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Jones, David

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

Cornelius Talade Atere [a], Tida Ge [a*], Zhenke Zhu [a], Chengli Tong [a], Davey L. Jones [a, b], Olga Shibistova [c, d], Georg Guggenberger [a, c], Jinshui Wu [a]

[a] *Key Laboratory of Agro-ecological Processes in Subtropical Region & Changsha Research Station for Agricultural and Environmental Monitoring, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan, 410125, China,*

[b] *School of Environment, Natural Resources & Geography, Bangor University, Gwynedd LL57 2UW, Wales, UK*

[c] *Institute of Soil Science, Leibniz Universität Hannover, 30419 Hannover, Germany*

[d] *VN Sukachev Institute of Forest, SB-RAS, 660036 Krasnoyarsk, Russian Federation*

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*Author for Correspondence:

Professor Tida Ge

Institute of Subtropical Agriculture, Chinese Academy of Sciences

Changsha, Hunan Province 410125, China

Email: gtd@isa.ac.cn

Tel: +86-731-84615224; Fax: +86-731-84619736

Abstract

This study aimed to better understand the stabilisation of rice rhizodeposition in paddy soil under the interactive effects of different N fertilization and water regimes. We continuously labelled rice ('Zhongzao 39') with $^{13}CO_2$ under a combination of different water regimes (alternating flooding-drying vs. continuous flooding) and N addition (250 mg N kg^{-1} urea vs. no addition, then followed ^{13}C incorporation into plant parts as well as soil fractions. N addition increased rice shoot biomass, rhizodeposition, and formation of ^{13}C (new plant-derived C) in the rhizosphere soils under both water regimes. By day 22, the interaction of alternating flooding-drying and N fertilisation significantly increased shoot and root ^{13}C allocations by 17% and 22% respectively, over the continuous flooding condition. The interaction effect also increased ^{13}C allocation to the rhizosphere soil by 46%. Alone, alternating water management increased ^{13}C deposition by 43%. In contrast, N addition increased ^{13}C deposition in rhizosphere soil macroaggregates under both water regimes, but did not increase macroaggregation itself. N treatment also increased ^{13}C deposition and percentage in microaggregates, as well as in the silt and clay-size fractions of the rhizosphere soil, a pattern that was higher under the alternating 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, they are desirable practices for improving rice cropping, capable of reducing cost, increasing water use efficiency, and raising C sequestration.

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

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.

Depending on plant species, age, and prevailing environmental conditions, rhizodeposition may release up to 40% of photosynthesized C (Lynch and Whipps 1990). The 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 of rhizodeposition is respired as CO_2. A small portion of low-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 mitigate the global increase in atmospheric CO_2 concentrations, we must better understand the management of SOC sources, pools, spatial distribution, and stabilisation processes (Miltner et al. 2012).

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

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; Xu et al., 2015). Drying-rewetting cycles, however, have major implications on below-ground 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 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 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). Despite these consequences, little is known about the combined effects of water management and N fertilisation on the partitioning and allocation of rice photosynthates in above- and belowground paddy soil systems.

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 bio-accessibility (von Lützow et al. 2007). Approximately 90% of surface SOC is associated 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 flocculation (Puget et al. 2000) makes them more stable, with longer turnover than 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 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, especially in relation to water management (Tian et al. 2013b) and N fertilisation.

This study aimed to investigate how water management (continuous versus alternating flooding-drying) and N fertilisation could interact to affect the partitioning and stabilisation of newly plant-derived C in the rice-soil system. Rice seedlings at the tillering stage were continuously labelled with $^{13}CO_2$, and the distribution of newly plant-derived C in above- and below-ground systems was investigated. We hypothesize that an alternating water regime and N fertilisation will increase rhizodeposition via enhanced root activity compared with continuous flooding. We also expect that the surge in microbial activities, and hence their increased use of rhizodeposits under flooding-drying episodes, will reduce C stabilisation. Finally, we hypothesize that N addition will increase rhizodeposition through enhancing photosynthesis, and the associated larger input of available OC will increase macroaggregation in rhizosphere soils under both water regimes.

Materials and methods

Site description and basic soil characteristics

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 mean annual temperature is 17.5°C, yearly precipitation is 1300 mm, annual hours of sunshine 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: 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}; and cation exchange capacity (CEC), 7.71 cmol kg^{1}. Particle-size analysis indicated a composition of 28% sand (>50 μm), 66% silt (2–50 μm), and 6% clay (<2 μm).

