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Irrigation management and phosphorus addition alter the abundance of carbon dioxide-fixing autotrophs in phosphorus-limited paddy soil

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Abstract

In this study, we assessed the interactive effects of phosphorus (P) application and irrigation methods on the abundances of marker genes (*cbbL*, *cbbM*, *accA*, and *aclB*) of CO_2-fixing autotrophs. We conducted rice-microcosm experiments using a P-limited paddy soil, with and without the addition of P fertiliser (P versus CK), and using two irrigation methods, namely alternate wetting and drying (AWD) and continuous flooding (CF). The abundances of bacterial 16S rRNA, archaeal 16S rRNA, *cbbL*, *cbbM*, *accA*, and *aclB* genes in the rhizosphere soil (RS) and bulk soil (BS) were quantified. The application of P significantly altered the soil properties and stimulated the abundances of Bacteria, Archaea, and CO_2-fixation genes under CF treatment, but negatively influenced the abundances of Bacteria and marker genes of CO_2-fixing autotrophs in BS soils under AWD treatment. The response of CO_2-fixing autotrophs to P fertiliser depended on the irrigation management method. The redundancy analysis revealed that 54% of the variation in the functional marker gene abundances could be explained by the irrigation method, P fertiliser, and the Olsen-P content; however, the rhizosphere effect did not have any significant influence. P fertiliser application under continuous flooding was more beneficial in improving the

abundance of CO_2-fixing autotrophs compared to the AWD treatment; thus, it is an ideal irrigation management method to increase soil carbon fixation.

Keywords: CO_2-fixing autotrophs; marker gene abundance; P fertiliser; irrigation management; rhizosphere effect

Introduction

Phosphorus (P) is regarded as a limiting factor resource in rice cultivation. It is an essential macronutrient that determines rice growth and productivity (Fageria et al. 2011); however, rice paddy fields are often P-deficient (Haefele et al. 2014), especially those located in the red-soil region of southern China (Li et al. 2012). To meet the P requirements of crops, P fertilisers have been increasingly applied in intensive rice-cropping systems (Lan et al. 2012; Shen et al. 2004), with P application rate rising up to 120 kg P ha^{-1} (Wang et al. 2012). Applying P fertiliser not only increases the availability of the nutrient to rice crop but also directly or indirectly changes the chemical, physical, and biological properties of soil (Griffiths et al. 2012; Yan et al. 2016). Such changes are believed to have a significant effect on the growth rates and ecological functions of various soil microbes involved in the biogeochemical carbon cycle (Černá et al. 2009; Su et al. 2015).

On the other hand, as a water-intensive crop, rice is mostly grown under irrigated conditions and therefore, water is another important limiting factor resource in this ecosystem (Cai and Chen 2000). The water regime and fertiliser uptake are related

physiological processes (Somaweera et al. 2016; Suriyagoda et al. 2014), which could interact to modify some soil properties. The bio-availability of soil P to plants is significantly related to the oxidation-reduction status of soil (Borggaard et al. 2004; Torrent. 1997), which can be influenced by irrigation management practices (Das et al. 2014; Haefele et al. 2006). Soil P availability, which is sensitive to water conditions, can influence plant growth (Hua et al. 2016), which, in turn, can affect nutrient turnovers in soil, especially in the rhizosphere soil (Jones et al. 2009; Richardson et al. 2009). It is possible that irrigation management together with P fertiliser application affect the activity, biomass and the composition of soil microbial communities.

Autotrophic microorganisms, capable of converting carbon dioxide (CO_2) into organic C, play an important role in the C cycle (Yuan et al. 2012a). This process is primarily catalyzed by the ribulose-1,5-biphosphate carboxylase/oxygenase (RubisCO) enzyme in the Calvin cycle (CBB) (Benson et al. 1950). The RubisCO enzyme exists in four forms (I–IV) (Tabita 1999); the *cbbL* and *cbbM* genes encode the large subunits of forms I and II of RubisCO, respectively, and are marker genes used in microbial ecology studies (Lynn et al. 2017; Wu et al. 2015; Xiao et al. 2014). In addition to the CBB cycle, four additional autotrophic CO_2-fixation pathways have been documented (Berg 2011). The reductive tricarboxylic acid (rTCA) cycle is a CO_2-fixation pathway of microaerophiles and anaerobes (Evans et al. 1966). The β-ATP citrate lyase enzyme is involved in this cycle and encoded by the marker gene *aclB* (Campbell et al. 2003). The reductive acetyl-CoA pathway is considered the

oldest pathway of strictly anaerobic bacteria and archaea (Berg. 2011; Wood et al. 1986), with the key enzyme being CO dehydrogenase/acetyl-CoA synthase, but without a proper marker gene. The 3-hydroxypropionate/malyl-CoA cycle is only found in a few species of the genus *Chloroflexus* (Holo. 1989) and the carboxylation reaction of this cycle is catalysed by biotin-dependent acetyl-CoA/propionyl-CoA carboxylase (Hügler. 2003). The marker gene *accC* encodes the subunit of biotin-dependent acetyl-CoA carboxylase and the primers developed to target this gene have low specificity (Auguet et al. 2008). The 3-hydroxypropionate/4-hydroxybutyrate pathway is a modified version of the 3-hydroxypropionate/malyl-CoA pathway exclusively found in *Crenarchaeota* (Berg et al. 2007). The acetyl CoA carboxylase (ACCase), encoded by the *accA* gene, is one of the key enzymes involved in CO_2 fixation in this cycle (Yakimov et al. 2009).

