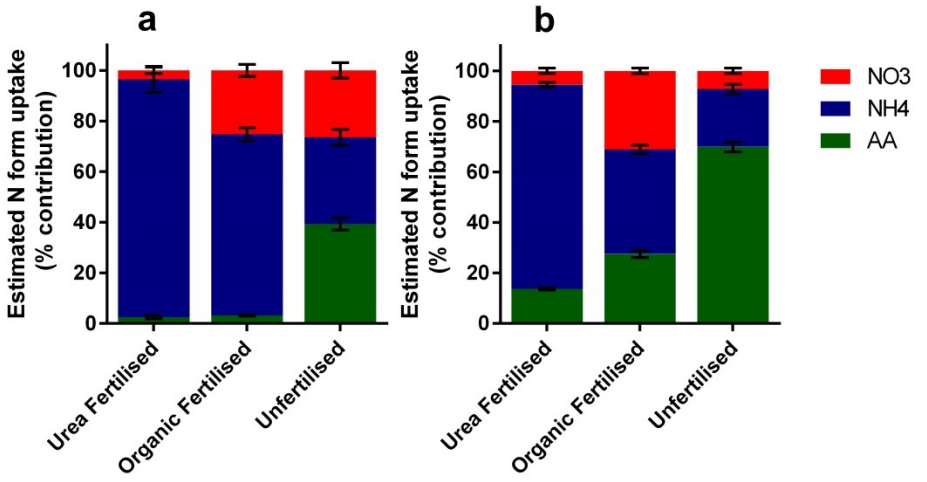

Figure 3: Estimated percentage contribution of soil N forms to plant nutrition based on a) the assumption that plant uptake will mirror soil availability as measured using KCl extraction, and b) based on a model using microdialysis fluxes from this study, combined with surface area-specific root uptake capacities for each N form calculated using data from Brackin *et al.* (2015).

In organic fertilised soil, amino acids contributed <3% of N in water and KCl extracts but 17% of N in the diffusive flux. In unfertilised soil, amino acids contributed ~40 % of N in water and KCl extracts and 70% of the flux. Using a model incorporating both soil diffusive fluxes and plant root uptake capacities (Brackin et al. 2015), we can roughly estimate potential plant N uptake from soil, which differs substantially from estimates based on the assumption that plant N uptake will mirror soil N form availability (Fig. 3). Use of high NH_4^+ and NO_3^- concentrations in fertilised soil is constrained by root N uptake capacity (Brackin et al. 2015), while the entire measured flux of amino acids can potentially be accessed by the plant. In this case, in the urea-fertilised and the organic-fertilised soils, 96% and 57% of the inorganic N flux is effectively unusable by plants at the time of sampling, as it is above the rate of root uptake capacity. While KCl extracts show inorganic N forms contributing 97.4%, 96.8% and 60.6% of the N in urea -fertilised, organic-fertilised and unfertilised soil respectively, our model suggests that their contribution to plant N nutrition is only 86.3%, 72.5% and 30.1% in the same soils. This suggests the overwhelming dominance of inorganic N in the two fertilised soils may not necessarily infer that plant N acquisition is equally dominated by these forms. Microdialysis probes acquire N in competition with soil microbes, although greater N consumption (or production) may occur in the active rhizosphere associated with a living root.

Conclusion

Microdialysis provides new ways to examine soil N dynamics and root-soil N interactions. Our results give insight to the development of depletion zones around roots, and indicate that the forms accessed by a root over time are likely to change. Importantly, flux measurements indicate that rapid production of amino acids results in relatively constant flux rates arriving at the root surface. From this and previous findings, we conclude that amino acids may contribute a greater proportion to plant N needs than would be assumed from quantification of N pools in soil extracts. An additional strength of microdialysis is that it can be directly related to root uptake kinetics for new insights into plant N uptake that consider rhizosphere and root dynamics. Future research will compare microdialysis- induced fluxes with other avenues for estimating nutrient fluxes over time such as lysimeters, resin bags and soil mineralisation incubation assays.

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