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1 **Composition and concentration of root exudate analogues regulate greenhouse gas fluxes from**
2 **tropical peat**

3

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10

11 **KEYWORDS:** Tropical peat; Carbon dioxide; Methane; Root exudates; Organic acids; Methanogenesis

12 Abstract

13 Tropical peatlands are a significant carbon store and source of carbon dioxide (CO₂) and methane (CH₄) to the
14 atmosphere. Plants can contribute to these gas emissions through the release of root exudates, including sugars
15 and organic acids amongst other biomolecules, but the roles of concentration and composition of exudates in
16 regulating emissions remains poorly understood. We conducted a laboratory incubation to assess how the type
17 and concentration of root exudate analogues regulate CO₂ and CH₄ production from tropical peats under anoxic
18 conditions. For CO₂ production, substrate concentration was the more important driver, with increased CO₂
19 fluxes following higher addition rates of four out of the six exudate analogues. In contrast, exudate type was the
20 more important driver of CH₄ production, with acetate addition associated with the greatest production, and
21 inverse correlations between exudate concentration and CH₄ emission for the remaining five treatments. Root
22 exudate analogues also altered pH and redox potential, dependent on the type of addition (organic acid or sugar)
23 and the concentration. Overall, these findings demonstrate the contrasting roles of composition and
24 concentration of root exudate inputs in regulating greenhouse gas emissions from tropical peatlands. In turn this
25 highlights how changes in plant communities will influence emissions through species specific inputs, and the
26 possible impacts of increased root exudation driven by rising atmospheric CO₂ and warming.

27 1. Introduction

28 Globally, peatlands are a significant source of methane (CH₄) emissions, contributing between 20 – 39% of
29 annual CH₄ production, as well as making a significant contribution to atmospheric carbon dioxide (CO₂)
30 emissions (Laanbroek, 2010). Tropical peatlands in particular are a significant carbon (C) store, accounting for
31 only 11% of total peatland area but containing approximately 104.7 Gt C (Page et al., 2011, Dargie et al., 2017).
32 Vegetation exerts a strong influence on tropical peatland greenhouse gas (GHG) emissions through inputs of
33 leaf, root and shoot litter, which can determine key peat properties (Wright et al., 2013). Plants also release root
34 exudates, which represent a significant source of labile C released at depth. This addition can impact peat
35 properties, but the precise effect on net GHG emissions is unclear (Kuzyakov and Domanski, 2000). Root
36 exudates have been ascribed a variety of functions, including as a means of chelating limiting minerals and
37 nutrients and as a chemoattractant (Dakora and Phillips, 2002, Strom et al., 2002), and have been shown to
38 directly affect properties such as pH (Dunfield et al., 1993, Yan et al., 1996). In turn, changes in nutrient
39 availability and pH, amongst other peat properties, can regulate GHG emissions, through processes mediated by
40 microbial communities (Sjögersten et al., 2011, Troxler et al., 2012). This represents an important process in the
41 context of land use change in tropical peatlands, as any process that alters plant communities may affect the
42 concentration and composition of exudate inputs, as well as alter peat properties (Tonks et al. 2017).
43 We previously showed that root exudate analogues significantly increase peat microbial community activity and
44 enhance the production of both CO₂ and CH₄ (Girkin et al., 2018a). However, the precise role of exudate
45 concentration in regulating net fluxes remains to be clarified. This is an important knowledge gap as rates of
46 root exudation are linked to rates of C fixation during photosynthesis, and therefore plant C inputs have a strong
47 regulatory role in ecosystems with high rates of net primary productivity, including in tropical forested
48 peatlands (Badri and Vivanco, 2009).
49 This study assesses how six different root exudate components, added at three different concentrations, regulate
50 GHG production from tropical peat. We hypothesised that: i) increased concentration of labile C addition
51 significantly increases net CO₂ and CH₄ production; ii) the extent of increases in CO₂ and CH₄ production will
52 vary between exudate types (i.e. sugars compared to organic acids); and iii) labile C additions alter soil pH and
53 redox, with responses depending on the concentration and type of substrate.

54 2. Methods

55 2.1. Study site

56 Peat samples were collected in February 2015 from the 80 km² ombrotrophic peatland at Changuinola, part of
57 the San San Pond Sak freshwater and marine wetland located in Bocas del Toro province, Panama. The central
58 peat dome is approximately 8 m deep and was initiated approximately 4000–5000 years ago (Phillips et al.,
59 1997). The site features seven distinct plant phasic communities beginning with a *Rhizophora mangle* mangrove
60 swamp on the coastal margins, which is succeeded by palm swamp dominated by *Raphia taedigera*, a mixed
61 forest stand, a monodominant *Camposperma panamensis* forest stand, and a *Myrica-Cyrilla* bog-plain (Phillips
62 et al., 1997). This vegetation gradient follows a pronounced decrease in nutrient availability from the margins to
63 the centre of the wetland (Sjögersten et al., 2011, Cheesman et al., 2012), and trends in microbial community
64 structure (Troxler et al., 2012).