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

$^{13}CO_2$ continuous labelling

Rice plants were subjected to $^{13}CO_2$ 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), $^{13}CO_2$ was produced in the chamber through the reaction of $NaH^{13}CO_3$ (50 atom percent ^{13}C, 1 mol L^{-1}) and H_2SO_4 (0.5 mol L^{-1}). The CO_2 concentrations inside the chamber were maintained between 360 and 380 μL CO_2 L^{-1} via further reactions or gas-flow diversion through CO_2 traps (1 M NaOH solution) that absorbed excess gas.

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

Sampling and harvesting

Plants and soil were harvested 14 and 22 d after labelling began. Shoots were severed from the roots at the stem base. Mesh with ingrowing roots was removed from the chambers and adhering soil was gently detached via gentle agitation for 1 min in 0.01 M $CaCl_2$ (pH 6.2), followed by thorough rinsing under running tap water. Soil inside and outside the mesh was considered 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 ^{13}C analyses. The remaining soil was stored at 4°C for aggregate fractionation and subsequent TC, TN, and ^{13}C determination.

Soil aggregate separation

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 separated following the method of Cambardella and Elliot (1993), as adapted by Six et al. (1998). A 50-g subsample of air-dried soil was placed on a 250-µm sieve nested into a 53-µm sieve. Both sieves were then placed in a bowl containing room-temperature deionized water, submerging the soil for 5 min. To achieve separation, the sieves were manually agitated up-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 aluminium pans. Floating organic materials (>250 µm) were decanted and discarded. Water plus soil that went through both sieves were considered the <53 µm (silt and clay-size) fraction, 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 rpm for 5 minutes, and the precipate was combined with the obtained sediment. The aggregates were oven-dried (60°C), weighed, finely ground, and stored in air-tight tubes at room temperature for TC, TN, and ^{13}C analyses.

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).

Dry shoots, roots, and soil samples were ground in a ball mill prior to analysis. The stable 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‰).

Calculations and statistical analyses

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

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

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

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

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

where $^{13}C_{amount\text{-}fraction}$ is the C amount in aggregates (mg C kg^{-1} soil), $C_{con\text{-}fraction}$ 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 ^{13}C incorporation in shoots, roots, and soil on each sampling day was calculated considering the total ^{13}C found in shoots, roots, and soil combined.

All data were expressed as the mean of three replicates ± 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 (SAS Institute Inc.).

Results

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 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'. The latter led to a 40% and 29% increase in shoot biomass from N0 on days 14 and 22, respectively. The interaction of N application and water regime also increased root biomass by 76% under 'Flooding' compared with 'Alternate' (Fertiliser × Water × Time, p = 0.037, Fig. 1a). N application also reduced the root/shoot biomass ratio (p = 0.0001, Fig. 1a) on both sampling dates and in both water regimes.

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).

In unfertilised soil, shoot C/N ratio increased from day 14 to 22 under both 'Alternate' and 'Flooding', whereas N treatment caused a corresponding decrease of 43% and 42% for the two water managements, respectively. This interaction between fertiliser and sampling date on C/N ratio was highly significant (p = 0.0001, Fig. 1d). Root C/N ratios exhibited similar 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 effect of fertiliser and sampling day (p = 0.003, Fig. 1d). The shoot and root ratios under 'Alternate' were lower by 7% (p = 0.03) and 13% (P = 0.007), respectively, than under 'Flooding'.

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

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

Compared with the control, N addition significantly increased the percentage of total ^{13}C 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 ^{13}C 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 ^{13}C 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 × Water × Time, $p = 0.001$, Fig. 2b), root ^{13}C percentage increased marginally from day 14 to 22 of 'Alternate', but decreased over the same period of 'Flooding'. Compared with the control, N addition significantly increased ($p = 0.04$, Fig. 2b) ^{13}C percentage in rhizosphere soil on day 14 of both water regimes (by 114% under 'Alternate' 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 belowground percentage. Neither water nor fertiliser regimes affected ^{13}C percentage in bulk soil.

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

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 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, microaggregate distribution was unaffected by any factor, including the rhizosphere, whereas 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 macroaggregates of bulk soil than of rhizosphere soil under both water regimes (Fig. S1b & d).

