

1 **Title:** Root exudates shift how N mineralization and N fixation contribute to the plant-available
2 N supply in low fertility soils

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14 **Abstract**

15 Nitrogen (N) availability is a primary constraint to plant productivity, especially in marginal
16 lands with inherently low fertility. Root exudates change with plant nutrient status, and are
17 expected to affect the microbially-mediated N transformations (gross N mineralization vs N
18 fixation) in low fertility soil (low soil organic matter). To explore this possibility, we sampled
19 soils from two monoculture switchgrass (var. *Cave-In-Rock*) plot with and without N addition at
20 two marginal land sites in Michigan, USA. In a two-week lab incubation, we quantified the
21 effect of different root exudates on gross N mineralization and N fixation by adding simulated
22 root exudates (carbohydrates, organic acids) at a rate of 100 $\mu\text{g C g}^{-1} \text{ day}^{-1}$. On average, adding
23 carbohydrates to low fertility soil increased the soil respiration by 254%, the dissolved organic
24 carbon (DOC) by 366% and reduced dissolved organic N (DON) by 40%. In contrast, soils
25 receiving organic acids had 159% more soil respiration, 163% higher DOC concentration and the
26 DON concentration increased by 49%. Analysis of the C recovery in measured pools revealed
27 that root exudates C inputs were nearly equivalent to the DOC, microbial biomass carbon
28 (MBC), and soil respiration in sandy soil, but only 45–74% of the root exudate C was recovered
29 in these pools in the sandy loam soil. This suggests that root exudate C may be adsorbed to
30 mineral particles in the sandy loam soil. Soil treated with organic acids had higher gross N
31 mineralization and N immobilization rates than soil with carbohydrates addition. Adding
32 carbohydrates significantly increased the free-living N fixation rates, compared to organic acid
33 addition. Changes in soil pH, and DON induced by root exudate addition had strong association
34 with N transformation rates and N availability. Gross N mineralization produced more plant-
35 available N than N fixation, as evidenced by higher inorganic N concentration in soils receiving
36 organic acids than carbohydrates. By quantifying how different root exudates affect the
37 contribution of N mineralization and N fixation to the plant-available N pool in low fertility
38 soils, this study enhances our understanding of the “C for N” exchange in the plant rhizosphere.

39

40 **Keywords:** Marginal lands, Root Exudates, N fixation, Gross N mineralization, Plant-microbe
41 interactions

42 1. Introduction

43 Nitrogen (N) mineralization and N fixation (free-living or symbiotic) are the two dominant
44 processes controlling plant available N (Coskun et al., 2017); however, both are energy intensive
45 processes (Smercina et al., 2019a). For example, N mineralization requires C for protease
46 enzyme production, and N fixation requires large amounts of ATP energy to break the N₂ triple
47 bond (Carnahan et al., 1960), which makes the energy or C supply for microbes an important
48 controller of process rates (Roley et al., 2018). Plant root exudates, composed primarily of low
49 molecular-weight organic compounds, including carbohydrates, organic acids, and amino acids
50 (Dakora and Phillips, 2002), can be directly taken up into microbial cells, providing an ideal C
51 source to support energy intensive N transformations. Therefore, root exudates have the potential
52 to stimulate SOM decomposition in relation to N acquisition or alter N fixation rates and
53 ultimately control soil N availability (Li et al., 2016; Meier et al., 2017). For example, Li et al.
54 (2016) found maize root exudates increase nodulation and stimulate nitrogen fixation through
55 enhanced gene expression, and Meier et al. (2017) found root exudates increase gross N
56 mineralization in both fertilized and unfertilized soils. Despite the importance of the connection
57 between root exudate C and processes controlling N availability, few studies have explored how
58 root exudates impact N mineralization or N fixation and to our knowledge no study has
59 simultaneously investigated the relative importance of N mineralization versus N fixation.

60 The amount and composition of root exudates is variable, depending on plant species
61 (Bürmann et al., 2005) and nutrient status (Smercina et al., 2020a). For example, previous work
62 has demonstrated that under different N conditions, a perennial grass excretes different root
63 exudate profiles; in N limited conditions organic acids were relatively more abundant than
64 carbohydrates, while the opposite was true in non-N limited conditions (Smercina et al., 2020a).
65 In general, carbohydrates-based metabolism is found more commonly in or is favored by a larger
66 proportion of soil microorganism, while organic acids are metabolized by a smaller group of
67 more specialized microorganisms (Bürmann et al., 2005; Landi et al., 2006). However, organic
68 acids were found to play a more important role than carbohydrates in shaping the structure of
69 microbial communities within the rhizosphere (Landi et al., 2006; Shi et al., 2011). This might be
70 due to shift in soil pH or solubilization of soil organic matter that affect the microbial community
71 composition (Rousk et al., 2009). For example, Shi et al. (2011) found significant change in soil
72 pH after organic acids addition compared with carbohydrates addition, which induced greater

73 increases than sugars in the soil microbial community richness. Therefore, different root
74 exudates might have different effects on N transformations through changes in both the structure
75 and function of soil microbial communities. Although some studies have focused on how root
76 exudates affect N mineralization or N fixation, results on how different root exudates affect these
77 N transformation processes are not consistent. For example, Rao et al. (1978) found evidence
78 that addition of different organic acids (succinate, butyrate, pyruvate, and acetate) stimulated N
79 fixation, while Bürgmann et al. (2005) showed that none of the organic acids treatments induced
80 N fixation. Furthermore, previous studies separately investigate the effects of root exudates on N
81 mineralization or N fixation (Meier et al., 2017; Smercina et al., 2019b), however, no studies
82 have measured both N mineralization and N fixation simultaneously in response to different root
83 exudates and assess the relative importance of N mineralization and N fixation in controlling the
84 soil N availability.

85 In agroecosystems, N addition has been shown to be a major control on N transformations.
86 N additions can either increase, decrease, or have no effect on soil gross N mineralization
87 (Cheng et al., 2019), and N addition has also been shown to either decrease or not change N
88 fixation rates. N addition can also alter N mineralization and N fixation by changing overall
89 microbial community structure (Reed et al., 2010). More specifically, N addition can change the
90 richness and abundance of N-fixing microbes (Freedman et al., 2013). For both N mineralization
91 and N fixation responses to N addition have been mixed, but a better understanding of how N
92 addition affects these N transformations will be necessary for successful and sustainable nutrient
93 management. In bioenergy cropping systems, nutrient management with an eye towards
94 sustainability is particularly important. Switchgrass (*Panicum virgatum L.*), a perennial, warm-
95 season, C4 grass, has been well-studied for potential as a cellulosic biofuel (Rodrigues et al.,
96 2017). N is the most commonly limiting nutrient for switchgrass, especially in marginal lands,
97 which are unsuitable for food crops due to the inherently low fertility soils and vulnerability to
98 environmental stress (Kang et al., 2013). A recent study found that switchgrass yields do not
99 respond to increasing N additions once switchgrass has been established and that it will remain
100 productive under very low N addition, even as N is being removed via annual harvest (Ruan et
101 al., 2016). Understanding how switchgrass maintains its N demands, and where this 'missing' N
102 is coming from in these low fertility, marginal lands, will allow us to optimize these bioenergy
103 cropping systems to enhance their long-term sustainability.

104 To elucidate the effects of different root exudates on the relative importance of N
105 mineralization and N fixation in the switchgrass rhizosphere, we sampled soils from
106 experimental monoculture switchgrass split-plots with or without N addition, at two marginal
107 land sites at the Great Lakes Bioenergy Research Center. During a two-week incubation, we
108 added different root exudate cocktails with varying carbohydrates or organic acids
109 concentrations into soils at a rate of 100 $\mu\text{g C g}^{-1}$ soil per day. After incubation, we used ^{15}N
110 incorporation and ^{15}N pool dilution methods to measure N fixation and gross N mineralization
111 and immobilization rates. We hypothesized: 1) both carbohydrates and organic acids addition
112 would increase N fixation and gross N mineralization rates because these are two energy
113 intensive processes; 2) organic acids would increase N fixation relatively more than N
114 mineralization because organic acids play a more important role than sugars in shaping microbial
115 community structure within the rhizosphere by recruiting more diazotrophs; and long-term N
116 fertilizer additions would alter microbial communities, with decreases in N fixation and
117 increasing gross N mineralization. Although this final hypothesis is supported by previous
118 studies, current work at our study sites suggests there may actually not be a typical fertilizer
119 response in these switchgrass systems (Smercina et al., 2021).

