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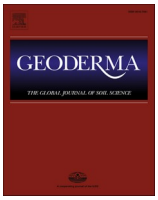

The influence of sucrose on soil nitrogen availability – A root exudate simulation using microdialysis

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

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

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ABSTRACT

Root exudates are thought to promote nitrogen (N) availability via rhizosphere interactions, but empirical evidence is difficult to obtain given the scale and temporary nature of these processes. Microdialysis has potential to simulate root exudation patterns and quantify the effects on N availability simultaneously, but this has so far not been attempted. In a conceptual root exudation study, we used sucrose as a simple C source to investigate if microdialysis could detect the effects of continuous localised C supply on soil inorganic N fluxes. Through retrodialysis we released sucrose and simultaneously monitored diffusive soil N fluxes over one week, followed by a further seven days without sucrose. Based on current understanding of rhizosphere N dynamics, we hypothesised that N fluxes are inversely related to sucrose release, and upon ceasing release, N fluxes would increase. Using a 5 mM sucrose perfusate, C releases resulted in decreased N fluxes, but contrary to our hypothesis, N fluxes did not increase after ceasing sucrose release (*c.f.* control soil). Diffusive sucrose efflux from microdialysis probes increased in soils amended with N-rich litter suggesting that microbial activity and associated sucrose consumption altered sucrose concentration gradients. The fluxes of sucrose breakdown products glucose and fructose were greatest in litter treatments receiving sucrose, indicative of increased invertase activity in the presence of fresh organic matter. In the short term (days), sucrose release did not prompt an increase in inorganic N availability, possibly because of stimulated microbial growth and increased N demand under C-rich conditions. Our study confirms that microdialysis allows time-sensitive insight into the dynamic interactions of carbon and N in the rhizosphere.

1. Introduction

Roots exude a wide range of biochemical compounds into the soil (Sasse et al., 2018; Canarini et al., 2019). Root exudates are considered instrumental in the formation of the rhizosphere – a zone extending millimetres to centimetres from the root surface and inhabited by diverse communities of microbes and microfauna. By providing a continuous source of energy in the form of immediately metabolisable carbon (C), roots increase microbial growth and activity, which in turn can promote the decomposition of soil organic matter (SOM) and increase the availability of essential nutrients such as nitrogen (N) (Kuzyakov et al., 2007; Dijkstra et al., 2013; Yin et al., 2013).

Exudation patterns and N availability likely differ over the length of a growing root, as changes in the quality and availability of exudates shift the response of microbial communities (Jaeger et al., 1999; Kuzyakov and Xu, 2013). Physiological mechanisms controlling exudation from plant roots can vary for specific compounds (reviewed by Canarini et al., 2019), with organic acids and other secondary metabolites exuded in mature sections of the root via symplastic flow and active transport across the plasmamembrane (Sasse et al., 2018; Canarini et al., 2019). At undifferentiated root tips where apoplastic barriers to diffusion are undeveloped, passive diffusion releases primary metabolites (sugars, amino acids) (Dennis et al., 2010; Doan et al., 2017; Sasse et al., 2018; Canarini et al., 2019). Thus, the soil surrounding a root tip will experience significant temporal and spatial shifts in the availability of exudates as a root grows through and beyond the site. Additionally, there is turnover, succession and predation of microbial communities, with consequences for N availability (Bukovská et al., 2018; Rüger et al., 2021). For instance, the zone around root tips has been indicated as a site of decreased plant N availability due to N

* Corresponding author at: Swedish University of Agricultural Science, Department of Forest Ecology and Management, Skogsmarksgränd 17, 901 83 Umeå, Sweden.
E-mail address: scott.buckley@slu.se (S. Buckley).

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immobilisation, as C-rich exudates provide ideal substrates for rapid microbial growth and associated uptake of available N (Chapin et al., 2011). However, as roots tips grow beyond a site and microbes become increasingly N-limited, greater decomposition of SOM by microbes, along with microfaunal predation and biomass turnover, may contribute to an increase in available N in mature root zones (Jaeger et al., 1999; Bonkowski, 2004; Kuzyakov and Xu, 2013).

Root exudation of C compounds may be important in N-limited soils like those of boreal forests, where depolymerisation of SOM-bound organic N likely form a significant bottleneck to N availability (Schimel and Bennett, 2004). Given a strong N-limitation and a fungal-dominated microbial community, it is predicted that root exudates promote greater N availability through faster SOM decomposition (Dijkstra et al., 2013). However, studies exploring the influence of exudation on N availability in boreal and temperate forests have shown varied results, including greater N mineralisation and availability (Nave et al., 2011; Phillips et al., 2011; Yin et al., 2013; Meier et al., 2017) and neutral or decreased N availability (Dannenmann et al., 2009; Winkler et al., 2010; Liese et al., 2018; Wild et al., 2017). In these studies, factors such as microbial community structure (Liese et al., 2018), stoichiometry and supply rates of exudates (Bengtson et al., 2012; Drake et al., 2013) are likely to have strong influences on how much SOM decomposition occurs, and how much N is tightly bound in microbial biomass. Overall, N and C cycling in the rhizosphere remains insufficiently understood; and arguably this is largely due to technical difficulties in quantifying N fluxes at small scales and high temporal resolution.

Common methods to study the influence of root exudates on soils can lead to biases. For instance, while tree girdling limits the supply of root-derived carbohydrates into soils, it also induces dieback and increased turnover of root biomass (Nave et al., 2011), providing C and N sources for microbes. Further, the direct supply of 'exudates' to the soil via pulse injections misrepresents the exudation patterns from roots with unrealistically high supply rates and water regimes aimed to promote microbial responses that are not necessarily representative of natural rhizosphere conditions. Finally, methods of destructively sampling soil, including rhizosphere soil, can transform N pools during soil storage and processing (Jones and Willett, 2006; Ros et al., 2009; Rousk and Jones, 2010; Inselsbacher et al., 2011). A potentially more sophisticated approach is minimally invasive methods such as microdialysis (Demand et al., 2017; McKay Fletcher et al., 2019) and microlysimeters (Kuzyakov et al., 2007; Drake et al., 2013; Meier et al., 2017; Baumert et al., 2018; Zhang et al., 2019). These methods may offer more realistic perspectives on exudation as they mimic the spatial and temporal scales experienced by plant roots, while minimising soil disturbance and solute transformations during sampling.

Microdialysis is a less commonly used tool capable of collecting solutes via induced diffusion (Miró and Frenzel, 2005; Inselsbacher et al., 2011), and its application in soil sciences has been summarised in our recent review (Buckley et al., 2020). Briefly, a perfusion fluid (often water) is pumped at slow flow rates behind a thin semi-permeable membrane, inducing soil solutes to diffuse across the membrane for collection over time. This results in a measurement of flux per unit membrane surface area. By including a solute in the perfusion fluid, the reverse action is also possible, enabling diffusion of solutes into the soil solution – whilst simultaneously sampling solutes from the surrounding environment. This 'retrodialysis' or 'reverse microdialysis' technique was used to mimic exudation of organic acids to examine their influence on soil phosphate availability in near real-time, showing increased phosphate fluxes with greater citrate effluxes, and responses to soil type, sampling time and phosphate depletion (Demand et al., 2017; McKay Fletcher et al., 2019, Schack-Kirchner et al., 2020).

