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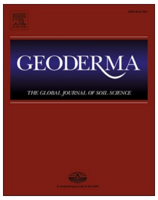

Microdialysis fluxes of inorganic nitrogen differ from extractable nitrogen by minimising disturbance of mineral-associated sources

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ABSTRACT

Measuring soil nitrogen (N) provides important information for ecosystem productivity and improving N use efficiency in agricultural systems. Conventional means of sampling N using soil extractions disturb soil structure and function, and likely distort accurate quantification. In situ microdialysis is a novel sampling method that generates differing N profiles compared to soil extractions. Here we test the hypothesis that differences observed between sampling methods are due to the minimal disturbance and sampling of a mobile N fraction when using microdialysis, with discernible patterns expected across soils with distinct clay and organic matter contents. In a short-term laboratory microcosm experiment with 21 sugarcane cropping soils, we compared salt (potassium chloride; KCl) or aqueous (H_2O) extractants and microdialysis. KCl-extractable ammonium (NH_4^+) was highly correlated with the content of clay, total N and carbon, indicative of bound N being solubilised. In contrast, NH_4^+ contributed significantly less to microdialysis fluxes and was not correlated with the measured soil properties, which we attribute to minimal disturbance of bound N·H_2O extracts sampled proportionally more NH_4^+ than microdialysis but were significantly correlated with fluxes. This suggests that while microdialysis and H_2O extraction sample from a dissolved N pool, H_2O extracts sample from an additional pool of loosely-bound NH_4^+. Nitrate (NO_3^-) measures were correlated between methods, but shared no relationship with the measured soil properties, indicating that NO_3^- sampling is less affected by the disturbance introduced by extractions. We conclude that sampling inorganic N is biased by the degree to which soil sampling methods disturb adsorbed N sources with implications for interpreting soil N measurements.

1. Introduction

Nitrogen is present in soil in a wide diversity of compounds, spanning organic and inorganic forms. Knowledge about soil N concentrations and fluxes is critically important for understanding ecosystem productivity, soil health, and for improving N use efficiency in agricultural systems which is a global priority (Udvardi et al., 2021). Measures of exchangeable and soluble N, as generated by salt-based and water-based extractions, are commonly used to identify the forms and concentrations of N that is potentially accessible to plant roots and soil microorganisms at a static timepoint. As extractions introduce high a degree of disturbance to soil structures, extractions may under- or over-represent soluble and fixed N fractions in soil (Ros et al., 2009). Examining how different types of sampling methods measure N pools across diverse soil types could help to better understand the true size and nature of soil N, as well as the innate biases of each method.

One approach is to compare extractions to in situ sampling methods which minimise structural disturbance of soils. Microdialysis is an in situ sampling method to measure N fluxes in soils (reviewed by Buckley et al., 2020). The technique allows for fine-scale sampling of metabolites via induced diffusion, using small probes fitted with semi-permeable membranes which minimise disturbance. Microdialysis fluxes (per unit surface area and time) are relatable to ecological processes, including root uptake rates of nutrients and gas fluxes (Brackin et al., 2015; Leitner et al., 2017), and allow for near-continuous non-destructive sampling. Fluxes are temporal and related to probe surface areas, and thereby contrast with static concentrations (per unit soil dry weight) that are measured by soil extractions, which presents difficulties when comparing microdialysis fluxes to soil studies that are predominantly based on extractions (Buckley et al., 2020). Previous studies have