120

121 2. Materials and Methods

122 2.1 *Soil sampling*

123 Soils were collected from two marginal land sites in Michigan, USA, Lux Arbor (LUX;
124 42.476365, -85.451887) and Lake City (LC; 44.296098, -85.199612). These field sites are
125 maintained as part of the Great Lakes Bioenergy Research Center's Marginal Land Experiment
126 (MLE; <https://www.glbrc.org/>). Soils at LUX are Typic Hapludalf loams (510 g sand kg^{-1} , 32 g
127 silt kg^{-1}) with 7.7 g C kg^{-1} and 0.6 g N kg^{-1} . Soils at LC are Oxyaquic Haplorthod sandy loams
128 (850 g sand kg^{-1} , 80 g silt kg^{-1}) with 9.2 g C kg^{-1} and 0.6 g N kg^{-1} (Kasmerchak and Schaetzl,
129 2018). Each field site has four replicate split plots of switchgrass (*Panicum virgatum* L. cv.
130 Cave-in-Rock) monoculture which have been maintained since 2013. Split plots are divided in
131 half, into two N addition levels (Nlevel), + 56 kg urea-N $\text{ha}^{-1} \text{yr}^{-1}$ and no added N. We sampled
132 soils from both N levels to test the root exudates effect on soil N transformation processes with
133 different N availability in different soils and climate conditions.

134 In October 2019, soils were collected using a PVC auger (5 cm in diameter) to a depth of 10
135 cm at least 1 m from the edges of switchgrass plots. Soils were collected close to (within 10 cm
136 of the crown) a single switchgrass plant in order to generate soil samples completely under the
137 influence of switchgrass roots. Three soil samples were collected from each split-plot and were
138 combined to form a composite sample. Soils were kept in cooler until returning to the lab where
139 they were stored at 5 °C until analysis. Visible roots and litter residues were manually removed
140 from each soil sample and soils were sieved (2 mm mesh) and homogenized. Soil moisture was
141 determined using 8 g of fresh soil dried at 60 °C for at least 24 h.

142

143 *2.2 Artificial root exudate cocktail and soil incubation*

144 To test the effect of root exudate excreted by switchgrass under different N scenarios, four
145 root exudate cocktails were prepared with nano-pure water as outlined in Table 1. The
146 composition and concentration of carbohydrate and organic acids in these solutions were based
147 on previous work identifying switchgrass root exudate profiles under both high and low N
148 conditions (Smercina et al., 2020a). 40 g dry mass equivalent soil was put into a 4 oz glass jar for
149 each of the four split-plot field replicates from each site and for each root exudate treatment plus
150 a water only control (n = 2 sites*4 blocks*2 split-plots*5 exudate or water treatments = 80 jars).
151 Root exudate cocktails were applied daily to soils using a syringe and 21-gauge needle at a rate
152 of 100 µg C g⁻¹ dry soil. We applied exudate cocktails daily to better mimic the continuous
153 secretion of root exudates in the field. Soils were incubated at 22 °C with jars open slightly every
154 night to ventilate and dry in order to keep the moisture between 55–70% water holding capacity.

155

156 *2.3 Soil respiration rates*

157 Every day before adding the root exudate cocktails, the background soil respiration rate was
158 monitored by a gas flow system attached to a LI-820 Infrared Gas Analyzer (LI-Cor Biosciences,
159 Lincoln, NE, USA) by taking two gas samples 60-120 min apart from the headspace of the
160 sealed jars. After adding the root exudate cocktails, CO₂ concentration in the headspace of the re-
161 sealed jars was monitored by taking headspace gas samples at 0.5 h, 4 h, and 8 h after cocktail
162 addition. Soil respiration rates between two measured time points were calculated based on linear
163 interpolation. Cumulative CO₂-C respired each day, or the ~24 hours that elapsed between root

164 exudate additions, was calculated by multiplying the average daily respiration rate (average of
165 rate measured before and after root exudate addition) by time between root exudate additions.

166

167 *2.4 Nitrogen fixation rates*

168 We measured N fixation rates based on the net changes in ¹⁵N of soil incubated with ¹⁵N₂ gas
169 (Smercina et al., 2019b). At the end of incubation, triple the daily rate of root exudates (300 µg C
170 g⁻¹ dry soil) were added to each soil in enough water to bring the soil to 65% water holding
171 capacity. Then, a 10 g soil subsample was weighed into a 22 ml glass scintillation vial. Vials
172 were then capped with aluminum rings and septa and evacuated to remove ambient atmosphere.
173 After evacuation, vials immediately received 1 ml of 98 atom% acid-washed ¹⁵N₂ gas (Sigma-
174 Aldrich, Inc., St. Louis, MO, USA) and reference vials received UHP-N₂. Following N₂ addition,
175 0.6 ml of oxygen was added to the vials to achieve a 5% oxygen headspace (Smercina et al.,
176 2019a). Vial were brought to a final pressure of 1 atm with 10.4 ml UHP-He. Soils were
177 incubated at room temperature for three days. After incubation, vials were uncapped, and
178 samples were placed in a 60 °C drying oven for 48 h, then ground into a fine powder on a roller
179 mill, weighed into tin capsules, and analyzed following standard procedures at Washington State
180 University's Stable Isotope Core Laboratory (Pullman, WA). Briefly, tinned samples were
181 combusted to N₂ with an ECS 4010 elemental analyzer (Costech Analytical, Valencia, CA) and
182 analyzed on a Delta PlusXP continuous flow isotope ratio mass spectrometer (ThermoFinnigan,
183 Bremen, Germany) equipped with a 3 m GC column. Details can be found in Smercina et al.
184 (2019a). N fixation rates were calculated as µg N fixed g⁻¹ soil day⁻¹ using the equation:

$$185 \frac{AE_i * TN_i}{AE_{atm} * t}$$

186 where AE_i represents atom percent excess of sample against an unenriched reference sample,
187 TN_i represents total nitrogen content in sample, AE_{atm} represents atom percent excess in the vial
188 atmosphere (98 atom% in our case), and *t* is incubation time in days (Smercina et al., 2019b).

189

190 *2.5 Gross N mineralization and N immobilization rates*

191 Gross N mineralization and gross N immobilization rates were determined using the ¹⁵N
192 isotope pool dilution method as described by Hart et al. (1994). Briefly, 8 g soil subsamples were
193 weighed into 50 ml polypropylene centrifuge tubes in triplicate. The first of the triplicate soil

194 samples was then immediately extracted with 40 ml of 1 M KCl by placing on a shaker table at
195 ~200 rpm for 30 minutes, centrifuging and finally filtering through Whatman #4 filter paper.
196 These soil extracts were used to determine the amount of inorganic ^{15}N needed for the pool
197 dilution. The remaining two of the triplicate soil subsamples had 0.5 ml of $(^{15}\text{NH}_4)_2\text{SO}_4$ (99%
198 atom% ^{15}N , Cambridge Isotope Laboratories, MA, USA) added, bringing the final water content
199 to 75% of WHC. The ^{15}N addition increased the ambient NH_4^+ pool size by about 2 mg of N kg^{-1}
200 of dry soil (Hart et al., 1994). Labelled soils were mixed vigorously to distribute the tracer
201 solution homogenously and then incubated at room temperature. These soil incubations were
202 terminated after 6 h (t_1) and 30 h (t_2) by extraction with 1 M KCl as described above. Soil extract
203 NH_4^+ contents were measured colorimetrically in 96-well plates as described by Sinsabaugh et
204 al. (2000). 25 ml soil extract was pipetted into 90 ml polypropylene specimen cups. We added
205 additional NH_4Cl to increase the total N concentration in each cup to 40.5 μg if necessary. The
206 cups were capped immediately after addition 0.4 g MgO powder and an acid trap. Each acid trap
207 consisted of two 4 mm diameter Whatman quartz microfiber filter paper circles soaked with 10
208 μl of 2.5 M KHSO_4 , that were wrapped in an 8 cm long strip of Teflon tape and sealed with the
209 inverted cap of a micro-centrifuge tube. Each cup was gently swirled at least once per day for six
210 days. After six days, the acidified quartz fiber filter paper was picked out from the Teflon tape
211 and placed inside silver capsules and dried overnight at 60 °C. Isotope composition of the N on
212 the acid trap discs was determined at the Washington State University's Stable Isotope Core
213 Laboratory as described above. Gross N mineralization and N immobilization rates were
214 calculated according to the equations described by Bengtson et al. (2005).