Retrodialysis has not been used to examine the influence of exudates on N fluxes, and so our primary aim was to test the viability of the technique to explore how C release affects inorganic N availability in soil microcosms. As the composition of plant root exudates varies widely (Dakora and Phillips, 2002; Sasse et al., 2018; Vives-Peris et al., 2020), we chose sucrose as a simplified starting model exudate, known to be exuded from living root tips (Jaeger et al., 1999; Farrar et al., 2003), using two concentrations covering the range previously detected in the root cytosol (Lohaus et al., 1994). We simulated the growth of a root tip through and beyond a soil region by releasing sucrose daily for seven days, before ceasing releases for a further seven days. Based on current models for root N availability (Jaeger et al., 1999; Bonkowski, 2004; Kuzyakov and Xu, 2013), we hypothesised that the delivery of labile C would result in short-term reductions in N fluxes as microbes utilise available N for growth. Upon ceasing the release, we hypothesise that N fluxes would increase, presumably through greater microbial turnover and enhanced SOM decomposition. As boreal forest soils are often N limited, we also added an N-rich plant litter to some microcosms to artificially increase total N and microbial activity, and to improve the potential for detecting N fluxes with microdialysis.

2. Materials and methods

2.1. Soil collection and litter preparation

Organic matter rich soil (podsol O-horizon) was collected from the top 10 cm of a boreal forest site near Umeå, Sweden (approximately 63°50′16.5″N, 20°17′52.5″E) in late April 2016, avoiding the superficial S layer (excluding mosses, lichens and fresh plant litter), and collecting material from the F and H layers. The organic layers were chosen for study as they represent a significant hotspot for microbial activity (Högberg et al., 2017), and for fine root distribution in podzolic boreal soils (Lim et al., 2015). Upon transfer to the laboratory, the soil was sieved through a 5 mm mesh to remove large particulates and roots and stored at 4° C until commencing the first experiment 14 days later and the second experiment after a further 7 days. The soil contained 1.44% N and 44.8% C (C/N 31.1).

Pea plants (*Pisum sativum*) were grown in a glasshouse for 4 weeks with replete nutrients and water. Plant stems and leaves were harvested, dried in an oven at 50 °C, milled (particles size < 2 mm) and stored in an airtight container until use. Pea litter was chosen to artificially increase N availability and promote microbial activity when added to microcosm (see section 2.2). Litter contained 2.8% N and 41.8% C (C/N 14.8).

2.2. Microcosm preparation

Two experiments were conducted in succession, each using sucrose solutions of differing concentration – the first using a 0.5 mM sucrose solution, and the second a 5 mM solution (Fig. 1). The second experiment commenced within a week of completing the first experiment.

For each experiment, microcosms were prepared as follows. Twenty-seven g soil (dry weight) was placed in microcosms consisting of modified 50 mL centrifuge tubes, with two layers of circular wire mesh inserted before the convex tube bottom to allow for an air gap that promotes aerobic conditions at the lowest soil depths (Inselsbacher et al., 2009). Soil weights were chosen based on the amount of soil that would reasonably fill the microcosm. Soils were watered to 70 % water-holding capacity (gravimetrically) and incubated at room temperature for three days.

Pea litter (0.3 g dry weight microcosm^{-1}; approx. 8.4 mg N microcosm^{-1}) was mixed into half of the microcosms by tipping soils into plastic tubs, mixing litter homogenously by hand, before returning mixtures to the microcosms. This amount of litter was chosen based on prior unpublished microcosm studies, where the dose provided a significant amount of N-rich litter to promote microbial responses (based on respiration, enzyme activity and microbial biomass), but not to significantly alter water holding capacity or bulk density. No-litter microcosms were also hand-mixed to emulate the disturbances introduced by removing and mixing soils. Half of the microcosms for Litter and No-Litter treatments were then perfused (via microdialysis probes) with either sucrose solution, or with water only, using a microdialysis setup

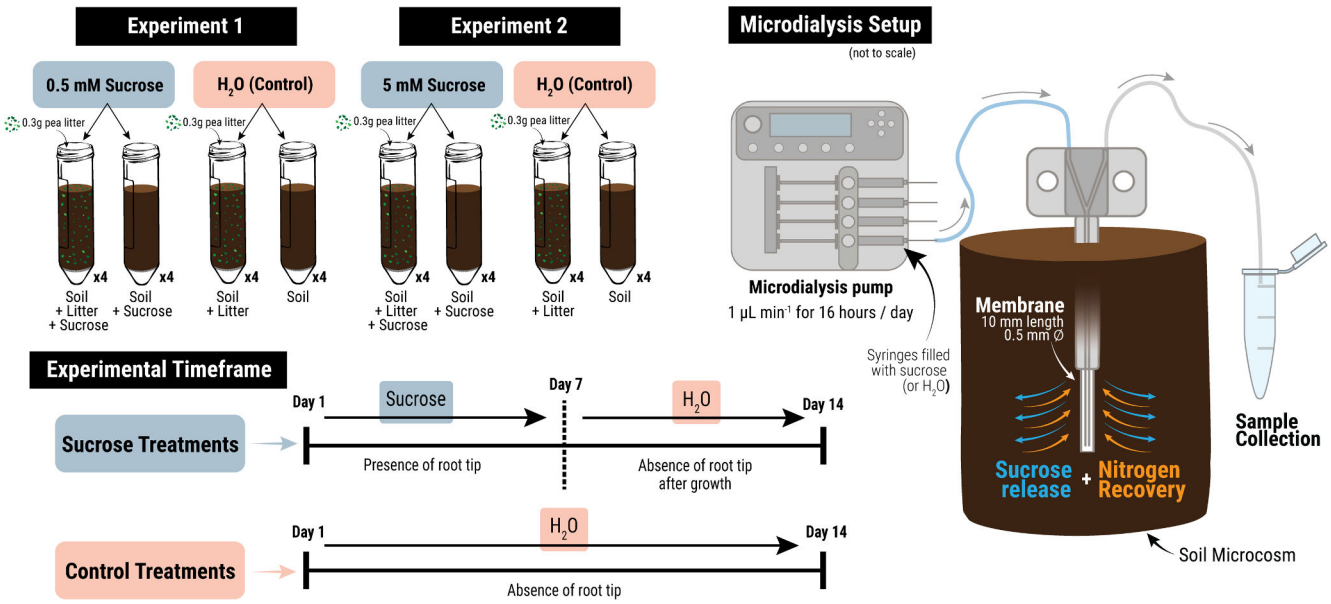

Fig. 1. A conceptualisation of the experimental design, and the microdialysis technique used to examine the influence of sucrose exudation on nitrogen fluxes. Sucrose (or H_2O as control) was released via microdialysis membranes into soil microcosm with and without N-rich pea litter over a 14-day experiment. After day 7, sucrose was swapped for H_2O for the remaining 7 days.

described in section 2.3. For each litter and sucrose treatment, n = 4. Final treatments for each experiment were as follows: Soil only (Soil); Soil + Sucrose; Soil + Litter; Soil + Litter + Sucrose. Non-sucrose treatments (Soil; Soil + Litter) were controls for sucrose treatments.

