Preprint

Root exudate stoichiometry is a key driver of soil N cycling: implications for forest responses to global change

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4 figures
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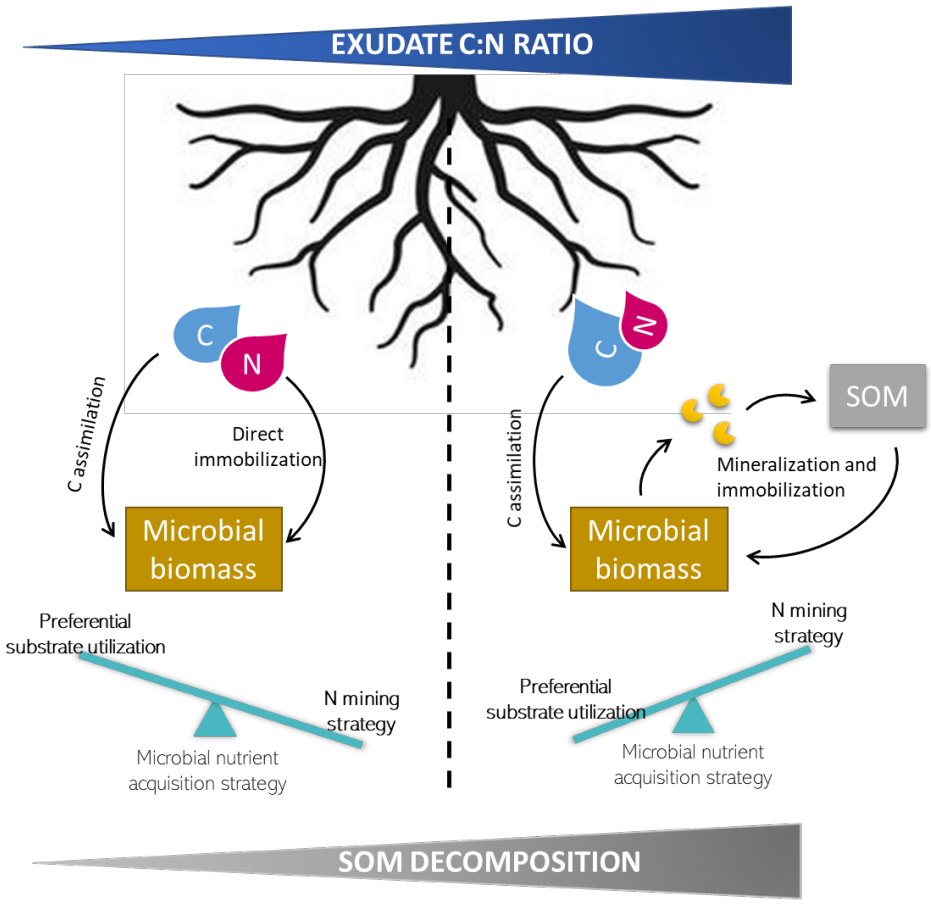
28 # Graphical abstract



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Abstract

Root exudate profile is expected to be altered by global change drivers, with significant implications for plant nutrition. Exposure to elevated atmospheric carbon dioxide (eCO_2) increases the quantity and alters the quality of exudates, which likely affects microbial activity and nitrogen (N) cycling. However, it is uncertain whether such changes will result in greater N availability for plants. In this field experiment, we used an automated root exudation system in a forest soil to mimic the increase in exudate C:N ratio observed under eCO_2. After six months of continuous application, we measured N transformation rates in O-horizon soils and in root and fungi exclusion soil bags (41 µm and 1 µm mesh sizes) to partition the role of fungi and bacteria. Increasing exudate C:N ratio stimulated gross N mineralization, especially in the rhizosphere, by shifting microbial nutrient acquisition strategy towards a N-mining strategy. High exudate C:N ratio increased nitrification in the absence of roots when both fungi and bacteria were present. These results demonstrate that N transformations are driven more by the C:N stoichiometry than by labile C alone in root exudates, and are largely influenced by the rhizosphere environment. Exudate stoichiometry thus may play a key role in alleviating N limitation under future atmospheric CO_2 concentration.

Keywords: root exudation; gross N mineralization; gross nitrification; elevated CO2; exudate stoichiometry

1. Introduction

Root exudation mediates interactions between plants and soil microbes, which are crucial for plant nutrition (Haichar et al., 2014; Shi et al., 2011; Zhalnina et al., 2018). Exudates consist of sugars, amino acids, organic acids (Smith, 1976) and numerous secondary metabolites (Salem et al., 2022), which are released both passively and actively by roots (Yan et al., 2023). Root exudates stimulate microbial activity, resulting in the release of bioavailable nutrients, such as nitrogen (N), during the decomposition of plant material and soil organic matter (SOM; Churchland and Grayston, 2014; Jacoby et al., 2017). Plants secrete between 1 to 10% of the carbon (C) assimilated via photosynthesis into the soil as root exudates (Brunn et al., 2022; Chari et al., 2024; Jones et al., 2004; Phillips et al., 2008).

