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Using microdialysis with a deuterium oxide tracer to estimate water exchange, water content and active surface area of the probe

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ABSTRACT

Microdialysis is a useful tool for measuring *in situ* fluxes of soil compounds with minimal disturbance of soil structure and function. Fluxes of sampled compounds are commonly calculated per unit of membrane surface area, assuming that the entire membrane surface is capable of exchange – which is unlikely given varying soil moisture and the occlusion of membrane pores by the soil solid phase. We present a method to quantify the degree of connectivity of the microdialysis probe membrane to the surrounding soil by means of water exchange between a microdialysis perfusate and soil solution using deuterium (^2H_2O; equilibrated to DHO) as an internal standard. We applied the method to a range of probe membrane surface areas and soil moisture conditions to generate empirical models that estimate membrane surface area active in exchange. Our results suggest that even in a saturated sandy soil, active membrane surface areas reach only 40.3% of the probe surface area, perhaps due to occlusion by soil particles. However, when accounting for volumetric water content of the soil, active surface areas approached 80–90% of the area likely in contact with water, indicating that sampling efficiency of water-filled pores may still be high, particularly at slow flow rates. Furthermore, our method enables assessment of local soil water content around the probe. Models estimating soil water content were applied to field measurements of DHO exchange in three soil horizons (Organic, B1, B2) at two boreal sites, and *in situ* estimates were similar to those from conventional soil moisture methods when models were calibrated with the same soil type. We present DHO exchange as a powerful method for improving microdialysis flux interpretations in future studies, and for exploring small-scale water variability in relatively undisturbed soils.

Microdialysis is an emerging tool for sampling soil compounds with minimal disturbance, and with high temporal and spatial resolution (Buckley et al., 2020). The technique involves inserting small probes into the soil and pumping a perfusate (usually water) at a slow flow rate ($\leq$5 μL min^{-1}) behind a semi-permeable membrane, inducing the diffusion of soil compounds across the membrane and into the perfusate. Microdialysis thus provides a measurement of solute flux per unit of membrane surface area (e.g. nmols m^{-2} s^{-1}) that is roughly analogous to root uptake. The technique can be useful in measuring solute availability and providing context to other soil flux measurements (Brackin et al., 2015; Leitner et al., 2017a; Leitner et al., 2017b).

Current microdialysis flux measurements assume full membrane exchange with the external environment. This may be true in a fully aqueous environment (e.g. water beaker), where membrane connectivity to external solution is non-limiting. However, soils feature solid mineral and organic particles and variable moisture content which may block or interfere with membrane exchange, limiting the actively exchanging surface area – resulting in an underestimation of potential solute fluxes. On the other hand, raising soil moisture content can increase microdialysis-derived flux rates as hydraulic barriers to diffusion are alleviated (Miró et al., 2010; Buckley et al., 2019; Müller et al., 2023). As water availability can vary even at small spatial scales (Tian et al., 2021), measurement of soil moisture around the probe site would improve interpretation and comparability of flux measurements.

We hypothesized that using an isotopically-labelled perfusate with diluted deuterium oxide (DHO) could quantify water exchange during microdialysis sampling (Bungay et al., 1990). We applied this to different membrane surface areas and soil moistures to create models