2.3. Microdialysis sampling and retrodialysis

Four infusion pumps (CMA 4004; CMA Microdialysis AB, Solna, Sweden) were equipped with a total of 16 micro-syringes (2.5 mL; CMA Microdialysis AB, Solna, Sweden). Each syringe was connected to a microdialysis probe (CMA 20; 20 kDa, 10 × 0.5 mm membrane) through-connected to a 1.5 mL collection tube kept on ice during sample collection.

Probes were inserted into individual soil microcosms using an introduction needle to create a small hole. Each day for 7 days, probes were perfused with MilliQ water or sucrose solution (0.5 or 5 mM) at a flow rate of 1 µL min^{-1} for 16 h. During each daily run, sucrose was released via microdialysis membranes whilst soil N was sampled, both occurring simultaneously using the same membrane. These sucrose concentrations were chosen for their coverage of the concentrate range detected in the root cytosol (Lohaus et al., 1994). The daily sampling/release time was chosen primarily for practical reasons (to allow for respiration measurements and soil moisture adjustments without interference from exterior connections) but may also resemble shifts in exudation rates due to diurnal changes in root sugar concentrations (Gibon et al., 2004; Malinova et al., 2014; Brauner et al., 2018). The 7-day release period was chosen to mimic the presence of a root tip within a 10 mm-long soil zone during elongation, and sits within a range of observed tree root elongation rates which vary widely from 0.03 to 12.72 mm day^{-1} depending on growth medium, root order, mycorrhizal status, nutrient and soil moisture conditions (Shaw et al., 1995; Triboulot et al., 1995, 1997; Ludovici and Morris, 1997; Cuesta et al., 2010; Nakano et al., 2012; Ding et al., 2020). At the end of each run, dialysates were frozen at −20 °C for later analysis of N and sugar content. Probes were left in place for the entirety of the experiment. On the eighth day, probes previously perfused with sucrose solution were switched to MilliQ deionised water for the remainder of the experiment. Dialysates were collected for analysis every 1–2 days (daily between days 7–9 as perfusates were changed). New probes were used for each experiment.

To test the influence of sucrose release on concomitant N fluxes, nitrate (NO_3^-) was sampled *in vitro* (100 mL beaker) from five standard solutions ranging from 15.625 µM to 5 mM NO_3^-, whilst perfusing with 5 mM sucrose or water only, using a 1 µL min^{-1} flow rate. 100 µL samples were collected for each standard solution and perfusate treatment (n = 4).

2.4. Measurement of CO_2 respiration

CO_2 respiration was measured daily from day 1 (after the first 24 h) as per Brackin et al. (2013), with four microcosms per treatment measured using a cresol red indicator in 1% agar (Rowell, 1995; Campbell et al., 2003) set in wells of a breakable 96-well microtitre plate (Costar EIA/RIA 1 X 8 Stripwell Plate, Corning, USA). Individual wells were fixed with temporary adhesive into the headspace of the microcosm tubes and sealed for 1–2 h. Wells were replaced into the 96-well plate arrangement and read at 520 nm using a spectrophotometer.

2.5. Analysis of nitrogen compounds and soluble sugars

Dialysates were analysed for ammonium (NH_4^+) using reverse-phase liquid chromatography, by means of a Waters Ultra Performance (UPLC) system equipped with a Waters Tuneable UV detector. Samples were prepared as per Inselsbacher et al. (2011). NO_3^- was determined via the reduction of NO_3^- to NO_2^- with vanadium (III) chloride, followed by the Griess reaction, and measured colourmetrically (Miranda et al., 2001).

Soluble sugars were measured using a three-step enzymatic and spectrophotometric method which quantifies concentrations of sucrose, fructose, and glucose (Stitt et al., 1989). Sucrose effluxes were calculated as the difference between the starting sucrose concentration in perfusates and the final dialysate concentration.

2.6. Data analysis

Diffusive flux (D) was calculated using the following equation (Inselsbacher et al., 2011):

$$D = C_{dial} \cdot v/A_m \cdot t \quad (1)$$

C_{dial} is the solute concentration in the final dialysate, A_m is the

surface area of the membrane (in metres^2), v is the volume of the dialysate, and t is time (seconds).

Sucrose effluxes (E) were calculated using the following equation (modified from Eqn. 1):

$$E = (C_{perf} - C_{dial}) \cdot v / A_m \cdot t \quad (2)$$

where C_{perf} is the sucrose concentration in the initial perfusate (before pumping).

Sucrose effluxes. NH_4^+, NO_3^- and CO_2 respiration data was analysed using Repeated Measures Two-Way ANOVA (or Mixed Effects models for NH_4^+ data), followed by a Bonferroni Post-Hoc test to determine daily differences between treatments. Analyses were split between the first 7 days (when sucrose treatments received sucrose), and the second 7 days (when sucrose was exchanged for MilliQ water). For N fluxes and CO_2 respiration, we focused primarily on post-hoc comparisons between a sucrose treatment and its control: Soil *vs.* Soil + Sucrose; Soil + Litter *vs.* Soil + Litter + Sucrose. For sucrose effluxes, we compared sucrose treatments directly: Soil + Sucrose *vs.* Soil + Litter + Sucrose. Glucose/fructose fluxes were analyses used Repeated Measures Two-Way ANOVA, followed by a Tukey's post-hoc test to compare daily means of all treatments to each other. Linear regression was used to determine differences in relative recoveries of NO_3^- from standard solution, using perfusates with and without sucrose. All analyses were conducted using Graphpad Prism 9 (Graphpad Software, Inc.).

3. Results

3.1. Nitrogen fluxes

In general, inorganic N fluxes in litter-amended soils were greater than in non-litter treatments regardless of sucrose perfusate concentration (Fig. 2). In the 0.5 mM sucrose experiment, NH_4^+ fluxes (Fig. 2, A) and NO_3^- fluxes (Fig. 2, B) in treatments receiving sucrose were similar to their non-sucrose controls ($p > 0.05$). In the 5 mM sucrose experiment, all treatments receiving sucrose had significantly lower NH_4^+ fluxes (Repeated Measures ANOVA; litter treatment $F_{(1,6)} = 8.656$, $p = 0.0027$; soil only $F_{(1,6)} = 23.8$, $p = 0.0028$) than their non-sucrose controls over the first 7 days of the experiment, although individual daily comparisons where not always significantly different (Fig. 2, C, D). The same was true for NO_3^- fluxes in litter treatments (Repeated Measures ANOVA; $F_{(1,6)} = 16.47$, $p = 0.0097$) where fluxes were approximately half those in non-sucrose litter soil, but daily differences were only significant at days 3 and 7 (Fig. 2, D). During the second week, when sucrose in perfusates were exchanged for water, inorganic N fluxes were similar in sucrose and non-sucrose treatments within each litter group ($p > 0.05$; Fig. 2, C, inset).