N application significantly increased ($p = 0.04$, Fig. 3a) ^{13}C in rhizosphere soil macroaggregates on day 14 of both water regimes. Approximately 2.1 and 1.7 mg ^{13}C 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 ^{13}C 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).

Compared with bulk soil, microaggregate ^{13}C-SOC was slightly higher in rhizosphere soil on day 14 of all treatments (Fig. 3a). By day 22, only 'Alternate' rhizosphere soil exhibited significantly higher microaggregate ^{13}C-SOC (62% increase over the control) under N application (Fig. 3c). Similarly, ^{13}C-SOC was significantly higher in the silt and clay-size 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 ^{13}C-SOC (157% increase over the control) under N application.

The percentage of ^{13}C-SOC in aggregate fractions exhibited similar patterns as the ^{13}C-SOC amount, with higher values of both in the macroaggregate fraction, followed by the silt and clay-size fraction, and then the microaggregate fraction (Fig.3b & d). On day 14 of both water regimes, N application significantly heightened macroaggregate ^{13}C-SOC percentage in rhizosphere soil (310% and 339% increases over N0 in 'Alternate' and 'Flooding', respectively; $p < 0.0001$, 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 under 'Alternate' (47% increase over N0 and 69% increase over 'Flooding'; Water × Rhizosphere, $p = 0.004$, Fig. 3d).

Regardless of fertilisation or water regime, microaggregate ^{13}C-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 ^{13}C-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 ^{13}C-SOC (in aggregate fractions and total soil).

Discussion

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.

While N application generally increased biomass over time, the effect of different water regimes altered that increase. Continuous flooding initially (day 14) caused higher shoot and root biomass than the alternating condition, but by day 22, this pattern was reversed. Several 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 roots (Schumacher and Smucker 1985), resulting in the initially higher biomass under continuous flooding. However, as time passed, less efficient nutrient use under flooding could 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 Rubisco activity (Wang et al. 2012; Lin et al. 2013), both of which are involved in photosynthesis. The growth-promoting activity (on plant development and improved nutrient availability) of some rhizobacteria could also be more favoured under alternate water regime, 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 with N addition, suggesting that shoots respond better to N. The decrease of root system in the 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 demand, explaining the significant increase in shoot and root TN at the second sampling date for both water regimes; the more pronounced effect in 'Alternate' conformed with our observations of late biomass increase under this water regime (Ye et al. 2013). Patterns in C/N 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, implying better nutrient uptake.

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

While ^{13}C 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).

The importance of roots is clearly demonstrated by the correlation between root biomass 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 penetrate fine pores such as those of the rhizosphere bags (Oades 1984). Substantial fungal growth even under the continuous flooding system could be possible through diffusion of atmospheric oxygen through rice aerenchyma to the root system, which could provide some pockets of aerobiosis (Yuan et al. 2016). . Looking within water regimes, however, translocation and rhizodeposition were superior under flooding-drying compared with continuous flooding. Rhizodeposition was thus considerably increased through the combination of N addition with the alternating water regime, supporting 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.

2013) and the consequent increase in overall exudate production. The oxic environment provided by the drying portion of the 'Alternate' treatment may enhance root growth and activity as well, improving exudation and nutrient uptake (Zhang et al. 2009; Mishra and 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 al. (2009) observed that while rice photosynthetic rate was not significantly reduced during the drying period of an alternating water regime, re-watering significantly increased the rate. The 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 continuous flooding have been well documented. Finally, high shoot respiration under 'flooding' (Swinnen et al. 1994; Kuzyakov et al. 2001) may reduce available shoot C for subsequent transfer belowground.

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, the shoot percentage of recent photoassimilate differed significantly from control, whereas the 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, continuous flooding caused an increase in the shoot percentage of recent photoassimilates from the first to the second sampling date, while the root percentage decreased (Fig. 2b). These outcomes are attributed to continuous root-oriented translocation of recent photoassimilate and rhizodeposition (Tian et al. 2013b) under the alternating flooding-drying regime, versus reduced translocation and rhizodeposition through prolonged anaerobiosis (Schumacher and Smucker 1985; Henry et al. 2007) under continuous flooding.