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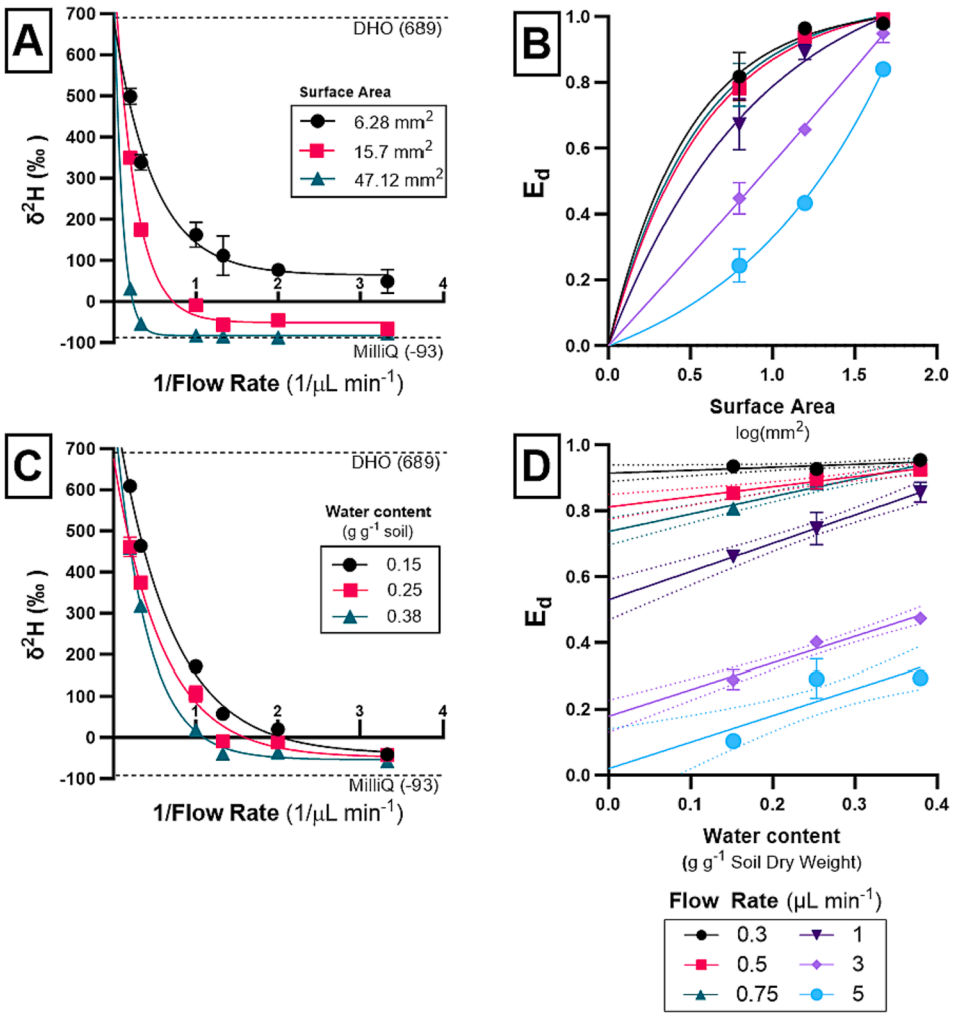

Fig. 1. A) δ^2H measured in dialysates sampled from a MilliQ water solution, using different flow rates (0.3 – 5 µL min^{-1}, expressed as 1/flow rate) and membranes with differing surface areas (6.28; 15.7; 47.12 mm^2). For each flow rate and membrane type, n = 4. B) Non-linear models plotting relative DHO exchange (E_d) versus surface area each flow rate. C) δ^2H measured in dialysates sampled from a MilliQ water solution, using different flow rates (0.3 – 5 µL min^{-1}, expressed as 1/flow rate) at three water-holding capacities (WHC). D) Linear models plotting relative DHO exchange (E_d) versus soil water content for each flow rate. Equations for models in B and D are provided in Supplementary Table S1.

estimating 1) active membrane surface areas; and 2) *in situ* water content.

To estimate active membrane surface areas, we quantified water exchange in a fully aqueous environment (a beaker of MilliQ water, – 93 ‰). Under these conditions we assume that 100 % of a membrane's surface area is exchanging, as external resistances to diffusion and contact are effectively nil (Bungay et al., 1990). We used a variety of flow rates (0.3 – 5 µL min^{-1}), while pumping an equilibrated DHO perfusate (680 ‰) (Fig. 1, A). We used three commercial microdialysis membranes with different known surface areas (CMA20, 20 kDa MWCO; 6.28 mm^2, 15.7 mm^2 and 47.12 mm^2; CMA Microdialysis AB, Solna, Sweden). For each flow rate, pumps were run until 100 µL of dialysate was obtained, and between sampling at each flow rate, water in beakers was replaced with fresh water to remove influence of previous runs on DHO background. DHO composition in dialysates was then analysed using TC/EA-IRMS using a method outlined by Gehre et al. (2004) (further details provided in Supplementary Materials). Relative DHO exchange (E_d) was calculated using the following equation (Bungay et al., 1990):

$$E_d = \left(C_d^{out} - C_d^{in}\right) / \left(C_e - C_d^{in}\right) \quad (1)$$

where C_d^{out} is the δ^2H of the final dialysate, C_d^{in} is the δ^2H of the starting perfusate, and C_e is the δ^2H of the external medium (MilliQ water). We then generated non-linear regressions of E_d versus surface area (Fig. 1, B), which we assume represents relative DHO exchange possible when the entire membrane surface of a given length is actively exchanging.

