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1 **Rice rhizodeposition and carbon stabilisation in paddy soil is regulated via drying-**
2 **rewetting cycles and nitrogen fertilisation**

3

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25 **Abstract**

26 This study aimed to better understand the stabilisation of rice rhizodeposition in paddy soil
27 under the interactive effects of different N fertilization and water regimes. We continuously
28 labelled rice ('Zhongzao 39') with $^{13}\text{CO}_2$ under a combination of different water regimes
29 (alternating flooding-drying vs. continuous flooding) and N addition (250 mg N kg⁻¹ urea vs.
30 no addition, then followed ^{13}C incorporation into plant parts as well as soil fractions. N addition
31 increased rice shoot biomass, rhizodeposition, and formation of ^{13}C (new plant-derived C) in
32 the rhizosphere soils under both water regimes. By day 22, the interaction of alternating
33 flooding-drying and N fertilisation significantly increased shoot and root ^{13}C allocations by
34 17% and 22% respectively, over the continuous flooding condition. The interaction effect also
35 increased ^{13}C allocation to the rhizosphere soil by 46%. Alone, alternating water management
36 increased ^{13}C deposition by 43%. In contrast, N addition increased ^{13}C deposition in rhizosphere
37 soil macroaggregates under both water regimes, but did not increase macroaggregation itself.
38 N treatment also increased ^{13}C deposition and percentage in microaggregates, as well as in the
39 silt and clay-size fractions of the rhizosphere soil, a pattern that was higher under the alternating
40 condition. Overall, our data indicated that combined N application and a flooding-drying
41 treatment stabilised rhizodeposited C in soil more effectively than other tested conditions. Thus,
42 they are desirable practices for improving rice cropping, capable of reducing cost, increasing
43 water use efficiency, and raising C sequestration.

44 Key words: Paddy soils, ^{13}C continuous labelling, Carbon stabilisation, Root exudation,
45 Rhizodeposition, Recent assimilates.

46

47 **Introduction**

48 Numerous studies have investigated the allocation and stabilisation of OC
49 photosynthesized by upland crops (e.g., wheat, maize, ryegrass, and barley) (Liljeroth et al.
50 1990; Henry et al. 2007; Canarini and Dijkstra 2015; He et al., 2015; Mwafulirwa et al. 2016).
51 However, sufficient attention has not been paid to the dynamics of photosynthesized C in rice
52 paddy soil, especially considering the economic and ecological importance of rice (with a
53 projected 800 million tons required to meet the global demand in 2030) and thus the large global
54 coverage of paddy soil.

55 Depending on plant species, age, and prevailing environmental conditions,
56 rhizodeposition may release up to 40% of photosynthesized C (Lynch and Whipps 1990). The
57 released C serves as an energy source for soil microorganisms (Lynch and Whipps 1990; Ge et
58 al. 2012) to cycle soil organic matter (SOM) and nutrients (Mwafulirwa et al. 2016), and part
59 of rhizodeposition is respired as CO₂. A small portion of low-
60 molecular-weight compounds such as sugars and organic acids, released as exudates (Jones and
61 Darrah 1994) may be reabsorbed for plant cellular metabolism (Sherson et al. 2003). Thus, to
62 mitigate the global increase in atmospheric CO₂ concentrations, we must better understand the
63 management of SOC sources, pools, spatial distribution, and stabilisation processes (Miltner et
64 al. 2012).

65 The rice crop depends on water management and fertilizer N inputs; rice production
66 consumes an estimated 90% of the total irrigated water allocated to crops (Bhuiyan 1992).
67 However, regular irrigation requires enormous energy input, and supplying fresh water for
68 continuously flooded paddies is increasingly unsustainable due to competitive demands from
69 urban and industrial fronts (Bouman and Tuong 2001; Fan et al. 2012). Furthermore, a predicted
70 increase in droughts through many subtropical regions have heightened concern for more
71 efficient water management in rice cultivation (IPCC 2012). These concerns, in addition to

72 perceived benefits of improved yield and water-use efficiency, has caused the alternation
73 between flooding and drying periods to become popular in rice cultivation ([Belder et al. 2004](#);
74 [Xu et al., 2015](#)). Drying-rewetting cycles, however, have major implications on below-ground
75 plant-soil-microbe interactions, such as instantaneous C and N mineralization ([Austin et al.](#)
76 [2004](#); [Schimel et al. 2007](#)), as well as shifts in microbial use and stabilisation of rhizodeposited
77 nutrients ([Canarini and Dijkstra 2015](#)). Soil rewetting is also linked closely to N loss through
78 gaseous emission and leaching ([Austin et al. 2004](#)). Further, increased photosynthate
79 partitioning and allocation belowground have been reported in crested wheatgrass under water
80 stress ([Henry et al. 2007](#)) as well as in rice under drying-rewetting ([Tian et al. 2013a, 2013b](#)).
81 Despite these consequences, little is known about the combined effects of water management
82 and N fertilisation on the partitioning and allocation of rice photosynthates in above- and
83 belowground paddy soil systems.

84 Studies on the distribution of OC derived from rhizodeposits are crucial for sustainable
85 crop productivity because the spatial arrangement of associated soil particles determines SOC
86 bio-accessibility ([von Lützow et al. 2007](#)). Approximately 90% of surface SOC is associated
87 with aggregates ([Jastrow et al. 1996](#)), and 20–40% of this large subset is intra-microaggregate
88 SOC ([Carter 1996](#)). The formation of microaggregates through biotic glues and abiotic clay
89 flocculation ([Puget et al. 2000](#)) makes them more stable, with longer turnover than
90 macroaggregates formed under more transient biogenic processes. Hence, soil OC and N were
91 reported to increase initially in macroaggregates, followed by a gradual decrease and
92 preferential redistribution into microaggregates ([Tisdall and Oades 1982](#); [Six et al. 1998](#)). Yet
93 little is known about the distribution of rice-derived C across different aggregate fractions,
94 especially in relation to water management ([Tian et al. 2013b](#)) and N fertilisation.

95 This study aimed to investigate how water management (continuous versus alternating
96 flooding-drying) and N fertilisation could interact to affect the partitioning and stabilisation of
97 newly plant-derived C in the rice-soil system. Rice seedlings at the tillering stage were
98 continuously labelled with $^{13}\text{CO}_2$, and the distribution of newly plant-derived C in above- and
99 below-ground systems was investigated. We hypothesize that an alternating water regime and
100 N fertilisation will increase rhizodeposition via enhanced root activity compared with
101 continuous flooding. We also expect that the surge in microbial activities, and hence their
102 increased use of rhizodeposits under flooding-drying episodes, will reduce C stabilisation.
103 Finally, we hypothesize that N addition will increase rhizodeposition through enhancing
104 photosynthesis, and the associated larger input of available OC will increase macroaggregation
105 in rhizosphere soils under both water regimes.