The CO_2-fixation by the autotrophic community depends on various factors, including fertiliser management (Su et al. 2015; Yuan et al. 2012b), soil type (Lynn et al. 2017), crop rotation (Wu et al. 2015), tillage (Ge et al. 2016), rhizosphere effect (Yousuf et al. 2013), and growth stage (Xiao et al. 2014). However, no previous study has assessed the effects of P fertiliser addition combined with irrigation management on the abundance of CO_2-fixation autotrophs. Understanding these effects can provide insights on the relationship between some agricultural management practices and CO_2 fixation by microbial communities of paddy soils.

We hypothesize that the interactive effect of P fertiliser and irrigation management on CO_2-fixing autotrophs can be significant. To test this hypothesis, we

conducted experiments with rice microcosms to investigate the effects of the addition of P fertiliser on CO_2-fixing autotrophs in a P-limited paddy soil under continuous flooding or alternative wetting and drying treatment. We quantified the copy numbers of the bacterial 16S rRNA gene, archaeal 16S rRNA gene, and four functional genes for three CO_2-fixation pathways (the CBB cycle, the rTCA cycle, and the 3-hydroxypropionate/4-hydroxybutyrate cycle) in both bulk and rhizosphere soils. In addition, we assessed the soil physicochemical properties and their potential influence on CO_2-fixing autotrophic microorganisms.

Materials and methods

Soil sampling

Surface (0 to 20 cm) soil was sampled in a P-limited paddy field on 4 April 2015 during the paddy fallow season; the field is located at the Changsha Agricultural and Environmental Monitoring Station (113°19′52″E, 28°33′04″N), Hunan Province, China. This region is characterized by a subtropical humid monsoon climate, with an annual precipitation of 1,330 mm, air temperature of 17.5°C, 1,663 h of sunshine, and a frost-free period of 274 days. The paddy soil, derived from granite red soil, is classified as Stagnic Anthrosols (Gong et al., 2007). The main soil properties were: 5.43 pH, 14.26 g kg^{-1} soil organic matter (SOC), 1.45 g kg^{-1} total N (TN), 0.75 g kg^{-1} total P (TP), 4.96 mg kg^{-1} available P (Olsen-P), and 7.71 cmol kg^{-1} cation exchange capacity (CEC). After removing all visible plant roots, the soil sample was sieved (<

2 mm), flooded with distilled water, and pre-incubated for 14 days at 25°C prior to use.

Rice microcosm

We added 160 mg K kg^{-1} dry soil as potassium chloride and 250 mg N kg^{-1} dry soil as ammonium sulfate to the pre-incubated paddy soil as base fertilisers. Subsequently, the soil sample was divided into two parts; one part was treated with monopotassium phosphate to reach a P content of 80 mg kg^{-1} dry soil. The other part was used as the control soil sample and no P was added. Subsequently, we packed either 1.0 kg of the P-treated soil or 1.0 kg of the control soil into PVC pots (diameter 17.2 cm and height 20 cm), corresponding to the P-treated-pot (P) or to the control pot (CK), respectively. Nylon rhizosphere bags (φ5 cm×17 cm, mesh 48 μm), each containing 500 g soil, with or without added P, were placed in the centre of the corresponding P or CK pot to create the rhizosphere soil. Two-week-old rice seedlings were subsequently transplanted into the rhizosphere bags, with or without added P, with each pot having three rice seedlings. The rice pots were then flooded with distilled water to develop 2–3 cm deep standing water.

All the rice pots were placed randomly in a growth chamber (80 ×250 cm, height 120 cm) and incubated for 14 days as described by Ge et al. (2012). Half of the CK and P-treated rice pots were flooded continuously (CF) with 2–3 cm water above the soil surface throughout the incubation, whereas the other half were subjected to

alternate wetting and drying (AWD). The latter soil was covered with 2–3 cm of water at the beginning of the experiment. Then, the soil was air-dried to achieve 70–75% of water holding capacity (WHC) for 3–4 days and it was re-flooded again. The drying-rewetting cycle was repeated three times during the experiment. Therefore, we had a total of four treatments: (1) rice grown in soil with no added P under continuous flooding; (2) rice grown in soil with no added P under alternate wetting and drying; (3) rice grown in P-added soil under continuous flooding; and (4) rice grown in P-added soil under alternate wetting and drying. Each treatment was repeated three times. Destructive soil sampling was conducted 14 days after rice transplanting. The rice grew well and did not suffer from nutrient limitation during sampling (Fig S1). We sampled rhizosphere soil (RS) (the soil in the rhizosphere bag) and the bulk soil (BS) (the soil out of the bag). Each soil sample was divided into two sub-samples, one was stored at 4°C for physicochemical analysis and the other was frozen at -80°C for molecular analysis.