We then applied the technique (pumping a DHO perfusate at the same flow rates to estimate E_d), to estimate active membrane surface areas in soil microdialysis using 47.12 mm^2 membranes inserted into 5 mL microcosms containing 7 g of a sandy mineral soil (bulk density of 1.4 g cm^{-3}) sampled from the B horizon of a boreal podsol at Rosinedal Research Area (see Lim et al. (2015) for a detailed site description, and Supplementary Methods for details of soil processing). Soil was dried to remove extant water, and then pre-moistened 48 h before sampling with MilliQ water (- 93 ‰) to moisture contents of 0.15, 0.25 and 0.38 g g^{-1} soil dry weight (DW) (Fig. 1, C), providing moisture conditions below and at field capacity, and a saturated condition, respectively – and a gradient of increasingly optimal conditions for water diffusion and exchange. We used one probe per microcosm, and new microcosms were used for each replicate to remove the influence of previous runs on background DHO composition (n = 4 for each moisture condition and flow rate). Values for E_d were then applied to flow rate models in Fig. 1 C to estimate actively exchanging membrane surface areas.

The highest active surface area was achieved at the saturated condition (0.38 g g^{-1} DW) and at 0.3 µL min^{-1} flow rate (19 ± 0.4 mm^2;

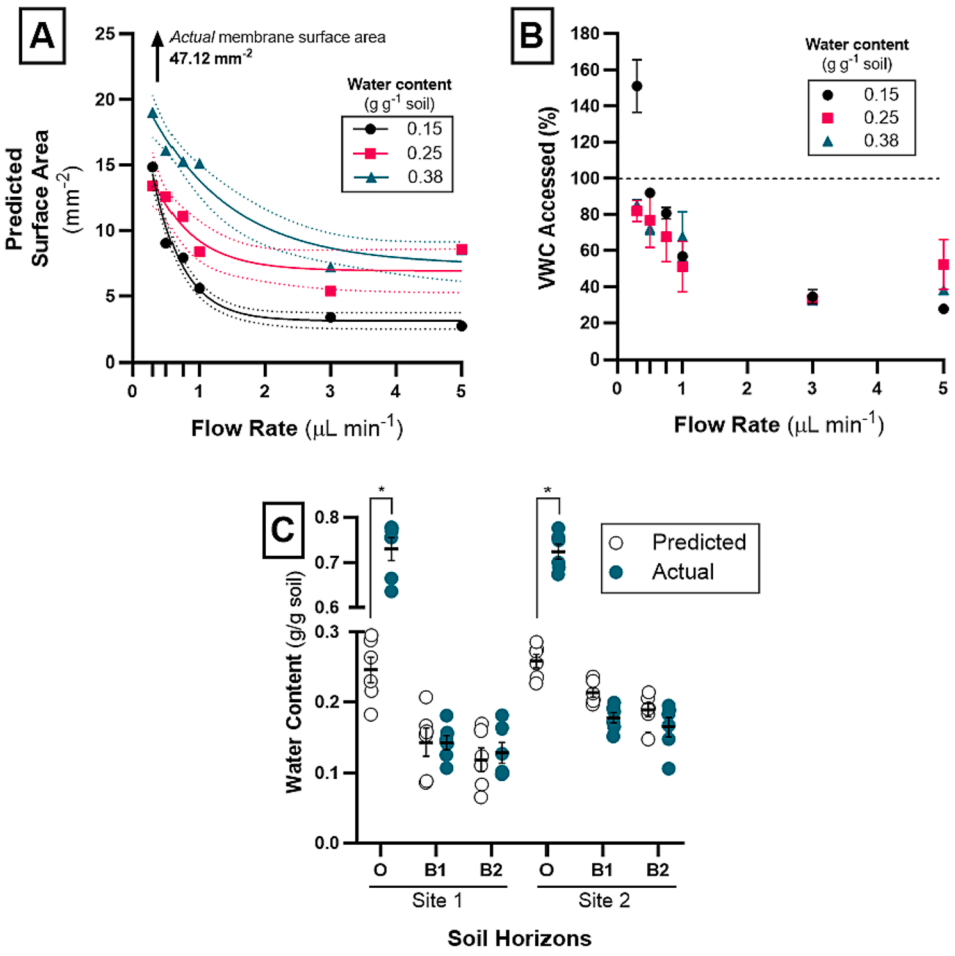

Fig. 2. A) Predicted mean active membrane surface areas at three different water contents (0.15, 0.25 and 0.38 g g^{-1} soil) at flow rates between 0.3 and 5 µL min^{-1}. B) Proportion of volumetric water content accessed by membrane surface areas at differing flow rates, calculated in graph A. Dotted line represents 100 % of water available across the membrane surface area. C) Values for water content measured from two field sites at three soil horizons (O - organic; B1, B2). Predicted values are those estimated using 3 µL min^{-1} model in Fig. 1 D; actual values are those obtained using conventional soil moisture measurements. Asterisk denotes significant difference between predicted and actual values for organic horizons (Unpaired T-test, * - $p < 0.01$).