Although much work has focused on quantifying the amount of C released into the soil as root exudates (Brunn et al., 2022; Chari et al., 2024) and its effect on microbial activity, research into root exudate quality (i.e. chemical composition) remains rare. Nonetheless, emerging evidence reveals that root exudate composition alters bacterial diversity (Wen et al., 2022) and SOM dynamics (Bradford et al., 2013; Carrillo et al., 2014; Chari and Taylor, 2022). Knowledge gaps around root exudates are further exacerbated by the limited number of *in-situ* studies (Liu et al., 2024; Lopez-Sangil et al., 2017; Yan et al., 2023). Most research has been performed in laboratory settings, and were mainly restricted to the addition of simple sugars, which limits our understanding under field conditions (Yan et al., 2023).Thereby, field-based studies are needed to clarify the role of root exudates in plant productivity, particularly within the context of global change that may exacerbate nutrient limitation (Dijkstra et al., 2013; Phillips et al., 2009; Zhang et al., 2016).

Global change drivers (i.e. drought, elevated CO_2, warming etc.) are expected to specifically alter root exudation profile (Dong et al., 2021; Hou et al., 2025; Liu et al., 2022). Especially, elevated atmospheric CO_2 concentration (eCO_2) often increases the quantity of root exudates, measured as C input to the soil (Dong et al., 2021; Norby et al., 2024; Phillips et al., 2011),

74 which is believed to accelerate N cycling in N-limited forests (Phillips et al., 2011). However,
75 a recent study conducted at BIFoR-FACE – a free-air CO_2 enrichment (FACE) experiment in
76 a mature oak woodland – highlighted that the C:N ratio of the exudates collected from the
77 organic layer also changed under eCO_2 (Reay et al., 2022). This increase in exudate C:N ratio
78 could influence N transformations and potentially explain the enhancement of N mineralization
79 and reduction of nitrification observed in the rhizosphere under eCO_2 (Rumeau et al., 2024;
80 Sgouridis et al., 2023). Higher exudate C:N ratio could induce faster N mineralization from
81 SOM (Du et al., 2020) and higher microbial retention of free amino acids (FAA) due to microbial
82 N limitation (Mooshammer et al., 2014), although it could also reduce microbial activity if lower
83 N availability limits enzyme production (Drake et al., 2013; Liu et al., 2022). Meanwhile, the
84 response of gross nitrification to a change in exudate C:N ratio remains unexplored to our
85 knowledge. Fungal heterotrophic nitrification, predominant in acidic forest soil (Li et al., 2018),
86 may be enhanced by greater C availability (Zhang et al., 2019), leading to higher N losses via
87 leaching or N_2O emissions offsetting benefits from C storage. Thus, quantifying the response
88 of N transformation rates and bacteria and fungi contributions is crucial to assess how changes
89 in root exudation profile may alleviate soil N limitation, essential for predicting future forest C
90 capture.

91 Our study investigated the effects of changes in soil exudate C:N (low C:N, high C:N and water
92 control) ratio on soil N transformations after applying a complex exudate “cocktail” (comprising
93 two sugars, four amino acids and five organic acids) for six months using a novel automated
94 root exudation system (ARES; Lopez-Sangil et al., 2017). The study was conducted adjacent
95 to a FACE experiment in a mature oak woodland, and the changes in exudate C:N ratio
96 reflected those of *in-situ* exudation rates by oak roots under eCO_2 enrichment (Norby et al.,
97 2024; Reay et al., 2022). To determine potential drivers of N transformations and of tree-
98 microbe interactions, we partitioned the contribution of fungi, bacteria and roots to N
99 transformation processes using exclusion bags with different mesh sizes. We hypothesized
100 that 1) gross N mineralization and NH_4^+ immobilization will be enhanced in both exudate

treatments (high and low C:N) compared to control with a more pronounced response in the high C:N ratio treatment due to higher microbial N limitation. 2) Gross nitrification will be enhanced by C addition independently from the C:N ratio because C availability stimulates heterotrophic fungal activity in acidic soil. To test our hypotheses, we analysed soil N concentration and gross N transformations following six months of experimental exudate treatments and compared the responses in the soil O horizon, and in soil bags excluding roots or roots and fungi.

2. Materials & Methods

2.1. Experimental set-up

This experiment was conducted at the Birmingham Institute of Forest Research (BIFOR) facility in Staffordshire, UK (52º 48' 3.6" N, 2º 18' 0"W), adjacent to a Free Air CO_2 Enrichment (FACE) experiment, which was established in 2017 (Hart et al., 2020). The forest is dominated by oak trees (*Quercus robur L.*) with common hazel (*Corylus avellana* L.), sycamore (*Acer pseudoplatanus* L.*)* and hawthorn (*Crataegus monogyna* L.) trees in the understorey and as sub-dominants, respectively. The soil at site, classified as Ortic Luvisol (Hart et al., 2020), set with an organic soil layer (O) of about 7 cm depth overlying an A horizon (15 to 25 cm deep) and a B horizon transiting into sandstone geology. The O-layer is a sandy loam (41% sand, 43% silt, 16% clay), with a pH of 3.8. More details can be found in Rumeau et al. (2024).

The present study consisted of four experimental blocks, each divided into three plots of 0.96 m^2 in which three different exudate treatments (Table 1) were administered: control (water + $CaCl_2$ to maintain ionic strength), low C:N (5% of forest NPP as root exudate solution with a C:N ratio of 4.4), high C:N (5% of forest NPP as root exudate solution with a C:N ratio of 7.4). Each block was located near an oak tree to ensure the predominance of oak roots and an Automated Root Exudate System (ARES; Lopez-Sangil et al. 2017) was set up in each plot. The ARES covered an area of *c*. 0.96 cm^2 and comprised a mini drip irrigation system with 24

spikes inserted into the O horizon to c. 2 cm depth to allow the diffusion of the exudates into the soil profile.