106

107 **Materials and methods**

108 **Site description and basic soil characteristics**

109 Soil was collected from a rice field located at Changsha Research Station for Agricultural and
110 Environmental Monitoring (113°19'52"E, 28°33'04"N, 80ma.s.l.), in subtropical China. The
111 mean annual temperature is 17.5°C, yearly precipitation is 1300 mm, annual hours of sunshine
112 are 1663, and the frost-free period is up to 274 d. The soil type is a typical Stagnic Anthrosol
113 (Gong et al. 2009) developed from granitic red soil (Alisol). Soil properties were as follows:
114 pH 5.43 (1:2.5, soil/water ratio); organic C, 14.26 g kg⁻¹; total N, 1.45 g kg⁻¹; total P, 0.75 g kg⁻¹;
115 and cation exchange capacity (CEC), 7.71 cmol kg⁻¹. Particle-size analysis indicated a
116 composition of 28% sand (>50 µm), 66% silt (2–50 µm), and 6% clay (<2 µm).

117

118 **Experimental set-up**

119 The experiment was factorial and arranged in a completely randomised design, comprising two
120 main factors (water management and N fertiliser) at two levels each. Each of the four treatments
121 was replicated six times (3 replicates to be harvested at two sampling points). Soil samples
122 (water content, 14.8%) were collected from the plough layer (0–20 cm), sieved (<4 mm) to
123 remove coarse plant residues, and then air-dried.

124 Twenty-four pots (11 cm inner diameter and 20 cm height) were each filled with 1.26
125 kg (oven-dried basis) soil each. A rhizosphere bag (mesh 30 μm ; 3.5 cm \times 15 cm) each which
126 allows the passage of nutrients and water, but not root (Su and Zhu, 2008; Finzi et al., 2015)
127 was filled with 0.34 kg soil and buried in each pot. Two rice 2-line hybrid ('Zhongzao 39')
128 seedlings at the third tillering stage were transplanted into each bag on 29 June 2015. All pots
129 were sprayed with NaH_2PO_4 (20 mg P kg^{-1} soil) and KCl (80 mg K kg^{-1} soil), then divided
130 evenly into two water management conditions: continuous flooding ('Flooding') or alternating
131 flooding-drying ('Alternate'). At the start of both treatments, soil was covered with 2–3 cm of
132 distilled water. Under 'Alternate' treatment, pots were subsequently dried for 3–4 days until the
133 soil water content reached 70–75% of the water holding capacity (WHC), and then flooded
134 again. Three drying-rewetting cycles occurred in the entire experiment. Fertilising conditions
135 consisted of either no N addition (N0) or 250 mg N kg^{-1} (562.2 kg N ha^{-2}) as urea (N250). Each
136 condition comprised 12 pots (six each from 'Alternate' and 'Flooding'). An additional 24 pots
137 were used as the unlabelled controls. The unlabelled controls were references for determining
138 natural ^{13}C abundance and calculating ^{13}C atom percent excess. Controls were placed outside,
139 10–15 m away from labelled chambers, but given the same water and fertilizer treatments as
140 labelled groups.

141

142 **¹³CO₂ continuous labelling**

143 Rice plants were subjected to ¹³CO₂ continuous labelling in a climate-controlled, air-tight glass
144 chamber (80 × 250 × 120 cm height), placed in a rice field for exposure to natural sunlight. As
145 described by Ge et al. (2012, 2015), ¹³CO₂ was produced in the chamber through the reaction
146 of NaH¹³CO₃ (50 atom percent ¹³C, 1 mol L⁻¹) and H₂SO₄ (0.5 mol L⁻¹). The CO₂ concentrations
147 inside the chamber were maintained between 360 and 380 μL CO₂ L⁻¹ via further reactions or
148 gas-flow diversion through CO₂ traps (1 M NaOH solution) that absorbed excess gas.

149 Two fans continuously circulated air in the labelling chamber. Two
150 temperature/humidity sensors (SNT-96S, Qingdao, China) monitored conditions inside and
151 outside the chamber; to maintain chamber temperatures at 1°C lower than ambient temperatures,
152 a data-logger script activated air conditioning whenever the former was 1°C higher than the
153 latter.

154

155 **Sampling and harvesting**

156 Plants and soil were harvested 14 and 22 d after labelling began. Shoots were severed from the
157 roots at the stem base. Mesh with ingrowing roots was removed from the chambers and adhering
158 soil was gently detached via gentle agitation for 1 min in 0.01 M CaCl₂ (pH 6.2), followed by
159 thorough rinsing under running tap water. Soil inside and outside the mesh was considered
160 rhizosphere soil and bulk soil, respectively. Roots, shoots, and a soil sub-sample were weighed,
161 oven-dried to a constant weight at 60°C, pulverized, and ball-milled for TC, TN, and ¹³C
162 analyses. The remaining soil was stored at 4°C for aggregate fractionation and subsequent TC,
163 TN, and ¹³C determination.

164

165 **Soil aggregate separation**

166 Wet-sieving of air-dried soils has been used for aggregate separation in paddy soils (Pan et al.
167 2008). Due to high clay content that might make fresh soil too sticky for separation, we
168 separated following the method of Cambardella and Elliot (1993), as adapted by Six et al.
169 (1998). A 50-g subsample of air-dried soil was placed on a 250- μm sieve nested into a 53- μm
170 sieve. Both sieves were then placed in a bowl containing room-temperature deionized water,
171 submerging the soil for 5 min. To achieve separation, the sieves were manually agitated up-
172 and-down by 3 cm, for 50 times (2 min). Subsequently, stable macroaggregates ($>250 \mu\text{m}$) and
173 large microaggregates (250–53 μm) were gently backwashed off the sieves into pre-weighed
174 aluminium pans. Floating organic materials ($>250 \mu\text{m}$) were decanted and discarded. Water
175 plus soil that went through both sieves were considered the $<53 \mu\text{m}$ (silt and clay-size) fraction,
176 although this fraction could also consist of small microaggregates (Chenu and Plante 2006;
177 Virto et al. 2008). The suspension was decanted (leaving the sediment), centrifuged at 3000
178 rpm for 5 minutes, and the precipate was combined with the obtained sediment. The aggregates
179 were oven-dried (60°C), weighed, finely ground, and stored in air-tight tubes at room
180 temperature for TC, TN, and ^{13}C analyses.

181

182 **Analytical methods**

183 Soil physicochemical properties were determined using standard methods: pH with a pH meter
184 (Delta 320; Mettler-Toledo Instruments Co., Ltd., China) in 1:2.5 soil/water ratio; soil particle
185 size with a laser particle size analyser (Mastersizer 2000; Malvern Instruments Ltd., UK).

186 Dry shoots, roots, and soil samples were ground in a ball mill prior to analysis. The stable
187 C isotope ratio ($^{12}\text{C}/^{13}\text{C}$) and the total C and N content of all samples were measured with an

188 isotope ratio mass spectrometer (IRMS, MAT253; Thermo-Fisher Scientific, Waltham, MA,
189 USA), coupled with an elemental analyser (FLASH 2000; Thermo-Fisher Scientific, Waltham,
190 MA, USA). The $^{12}\text{C}/^{13}\text{C}$ ratio was expressed as parts per thousand relative to the international
191 standard, Peedee Belemnite (PDB), using delta units ($\delta\text{‰}$).

192

193 **Calculations and statistical analyses**

194 ^{13}C content ($^{13}\text{C}_{\text{sample}}$) (mg C pot^{-1} or mg C m^{-2}) was calculated using the following equation:

$$195 \quad ^{13}\text{C}_{\text{sample}} = [(\text{atomic } ^{13}\text{C}\%)_l - (\text{atomic } ^{13}\text{C}\%)_{nl}]_{\text{sample}} \times \text{TC}_{\text{sample}} / 100 \quad (1)$$

196 where subscripts 'l' and 'nl' are labelling and non-labelling, respectively; 'TC' is the total C
197 content in a sample.