The percentage of recent photoassimilates found in soil was higher under 'Alternate' (17.7%) than under 'Flooding'. This outcome was likely due to improved root activity for nutrient uptake (Mishra and Salokhe 2011), more efficient nutrient use (Lin et al. 2013), 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 previous findings. According to Kuzyakov and Domanski (2000) and Tian et al. (2013b), 5–10% of the net photosynthesized C allocated to roots can be recovered from the soil during 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 ^{14}C can more quantitatively estimate rhizodeposited C (Wichern et al. 2011), when compared short-term pulse labelling.

Impact of drying-rewetting cycles and nitrogen fertilisation on recent assimilate (^{13}C) allocation to aggregate fractions

We observed reduced macroaggregation in rhizosphere soil versus bulk soil irrespective of N 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 SOM decomposition, due to physical root-soil interactions (Huck et al. 1970). Growing roots prefer wider soil pores than their own diameters (Russel 1977). Under less ideal conditions, roots will displace soil particles (Whiteley and Dexter 1984; Helal and Sauerbeck 1989) while enlarging narrow pores, thus crushing some soil aggregates. Although N addition improved macroaggregate SOC concentration in the rhizosphere soil, we generally observed closer associations between SOC and the other two fractions (silt-and-clay and microaggregates). This

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 all rhizosphere soil aggregate fractions compared with 'Flooding', indicating enhanced rhizodeposition and stabilisation of recent assimilates. This finding did not support our second hypothesis, but corroborated some previous data showed that ^{14}C incorporation into macroaggregates increased with time in the non-flooded treatment versus the flooded treatment (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 because high soil N enhances photosynthetic capability (Lin et al. 2013) and reduces the 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 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).

Subsequently, we observed higher recent-assimilate incorporation into the silt and clay-size than the microaggregate fraction. This result contradicts the concept of aggregate hierarchy (Tisdall and Oades 1982; Six et al. 1999), where the photoassimilate content is expected to 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 fractions in this study, and 2) silt and particularly clay both have high surface areas for increased fresh C absorption (Sposito et al. 1999). Moreover, intra-microaggregate pores are small and new C may not be able to easily diffuse through them. Therefore, the effective surface area of microaggregates is smaller, and this fraction could also have slower turnover rates than both macroaggregate-linked OC (Puget et al. 2000) and the silt and clay-size fraction.

Conclusions

The results of our study showed that the interactive effects of water regimes and N fertilisation increased rice shoot biomass, as well as the allocation and stabilisation of newly the plant-derived C in the rice-soil system. Moreover, N application was more effective in the alternating flooding-drying treatment than in continuous flooding, causing a larger increase to recent assimilate deposition in rhizosphere soil macroaggregates, microaggregates, and silt and clay-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 addition to benefits such as cost reduction, water use efficiency, and yield increase, the positive 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 paddy soil under combined effect N addition and water regimes, and thus established the

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

Acknowledgments

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Figure captions

Fig. 1 Effects of drying-rewetting cycles and N fertilisation on rice shoot and root biomass (dry 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 ^{13}C 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; D14: sampling on day 14 of continuous labelling; D22: sampling on day 22 of continuous labelling. Error bars represent one standard error of the mean (n = 3). Different 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

Fig. 2 Effects of drying-rewetting cycles and N fertiliser application on ^{13}C content (a) and percentage (b) in shoot, root, rhizosphere soil (RS), and bulk soil (BS) at days 14 and 22 of the ^{13}C-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; D14: sampling on day 14 of continuous labelling; D22: sampling on day 22 of 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 (Rhzisphere soil), or BS (bulk soil) parameter across treatments; curved arrows link the letters to the corresponding sections of the bars they represent

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 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. 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 µm) across treatments