To determine the role of roots and fungi in N transformation processes, exclusion bags were installed in the organic layer (right below the soil surface) of each plot right before the beginning of the experiment. The bags measured 4-cm diameter and 10-cm depth and consisted of 100 g of autoclaved sieved soil from the experimental site. The mesh size was of either 41 µm to exclude roots but allow both bacteria and fungal mycelium to access the soil inside the bags, or 1 µm to exclude roots and fungi, allowing only bacteria to access the soil (Adamczyk et al., 2019). This mesh selection was based on the typical size of fungi (< 40 µm) and bacteria (< 0.5 µm) (Christensen et al., 1999; Swift, et al., 1979). However, while this method has been used in several studies (Adamczyk et al., 2019; Brooks et al., 2011; Liang et al., 2021; Nottingham et al., 2013), it has limitations. Some bacteria can exceed 1 µm particularly within the Gammaproteobacteria order and be excluded (Luan et al., 2020). Additionally, while autoclaving is a widely used sterilization technique, it may not completely eliminate fungal spores, and some fungi may revive from spores in the 1 µm bag. Another consideration is that autoclaved soil from the exclusion bags may behave differently compared to undisturbed O-horizon soil, preventing direct comparisons. Nonetheless, both 1 µm and 41 µm bags were prepared in the same way, ensuring their comparability under the treatment effects scenarios.

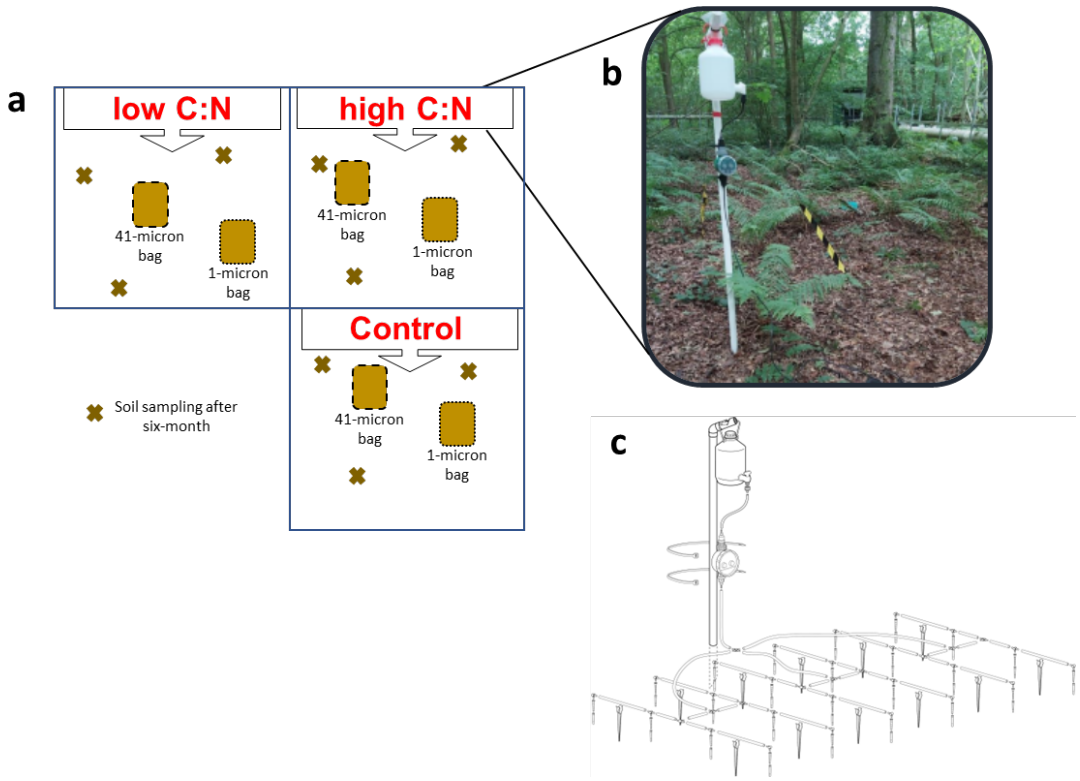


Figure 1: a) Schematic of one experimental block with the three root exudate treatment plots, showing the location of soil sampling and exclusion bags, b) Picture of one of the automated root exudation systems (ARES), c) Schematical diagram representing the ARES system from Lopez-Sangil et al. (2017).

2.2. Exudate solutions and delivery

Standard exudation solutions representing control or eCO_2 treatments were prepared weekly in the laboratory by combining sugars, amino acids and organic acids (see composition in Table 1). These solutions were made using distilled water in acid-washed containers to minimize the risk of bacterial pollution in the exudate solutions. The total C concentration was adjusted to represent approximatively 5% of the forest NPP (i.e. 60 g C m^{-2} y^{-1}; Norby et al., 2024). However, due to the complexity of the solution composition, there was a slight difference in total C between the two exudate treatments (see Table 1). Exudate treatments were continuously delivered to the soils at a rate of 0.29 L d^{-1} through 24 needles over a 0.96 m^2 area by an automated root exudation system (ARES; Figure 1), which operated for 6 months from May to December 2022. Over six months of treatments, soils received an

161 additional 10% of water compared to normal precipitation of 1152 mm per year in 2022 (rain
162 gauges at site TR-525M; Dallas, Texas). A full description of the ARES can be found in Lopez-
163 Sangil et al. (2017).

