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

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25 Abstract

This study aimed to better understand the stabilisation of rice rhizodeposition in paddy soil 26 under the interactive effects of different N fertilization and water regimes. We continuously 27 labelled rice ('Zhongzao 39') with ¹³CO₂ under a combination of different water regimes 28 (alternating flooding-drying vs. continuous flooding) and N addition (250 mg N kg⁻¹ urea vs. 29 no addition, then followed ¹³C incorporation into plant parts as well as soil fractions. N addition 30 increased rice shoot biomass, rhizodeposition, and formation of ¹³C (new plant-derived C) in 31 the rhizosphere soils under both water regimes. By day 22, the interaction of alternating 32 flooding-drying and N fertilisation significantly increased shoot and root ¹³C allocations by 33 17% and 22% respectively, over the continuous flooding condition. The interaction effect also 34 increased ¹³C allocation to the rhizosphere soil by 46%. Alone, alternating water management 35 increased ¹³C deposition by 43%. In contrast, N addition increased ¹³C deposition in rhizosphere 36 37 soil macroaggregates under both water regimes, but did not increase macroaggregation itself. N treatment also increased ¹³C deposition and percentage in microaggregates, as well as in the 38 silt and clay-size fractions of the rhizosphere soil, a pattern that was higher under the alternating 39 40 condition. Overall, our data indicated that combined N application and a flooding-drying treatment stabilised rhizodeposited C in soil more effectively than other tested conditions. Thus, 41 they are desirable practices for improving rice cropping, capable of reducing cost, increasing 42 43 water use efficiency, and raising C sequestration.

Key words: Paddy soils, ¹³C continuous labelling, Carbon stabilisation, Root exudation,
Rhizodeposition, Recent assimilates.

46

47 Introduction

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

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

The rice crop depends on water management and fertilizer N inputs; rice production consumes an estimated 90% of the total irrigated water allocated to crops (Bhuiyan 1992). However, regular irrigation requires enormous energy input, and supplying fresh water for continuously flooded paddies is increasingly unsustainable due to competitive demands from urban and industrial fronts (Bouman and Tuong 2001; Fan et al. 2012). Furthermore, a predicted increase in droughts through many subtropical regions have heightened concern for more 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 between flooding and drying periods to become popular in rice cultivation (Belder et al. 2004; 73 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. 2004; Schimel et al. 2007), as well as shifts in microbial use and stabilisation of rhizodeposited 76 77 nutrients (Canarini and Dijkstra 2015). Soil rewetting is also linked closely to N loss through gaseous emission and leaching (Austin et al. 2004). Further, increased photosynthate 78 79 partitioning and allocation belowground have been reported in crested wheatgrass under water stress (Henry et al. 2007) as well as in rice under drying-rewetting (Tian et al. 2013a, 2013b). 80 Despite these consequences, little is known about the combined effects of water management 81 82 and N fertilisation on the partitioning and allocation of rice photosynthates in above- and belowground paddy soil systems. 83

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

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 continuously labelled with ¹³CO₂, and the distribution of newly plant-derived C in above- and 98 below-ground systems was investigated. We hypothesize that an alternating water regime and 99 N fertilisation will increase rhizodeposition via enhanced root activity compared with 100 continuous flooding. We also expect that the surge in microbial activities, and hence their 101 102 increased use of rhizodeposits under flooding-drying episodes, will reduce C stabilisation. Finally, we hypothesize that N addition will increase rhizodeposition through enhancing 103 104 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 Environmental Monitoring (113°19'52"E, 28°33'04"N, 80ma.s.l.), in subtropical China. The 110 mean annual temperature is 17.5°C, yearly precipitation is 1300 mm, annual hours of sunshine 111 112 are 1663, and the frost-free period is up to 274 d. The soil type is a typical Stagnic Anthrosol (Gong et al. 2009) developed from granitic red soil (Alisol). Soil properties were as follows: 113 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⁻¹ 114 ¹; and cation exchange capacity (CEC), 7.71 cmol kg¹. Particle-size analysis indicated a 115 composition of 28% sand (>50 μ m), 66% silt (2–50 μ m), and 6% clay (<2 μ m). 116