215

216 *2.6 Soil pH, microbial biomass, dissolved organic C and N, ammonium and nitrate*

217 Soil pH at the beginning and after incubation were measured using S20 SevenEasy pH
218 meter (Mettler Toledo, OH, USA). At the end of the incubation, soil microbial biomass carbon
219 (MBC) and microbial biomass nitrogen (MBN) were analyzed using the chloroform fumigation-
220 extraction method (Vance et al., 1987). Briefly, 40 ml of 0.5 M K_2SO_4 was added to 8 g dry mass
221 equivalent soil, one unfumigated and one directly fumigated for 24 h with 2-ml of ethanol-free
222 chloroform to lyse microbial cells and accumulate microbial C and N (Wepking et al., 2019).
223 Soils plus K_2SO_4 were placed horizontally on an orbital shaker set at 250 rpm for 1 h. Total
224 organic C and N in the resulting soil extracts was then determined using a Vario Select TOC/TN

225 analyzer (Elementar, Ronkonkoma, NY). Microbial biomass was estimated as the difference
226 between the quantity of C and N between the fumigated and un-fumigated samples. Microbial
227 biomass C and N were calculated as fumigated extractable organic C (DOC) or total dissolved N
228 (TDN) minus un-fumigated DOC and TDN, divided by an efficiency factor of 0.45 (Jenkinson et
229 al., 2004). Dissolved organic N (DON) was calculated by subtracting total inorganic N from
230 TDN. The inorganic N (ammonium and nitrate) was measured using colorimetric methods.
231 Ammonium was measured in clear 96-well plates as described by Sinsabaugh et al. (2000).
232 Nitrate analysis protocol was adapted from the Nitrate Elimination Co. Inc. (NECi) Method N07-
233 0003 (<http://www.nitrate.com/node/164>). This method uses nitrate reductase (E.C. 1.7.1.1) to
234 convert NO_3^- to NO_2^- so that standard colorimetric chemistry (sulfanilamide-NED) can be used
235 to determine NO_2^- in clear 96-well plates as described by (Doane and Horwath, 2003).
236 Absorbance was subsequently measured at 660 nm or 540 nm for ammonium and nitrate analysis
237 respectively, on a spectrophotometric microplate reader (Synergy H1, BioTek, Winooski, VT).

238

239 2.7 Statistical analyses

240 Prior to statistical tests, all variables were visually checked using histograms, density, and
241 Q-Q plots. Data was tested for normality (Shapiro–Wilk test) and homogeneity of variance
242 (Levene’s test). Variables that did not meet the assumption of parametric statistical tests
243 (normality and homoscedasticity of errors) were log-transformed or square root-transformed to
244 fulfill assumptions of normality and homogeneity where necessary. We tested for fixed effects of
245 sampling site, root exudates treatment and their interaction using linear mixed effect models
246 using the R package *nlme* to analyze the responses of soil variables (pH, DOC, DON, DOC:
247 DON, MBC, MBN, MBC: MBN, inorganic N), total cumulative CO_2 -C respired, percentage of
248 root exudate C recovery and soil N transformation processes (gross N mineralization, gross N
249 immobilization, N fixation). Replicate field blocks were treated as a random effect. Effects of
250 root exudate treatment on average respiration rate on days 1, 8, 10, 15 were tested by one-way
251 ANOVA. Statistical comparisons among different root exudate treatments were performed using
252 a “Bonferroni” pairwise post-hoc test. Correlations between different soil properties and soil N
253 transformation processes were examined with *corrplot* package in R. Linear mixed effect model
254 results showed that there were significant sampling site effects on most of the variables (Table
255 S1) but only a few variables were significantly affected by the field level N addition. Therefore,

256 all the figures are presented by different sites and grouped with N addition treatment. Finally, we
257 built a structural equation model (SEM) to evaluate the direct and indirect pathways that regulate
258 N transformation processes and inorganic N, and to evaluate how these factors contribute to the
259 standardized total effects (direct effects plus indirect effects). Predicted causal relationships
260 between variables were based on prior knowledge of how different soil variables affect N
261 transformation processes. The adequacy of the model was determined by the χ^2 -test, goodness of
262 fit (GIF > 0.90) index, and root mean squared error of approximation (RMSEA < 0.08) index
263 (Liu et al., 2017). The SEM analysis was conducted using Amos 21.0 (Amos Development
264 Corporation, Chicago, IL). All the statistical analyses, except SEM, were performed using R
265 statistical software v3.2.4 (R Development Core Team, 2016). The significance of the analyses
266 was set at $p < 0.05$ level. *ggplot2* package was used for plotting graphs (Wickham, 2016).

267

268 3. Results

269 3.1 Root exudate addition induced significant changes in soil pH and different C pools

270 Root exudate type significantly affected the soil pH (Table 2, Fig. S1). Overall, soil pH
271 increased by 1–3 units with all treatments containing organic acids (OA) compared to control at
272 both sites at the end of the incubation (Fig. 1a). In contrast, carbohydrate (Crb) treatment had no
273 significant effect on soil pH at either site, and these changes in pH were not affected by N
274 addition. Adding root exudates significantly increased soil respiration throughout the incubation
275 (Fig. 1b; Figs. S2-3). On average, adding carbohydrates stimulated soil respiration to a greater
276 extent than organic acids (Fig. 1b). Soil respiration in Crb and high carbohydrate and low
277 organic acid (HCLOA) treatments were increased by 249% and 243% respectively, while OA
278 and low carbohydrate and high organic acid (LCHOA) treatments increased by 154% and 188%
279 respectively compared to control (Fig. 1b). Every day after root exudate addition, there was a
280 respiration pulse that resulted in 2x greater respiration rates compared to just prior to root
281 exudate additions, across all treatments and sites (Fig. S2). MBC increased significantly after
282 Crb addition compared with control in Lake City under field N addition treatment. In contrast, no
283 significant effect of root exudate type on MBC was found in Lux Arbor soil. Root exudate type
284 had no significant effect on MBN at either site. However, N addition significantly increased
285 MBN in Lake City, but significantly decreased MBN in Lux Arbor soils.

286 In Lake City soils, DOC was significantly greater across all root exudate types compared
287 with control and OA was significantly greater than Crb and HCLOA (Fig 2a). In Lux Arbor
288 soils, there was a significant interaction between root exudate and N addition on DOC (Fig. 2a).
289 With no N addition, all root exudate treatments had significantly increased DOC compared with
290 control, with no differences among root exudate treatments; with N addition, all root exudate
291 treatments but not Crb treatments, had significantly increased DOC compared with control (Fig.
292 2a). DON at Lake City was greatest in OA and LCHOA and lowest in Crb and HCLOA
293 treatments (Fig. 2b) with no significant effects of N addition. In Lux Arbor soils, Crb and
294 HCLOA treatments decreased DON compared with OA and overall, DON was significantly
295 reduced with N addition. The ratio of DOC:DON was significantly widened by all root exudate
296 additions compared to control at both sites (Fig. 2c). There were significantly different C
297 recovery patterns between the two sites (Fig. 3; Table S1, $p < 0.001$). In Lake City soils, the total
298 C recovered in DOC, MBC, and respired C pools accounted for 92%–106% of the total added
299 root exudate C with no significant differences in total C recovered between root exudate
300 treatments or N addition levels (Fig. 3; Table S1). In contrast, in Lux Arbor soils, only 45%–74%
301 of the added root exudate C was recovered in these same three C pools, with no significant
302 differences in recovery between different root exudate treatments, while the total C recovered
303 was significantly higher without N addition compared to N addition (Table 2).