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compared microdialysis fluxes and extraction concentrations using N proportions, noting that the contribution of inorganic and organic N in microdialysis fluxes can differ significantly from soil extractions. For instance, ammonium (NH_4^+) often contributes proportionally less, and organic N (particularly, uncharged or negatively-charged amino acids) proportionally more to the N quantified in microdialysis fluxes compared to the N forms detected in water or weak salt extractions (Inselsbacher and Näsholm, 2012; Inselsbacher et al., 2014). We hypothesise that these differences are the combined result of (i) minimal disturbances of N pools that are adsorbed to mineral surfaces or organic matter which are solubilised in soil extractions but not with microdialysis, and (ii) sampling bias of microdialysis towards highly mobile compounds which diffuse more easily towards the probe (Ros et al., 2009; Buckley et al., 2020). As microdialysis requires hydraulic connectivity with the external environment to sample solutes via exchange with soil water, we further hypothesise that microdialysis would likely sample from a similar water-soluble pool as H_2O extractions but without the additional disturbances of extractions on soil aggregates or organic structures. Thus, microdialysis would least resemble salt-based extractions that desorb bound N from charged soil surfaces via ionic exchange, and more resemble water extracts. However, limited hydraulic connectivity may also lead to the spatial occlusion of some N compounds (including labile forms such as nitrate, NO_3^-), resulting in a microdialysis-generated N profile that differs greatly from both types of extraction (Darrouzet-Nardi and Weintraub, 2014).

Because of its reliance on diffusion to sample solutes, we also assume that microdialysis can integrate factors that affect the diffusion of N through soil, including sorption/desorption dynamics from mineral surfaces (Tinker and Nye, 2000; Buckley et al., 2020). Solute charge likely affects N sampling with microdialysis, with positively-charged ions less likely to be sampled by microdialysis than extractions (Inselsbacher et al., 2011) with positive charge adsorbed by negatively-charged soil sites (Nieder et al., 2011). These interactions would then be expected to increase in soil with greater contents of clay or organic matter. In contrast, negative ions such as NO_3^- and acidic amino acids may be more easily sampled by microdialysis due to limited interaction with soil surfaces (Inselsbacher et al., 2011). So far, the influence of soil properties on the fluxes of different N ions has not been examined systematically.

To investigate how extractions and microdialysis differ when sampling inorganic N in different soil types, we sampled inorganic N (NH_4^+, NO_3^-) in 21 agricultural soils with microdialysis, as well as H_2O and potassium chloride (KCl) extractions in a laboratory-based microcosm experiment. Soils were collected from the upper 20 cm of tropical and subtropical sugarcane farms in Queensland, Australia, as part of a separate study evaluating soil N mineralisation indices (Allen et al., 2019). All soils had a management history of no N fertiliser applied for at least 12 months and were collected under fallow crops grown between sugarcane ratoon cycles. The soils had diverse physical properties (e.g., clay content 4.6–54 %) and chemical properties (pH 5–7.65; total carbon (C) 0.7–3.4%; total N 0.05–0.24%) (see Supplementary Fig. 1) allowing to systematically examine how soil properties correlate to NH_4^+ and NO_3^- sampled in fluxes and extractions. Soils were stored in resealable plastic bags at 4 °C for three months before handling for microdialysis and extractions. Soil from each site was sampled using KCl extractions, H_2O extractions and microdialysis ($n = 4$) after a short two-day incubation with soils stabilised at 70% water-holding capacity. Detailed descriptions of microcosm preparation, sampling methods and analyses are outlined in Supplementary Methods. Nitrite was measured (see Supplementary Methods) but was commonly not detectable or negligible (data not shown) and so nitrite was excluded as an inorganic N compound for investigation.

Extractions sampled proportionally more NH_4^+ than microdialysis fluxes (One-Way ANOVA, $F_{2,60} = 12.17$, $p < 0.001$; Fig. 1, A),