We also examined the influence of sucrose exudation on *in vitro* NO_3^- recovery. Linear regression of relative recoveries using perfusates with and without 5 mM sucrose had similar slope ($p > 0.05$) and y-intercept ($p > 0.05$) (Supplementary Fig. 1).

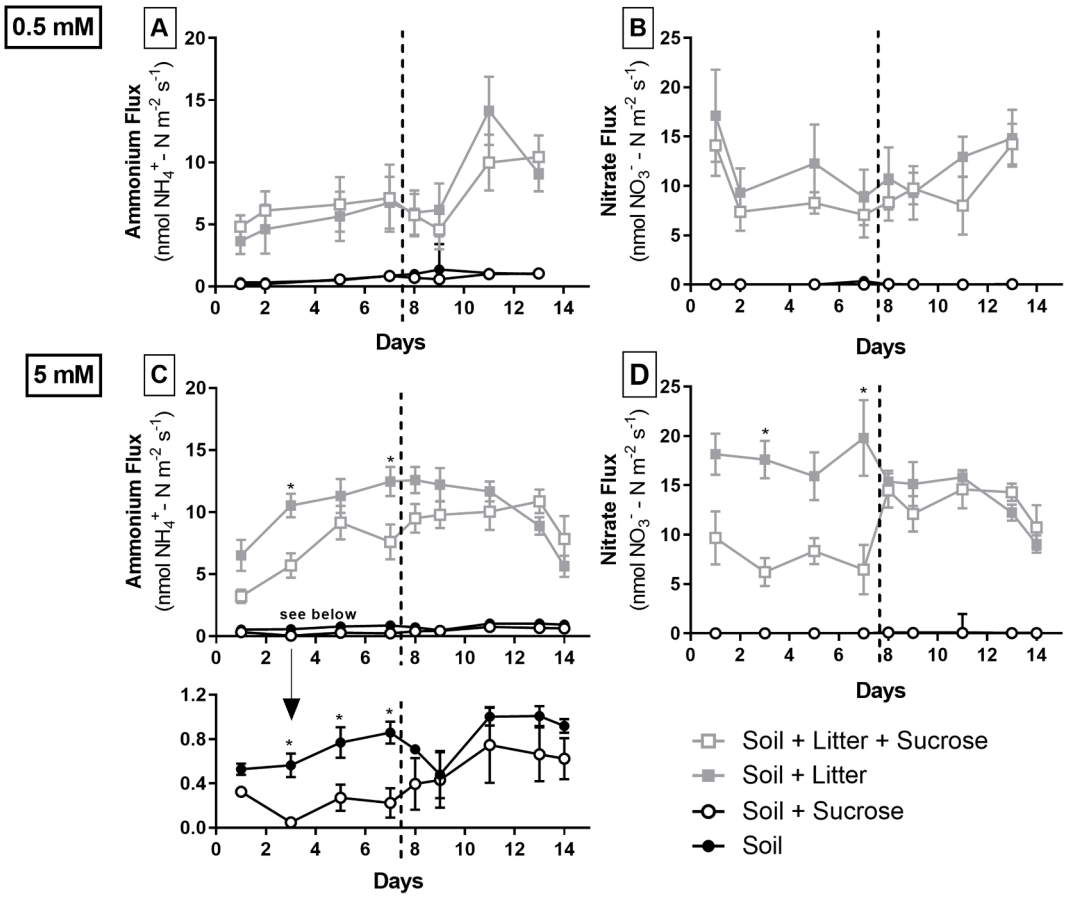

Fig. 2. Fluxes (nmols m^{-2} s^{-1}) of ammonium (A, C) and nitrate (B, D) measured in soils supplied with and without plant litter and sucrose, sampled with microdialysis using two sucrose concentrations as perfusate (0.5 mM – A, B; 5 mM – C, D) over a 14-day incubation. For sucrose treatments, the dotted line after day 7 indicates the point at which sucrose perfusates were exchanged with MilliQ water. Error bars represent ± 1 SEM; for all data points, n = 4. Asterisks (*) denote significant daily differences (Bonferroni Post-hoc test, $p < 0.05$) sucrose and non-sucrose treatments within litter and no-litter soil groups.

3.2. Sucrose efflux

Sucrose effluxes were observed with both sucrose concentrations, but patterns of efflux differed with concentration, as well as soil treatment. With 0.5 mM sucrose concentration (Fig. 3), effluxes peaked at day three in the litter treatment, at 4.2 ± 0.48 μg C m^{-2} s^{-1} (30 ± 3.5 nmols sucrose m^{-2} s^{-1}). Mean effluxes were generally greater in the litter treatment, although these differences were not significant ($p > 0.05$). After day seven (when sucrose was excluded from perfusates), sucrose fluxes were undetectable. No sucrose fluxes were observed in non-sucrose treatments.

With 5 mM sucrose concentrations (Fig. 3), effluxes also peaked at day three in the Soil + Litter + Sucrose treatment, at 38.2 ± 3.5 μg C m^{-2} s^{-1} (265 ± 24 nmols sucrose m^{-2} s^{-1}), representing a five-fold increase from day one, and were significantly greater than Soil + Sucrose for days three and five (Bonferroni post-hoc test, $p < 0.05$); this value also represents a nine-fold increase from the greatest peak with 0.5 mM sucrose as perfusate. Sucrose effluxes in the litter treatment decreased to similar rates as the non-litter treatment on day seven. After day seven (when sucrose was excluded from perfusate), sucrose fluxes were undetectable. In non-sucrose treatments, minor influxes of sucrose were detected on day 1 (0.01 – 0.07 μg C m^{-2} s^{-1}) but were not detected on following days.