304 *3.2 Root exudates shift how N mineralization and N fixation contribute to plant-available N*

305 There is a strong correlation between gross N mineralization and gross N immobilization
306 (Fig. S5). In Lake City soils, overall, there were no significant root exudate type or N addition
307 effects on either gross N mineralization or gross N immobilization (Fig. 4ab). In Lux Arbor soils,
308 Gross N mineralization and gross N immobilization were significantly increased with OA
309 compared with Crb and HCLOA treatments (Fig. 4ab), and both gross N mineralization and
310 gross N immobilization were reduced with N addition. No significant root exudate type or N
311 addition effects were found on net N mineralization at either site (Fig. 4c). N addition and root
312 exudate type had varied effects on N fixation rates (Table 2; Fig. 5). In Lake City soils, N
313 fixation were lowest in OA compared to all other root exudate types (Fig. 5). In Lux Arbor soils,
314 there was a significant interaction between root exudate type and N addition such that soils with
315 no N addition (+0N) had the highest N fixation with Crb addition. In Lux Arbor soils receiving
316 N (+56N), N fixation was higher only in HCLOA compared to OA. The ratio of gross N

317 mineralization and N fixation (N_{\min} : N_{fix}) was not significantly affected by N addition at either
318 site but did vary by root exudate type (Fig. 6a; Table 2). In both sites, the N_{\min} : N_{fix} ratio was
319 wider after OA compared to Crb and HCLOA additions. In Lake City, inorganic N was not
320 significantly affected by either root exudate type or N addition (Table 2). In contrast, root
321 exudate type had a significant effect on inorganic N in Lux Arbor soils (Fig. 6b), with greater
322 inorganic N with OA addition compared with Crb and HCLOA root exudate types.

323 *3.3 Root exudate induced pH and DON change had strong correlation with N transformations* 324 *and soil N availability*

325 Correlation analysis showed that gross N mineralization or gross N immobilization were
326 significantly positively correlated with soil pH at the end of incubation (pH_{end}), DOC, DON, and
327 inorganic N, but negatively correlated with soil respiration (Fig. S6). N fixation was negatively
328 affected by increasing pH_{end} and DON and positively correlated with soil respiration. N_{\min} : N_{fix}
329 was positively correlated with DOC, DON, and soil pH_{end} , and negatively correlated with soil
330 respiration rate. Net N mineralization was only positively correlated with DON. In addition,
331 inorganic N had positive correlations with soil pH_{end} , DOC, and DON (Fig. S6). Changes in soil
332 pH induced by root exudate additions and subsequent changes of dissolved organic C and N
333 (DOC, DON) were the main influencing factors in controlling the different N transformation
334 processes and soil inorganic N availability (Fig. 7). Gross N mineralization was mainly
335 associated with soil pH_{end} and DON, while N fixation was indirectly associated by DOC and
336 directly correlated with microbial activity (soil respiration). Finally, gross N mineralization was
337 more important than N fixation in controlling inorganic N availability in these marginal land
338 soils.

339

340 4. Discussion

341 *4.1 Root exudates shift the relative contribution of N mineralization and N fixation to N* 342 *availability*

343 Soil N availability is determined by the balance of N mineralization and N fixation.
344 However, previous studies solely focus on how root exudates affect either N mineralization or N
345 fixation processes (Bürgmann et al., 2005; Le Roux et al., 2008; Meier et al., 2017). How root
346 exudates (carbohydrates *versus* organic acids) affect the relative importance of N mineralization
347 and N fixation is unknown. This study, to the best of our knowledge, is the first to quantify how

348 different root exudates affect the relative importance of N mineralization and N fixation in
349 controlling soil N availability (Fig. 8). Because we lack gross N mineralization measurements
350 with water only addition, we cannot test the hypothesis that all root exudate additions increase N
351 mineralization rates, but we found higher gross N mineralization after adding organic acids
352 compared with carbohydrates. We also found a significant increase in DON after adding organic
353 acids (Fig. 2b), which might be due to dissociation of mineral-associated OM. The increase of
354 soil pH with OA addition, which we discuss below (4.2), is evidence for dissociation of mineral-
355 associated OM. In any case, the increase in DON after organic acids addition compared with
356 carbohydrates, provided more substrate for N mineralization.

357 Adding carbohydrates significantly increased soil N fixation, which is in agreement with
358 previous studies (Rao, 1978; Bürgmann et al., 2005; Li et al., 2016) and partly supports our first
359 hypothesis that all root exudate additions will increase N fixation. This is not surprising because
360 N fixation is an energy intensive process, and carbohydrates used in this study are compounds
361 that can be directly taken up into microbial cells without release of additional extracellular
362 enzymes and will yield relatively high amounts of energy to support N fixation (Shi et al., 2011).
363 For example, Rao et al., (1978) previously showed that addition of glucose and sucrose
364 stimulated N fixation. Similarly, Bürgmann et al. (2005) found that all sugar treatments
365 significantly increased acetylene reduction, which is used as an indicator of increased
366 nitrogenase activity and N fixation. Additionally, they also found the fastest increase and highest
367 rates of acetylene reduction in treatments with glucose compared to fructose and sucrose, which
368 suggests that N fixing microorganisms have different carbohydrate preferences. In contrast to our
369 hypothesis, organic acids addition did not increase N fixation, but induced a slight decrease of N
370 fixation, which is surprising because organic acid is also an ideal C source for the higher energy
371 intensive N fixation. The only reason that there was no response of N fixation to organic acid
372 addition is that N fixers is not preferring the organic acid that we added. Acetate acid and
373 succinic acid were the two abundant organic acid that were excreted by switchgrass under N
374 limiting condition (Smercina et al., 2020a), which we presumably thought to recruit N fixers
375 around the rhizosphere to increase the available N for the plant. A previous study also showed
376 that none of the added organic acid induced N fixation (Bürgmann et al., 2005), which is also
377 unexpected because that both citric and malic acid were consumed, and nitrate was depleted in a
378 similar pattern as compared with sugar-containing treatments. Additionally, organic acid

379 accumulation (high level of malate) under phosphorus deficiency condition were also found to
380 associate with reduced N fixation (Le Roux et al., 2008). In contrast, Rao et al., (1978)
381 previously showed that addition of several organic acids (succinate, butyrate, pyruvate, propanol,
382 acetate) stimulated N fixation, however, their experiment is under anaerobic condition, which is
383 not comparable with our study that was conducted under aerobic condition. Overall, our results
384 combined with previous studies suggested that N fixers have a preference use of carbohydrates
385 over organic acids.

386 We found a significant increase of the ratio of gross N mineralization to N fixation ($N_{\min}:$
387 N_{fix}) after adding organic acids compared to carbohydrates, regardless of the concentration of
388 organic acids (Fig. 8), and therefore a higher N availability in organic acids treatment than in
389 carbohydrates treatment. This result is in contrast with our second hypothesis that organic acids
390 will increase N fixation more than N mineralization because organic acids are usually used by
391 specialized microorganisms under N limiting conditions, which are thought to recruit N fixers
392 (Smercina et al., 2020a). For example, Shi et al. (2011) found significant increase of N fixers
393 (*Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Sphingomonadales* etc.) with root exudate cocktails
394 with organic acids rather than with sugar additions. However, one of the organic acids used in
395 our study, acetate acid, excreted under low N conditions by switchgrass, has been shown to
396 completely inhibit nitrogenase activity of diazotrophs in pure cultures (Dobereiner et al., 1975).
397 In addition, we found direct evidence that there was more DOC left (not respired) in the organic
398 acid treatment than in the carbohydrate treatment after the incubation, which suggests that
399 microorganisms in these soils may prefer carbohydrates as a C source over organic acids. This
400 evidence can help to explain why the organic acids addition did not increase N fixation, and is in
401 line with previous opinions that carbohydrates are favored by a larger proportion of soil
402 microorganism, while organic acids are preferred by specialized microorganisms (Landi et al.,
403 2006). Therefore, the secretion of organic acids by switchgrass might not be used for supporting
404 N fixation, instead being used for stimulating N mineralization by destabilizing previously
405 protected soil organic matter. Overall, our results suggest that N mineralization may play a more
406 important role in controlling N availability than N fixation after addition of organic acids, while
407 N fixation maybe more important in providing N to switchgrass when they excrete more
408 carbohydrates in these marginal land soils.