65 Six peat samples were collected from six plots in the mixed forest stand (09° 18' 13.00"N, 82° 21' 13.80"W)
66 located approximately 600 m from the coast. Samples were collected from two points within each plot, located
67 no more than 1 m apart, under both *R. taedigera* and *C. panamensis* plants, from a depth of 10–20 cm using a
68 hand trowel to reduce the effect of inputs from recent litterfall and sample from a depth likely to receive regular
69 inputs of exudates. Previously, variation in peat properties between the same set of samples, including pH,
70 conductivity, redox potential, organic matter and gravimetric water content, was found to be low, with no
71 statistically significant differences (Girkin et al., 2018a). Samples were sealed in zip-lock bags and transported
72 to the Smithsonian Tropical Research Institute station in Bocas del Toro and refrigerated for four weeks 4 °C
73 prior to transportation to the University of Nottingham, UK. Samples from the two points were homogenised to
74 create a composite. Samples were not sieved but larger roots were removed by hand.

75

76 2.2. Experimental design

77 2.2.1. Root exudate compound selection

78 Root exudate compounds (RECs) were selected using data from a previously reported literature survey of
79 common sugars and organic acids from 33 tree species (Girkin et al., 2018a). The selected additions were
80 glucose, sucrose and fructose sugars, and acetate, formate and oxalate organic acids. Compounds were added at
81 three addition rates: of 0.1, 0.2 and 0.3 mg C g⁻¹ day⁻¹ (calculated using peat dry weight equivalent). These rates
82 were selected to match previously reported root exudate input rates and represent low, medium and high plant
83 photosynthetic activities in forest ecosystems, although no reported data was available specifically for tropical

84 forested peatlands (Grayston and Campbell, 1996, Baudoin et al., 2003, Shi et al., 2011, Basiliko et al., 2012).
85 All REC solutions were prepared by dissolving the sugar or organic acid in DI water and adjusting the pH to 5.5
86 using NaOH and HCl, to match *in situ* measurements, and prevent a reduction in pH on treatment addition
87 (Renella et al., 2006). Following preparation, REC solutions were sterilised by autoclaving and stored at 4 °C.

88

89 **2.3. Incubation**

90 Peat samples (7.5 g dry weight equivalent) were placed in 120 ml glass serum bottles (Kinesis, St. Neots, UK),
91 and saturated with DI water to give a total occupied volume of 40 ml, leaving 80 ml headspace. This approach
92 was adopted to simulate the water-saturated and anoxic conditions found at the site. Serum bottles were flushed
93 for two minutes with nitrogen to displace headspace gases, before sealing with a rubber septa (13 × 19 × 12 mm;
94 Rubber B.V., Hilversum, NL), and an aluminium crimp. Each of the 18 treatments and the control were
95 replicated six times, resulting in 114 replicates. Serum bottles were placed in a 28 °C temperature control room
96 for two weeks for acclimation prior to beginning the experiment. Serum bottles were subsequently opened,
97 flushed with nitrogen for two minutes, and then re-sealed. Headspace gas samples were collected after seven
98 days incubation, prior to the addition of REC solutions. REC solutions were added at a rate of 1 ml per day, over
99 14 days, with 1 ml autoclaved de-ionised water as a control, between days 8 and 22. Headspace gas samples
100 were collected on days 15 and 22 (during exudate addition) and on days 30, 38, 45 and 52. At the conclusion of
101 the experiment bottles were opened to characterise peat properties.

102 During headspace sampling, gas samples (5 ml) were extracted by syringe and analysed by gas chromatography
103 (GC-2014, Shimadzu UK LTD, Milton Keynes, UK). CO₂ and CH₄ concentrations were determined using a
104 single injection system, with a 1 ml sample loop that passed the gas sample using H₂ as carrier through a non-
105 polar methyl silicone capillary column (CBP1-W12-100, 0.53 mm I.D., 12 m, 5 mm; Shimadzu UK LTD,
106 Milton Keynes, UK). Thermal conductivity (TCD) and flame ionization (FID) detectors were used to measure
107 CO₂ and CH₄, respectively (Wright et al. 2011). Gas concentrations were adjusted for incubation temperature
108 (28 °C) and changes in pressure and headspace volume within the serum bottles, according to the ideal gas law.
109 The rate of potential gas production, expressed as μg CO₂ g⁻¹ hr⁻¹ or μg CH₄ g⁻¹ hr⁻¹, was calculated assuming a
110 linear accumulation rate of gases in the headspace (Hogg et al., 1992).

111

112 **2.4. Peat characterization**

113 Three composite sub-samples from each plot were used to characterize peat physiochemical properties prior to
114 beginning the incubation. Gravimetric water content was determined by analysis of the mass of water lost from
115 10 g wet weight peat oven dried at 105 °C for 24 hours. Organic matter content was determined as the mass lost
116 after ignition for 7 hours at 550 °C. Bulk density was measured by collecting 10 cm × 10 cm × 20 cm sections
117 from the peat surface, and oven drying at 105 °C for 24 hours. Total peat carbon (C) and total nitrogen (N) were
118 determined from 0.2 g dry, homogenised peat combusted using a total element analyser (Thermo Flash EA
119 1112, CE Instruments, Wigan, UK). Solution pH and redox potential were measured using a Hanna 209 meter
120 coupled with pH and redox probes at the conclusion of the experiment.

