Microdialysis - a sensitive method for estimating plant-available N released during litter decomposition

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Abstract

Given the importance of soil nitrogen (N) availability in controlling N supply of plants and microbes, accurate estimates of soil N forms are vital. However, common extraction methods disrupt the soil environment, biasing estimates of soil N availability. Microdialysis offers an alternative by sampling N fluxes with minimal disturbance, and here we compare ex situ soil microdialysis with traditional potassium chloride or water extractions in the context of crop litter decomposition. We amended soil microcosms with sugarcane (0.68% N) or soybean (2.51% N) litter at realistic rates (0.72, 5 and 14.3 mg C g^{-1} soil), quantified microbial activity parameters throughout a 30-day incubation period, and sampled N at day 30. In contrast to soil extractions, the diffusive fluxes generated with microdialysis facilitated a high-resolution snapshot of N availability. Microdialysis revealed that N was immobilised in the presence of sugarcane litter and was mineralised with soybean litter. Nitrogen immobilisation or mineralisation increased mostly with litter dose (although sensitivity varied somewhat between treatments) and in agreement with observed microbial activities. Such N processes were not apparent in soil extractions, indicating uniform N concentrations and forms across litter treatments. The only exception was the high soybean-amended treatments, in which total N increased. Our findings challenge the effectiveness of soil extractions to estimate plant-available N and resolution of N cycling processes in soils. Conversely, microdialysis represents a sensitive method for estimating the fine-scale N fluxes that are relevant to plants and insight into the factors regulating N cycling.

Key Words

Soil nitrogen, nitrogen availability, microdialysis, soil extractions

Introduction

Soil N availability is an important parameter for plant and microbial nutrition. Knowledge of available N forms, and the rates at which plants and microbes acquire them, is crucial to improving N management practices in agricultural systems, and to predict responses to increasing amounts of anthropogenic N deposition in natural systems. Factors driving N availability are complex, and methods of sampling and estimating available soil N can add additional complexity. Extractions of soil N with salt solutions (KCl, K_2SO_4) or water are cost-effective for sampling exchangeable and dissolved N, but are destructive methods which can confound estimates of soil N and are not a direct measure of the plant-available N pool. For instance, extraction methods can release N from previously protected soil components such as fine roots and hyphae (Hobbie and Hobbie, 2013), and can facilitate mineralising organic N to NH_4^+ and NO_3^-, thereby providing poor estimations of N pools (Inselsbacher, 2014). Variations in extraction methods, such as shaking time, temperature and extractant concentrations, can further confound N estimates (Jones and Willett, 2006, Ros et al., 2009). This highlights the need for standardised methods of sampling soil N, which are both repeatable and minimally disturb soil structures.

A number of methods exist which attempt to sample soil N with minimal disturbance; we focus here on microdialysis is recently adopted method (Inselsbacher et al., 2011). The core function of microdialysis is centred around small probes (featuring semi-permeable polyarylethersulfone (PAES) membranes, 0.5 mm x 10 mm with a 20 kDa molecular weight cut-off that excludes microbes), which can be inserted into soils with minimal disturbance. A perfusate (such as water) is pumped behind the membrane, inducing a diffusive flux (DF) of soluble soil compounds across the membrane along a concentration gradient. The resulting dialysates that contain the target compounds are collected for later analysis in a cooled fraction sampler. The small probe size, and passive nature of sampling minimises artefacts that caused by disturbance and different extraction methods. Here, we compare the effectiveness of microdialysis and traditional soil extractions to estimate available N in the context of a litter decomposition experiment. We hypothesised that different patterns of induced microbial activity ensue in response to different litter quality and quantity; N immobilisation in response to sugarcane litter with high C/N ratio, and N mineralisation in response to soybean litter with low C/N ratio).

Methods

Unsieved agricultural soil (chromosol; 60 g dry soil equivalent) was loosely packed into microcosms (Inselsbacher et al. (2009), and pre-incubated at 27°C for 4 days at 70% water-holding capacity (WHC). Sugarcane litter (C/N: 63.1) and soybean litter (C/N: 14.8) was mixed homogenously with soils before re-packing into microcosms. Litter was added at three rates of C, corresponding to differing rates of N addition (Table 1), a control received no litter. Microcosms were randomised in custom foam blocks and incubated at 27°C and 80% humidity for 30 days, and watered every 1-2 days to maintain ~70% WHC.

Table 1. Amounts of C and N added to soil microcosms per litter treatment.

Litter Treatment	Sugarcane (mg N g^{-1} soil)	Soybean (mg N g^{-1} soil)
Control	0	0
Low (0.72 mg C g^{-1} soil)	0.01	0.12
Medium (5mg C g^{-1} soil)	0.08	0.83
High (14.3 mg C g^{-1} soil)	0.23	2.38

Five microcosms (per litter treatment) were destructively harvested at days 0, 3, 8 and 30 for analysis of soil biological and chemical properties. Microbial biomass-N (MB-N) was determined as per Joergensen and Brookes (1990), using 5 g of unsieved soil, and a three-day fumigation with $CHCl_3$ before extraction with 10mL 0.5 M K_2SO_4. A K_{EN} of 0.54 was used to estimate the fraction of MB-N mineralised (Brookes et al., 1985). Protease activity was determined as per Kandeler (1996). CO_2 respiration was measured as per Brackin et al. (2013), for 7 microcosms (per treatment), measured every 1-2 days.

