Carbon allocation and fate in paddy soil depending on phosphorus fertilisation and water management: results of 13C continuous labelling of rice

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Carbon allocation and fate in paddy soil depending on phosphorus fertilisation and water management: results of $^{13}$C continuous labelling of rice

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We grew rice in P-deficient subtropical paddy soil in a field study and used $^{13}$CO$_2$ continuous labelling to investigate photosynthetic C partitioning and allocation under FLOOD versus WET/DRY conditions, with and without P fertilisation (80 mg P kg$^{-1}$). The plants and soil were sampled after each of three WET/DRY cycles to determine $^{13}$C allocation in above-/belowground plant biomass, microbial biomass, the rhizosphere, and bulk soil. Irrespective of water management, P fertilized plants had higher biomass and P content and more total $^{13}$C in the rice-soil system, especially the $^{13}$C incorporation into the shoots (51–96%), than samples without P fertilisation. Root and bulk-soil $^{13}$C were largely independent of both P fertilisation and water management. However, by the third sampling, P fertilisation had increased the amount of $^{13}$C and microbial biomass $^{13}$C in the rhizosphere soil by 28% (WET/DRY) and 95%, (FLOOD), and by 47% (WET/DRY) and 50% (FLOOD), respectively. The WET/DRY had significantly higher microbial biomass and 13C contents than FLOOD only in the rhizosphere soil. These results indicate that a well-established aboveground plant biomass following P fertilisation is required to increase belowground C allocation. Thus, WET/DRY, like FLOOD can provide moisture sufficient for unhindered P availability in rice-paddy system.

Key words: Microbial biomass, phosphorus deficiency, paddy soil, rice photosynthesised C, water management

**Running head:** Atere et al. – Rice C allocation depending on P and water management

**Abbreviations:** RS, Rhizosphere soil; DOC, dissolved organic carbon; TC, total carbon; TN,
total nitrogen; **MBC**, microbial biomass carbon;  
**SOC**, soil organic carbon

**INTRODUCTION**

Photosynthetic carbohydrates transfer from the leaves to the roots and the rhizosphere provide necessary energy for plant and microbial respiration, thus driving soil C dynamics (Kuzyakov and Gavrichkova 2010; Tian et al. 2013a). Aboveground processes influence C input, whereas belowground root production and associated microbial activity and processes drives the formation of soil organic matter (Milchunas et al. 1985; Kleber et al. 2007; Alvarez-Flores et al. 2014). Newly photosynthesised C is a major contributor of C input into the soil and the formation of soil organic C (SOC) (Sanderman et al. 2010). Soil organic C plays a key role in global C cycles and climate-change modulation. Thus, a quantitative assessment of belowground C allocation and partitioning in the plant, soil, and microbial components is critical for understanding their respective roles in C cycling.

In agriculture, changes in ecosystems (e.g. altering land use) and crop management affect soil C content. For example, photosynthesis-derived C is one pathway which affects SOC, and its allocation and partitioning in the plant-soil-microbial system depends on fertilisation (Saggar et al. 1997; Ge et al. 2017), plant development (Van Veen et al. 1989; Nguyen 2003; Watanabe et al. 2004a; Bais et al. 2006), and crop type (Zagal 1994). Therefore, efforts to increase crop yields should simultaneously increase belowground carbon stocks.

The application of P fertiliser is one of the most important crop management practices (Cordell et al. 2009; Singh et al. 2013). As a nucleic acid component, P regulates plant performance and energy storage as well as ecosystem C and N cycles (Lin et al. 2009; Wang et al. 2013b; Zeng and Wang 2015). Meanwhile, P is frequently lost from soil through removal during harvest, soil erosion, leaching to groundwater, and occlusion in highly
weathered soils (Falkowski et al. 2000; Vitousek 2004; Elser et al. 2007). Thus, P is often a limiting nutrient for crop production (Vitousek and Sanford, 1986; Tanner et al. 1998; Hall and Matson 1999; Sánchez 2010) and microbial activity (Liu et al. 2011; Mori et al. 2013). The critical P value for crop yield is known to vary with factors such as soil properties, crop type and target yield, and climate among others (Colomb et al. 2007; Tang et al. 2009). In China, for example, the critical soil Olsen P levels for optimal rice yield range from 10–20 mg kg\(^{-1}\) in the south, and from 11–21 mg kg\(^{-1}\) in the southwest, respectively (Zhang et al. 2008; Bai et al. 2013). Further, an optimum soil Olsen P of 20 mg P kg\(^{-1}\) is considered as the minimum limit for most field crops (Li et al. 2011). Based on the differences in soil properties, however, soil Olsen P levels of 9 mg kg\(^{-1}\) and 39 mg kg\(^{-1}\) are recommended for crop growth in Northeast and South China, respectively (Li et al. 2015). Numerous studies have examined the effects of fertilisation on the allocation of plant-derived C and soil microbial activity in upland soils. For example, high N fertilisation rates through increased shoot and root biomass resulted in higher rice rhizodeposition in paddy soil (Liu et al. 2014; Ge et al. 2015; Ge et al. 2017). Further, a higher rate of 35 kg P ha\(^{-1}\) resulted in the largest soybean yield, while a lower rate of 18 kg P ha\(^{-1}\) led to the highest soil microbial P (Liu et al. 2008). The addition of P also improved plant root growth and N uptake (Bowatte et al. 2006). Supplying P to a P-limited ecosystem might alter the plant leaf N:P ratio, a value indicative of net primary production and soil C dynamics because it correlates with plant photosynthetic capacity, photosynthetic N-use efficiency, and relative growth rate (Wright et al. 2005; Wang et al. 2013b). Comparatively, fewer studies have addressed the effects of P fertilisation on the flow of plant-derived C to paddy soils. One such study demonstrated that a low P application rate (0.5 kg ha\(^{-1}\)) , by increasing root biomass, stimulated rice root exudation by 1.3–1.8 or 2.1–2.4 fold as compared to a medium P (5 kg ha\(^{-1}\)) or high P supply (10 kg ha\(^{-1}\)) (Lu et al. 1999). Overall, the effects of P fertilisation could vary widely with ecosystems, crop types,
and land management practices. Hence, in this study, we investigated the effect of P fertilisation in rice-paddy soil systems.