198 The amount of ^{13}C in aggregates and free silt and clay-size fraction was calculated as
199 follows:

$$200 \quad ^{13}\text{C}_{\text{amount-fraction}} = ^{13}\text{C}_{\text{con-fraction}} \times M_{\text{fraction}} / 100 \quad (2)$$

201 where $^{13}\text{C}_{\text{amount-fraction}}$ is the C amount in aggregates (mg C kg^{-1} soil), $\text{C}_{\text{con-fraction}}$ is the organic C
202 concentration of aggregates (mg C kg^{-1} fraction), and M_{fraction} is the mass percentage of
203 aggregates in whole soil (%).

204 Because macroaggregates and microaggregates were similar in particle size distribution,
205 the organic C in aggregates was not corrected for sand content in any treatment.

206 The percentage of ^{13}C incorporation in shoots, roots, and soil on each sampling day was
207 calculated considering the total ^{13}C found in shoots, roots, and soil combined.

208 All data were expressed as the mean of three replicates \pm SE. Multivariate ANOVA with
209 Duncan tests was used to test differences in the measured variables among different treatments

210 at a 5% probability level. Statistical analyses were performed in SAS 9.1 for Windows (SAS
211 Institute Inc.).

212

213 **Results**

214 **Effect of water and N treatment on plant biomass, TC, and TN**

215 N (N250) application significantly increased shoot biomass ($\text{g}\cdot\text{m}^{-2}$; $p = 0.0001$, **Fig. 1a**) under
216 both ‘Alternate’ and ‘Flooding’ regimes. Under the former, shoot biomass reached its maximum
217 value on day 22, with a 49% increase over the control (N0) and 19% increase over ‘Flooding’.
218 The latter led to a 40% and 29% increase in shoot biomass from N0 on days 14 and 22,
219 respectively. The interaction of N application and water regime also increased root biomass by
220 76% under ‘Flooding’ compared with ‘Alternate’ (Fertiliser \times Water \times Time, $p = 0.037$, **Fig.**
221 **1a**). N application also reduced the root/shoot biomass ratio ($p = 0.0001$, **Fig. 1a**) on both
222 sampling dates and in both water regimes.

223 N addition increased shoot TC ($p = 0.0001$, **Fig. 1b**) on day 22 of ‘Alternate’ (by 52%),
224 as well as on days 14 (43%) and 22 (28%) of ‘Flooding’. The interaction of water, fertiliser,
225 and sampling day significantly affected shoot TC ($p = 0.005$, **Fig. 1b**). N application did not
226 significantly alter root TC under ‘Alternate’ irrigation, but reduced root TC by 24% ($p = 0.01$)
227 on day 22 under ‘Flooding’. N treatment thus decreased the root/shoot TC ratio ($p = 0.0001$,
228 **Fig. 1b**) across all sampling days and water regimes.

229 Shoot TN was not affected by N application on day 14. The interaction of fertiliser and
230 water management significantly increased shoot TN ($P = 0.02$, **Fig. 1c**) on day 22 of both water
231 regimes, but by 33% more ($p = 0.023$) in ‘Alternate’ than in ‘Flooding’. The interactions of
232 fertiliser and sampling date ($p = 0.0001$, **Fig. 1c**), as well as of water management and sampling

233 date ($p = 0.03$), significantly affected root TN. While N application decreased root TN on day
234 14 of both water regimes, an increase occurred on day 22, again with the change being more
235 pronounced under ‘Alternate’ than under ‘Flooding’ (a 43% difference). Further, N application
236 reduced the root/shoot N ratio across both sampling dates and water regimes ($P = 0.0001$, **Fig.**
237 **1c**).

238 In unfertilised soil, shoot C/N ratio increased from day 14 to 22 under both ‘Alternate’
239 and ‘Flooding’, whereas N treatment caused a corresponding decrease of 43% and 42% for the
240 two water managements, respectively. This interaction between fertiliser and sampling date on
241 C/N ratio was highly significant ($p = 0.0001$, **Fig. 1d**). Root C/N ratios exhibited similar
242 patterns. Under N addition, root C/N decreased from day 14 to 22 by 69% and 72% for
243 ‘Alternate’ and ‘Flooding’ water regimes, respectively, indicating a significant interactive
244 effect of fertiliser and sampling day ($p = 0.003$, **Fig. 1d**). The shoot and root ratios under
245 ‘Alternate’ were lower by 7% ($p = 0.03$) and 13% ($P = 0.007$), respectively, than under
246 ‘Flooding’.

247

248 **^{13}C amount (mg C pot^{-1}) and percentage (%) in the rice-soil system**

249 Although N addition increased shoot ^{13}C amount under both ‘Alternate’ and ‘Flooding’
250 compared with the control, a similar increase was not observed in roots (**Fig. 2a**). The
251 interaction of fertiliser \times sampling date was significant ($p = 0.0001$, **Fig. 2a**); shoot ^{13}C increased
252 by 40% and 98% on days 14 and 22 under ‘Alternate’, but by 53% and 32% under ‘Flooding’.
253 Hence, ‘Alternate’ resulted in a 17% increase over ‘Flooding’ on day 22 (Fertiliser \times Water \times
254 Time, $p = 0.001$, **Fig. 2a**).

255 Root ^{13}C increase between sampling days (14 and 22) was greater under ‘Alternate’ than
256 ‘Flooding’; N addition did not significantly affect this pattern. N addition increased ($p = 0.0001$,
257 Fig. 2a) rhizosphere soil ^{13}C by 174% and 104% on days 14 and 22 under ‘Alternate’,
258 respectively, and by 65% and 95% under ‘Flooding’. The difference in rhizosphere soil ^{13}C
259 between the water regimes on day 22 was significant (Water, $p = 0.02$, Fig. 2a). Neither N
260 addition nor water management significantly affected ^{13}C incorporation into bulk soil. Notably,
261 the ^{13}C content (mg C kg^{-1} soil) in rhizosphere soil was 3 to 21 times higher than in the bulk
262 soil, depending on sampling day, N application, and water treatment (Table S1).

263 Compared with the control, N addition significantly increased the percentage of total
264 ^{13}C allocated to the shoot ($p = 0.0001$, Fig. 2b), but only on day 14 of ‘Alternate’ (by 29%) and
265 day 22 of ‘Flooding’ (by 17%). N-induced changes in shoot ^{13}C percentage across sampling
266 dates was only significant under ‘Flooding’ (a 28% increase, $p = 0.002$). A significant
267 interaction of water regime, fertiliser, and sampling date ($p = 0.02$, Fig. 2b) led to a 15%
268 increase in ^{13}C percentage on day 14 of ‘Alternate’ compared with ‘Flooding’, a pattern that
269 reversed on day 22 with ‘Flooding’ increasing by 19% over ‘Alternate’.