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

Figures

Fig. 1

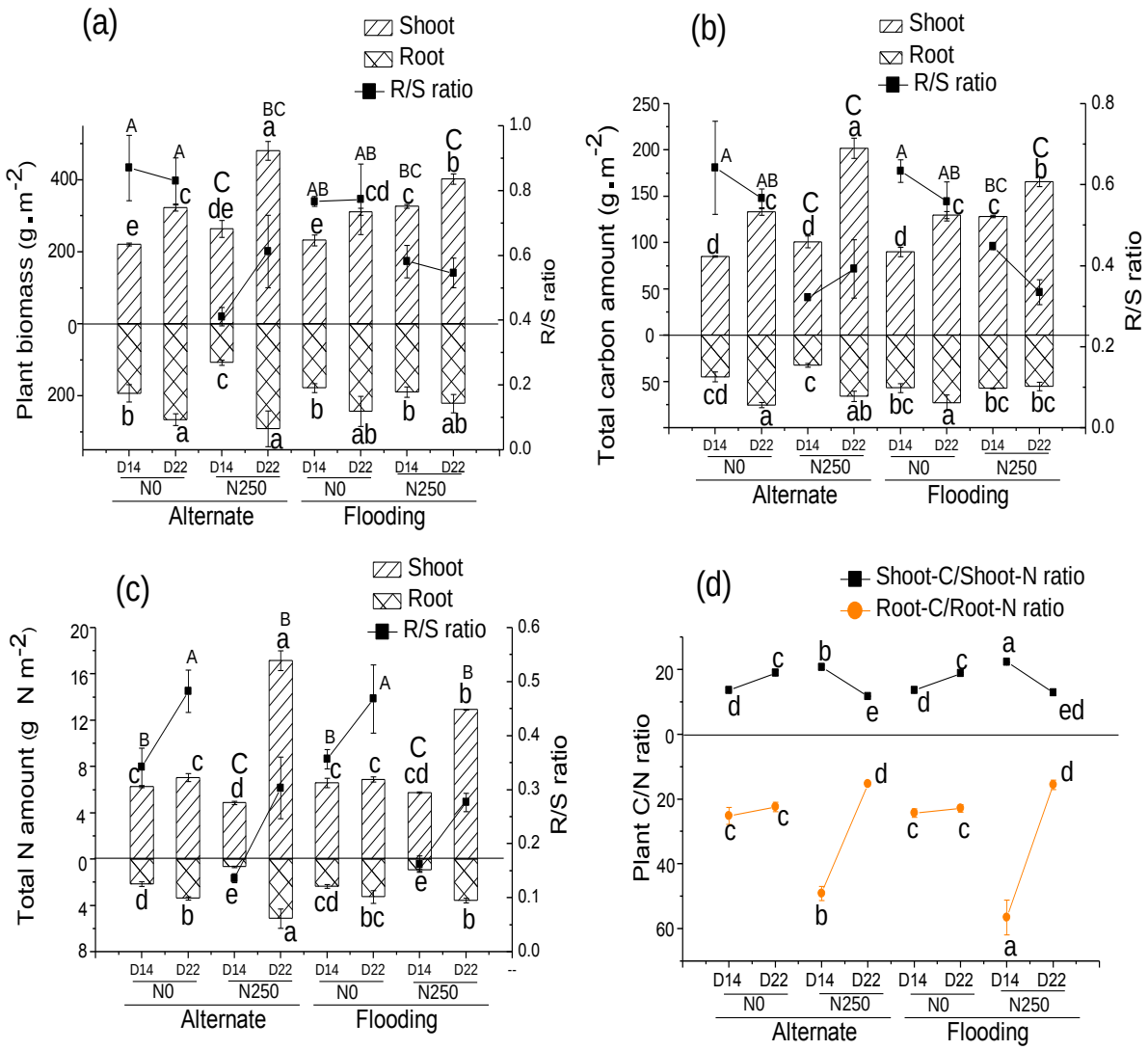


Fig. 2

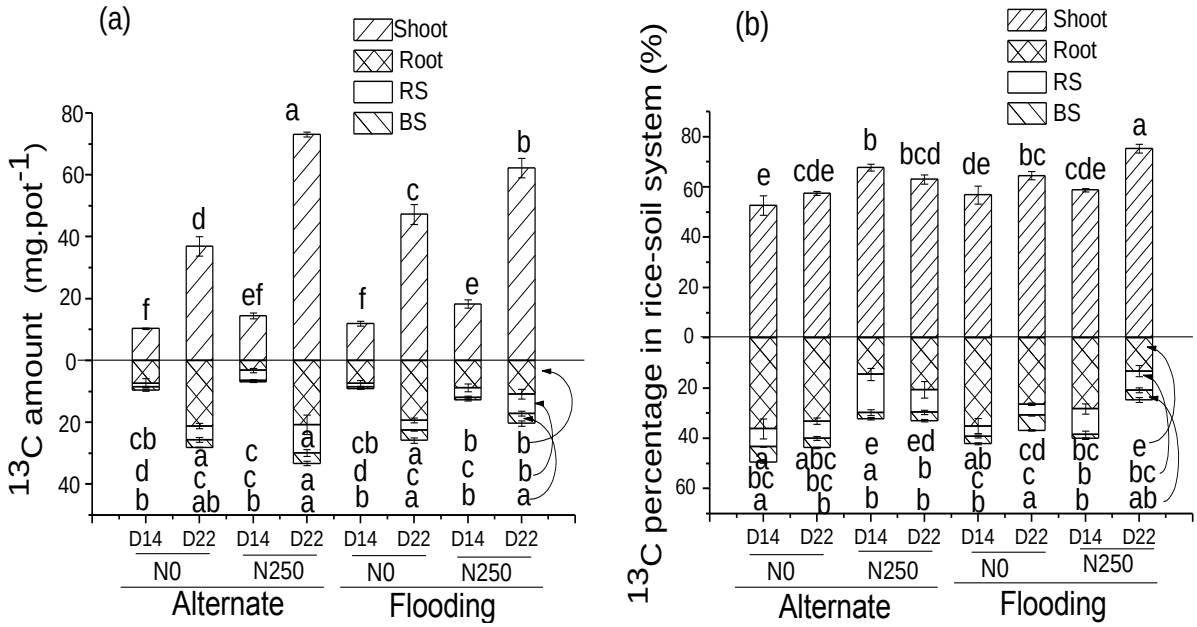

(a)
Shoot
Root
RS
BS
^{13}C amount (mg.pot^{-1})
80
60
40
20
0
20
40
f
d
ef
a
f
c
e
b
cb
d
b
a
c
ab
c
c
b
a
a
a
cb
d
b
a
c
a
b
c
b
b
b
a
D14
D22
D14
D22
D14
D22
D14
D22
N0
N250
N0
N250
Alternate
Flooding
(b)
Shoot
Root
RS
BS
^{13}C percentage in rice-soil system (%)
80
60
40
20
0
20
40
60
e
cde
b
bcd
de
bc
cde
a
a
bc
a
abc
bc
b
e
a
b
ed
b
b
ab
c
b
cd
c
a
bc
b
b
e
bc
ab
D14
D22
D14
D22
D14
D22
D14
D22
N0
N250
N0
N250
Alternate
Flooding