Water management is another major crop management practice that requires particular attention. Irrigated agriculture accounts for over 70% of total water use globally, and irrigated land produces more than 75% of China’s grain (Xiong et al. 2010). Nevertheless, increasing competition in water use from the urban and industrial sectors have heavily decreased the fresh water available for irrigation (Xiong et al. 2010; Wang et al. 2013a). This problem is likely to worsen because non-agricultural sectors typically receive more attention (van der Hoek et al. 2001). Thus, effective water management in agriculture is critical for sufficient crop productivity under decreasing water availability.

Paddy soil is an ideal environment for investigating the interactive effects of P input and water management on photosynthesised C. As the world’s largest anthropogenic wetland (Kögel-Knabner et al. 2010), paddy soils occupy one-third of the world’s total cultivated land and provide over 75% of global rice production (van der Hoek et al. 2001). They play a key role in terrestrial C sequestration; China’s paddy soils for instance, are believed to have 3.0 Pg SOC sequestration potential (Pan et al. 2003; Tian et al. 2013b). Both intensive P (and N) fertilization and substantial amounts of water through irrigation are commonly needed to achieve high yields in rice cultivation (Zhang et al. 2015). Besides fertilization, several water management strategies, including continuous flooding (FLOOD), alternate wetting and drying (WET/DRY), and mid-season flooding and drainage, have been adopted for rice cultivation (Yao et al. 2012a; Tian et al. 2013a, b; Xu et al. 2015; Peyron et al. 2016; Atere et al. 2017). In particular, WET/DRY, when compared to FLOOD management, appears to increase rhizodeposition via higher root activity, more efficient nutrient use, and greater biomass production (Mishra and Salokhe 2011; Tian et al. 2013b; Atere et al. 2017). However, the higher microbial activity and rhizodeposit use found under WET/DRY

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conditions than under FLOOD conditions (Tian et al. 2013a) could cause substantial loss of rhizodeposited C through microbial respiration (Leake et al. 2006; Zhu and Cheng 2012). Despite these reports, there is limited information on the influence of combined water management and P fertilisation of rice C allocation in paddy soils.

During rice cropping seasons, paddy systems typically experience anaerobic conditions under flooding, with frequently fluctuating redox potential. Flooding depletes oxygen from bulk topsoil because oxygen diffuse slower through water than air (Gao et al. 2002; Tanji et al. 2003). Moreover, post-flooding pH is always nearly neutral, irrespective of initial pH (Sahrawat 2005). Variation in both soil pH and anoxic conditions can directly or indirectly influence P solubility and sorption/desorption (Maranguit et al. 2017). For example, under flooded conditions, ferric (Fe$^{3+}$) phosphate is reduced to ferrous (Fe$^{2+}$) phosphate, leading to increased P availability from insoluble Fe compounds (Snyder and Slaton 2002). Photosynthetic C allocation/partitioning may alter in response to the individual and combined effects of water management (FLOOD versus WET/DRY) and P fertilisation. Considering the negative consequences under global climate change, there is an increasing need for better management of soil C. To enable this, we must improve the current knowledge of how anthropogenic P input (e.g. fertilization) and water management regulate the dynamics of plant-derived C. Numerous studies have showed the effectiveness of the $^{13}$C-CO$_2$ continuous labelling technique in quantifying photosynthetic C assimilation and partitioning and rhizodeposition (Meharg and Killham 1991; Kuzyakov and Schneckenberger 2004). Continuous, unlike pulse labelling has the additional advantage of being homogeneous in all plant C pools (Martin et al. 1992). Hence, in this study, we employed continuous $^{13}$C-CO$_2$ labelling at the vegetative stage of rice growth. This stage of rice growth often requires good fertilisation to supply adequate nutrients for plant performance and is known for allocating more assimilates belowground when compared to other growth stages (Nguyen 2003). The
important questions for this study are: how does P fertilisation affect the allocation of rice-derived C in paddy soils; and does this effect vary under FLOOD versus WET/DRY water management? Thus, the objectives were to investigate the combined effects of P fertilisation and two water-management practices (FLOOD versus WET/DRY) on (i) above- and belowground rice biomass, and (ii) C allocation among the shoots, roots, rhizosphere, bulk soil, and microbial biomass. We hypothesised that: (1) P input into P-limited soil through increased plant growth will lead to an overall higher belowground allocation of photoassimilated C by rice compared to plants without P fertilisation. (2) Less photoassimilated C will remain in the soil under WET/DRY conditions than in FLOOD conditions. (3) WET/DRY management (due to increased aerobic conditions) and P fertilisation lead to a higher microbial assimilation of plant-derived C than FLOOD management.