Soil physicochemical analysis

The soil pH was determined in a 1:2.5 (wt/vol) soil-to-water suspension using a pH meter (Delta 320, Mettler-Toledo Instruments Ltd., China). The soil organic carbon (SOC) and total nitrogen (TN) contents were measured by dry combustion using an elemental analyser (Vario MAX C/N, Elementar, Germany). Exchangeable ammonium ($N\text{-}NH_4^+$) and nitrate ($N\text{-}NO_3^-$) were extracted with 1 M KCl and

determined by an automated continuous flow analyser (FIAstar 5000, Foss, Sweden). Olsen-P was extracted with $NaHCO_3$ (0.5 M, pH 8.5) and measured by a colorimetric method (Murphy and Riley 1962). Soil microbial biomass carbon (MBC) was measured by the chloroform fumigation-extraction method described by Wu et al. (1990).

Soil DNA extraction

Soil DNA was extracted from 0.5 g of frozen soil (-80°C) using the FastDNA Spin Kit for soil (MP Biomedicals, OH, USA), following the manufacturer's instructions. Subsequently, the DNA extracts were diluted with nuclease-free water before the integrity and quality check. The integrity of the DNA was evaluated with 1% agarose gel. The DNA concentration and quality were determined using NanoDrop spectrophotometer (NanoDrop ND-1000, PeqLab, Germany).

Quantification of genes

The abundances of the bacterial 16S rRNA gene, archaeal 16S rRNA gene, and functional genes (*cbbL*, *cbbM*, *accA*, *aclB*) involved in autotrophic CO_2-fixation pathways were estimated by quantitative PCR (qPCR), using 341f/515r (López-Gutiérrez et al. 2004), 364f/934r (Großkopf et al. 1998), K2f/V2r (Tolli and King. 2005), cbbM-f/cbbM-r (Alfreider et al. 2003), Crena_529F/Crena_981R

(Yakimov et al. 2009), and 892F/ 1204R (Campbell et al. 2003) primers, respectively. The detailed sequences of the primer sets used in this study are listed in Table S1.

We carried out qPCR in 10 μL reaction mixtures containing 1×SYBR Premix Ex Taq (Takara, Dalian, China), 1 μL diluted template DNA of approximately 5 ng, 0.15 μM respective primer, and nuclease-free water. Reactions were repeated three times for each sample using an ABI 7900 Real-Time PCR System (PerkinElmer, Applied Biosystems, USA). The conditions were as follows: initial denaturation at 95°C for 30 s, 40 cycles of denaturation at 95°C for 15 s, annealing at 50–62°C for 30 s (Table S1), and extension at 72°C for 20 s. Series of ten-fold dilutions of plasmids that contained cloned genes of bacterial 16S rRNA, archaeal 16S rRNA, *cbbL*, *cbbM*, *accA*, and *aclB* were run in parallel with the template DNA to prepare the respective standard curves for quantification. At the end of each qPCR run, melting curve analysis was performed to check the specificity of the amplification. The copy numbers of the relative genes were calculated using the SDS 2.3 software within the qPCR system.

Statistical analysis

The effects of environmental variables (P addition of P fertiliser, irrigation management, rhizosphere effect) and their interactions on the abundances of Bacteria, Archaea, and CO_2 fixing autotrophs were tested by univariate analysis of variance (UANOVA) in SPSS 13.0 for Windows (IBM, Armonk, NY, USA), considering the

significant difference at $p<0.05$. The influence of soil properties (pH, MBC, DOC, $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, and Olsen-P) and the environmental variables on the abundances of microbial functional genes was evaluated by redundancy analysis (RDA) using CANOCO 5.0 for Windows (Microcomputer Power, Ithaca, NY, USA). A forward selection procedure was conducted to select soil properties and environmental variables that significantly affected microbial functional gene abundances. Following the forward selection, the pure contribution of each significant parameter, as well as their interactive contributions to changes in the abundances of microbial functional communities was further determined with the variance partitioning analysis by CANOCO 5.0. The significance test was conducted using the Monte Carlo permutation test, with 999 unrestricted permutations ($p<0.05$).

Results

Abundances of Bacteria and Archaea

The number of bacterial 16S rRNA gene copies outnumbered the archaeal 16S rRNA gene copies by one to two orders of magnitude. The abundances of both genes were influenced significantly by P addition (Table 1). The copy numbers of the archaeal 16S rRNA genes in BS and RS were higher in P treatment than in CK treatment, irrespective of irrigation methods, with an average increase of 167% (Fig 1). On the contrary, the effect of P addition on the number of bacterial 16S rRNA gene copies depended on the irrigation management method. Under CF treatment, P

addition significantly increased the bacterial abundance (101% on average), regardless of the rhizosphere effect (Fig 1), whereas the opposite (an average decrease of 21%) was observed in the AWD treatment.

The UANOVA showed a statistically significant rhizosphere effect on the abundances of the bacterial 16S rRNA and archaeal 16S rRNA genes (Table 1). RS harboured a significantly higher number of bacterial 16S rRNA gene copies than BS, whereas an opposite pattern was observed for the number of archaeal 16S rRNA gene copies, which was on average 44% lower in RS than in BS (Fig 1). Although no significant differences in gene copy numbers were observed between the two types of irrigation management methods, the interaction of the water treatment method and the rhizosphere effect significantly affected the gene abundances (Table 1).

