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Development of online microdialysis-mass spectrometry for continuous minimally invasive measurement of soil solution dynamics

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ABSTRACT

The rate that amino acids are removed from the soil solution is poorly known but vitally important. It is possible to determine the time course of soil solution concentrations by extracting soils at different time points after adding labelled compounds, but this approach either lacks sufficient temporal resolution or generates large number of samples that require subsequent offline analysis. The aim of this study was to develop online microdialysis-mass spectrometry to enable the minimally invasive measurement of the time-course of isotope labelled amino acid added to soil. The method was subsequently tested by examining the fate of isotope labelled L- and D-alanine added to sterile and non-sterile soils. One concern with application of microdialysis to soil is if calibrations are affected by inorganic ion composition of the perfusate and the external (soil) solution. Tests showed that the presence/absence of inorganic ions in perfusate and external solution did not affect dialysate concentrations, suggesting that perfusing with an artificial soil solution matching the inorganic ion composition of the external solution does not convey any benefits. Hence water was used as perfusate for development of online microdialysis-mass spectrometry. The online system took around one minute to equilibrate to step-changes in concentration and had detection limits around 0.5 μmol L^{-1} for alanine. Addition of isotope labelled alanine to soils led to an almost instantaneous increase and subsequent decrease in dialysate alanine concentration. With sterile soils there was a slow abiotic decrease in dialysate concentrations, presumably due to development of a depletion shell around the microdialysis probe and adsorption of alanine to the soil. For non-sterile soils there was an additional more rapid biotic decrease in dialysate concentrations that presumably reflected microbial uptake. For L-alanine added to non-sterile soil much of the compound was taken up before it reached the probe surface and concentrations decreased to below detection limits within 5–20 min. Thus microdialysis afforded a graphic illustration of the ephemeral nature of intact L-alanine in non-sterile soil, while parallel measurements showed that added D-alanine was removed from soil solution several times more slowly.

1. Introduction

Nitrogen commonly limits productivity of terrestrial ecosystems (Vitousek and Howarth, 1991) and consequently there is intense interest in the pools and fluxes of N in soil. Soil solutions typically contain a large pool of oligomers and polymers of amino acids (Michalzik and Matzner, 1999; Yu et al., 2002; Andersson and Berggren, 2005; Kögel-Knabner, 2006; Farrell et al., 2011; Hill et al., 2011b, 2012) with the bottleneck in N cycling being the rate these high molecular weight organic N compounds are depolymerised by extracellular enzymes into small peptides and amino acids (Schimel and Bennett, 2004). There is rapid uptake and strong competition for amino acids by plants and microbes (Jones and Murphy, 2007; Boddy et al., 2008; Jones and Kielland, 2012; Roberts and Jones, 2012; Warren, 2013b), which leads to amino acids having a short residence time and low steady-state concentrations in the free and adsorbed pools (van Hees et al., 2005; Jones and Willett, 2006).

The metabolism of low molecular weight compounds such as amino acids has been explored using a variety of techniques. The most common approach is to add isotope labelled amino acid to soil and quantify evolved $^{14}CO_2$ (e.g. Jones et al., 2004; Boddy et al., 2007; Glanville et al., 2012) or $^{13}CO_2$ (Warren, 2012). Typically studies observe that mineralisation has a biphasic response (e.g. Oburger and Jones, 2009; Glanville et al., 2012). The dynamics of soil solution concentrations after adding substrate are not as well-known as rates of mineralisation, though are vitally important for interpreting isotope labelling experiments. For example, in experiments where soil is injected with isotope labelled amino acid(s) to investigate amino acid uptake by plants one needs to know the rate added amino acids are removed from solution and concentrations at root surfaces (Hobbie and Hobbie, 2013). For soils at steady state the half-life of amino acids in solution can be calculated from isotope pool dilution estimates of gross

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fluxes of amino acids (Wanek et al., 2010; Hu et al., 2017). For non-steady-state experiments, directly measuring the rate added label is removed from soil solution is logistically challenging. Studies examining amino acid uptake by plants have typically sampled soils ≫ 30 min after adding label by which time amounts of labelled amino acid in free solution are typically below detection limits and the bulk of label is in microbial biomass and/or has been mineralised (McFarland et al., 2002; Warren, 2009b, 2012). In one of the few studies to directly measure depletion of soil solution amino acids at high temporal resolution, it was found that the soil solution half-life of added amino acids was 4–7 min (Jones et al., 2004) which neatly explains why previous studies did not detect labelled amino acids in soil solution after 30 or more minutes.

The approach used by Jones et al. (2004) to measure soil solution half-life involved extracting separate sub-samples at specific time points (e.g. 0, 5, 15, 30, 60 min), which is labour intensive and prone to artefacts. The approach is labour intensive because the rapidity of amino acid metabolism means that samples need to be collected at short time intervals (e.g. every 5 min for an hour), and thus a small experiment yields a large number of samples. Artefacts associated with extraction (Jones and Willett, 2006; Inselsbacher et al., 2011; Hobbie and Hobbie, 2012; Inselsbacher, 2014) are problematic because they could alter estimates of amino acid concentrations in soil solution. In addition to this general problem there are specific concerns about use of extractions for time-course experiments. Extraction with water or salt solution does not yield discrete time-points because extraction does not lead to an immediate cessation of metabolic activity, and thus during the extraction process (which typically lasts > 5 min) there will be artefactual changes in compound concentrations (Rousk and Jones, 2010; Inselsbacher, 2014).