MATERIALS AND METHODS

Site Description

Typical Stagnic Anthrosol (Gong et al. 2009) was collected from the plough layer (0–20 cm) of a rice field at Changsha Research Station for Agricultural and Environmental Monitoring (113°19′52″E, 28°33′04″N, 80 m a.s.l.), China. The climate of this area is subtropical, with a mean annual temperature of 17.5°C, an annual precipitation of 1,300 mm, 1,663 annual sunlight hours, and a frost-free period of up to 274 d. Soil properties were as follows: pH 5.43 (1:2.5, soil/water ratio), 14.26 g organic C kg⁻¹, 1.45 g total N kg⁻¹, 0.75 g total P kg⁻¹, 12.75 mg Olsen P kg⁻¹ and 7.71 cmol CEC kg⁻¹. Particle-size distribution was 280 g kg⁻¹ sand (>50 µm), 660 g kg⁻¹ silt (2–50 µm), and 60 g kg⁻¹ clay (<2 µm).
Experimental Design

Soil was sieved (<4 mm) to remove coarser particles, and then air-dried. Soil was treated with a basal application of urea (250 mg N kg\(^{-1}\), equivalent to 560 kg N ha\(^{-1}\)), KCl (80 mg K kg\(^{-1}\), equivalent to 179 kg K ha\(^{-1}\)), and dicyandiamide (nitrification inhibitor, 26 mg N kg\(^{-1}\) equivalent to 58 kg P ha\(^{-1}\)) before being measured into pots and rhizosphere bags. Thirty-six pots (11 cm inner diameter and 20 cm height) were individually filled with ~1.26 kg (oven-dry basis) soil. A rhizosphere bag (30 µm mesh, 3.5 cm × 15 cm) filled with 0.34 kg soil was buried in the middle of each pot. The bag allows the movement of nutrients and water but does not allow root penetration into the bulk soil (Su and Zhu 2008; Finzi et al. 2015). Soil in half (18) of the pots also received 80 mg P kg\(^{-1}\) (179 kg P ha\(^{-1}\)) soil as NaH\(_2\)PO\(_4\), which was applied the same way as the basal nutrients. Two rice (\textit{Oryza sativa} L.) 2-line hybrid (‘Zhongzao 39’) seedlings at the third tillering stage were transplanted into each bag. Soils in all pots were initially saturated with distilled water (with a 2–3 cm water layer above the soil surface) and divided evenly between WET/DRY or FLOOD water management. Under the WET/DRY conditions (which commenced at the start of \(^{13}\)CO\(_2\) labelling), soils were subsequently dried for 3–4 days until a 70–75% water holding capacity was reached (WHC), and then flooded again. Thus, as is mostly practised under WET/DRY management, the soil was maintained at a level of near-saturation to saturation throughout this study (Carrijo et al. 2017). The initial water level of 2-3 cm was maintained for the FLOOD treatment throughout the experiment. A total of three drying-rewetting cycles were implemented before the study was terminated at the plant maximum tillering stage. Two P fertilisation rates were also examined: no P addition (-P) or 80 mg P kg\(^{-1}\) soil as NaH\(_2\)PO\(_4\) (+P). Each P rate was assigned to 18 pots (nine pots for each water management system). The experimental set-up followed a full factorial design, comprising two main factors (water management and P fertilisation) at two levels each. Thus, there were four treatments having three replicates each.
**$^{13}$CO$_2$ Continuous Labelling**

To trace above- and belowground rice-photosynthesised C allocation, a uniform $^{13}$C labelling of rice plants was done following Ge et al. (2012, 2015). Briefly, rice plants (in pots) were placed in a climate-controlled, air-tight glass chamber (80 × 250 × 120 cm height) that was set in a rice field to experience a natural environment. The rice shoots in the chamber were exposed to $^{13}$CO$_2$ through the reaction of NaH$^{13}$CO$_3$ (50 atom percent $^{13}$C, 1.0 M) with H$_2$SO$_4$ (0.5 M). The CO$_2$ concentration within the chamber was maintained at 360–380 µL CO$_2$ L$^{-1}$ by further reactions or gas-flow diversion through CO$_2$ traps (1.0 M NaOH solution) that absorbed the excess gas. Two fans continuously circulated the air in the labelling chamber and two temperature/humidity sensors (SNT-96S, Qingdao, China) monitored conditions inside and outside the chamber. The chamber temperatures were kept within 1°C of the external ambient temperature using a data-logger script that activated air conditioning whenever the threshold was exceeded. The labelling lasted for 22 days covering three complete drying-rewetting cycles. An additional 36 pots outside the labelling chambers, about 10–15 m away and under the same water and fertilisation management conditions, served as the unlabelled controls to determine the natural abundance of $^{13}$C. Only the vegetative stage of rice growth was tested in the present study as photosynthetic C allocation varies with plant age (Lu et al. 2002a).

**Sampling and Measurements**

Plants and soil samples were collected at the end of every drying-rewetting cycle just before re-watering in the DRY/WET treatments. These corresponded to 6, 14, and 22 days after the start of continuous labelling. Shoots were harvested by severing at the stem base. The
rhizosphere bags with enclosed roots were then removed from the pots. Roots were carefully separated, gently agitated (for 1.0 min with 0.01 M CaCl₂, pH 6.2) to remove adhering soil, and then thoroughly rinsed under running tap water. The roots, shoots, and two soil sub-samples (rhizosphere soil [RS] inside the bag and bulk soil outside the bag) were weighed, oven-dried at 60°C to a constant weight, pulverized, and ball-milled for total C (TC) and ¹³C analyses. The remaining soil was temporarily stored at 4°C for further total N (TN), TC, and ¹³C determination.