On day 30, ten microcosms per litter treatment were destructively harvested, with 5 g of unsieved soil extracted with 10 mL of 1M KCl or distilled H_2O for one hour on an orbital shaker, centrifuged, and supernatant collected for N analysis. Additionally, diffusive flux (DF) of four microcosms (per litter treatment) were non-destructively sampled using a microdialysis method described previously (Brackin et al., 2015), except for use of an alternative syringe pump (CMA 4004, CMA Microdialysis AB, Kista, Sweden), and a sampling flow rate of 1µl/min for five hours. NO_3^- concentrations of all samples were determined as per Miranda et al. (2001). NH_4^+ and amino acids were determined using a UPLC (Ultra Pressure Liquid Chromatography, Waters, Milford, USA; Holst et al. (2012).

Results

Across all sampling methods, Soy High treatments contained the highest concentrations and fluxes of total LMW-N (Figure 1, top). Concentrations of N were generally uniform in KCl and H_2O extracts, with the exception of higher concentrations in Soy High treatments. DF measurements (Figure 1, C: bottom) showed a trend of increasing N fluxes in soy litter treatments, with the effect increasing with litter dose ($r^2 = 0.7862$, $p < 0.001$); although N release in Soy Low treatments were not significantly greater than controls (Fig 1, C; top) indicating either a limitation in sensitivity of the method or threshold of mineralisation. Cane litter treatments did not show a dose-response ($r^2 = 0.2205$, $p = 0.077$), which mirrored soil extractions; however diffusive fluxes showed a (non-significant) trend of decreasing mean N fluxes, which may be resolved with greater sample replication (Fig 1, C; top). Contributions of N forms to total LMW-N differed between methods. Extractions (Figure 1, A & B: bottom) estimated NO_3^- as the dominant N species in all but Soy High treatments which showed NH_4^+ as dominant N form. NO_3^- also dominated N fluxes in controls, Cane Low and all soy litter treatments (Figure 1, C: bottom), but amino acids accounted for the greatest N fluxes in Cane Medium and Cane High treatments (Figure 1, C: bottom).

CO_2 respiration rates were highest in the Soy High treatments over 30 days (Figure 2, A), significantly greater ($p < 0.001$) than Soy Medium and Cane High treatments, the two most similar treatments. Protease activity was highest in the soy high treatment at all time points ($p < 0.001$), peaking at day 8 (Figure 2, C). The Soy Medium treatment had moderately high protease activity that was significantly higher ($p < 0.05$) than cane treatments (except Cane High treatment and controls). Microbial biomass (MB)-N measurements, showed an overall trend across treatments, with initial increases in MB-N peaking at day 3, followed by a decrease by day 8, after which measurements stabilised with a (non-significant) trend towards a steady increase (Figure 2, B). MB-N was greatest in the Soy High treatment ($p < 0.01$); MB-N of Soy Medium and Cane High treatments were also significantly higher than controls on day 3 ($p < 0.01$); however, Cane High biomass declined to control levels by day 30.