121

122 **2.5. Statistical analysis**

123 A repeated measurements ANOVA was used to assess differences in CO₂ and CH₄ fluxes between treatments,
124 using a combined variable comprising REC compound and concentration of addition as a fixed effect. This
125 approach prevented aliasing from the control treatment. Subsequently, a one-way ANOVA was used to assess
126 differences in cumulative CO₂ and CH₄ production, with a post-hoc Bonferroni test used to assess differences
127 between treatments. Differences in redox potential and pH were assessed using a one-way ANOVA. CO₂ and
128 CH₄ fluxes were log-transformed to meet test assumptions. Significance was assessed at $p < 0.05$. All statistical
129 analyses were carried out in Genstat v17.1, and figures were produced using Graphpad Prism v7.01.

130

131 **3. Results**

132 **3.1. Peat properties**

133 The peat was strongly acidic (pH 5.3) and waterlogged, with high gravimetric moisture (81.7%) and low bulk
134 density (0.1 g cm⁻³) (Table 1). Organic matter content was high (92.2%), as was total carbon and nitrogen, with
135 a C:N of 16.9. In general, peats showed limited variability in properties between replicates.

136

137 **3.2. Exudate influence on greenhouse gas fluxes**

138 All REC additions were associated with a significant increase in CO₂ fluxes ($F_{18,90} = 12.72$, $p < 0.001$, Fig. 1a-f),
139 with a significant increase in fluxes over time ($F_{6,570} = 1498.4$, $p < 0.001$). In addition, there was a significant
140 interaction between treatment and time ($F_{108,570} = 8.97$, $p < 0.001$). The greatest mean CO₂ flux was from the 0.2
141 mg C g⁻¹ oxalate addition (8.92 μg CO₂ g⁻¹ hr⁻¹). In general, increased exudate concentration yielded greater CO₂
142 fluxes. The exception was formate, for which the highest mean CO₂ flux occurred under the lowest exudate

143 concentrations, suggesting inhibition at higher concentrations. The most rapid increases in fluxes occurred with
144 0.3 mg sugar additions, but this effect was transitory, observable only for the duration of exudate addition. By
145 day 52 there were only limited differences in fluxes between concentrations. With organic acid additions, there
146 were fewer discernible differences in response among different concentrations. In general, the greatest increase
147 in fluxes occurred during the 14 day treatment period. Cumulative CO₂ production also differed significantly
148 between treatments ($F_{18,90} = 12.00$, $p < 0.001$, Fig. 3a). A post-hoc Bonferroni test indicated that oxalic
149 treatments were associated with the greatest cumulative fluxes.

150 With the exception of the 0.3 mg formate addition, all treatments significantly increased CH₄ fluxes compared
151 to the control ($F_{18,90} = 3.86$, $p < 0.001$, Fig. 2a-f), with a significant increase in fluxes over time ($F_{6,570} = 491.8$, p
152 < 0.001). In addition, there was a significant interaction between treatment and time ($F_{108,570} = 6.68$, $p < 0.001$).
153 Lower concentrations were generally associated with greater increases in CH₄ fluxes, an observation consistent
154 for all sugar treatments and formate addition (Fig. 3b). The 0.2 mg C g⁻¹ formate addition had a mild inhibitory
155 effect up to day 38 compared to the 0.1 mg C g⁻¹ addition, with a reduction of fluxes compared to the control of
156 up to 22% but by day 52, fluxes were 85% higher than the control. By comparison, the addition of 0.1 mg C g⁻¹
157 formate resulted in fluxes up to 190% higher than the control by day 52. Greatest CH₄ production was
158 consistently associated with acetate addition, with up to 426% increase in production relative to the control for
159 the 0.1 mg C g⁻¹ addition, 411% for 0.2 mg C g⁻¹, and 377% for 0.3 mg. Cumulative CH₄ fluxes were more
160 sensitive to the concentration of the REC addition than CO₂ fluxes, with reduced fluxes at higher concentrations
161 for all treatments, with the exception of acetate ($F_{18,90} = 4.52$, $p < 0.001$, Fig. 3b).

162

163 3.3. Exudate effects on peat properties

164 REC addition significantly altered peat pH, with the effect dependent on both concentration and the compound
165 added ($F_{18,87.5} = 92.1$, $p < 0.001$, Fig. 4a). Low concentration sugar additions (0.1 mg) reduced pH to 4.8 – 5.1.
166 High concentration additions (0.3 mg) caused a greater reduction in pH to 3.6. In contrast, organic acid additions
167 increased pH, with no significant effect of increased concentration on pH.

168 REC addition significantly affected redox potential, with extent of the response affected by both the type of
169 REC addition and concentration ($F_{18,88.3} = 152.84$, $p < 0.001$, Fig. 4b). All sugar additions increased redox
170 potential compared to the control, with more pronounced increases at higher concentrations. In contrast, all
171 organic acid additions significantly decreased redox potential, with the greatest decreases generally found at the

172 highest REC concentrations. The exception to this pattern was 0.2 mg C g⁻¹ addition of oxalate which resulted in
173 a slightly higher redox potential than 0.1 and 0.3 mg C g⁻¹ additions.