118 Experimental set-up

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

Twenty-four pots (11 cm inner diameter and 20 cm height) were each filled with 1.26 124 125 kg (oven-dried basis) soil each. A rhizosphere bag (mesh 30 μ m; 3.5 cm \times 15 cm) each which allows the passage of nutrients and water, but not root (Su and Zhu, 2008; Finzi et al., 2015) 126 127 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 were sprayed with NaH₂PO₄ (20 mg P kg⁻¹ soil) and KCl (80 mg K kg⁻¹ soil), then divided 129 130 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 distilled water. Under 'Alternate' treatment, pots were subsequently dried for 3-4 days until the 132 soil water content reached 70-75% of the water holding capacity (WHC), and then flooded 133 again. Three drying-rewetting cycles occurred in the entire experiment. Fertilising conditions 134 consisted of either no N addition (N0) or 250 mg N kg⁻¹ (562.2 kg N ha⁻²) as urea (N250). Each 135 condition comprised 12 pots (six each from 'Alternate' and 'Flooding'). An additional 24 pots 136 137 were used as the unlabelled controls. The unlabelled controls were references for determining natural ¹³C abundance and calculating ¹³C atom percent excess. Controls were placed outside, 138 10-15 m away from labelled chambers, but given the same water and fertilizer treatments as 139 140 labelled groups.

142 ¹³CO₂ continuous labelling

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

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

154

155 Sampling and harvesting

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

165 Soil aggregate separation

166 Wet-sieving of air-dried soils has been used for aggregate separation in paddy soils (Pan et al. 2008). Due to high clay content that might make fresh soil too sticky for separation, we 167 168 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 µm) and large microaggregates (250-53 µm) were gently backwashed off the sieves into pre-weighed 173 aluminium pans. Floating organic materials (>250 µm) were decanted and discarded. Water 174 plus soil that went through both sieves were considered the <53 µm (silt and clay-size) fraction, 175 176 although this fraction could also consist of small microaggregates (Chenu and Plante 2006; Virto et al. 2008). The suspension was decanted (leaving the sediment), centrifuged at 3000 177 rpm for 5 minutes, and the precipate was combined with the obtained sediment. The aggregates 178 were oven-dried (60°C), weighed, finely ground, and stored in air-tight tubes at room 179 temperature for TC, TN, and ¹³C analyses. 180

181

182 Analytical methods

Soil physicochemical properties were determined using standard methods: pH with a pH meter
(Delta 320; Mettler-Toledo Instruments Co., Ltd., China) in 1:2.5 soil/water ratio; soil particle
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 $({}^{12}C/{}^{13}C)$ and the total C and N content of all samples were measured with an isotope ratio mass spectrometer (IRMS, MAT253; Thermo-Fisher Scientific, Waltham, MA, USA), coupled with an elemental analyser (FLASH 2000; Thermo-Fisher Scientific, Waltham, MA, USA). The ${}^{12}C/{}^{13}C$ ratio was expressed as parts per thousand relative to the international standard, Peedee Belemnite (PDB), using delta units (δ %).

192

193 Calculations and statistical analyses

194 13 C content (13 C_{sample}) (mg C pot⁻¹ or mg C m⁻²) was calculated using the following equation:

195
$${}^{13}C_{sample} = [(atomic \, {}^{13}C\%)_{l} - (atomic \, {}^{13}C\%)_{n1}]_{sample} \times TC_{sample} / 100$$
 (1)

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

The amount of ¹³C in aggregates and free silt and clay-size fraction was calculated as
 follows:

200
$${}^{13}C_{amount-fraction} = {}^{13}C_{con-fraction} \times M_{fraction}/100$$
 (2)

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

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

- The percentage of ¹³C incorporation in shoots, roots, and soil on each sampling day was calculated considering the total ¹³C found in shoots, roots, and soil combined.
- All data were expressed as the mean of three replicates \pm SE. Multivariate ANOVA with Duncan tests was used to test differences in the measured variables among different treatments

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

212

213 **Results**

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

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

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

Shoot TN was not affected by N application on day 14. The interaction of fertiliser and water management significantly increased shoot TN (P = 0.02, Fig. 1c) on day 22 of both water regimes, but by 33% more (p = 0.023) in 'Alternate' than in 'Flooding'. The interactions of fertiliser and sampling date (p = 0.0001, Fig. 1c), as well as of water management and sampling date (p = 0.03), significantly affected root TN. While N application decreased root TN on day 14 of both water regimes, an increase occurred on day 22, again with the change being more pronounced under 'Alternate' than under 'Flooding' (a 43% difference). Further, N application reduced the root/shoot N ratio across both sampling dates and water regimes (P = 0.0001, Fig. 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 'Alternate' and 'Flooding' water regimes, respectively, indicating a significant interactive 243 effect of fertiliser and sampling day (p = 0.003, Fig. 1d). The shoot and root ratios under 244 'Alternate' were lower by 7% (p = 0.03) and 13% (P = 0.007), respectively, than under 245 'Flooding'. 246