**Analytical Procedures**

Soil pH was determined using a pH meter (Delta 320; Mettler-Toledo Instruments Co., Ltd., China) in a 1:2.5 soil/water ratio. Soil-particle size distribution was measured with a laser particle-size analyser (Mastersizer 2000; Malvern Instruments Ltd., UK). The stable C isotope ratio (¹²C/¹³C) and total C content of all plant and soil 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; measurement precision: 0.5‰). Soil microbial biomass C (MBC) was measured with the fumigation-extraction method (Wu et al. 1990). Resultant extracts were freeze-dried, then ground to a fine powder with a pestle and mortar for isotope measurements. Available Fe (II) was extracted with HCl (0.5 M) and determined via a 1,10- phenanthroline colorimetric assay (Fadrus and Malý 1975). Dissolved organic C (DOC) was extracted with 0.5 M K₂SO₄ for 1.0 h, centrifuged at 3,000 rpm for 15 min, and then filtered through Walkman 57 filter paper. Extracts were freeze-dried and used for ¹³C measurement.

**Calculations and Statistical Analyses**
The $^{13}$C amount ($^{13}$C$_{\text{sample}}$) (mg C pot$^{-1}$ or mg C m$^{-2}$) was calculated using the following equation:

$$^{13}$C$_{\text{sample}} = [(\text{atomic } ^{13}$C\%)$_{\text{l}} - (\text{atomic } ^{13}$C\%)$_{\text{nl}}]_{\text{sample}} \times TC_{\text{sample}}/100 \text{ (Lu et al., 2002)} \text{(1)}$$

where subscripts ‘l’ and ‘nl’ indicate labelled and non-labelled samples, respectively, while ‘TC’ is the total C content in a labelled sample.

The proportion of $^{13}$C (%) incorporation in shoots, roots, soil, and microbial biomass per sampling day was expressed as the ratio of $^{13}$C distribution in each pool to the total $^{13}$C in all three pools combined, multiplied by 100.

The rhizosphere effect (RE) was calculated for each treatment per sampling day as the ratio of rhizosphere soil $^{13}$C and bulk soil $^{13}$C.

$^{13}$C incorporated in the microbial biomass ($^{13}$C-MBC) was calculated as the difference in $^{13}$C between fumigated and unfumigated soil extracts, divided by 0.45 (Lu et al. 2002b; Ge et al. 2016):

$$^{13}$C-MBC = \frac{[(\text{Atomic } ^{13}$C\%)$_{\text{f, L}} - (\text{Atomic } ^{13}$C\%)$_{\text{f, UL}}] \times C_{\text{f}} - [(\text{Atomic } ^{13}$C\%)$_{\text{uf, L}} - (\text{Atomic } ^{13}$C\%)$_{\text{uf, UL}}] \times C_{\text{uf}}}{100/0.45} \text{ (Lu et al., 2002)} \text{(2)}$$

where (atomic $^{13}$C\%) is the percentage of atomic $^{13}$C in soil extracts and C is the total C of soil extracts. Subscripts ‘f’, ‘uf’, ‘L’, and ‘UL’ indicate fumigated, unfumigated, labelled, and unlabelled samples, respectively.

All data are expressed as the mean of three replicates ± SE. Multivariate ANOVAs with Duncan’s tests were used to determine differences at $p < 0.05$ in the variables’ response to water management and P fertilization. Data were analysed using the PROC MIXED procedure in SAS 9.2 software (SAS Institute Inc., Cary, NC) considering water and P treatments and their interactions as fixed factors, replicates/blocks as an error source.
RESULTS

Responses of Plant Biomass, Total P, and C/P Ratio to P Fertilisation and Water Management

Shoot biomass was higher with than without P fertilisation by differences that ranged from 16–42% in the first sampling to 41–71% in the third sampling (p < 0.05, Fig. 1a–c). Root biomass did not differ between fertiliser and water management levels in the first two sampling events (Fig. 1d & e). During the third sampling, however, P fertilisation increased root biomass by 34–66% (p < 0.05, Fig. 1f). Because shoot biomass increased relatively more than root biomass, the root/shoot biomass ratio was lower with than without P fertilisation, especially in the first sampling event (p = 0.03). Shoot and root total P (TP) was higher with than without P fertilisation in all sampling events (Fig. 2a–c). While the increase due to P fertilisation from the three sampling events ranged from 80–147% in the shoot TP (p < 0.01), the increase (34–147%) in the root TP was more prominent in the third sampling (p = 0.04). This increase in plant TP resulted in a decrease in the shoot and root C/P ratio with P fertilisation in all sampling events (p ≤ 0.05, Fig. 2d–f). All the above P-induced differences were independent of water management (Tables S1 & S2).

13C Distribution in the Rice-Soil System, and Fe2+ in Soil Solutions Following P Fertilisation and Water Management

The amount of shoot 13C was 51–96% higher with than without P fertilisation across all sampling events (p ≤ 0.05, Fig. 3a–c). In contrast, root 13C remained largely unaffected by fertilisation (Fig. 3a–c). The amount of rhizosphere soil 13C was unaffected by P fertilisation in the first two sampling events (Fig. 3d & e). This trend, however, changed in the third sampling event, with increases of 28% (WET/DRY) and 95% (FLOOD) with P fertilisation (p < 0.05, Fig. 3f). Bulk-soil 13C was independent of P fertilisation across all sampling
events. The overall $^{13}$C amount in the rice-soil system increased until the third sampling and was higher with than without P fertilisation, regardless of water management ($p < 0.05$, Fig. S1, Table S8).