270 At N addition (Fertiliser \times Water \times Time, $p = 0.001$, Fig. 2b), root ^{13}C percentage
271 increased marginally from day 14 to 22 of ‘Alternate’, but decreased over the same period of
272 ‘Flooding’. Compared with the control, N addition significantly increased ($p = 0.04$, Fig. 2b)
273 ^{13}C percentage in rhizosphere soil on day 14 of both water regimes (by 114% under ‘Alternate’
274 and 95% under ‘Flooding’). The total percentage on day 14 in bulk soil+rhizosphere soil was
275 17.7% (Alternate) and 11.8% (Flooding), respectively representing 54.8% and 29.4% of the
276 belowground percentage. Neither water nor fertiliser regimes affected ^{13}C percentage in bulk
277 soil.

278

279 **Mass percentage of aggregates (%), SOC content (g C kg⁻¹ soil), and ¹³C amount (mg C**
280 **pot⁻¹) and percentage (%) in aggregate fractions**

281 Overall, aggregate percentage and SOC distribution across all treatments and sampling dates
282 were greatest in the silt and clay-size fraction, followed by microaggregates, then by
283 macroaggregates (Fig. S1a-d). Macroaggregates were more prominent in bulk soil compared
284 with rhizosphere soil, regardless of N addition or water management (Fig. S1a & c). In contrast,
285 microaggregate distribution was unaffected by any factor, including the rhizosphere, whereas
286 the silt and clay-size fraction was higher in rhizosphere soil than in bulk soil. The SOC
287 distribution across the fractions mirrored aggregate distribution, being higher ($p < 0.01$) in
288 macroaggregates of bulk soil than of rhizosphere soil under both water regimes (Fig. S1b & d).

289 N application significantly increased ($p = 0.04$, Fig. 3a) ¹³C in rhizosphere soil
290 macroaggregates on day 14 of both water regimes. Approximately 2.1 and 1.7 mg ¹³C pot⁻¹
291 were allocated to macroaggregates under ‘Alternate’ and ‘Flooding’, respectively, reflecting a
292 347% and 254% increase from the control. In contrast, by day 22, N application only increased
293 macroaggregate ¹³C in rhizosphere soil (53 mg C pot⁻¹; $p = 0.002$) under ‘Alternate’ (97%
294 increase over the control), compared with ‘Flooding’ (43% increase over the control) (Water ×
295 Rhizosphere, $p = 0.01$, Fig. 3c).

296 Compared with bulk soil, microaggregate ¹³C-SOC was slightly higher in rhizosphere
297 soil on day 14 of all treatments (Fig. 3a). By day 22, only ‘Alternate’ rhizosphere soil exhibited
298 significantly higher microaggregate ¹³C-SOC (62% increase over the control) under N
299 application (Fig. 3c). Similarly, ¹³C-SOC was significantly higher in the silt and clay-size
300 fraction of rhizosphere soil (Fertiliser × Rhizosphere, $p = 0.005$, Fig. 3a) across all N and water

301 treatments. However, by day 22, only rhizosphere soil in 'Alternate' showed significantly
302 higher ^{13}C -SOC (157% increase over the control) under N application.

303 The percentage of ^{13}C -SOC in aggregate fractions exhibited similar patterns as the ^{13}C -
304 SOC amount, with higher values of both in the macroaggregate fraction, followed by the silt
305 and clay-size fraction, and then the microaggregate fraction (Fig.3b & d). On day 14 of both
306 water regimes, N application significantly heightened macroaggregate ^{13}C -SOC percentage in
307 rhizosphere soil (310% and 339% increases over N0 in 'Alternate' and 'Flooding', respectively;
308 $p < 0.0001$, Fig. 3b), although the change was more dramatic in 'Alternate' (80% increase over
309 'Flooding'; $p = 0.02$, Fig. 3b). By day 22, a significant increase in percentage was only observed
310 under 'Alternate' (47% increase over N0 and 69% increase over 'Flooding'; Water \times
311 Rhizosphere, $p = 0.004$, Fig. 3d).

312 Regardless of fertilisation or water regime, microaggregate ^{13}C -SOC percentage was
313 greater on day 14 in rhizosphere soil (0.73–1.09%) than in bulk soil (0.20–0.47%). In contrast,
314 N application raised silt and clay-size fraction ^{13}C -SOC percentage in rhizosphere soil over bulk
315 soil ($p < 0.01$) across both sampling dates and water regimes (Fig. a & d). Further, positive
316 correlations ($R^2 = 0.33$ – 0.39 , $p < 0.001$, Fig. 4) were found between root biomass and ^{13}C -SOC
317 (in aggregate fractions and total soil).

318

319 Discussion

320 Effects of drying-rewetting cycles and N fertilisation on biomass, TC and TN in rice

321 Both individual and interaction effects of water management and N fertiliser application
322 significantly increased rice biomass, as well as TC and TN content. These increases were
323 greater in the shoot and especially, under flooding-drying water management.

324 While N application generally increased biomass over time, the effect of different water
325 regimes altered that increase. Continuous flooding initially (day 14) caused higher shoot and
326 root biomass than the alternating condition, but by day 22, this pattern was reversed. Several
327 possible explanations can explain these results. First, reduced translocation below-ground and
328 rhizodeposition under flooded conditions can leave more materials for growth in shoots and
329 roots ([Schumacher and Smucker 1985](#)), resulting in the initially higher biomass under
330 continuous flooding. However, as time passed, less efficient nutrient use under flooding could
331 lead to relative biomass reduction compared with the alternating system. Efficient N use in
332 plants is linked to enhanced photosynthetic capability through increasing chlorophyll and
333 Rubisco activity ([Wang et al. 2012](#); [Lin et al. 2013](#)), both of which are involved in
334 photosynthesis. The growth-promoting activity (on plant development and improved nutrient
335 availability) of some rhizobacteria could also be more favoured under alternate water regime,
336 leading to higher shoot and root biomass ([Pii et al. 2015](#)).

337 Regardless of the water regime, we noticed that the root/shoot biomass ratio decreased
338 with N addition, suggesting that shoots respond better to N. The decrease of root system in the
339 presence of N and its increase under N limitations has been well established ([Voisin et al. 2002](#);
340 [Shangguan et al. 2004](#)). In the present study, continued growth then led to even higher N
341 demand, explaining the significant increase in shoot and root TN at the second sampling date
342 for both water regimes; the more pronounced effect in ‘Alternate’ conformed with our
343 observations of late biomass increase under this water regime ([Ye et al. 2013](#)). Patterns in C/N
344 ratios (initially high, followed by subsequent decrease) also support a gradual increase in N
345 uptake. Again, this change was more obvious under the ‘Alternate’ water management,
346 implying better nutrient uptake.

347

348 **Effects of drying-rewetting cycles and N fertilisation on recent photoassimilate (¹³C)**
349 **deposition (mg C pot⁻¹) in the rice-soil system**

350 While ¹³C assimilation rose in response to shoot biomass increase under both water regimes,
351 the effect was much stronger under alternating than continuous flooding. The relative decreased
352 allocation of recent photoassimilate to the root under ‘Flooding’ compared with ‘Alternate’
353 could be due to prolonged anaerobic conditions that reduced root respiration, and hence, the
354 demand and shoot-to-root translocation of recent photoassimilate ([Schumacher and Smucker](#)
355 [1985](#)).