174

175 **4. Discussion**

176 We previously showed that the addition of RECs in combination increased net CO₂ and CH₄ fluxes more than
177 higher concentration additions comprising fewer individual components (Girkin et al., 2018a). For example, the
178 addition of 0.3 mg C g⁻¹ comprising three sugars and one organic acid added to an anoxic peat soil resulted in
179 lower cumulative fluxes than a 0.2 mg C g⁻¹ addition comprising four organic acids. In this study, we
180 demonstrate that low concentrations of specific RECs may have a disproportionately important effect on GHG
181 emissions, as higher REC concentrations were not necessarily associated with the greatest CO₂ and CH₄
182 production.

183 All sugar solution additions and oxalate additions increased CO₂ fluxes more rapidly than acetate and formate
184 additions, and were associated with greater cumulative production. Previous incubation experiments
185 demonstrated the rapid use of sugars by peat microbial communities (Jones & Murphy, 2007) and increased
186 activity of hydrolytic enzymes (Shi et al. 2011). Acetate, the most important substrate for methanogenesis, was
187 associated with the greatest CH₄ efflux, with increases in production occurring more rapidly than other
188 additions. Acetate has been estimated to contribute to up to two-thirds of net CH₄ production, with formate
189 recognised as the second most important substrate (Ferry, 1992, Fox & Comerford, 1990).

190 For all REC additions, CO₂ production increased more rapidly than CH₄ production, in keeping with previous
191 incubation studies of tropical peats (Avery et al., 2003, Galand et al., 2005), an effect driven by the preferential
192 depletion of a series of terminal electron acceptors during C mineralisation (Lipson et al., 2010). In four of six
193 additions, higher concentration additions yielded greater CO₂ production. CH₄ production was more dependent
194 on the type of addition than the concentration, but with some inhibition of fluxes at higher concentrations for
195 four of six treatments. In both cases, the extent of decomposition and net fluxes of both gases arising from labile
196 C additions may be constrained in part by nutrient availability (Hoyos-Santillan et al., 2018), and differences in
197 inherent organic matter properties (Upton et al., 2018).

198 Studies using ¹³C and ¹⁴C isotope methodology have demonstrated that labile C additions can significantly
199 enhance the decomposition of older, more recalcitrant organic matter in a process termed priming, and that the
200 effect is frequently determined by the chemical composition of the additions (Verma et al., 1995, Hamer and
201 Marschner, 2002). This has been speculated as being driven by a combination of the activation of specific

202 microbial groups and the behaviour of the individual organic molecules added. Conversely, it has been reported
203 that some additions, for example oxalate, can bind to lignin structures, reducing availability for enzyme activity
204 (Piccolo et al., 1996). Part of the difference in fluxes between organic acid treatments may therefore be due to
205 different rates of organic acid adsorption, which can reduce C mineralisation, rates of decomposition and overall
206 microbial growth (Lopez-Hernandez et al., 1986). Monovalent organic acids, including acetate and formate, are
207 more weakly adsorbed by soils compared to divalent organic acids such as oxalate (Jones et al. 2003), although
208 these processes can be very slow, occurring at the rate of hours to months (Van Hees et al., 2005). Microbial
209 uptake of low weight molecular compounds, including organic acids and sugars, is a much more rapid process
210 which occurs over several minutes (van Hees et al., 2005). A combination of differences in the relative
211 adsorption of organic acids versus microbial uptake likely explains the resulting differences in GHG fluxes
212 between REC addition types. It is also plausible that some parts of the microbial community may also be more
213 dependent on the specific exudates released by the plant species and therefore some of the differences in
214 response to contrasting REC additions may be because the community is not fully adapted for its decomposition
215 (DeAngelis et al., 2009, Schimel & Schaefer, 2012). Over time, changes in microbial community composition
216 may explain the increase in CH₄ production in oxalate treatments by day 56.

217 Organic acid additions have been reported to inhibit methanotroph activity under aerobic conditions (Wieczorek
218 et al., 2011), inhibit enzyme activities, and alter bacterial taxa diversity and abundance (Shi et al., 2011). High
219 concentrations of acetate can have an inhibitory effect at pH < 4.5 due to the protonated forms disturbing
220 microbial cell membranes (Russell, 1992). As higher concentrations of formate were only associated with
221 reduced CH₄ fluxes, and these treatments were associated with an increase in pH, it is possible that the
222 methanogenic community was particularly sensitive to this perturbation, although previous studies have
223 indicated that methanogen activity increases at higher pH (Ye et al. 2012). Autoclaving may have resulted in the
224 thermal decomposition of formate, resulting in carbon monoxide (CO) formation, which can inhibit
225 methanogenesis (Oelgeschläger & Rother, 2009). However, this process is unlikely to fully account for the
226 observed results, as CO toxicity would also have inhibited CO₂ production which did not differ significantly
227 between the three formate concentrations, or compared to high concentration oxalate additions. High formate
228 concentrations have been reported to inhibit acetoclastic methanogenesis (the dominant CH₄ production
229 pathway), which have resulted in reduced cumulative CH₄ production (Guyot, 1986, Guyot & Brauman, 1986).
230 Subsequently, the gradual consumption of formate may have resulted in a reduction in inhibition and account for
231 the increased CH₄ production for the 0.1 mg C g⁻¹ treatment between 45 and 52 days (Figure 2e).