164 **Table 1:** Composition of the experimental exudate cocktails with concentrations expressed in
165 mg L^{-1}. The control treatment (water) only contained calcium chloride (0.18 g L^{-1} $CaCl_2$) to
166 maintain consistent ionic strength across treatments.

Compound	Control	low C:N	high C:N
Sugars			
Glucose	0	260	390
Sucrose	0	260	390
Organic acids			
Oxalic acid	0	220	330
Sodium acetate	0	160	240
Fumaric acid	0	80	120
Citric acid	0	80	120
Sodium succinate	0	100	150
Amino acids			
Glutamic acid	0	600	300
Alanine	0	500	250
Glycine	0	500	250
Valine	0	500	250
Total C	0	1222	1023
Total N	0	276	138
C:N ratio		4.4	7.4

167

168 ### 2.3. Soil sampling

169 In December 2022, after six months of exudate treatments, soil cores (5 cm diameter) from
170 the organic layer (depth *c*. 7 cm) were collected in three random locations in each plot, which
171 were then sieved (< 2 mm) and combined to make one composite sample per plot. On the

same day, the exclusion bags were retrieved. All samples were brought back to the laboratory on ice and were kept at 4°C for maximum one week until analysis.

2.4. Soil nutrient concentrations

Soil extractable ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) concentrations were determined by extracting 2 g of wet soil with 10 ml 0.5 M potassium sulphate (K_2SO_4). We used K_2SO_4 because it provides better precision with our instrument compared to KCl, and concentrations obtained with both extractants are known to be highly correlated (Zhong and Makeschin, 2003). The extracts were filtered at 0.45 µm with syringe filters and analyzed by continuous flow colorimetry using the Berthelot reaction method for NH_4^+-N and the cadmium-reduction reaction method for NO_3^--N (SA 3000 analyser, Skalar Analytical, Breda, Netherlands). The limit of detection was 0.02 mg N L^{-1} for both NH_4^+-N and NO_3^--N. The concentrations were blank corrected, and the relative standard deviation (RSD) calculated on quality control (QC) samples was < 2%.

Soil free amino acid (FAA) concentration was determined using the *o*-phthaldialdehyde and β-mercaptoethanol (OPAME) fluorescence assay described by Jones (2002) and adapted to a microplate assay by (Darrouzet-Nardi et al. (2013). Briefly, 2 g of wet soil was extracted in 10 ml of 1 M potassium chloride (KCl), shaken, filtered at 0.45 µm, and 50 µl was added to a microplate with 100 µl of OPAME working reagent. As NH_4^+-N reacts to fluorescence within 1 hour, fluorescence was measured after 1 h of incubation at ambient temperature when the fluorescence from reaction with soil NH_4^+-N is negligible. Gravimetric soil water concentration was measured by drying 5 g of soil at 105°C for 48 h in order to mass correct soil nutrient concentration.

2.5. Gross N transformations

To determine N transformations, gross mineralisation and nitrification rates were assessed by the ^{15}N pool dilution method (Davidson et al., 1991; Hart et al., 1994) within a week of sampling. For this, 10 g of each soil sample was weighed in quadruplet into specimen cups.

198 Before ^{15}N labelling, all samples were acclimated in the dark at 15°C for 2 days, chosen to
199 match summer soil temperature at the field site. To achieve a 20% enrichment in ^{15}N atom
200 percentage (at. % ^{15}N) in all samples within a 5% increase in moisture, a range of labelling
201 solutions were prepared to deal with the different soil concentrations in NO_3^--N and NH_4^+-N.
202 Solutions were prepared using $K^{15}NO_3$ (98 at.% ^{15}N, Merck) or $^{15}NH_4Cl$ (98 at. % ^{15}N, Merck).
203 The labelling solutions were added dropwise, and soils were gently stirred with a spatula to
204 ensure a uniform distribution of labels before closing the specimen cups. Soil subsamples
205 were extracted with 1 M KCl immediately after labelling and again following a 24 h incubation
206 at 15°C in the dark. An aliquot of extract was then analysed for NH_4^+-N and NO_3-N, as
207 described above. The rest of the soil extract was analysed followed the gas diffusion
208 procedure (Brooks et al., 1989) to determine the ^{15}N concentration in the NH_4^+-N and NO_3^--N
209 pools. Briefly, acid traps were prepared with 8-mm diameter Whatman^{TM} 3 filter discs soaked
210 with 10 µL of 2.5 M $KHSO_4$ and sealed in PTFE tape. NH_4^+-N was diffused by simultaneously
211 adding 0.2 g of MgO and an acid trap to the soil extract. To ensure complete diffusion, extracts
212 were shaken at low speed for 7 days (Brooks et al., 1989; Sørensen and Jensen, 1991). To
213 diffuse NO_3^--N, NH_4^+-N was first removed from the solution using the above procedure but the
214 filters were discarded. NO_3^- was then diffused by adding 100 µL of 30 % Brij-35 and 0.4 g of
215 Devarda's alloy, which reduces NO_3^--N to NH_4^+-N. A second acid trap was placed into the
216 specimen cups and samples were shaken for another 7 days at room temperature. All acid
217 traps were then dried in a desiccator, encapsulated in tin capsules and analysed via a
218 continuous flow isotope ratio mass spectrometer (IRMS; Elementar Isoprime Precision;
219 Elementar Analysensysteme GmbH, Hanau, Germany) coupled with an elemental analyser
220 (EA) inlet (vario PYRO cube; Elementar Analysensysteme GmbH, Hanau, Germany).