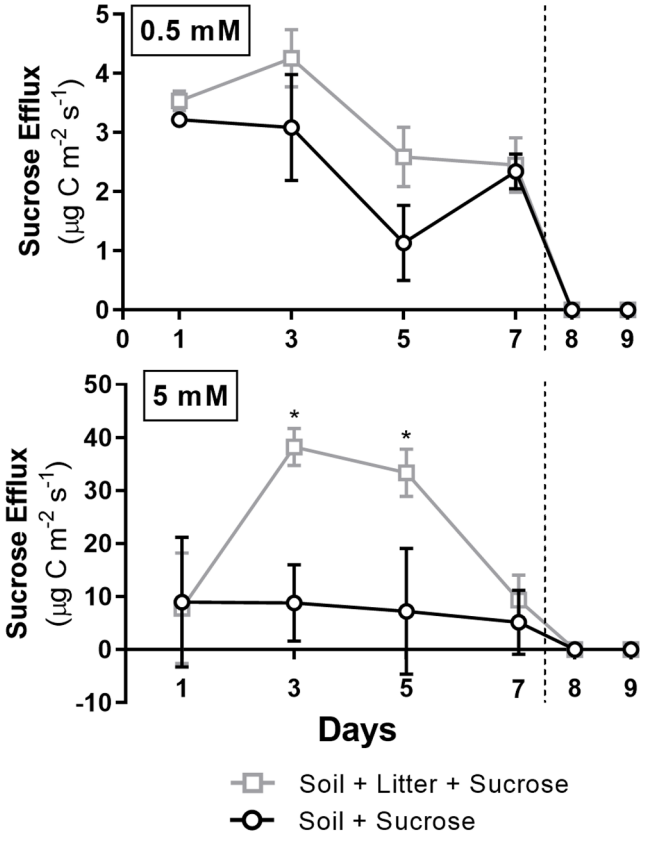

Fig. 3. Rates of sucrose efflux (μg C m^{-2} s^{-1}) from probes perfused with two concentrations of sucrose solution; 0.5 mM and 5 mM, in soils with and without plant litter, over the first nine days of each experiment. Negligible or nil sucrose fluxes were detected in control soils (which received no sucrose) and are not shown. The dotted line at day 7 indicates the point at which sucrose perfusates were exchanged with MilliQ water. Error bars represent ± 1 SEM; for all data points, n = 4; asterisks (*) denote significant daily differences between shown treatments (Bonferroni post-hoc test, $p < 0.05$).

3.3. Glucose and fructose fluxes

In the 0.5 mM sucrose experiment, the greatest fluxes of glucose (Fig. 4, A) and fructose (Fig. 4, B) were also observed in the Soil + Litter + Sucrose treatment. However, these fluxes peaked at day one (glucose: 6.3 ± 1.7 nmols m^{-2} s^{-1}; fructose: 6.3 ± 1.6 nmols m^{-2} s^{-1}), dropping by approximately half by day three and stabilising through day seven. In contrast, sugar fluxes in the Soil + Sucrose treatment were near-undetectable on day one but steadily increased with time, peaking at day seven (glucose: 1.1 ± 0.6 nmols m^{-2} s^{-1}; fructose: 1.1 ± 0.5 nmols m^{-2} s^{-1}). No sugar fluxes were detectable after day 7, when sucrose was excluded from perfusates. Sugar fluxes were rarely detected in non-sucrose treatments, except for transient fluxes within litter-amended soil on day one.

In the 5 mM sucrose experiment, fluxes of glucose (Fig. 4, C) and fructose (Fig. 4, D) were greatest in Soil + Litter + Sucrose treatment, and steadily increased with time, peaking at day seven (glucose: 14.7 ± 4.1 nmols m^{-2} s^{-1}; fructose: 17.4 ± 7.5 nmols m^{-2} s^{-1}). Glucose fluxes were rarely observed in the Soil + Sucrose treatment, although some were apparent at day seven (1.4 ± 1.2 nmols m^{-2} s^{-1}). However, fructose fluxes were relatively stable in the Soil + Sucrose treatment from day three but did not accumulate as within the litter-amended soil. Very little glucose and fructose fluxes were observed in non-sucrose treatments, although some were detected within litter-amended soils on day one (glucose: 1.8 ± 1 nmols m^{-2} s^{-1}; fructose: 1.7 ± 1.3 nmols m^{-2} s^{-1}). No sugar fluxes were detectable after day 7, when sucrose was excluded from perfusates.

3.4. CO_2 respiration

Soils amended with litter had significantly greater CO_2 respiration rates than unamended soil, apparent as soon as day two in both experiments (Fig. 5). In the 5 mM Sucrose experiment, litter treatments peaked at day 2 at 96.7 ± 4.9 (Soil + Litter + Sucrose) and 96.5 ± 5.1 μg CO_2-C g^{-1} soil DW h^{-1} (Soil + Litter); soil only treatments peaked at day one, at 47.1 ± 1.6 μg CO_2-C g^{-1} soil DW h^{-1} (Soil + Sucrose) and 41.9 ± 3.8 μg CO_2-C g^{-1} soil DW h^{-1} (Soil Only). Rates for soil only treatments levelled out from around day 6, however Litter treatments rapidly dropped from peak respiration at day two, before increasing again at day 5 (approximately 82 μg CO_2-C g^{-1} soil DW h^{-1}) before steadily decreasing until day 14.

In the 0.5 mM sucrose experiment, litter treatments also peaked at day 2, at 92 ± 9 (Soil + Litter + Sucrose) and 81.8 ± 18.3 μg CO_2-C g^{-1} soil DW h^{-1} (Soil + Litter); soil only treatments peaked at day one, at 33.9 ± 0.5 μg CO_2-C g^{-1} soil DW h^{-1} (Soil + Sucrose) and 33.6 ± 0.9 μg CO_2-C g^{-1} soil DW h^{-1} (Soil Only). Rates for soil only treatments remained relatively stable from day 2, and litter treatments decreased from the peak at day 2, stabilising from day 6 until day 14 – and unlike the 5 mM experiment, no obvious second peak was observed.

In the 5 mM sucrose experiment, Soil + Sucrose had significantly greater respiration rates than the Soil control during the first 7 days (Repeated Measures ANOVA; $F_{(1, 6)} = 8.458$, $p = 0.027$), but were similar during the second week (with a water perfusate). No significant differences were observed between litter treatments (Soil + Litter; Soil + Litter + Sucrose) during either stage of the experiment.

With 0.5 mM sucrose, all treatments had similar respiration rates to controls over the two weeks of the experiment (Fig. 5, 0.5 mM).

3.5. Comparing control treatments between experiments

Control treatments (Soil + Litter; Soil only) of each experiment were further compared to determine the baseline respiration, N fluxes and soluble sugar fluxes between each experiment, given that each experiment was performed separately, and as the soil was stored for a further three weeks between the beginning of experiment 1 (0.5 mM sucrose treatment) and experiment 2 (5 mM sucrose treatment).