The proportion of $^{13}$C allocated to the shoots was higher with than without P fertilisation in all sampling events, increasing by 17–32% in the first two samplings ($p < 0.01$, Fig. 4a–c). The allocation of $^{13}$C to the roots in as a % of assimilated C (proportion) did not follow a definite pattern and was mostly similar with and without P fertilisation. The proportion of $^{13}$C allocated to the rhizosphere soil decreased by 37–56% with P fertilisation in the earlier two sampling events (Fig. 4a & b). Meanwhile, there was a relative increase of the proportion with P addition in the third sampling compared to the earlier two samplings events (Fig. 4c), due to the higher $^{13}$C amount in the rhizosphere (Fig. 3f). Thus, $^{13}$C proportion was similar between P and no P in the third sampling event. In the bulk soil, the allocation proportions of $^{13}$C were similar between the P and no P fertilization treatments in most sampling events.

Rhizodeposition per unit rice biomass C ($^{13}$C-SOC in rhizosphere/rice biomass C) was lower with than without P fertilisation in the first two sampling events ($p = 0.01$; Fig. 5a & b). But, with increased rhizodeposition under P fertilisation (Fig. 3f), the trend changed in the third sampling event, giving similar results (rhizodeposition per unit rice biomass) between the P and no P fertilization treatments (Fig. 5c). The rhizosphere effect was higher with P fertilisation (20–108%; $p < 0.05$) than without P in every sampling event. All measured plant and soil $^{13}$C levels were similar between the two water management treatments.

Rhizosphere $^{13}$C-DOC was 17–89% higher with than without P fertilisation ($p < 0.05$, Fig. S2a–c), and 29–98% higher ($p < 0.05$) under FLOOD conditions than WET/DRY conditions. The effects of P fertilisation and water management on bulk-soil $^{13}$C-DOC, though not as pronounced, were similar to those in the rhizosphere soil.

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The Fe$^{2+}$ in soil solution was 1.1–4.0 fold lower in the rhizosphere soil and 0.9–4 fold lower in the bulk soil ($p < 0.05$, Fig. S2d–f) with than without P fertilisation. Fe$^{2+}$ was also higher under FLOOD conditions than under WET/DRY conditions ($p < 0.05$).

**Effects of P Fertilisation and Water Management on MBC**

Compared to samples without added P, P fertilisation significantly increased the MBC in the rhizosphere by 17–131% and in the bulk soil by 13–72% over the period covering the second and third sampling events ($p < 0.05$; Fig. 6a & c). Additionally, water management and its interaction effect with P fertilisation resulted in higher MBC content in WET/DRY soil than in FLOOD soil by 53–130% (rhizosphere soil) and 67–83% (bulk soil) ($p < 0.05$, Table S6).

**Effects of P Fertilisation and Water Management on $^{13}$C in Microbial Biomass**

Rhizosphere $^{13}$C-MBC was lower with than without P fertilisation in the first sampling event, but the trend gradually reversed until P fertilisation increased $^{13}$C-MBC by 47–50% in the third sampling event ($p < 0.05$) (Figs. 7a–c). Additionally, $^{13}$C-MBC was higher under WET/DRY conditions than under FLOOD conditions, especially in the first and third sampling event ($p < 0.05$). Bulk-soil $^{13}$C-MBC was similar regardless of P fertilisation or water management.

The proportion (%) of $^{13}$C followed a similar pattern as its content ($\mu$g C Kg$^{-1}$) in MBC. The proportion of $^{13}$C-MBC in the rhizosphere was lower with than without P fertilisation in the first sampling, but then increased until it was 43–45% higher ($p < 0.05$) in the third sampling event (Figs. 7d–f). The proportion of $^{13}$C-MBC in the bulk-soil was also higher under P fertilisation in the third sampling event ($p < 0.05$). Further, a higher proportion
of $^{13}$C was observed in the MBC under WET/DRY conditions than under FLOOD conditions in the first and third sampling events ($p < 0.05$).

**Relationships Between Plant Biomass and $^{13}$C-SOC and Between $^{13}$C-SOC and $^{13}$C-MBC**

The $^{13}$C-MBC was positively correlated with $^{13}$C-SOC in both the rhizosphere soil [$R^2 = 0.38$ (-P); $R^2 = 0.50$ (+ P)], and the bulk soil [$R^2 = 0.29$ (-P); $R^2 = 0.71$ (+P)] ($p < 0.01$) (Fig. 8a & b). The $^{13}$C-SOC was positively correlated with plant biomass production in the shoot [$R^2 = 0.82$ (-P), $R^2 = 0.84$ (+P), and in the root [$R^2 = 0.79$ (-P); $R^2 = 0.89$ (+P)] ($p < 0.0001$) (Fig. 8c & d).