Abundances of marker genes related to autotrophic CO_2 fixation

In all treatments, the *cbbL* gene copies, ranging from 1.86×10^8 to 1.93×10^9 copies g^{-1} dry soil, were higher than those of the *cbbM* gene (5.49×10^7 to 1.70×10^8 copies g^{-1} dry soil), *accA* gene (6.96×10^7 to 2.88×10^8 copies g^{-1} dry soil), and *aclB* gene (4.30×10^6 to 2.15×10^7 copies g^{-1} dry soil). The P application changed the abundances of all marker genes involved in autotrophic CO_2 fixation, according to the irrigation treatment (Table 1; Fig. 2). As regards CF treatment, the abundances of CO_2 fixation genes were increased significantly by P addition 14 days after transplanting ($P<0.05$), 68% and 156% increase for the *cbbL* gene, 2% and 99% for the *cbbM* gene, 82% and 71% for the *accA* gene, and 149% and 142% for the *aclB* gene in BS and

RS, compared to the CK treatment. However, in the AWD treatment, the effects of P fertiliser on the CO_2-fixation functional genes differed between BS and RS. Phosphorus application decreased the number of *cbbL*, *cbbM*, *accA*, and *aclB* genes in BS of the CK treatment, whereas it increased the gene abundances in RS compared to the CK treatment, except for the *cbbM* gene (Fig. 2).

The influence of the rhizosphere effect was only significant for the *cbbL* gene (Table 1), with the gene abundance being on average 260% greater in RS than in BS (Fig. 2). The irrigation method significantly affected the abundances of all functional genes involved in the autotrophic CO_2 fixation (Table 1). For example, the abundances of the *cbbL*, *accA*, and *aclB* genes in BS treated with P fertiliser were higher in the CF than in the AWD treatment. However, the *cbbM* gene showed an opposite pattern (Fig 2). The UANOVA showed that the interaction between the added P, the rhizosphere effect, and the irrigation management method significantly affected the abundances of most functional genes involved in autotrophic CO_2 fixation (Table 1).

Relationships among microbial functional genes, management practices, and soil properties

The first two axes of the RDA explained 42.97% and 16.44%, respectively, of the total variation in autotrophic CO_2-fixation gene abundances (Fig 3). The Olsen-P (P=0.001), irrigation management method (P=0.001), and P addition (P=0.001)

significantly influenced the abundances of autotrophic CO_2-fixing microorganisms. Variance partitioning analysis showed that these variables independently explained 12.8%, 42.1%, and 17.3% of the total observed variation, respectively, leaving 46% of the variation unexplained (Fig 4). The interactions among Olsen-P, irrigation management method, and P addition had a smaller effect than each individual property, except for the shared variation between Olsen-P and irrigation management method (-13.7%).

Discussion

To quantify the response of the abundances of microbial communities involved in the CO_2-fixation to P fertiliser addition may help understand the potential ecological functions of these communities (Su et al. 2015). The addition of P fertiliser can change the soil properties such as enzyme activities and the amount of available nutrients, which may affect the size, activity, and composition of soil microbial communities sensitive to such changes (Krey et al. 2013; Malik et al. 2012; Van Geel et al. 2016). Therefore, the differences in soil properties, such as nutrient concentrations (Table S2), may explain the differences in the abundances of bacterial, archaeal and CO_2-fixing autotrophic communities in CK and P soils as shown by the qPCR and CCA analyses. The increase in the abundance of functional microbial genes in soils amended with P fertiliser is likely caused by higher organic C inputs to soil owing to the stimulation of root growth and root exudation by added fertiliser (Tan et

al. 2013; Wang et al. 2016). In this study, the application of P increased the abundances of autotrophic CO_2-fixing microorganisms in RS except the *cbbM* genes but decreased the copy numbers of functional marker genes in BS under the AWD treatment. Probably the abundance of *cbbM* genes decreased in RS following fertilizer application in the AWD treatment because of the competition among different functional microorganisms in the microenvironment (Hibbing et al. 2010). In AWD soil, P application might have mitigated the P shortage pressure on functional groups, such as denitrifiers, and stimulated their growth under aerobic conditions (Wei et al. 2017). It might have inhibited the growth of the *cbbM*-harboring autotrophs probably because these species were less competitive. However, the underlying mechanisms need further investigation.

The effect of P application on the abundance of CO_2-fixing autotrophic communities also depended on the irrigation condition. The irrigation management method explained 42.1% of the variations in the abundances of the four marker genes. It is well established that water conditions can affect activity, biomass, and composition of soil microbial communities (Kavamura et al. 2013; Tian et al. 2013a). Irrigation condition is a crucial factor that impacts microbial biomass, through its effects on the dynamics of available C in rice cropped soils (Tian et al. 2013b). The available C may also affect the biomass and the distribution of CO_2-fixing autotrophic communities because most of these populations are facultative autotrophs, capable of using organic substrates as a carbon and an energy source (Hügler and Sievert. 2011). In addition, water conditions may affect the activity of some enzymes involved in

microbial CO_2 fixation, showing different affinities for oxygen (Hügler and Sievert. 2011); for example, the ATP-citrate lyase is sensitive to oxygen and the RubisCO is tolerant to oxygen (Berg. 2011). Accordingly, the marker genes involved in four CO_2-fixation pathways responded differently to irrigation management methods. Compared to P fertilizer addition, irrigation management contributed more to variations in the abundances of CO_2-fixing autotrophs (Fig. 5), probably because the irrigation management might have influenced the turnover and bio-availability of soil P (Das et al. 2014; Haefele et al. 2006), which are quite important for the growth of CO_2-fixing autotrophs.