Fig. 3

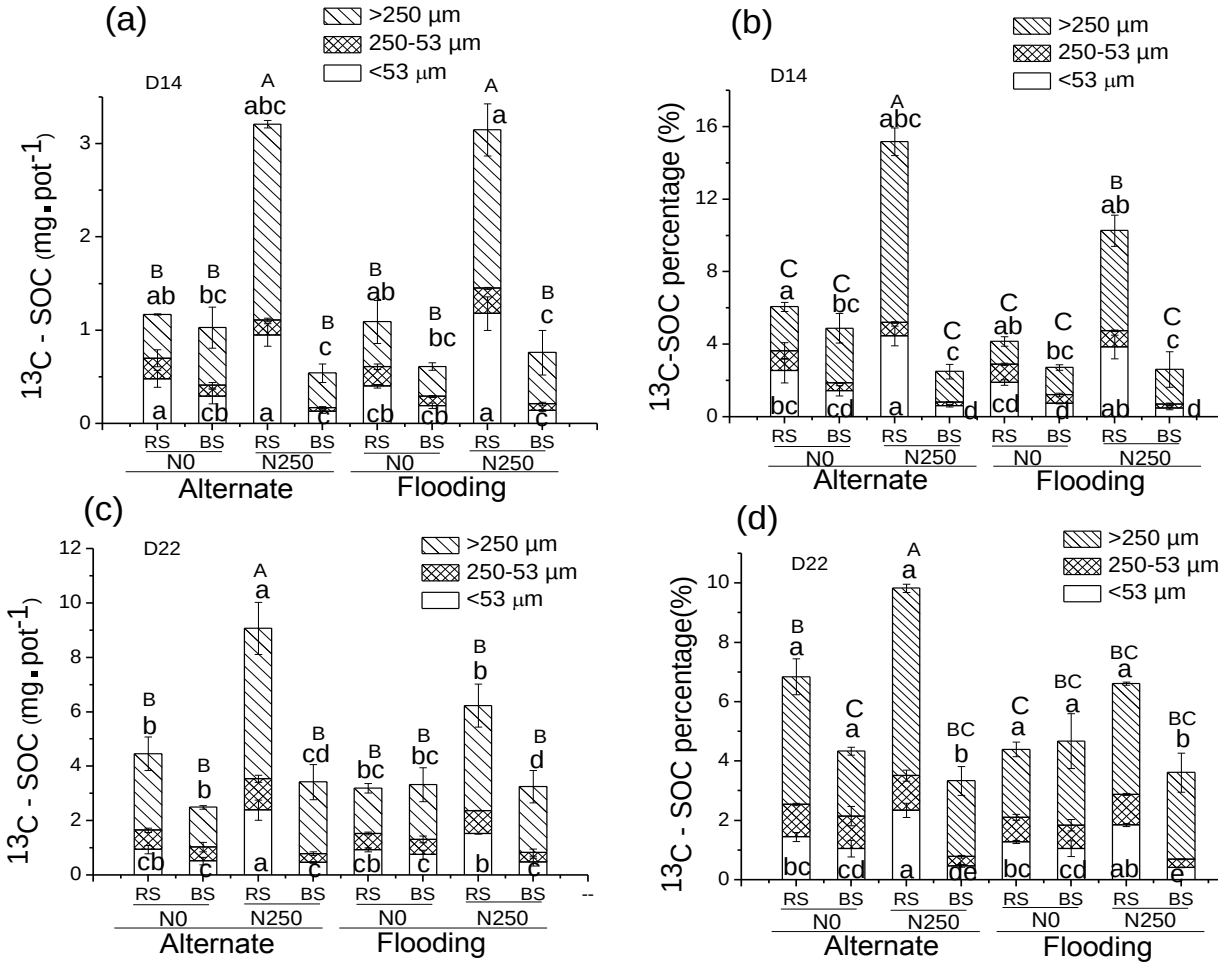
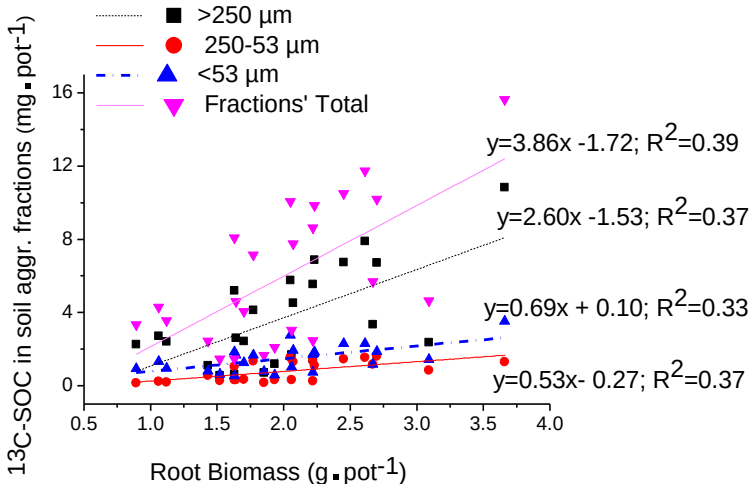

(a)
D14
>250 μm
250-53 μm
<53 μm
13C - SOC (mg▪pot-1)
RS BS RS BS RS BS RS BS
N0 N250 N0 N250
Alternate Flooding
(b)
D14
13C-SOC percentage (%)
(c)
D22
13C - SOC (mg▪pot-1)
(d)
D22
13C - SOC percentage(%)