409 4.2 *Root exudates mediate N transformations and N availability through different mechanisms in*
410 *low fertility soils*

411 We found that root exudates induced changes in soil pH and subsequent changes of DON
412 and soil respiration had strong associations with different N transformation processes (Fig. 7).
413 The pH-induced increase in N mineralization can be explained by the dissociation of the mineral-
414 associated OM followed by increases in solubility of OM in soils with increased pH (Keiluweit
415 et al., 2015; Curtin et al., 2016; Jilling et al., 2018). This is supported by previous studies that
416 reported gross N mineralization increased with increases of soil pH (Cheng et al., 2013). In our
417 study, we found a significant increase of soil pH after adding organic acids, regardless of organic
418 acids concentration, while carbohydrates did not change the soil pH (Fig. 1). This result is
419 unexpected, because usually the secretion of organic acids in the plant rhizosphere will decrease
420 the soil pH due to its acidity (Hinsinger et al., 2003). The increase of soil pH after adding organic
421 acids can be explained by the degradation of carboxylic acids by soil microbes which consumes
422 H^+ and liberates OH^- and CO_2 (Gramss et al., 2003). Furthermore, the acetate that we used in
423 root exudate cocktails, and is commonly found in the rhizosphere, will also directly lead to
424 increases of soil pH to some extent (Yu and Huang, 2009). This result is also consistent with
425 several previous studies (Shi et al., 2011; Keiluweit et al., 2015; Girkin et al., 2018ab), which
426 also found organic acids addition increased the soil pH in lab incubation studies. For example,
427 Shi et al. (2011) found a 1–3 unit increase of soil pH after adding a root exudates cocktail
428 containing organic acids, which is comparable with our results.

429 In our study, the increases in pH were accompanied by increased DON (Fig. 2b), which
430 provides more available organic substrate for N mineralization. Keiluweit et al. (2015) also
431 provides direct evidence that organic acids (acetic acid) addition significantly reduces the
432 amount of Fe and Al in metal-organic complexes and disrupts mineral-organic associations.
433 Additionally, soil pH increase can enhance N mineralization by increasing the activity of soil
434 enzymes involved in the N cycle (Vázquez et al., 2019). Furthermore, at higher pH, the
435 molecular formulae of dissolved organic matter can have a lower degree of unsaturation and
436 oxygenation, lower molecular size, and higher abundance of N-containing compounds available
437 for higher biological decomposition activity (Roth et al., 2015).

438 The additional, and more biologically accessible dissolved organic matter (DOC more
439 specifically in this case) also had a positive impact on N fixation as can be seen by the strong

440 relationship between soil respiration and N fixation (Fig. 7). N fixation is an extremely expensive
441 process energetically, requiring 16 ATP to break apart one di-nitrogen molecule (Smercina et al.,
442 2019a). Therefore, respiratory costs for N fixation are high and are reflected in the relatively
443 higher respiration rates associated with N fixation that are fueled by increased DOC availability.
444 Overall, pH changes and subsequent increases in the bioavailability of dissolved organic matter
445 (both DON and DOC) that were induced by different root exudate additions altered the relative
446 importance of N mineralization and N fixation and as a result affected soil inorganic N
447 availability in those low fertility soils.

448 *4.3 C recovery patterns of added RE in marginal land soils*

449 After two weeks incubation, we found different C recovery patterns between the two
450 marginal land sites (Fig. 3). In Lake City soils, when MBC, DOC, and cumulative C respired
451 were summed, we were able to account for 92–106% of the C we added as root exudates. In
452 contrast, we found the recovered C in Lux Arbor soils only accounted for 45–74% of the total
453 root exudate added C (Fig. 3). The potential mechanism explaining the low C recovery in these
454 soils are that 1) the root exudate C was directly adsorbed onto mineral surfaces (Curtin et al.,
455 2016; Yu et al., 2017) or 2) a faster microbial biomass turnover rate in the Lux Arbor soils would
456 lead to greater SOC through microbial necromass stabilization (Sokol and Bradford, 2019).
457 Earlier work by Carter (1978) showed that DOC could be directly adsorbed onto CaCO₃. In our
458 study, we found that carbonate content is higher in Lux Arbor (2.4%) compared to Lake City
459 (1%) soils (data not shown). The high proportion of carbonate in Lux Arbor may have led to
460 adsorption of some of the added root exudate C and partially explain the lower C recovery in
461 these soils. In addition, there is more silt and clay and less sand in Lux Arbor compared to Lake
462 City soils (Kasmerchak and Schaetzl, 2018). Sandier soils are often more limited in their
463 capacity to build soil organic matter due to a limited capacity to protect C long-term by various
464 mechanisms such as direct mineral surface interactions (Plante et al., 2006; Jilling et al., 2020).
465 Therefore, the difference in soil texture may explain part of the low recovery of root exudate C in
466 Lux Arbor as more clay surfaces available in these soils would also lead to greater potential for
467 adsorption.

468 *4.4 N addition have no significant effects on N transformations*

469 Field level N additions had no significant effects on either N mineralization or N fixation
470 rates without adding any root exudate, which is surprising, as we hypothesized that N addition

471 would decrease N fixation and increase N mineralization. N additions have been shown to be a
472 major control on N transformations, but no change in N fixation or N mineralization with N
473 addition in our study is in contrast with previous studies (Cheng et al., 2019; Fan et al., 2019).
474 These surprising results may be explained by the following reasons: first, the amount of N added
475 in the field was relatively small at a dose of 56 kg N ha⁻¹ year⁻¹, which is equal to the amount of
476 N that is removed, on average, through annual switchgrass biomass harvests. This is a pretty low
477 amount of N compared with other studies where N additions are as high as 140 kg N ha⁻¹ year⁻¹
478 (Cheng et al., 2019). Also, N addition effects may disappear or attenuate to a negligible extent by
479 the time we sampled the soil in October as the N was added as urea in May. Indeed, in a recent
480 study at the same sites, N fixation rates were significantly different between N addition and no N
481 addition treatments immediately after N addition, with differences disappearing for the
482 remainder of the growing season (Smercina et al., 2021). In addition, previous results at the same
483 site have shown there are few impacts of N addition on the microbial community (metagenome,
484 unpub. results) including diazotrophs (Smercina et al., 2020b; 2021). In conclusion, the relatively
485 low amount of N added, and disappearance of N affects over time resulted in no response of N
486 transformation processes to N fertilizer additions in these switchgrass system soils.

487 To summarize, this is the first study to quantify how different root exudates affect the
488 relative importance of N mineralization and N fixation in marginal land soils. We showed that
489 root exudates addition induced changes in pH and DON that in turn shift how N mineralization
490 and N fixation contribute to plant available N in low fertility soils (Fig. 8). N mineralization
491 appears to play a more important role than N fixation in regulating soil N availability. In
492 addition, we found contrasting root exudate C retention in different soils, which were
493 presumably caused by different soil texture and mineralogy. Future studies should also explore
494 whether different N transformation processes will respond similarly to different types of organic
495 acids or carbohydrates. It is also worthwhile to investigate the underlying mechanisms of how
496 different soil textures and mineralogy affect root exudate C sorption and desorption processes
497 and contributions to soil C accumulation. By quantifying how different root exudates affect the
498 contribution of N mineralization and N fixation to the plant-available N pool in low fertility
499 soils, this study enhances our understanding of the “C for N” exchange in the plant rhizosphere,
500 which will be useful for reaching long-term sustainability goals in cropping systems. .

501

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511

512 **Conflict of interest**

513 The authors declare no conflict of interest

514 **References**

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643 **Table legends:**

644 **Table 1** Composition and rates of carbohydrates and organic acids in root exudate cocktails.

645 **Table 2** Summary (F-values) of two way-ANOVA for the effects of N addition (Nlevel), root exudate
646 types (RE) and their interactions on soil properties and N transformation processes in two sites

647 **Table 1.** Composition and rates of carbohydrates and organic acids in root exudate cocktails.

Treatment	Daily C input ($\mu\text{g C g}^{-1}$ soil)	Ratio of Crb and OA	Carbohydrate		Organic acid	
			($\mu\text{g C g}^{-1}$ soil)		($\mu\text{g C g}^{-1}$ soil)	
			Arabinose	Fructose	Sodium Acetate	Succinic acid
W [†]	0	0: 0	/	/	/	/
Crb	100	100: 0	50	50	/	/
OA	100	0: 100	/	/	50	50
HCLOA	100	82: 18	41	41	9	9
LCHOA	100	30: 70	15	15	35	35

648 [†]W, control treatment with only nano-pure water; Crb, only carbohydrate; OA, only organic acid; HCLOA, high proportion of carbohydrate and low proportion of
649 organic acid; LCHOA, low proportion of carbohydrate and high proportion of organic acid.

650 **Table 2.** Summary (F-values) of two way-ANOVA for the effects of N addition (Nlevel), root exudate types (RE) and their interactions on soil properties
 651 and N transformation processes in two sites.