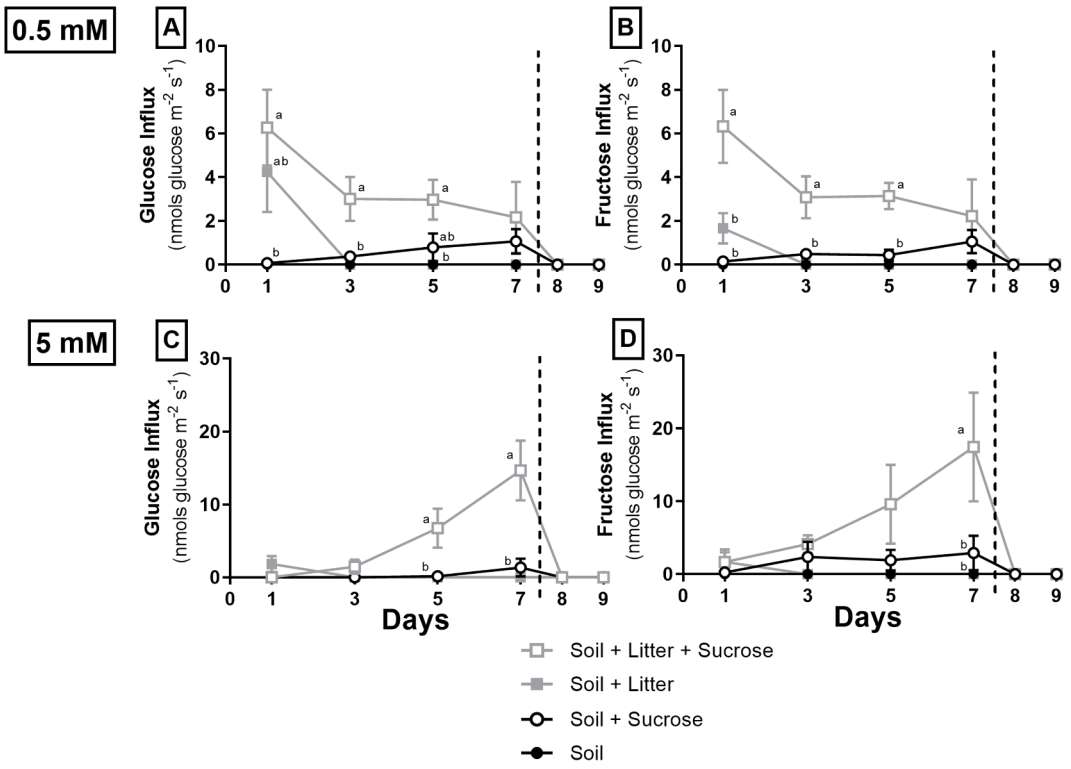

Fig. 4. Fluxes (in nmols m^{-2} s^{-1}) of glucose (A, C) and fructose (B, D), in soils amended with/without plant litter, and perfused with/without sucrose (0.5 mM – A, B; 5 mM – C, D). For sucrose treatments, the dotted line after day 7 indicates the point at which sucrose perfusates were exchanged with MilliQ water. Letters denote significant daily differences between treatments (Tukey's post-hoc test, $p < 0.05$). Error bars represent ± 1 SEM; for all data points, n = 4.

For respiration measurements, each set of controls was significantly elevated during the 5 mM sucrose experiment (Repeated Measures ANOVA; Soil only controls: $F_{(13,\ 78)} = 3.328$, $p = 0.0005$; Litter controls: $F_{(13,\ 78)} = 2.994$, $p < 0.0013$) compared to the 0.5 mM sucrose experiment. During the first 7 days, respiration in soil-only controls were 1.14 ± 0.07-fold higher, and litter controls were 1.3 ± 0.15-fold higher (Supplementary Fig. 2). Larger differences were observed between days 5 and 7 between litter controls, when a second peak was observed in the 5 mM sucrose experiment that increased respiration rates by 1.95-fold compared to the 0.5 mM experiment.

NH_4^+ fluxes in the litter control of the 5 mM sucrose experiment were significantly elevated compared to the 0.5 mM litter control (Repeated Measures ANOVA, $F_{(7,\ 41)} = 3.040$, $p = 0.0114$) which was on average 1.9 ± 0.1-fold greater during the first 7 days. In contrast, the soil-only controls were not significantly different between experiments (Repeated Measures ANOVA, $F_{(7,\ 41)} = 30.8655$, $p > 0.05$). Likewise, NO_3^- fluxes were also elevated in the litter control of the 5 mM sucrose experiment (Repeated Measures ANOVA, $F_{(7,\ 41)} = 2.692$, $p = 0.0213$; Supplementary Fig. 3 – bottom graph), which was 1.6 ± 0.2-fold greater during the first 7 days. However, soil-only controls were similar (Repeated Measures ANOVA, $F_{(7,\ 41)} = 0.9534$, $p > 0.05$; Supplementary Fig. 3 – bottom graph).

Glucose and fructose concentrations in all controls were similar between experiments (Repeated Measures ANOVA, $p > 0.05$).

4. Discussion

4.1. Microdialysis as a tool for emulating root release of carbon

Microdialysis can release and acquire soil solutes at a scale that mimics root exudation and uptake processes. This has proven effective for examining organic acid release and corresponding increase in phosphorus bioavailability, and we show here that the technique can also quantify the impact of C release on N availability. By mimicking root exudation patterns and concomitantly monitoring N fluxes with high temporal resolution, we could test hypotheses relating to rhizosphere C and N dynamics with a new lens. We observed that sucrose release via diffusion is a dynamic, rather than a static process, likely caused by fluctuating sink strength of microbe populations in the probosphere. We find that N influxes are significantly reduced in the presence of sucrose, confirming our hypothesis that releasing microbes from C limitation would increase their demand for N. Together, these findings confirm that microdialysis can detect fine-scale processes that mimic root C exudation and N release. It confirms the notion that C release at small spatial scales is a dynamic temporal process that causes immediate consequences for N availability as a root would experience it (Canarini et al., 2019).

However, we also find that these shifts in N availability were only statistically significant when we used our highest sucrose concentration (5 mM discussed further in sections 4.2 and 4.3). One explanation is that the dose effect of the greater sucrose concentration was the cause. However, CO_2 respiration rates in (no sucrose) control treatments were significantly elevated during the first week of the 5 mM sucrose experiment (compared to the 0.5 mM experiment), as were N fluxes between the litter controls. This means we cannot rule out the possibility that greater microbial activity may have contributed to the pronounced responses in N fluxes rather than the greater sucrose concentration alone. Although we do see a significant effect of sucrose release with a 5 mM sucrose concentration, we cannot confirm if a C dose response exists with the current study as it is conceivable that given higher or lower microbial activity, a different outcome could result from either sucrose concentration. Nevertheless, we have previously argued that microdialysis affords an integrated perspective of N availability, as microdialysis fluxes are likely to be influenced by soil factors that alter diffusion of solutes, including microbial activity (Buckley et al., 2020). So, it is important that we consider microbial factors such as community assemblage, biomass and enzyme activity more deeply in future studies, and interpret our results through these influences.