232 All organic acid additions increased pH significantly compared to the control, whereas sugar additions
233 decreased pH. Microbial degradation of carboxylic acids consumes H^+ , liberating OH^- and CO_2 (Gramss et al.,
234 2003), while the utilisation of sugars generates H^+ (Srinivasan and Mahadevan, 2010). Increases in pH after
235 organic acid additions are associated with significant shifts in microbial communities (Shi et al., 2011) and
236 increases in CO_2 (Yan et al., 1996) and CH_4 production (Wang et al., 1993).

237 Redox potential increased with sugar addition, and decreased with organic acid addition. Addition of labile plant
238 residues can also reduce redox potential as high respiration depletes oxygen (Fig. 4a) (Flessa and Beese, 1995).
239 Changes in pH, redox potential and conductivity are closely coupled, because redox reactions frequently involve
240 the transfer of H^+ due to changes in the oxidative state of Fe, Mn or N (Husson, 2013). Combined, changes in
241 pH and redox potential can affect microbial community structure and activity may account for the inhibition of
242 GHG production at higher REC concentrations. Tropical peatland microbial communities are likely to be
243 relatively well-adapted to changing redox potential due to frequent fluctuations in water table height, altering
244 the balance between anoxic conditions favouring methanogens and CH_4 production, methanotrophs and CH_4
245 oxidation, and heterotrophic respiration (Tokarz & Urban, 2015).

246 *In situ*, root inputs of exudates contribute significantly to net GHG fluxes. For example, approximately two-
247 thirds of CO_2 emissions from the Changuinola mixed forest stand are root-derived, an estimate which includes
248 components from both root respiration and microbial use of exudates (Girkin et al., 2018b). This is particularly
249 important in the context of land use change in tropical peatlands, for example the expansion of plantation
250 agriculture in Southeast Asia. Malaysia alone has undergone a 150% increase in land area planted by oil palm,
251 with significant expansion onto peatlands (FAO, 2016, Pirker et al., 2016). While this changes peat physical
252 properties (Tonks et al. 2017), our results suggest that any changes in plant community composition that alter
253 root exudate profiles may result in substantial changes to GHG emissions. However, due to the sparsity of
254 studies assessing root exudate profiles of tropical plant species, particularly palms, the precise effect on GHG
255 fluxes remains to be elucidated. Climate change may also significantly affect *in situ* root exudation. Elevated
256 atmospheric CO_2 has been found to increase rates of root exudation in wetland ecosystems (Sanchez-Carrillo et
257 al., 2018). Increases in temperature have also been reported to increase rates of exudation in some tree species
258 (Uselman et al., 2000), and alter the composition of exudate profiles (Vančura, 1967, Badri & Vicanco 2009).

259 Our results demonstrate that the type and concentration of root exudates influence CO_2 and CH_4 production. For
260 CO_2 production, substrate concentration was the most important driver of fluxes over the short term, whereas for
261 CH_4 production the most critical driver is exudate type, with peat CH_4 fluxes most sensitive to acetate addition.

262 Moreover, there is an inverse relationship between REC addition concentration and CH₄ fluxes. These effects
263 are most likely driven by differing levels of adsorption and shifts in peat properties following addition. These
264 findings are particularly important in the context of understanding how plant inputs are able to regulate GHG
265 emissions from tropical peatlands, because any process which alters plant community composition may alter
266 root exudate profiles.

267

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274

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440 **Tables and figures**

441 Table 1: *In situ* site properties of the mixed forest stand on the Changuinola peat deposit. Means \pm 1 SEM.

442

443 Fig. 1: CO₂ flux derived from (a) fructose, (b) glucose and (c) sucrose, (d) acetate, (e) formate and (f) oxalate
444 addition at 0.1 – 0.3 mg g⁻¹ day⁻¹. Means \pm 1 SEM (n = 6). SEM not shown if smaller than symbol.

445

446 Fig. 2: CH₄ flux derived from (a) fructose, (b) glucose and (c) sucrose, (d) acetate, (e) formate and (f) oxalate
447 addition at 0.1 – 0.3 mg g⁻¹ day⁻¹. Means \pm 1 SEM (n = 6). SEM not shown if smaller than symbol.