Fig. 4

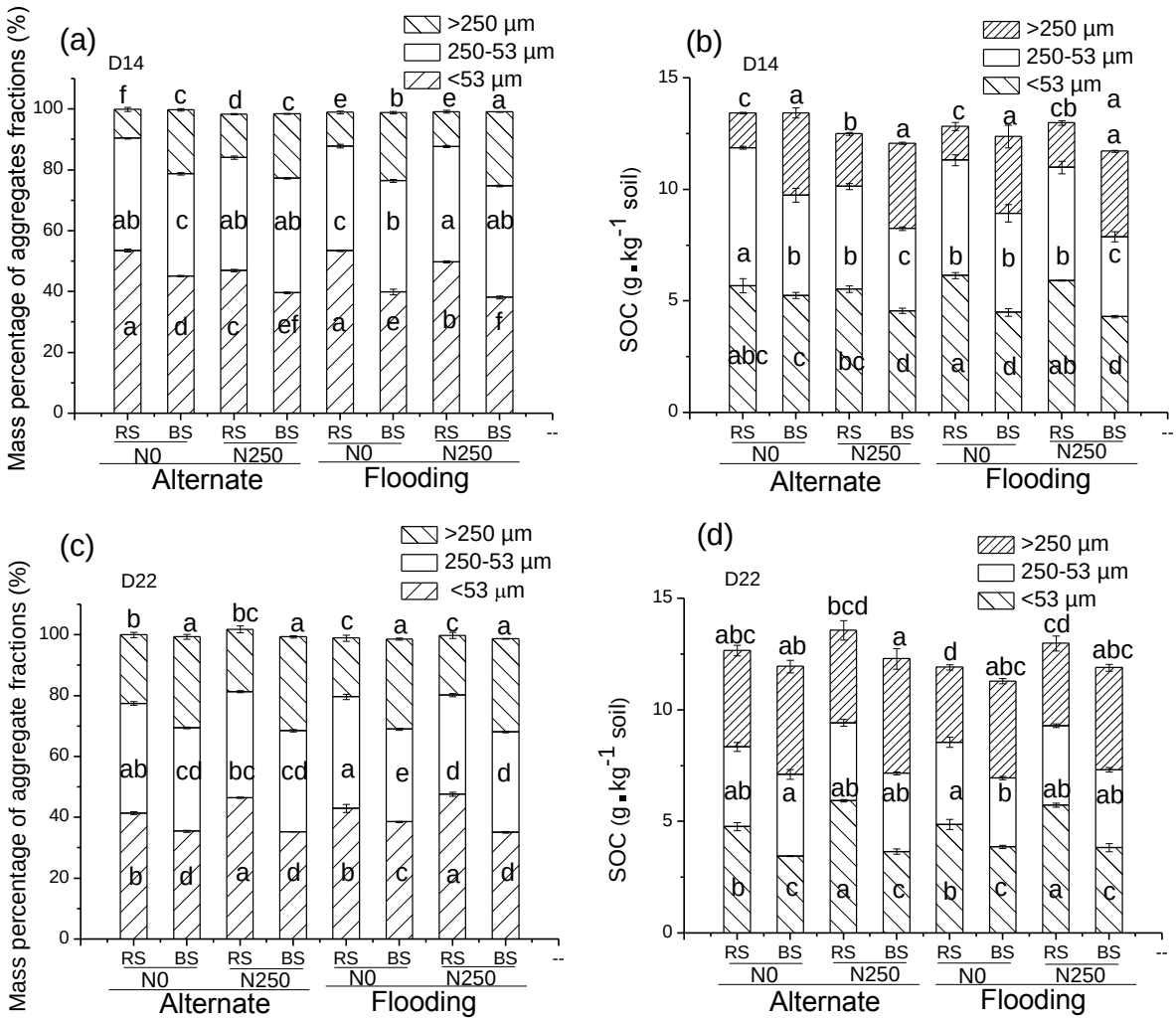

>250 µm
250-53 µm
<53 µm
Fractions' Total
y=3.86x -1.72; R2=0.39
y=2.60x -1.53; R2=0.37
y=0.69x + 0.10; R2=0.33
y=0.53x- 0.27; R2=0.37
13C-SOC in soil aggr. fractions (mg ▪ pot-1)
Root Biomass (g ▪ pot-1)
0
4
8
12
16
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0

Supplementary material

Table S1. ^{13}C-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 ^{13}C-continuous labelling experiment. Alternate: alternating flooding-drying water management; Flooding: continuous flooding; N0: no N fertiliser application; N250: urea at 250 mg N kg^{-1} soil; RS: rhizosphere soil; BS: bulk soil

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

Fig. S1. Effects of drying-rewetting cycles and nitrogen (N) fertiliser application on mass percentage and SOC, respectively, of rhizosphere soil and bulk soil aggregate fractions at two sampling days 14 (a & b) and 22 (c & d) in a ^{13}C-continous 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. 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 μm) across different treatments



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