Response variable/Site	Lake City			Lux Arbor		
	N addition (Nlevel)	Root exudate types (RE)	Nlevel × RE	Nlevel	RE	Nlevel × RE
pH _s	< 0.001	3.58*	0.16	0.48	7.83***	0.02
pH _{end}	< 0.001	128***	0.71	4.47***	187***	0.96
ΔpH	0.43	42.5***	1.54	0.23	52.6***	5.46***
MBC	1.95	4.30**	0.31	1.24	0.49	0.17
MBN	47.1***	2.89*	1.17	15.7***	0.12	0.82
MBC: MBN	2.16	2.96*	0.47	10.9**	0.68	0.27
DOC	0.23	24.5***	0.10	31.9***	27.9***	4.49**
DON	0.74	32.4***	0.19	24.1***	16.4***	0.68
DON: DON	0.03	9.19***	0.23	1.02	32.0***	2.25
Respiration rate	3.90	132***	0.15	13.2***	213***	0.27
Cumulative CO ₂	3.90	132***	0.15	13.2***	213***	0.27
C recovery	0.89	0.82	0.25	4.92*	0.43	0.63
Inorganic N	2.65	0.28	0.66	< 0.01	6.71**	0.95
GrossN _{min}	2.99	1.73	1.02	4.62*	13.9***	0.29
GrossN _{immob}	2.15	1.44	1.11	4.97*	12.6***	0.39
NetN _{min}	5.87*	1.01	1.77	0.21	1.26	1.26
N fixation	4.23*	7.20***	0.57	2.87	15.7***	7.23***
N _{min} : N _{fix}	1.07	8.54***	2.39	1.36	23.7***	1.11

652 Abbreviations: ΔpH was calculated by subtracting the initial soil pH (pH_s) from soil pH after incubation (pH_{end}); MBC, microbial biomass carbon; MBN,
 653 microbial biomass nitrogen; MBC: MBN, the ratio of MBC and MBN; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DOC: DON,
 654 the ratio of DOC and DON; GrossN_{min}, Gross N mineralization; GrossN_{immob}, gross N immobilization; NetN_{min}, net N mineralization; N_{min}: N_{fix}, the ratio
 655 of gross N mineralization and N fixation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

656 **Figure legends:**

657 **Fig. 1 Changes in soil pH (ΔpH = difference in pH before and after incubation) and average soil**
658 **respiration rate among different root exudates and N addition levels.** Values are average ($\pm\text{SE}$) of
659 four field replicates. The red dashed line at zero indicates no change in pH. The effects of N addition
660 (Nlevel) and root exudate types (RE) and their interaction were tested by using two-way ANOVA; ns
661 represent non-significant difference, *, **, *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.
662 Different lower-case letters represent significant differences among root exudate (RE) treatments, with
663 different color letters representing differences by N addition level: +0N (black); +56N (red). W: nano-
664 pure water control; Crb: carbohydrate treatment; OA: organic acid treatment; HCLOA: high fraction of
665 Crb and low fraction of OA treatment; LCHOA: low fraction of Crb and high fraction of OA treatment.

666 **Fig. 2 Dissolved organic carbon (DOC, a) dissolved organic nitrogen (DON, b), and the ratio of**
667 **DOC: DON (c) among different root exudate types and N addition levels in Lake City and Lux**
668 **Arbor soils.** Values are average ($\pm\text{SE}$) of four field replicates. The effects of N addition (Nlevel) and root
669 exudate types (RE) and their interaction were tested by using two-way ANOVA; ns represent non-
670 significant difference, *, **, *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Different
671 lower-case letters represent significant differences among root exudate (RE) treatments, with different
672 color letters representing differences by N addition level: +0N (black); +56N (red).

673 **Fig. 3 Measured C pools after incubation with root exudate addition relative to the water only**
674 **treatment in Lake City and Lux Arbor soils receiving two levels of N addition 0 and 56 kg N ha⁻¹.** C
675 pools include dissolved organic carbon (DOC), microbial biomass carbon (MBC) and cumulative C
676 respired (RsCumC). The red dashed line indicates the total amount of root exudate C added over the two-
677 week incubation. Note: negative MBC means a decrease of MBC in the root exudate treatment relative to
678 the water control. Although partitioning between C pools differed, there were no significant differences in
679 total C recovered across root exudate (RE) treatments.

680 **Fig. 4 Gross N mineralization (N_{min}), gross N immobilization (N_{immob}), and net N mineralization**
681 **(NetNmin) among different root exudate types and N addition levels in Lake City and Lux Arbor.**
682 Values are average ($\pm\text{SE}$) of four field replicates. The effects of N addition (Nlevel) and root exudate
683 types (RE) and their interaction were tested by using two-way ANOVA; ns represent non-significant
684 difference, *, **, *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Different lower-case
685 letters represent significant differences among root exudate (RE) treatments, with different color letters
686 representing differences by N addition level: +0N (black); +56N (red).

687 **Fig. 5 Effects of different root exudate types and N addition levels on N fixation rates in Lake City**
688 **and Lux Arbor soils.** Values are average (\pm SE) of four field replicates. The effects of N addition
689 (Nlevel) and root exudate types (RE) and their interaction were tested using two-way ANOVA; ns
690 represents non-significant difference, *, **, *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.
691 Different lower-case letters represent significant differences among root exudate (RE) treatments, with
692 different color letters representing differences by N addition level: +0N (black); +56N (red).

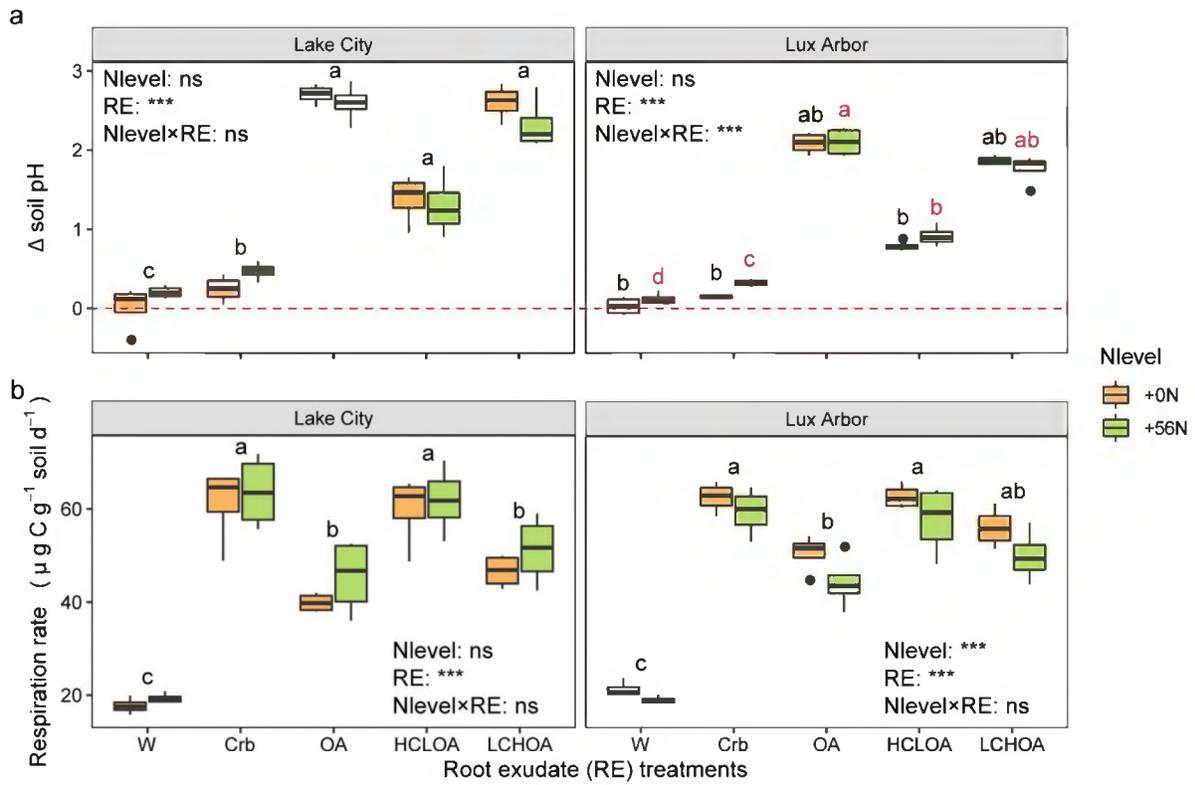
693 **Fig. 6 The ratio of gross N mineralization and N fixation ($N_{\min}: N_{\text{fix}}$) (a), and inorganic N (b) among**
694 **different root exudate types and N addition levels in Lake City and Lux Arbor soils.** Values are
695 average (\pm SE) of four field replicates. The effects of N addition (Nlevel) and root exudate types (RE) and
696 their interaction were tested using two-way ANOVA; ns represent non-significant difference, *, **, ***
697 represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Different lower-case letters represent significant
698 differences among root exudate (RE) treatments.