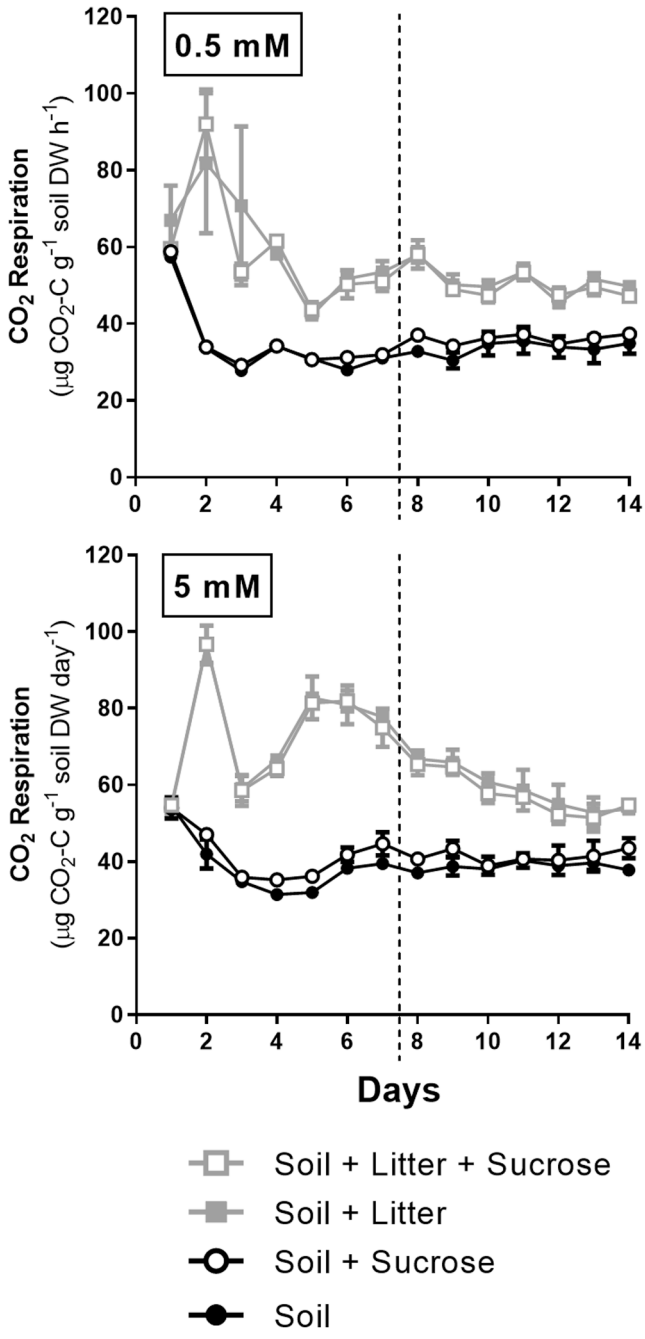

Fig. 5. CO_2 respiration (µg CO_2-C g^{-1} dry soil h^{-1}) measured in soils with and without plant litter, and supplied with sucrose exudates via microdialysis, with perfusate concentrations of 0.5 mM (top graph) and 5 mM (bottom graph). For sucrose treatments, the dotted line after day 7 indicates the point at which sucrose perfusates were exchanged with MilliQ water. Error bars represent ± SEM, for each point, n = 4.

The differences in baseline microbial respiration were likely due to changing soil microbial communities and their substrates during storage (stored fresh at 4 °C), despite the quick turnaround between experiments. Such problems of experimental replicability are a common experience for soil scientists, with knowledge gaps on how storage and treatment of sampled soils affect soil processes over time. Similarly, microdialysis measurements can have high variability due to the innate heterogeneity of soil microsites – even in homogenised soils (Inselsbacher et al., 2011; Demand et al, 2017). Overcoming variability with greater biological replication can be a challenge with microdialysis, particularly when pump units and probes can be costly, and sampling times (especially over longer timescales) can limit practical replication. Moving forward, improving repeatability and replication could be accomplished through modifications to microdialysis pumps (such as syringe extensions) to enable more probes to be used simultaneously with fewer required pumps.

4.2. *Nitrogen dynamics during and after sucrose release*

With 5 mM sucrose perfusate, the subsequent sucrose releases can result in significant decreases in N fluxes within the first day of sucrose release. Although we did not directly measure the microbial activity around the microdialysis probes, we interpret these reductions in N fluxes as microbial N immobilisation. This would corroborate a commonly-held hypothesis that C exudation promotes N immobilisation surrounding the root tip as microbes use labile C and N for growth (Kuzyakov and Xu, 2013). The lack of the same response with a 0.5 mM sucrose perfusate could suggest a dose dependency, and that lower C release insufficiently alters microbial activity to cause N immobilisation – but as we have discussed previously, further work is required to confirm this notion.

It has also been suggested that, as microbial activity increases with greater C availability, so does predation and microbial turnover, along with N-limitations that drive N recovery from SOM – all of which could in turn make N available for root uptake (Bonkowski, 2004; Kuzyakov and Xu, 2013), particularly in mature root zones as root tips develop and extend beyond the initial site. We assume that by stopping sucrose release after day 7, we mimic root growth through the soil and observe the environment that mature roots encounter. Although N fluxes increased at day 7 in soils previously exposed to sucrose release, the N flux rates were similar to control soils and suggests that – at least in the shorter term (7 days) – immobilised N is not appreciably liberated in the days following release. This finding supports the observation that considerable N retention occurs in response to shifting C supply in organic soil horizons of boreal forests (Blaško et al., 2015; Wild et al., 2017), but is contrary to studies that show increased N mineralisation rates with increased exudation in temperate forest systems (Bengtson et al., 2012; Meier et al., 2017), but which consider much longer term perspectives of soil processes (weeks to months). Studies with primary focus on sucrose additions in forest soils show reduced N availability with increased microbial and microfaunal activity (Teklay et al., 2010; Nieminen and Pohjola, 2014, Chapman et al., 2016) – but may also result in greater N availability over similarly long timeframes (Nieminen and Pohjola, 2014). Taken together, our study may simply be too short to quantify the turnover of immobilised N over time (after weeks to months). Inducing N immobilisation may then efficiently minimise N losses from soils in the short-term (Fisk et al., 2015), with N liberated from biomass in the longer term. If mycorrhizal fungi are partly responsible for immobilising available N, this could also result in N delivery to plants, although this may depend on the overall N supply (Näsholm et al., 2013; Henriksson et al., 2021). More clues could be gained from spatial gradients of microbial activity and turnover processes that extend from rhizoplane and rhizosphere to the soil, as demonstrated in the variable spatial patterns of enzyme activity shown with 2D zymography following the *in situ* release of simulated exudates (Zhang et al., 2019). It is perceivable that there are distant sites of microbial turnover and N release that do not immediately benefit one root, but perhaps a co-located root, influencing N availability and plant uptake at a scale beyond a single root (Schenk, 2006; Mommer et al., 2016).

We also acknowledge that sucrose contributes only a fraction of the diversity of compounds released by roots. Root exudates can contain N compounds which may have a substantial impact on microbial activity and net N mineralisation from soil organic matter through optimisation of microbial stoichiometry (Drake et al., 2013). Such N-rich root exudates (e.g. amino acids) may be important for stimulating microbial activity and subsequent greater N recovery by roots. Organic acids and

secondary metabolites (including those containing N), exuded from more mature root regions (Sasse et al., 2018), may also contribute to sustaining or modifying microbial responses that can promote biomass turnover, or encourage greater decomposition of SOM. Given the ease of modifying perfusates to alter the composition of exudates with microdialysis and quantifying resultant N fluxes, there is much potential to explore these temporal dynamics.