356 The importance of roots is clearly demonstrated by the correlation between root biomass
357 and recent photoassimilates in soil aggregates. The transfer of recent assimilates from
358 rhizosphere soil to bulk soil may be possible with help of fungi hyphae, which are able to
359 penetrate fine pores such as those of the rhizosphere bags ([Oades 1984](#)). Substantial fungal
360 growth even under the continuous flooding system could be possible through diffusion of
361 atmospheric oxygen through rice aerenchyma to the root system, which could provide some
362 pockets of aerobiosis ([Yuan et al. 2016](#)).

363 Looking within water regimes, however, translocation and rhizodeposition were superior under
364 flooding-drying compared with continuous flooding. Rhizodeposition was thus considerably
365 increased through the combination of N addition with the alternating water regime, supporting
366 our first hypothesis.

367 Besides greater root biomass, N-induced increases to shoot biomass and reduced N loss
368 under the ‘Alternate’ water regime could also improve assimilate translocation to the root. For
369 example, [Liljeroth et al. \(1990\)](#) reported increases in wheat root exudates with higher N
370 availability, a pattern attributable to the enhanced photosynthesis of high-N plants ([Lin et al.](#)

371 2013) and the consequent increase in overall exudate production. The oxic environment
372 provided by the drying portion of the ‘Alternate’ treatment may enhance root growth and
373 activity as well, improving exudation and nutrient uptake (Zhang et al. 2009; Mishra and
374 Salokhe 2011). Therefore, a positive correlation typically exists between increased exudates
375 and root tip number (Thornton et al. 2004; Pausch and Kuzyakov 2011). In addition, Zhang et
376 al. (2009) observed that while rice photosynthetic rate was not significantly reduced during the
377 drying period of an alternating water regime, re-watering significantly increased the rate. The
378 decrease and suppression, respectively, of root respiration (Schumacher and Smucker 1985)
379 and growth (Tian et al. 2013a & b; Zhu et al. 2016) under anaerobic condition such as that of
380 continuous flooding have been well documented. Finally, high shoot respiration under
381 ‘flooding’ (Swinnen et al. 1994; Kuzyakov et al. 2001) may reduce available shoot C for
382 subsequent transfer belowground.

383 The percentage of recent photoassimilates in shoots and roots differed noticeably with
384 N treatment under the two water regimes. In the first sampling of the ‘Alternate’ management,
385 the shoot percentage of recent photoassimilate differed significantly from control, whereas the
386 root percentage was not significant. Both the shoot and the root percentage of the recent
387 photoassimilates, however, slightly increased until the second sampling day. In contrast,
388 continuous flooding caused an increase in the shoot percentage of recent photoassimilates from
389 the first to the second sampling date, while the root percentage decreased (Fig. 2b). These
390 outcomes are attributed to continuous root-oriented translocation of recent photoassimilate and
391 rhizodeposition (Tian et al. 2013b) under the alternating flooding-drying regime, versus
392 reduced translocation and rhizodeposition through prolonged anaerobiosis (Schumacher and
393 Smucker 1985; Henry et al. 2007) under continuous flooding.

394 The percentage of recent photoassimilates found in soil was higher under ‘Alternate’
395 (17.7%) than under ‘Flooding’. This outcome was likely due to improved root activity for
396 nutrient uptake (Mishra and Salokhe 2011), more efficient nutrient use (Lin et al. 2013),
397 increased biomass, and better translocation of photoassimilates below-ground (Tian et al.
398 2013b) under the ‘Alternate’ regime. We also noted that our values were generally higher than
399 previous findings. According to Kuzyakov and Domanski (2000) and Tian et al. (2013b), 5–
400 10% of the net photosynthesized C allocated to roots can be recovered from the soil during
401 plant vegetative growth. The use of continuous labelling rather than the pulse labelling common
402 to previous studies could have been a factor in our higher percentage. Continuous labelling with
403 ¹⁴C can more quantitatively estimate rhizodeposited C (Wichern et al. 2011), when compared
404 short-term pulse labelling.

405

406 **Impact of drying-rewetting cycles and nitrogen fertilisation on recent assimilate (¹³C)**
407 **allocation to aggregate fractions**

408 We observed reduced macroaggregation in rhizosphere soil versus bulk soil irrespective of N
409 or water regime (Fig. S1a and c), in contrast to our hypothesis of enhanced rhizosphere soil
410 macroaggregation. This outcome could result from the destabilising effects of growing roots on
411 SOM decomposition, due to physical root-soil interactions (Huck et al. 1970). Growing roots
412 prefer wider soil pores than their own diameters (Russel 1977). Under less ideal conditions,
413 roots will displace soil particles (Whiteley and Dexter 1984; Helal and Sauerbeck 1989) while
414 enlarging narrow pores, thus crushing some soil aggregates. Although N addition improved
415 macroaggregate SOC concentration in the rhizosphere soil, we generally observed closer
416 associations between SOC and the other two fractions (silt-and-clay and microaggregates). This

417 higher SOC content under the finer fractions could be explained by the faster turnover rate of
418 macroaggregate-associated SOC (Tisdall and Oades 1982; Baldock and Skjemstad 2000),
419 especially because the seasonal puddling of paddy soils (in preparation for rice cultivation)
420 tends to reduce macroaggregate portions. Our results support previous work showing that N
421 addition may reduce native SOC decomposition (Liljeroth et al. 1990). In this study, more
422 native SOC was stabilised and protected against microbial decomposition through association
423 with the silt and clay-size fraction.

424 Under 'Alternate', N addition increased recent assimilate deposition and percentage in
425 all rhizosphere soil aggregate fractions compared with 'Flooding', indicating enhanced
426 rhizodeposition and stabilisation of recent assimilates. This finding did not support our second
427 hypothesis, but corroborated some previous data showed that ^{14}C incorporation into
428 macroaggregates increased with time in the non-flooded treatment versus the flooded treatment
429 (Tian et al. 2013b). Both N addition (Liljeroth et al. 1990) and non-flooded conditions (Tian et
430 al. 2013b) increase photosynthesized C rhizodeposition. The former may exert such an effect
431 because high soil N enhances photosynthetic capability (Lin et al. 2013) and reduces the
432 competition between roots and microorganisms for exudates (Ge et al. 2015). The latter may be
433 effective because drying conditions improve root activity and architecture (Mishra and Salokhe
434 2011; Thakur et al. 2011).

435 Though N fertilisation affects the allocation of recent assimilates, roots exert a strong
436 influence on soil C dynamics irrespective of N, as evidenced by the higher assimilate percentage
437 in rhizosphere soil over bulk soil (microaggregates and silt and clay-size fractions) under both
438 water regimes. A larger portion of recent photoassmilates was deposited into macroaggregates,
439 corroborating previous work that newly plant-derived C is initially incorporated into this
440 fraction (Jastrow 1996; Tian et al. 2013b; Six et al. 2000).

441 Subsequently, we observed higher recent-assimilate incorporation into the silt and clay-
442 size than the microaggregate fraction. This result contradicts the concept of aggregate hierarchy
443 (Tisdall and Oades 1982; Six et al. 1999), where the photoassimilate content is expected to
444 decrease with decreasing aggregate size. This deviation from expected outcomes may be caused
445 by two factors: 1) the silt and clay-size fraction exhibited the highest weight percentage of all
446 fractions in this study, and 2) silt and particularly clay both have high surface areas for increased
447 fresh C absorption (Sposito et al. 1999). Moreover, intra-microaggregate pores are small and
448 new C may not be able to easily diffuse through them. Therefore, the effective surface area of
449 microaggregates is smaller, and this fraction could also have slower turnover rates than both
450 macroaggregate-linked OC (Puget et al. 2000) and the silt and clay-size fraction.