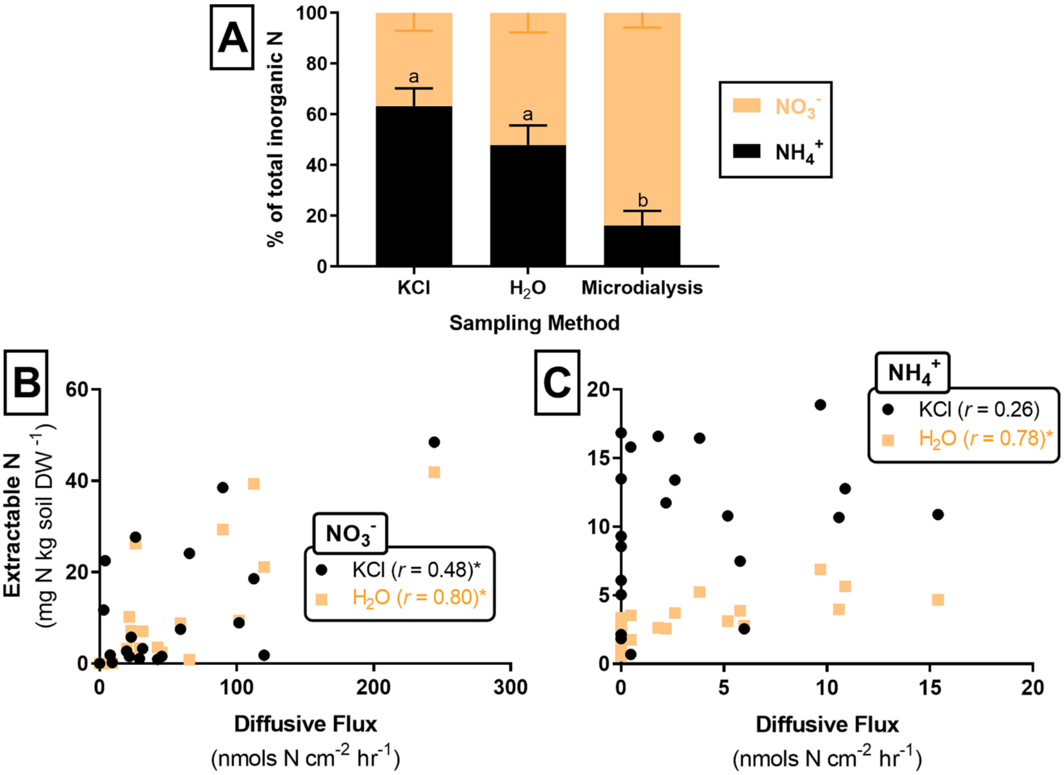

Fig. 1. A: Proportions of total inorganic N as NH_4^+ (in black) and NO_3^- (orange) as estimated by extractions (KCl, H_2O) and microdialysis and averaged from 21 soils. Error bars represent ± 1 SEM; letters denote statistical differences between sampling methods (Tukey's post hoc test, $p < 0.05$). **B, C:** Spearman correlations of NO_3^- and NH_4^+ fluxes (nmols N cm^{-2} hr^{-1}) versus extractable-N (KCl, in black circles; H_2O, in orange squares) across all soil sites with r values provided; * $p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

contributing 63.3 ± 7.0 % of total inorganic N (the sum of NH_4^+-N and NO_3^--N) in KCl extractions, and 48 ± 7.7 % in H_2O extractions, compared to 16.1 ± 5.8 % in fluxes. NH_4^+ sampled in KCl extractions shared no relationship with microdialysis fluxes (Spearman $r = 0.26$, $p = 0.24$; Fig. 1, B). H_2O also extracted greater proportions of NH_4^+ than microdialysis (Fig. 1, A), however both methods were positively correlated across soil types (Spearman $r = 0.78$, $p < 0.0001$; Fig. 1, B). Although relative NO_3^- contributions differed between microdialysis fluxes and extractions (Fig. 1, A), fluxes were positively correlated with both extraction methods (KCl extractions Spearman $r = 0.48$, $p = 0.029$; H_2O extractions Spearman $r = 0.80$, $p < 0.0001$; Fig. 1, C), suggesting that absolute measures of NO_3^- are similarly represented by all methods. Overall, inorganic N concentrations (particularly NO_3^-) were comparatively high in comparison to other unfertilised sugarcane soil (Brackin et al., 2015) and this may be attributable to the soil storage, which can promote disproportionate increases in NO_3^- (Bailey et al., 2021). Finally, we used Spearman r correlations to assess the relationship between soil parameters and the varied N methods considered in the study (Table 1). KCl-extractable NH_4^+ showed positive correlations with soil chemical properties, including total N, total C and potentially-mineralisable N, but negative correlations with pH and bulk density (Table 1). In contrast, neither H_2O-extractable NH_4^+ and NH_4^+ fluxes, nor any measurements of NO_3^- (regardless of sampling method) were correlated with the soil properties measured here.