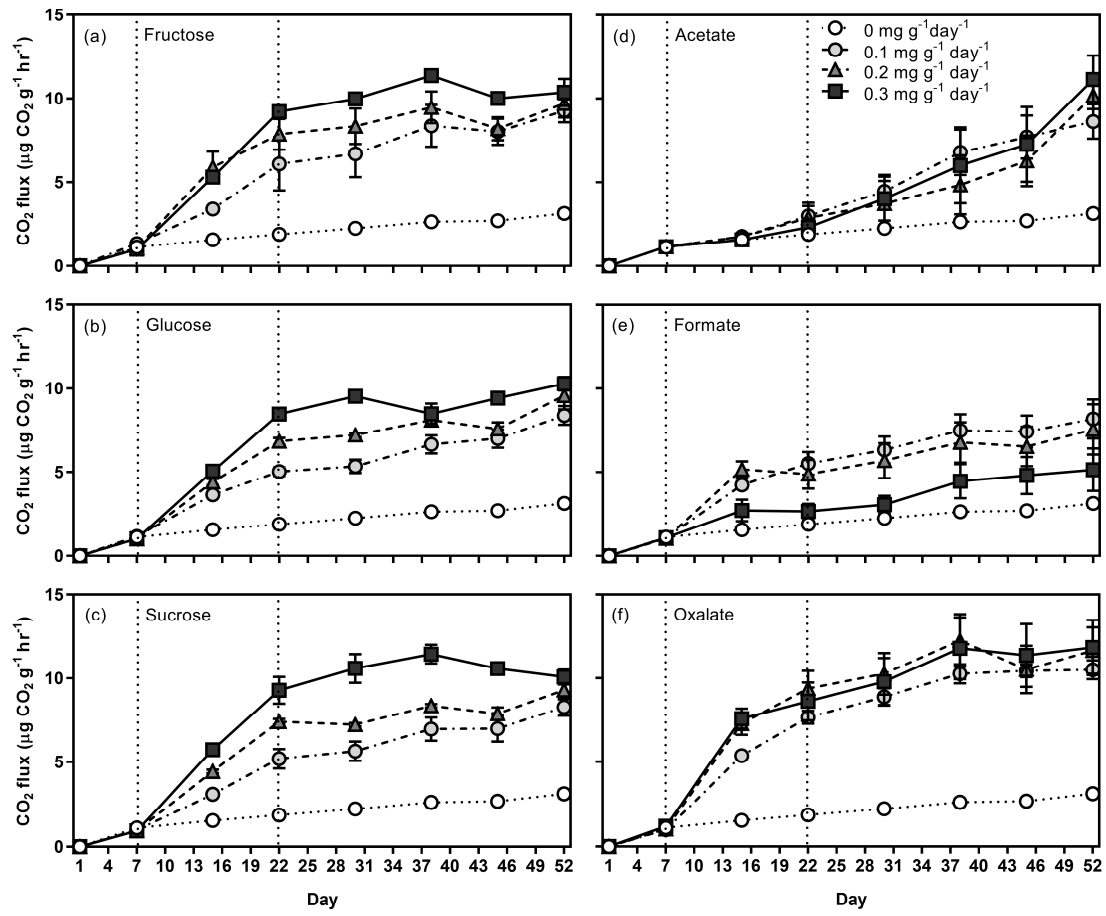
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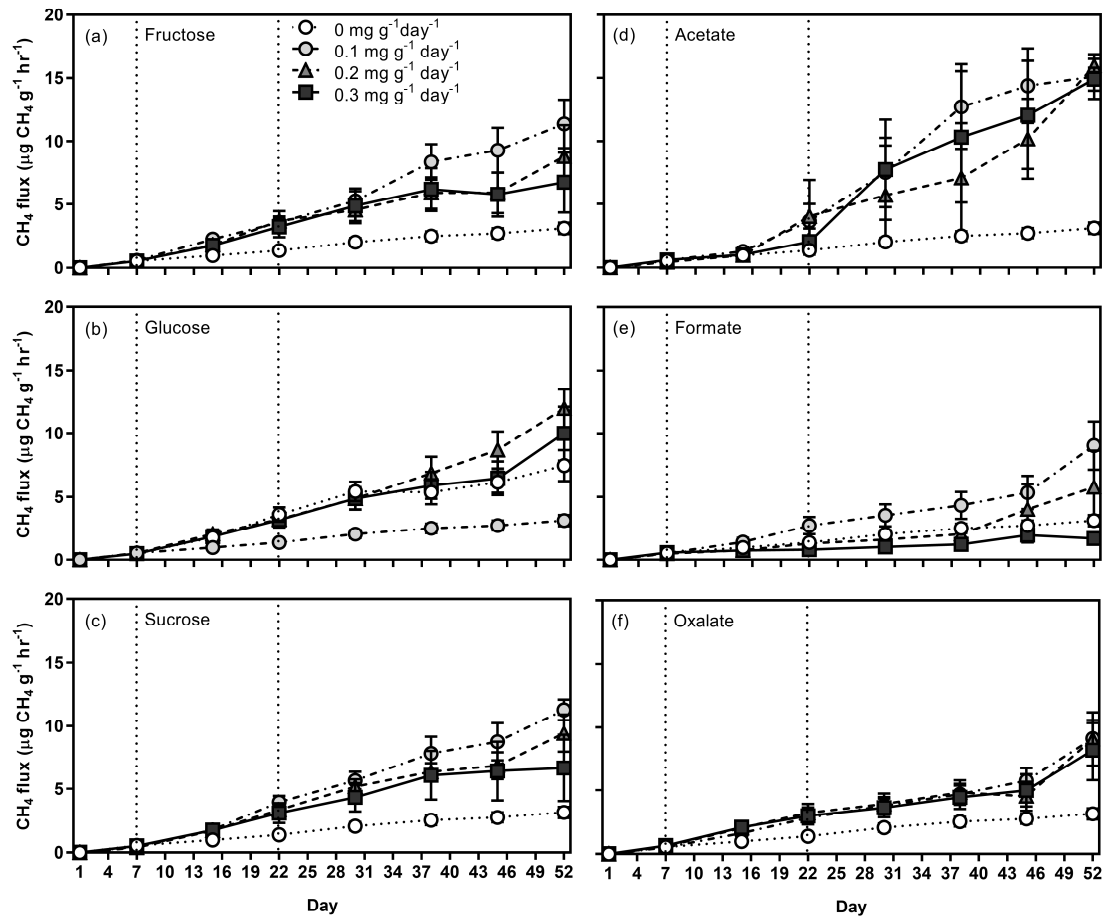
449 Fig. 3: Cumulative (a) CO₂ flux, (b) CH₄ flux derived from REC compound at addition rates of 0.1 – 0.3 mg g⁻¹
450 day⁻¹. Means \pm 1 SEM (n = 6). Letters indicate significant differences from a post-hoc Bonferroni test (p < 0.05)
451 for all compositions and concentrations.