451

452 **Conclusions**

453 The results of our study showed that the interactive effects of water regimes and N fertilisation
454 increased rice shoot biomass, as well as the allocation and stabilisation of newly the plant-
455 derived C in the rice-soil system. Moreover, N application was more effective in the alternating
456 flooding-drying treatment than in continuous flooding, causing a larger increase to recent
457 assimilate deposition in rhizosphere soil macroaggregates, microaggregates, and silt and clay-
458 size fractions. Thus, combining N application with a drying-rewetting water management
459 stabilized rhizodeposited C in soil more effectively than other tested conditions. Hence, in
460 addition to benefits such as cost reduction, water use efficiency, and yield increase, the positive
461 impact on C sequestration makes this combined management system desirable for rice
462 cropping. This study for the first time investigated rice C rhizodeposition and stabilization in
463 paddy soil under combined effect N addition and water regimes, and thus established the

464 superior effect of drying-rewetting water regime and N addition. However, because we air-dried
465 the soils prior to wet-sieving separa, future research study needs to look into the suitability of
466 this method for aggregate separation in paddy soils. This is more important because the method
467 has been mostly used for aerobic soils.

468

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667

668

669 **Figure captions**

670 **Fig. 1** Effects of drying-rewetting cycles and N fertilisation on rice shoot and root biomass (dry
671 weight) (a), C, and N contents (b, c), as well as plant (root and shoot) C/N ratio (d) at days 14
672 and 22 of the ¹³C continuous labelling experiment. Alternate: alternating flooding-drying water
673 management; Flooding: continuous flooding; No: no N fertiliser application; N250: urea at 250
674 mg N kg⁻¹ soil; D14: sampling on day 14 of continuous labelling; D22: sampling on day 22 of
675 continuous labelling. Error bars represent one standard error of the mean (n = 3). Different
676 lower- and uppercase letters respectively indicate significant differences (p < 0.05) of the shoot
677 and root parameters, as well as their root/shoot ratio across treatments

678

679 **Fig. 2** Effects of drying-rewetting cycles and N fertiliser application on ¹³C content (a) and
680 percentage (b) in shoot, root, rhizosphere soil (RS), and bulk soil (BS) at days 14 and 22 of the
681 ¹³C-continuous labelling experiment. Alternate: alternating flooding-drying water
682 management; Flooding: continuous flooding; No: no N fertiliser application; N250: urea at 250
683 mg N kg⁻¹ soil; D14: sampling on day 14 of continuous labelling; D22: sampling on day 22 of
684 continuous labelling. Error bars represent one standard error of the mean (n = 3). Different
685 lowercase letters indicate significant differences (p < 0.05) of the measured shoot, root, RS
686 (Rhizosphere soil), or BS (bulk soil) parameter across treatments; curved arrows link the letters
687 to the corresponding sections of the bars they represent

688

689 **Fig. 3** Effects of drying-rewetting cycles and N fertiliser application on ¹³C-SOC and
690 percentage, respectively, in rhizosphere-soil and bulk-soil aggregate fractions at day 14 (a & b)
691 and 22 (b & c) of the ¹³C-continuous labelling experiment. Alternate: alternating flooding-

692 drying water management; Flooding: continuous flooding; No: no N fertiliser application;
693 N250: urea at 250 mg N kg⁻¹ soil; RS: rhizosphere soil; BS: bulk soil. Error bars represent one
694 standard error of the mean (n = 3). Different lowercase letters indicate significant differences
695 (P < 0.05) of the parameter measured in soil aggregate fractions (>250, 250–53, or <53 μm)
696 across treatments

697

698 **Fig. 4** Correlations between root biomass and ¹³C-SOC content in soil aggregate fractions,
699 resulting from a ¹³C-continuous labelling experiment. All regression lines are significant at p <
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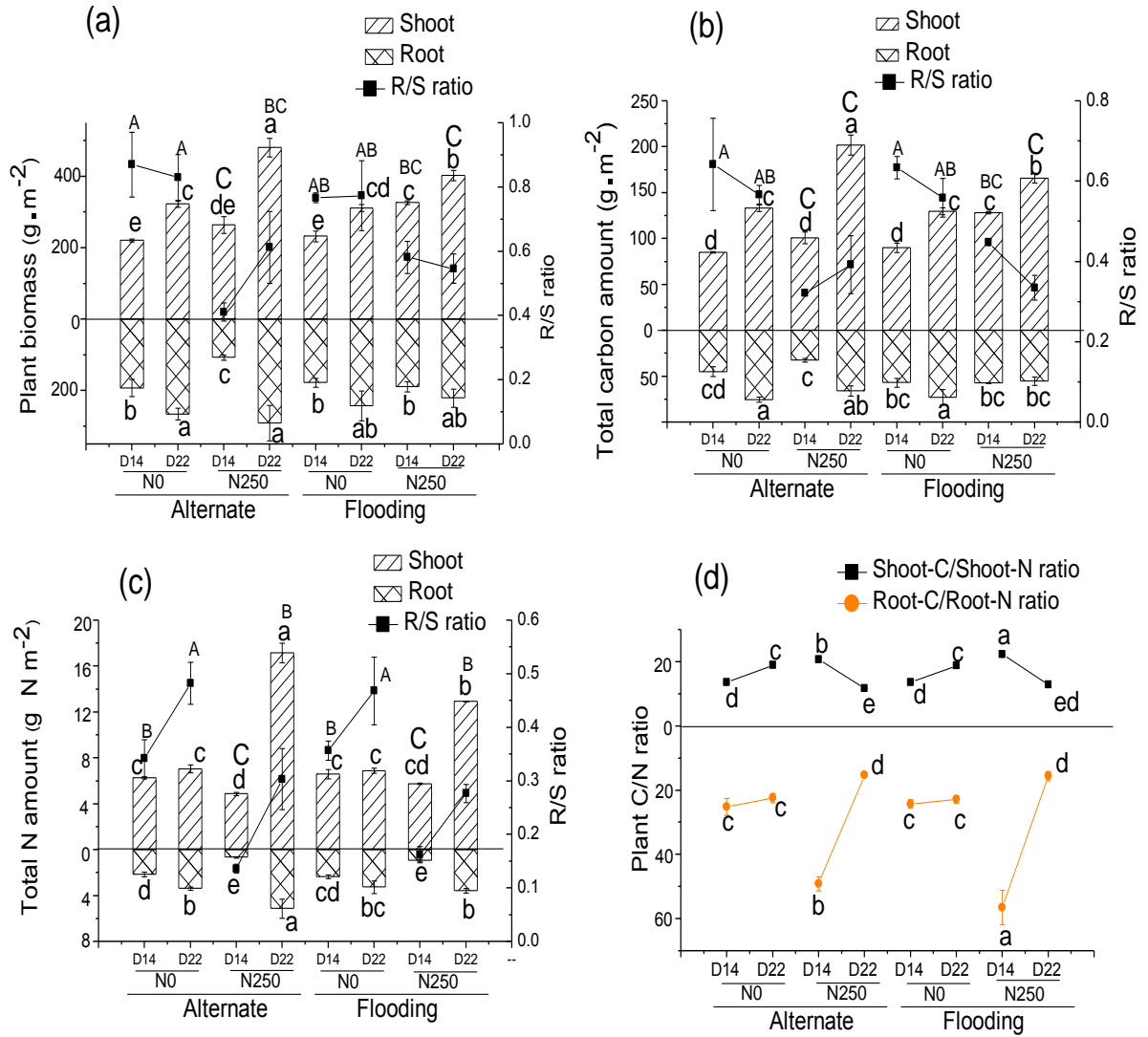
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706 **Figures**