**DISCUSSION**

**Effects of Water Management with Respect to P Fertilisation on Rice Growth and $^{13}$C Distribution in the Rice-Soil System**

Previous studies have reported rice photosynthetic-C dynamics in paddy soils under varying N fertilisation and water management strategies (Tian et al. 2013a, b; Atere et al. 2017), but studies examining P fertilisation and water management are limited. Previous research has demonstrated improved P solubility and diffusion under flooded conditions (Snyder and Slaton 2002; Shankar et al. 2005). Thus, plant growth and photoassimilate production would be increased, and more assimilates could be partitioned belowground and remain in the soil under FLOOD conditions. Moreover, organic C turnover is slower under FLOOD conditions than WET/DRY conditions (Tian et al. 2013b) because the wetter conditions suppress microbial activity (Li et al. 2011b; Qiu et al. 2017; Xu et al. 2017), providing further support
for our prediction. Improved rhizodeposition under WET/DRY conditions has been partly attributed to the stimulatory effect of water stress (Tian et al. 2013b) and improved oxygen availability for root activities (Mishra and Salokhe 2011). It was not clear, however, whether this improved rhizodeposition was accompanied by better retention of the rhizodeposited C in the soil under these conditions. This is because higher microbial activity and rhizodeposit use under WET/DRY conditions than under FLOOD conditions (Tian et al. 2013a) could both result in more stabilized microbial by-product production (Averill 2016) and/or substantial loss of rhizodeposited C through microbial respiration (Leake et al. 2006; Zhu and Cheng 2012). However, the evidence did not support our expectation of increased belowground assimilates under FLOOD conditions, as the different water management strategies displayed similar effects on rice growth parameters in our study (Fig. 1). The higher Fe$^{2+}$ levels under FLOOD conditions than under WET/DRY conditions (Fig. S2d–f) would seem to be an indication of more Fe-occluded P release and thus enhanced uptake by plant and resultant higher growth and photosynthate partitioning. However, a higher DOC was also observed under FLOOD conditions than under WET/DRY conditions (Figs. S2a–c). The DOC in the soil solutions, though it varied with soil properties, increased with an increase in the soil:solution ratio (Pampura and Ustinin 1996). High DOC content could mean more C loss in the form of CH$_4$ into the atmosphere (Lu et al. 2000; He et al. 2015), thereby reducing the synergetic effect of FLOOD + P fertilisation on belowground C allocation. On the other hand, P availability is a potential challenge in crops (soybeans, corn, wheat, etc.) following rice harvest due to drainage and adsorption of soluble phosphates to amorphous iron compounds (Snyder and Slaton 2002). However, this phenomenon seemed not to be a challenge under the WET/DRY conditions in the current study, indicating a sufficient (near-saturation) soil moisture status for the dissolution of Fe-occluded P. Thus, similar results for rice growth and
assimilate distributions were obtained under the two tested water management strategies, following P fertilisation.

**Effects of P Fertilisation on $^{13}$C Distribution in the Rice-Soil System**

We hypothesised that P input into P-limited soil would increase rice photosynthesised C and its belowground allocation. Overall, the total photosynthate amount in the plant-soil system generally increased with plant growth and was higher under P fertilisation, independent of water management (Fig. S1). The overall pattern deviated slightly in C allocation to the plant and soil pools (Fig. 3a–f). Specifically, recent photoassimilates were increased in the shoots (51–96%) under P fertilization (Fig. 3a–c), possibly to sustain P-induced increases in plant biomass (Fig. 1a–f & 2a–c). Photoassimilates can be retained for new shoot production or stored as starch in the aboveground plant part (Leake et al. 2006). Notably, the P-induced increase in biomass did not concurrently lead to more belowground translocation of photosynthates (Figs. 3d & e). While the amount of assimilates in the roots did not differ with fertilisation, higher levels of assimilates (28–95%) were only found in the rhizosphere soil at a later plant growth stage (third sampling) under P fertilisation (Fig. 3f). This result was also corroborated by the increased proportion of photoassimilates in the shoots, accompanied by no change in the roots (Figs. 4a–c), and the decrease in both the rhizosphere and the bulk soil in first two samplings (Figs. 4d–e) under P fertilisation. Further, the assimilate per unit biomass partitioned belowground was lower with than without P fertilisation (Figs. 5a–c). Thus, the earlier results (Figs. 3a–e) showed preferential partitioning of assimilates aboveground with P fertilisation. Only the result of the last sampling event (Fig. 3f) supported our hypothesis that P fertilisation would increase photoassimilate translocation belowground. However, such effects would not be apparent until later in the rice growth cycle. The observed trend in the earlier samplings could be due to a number of reasons. The
stronger rhizosphere effect on rhizodeposition observed under P compared with no P fertilisation (Figs. 5d–e) could indicate higher rhizo-microbial activity and thus increased soil respiration (Cleveland and Townsend 2006; Ouyang et al. 2015). Hence, comparatively fewer assimilates were transferred to the bulk soil with than without P fertilisation.

Furthermore, the DOC was higher with than without P fertilisation. The DOC in the rice root zone, being enriched by root-derived C, and its positive correlation with CH$_4$ emissions, could indicate higher C loss (Lu et al. 2000; Hu et al. 2018; Wu et al. 2018). Moreover, given the decreased Fe$^{2+}$ in the soil solution under P fertilisation, Fe$^{3+}$ could have occluded part of the applied P under any available oxic conditions in the soil, preventing immediate plant uptake. Further results (Fig. 8c & d) showed that $^{13}$C-SOC was positively correlated with shoot and root biomass under both P and no P fertilisation treatments, suggesting that even without P, rice was still able to shift shoot resources to the roots and soil for optimal functioning, resulting in effects comparable to P addition. A higher resource (photosynthates) allocation belowground in plants is a means of coping with stresses including low soil fertility (Saggar et al. 1997; Singh et al. 2013; Thuynsma et al. 2014). Plants can rely on soil microbiota to drive nutrient cycling under low nutrient availability, facilitating C flow from the roots to the soil (Li et al. 2011b; Richardson et al. 2011; Paterson et al. 2007). Thus, this microbial-driven nutrient supply may also have played a role in minimizing the differences between the P and no P fertilisation treatments in the earlier sampling events. The result from the third sampling, however, showed that under P fertilisation, higher belowground allocation of plant-assimilated C could be attained, following a well-established aboveground plant part. This conclusion is based on the increased plant biomass production also recorded at this sampling point, indicating the capability of the plants for overall higher assimilate production and allocation under larger biomass.
Effects of Water Management with Respect to P Fertilisation on MBC and $^{13}$C Allocation to Microbial Biomass