The Olsen-P concentration in soil was another important factor influencing the composition of CO_2-fixing autotrophic communities, probably because, as any microorganism, they require P for energy metabolism and nucleic acids and cell membrane synthesis (Richardson et al. 2009; Rodríguez and Fraga. 1999). Indeed, the increase in the Olsen-P content can stimulate the growth of CO_2-fixing autotrophs, as this form of P can be directly utilized by microorganisms (Malik et al. 2012). Consistent with a previous study (Saleque et al. 2004), we observed a significant increase in the Olsen-P content in all soils after the application of inorganic P (Table S2), but this increase was not followed by the increase in the abundances of CO_2-fixing microorganisms in BS under the AWD treatment. It may because the AWD treatment (the soil water content reached 70–75% of the WHC after drying for 3–4 days) limited the availability of Olsen-P to the soil microorganisms. In addition, although the Olsen-P is considered a labile form to be easily used by microorganisms,

it refers to a P extraction pool; thus it is possible that some of this P pool may not be easily degradable by soil microbes (Malik et al. 2012). The Olsen-P becomes less available when bound to metal oxides in soil (Stewart and Tiessen. 1987). In contrast to a previous study on CO_2 fixation gene abundances (Xiao et al. 2014), the rhizosphere effect was not significant in our study as revealed by the RDA analysis. P application can stimulate the growth of rice roots and thus, the increase of rhizodeposition (Zhong and Cai, 2007), which may increase the microbial abundance and activity in RS compared to BS. However, this is not likely to occur in our short-term experiment because of the low root biomass (Fig. S1). Future research is needed to verify this hypothesis by monitoring the quality and quantity of rhizodeposition.

A considerable percentage (46%) of the variation in the abundances of CO_2-fixation genes was not explained by the measured properties. It is possible that an undetermined soil property is important in affecting the abundances of the four marker genes. For instance, the quality and quantity of root exudation can affect the abundances of functional genes in rhizosphere soil (Baranya et al. 2015; Pathan et al. 2015; Yuan et al. 2016; Zhu et al. 2016) and this may also happen for the CO_2 fixing microorganisms that are facultative autotrophic. The quality and quantity of root exudation depends on different types and rates of fertiliser application and irrigation management (Zhong and Cai. 2007). Both acid and alkaline phosphomonoesterase activities of soils depend on the type and the rate of fertiliser application and irrigation treatment (Nannipieri et al. 2011; Tan et al. 2013) and they may also play a role in

affecting the abundance of CO_2-fixing autotrophs because both enzyme activities mineralize organic P to inorganic P.

Conclusions

The addition of P significantly changed the soil properties and the abundances of four marker genes involved in CO_2 fixation in P-limited paddy soils. The application of P increased the abundances of CO_2-fixing autotrophs in the continuous flooding treatment, but decreased the copy numbers of the functional marker genes in bulk soil under the alternate wetting and drying treatment. This indicates that the response of CO_2-fixing autotrophs to P fertiliser can be mediated by changes in the irrigation management system. Our findings revealed the positive and interactive effect of P application and continuous flooding on the abundance of CO_2-fixing autotrophs, suggesting that this combined management may potentially be more effective in increasing soil C sequestration than other tested conditions in P-limited paddy soils. However, the detection of genes does not imply that they are active; therefore, further research, including transcriptomics and proteomics studies, should be conducted to determine the activity and the expression of these CO_2 fixation genes.

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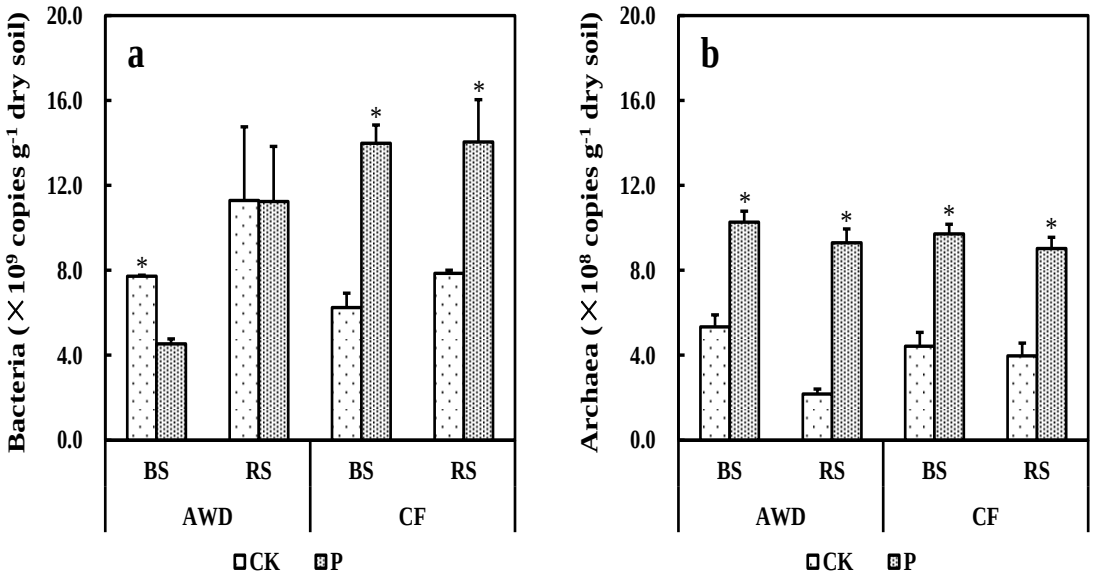

