Soil Biology & Biochemistry 114 (2017) 93–103

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Improving *in situ* recovery of soil nitrogen using the microdialysis technique

Scott Buckley [a, *], Richard Brackin [a], Torgny Näsholm [b, c], Susanne Schmidt [a], Sandra Jämtgård [b]

[a] School of Agriculture and Food Science, The University of Queensland, Brisbane, QLD, 4072, Australia
[b] Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden
[c] Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, SE-901 85 Umeå, Sweden

ARTICLE INFO

Article history:
Received 14 May 2017
Received in revised form
7 July 2017
Accepted 9 July 2017
Available online 16 July 2017

Keywords:
Soil nitrogen availability
N flux
Inorganic N
Amino acids
Organic nitrogen

ABSTRACT

Microdialysis is a technique that can be used to sample fluxes of nitrogen (N) in soils with minimal disturbance. To advance our understanding of the technique and improve N recovery, we compared a common membrane type (10 × 0.5 mm probe length and width, 20 kDa molecular weight cut-off; MWCO) with alternative length and MWCO configurations (30 mm; and 100 kDa MWCO). We hypothesised that the alternative membranes would improve recovery of low molecular weight N *via* increased surface area and membrane pore size. The test environments, sampled at fixed pump flow rates, were: (i) stirred 100 μM N standard solution containing organic (amino acid) and inorganic (ammonium, nitrate) N; (ii) soil spiked with 100 μM standard N solution; and (iii) *in situ* boreal forest soil. In general, long membranes recovered more N, but the magnitude of improved recovery varied with test environment. Long membranes recovered more inorganic N regardless of flow rate, except ammonium in stirred solution, where length had no effect at slow flow rates. Long membranes also recovered more organic N from stirred solution regardless of flow rate, and recovered most N at slow flow rates in spiked soil. Longer membranes recovered more amino acids *in situ* in forest soil, with improved resolution of individual amino acids, but were biased towards soluble, mobile forms. MWCO did not affect N recoveries, indicating that in the test conditions, membrane length had greater control than pore size. We discuss the bottlenecks of microdialysis application in soil research and conclude that optimised membrane configurations will advance its use as a tool for quantifying nutrient fluxes in soils.

1. Introduction

Microdialysis is a novel method for sampling solutes, initially developed for biomedical research to sample or deliver solutes within living tissue *via* diffusion, with minimal impact or disruption (Nandi and Lunte, 2009). Use of microdialysis has increased in environmental monitoring (Miró and Frenzel, 2004, 2005; Öhlund and Näsholm, 2004; Sulyok et al., 2005; Inselsbacher et al., 2011), and the low-impact nature of the technique is suitable for examining processes in undisturbed soil and nutrient availability at small spatial scales, such as the rhizosphere (Inselsbacher et al., 2011).

In-depth descriptions of the microdialysis technique have previously been presented (Miró and Frenzel, 2005; Nandi and Lunte, 2009; Inselsbacher et al., 2011). Briefly, soil solutes are sampled by passive diffusion across a small semi-permeable membrane, positioned in the soil with minimal disturbance to the surrounding soil structures. Diffusion is induced by the slow perfusion of water behind the membrane, allowing solutes to move across the membrane along a concentration gradient. The solute/water mixture (termed 'dialysate') is collected for analysis. Subsequent measures of solutes are termed a diffusive flux; *i.e.* the amount of solute which has passed across the membrane over the sampling period (often expressed in nmol m^{-2} s^{-1}). The technique is still considered novel for soil research, but has already provided valuable information on N availability and potential N acquisition by plants in natural and agricultural soils (Inselsbacher and Näsholm, 2012;

* Corresponding author.
E-mail address: s.buckley3@uq.edu.au (S. Buckley).

http://dx.doi.org/10.1016/j.soilbio.2017.07.009
0038-0717/

Inselsbacher et al., 2014; Oyewole et al., 2014; Shaw et al., 2014; Brackin et al., 2015; Buckley et al., 2016; Oyewole et al., 2016). As microdialysis avoids many of the disruptions that are introduced by aqueous soil extractions (Jones and Willett, 2006; Ros et al., 2009; Rousk and Jones, 2010; Hobbie and Hobbie, 2013; Inselsbacher, 2014), it may provide better estimates of plant-available N in soils – especially since estimates of soil nutrient fluxes can be related to both surface area and nutrient uptake capacity of roots (Brackin et al., 2015; Oyewole et al., 2016).

However, microdialysis sampling often provides low recoveries of target molecules from soil. Of the N compounds tested so far, organic N (in the form of amino acids) can constitute a considerable proportion of the low molecular weight N fluxes in soils (Inselsbacher et al., 2011; Inselsbacher and Näsholm, 2012; Inselsbacher et al., 2014; Brackin et al., 2015), but concentrations for individual amino acids in dialysates are often near the detection limits of analysis. To address this issue, this study explores ways the microdialysis technique can be optimised for increased N recovery and improved sensitivity.

Recovery of a solute (E_d) is a function of resistances to solute movement imposed by the soil environment (R_{ext}), the membrane itself (R_m) and the dialysate flowing behind the membrane (R_d) as follows (Bungay et al., 1990):

$$E_d = 1 - \exp(-1/Q_p(R_d + R_m + R_{ext})) \quad (1)$$

where Q_p is the perfusate flow rate. Improvements to recovery can be made by decreasing these resistances. Similarly, slower flow rates can improve recoveries, but at the cost and practicality of longer sampling times (Inselsbacher et al., 2011).