221 Gross mineralisation fluxes were calculated using the following equations developed by
222 Kirkham and Bartholomew (1954):

$$M\ (\mu g\ g^{-1}\ day^{-1}) = \frac{[NH_4^+]_0 - [NH_4^+]_t}{t} \times \frac{\log\left(\frac{APE_0}{APE_t}\right)}{\log\left(\frac{[NH_4^+]_0}{[NH_4^+]_t}\right)} \quad (1)$$

$$C\ (\mu g\ g^{-1}\ day^{-1}) = M - \frac{[NH_4^+]_t - [NH_4^+]_0}{t} \quad (2)$$

Where M= gross mineralization rate, C= NH_4^+-N consumption rate, t = time elapsed, [NH_4^+-N] = NH_4^+-N soil concentration (µg g^{-1}), APE_τ = ^{15}N atom percent excess of the NH_4^+-N pool, τ. Gross rates of nitrification and NO_3^--N consumption were calculated by replacing NH_4^+-N concentration by NO_3^--N concentration, and APE in the NH_4^+ pool by the APE in the NO_3^--N pool. The NH_4^+-N immobilization rate is equal to the difference between the NH_4^+-N consumption rate and gross nitrification. The NO_3^--N immobilisation rate is assumed equivalent to the gross NO_3^--N consumption rate.

2.6. Statistical analyses

Statistical analyses were carried out in R version 4.1.0 (R Core Team, 2021) with Rstudio software (RStudio Team, 2021). The effects of exudate treatments (low C:N and high C:N) on soil N concentrations and transformations were tested in the O-horizon soil independently using a one-way analysis of variance (ANOVA). When the ANOVA indicated a significant treatment effect at $P < 0.05$, we did a Tukey's post-hoc tests for multiple comparisons. While the effects of exudate treatments and microbial exclusions (1-µm bag, 41-µm bag) were tested with a two-way ANOVA followed by Tukey's post-hoc tests. The normality of the residuals and homogeneity of variances were inspected for all variables and log transformations were performed for the N cycling rates.

3. Results

3.1. Amino acids and inorganic soil N concentrations

The concentrations of NH_4^+ and NO_3^- in the O-horizon soils were similar between exudate treatments but both were significantly higher than in the control soils ($P < 0.05$; Figure 2a). NH_4^+ and NO_3^- concentrations in the exclusion bags did not differ between control and exudate treatments (Figure 2b). Surprisingly, FAA concentration was lower in the low C:N exudate treatment compared to the high C:N exudate treatment in both O-horizon soil (— 50%; $P = 0.09$) and 41 µm bags (— 27%; $P < 0.05$), although the amount of amino acids added in the low C:N exudate treatment was twice as high (Figure 2). Overall, NH_4^+, NO_3^- and FAA concentrations were higher in the 41 µm bag followed by the 1 µm bag (Figure 2b).