Microdialysis could be one means of probing soil solution concentrations at high temporal resolution. Microdialysis involves insertion of a small probe into the soil and subsequent perfusion of the probe lumen with a perfusate solution. The probe contains a semi-permeable membrane that allows small molecules to penetrate the membrane down a concentration gradient (Miro and Frenzel, 2005). The outflow from the probe (dialysate) contains the analytes of interest and can subsequently be analysed (Korf et al., 2010). Microdialysis was originally developed for *in vivo* sampling of brain neurotransmitters, but has subsequently been applied to other systems including soils (Miro and Frenzel, 2005; Sulyok et al., 2005; Inselsbacher et al., 2011; Inselsbacher and Näsholm, 2012). One of the major advantages of microdialysis is that it can estimate concentrations of analytes in the soil solution of scarcely disturbed soil – thereby avoiding many of the pitfalls associated with collection and extraction of soils (e.g. Inselsbacher et al., 2011; Hobbie and Hobbie, 2012; Inselsbacher and Näsholm, 2012). Microdialysis is intrinsically suited to continuous sampling, yet most commonly dialysates are collected over a period of tens of minutes and subsequently analysed offline as discrete samples.

If samples are collected by microdialysis and then subsequently analysed offline the temporal resolution may not be sufficient for compounds that turnover rapidly. In previous studies, for example, dialysates were pooled over 26- to 30-min intervals after adding substrates to soil (Inselsbacher et al., 2011; Ganeteg et al., 2017), which represents slower sampling than required for analysing dynamics of amino acids in soil solution (e.g. if the half-life of amino acids is 4–7 min: Jones et al., 2004). The frequency of sampling is ultimately limited by needing to collect a large enough dialysate volume for subsequent analysis. A minimum of 10 μL is typically required for sample handling and downstream analysis, which at microdialysis perfusion rates (1–4 $\mu L\,min^{-1}$) suggest the minimum time required to collect sample will be between 2.5 and 10 min. The frequency of sampling may be further limited by the ability of the lab to analyse the hundreds of samples that would result from sampling at high temporal resolution. For example, if samples are collected every 2.5 min, each hour of microdialysis generates 24 samples, such that for a replicated (n ≥ 3) experiment sampling for a total of 120 min more than 100 samples would be generated. Such a large number of samples would be impractical for analytes that require expensive or time-consuming downstream analysis (e.g. isotope labelled organic compounds best analysed by LC-MS or GC-MS).

One way of getting around the limitations posed by offline analysis is to directly couple microdialysis with an analytical detector. On-line coupling reclaims the unique property of microdialysis to yield continuous data at high temporal resolution while eliminating the need for manual handling of microliter volumes, storage of samples, and time-consuming off-line analyses (Jin et al., 2008). A variety of analytical detector can be directly coupled with microdialysis (e.g. LC, CE, biosensors: Jin et al., 2008), but mass spectrometry is an appealing option from the perspective of tracing low molecular weight organic compounds in soil. The reason for this is that mass spectrometry permits the quantification of isotopologues and thus lends itself towards experiments involving addition of isotope-labelled organic compounds. The on-line coupling of microdialysis with mass spectrometry was demonstrated at least 25 years ago (Deterding et al., 1992), but is yet to be applied to soil.

The aim of this study was to develop on-line coupling of microdialysis with mass spectrometry as a tool to trace soil solution concentrations of isotope-labelled amino acids added to soil. Amino acids are the focus because they are the largest input of organic N to soil, and are significant nutrient sources for microbes and plants (Jones et al., 2009). Studies to date have largely focussed on the fate of L-enantiomers of amino acids (Jones et al., 2004), but there is evidence that rates of mineralisation and/or microbial uptake are slower for D-than L-enantiomers (Hill et al., 2011a; Broughton et al., 2015; Formanek et al., 2015) although other studies suggested comparable cycling of D- and L-enantiomers of amino acids (Hu et al., 2017). Thus we applied online microdialysis-mass spectrometry to investigate how the rate of removal from soil solution compares for D-versus L-alanine.

2. Materials and methods

2.1. Soil

Experiments were carried out on soil collected from mesocosms that have been described previously (Warren, 2014c, 2016). Replicate 200-L mesocosms were established in June 2009 with a sandy loam soil pooled from A1 and A2 horizons of an abruptic lixisol in western Sydney (34.0 S, 150.6 E, 75 m above sea-level). Mesocosms were held within a sunlit polythene-covered greenhouse that transmitted around 70% of sunlight and maintained temperatures within 5 °C of ambient. In November 2009 mesocosms were planted with two perennial native grasses *Themeda triandra* and *Microlaena stipoides*. Soils used for microdialysis were collected in November 2017 from four mesocosms that had been kept well watered since establishment. Approximately 250 mL of surface (0–15 cm) soil from each of four mesocosms was pooled, homogenised, visible roots were removed and then approximately 45 mL of soil was packed into replicate 50-mL polypropylene centrifuge tubes (27 mm diameter, 115 mm heigh). The soil-filled centrifuge tubes (hereafter referred to as microcosms) were pre-incubated at 50% of field capacity at 22 °C for 4 days prior to experimentation. The soil used for microdialysis had a pH (H_2O) of 6.5, organic C of 1%, and particle size distribution of approximately 40% coarse sand, 42% fine sand, 10% silt and 8% clay.

2.2. Microdialysis apparatus and procedures

Microdialysis was carried out with commercially available equipment using a syringe pump (NE-1002X Microfluidics Syringe Pump, New Era Pump Systems, Farmingdale, NY, USA) driving 500-μL PTFE-tipped gas tight syringes (SGE, Ringwood, Vic, Australia). Approximately 20 cm of FEP tubing (internal volume = 3.6 μL)