Fig. 1 Abundance of (a) bacterial and (b) archaeal 16S rRNA genes in soils without (CK) or with P addition (P) under alternate wetting and drying (AWD) and continuous flooding (CF) irrigation management. Both bulk soil (BS) and rhizosphere soil (RS) were analyzed. Error bars represent standard errors of triplicate samples. Asterisks indicate significant differences.

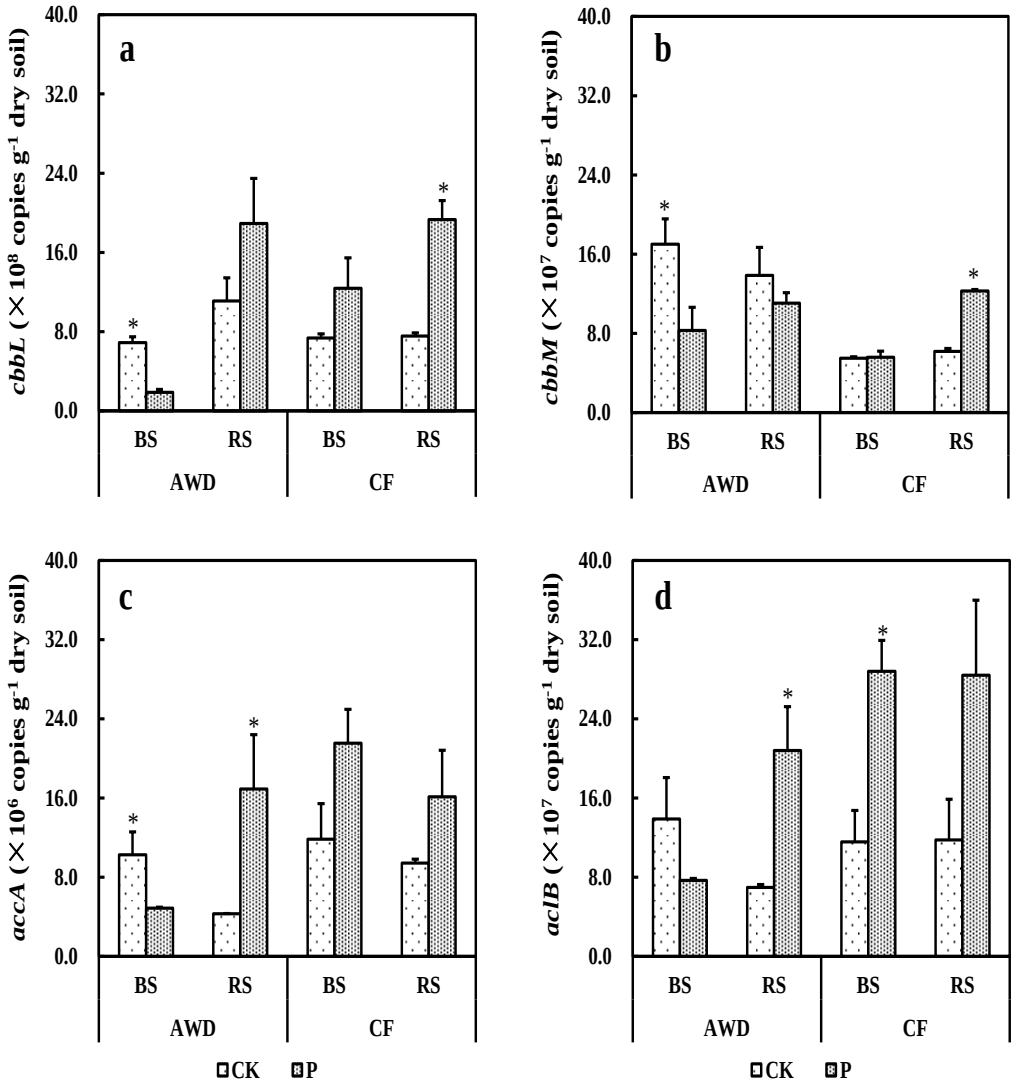

Fig. 2 Abundances of (a) *cbbL* gene, (b) *cbbM* gene, (c) *accA* gene, and (d) *aclB* gene in soils without (CK) or with P addition (P) under alternate wetting and drying (AWD) and continuous flooding (CF) irrigation management. Both bulk soil (BS) and rhizosphere soil (RS) were analyzed. Error bars represent standard errors of triplicate samples. Asterisks indicate significant differences.