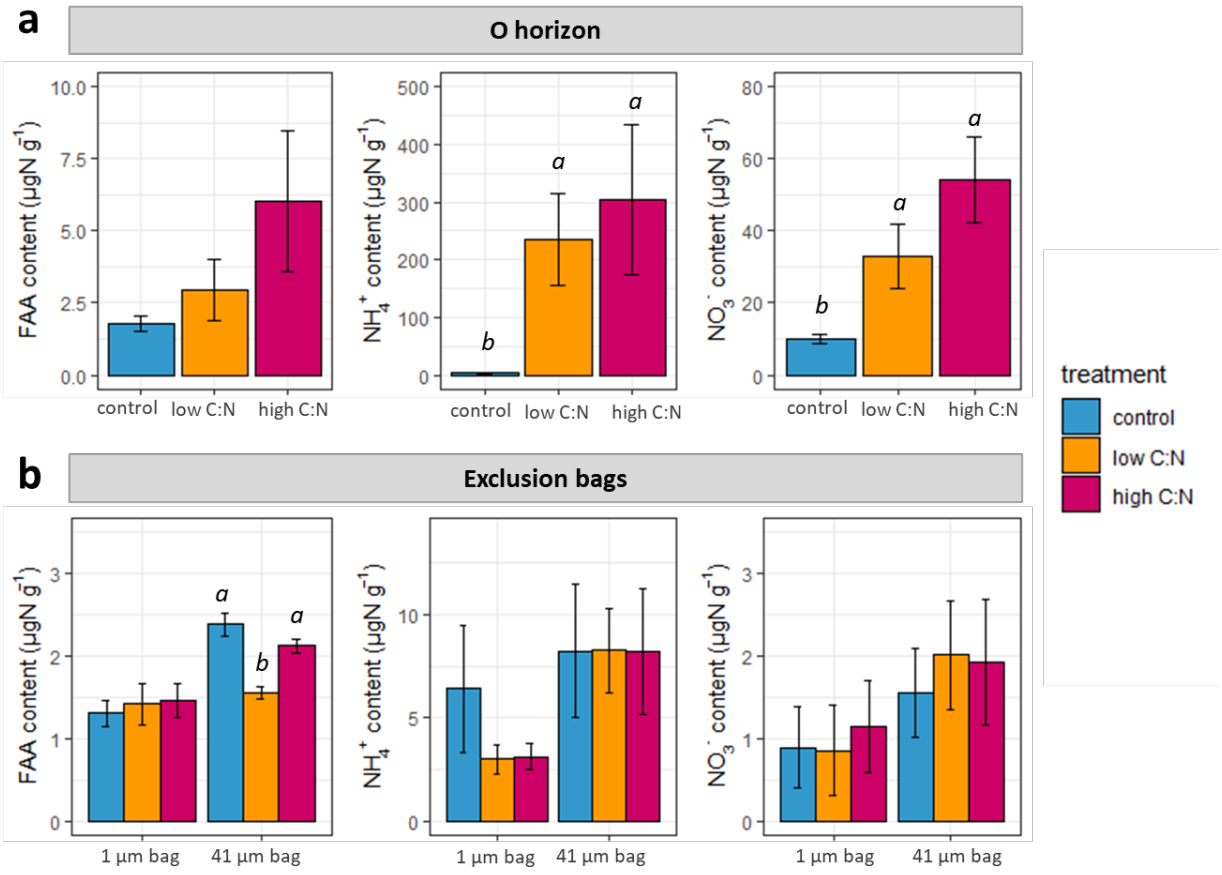
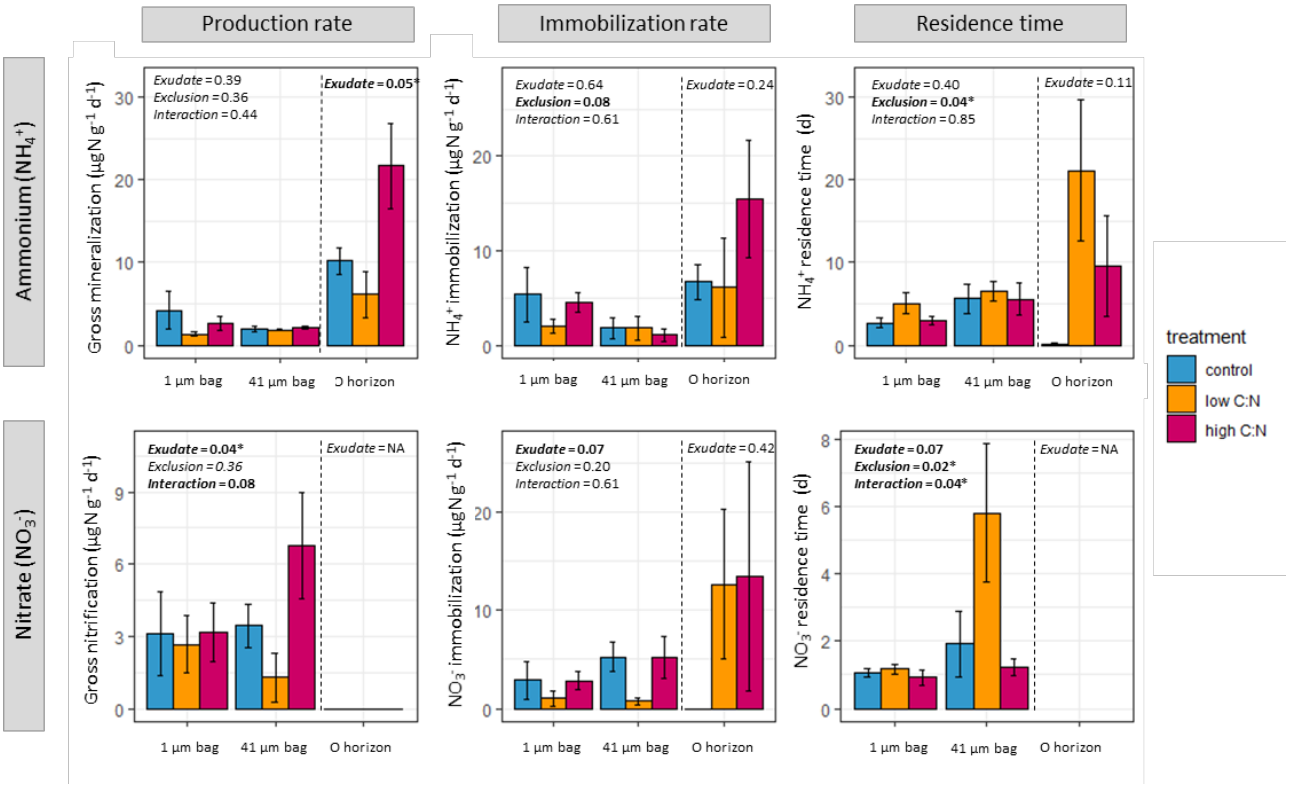


Figure 2: Nitrogen concentrations in experimental exudate treatments a) in the O-horizon soil and b) in exclusion bags. Letters (a,b) indicate significant difference between treatment for this type soil micro-habitat. Values are means ± SE for n = 4 per treatment. Note the difference in y axis ranges among panels.