699 **Fig. 7 Structural equation model (SEM) shows how root exudates affect N cycling.** (a) solid lines
700 represent positive relationships, and dashed line represent negative relationships; R^2 values associated
701 with response variables indicate the proportion of variation explained by relationships with other
702 variables. Numbers adjacent to arrows are standardized path coefficients (covariation coefficients)
703 proportional to thickness of the lines, Significance: *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p <$
704 0.001 level. pH_{end} , soil pH at the end of the incubation; DOC: dissolved organic carbon; DON, dissolved
705 organic nitrogen; R_s : soil respiration rate; InorgN: inorganic N; GrossN_{\min} , gross N mineralization; N_{fix} , N
706 fixation. (b) SEM standardized total, direct, and indirect effects of different soil properties on GrossN_{\min} ,
707 N_{fix} , as well as InorgN.

708 **Fig. 8 Conceptual diagram showed potential mechanisms of root exudates (organic acid *versus***
709 **carbohydrate) on different N transformation processes in plant rhizosphere under different N**
710 **condition.** The circle size represents the magnitude of different pool or the flow in organic acid or
711 carbohydrate treatment. Red arrows indicate negative effect, while green arrows indicate positive effect.
712 The width of the arrows showed relative strength of different processes. The bolded and enlarged text
713 (e.g., DOC) under N limiting condition on the left shows their higher increase with organic acid addition
714 compared with carbohydrate addition, with a similar situation for text on the right without N limitation.
715 DOC: dissolved organic carbon; DON, dissolved organic nitrogen; N_{\min} , gross N mineralization; N_{fix} , N
716 fixation; $N_{\min}: N_{\text{fix}}$, the ratio of gross N mineralization and N fixation; N availability represented by
717 inorganic N; Note that plant excrete more organic acid under N limiting conditions, whereas more
718 carbohydrate without N limitation (Smircina et al., 2020a). Compared with carbohydrate addition,
719 organic acid significantly increased soil pH, which induced release of mineral-associated OM and

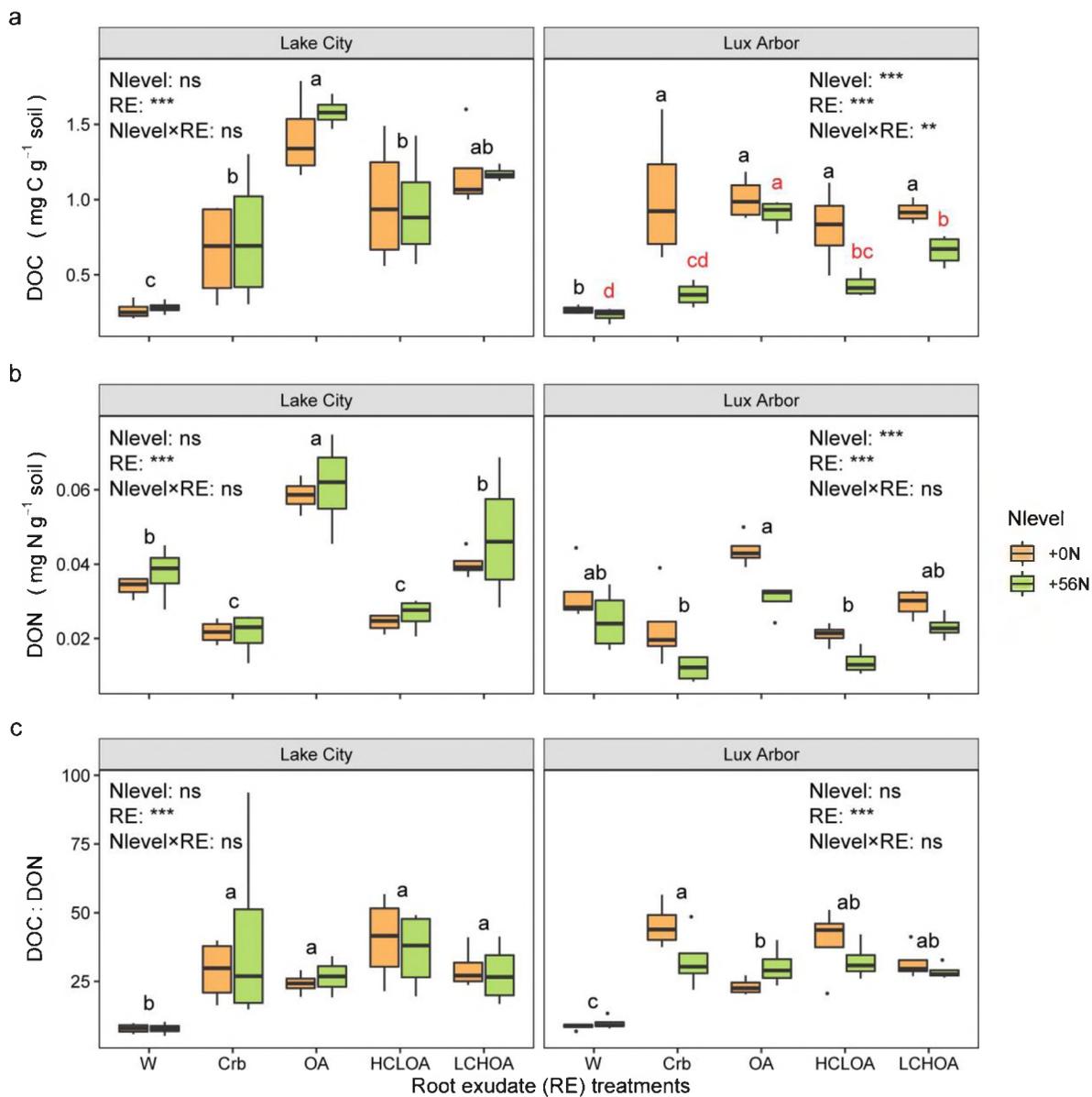
720 therefore, increased DON, that provide the substrate for N mineralization and increased the N_{min} , but the
721 increased DON downregulate the N fixation, overall, N_{min} outcompete N fixation in regulating the
722 availabilble N and increase the N availability under N limited condition. While carbohydrate addition
723 significantly increased soil respiration, which depleted DOC and DON, the decrease DON therefore
724 decrease the N_{min} but increase the N fixation. However, the influence of DON on N_{min} is high than N
725 fixation, which decreased the final N availability under N abundant condition.