4.3. Sucrose release is modified by microbial activity

By simulating the release of sucrose using microdialysis, we released C at a range of rates (1.1 – 38.2 μg C m^{-2} s^{-1}) that were similar to, or up to 7.8-fold higher than average rates recorded from forest trees (Supplementary Table 1), and within the same range as delivered with other *in situ* exudation methods (e.g. Meier et al., 2017, 20 – 200 μg C cm^{-2} day^{-1} = 2 – 23 μg C m^{-2} s^{-1}). Rates may have been dependent on the sucrose concentration used, given 5 mM sucrose perfusates reached maximum effluxes were an order of magnitude>0.5 mM sucrose rates – but further work that considers background microbial activity will be needed to examine this further. Additionally, soils amended with fresh plant litter promoted greater effluxes of sucrose – particularly in the 5 mM sucrose experiment. If we assume that fresh litter supported greater microbial growth compared to the no-litter control soil, then it is likely that larger microbial biomass increased sucrose consumption, driving greater release rates by modifying concentration gradients and increasing diffusion rates. This finding would support previous studies where greater exudation rates occurred in plants grown in non-sterile environments, and greater turnover of exudates resulted in increased efflux (Shepherd and Davies, 1994; Pii et al., 2015). With 5 mM sucrose, we noted a significant drop in sucrose effluxes on day 7. Given that glucose and fructose fluxes peaked on the same day, this could be interpreted as a repression of microbial invertase activity in the presence of higher concentrations of metabolisable sugars (Marques et al., 2015; Lincoln and More, 2017). This would decrease sucrose depolymerisation, promoting a localised sucrose accumulation that might slow effluxes by altering concentration gradients. This emphasises the transient and dynamic nature of exudation processes, which are not fixed rates of supply but continuously change as microbial activity shifts in response. Future studies using microdialysis would benefit from directly measuring microbial activity at similar spatial and temporal scales to correlate microbial responses to changes in N and C dynamics – for example, by monitoring exoenzyme activity *in situ* (Buckley et al., 2019; Zhang et al., 2019).

Although microbial consumption may have a strong influence over diffusional exudation, there are likely physiological controls that plant roots can deploy to modify efflux rates, which microdialysis fails to mimic. For instance, plants may repress genes related to passive efflux transmembrane carriers at phloem unloading sites - e.g. SWEET-related genes for sugars (Chen et al., 2015), or UMAMITs for amino acids (Besnard et al., 2016; Tegeder and Hammes, 2018). Re-uptake of nutrient-rich exudates may present another strategy for minimising losses through diffusion; for instance, expression of amino acid uptake transporters such as LHT1 and AAP5 (Svennerstam et al., 2011), which may enable amino acid uptake at much faster rates than efflux (Warren, 2015). Over longer timescales, C allocation to roots and exudation patterns may also change with plant age (Gransee and Wittenmayer, 2000), or in response to nutrient limitations (Carvalhais et al., 2011), influencing microbial supply and assemblage (Chaparro et al., 2013). On the other hand, microbes may release secondary metabolites which can increase the permeability of root membranes, further facilitating diffusional exudation (Phillips et al., 2011; Kudoyarova et al., 2014). Although microdialysis probes cannot sense their environment and respond as roots do, the technique does, by modifying perfusate concentrations and pump flow rates, allow for flexibility in exudate delivery. Future studies may examine how functional changes to net exudation alter soil dynamics in near real-time. We also acknowledge that the use of microdialysis requires moist soil to allow for sufficient diffusion of target compounds (Miró et al., 2010; Inselsbacher et al. 2011). Such conditions will also influence efflux rates by promoting greater hydraulic connectivity between the membrane and adjacent microbes – and as such may overrepresent fluxes present in drier soils.

4.4. CO_2 respiration rates from sucrose utilisation are masked by bulk soil volumes

We observed that Soil + Sucrose (supplied with 5 mM sucrose perfusates) had significantly elevated respiration rates than the soil-only control during the first 7 days, which may indicate an oversupply of C in relation to N availability promoting C loss through respiration, rather than retention within biomass (Schimel and Weintraub, 2003). However, this may not be so in an N-rich environment, for despite gross sucrose effluxes in 5 mM Litter + Sucrose treatment peaking at 14 μg C h^{-1} – at approximately 21% of the equivalent gross rate in CO_2 respiration (66 μg C h^{-1}), respiration rates were not elevated (*c.f.* Litter) in this treatment. This might indicate tight C retention from sucrose supply and biomass turnover when N availability is greater – if so, greater differences may become more apparent over longer timeframes, as can be the case with respiration shifts due to priming effects, as SOM and microbial biomass are cycled (Kuzyakov, 2010). However, we also expect that the soil volume influenced by the diffusion of sucrose to be quite small - potentially within an area<1 – 2 mm from the membrane surface (Demand et al., 2017). Such a highly localised supply of C would benefit a small proportion of the total microbial population within the microcosm, with any contributions to CO_2 respiration through consumption masked by those from the greater bulk soil volume. Capturing these dynamics likely requires sensitive methods and measurements to detect treatment differences – particularly if using lower sucrose concentrations to emulate smaller C releases. Longer-term studies, paired with more sensitive respiration analyses and characterisation of C consumption (e.g. isotopic tracing) will help quantifying rates of C use by soil microbes exposed to realistic exudation patterns.

5. Conclusions

We present microdialysis as a sensitive tool for representing continuous root C release patterns, and for examining their influence on N availability with high temporal resolution, and without the need for destructive harvesting of soil. We show that by releasing a simplified model exudate, the immediate influences on N fluxes can be monitored, and presents a viable technique for observing the role of more complex exudate mixtures in future studies. Sucrose release induced significant reductions in N fluxes, in line with previous models of root-microbe interactions. However, we did not observe increases in N fluxes in the days after ceasing exudation, indicating that microbial-N turnover and/or SOM mining was insufficient to enhance N availability over the duration of the experiment. We also demonstrate that diffusional exudation is highly dynamic, and we suggest this may be linked to microbial growth which could drive exudation from roots. However, we acknowledge that potential microbial differences induced during soil storage may have increased the response we observed when using 5 mM sucrose perfusates, and so it is difficult to determine if a C dose response is apparent in our study.

We also acknowledge that organic N compounds (such as amino acids) can also contribute significantly to N fluxes in soils (Inselsbacher and Näsholm, 2012; Brackin et al., 2015; Oyewole et al., 2016) and along with proteins and peptides, are likely products of soil organic matter decomposition (Farrell et al., 2011; Macdonald et al., 2014; Hill et al., 2019; Warren, 2021) and are quickly acquired by soil microbes. As proteolysis forms a bottleneck for N availability in N-limited soil systems such as the one studied here (Schimel and Bennett, 2004), C-rich root exudates may provide a critical ecosystem function by promoting microbial exoenzyme production and, consequently, increasing rates of

proteolysis and protein availability. Future studies examining the influences of a variety of carbon sources on a broader range of N fluxes (including organic N) in tandem with characterising soil organisms and their activity will shed light to this topic.

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.

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Appendix A. Supplementary data

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