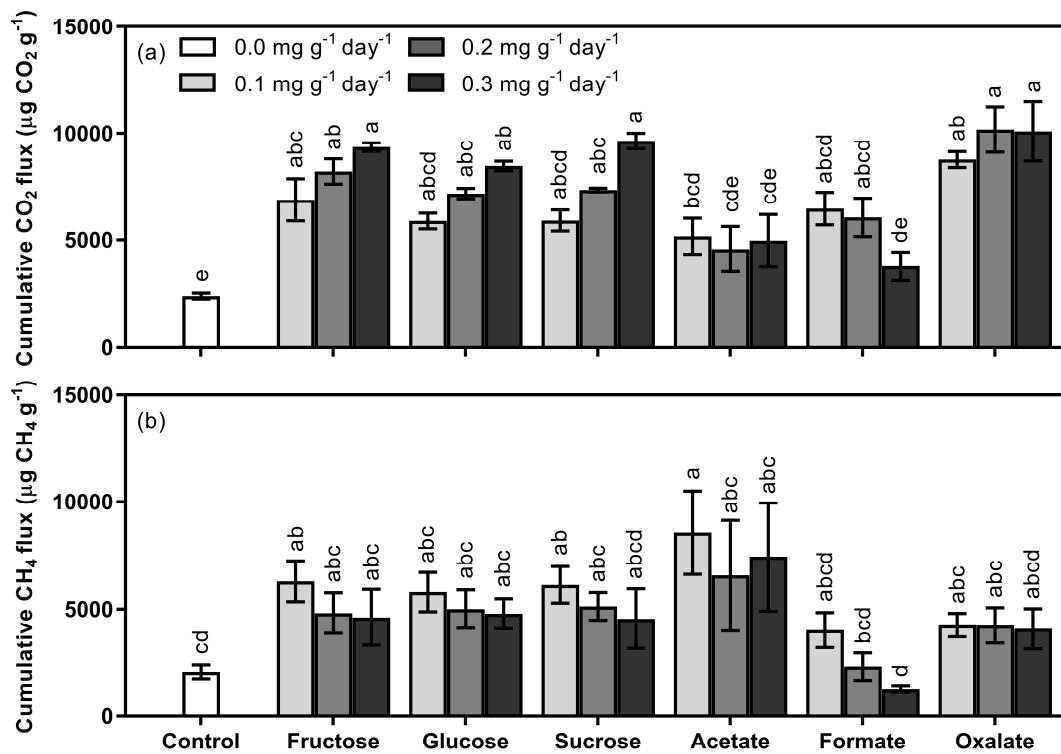
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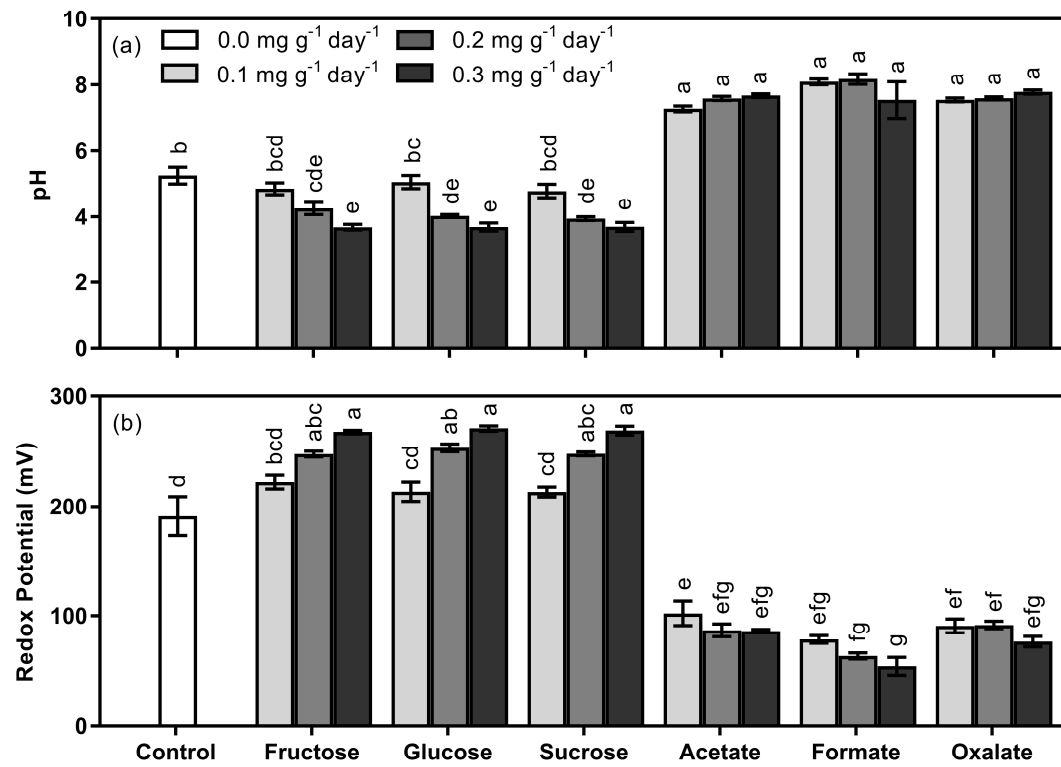
453 Fig. 4: Root exudate component influence on (a) pH, and (b) redox potential from addition rates of 0.1 – 0.3 mg
454 g⁻¹ day⁻¹. Means \pm 1 SEM (n = 6). Letters indicate significant differences from a post-hoc Bonferroni test (p <
455 0.05) for all compositions and concentrations.