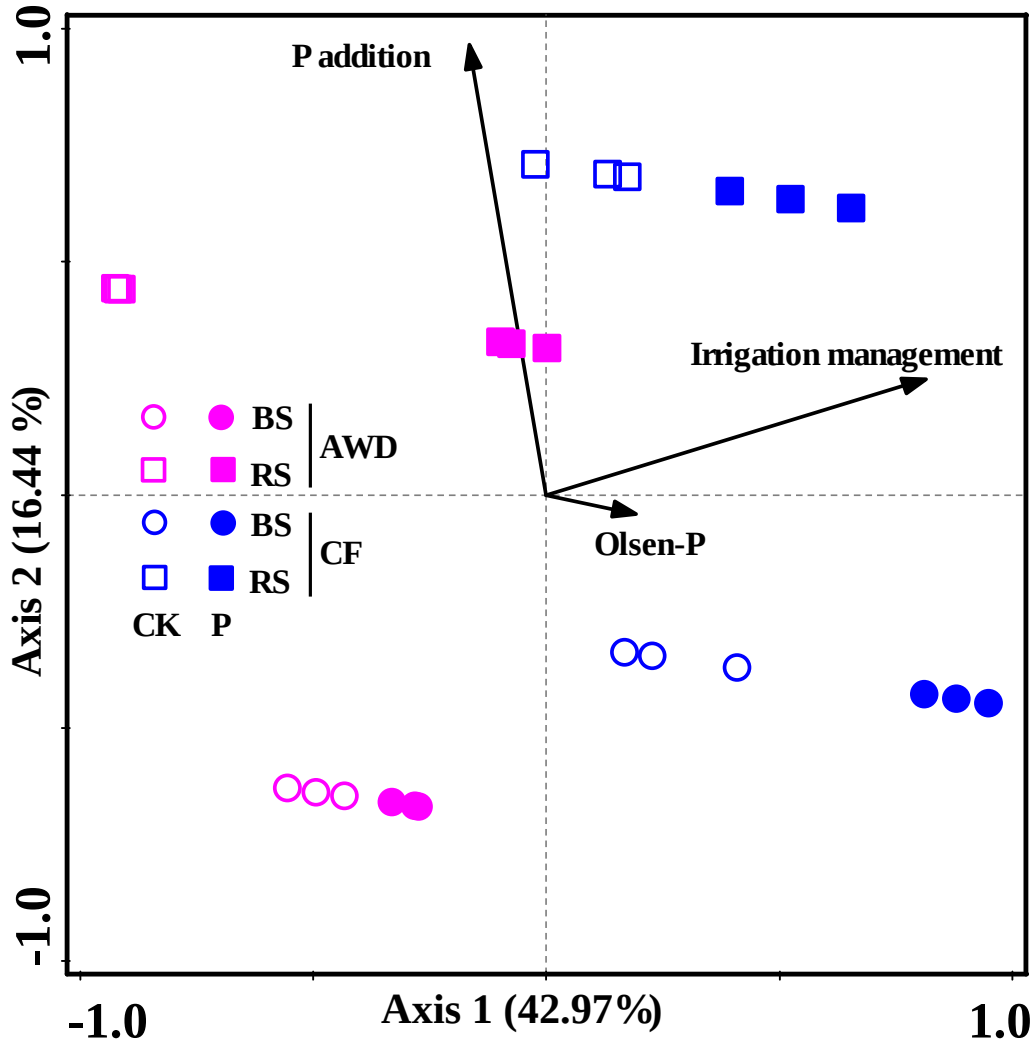

Fig. 3 RDA analysis of autotrophic CO_2 fixation gene abundances, soil properties, and environmental variables that significantly affected microbial variations. Pink and blue colours denote alternate wetting and drying (AWD) and continuous flooding (CF), respectively. The circle and square indicate bulk soil (BS) and rhizosphere soil (RS), respectively. The hollow and solid symbols indicate soils without (CK) and with P addition (P), respectively.

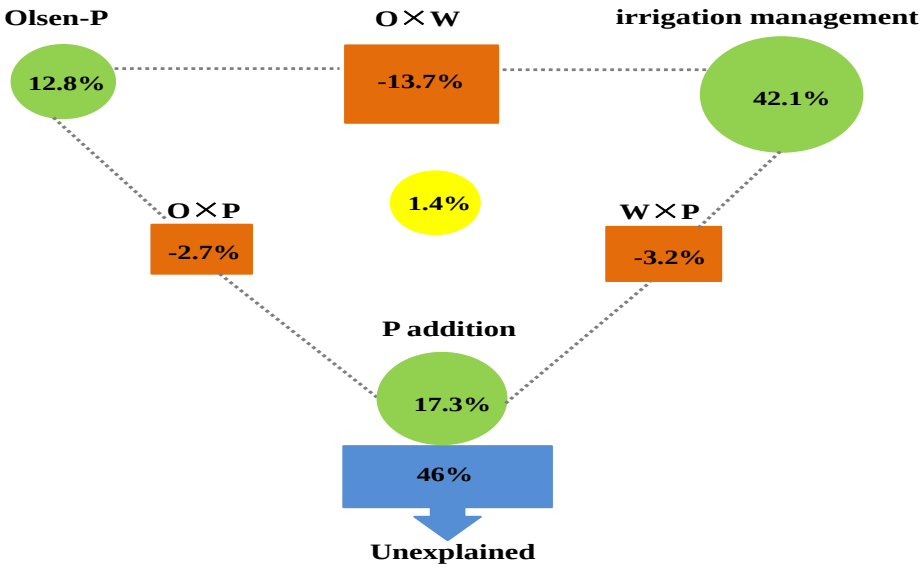

Fig. 4 Variation partitioning analysis of the pure effects of Olsen-P (O), irrigation management (W), P addition (P), and the interactions of a combination of these factors (O×W, O×P, W×P, and O×W×P) on the abundances of the autotrophic CO_2 fixing community. The circles on the edges of the triangle show the variation due to each factor alone. The square on the sides of the triangle and the circle at the centre of the triangle show the interactions of any two factors and the interactions among all the factors, respectively. The unexplained portion is shown by the square at the bottom of the triangle. The geometric areas of each diagram are proportional to the respective percentages of the explained variation by a single factor or a combination of factors.