The microbial uptake and use of C plays a key role in root-derived C stabilisation (He et al. 2015) and stable SOC formation in paddy soil (Zhu et al. 2016). In our study, the WET/DRY with P fertilisation treatment elevated MBC in both the rhizosphere and bulk soil (Figs. 6b & c). Earlier reports indicated that prolonged flood-related anaerobic conditions decrease some soil microorganisms and alter their community structure (Ge et al. 2012). As earlier samplings revealed very little photoassimilate incorporation into soil and microbial biomasses under P fertilisation, positive priming could have caused the subsequent elevation (17–131%) of MBC (Kuzyakov et al. 2000). The priming effect of the little rhizodeposited C (on native SOC), coupled with a more oxygenated environment provided by the WET/DRY condition, could enhance microbial decomposition of the native SOC.

Our third hypothesis was that the WET/DRY and P fertilisation treatment, would result in higher incorporation of photoassimilates into the microbial biomass than the FLOOD and no P treatment. Observed patterns in photoassimilate amount (Fig. 7a–c) and proportion (Fig. 7d–f) in the microbial biomass partially supported this hypothesis. The WET/DRY irrigation method indeed increased the incorporation of recent photoassimilates into the rhizosphere soil microbes (Figs. 7a–f), possibly due to a more aerobic environment that enhanced microbial activity and efficiency in the use of rhizodeposited C (Tian et al. 2013a). However, data from the first two samplings showed that P fertilisation lowered the microbial assimilation of rhizodeposited C (Figs. 7a–c). We attributed this contrary outcome to the decreased rhizodeposition under P fertilisation during earlier growth stages. But, over time, as plant growth continued and biomass production increased, heightened rhizodeposition occurred and thus more microbial incorporation of rhizodeposited C under P fertilisation
(Fig. 7c & f). This result is corroborated by data from P-fertilised soil, showing a stronger correlation between photoassimilates in the rhizosphere and in those in the microbial biomass than those from without P fertilisation. Thus, the interaction between P fertilisation and WET/DRY irrigation heightened microbial rhizodeposit use over the course of plant growth, compared to the interaction between P fertilisation and FLOOD irrigation. While there are limited studies on the combined effect of P fertilisation and water management, our study corroborated some past studies who found heightened microbial utilisation of rhizodeposits under alternating wet/dry conditions as compared with that under continuous flooding conditions (Tian et al. 2013a; Yao et al. 2012b; Yuan et al. 2016).

CONCLUSIONS

This study provided insight on how water management and P fertiliser could alter the dynamics of photosynthates in a P-limited rice-soil system. Phosphorus fertilisation increased the aboveground plant biomass and shifted photosynthetic allocation aboveground in the early vegetative growth stages of rice. Higher partitioning of assimilates belowground with than without P fertilisation occurred only at a later growth stage, along with a concurrent increase in plant biomass. Water management did not alter the response of rice (either growth or the allocation of photosynthetic C in the plant-soil system) to P fertilisation. However, WET/DRY conditions caused higher photoassimilate incorporation into microbial biomass than FLOOD conditions, irrespective of fertilisation. Therefore, rice photosynthetic C allocation and its response to P availability in paddy soil might not vary significantly between the WET/DRY and FLOOD water management strategies. In conclusion, P fertilisation, through establishing stronger aboveground plant parts can achieve increased belowground allocation of plant-assimilated C. The water saving irrigation technique tested in this study
(WET/DRY) should not reduce P availability or the content of rice-assimilated C in paddy soil.

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REFERENCES


Bai, Z., Li, H., Yang, X., Zhou, B., Shi, X., Wang, B., Li, D., Shen, J., Chen, Q., Qin, W.,


Pampura, T., and Ustinin, M. 1996. Effect of succinic acid produced by microorganisms and


dynamics of assimilated carbon in rice-soil system depending on water management.


Wang, J., Hui, D., Ren, H., Liu, Z., and Yang, L. 2013b. Effects of understory vegetation and litter on plant nitrogen (N), phosphorus (P), N:P ratio and their relationships with growth rate of indige


trait relationships. New Phytol. 166: 485–496.


Zagal, E. 1994. Carbon distribution and nitrogen partitioning in a soil-plant system with
barley (Hordeum vulgare L.) ryegrass (Lolium perenne) and rape (Brassica napus L.)
grown in a $^{14}$CO$_2$-atmosphere. Plant Soil **166**: 63–74.

Zeng, W., and Wang, W. 2015. Combination of nitrogen and phosphorus fertilization
enhance ecosystem carbon sequestration in a nitrogen-limited temperate plantation of

mycorrhiza and fertilizer management reduce phosphorus runoff from paddy fields? J.
Environ. Sci. (China) **33**: 211–218.

Zhang, W., Ma, W., Ji, Y., Fan, M., Oenema, O., and Zhang, F. 2008. Efficiency, economics,
and environmental implications of phosphorus resource use and the fertilizer industry in

Zhu, B., and Cheng, W. 2012. Nodulated soybean enhances rhizosphere priming effects on
soil organic matter decomposition more than non-nodulated soybean. Soil Biol.
Biochem. **51**: 56–65.