726 **Fig. 1**

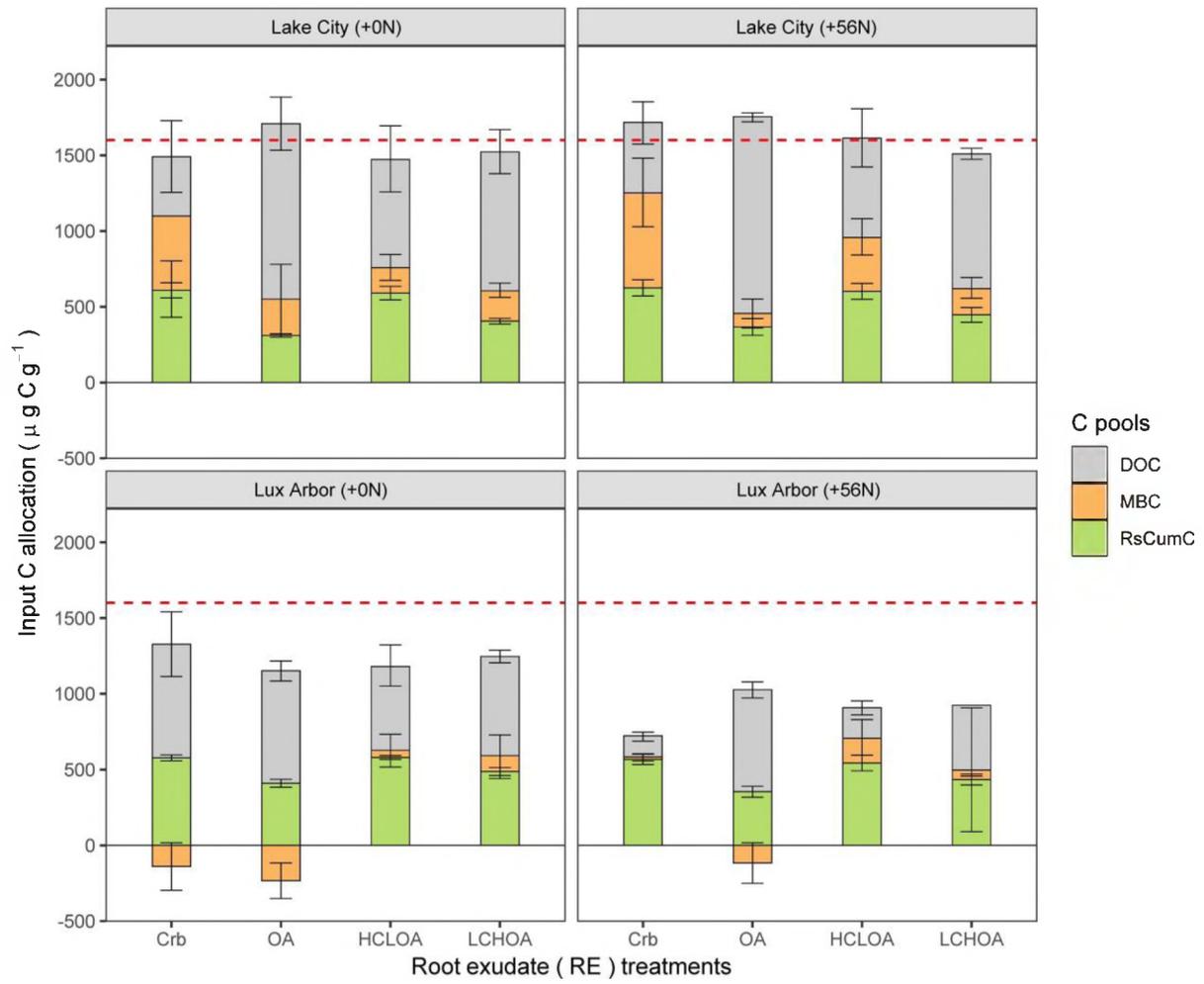


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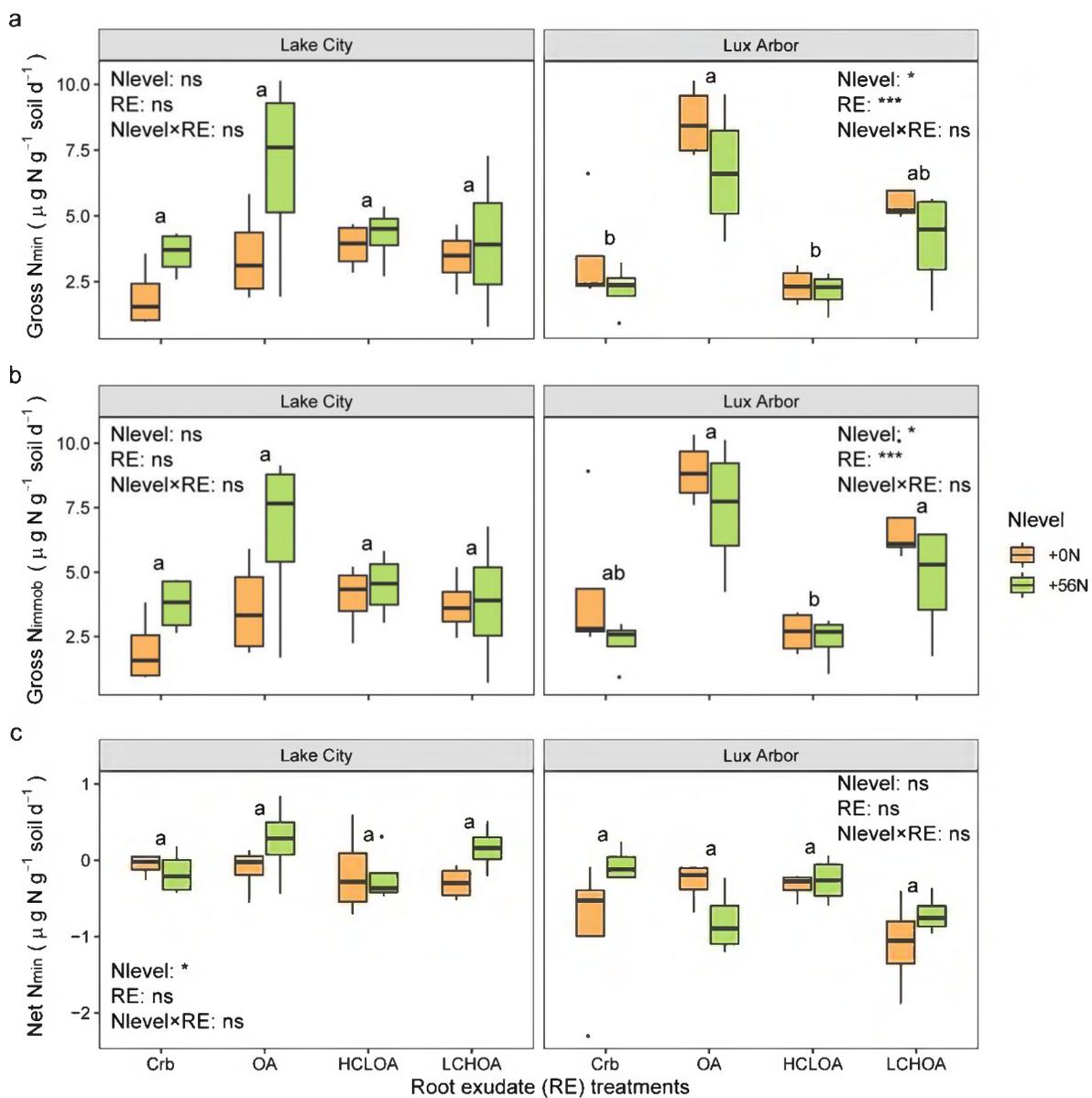
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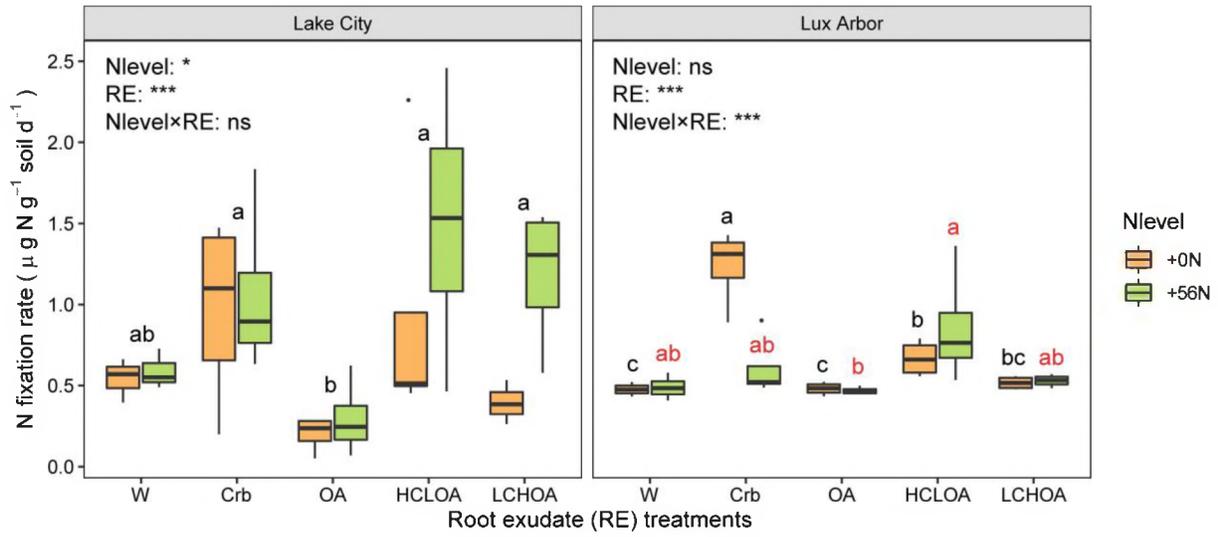
731 Fig. 3



732



735 Fig. 5



736