Table 1
Spearman correlations between KCl extractable-N, H_2O-extractable N, and N flux quantified with microdialysis versus soil parameters – clay content (%), bulk density (BD; in g soil DW cm^{-3}), electro-conductivity (EC), pH, total C and N (%), potentially-mineralisable N (PM-N; mg N kg^{-1} soil). Numbers represent Pearson r values; asterisks represent significant relationship (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Dashes represent non-significant correlations ($p \geq 0.05$).

	Clay %	BD	EC	pH	Total C	Total N	PM-N
KCl Extractions							
NH_4^+	0.51*	−0.57**	–	−0.73***	0.49*	0.65***	0.62**
NO_3^-	–	–	–	–	–	–	–
H_2O Extractions							
NH_4^+	–	–	–	–	–	–	–
NO_3^-	–	–	–	–	–	–	–
Microdialysis							
NH_4^+ (Flux)	–	–	–	–	–	–	–
NO_3^- (Flux)	–	–	–	–	–	–	–

Our findings suggest that the major difference in inorganic N measurements between microdialysis and extractions lies in the degree of disturbance of the soil matrix that may promote or minimise exchange with adsorbed NH_4^+, which is in turn dependent on soil factors that enhance N adsorption or storage, such as greater clay or soil organic matter content. As microdialysis fluxes of NH_4^+ related little to soil factors measured here, this indicates that the method minimises disturbance of adsorbed N sources, resulting in smaller NH_4^+ fluxes. In contrast, KCl extractions desorb bound NH_4^+ via K^+ exchange boosting the presence of NH_4^+. H_2O extractions represent a middle ground, sampling proportionately more NH_4^+ than microdialysis fluxes, but both methods sharing a correlative relationship. This suggests that microdialysis and H_2O extractions may sample from a related water-soluble N pool, but with H_2O extractions sampling from an additional pool of loosely-bound NH_4^+. In contrast, NO_3^- appears to be much less influenced by disturbance, likely through its negative charge leading to limited interaction with soil surfaces, and thus greater solubility. It is already well understood that extractions using salt solutions provide a measure of exchangeable soil N, biased towards the sampling of bound N sources which could release into the soil solution to supply plants and soil organisms (Ros et al., 2009). However, we also highlight that microdialysis fluxes present their own bias towards highly soluble compounds like NO_3^-. This bias has previously been argued to represent diffusible compounds most likely to be encountered by microorganisms and plant roots in situ (Inselsbacher et al., 2011; Buckley et al., 2020). We assume this is because fluxes account for in situ physical and chemical properties that influence diffusion through altered sorption rates and solute charge (Tinker & Nye, 2000). Yet in our study, we found inorganic N fluxes measured were not directly impacted by the physical or chemical soil properties considered (Table 1), implying that our overarching assumption may be incorrect – at least in the context of our short-term microcosm experiment using disturbed soils. Fluxes may be better correlated to properties immediately adjacent to the microdialysis probe, but suitable methods which can accurately evaluate these small soil volumes – within millimetres of the membrane surface (Buckley et al., 2020) – would be needed. Microbial activity was not evaluated here (except for potentially-mineralisable N), but it is likely that microbial mineralisation, ammonification and nitrification greatly contribute to measurable fluxes (Bailey et al., 2021), and should be considered in future studies.

We conclude that sampling of NH_4^+ is biased by the degree to which soil sampling methods disturb adsorbed N sources, with in situ microdialysis minimising disturbances and leading to smaller fluxes and proportion of NH_4^+. In contrast, NO_3^- is less influenced by the sampling method indicating that NO_3^- sampling is less affected by soil disturbance. Future studies comparing microdialysis to extractions would greatly benefit from observing the effects on organic N, which forms a large and chemically diverse pool of compounds of variable solubility and diffusive capacity in different soil environments, and relevant to plant and microbial nutrition (Farzadfar et al., 2021).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2023.116510.

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