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708 **Fig. 1**

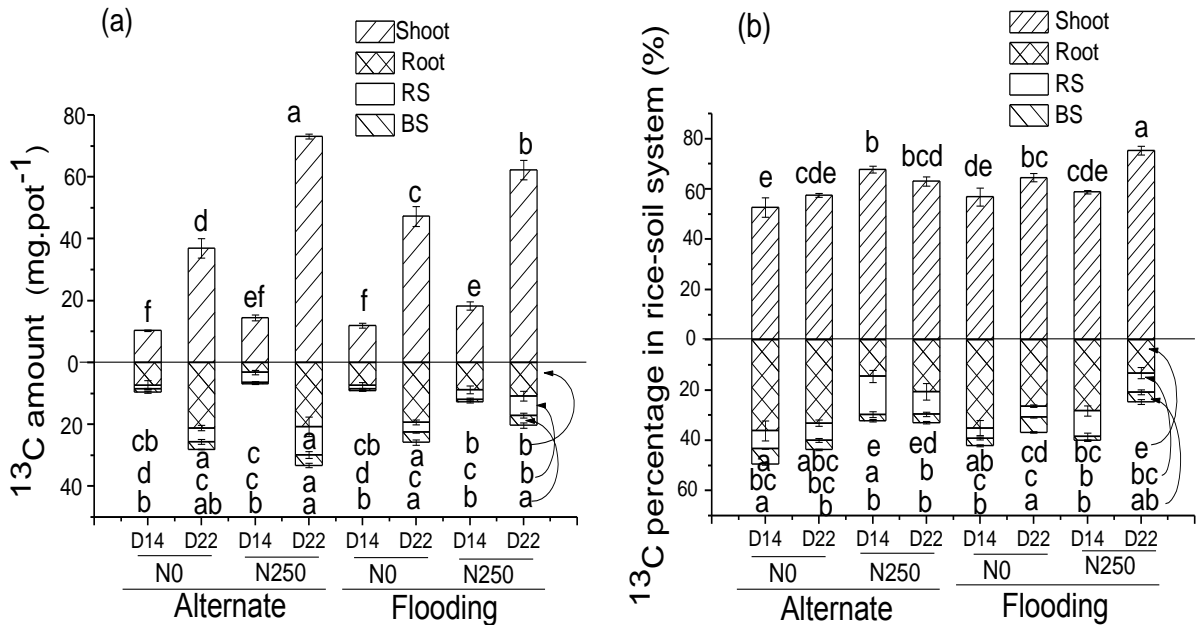


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711 **Fig. 2**

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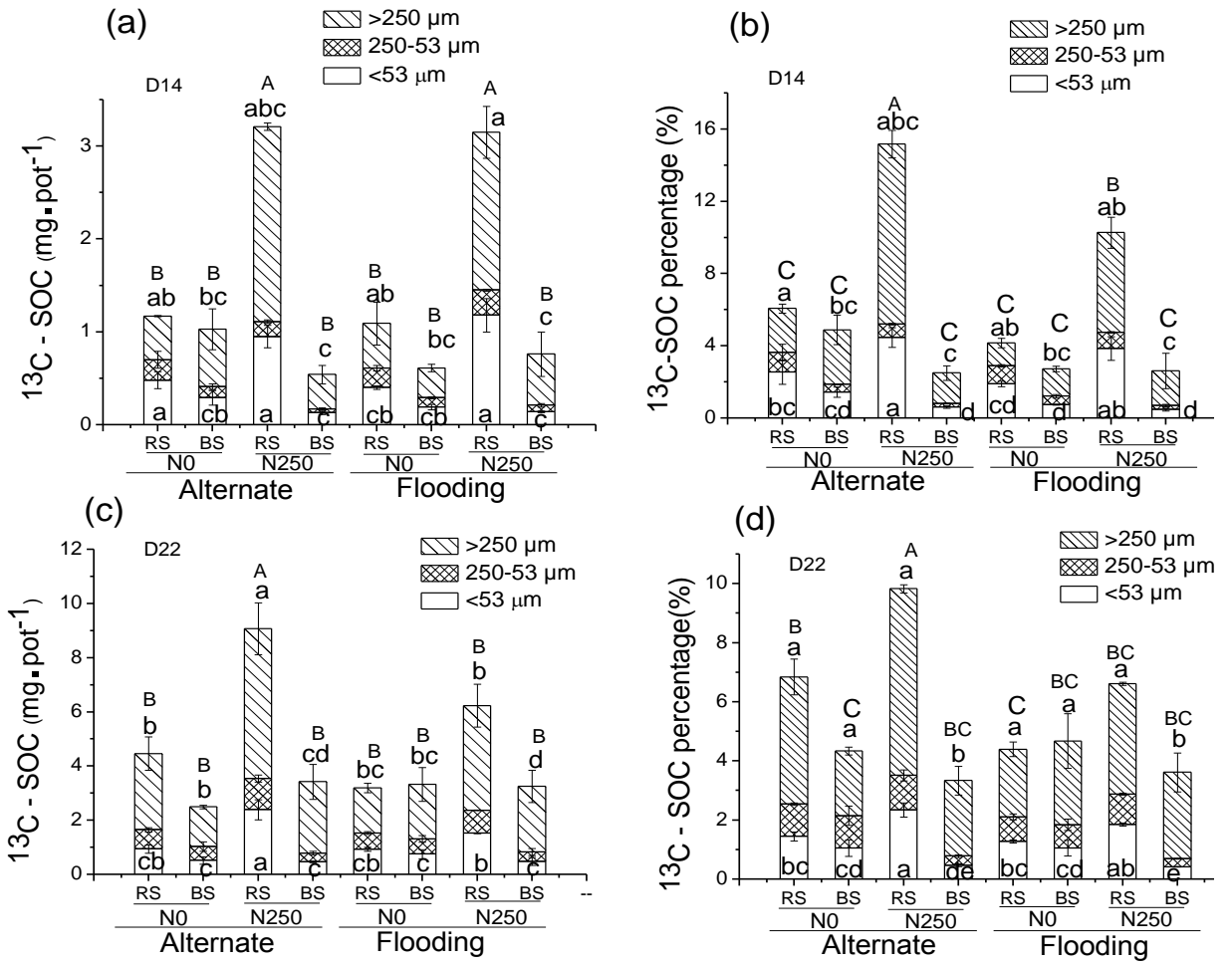
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724 **Fig. 3**