247

¹³C amount (mg C pot⁻¹) and percentage (%) in the rice-soil system

Although N addition increased shoot ¹³C amount under both 'Alternate' and 'Flooding' compared with the control, a similar increase was not observed in roots (Fig. 2a). The interaction of fertiliser × sampling date was significant (p = 0.0001, Fig. 2a); shoot ¹³C increased by 40% and 98% on days 14 and 22 under 'Alternate', but by 53% and 32% under 'Flooding'. Hence, 'Alternate' resulted in a 17% increase over 'Flooding' on day 22 (Fertiliser × Water × Time, p = 0.001, Fig. 2a).

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

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

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

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

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

N application significantly increased (p = 0.04, Fig. 3a) ¹³C in rhizosphere soil macroaggregates on day 14 of both water regimes. Approximately 2.1 and 1.7 mg ¹³C pot⁻¹ were allocated to macroaggregates under 'Alternate' and 'Flooding', respectively, reflecting a 347% and 254% increase from the control. In contrast, by day 22, N application only increased macroaggregate ¹³C in rhizosphere soil (53 mg C pot⁻¹; p = 0.002) under 'Alternate' (97% increase over the control), compared with 'Flooding' (43% increase over the control) (Water × 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 treatments. However, by day 22, only rhizosphere soil in 'Alternate' showed significantly
 higher ¹³C-SOC (157% increase over the control) under N application.

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

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

Both individual and interaction effects of water management and N fertiliser application significantly increased rice biomass, as well as TC and TN content. These increases were 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 rhizodeposition under flooded conditions can leave more materials for growth in shoots and 328 roots (Schumacher and Smucker 1985), resulting in the initially higher biomass under 329 330 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 plants is linked to enhanced photosynthetic capability through increasing chlorophyll and 332 333 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 availability) of some rhizobacteria could also be more favoured under alternate water regime, 335 336 leading to higher shoot and root biomass (Pii et al. 2015).

Regardless of the water regime, we noticed that the root/shoot biomass ratio decreased 337 with N addition, suggesting that shoots respond better to N. The decrease of root system in the 338 339 presence of N and its increase under N limitations has been well established (Voisin et al. 2002; Shangguan et al. 2004). In the present study, continued growth then led to even higher N 340 341 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 uptake. Again, this change was more obvious under the 'Alternate' water management, 345 implying better nutrient uptake. 346

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

While ¹³C assimilation rose in response to shoot biomass increase under both water regimes, the effect was much stronger under alternating than continuous flooding. The relative decreased allocation of recent photoassimilate to the root under 'Flooding' compared with 'Alternate' could be due to prolonged anaerobic conditions that reduced root respiration, and hence, the demand and shoot-to-root translocation of recent photoassimilate (Schumacher and Smucker 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 rhizosphere soil to bulk soil may be possible with help of fungi hyphae, which are able to 358 penetrate fine pores such as those of the rhizosphere bags (Oades 1984). Substantial fungal 359 360 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 of aerobiosis 362 pockets (Yuan et al. 2016). Looking within water regimes, however, translocation and rhizodeposition were superior under 363 364 flooding-drying compared with continuous flooding. Rhizodeposition was thus considerably increased through the combination of N addition with the alternating water regime, supporting 365 366 our first hypothesis.

Besides greater root biomass, N-induced increases to shoot biomass and reduced N loss under the 'Alternate' water regime could also improve assimilate translocation to the root. For example, Liljeroth et al. (1990) reported increases in wheat root exudates with higher N 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 and root tip number (Thornton et al. 2004; Pausch and Kuzyakov 2011). In addition, Zhang et 375 376 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) and growth (Tian et al. 2013a & b; Zhu et al. 2016) under anaerobic condition such as that of 379 380 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 subsequent transfer belowground. 382

383 The percentage of recent photoassimilates in shoots and roots differed noticeably with N treatment under the two water regimes. In the first sampling of the 'Alternate' management, 384 the shoot percentage of recent photoassimilate differed significantly from control, whereas the 385 386 root percentage was not significant. Both the shoot and the root percentage of the recent photoassimilates, however, slightly increased until the second sampling day. In contrast, 387 continuous flooding caused an increase in the shoot percentage of recent photoassimilates from 388 389 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 reduced translocation and rhizodeposition through prolonged anaerobiosis (Schumacher and 392 Smucker 1985; Henry et al. 2007) under continuous flooding. 393

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. 2013b) under the 'Alternate' regime. We also noted that our values were generally higher than 398 399 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 to previous studies could have been a factor in our higher percentage. Continuous labelling with 402 ¹⁴C can more quantitatively estimate rhizodeposited C (Wichern et al. 2011), when compared 403 404 short-term pulse labelling.