3.2. Effect of exudates on N cycling rates

In the O-horizon, the high C:N exudate treatment significantly enhanced gross N mineralization (P = 0.05), doubling the rate compared to the control and tripling it compared to the low C:N ratio treatment (Figure 3). A similar trend was noticeable on the NH_4^+ immobilization rate although not significant. Gross nitrification and NO_3^- immobilization rates were almost always undetectable in the O-horizon but were detectable in all exclusion bag samples (Figure 3). In the 41 μm bags, the high C:N exudate treatment enhanced gross nitrification (+95% relative to the control), whereas no such effect was observed in the 1 μm bags (Figure 3). By contrast, the low C:N exudate treatment slightly reduced gross mineralization and gross nitrification compared to the control (−46% and −40% respectively; Figure 3) in the exclusion bags and in the O-horizon. As a result of the lower mineralization and nitrification activity in the low C:N exudate treatment, the mean residence times of both NH_4^+ and NO_3^- were overall higher compared to the control (non-significant; Figure 3). The ratio of NH_4^+ immobilization to mineralization (I:Mi ratio) was unaffected by treatments (Figure 4a). But I:Mi was higher in the 1 μm bag compared to the 41 μm bag (P < 0.05), indicating that more NH_4^+ was immobilized per amount of NH_4^+ mineralized when roots and fungi were excluded (Figure 4a). By contrast, the ratio NO_3^- immobilization to nitrification (I:Ni) was lower in the two exudate treatments in the 41 μm bags compared to control (P < 0.05), indicating that less NO_3^- was immobilized for the same amount nitrified under exudate treatments (Figure 4b).



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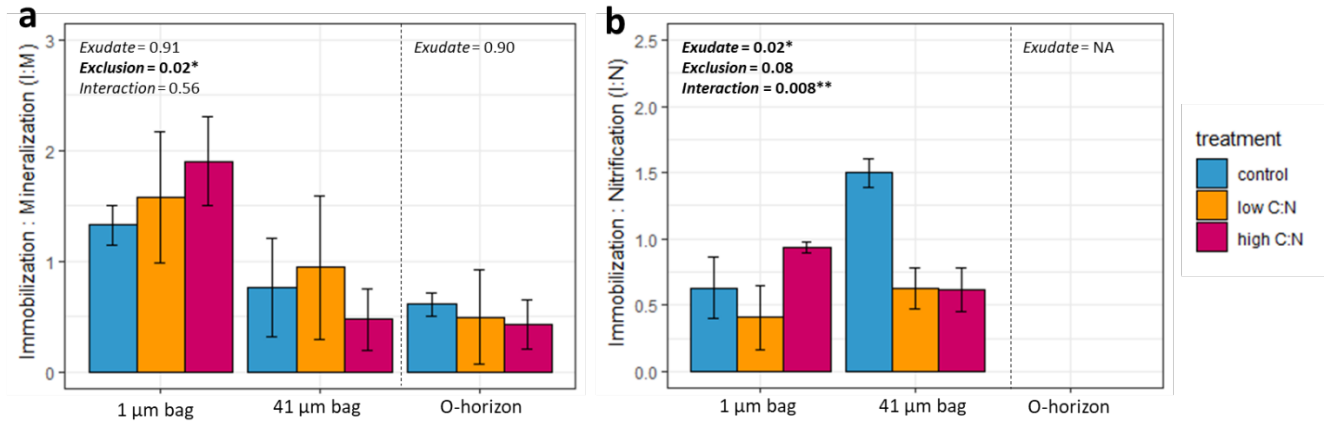
276 **Figure 3:** Ammonium and nitrate production rates, immobilization rates and mean residence time in
277 experimental exudate treatments and exclusion bags. Values are mean ± SE for n = 4 per treatment. A
278 dashed line separates the O-horizon and exclusion bag soils. Gross nitrification and NO_3^- residence
279 time were not detectable in the O-horizon. *P*-values for the effects of treatments, exclusions, and their
280 interactions in exclusion bags are shown on the left, while *P*-values for treatment effects in the O-horizon
281 are on the right of each plot.



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283 **Figure 4:** The ratio of consumption to production rates for a) NH_4^+ (NH_4^+ immobilization / gross
284 mineralization) and b) NO_3^- (NO_3^- immobilization / gross nitrification). Values are means ± SE for n =

4.A dashed line separates the O-horizon and exclusion bag soils. *P*-values for the effects of treatments, exclusions, and their interactions in exclusion bags are shown on the left, while *P*-values for treatment effects in the O-horizon are on the right of each plot.

4. Discussion

We assessed the effect of root exudate C:N ratio on N transformations using an automated root exudation system that delivered complex exudate cocktails. By simulating changes in root exudate composition under eCO_2, we were able to isolate the specific effects of altered root exudation on N transformations. Our results also provided insights into the microbial groups involved in these transformations. However, we suggest that in the future integrating microbiology measurements alongside the mesh bag method could enhance the and functional understanding of the microbials groups.

4.1. Higher exudate C:N ratio stimulated N mineralization

Higher gross mineralization under the high C:N treatment but not under the low C:N treatment suggests that gross mineralization is stimulated by N limitation in the high C:N treatment rather than by the amount of C supplied to microbes. The significant differences in N cycling indicate that C:N ratio, rather than minor variations in C concentration, drive the observed effects. This observation supports the “microbial N mining theory” stating that mineralisation of SOM in response to C addition (i.e. priming) occurs under low N availability as a strategy for microbes to acquire additional N (Chen et al., 2014; Craine et al., 2007; Dijkstra et al., 2013) and maintain their C:N stoichiometry (Hessen et al., 2004). This suggests that both C and N availability are essential regulators of N release from SOM, challenging the original theory focusing mainly on energy input from C (Jones et al., 2009, 2004). In our study, mineralization was five times faster under the high C:N treatment but this was only detectable in the presence of roots suggesting that priming under high C:N ratio is initiated by the rhizosphere microbes or by root-microbe competition for N (Yin et al., 2018). Our findings build on previous work on N availability in the rhizosphere under eCO_2 (Meier et al., 2015; Rumeau et al., 2024) by