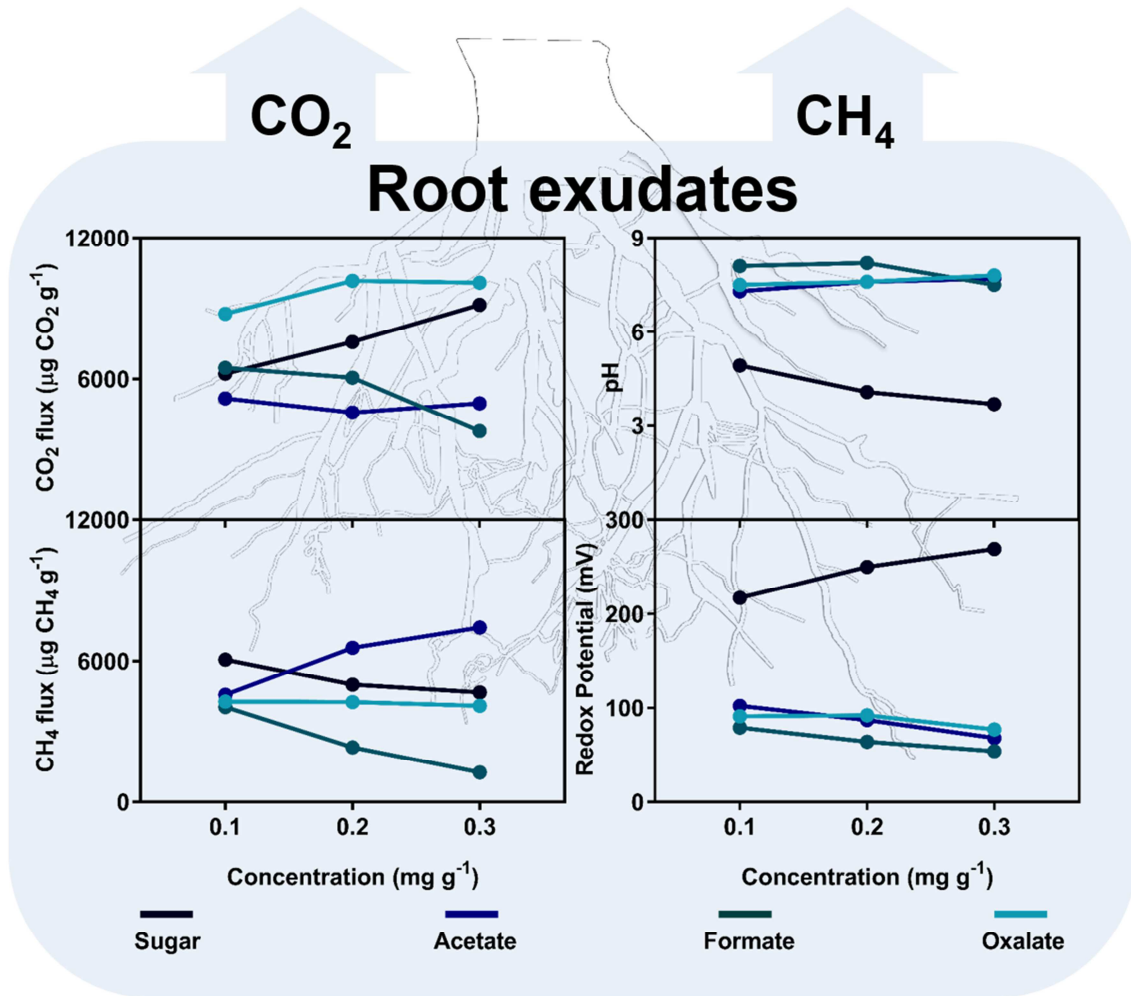
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Property	
Gravimetric water content (%)	81.7 ± 4.7
Organic matter content (%)	92.2 ± 1.7
Bulk density (g cm⁻³)	0.1 ± 0.0
pH	5.3 ± 0.1
C (%)	44.1 ± 1.2
N (%)	2.6 ± 0.1
C:N	16.9 ± 0.0

- CO₂ production increased at higher C input rates.
- CH₄ production was generally inhibited at higher C input rates.
- Acetate additions were associated with highest CH₄ production.
- Redox potential and pH showed concentration and composition dependent responses.

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