405

Impact of drying-rewetting cycles and nitrogen fertilisation on recent assimilate (¹³C) 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 macroaggregation. This outcome could result from the destabilising effects of growing roots on 410 411 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 macroaggregate SOC concentration in the rhizosphere soil, we generally observed closer 415 associations between SOC and the other two fractions (silt-and-clay and microaggregates). This 416

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

Under 'Alternate', N addition increased recent assimilate deposition and percentage in 424 425 all rhizosphere soil aggregate fractions compared with 'Flooding', indicating enhanced 426 rhizodeposition and stabilisation of recent assimilates. This finding did not support our second hypothesis, but corroborated some previous data showed that ¹⁴C incorporation into 427 428 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 al. 2013b) increase photosynthesized C rhizodeposition. The former may exert such an effect 430 431 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 effective because drying conditions improve root activity and architecture (Mishra and Salokhe 433 434 2011; Thakur et al. 2011).

Though N fertilisation affects the allocation of recent assimilates, roots exert a strong influence on soil C dynamics irrespective of N, as evidenced by the higher assimilate percentage in rhizosphere soil over bulk soil (microaggregates and silt and clay-size fractions) under both water regimes. A larger portion of recent photoassmilates was deposited into macroaggregates, corroborating previous work that newly plant-derived C is initially incorporated into this 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 by two factors: 1) the silt and clay-size fraction exhibited the highest weight percentage of all 445 446 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 stabilized rhizodeposited C in soil more effectively than other tested conditions. Hence, in 459 460 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 cropping. This study for the first time investigated rice C rhizodeposition and stabilization in 462 paddy soil under combined effect N addition and water regimes, and thus established the 463

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.

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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 and 22 of the ¹³C continuous labelling experiment. Alternate: alternating flooding-drying water 672 management; Flooding: continuous flooding; No: no N fertiliser application; N250: urea at 250 673 mg N kg⁻¹ soil; D14: sampling on day 14 of continuous labelling; D22: sampling on day 22 of 674 675 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 and root parameters, as well as their root/shoot ratio across treatments 677

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Fig. 2 Effects of drying-rewetting cycles and N fertiliser application on ¹³C content (a) and 679 680 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 mg N kg⁻¹ soil; D14: sampling on day 14 of continuous labelling; D22: sampling on day 22 of 683 684 continuous labelling. Error bars represent one standard error of the mean (n = 3). Different lowercase letters indicate significant differences (p < 0.05) of the measured shoot, root, RS 685 (Rhzisphere soil), or BS (bulk soil) parameter across treatments; curved arrows link the letters 686 687 to the corresponding sections of the bars they represent

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Fig. 3 Effects of drying-rewetting cycles and N fertiliser application on 13 C-SOC and percentage, respectively, in rhizosphere-soil and bulk-soil aggregate fractions at day 14 (a & b) and 22 (b & c) of the 13 C-continuous labelling experiment. Alternate: alternating floodingdrying water management; Flooding: continuous flooding; No: no N fertiliser application; N250: urea at 250 mg N kg⁻¹ soil; RS: rhizosphere soil; BS: bulk soil. Error bars represent one standard error of the mean (n = 3). Different lowercase letters indicate significant differences (P< 0.05) of the parameter measured in soil aggregate fractions (>250, 250–53, or <53 μ m) across treatments

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Fig. 4 Correlations between root biomass and ¹³C-SOC content in soil aggregate fractions,
resulting from a ¹³C-continuous labelling experiment. All regression lines are significant at p <
0.001
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708 Fig. 1







729 Fig. 4







732 Supplementary material

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

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: alternating flooding-drying water management; Flooding: continuous flooding; No: No N 742 fertiliser application; N250: Urea at 250 mg N kg⁻¹ soil; RS: rhizosphere soil; BS: bulk soil. 743 Error bars represent one standard error of the mean (n = 3). Different lowercase letters indicate 744 significant differences (P < 0.05) of the parameter measured in soil aggregate fractions (>250, 745 746 250–53, or <53 µm) across different treatments



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