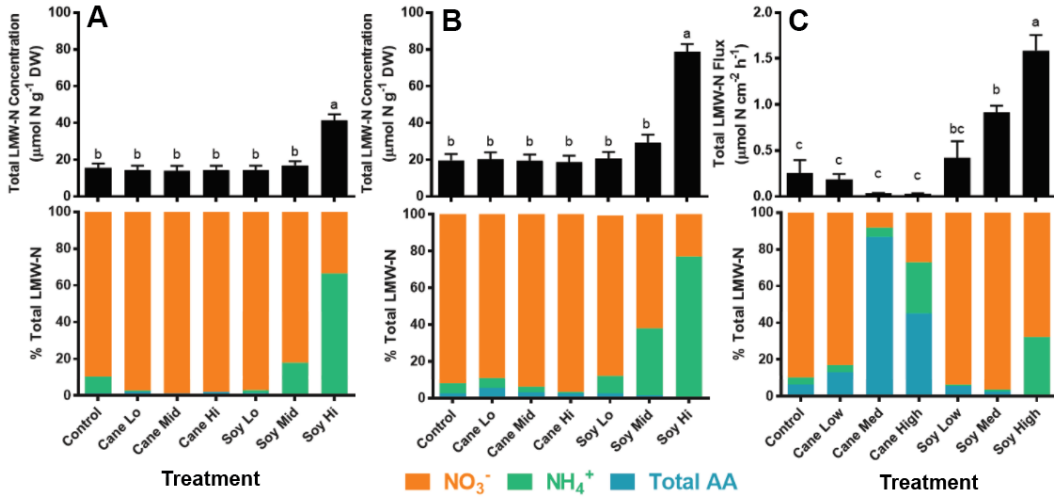

Figure 1. Total low molecular weight nitrogen (LMW-N) concentrations of water (H_2O) and potassium chloride (KCl) soil extractions, and proportions (%) of total LMW-N as nitrate (NO_3^-, orange), ammonium (NH_4^+, green) and total amino acids (Total AA, blue) for H_2O soil extractions (graph A), KCl soil extractions (graph B); total LMW-N diffusive fluxes and % of total LMW-N as NO_3^-, NH_4^+ and Total AA, as measured by microdialysis (graph C). All treatments reflect soil N after 30 days of incubation with sugarcane (Cane) or soybean (Soy) litter. For H_2O and KCl extractions (A, B) n = 10; for diffusive fluxes (C), n = 4. Letters represent significant differences ($p \leq 0.05$) between treatments. Error bars represent ± SE.

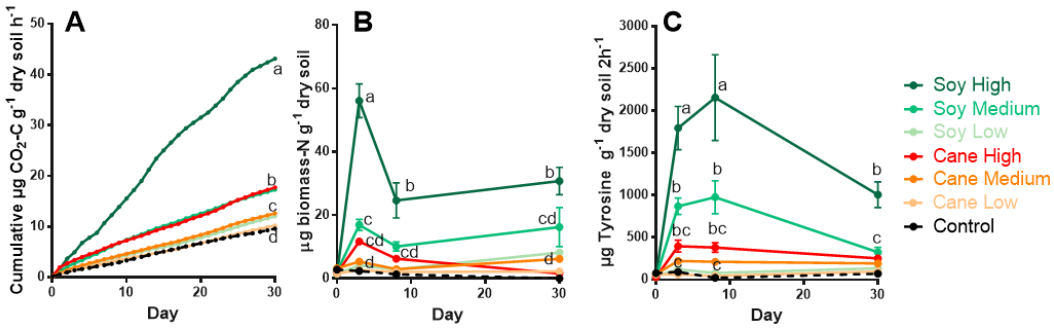

Figure 2. Measures of microbial activity in soils amended with sugarcane (Cane) and soybean (Soy) litter. A) CO_2 respiration (µg CO_2 g^{-1} dry soil h^{-1}); B) microbial biomass-N (µg biomass-N g^{-1} dry soil); C) protease activity (µg tyrosine g^{-1} dry soil $2h^{-1}$). For microbial biomass-N and protease activity, n = 5 for each data point. For cumulative CO_2, n = 7 for each data point. Letters represent significant differences ($p \leq 0.05$) between treatments at each time point, or at end of experiment (cumulative CO_2 respiration). Error bars represent ± SE.

Discussion

There is a need for protocols that better represent the undisturbed soil environment in which plants and microbes co-exist. The complex nature of N cycling requires sensitive methods capable of resolving minute changes in N pools over time, in context with the dynamic, transformational role of the microbial community. Here, we show that microdialysis provides a different view of N availability compared to conventional soil extractions, and more consistent with measures of microbial activity. Although statistically not significant with litter dosage with cane litter, a notable pattern of decreasing mean N fluxes suggest that N is immobilised, while significant N mineralisation occurred in a dose-response dependent manner with soy litter. The latter flux patterns are congruent with higher measures of microbial activity in soy litter treatments, which are likely to increase rates of N cycling (Bengtsson et al., 2003). In contrast, cane litter with high rates of C input appeared to rapidly immobilise available N in microbial biomass. H_2O and KCl extraction methods failed to show relative patterns of N availability in response to litter inputs, yielding almost uniform total LMW-N patterns. Only the highest soy litter input had elevated N concentrations indicative of a higher threshold of N sensitivity in extraction methods (Inselsbacher, 2014, Jones and Willett, 2006). Estimations of N pools are particularly important when identifying the diversity of N forms available to plants and microbes. Since amino acids accounted for the significant proportion of N fluxes in Cane Medium and High treatments, organic N may dominate plant-available N pools during periods of high microbial N immobilisation. Because soil extractions did not reveal such patterns, current concepts of N source use generally assume exclusive use of inorganic N by plants (reviewed by Paungfoo-Lonhienne et al., 2012). However, we argue that a broader view of N source use by crops and microbes can be derived from

alternative methods such as microdialysis, tension lysimeters and ion exchange membranes and resins, which measure N availability in context with microbial influences and potential N pools (Jämtgård et al., 2010, Stackpoole et al., 2011).

It is important to note that diffusive fluxes and N concentrations in soil extracts are differing measures – the former is a temporal measure of dissolved N, the latter a static measure of absolute N concentrations. Future studies will compare diffusive fluxes to soil extractions over multiple time-points, providing a fairer basis for comparison, and alignment with microbial processes. Nevertheless, microdialysis represents a promising method for estimating plant-available N, with potential for genuine insight into spatial and temporal factors affecting N cycling in undisturbed soil environments.

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