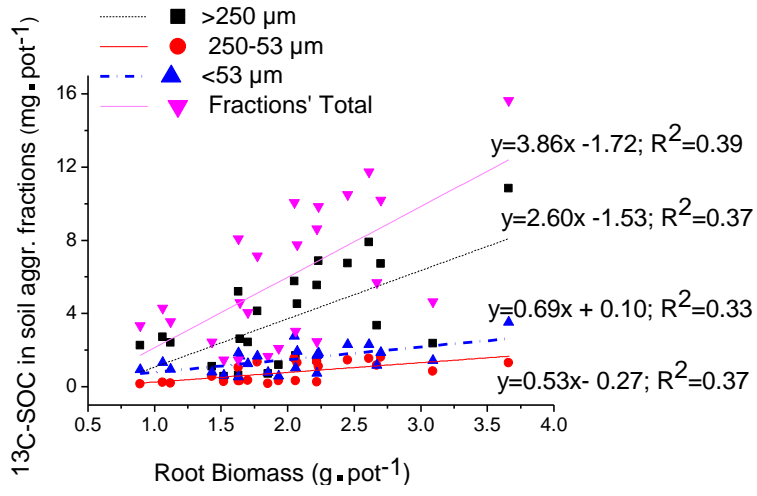
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729 **Fig. 4**



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732 **Supplementary material**

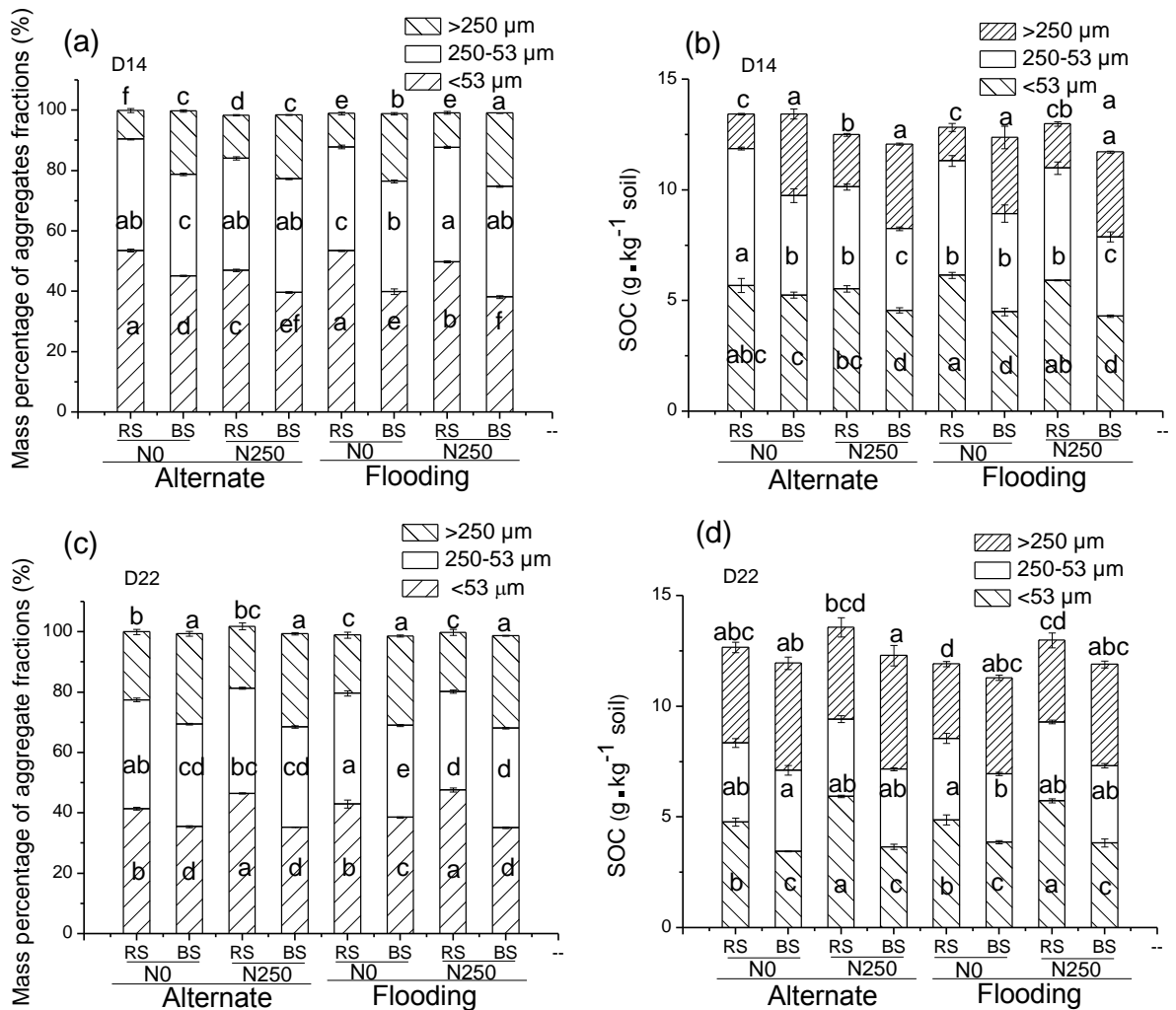
733 Table S1. ¹³C-SOC (mg C kg⁻¹ soil) in rhizosphere soil (RS) or bulk soil (BS) aggregate
 734 fractions at day 14 (a & b) and 22 (b & c) of the ¹³C-continuous labelling experiment. Alternate:
 735 alternating flooding-drying water management; Flooding: continuous flooding; N0: no N
 736 fertiliser application; N250: urea at 250 mg N kg⁻¹ soil; RS: rhizosphere soil; BS: bulk soil

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Water regime	level	Day	Sum of aggregate fractions		>250 μm		250–53 μm		<53 μm	
			RS	BS	RS	BS	RS	BS	RS	BS
Alternate	N0	D14	3.44	0.97	1.39	0.59	0.63	0.11	1.41	0.28
		D22	13.12	2.33	8.28	1.37	2.08	0.63	2.75	0.62
	N250	D14	6.27	0.43	4.11	0.30	0.31	0.03	1.85	0.11
		D22	17.82	2.76	10.85	2.13	2.26	0.26	4.69	0.38
Flooding	N0	D14	3.21	0.57	0.74	0.30	0.62	0.09	1.16	0.18
		D22	9.41	3.11	4.90	1.88	1.78	0.53	2.74	0.70
	N250	D14	6.18	0.60	4.12	0.44	0.54	0.11	2.31	0.11
		D22	12.22	2.59	7.56	1.92	1.67	0.28	2.99	0.38

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739 Fig. S1. Effects of drying-rewetting cycles and nitrogen (N) fertiliser application on mass
 740 percentage and SOC, respectively, of rhizosphere soil and bulk soil aggregate fractions at two
 741 sampling days 14 (a & b) and 22 (c & d) in a ¹³C-continous labelling experiment. Alternate:
 742 alternating flooding-drying water management; Flooding: continuous flooding; No: No N
 743 fertiliser application; N250: Urea at 250 mg N kg⁻¹ soil; RS: rhizosphere soil; BS: bulk soil.
 744 Error bars represent one standard error of the mean (n = 3). Different lowercase letters indicate
 745 significant differences (P < 0.05) of the parameter measured in soil aggregate fractions (>250,
 746 250–53, or <53 μm) across different treatments



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