Zhu, Z., Zeng, G., Ge, T., Hu, Y., Tong, C., Shibistova, O., He, X., Wang, J., Guggenberger,
G., and Wu, J. 2016. Fate of rice shoot and root residues, rhizodeposits, and microbe-
assimilated carbon in paddy soil - Part 1: Decomposition and priming effect.
Biogeosciences **13**: 4481–4489.
**Figure captions**

**Fig. 1** Biomass of rice plants (shoots, a–c; roots, bars of d–f and root/shoot biomass ratio, triangle symbols of d–f) following P fertilisation under two water regimes (alternate wetting and drying: WET/DRY versus continuous flooding: FLOOD) and $^{13}$C continuous labelling. Sampling events S1, S2, and S3 correspond to the end of the first, second, and third drying-rewetting cycles (6, 14, and 22 days after the start of continuous labelling), respectively. +P or –P, with or without 80 mg P kg$^{-1}$ soil as NaH$_2$PO$_4$. Data (means ± SE, n = 3) that do not share a lowercase letter indicate differences ($p < 0.05$) between variables, in response to water management and P fertilization.

**Fig. 2** Total phosphorus (TP) (a–c) and C/P molar ratio (d–f) in plant biomass following P fertilisation under two water regimes (alternate wetting and drying: WET/DRY versus continuous flooding: FLOOD) and $^{13}$C continuous labelling. All variables are as defined in Fig. 1. The sections of the graphs below ‘zero’ are positive.

**Fig. 3** Total $^{13}$C in the rice-soil system: rice (a–c), and soil (d–f) following P fertilisation under two water regimes (alternate wetting and drying: WET/DRY versus continuous flooding: FLOOD) and $^{13}$C continuous labelling. All variables are as defined in Fig. 1. The section of the graph below ‘zero’ is positive.

**Fig. 4** $^{13}$C percentage in rice-soil system (a–c) following P fertilisation under two water regimes (alternate wetting and drying: WET/DRY versus continuous flooding: FLOOD) and
$^{13}$C continuous labelling. All variables are as defined in Fig. 1. The section of the graph below ‘zero’ is positive

**Fig. 5** $^{13}$C in rhizosphere soil/rice biomass C (a–c) and the rhizosphere effect (d–f) following P fertilisation under two water regimes (alternate wetting and drying: WET/DRY versus continuous flooding: FLOOD) and $^{13}$C continuous labelling. All variables are as defined in Fig. 1

**Fig. 6** Microbial biomass carbon (MBC) (a–c) following P fertilisation under two water regimes (alternate wetting and drying: WET/DRY versus continuous flooding: FLOOD) and $^{13}$C continuous labelling. All variables are as defined in Fig. 1. The sections of the graphs below ‘zero’ are positive

**Fig. 7** $^{13}$C-MBC (microbial biomass C) (a–c) and $^{13}$C recovery in MBC (d–f) following P fertilisation under two water regimes (alternate wetting and drying: WET/DRY versus continuous flooding: FLOOD) and $^{13}$C continuous labelling. All variables are as defined in Fig. 1. The sections of the graphs below ‘zero’ are positive

**Fig. 8** Relationships between $^{13}$C-MBC and $^{13}$C-SOC in the rhizosphere (a) and in the bulk soil (b), between $^{13}$C-SOC and shoot biomass (c), and between $^{13}$C-SOC and root biomass (d) with and without P fertilisation. Variables are as defined in Fig. 1. All comparisons occurred under both alternate wetting and drying: WET/DRY and continuous flooding: FLOOD. All regression lines are significant at $p < 0.01$
Fig. 1

(a) S1  
Shoot

(b) S2

(c) S3

Plant biomass (g m⁻²)

(d) S1  
Root

R/S ratio

(e) S2

(f) S3

Root/Shoot ratio

WET/DRY FLOOD

- P  + P

WET/DRY FLOOD

- P  + P

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Fig. 2

(a) S1

(b) S2

(c) S3

(d) S1

(e) S2

(f) S3

Plant total phosphorus (g m⁻²)

Plant C/P (molar ratio)

Shoot

Root

WET/DRY

FLOOD

0

0.5

1.0

1.5

0

500

1000

1500

0

500

1000

1500

0

500

1000

1500
Fig. 4

(a) S1

(b) S2

(c) S3

Proportion of $^{13}$C (%)
Fig. 6

(a) S1

(b) S2

(c) S3

MBC (mg C kg⁻¹ soil)

WET/DRY FLOOD
Rhizosphere soil
Bulksoil

- P +P - P +P - P +P - P +P

0 500 1000 1500 2000

For Review Only
Fig. 7

(a) $S_1$

(b) $S_2$

(c) $S_3$

(d) $S_1$

(e) $S_2$

(f) $S_3$

$^{13}$C-MBC (µg C kg$^{-1}$)

Proportion of $^{13}$C in MBC (%)
Fig. 8

(a) Rhizosphere soil
- P: $y = 0.11x + 0.36$; $R^2 = 0.38$
+ P: $y = 0.13x + 0.36$; $R^2 = 0.50$

(b) Bulk soil
- P: $y = 0.02x + 0.07$; $R^2 = 0.29$
+ P: $y = 0.04x + 0.05$; $R^2 = 0.71$

(c) Shoot
- P: $y = 4.17x - 6.11$; $R^2 = 0.82$
+ P: $y = 3.26x - 7.32$; $R^2 = 0.84$

(d) Root
- P: $y = 5.96x - 3.68$; $R^2 = 0.78$
+ P: $y = 4.84x - 3.31$; $R^2 = 0.89