311 demonstrating that N cycling is not only influenced by root exudates but also by the interaction
312 between exudates and rhizosphere communities. However, our experiment does not allow us
313 to quantify the relative contribution of ECM fungi and rhizosphere bacteria in the enhanced
314 mineralization. Furthermore, our exudate treatments did not stimulate microbes to retain more
315 N, as the I:Mi ratio was unaffected by the treatments. The difference in N retention between
316 the exclusion bags may indicate different N acquisition strategies (assimilatory vs
317 dissimilatory) between bacteria and fungi, but this requires further investigation by coupling it
318 with microbial composition measurements. These findings demonstrate that the increase in
319 root exudate C:N ratio, previously observed under eCO_2 (Reay et al., 2022), rather than the
320 increase in the amount of root exudates, enhances gross N mineralization by the rhizosphere
321 microbes without disrupting the balance between mineralization and immobilization, thereby
322 resulting in greater N availability for trees.

323 **4.2. Exudate C:N ratio shifted microbial nutrient acquisition strategies**

324 The lower-than-expected concentration of FAA in the low C:N exudate treatment, coupled with
325 the low rates of inorganic N immobilization, suggests that microbes in this treatment
326 preferentially assimilated the added amino acids rather than inorganic N (e.g., ammonium or
327 nitrate). Our hypothesis is further supported by previous studies demonstrating that soil
328 microbes prefer to assimilate intact amino acids over inorganic N (Knowles et al., 2010;
329 Mooshammer et al., 2014). Hence, increasing exudate C:N ratio, rather than the quantity of
330 exudate-C, induced microbes to switch from preferential substrate utilization (Dijkstra et al.
331 2013) strategy, wherein microbes utilize exudates, to a N-mining strategy, wherein microbes
332 mine nutrients from SOM (Cheng, 1999). This shift is evidenced in the high C:N ratio treatment,
333 where SOM decomposition is favoured (N-mining), versus the low C:N ratio treatment, where
334 reduced SOM decomposition suggests direct AA uptake from exudates (preferential substrate
335 utilization). Consequently, our results suggest that exudate composition plays a crucial role
336 on microbial mechanisms of soil SOM storage, by prompting the switch from preferential

substrate utilization and SOM accumulation at high amino acid concentrations (relative to C) to SOM decomposition via N mining at low amino acid concentrations (relative to C).

4.3. High exudate C:N ratio and root exclusion stimulated nitrification

We measured greater nitrification rates in the 41 µm exclusion bags under the high C:N treatment but not in the low C:N treatment, contrary to our hypothesis. This suggests that C addition enhanced nitrification, but only under low N concentration. As higher nitrification rates were only measured in the 41 µm bags, it indicates increased nitrification when fungi and bacteria were present. Likely, high C (relative to N) specifically stimulated fungal heterotrophic nitrification, which consistent with previous observation highlighting the role of C:N ratio on heterotrophic nitrification (Zhang et al., 2019). Furthermore, nitrification was suppressed in the O-horizon at the end of the experiment but not in the exclusion bags. Yet, high nitrate levels in the O-horizon soil under exudate treatments indicate that nitrification was not fully suppressed throughout the experiment. Nitrification suppression in the presence of roots has been previously noticed at site (Rumeau et al., 2024) and could be due to root uptake of ammonium or the release of biological nitrification inhibitors by trees as a strategy to conserve N (Andrianarisoa et al., 2010; Florio et al., 2021; Paavolainen et al., 1998). Biological inhibitors are a likely explanation for lower overall nitrification rates, as our exudate cocktail did not include inhibitory compounds. Thus, our root exudate cocktails may have stimulated nitrification when roots were excluded, likely as a response to the addition of high labile C sources (relative to N) which are known to enhance heterotrophic nitrification (Zhang et al., 2019). However, increased nitrification in response to altered exudate quality are unlikely to play a major role in N-limited forest soils under eCO_2, where trees invest in larger root biomass mitigating nitrification (Norby et al., 2004; Rumeau et al., 2024; Ziegler et al., 2023).

5. Conclusion

Together our results demonstrate that N transformations are influenced more by the C:N stoichiometry than simply by the presence of labile C in root exudates, and are largely

dependent on the rhizosphere environment and its microbes. In response to increasing exudate C:N ratio, soil microbes adopted an “N mining strategy”, using exudate C to mine for N via SOM decomposition. This shift was evidenced in the presence of roots as they compete with microbes for available N. Our findings reveal that both C and N concentrations in exudates are critical drivers of rhizosphere N cycling unveil a key mechanism by which trees regulate soil N availability under eCO_2.

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Competing interests

No competing interests to disclose.

Author contributions

J.P., S.U., M.K.R., I.H., L.H. and E.S. conceptualized the work. M.R., J.P., G.F. and F.S. performed the investigation. F.S. and S.U. provided the resources. M.R. worked on the methodology, performed the formal analysis, the data visualization and wrote the original draft and performed the data visualization. J.P., S.U., L.H., I.H., M.K.R., Y.C., E.M. and A.R.M. contributed to manuscript revisions.

Data availability

Data will be made available upon request.

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