Fig. 2, A). This was expected given that higher water content optimises connectivity between membranes and soil water, and slower flow rates allow longer exposure times for perfusates to exchange with soil water. However, this represented only 40.3 % of the membrane surface area available, and these estimates decreased with faster flow rates and drier soil. In the drier soil, using a flow rate of 5 µL min^{-1}, we predicted that only 5.8 % of the membrane (2.72 ± 0.02 mm^2) was actively exchanging with soil solution, indicating it is problematic to assume that the entire membrane surface is actively exchanging in a soil environment. Given that most soil studies use a 5 µL min^{-1} flow rate (Buckley et al., 2020) and are below saturated conditions, it is likely many in our community are significantly underestimating microdialysis-derived solute fluxes in soils. This is consistent with the concept that solute concentrations in soil water might increase as soil water films evaporate (Bechtold et al., 2011), increasing localised diffusive fluxes when accessed (by a microdialysis probe, or a plant root), but at the expense of surface area access, which limits overall uptake and exchange.

Interestingly, when we calculated the membrane surface area likely in contact with water in the soil (volumetric water content of the soil (%) × total membrane surface area; 47.12 mm^2), surface areas approached approximately 80——90 % of the accessible water at relatively low flow rate, regardless of the water availability (Fig. 2, B). This suggests that probes can exchange relatively efficiently with what water is spatially available, particularly at slower flow rates. It also highlights how spatial access to water in soil environments is useful for determining the true efficiency of microdialysis sampling. One outlier was noted at 0.3 µL min^{-1} in the 0.15 g g^{-1} DW moisture condition (Fig. 2, B), potentially due to loss of perfusate water during sampling via ultrafiltration (Müller et al., 2023; see Supplementary Fig. S1), which may have facilitated greater connection between membranes and soil water, increasing estimates of active surface areas.

The second component of our study estimates *in situ* water content. To do this we generated linear regressions (Fig. 1, D) based on E_d at different soil moisture contents and flow rates when using a 47.12 mm^2 membrane (Fig. 1, C) in the microcosm study described above. To test these regressions, we measured E_d *in situ* at two sites at the Rosinedal Research area (see Supplementary Materials for more information). At each site, six randomly chosen pits (60 cm deep) were dug, and microdialysis membranes (47.12 mm^2 surface area; n = 6) were inserted into the organic, B1 and B2 horizons. Before sampling, several drops of MilliQ water (approximately 110 µL) were added as a precaution to ensure some hydraulic connectivity. The probes were then perfused with DHO for 45 min at 3 µL min^{-1}. Soil samples (n = 6) were destructively taken to measure soil moisture (using soil weights before and after oven-drying) at each soil pit and horizon. The 3 uL min^{-1} regression in Fig. 1D was used to predict soil water content from E_d values and results were compared to destructive soil moisture measurements (Fig. 2, C).

Our models accurately predicted water content in the B horizons at both sites (Fig. 2, C - B1 and B2), but underestimated water content in the organic horizons (Fig. 2, C - O). As our models were calibrated using

the B horizon soil, the disparity is likely due to the vast differences in soil texture and water-holding capacity between each horizon. This emphasizes the need to calibrate empirical models for each soil type, particularly when soils significantly differ in physical properties affecting water-holding capacities, and perhaps even extant DHO concentrations (such as soil organic matter). We also predicted slightly greater water content in the B horizons at site 2 (Fig. 2 C), possibly due to limitations in our model's ability to predict water content below our lowest soil moisture treatment (0.15 g g^{-1} soil DW). However, they could also be explained our addition of water drops at the probe site, which would have increased water availability locally. Nevertheless, our technique can predict water content *in situ* for calibrated soils, offering insight into small-scale soil moisture variability relevant to biogeochemical processes.

The use of perfusate $^{2}H_2O$ enables assessment of the efficacy of sampling soil solutions using microdialysis, and helps to correct for the impacts of variable soil water availability on solute fluxes. DHO exchange can also aid in quantifying probe performance over extended periods, potentially identifying issues such as faults, or decreased sampling efficiency due to membrane fouling. As such, the technique will improve comparability across studies using soil microdialysis. Finally, information pertaining to spatial and temporal variations in probe performance may also be of relevance for understanding constraints to root-soil exchange of solutes.

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.2023.116689.

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