For low molecular weight compounds in soil, $R_{ext} >> R_m >> R_d$; that is, the resistances to solute movement within the soil have greater control over solute recovery than membrane or dialysate resistances (Hsiao et al., 1990; Miró et al., 2010). R_{ext} includes environmental factors such as impedances to solute movement by the soil solid phase (Tinker and Nye, 2000), and biological processes which dictate the production and removal of compounds from the matrix; for instance, microbial immobilisation and mineralisation (Schimel and Bennett, 2004). However, whilst aiming for minimal soil disturbance, it is undesirable to modify the soil matrix to reduce R_{ext}. R_d includes resistances introduced by the perfusate, such as viscosity, temperature and solutes already present in the perfusate (Miró et al., 2010), but these generally have minor effects on recovery (Bungay et al., 1990). R_m remains as a means of increasing solute recoveries, achievable by modifying physical attributes of the membrane.

R_m can be described as follows (Bungay et al., 1990; Hsiao et al., 1990):

$$R_m = ln(r_o/r_i)/2\pi L\, D_m \varnothing_m \quad (2)$$

where r_o is the membrane's outer radius, r_i is the membrane's inner radius, L is the membrane length, $D_m\, \varnothing_m$ is the diffusion coefficient of the membrane for a specific solute. From this equation, physical and practical characteristics of the membrane, including radius, length (L), and diffusion coefficient ($D_m\, \varnothing_m$), could be modified to reduce R_m. The effect of greater membrane length on solute recovery has been shown (Tossman and Ungerstedt, 1986; Eliasson, 1991; Kjellström et al., 2000; Miró and Frenzel, 2005); yet few studies use membranes longer than 10 mm – particularly in environmental sampling – leading to the assumption that longer membranes may lack robustness for field use (Miró and Frenzel, 2005). Here, we compare the effectiveness of conventional membranes (10 mm length, 20 kDa MWCO) to a custom-made 30 mm membrane with the same aperture (Fig. 1).

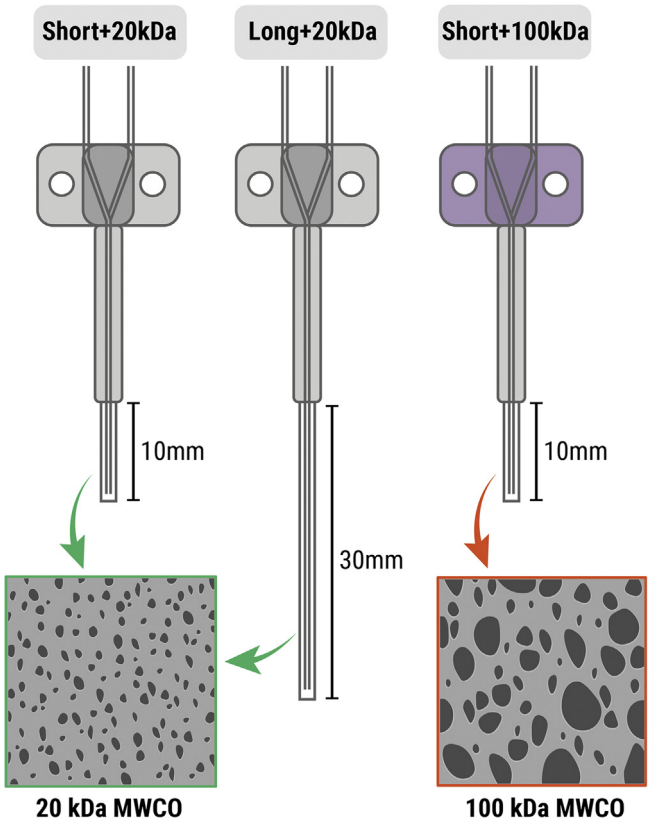

Fig. 1. Three types of membranes with different molecular weight cut-off (MWCO) or length were used. Short+20 kDa membranes represent a common configuration used in soil N sampling.

Increasing $D_m\, \varnothing_m$ should also increase recoveries, particularly when – for a membrane of a fixed radius and length – $D_m\, \varnothing_m$ will have the greatest influence over R_m (Bungay et al., 1990). $D_m\, \varnothing_m$ is modified by membrane porosity and tortuosity, but both are difficult parameters to measure directly so that studies empirically derive an effective diffusion coefficient (D_{eff}) for a given membrane and compound (Torto et al., 1999). Quantifying D_{eff} can be made more difficult by membrane 'fouling', the adsorption of solutes (most typically larger organic molecules such as proteins) to membranes, which block or interfere with the passage of smaller solutes (Rosenbloom et al., 2005). Fouling can affect diffusion of target solutes over time (Torto et al., 1999; Snyder et al., 2001), and is likely a phenomenon in environmental microdialysis sampling where heterogeneous solutions of diverse organic molecules predominate (Torto et al., 1998).

Although 20 kDa MWCO membranes would not typically hinder low molecular weight (LMW) solutes during sampling (Bungay et al., 1990), fouling may decrease membrane functionality. While we do not directly measure $D_m\, \varnothing_m$, or the effect of fouling on R_m, we hypothesize that a larger membrane pore size (characterised by a larger MWCO) may improve N recoveries through decreased solute hindrance, and/or tortuosity of solute movement, during exposure to soil environments likely prone to membrane fouling. Most recent N studies have utilised ≤20 kDa MWCO membranes, but 100 kDa MWCO membranes have also been used to estimate N fluxes across a grassland soil gradient (Shaw et al., 2014). We therefore investigate the effectiveness of a commercially-available 100 kDa MWCO membrane to recover N from soil (Fig. 1). Three test environments were chosen to represent different levels of experimental control: a