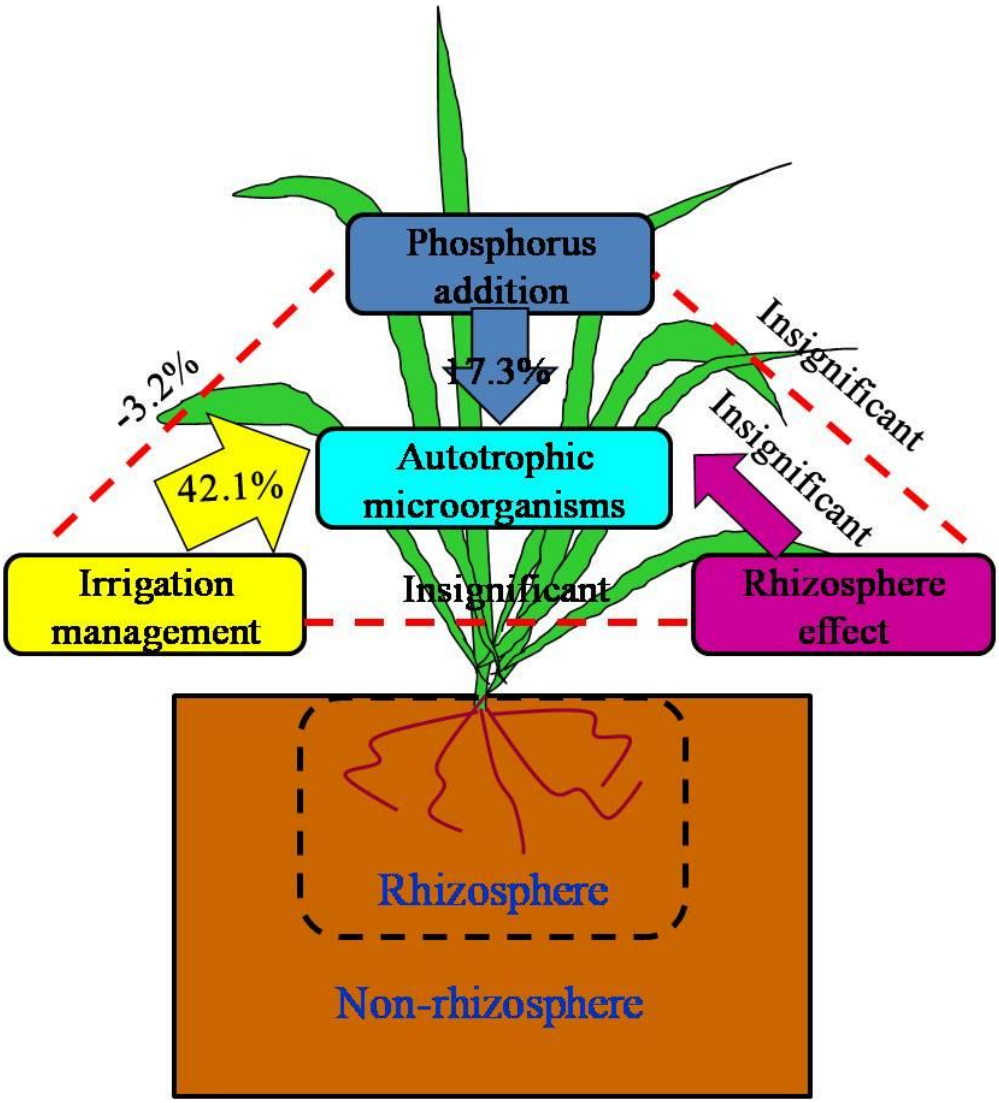

Fig. 5 A conceptual diagram of the pure and interacted contributions of phosphorus addition, irrigation management, and rhizosphere effect to the abundances of the autotrophic CO_2 fixing community.

Table 1 Three-way analysis of the influences of P addition (P), rhizosphere effect (R), irrigation management (W), and their interactions on the abundances of 16S rRNA genes and autotrophic CO_2 fixation genes

Source of variance	Variable					
	bacterial 16s rRNA	archaeal 16S rRNA	*cbbL*	*cbbM*	*accA*	*aclB*
P	**0.020**	**0.000**	0.147	0.179	**0.006**	**0.000**
R	**0.004**	**0.001**	**0.000**	0.082	0.818	0.532
W	0.054	0.983	**0.001**	**0.000**	**0.008**	**0.004**
P × R	0.555	0.133	**0.000**	**0.006**	0.060	0.054
P× W	**0.000**	0.183	**0.000**	**0.000**	0.232	**0.013**
R × W	**0.030**	**0.028**	**0.000**	0.056	0.080	0.500
P × R × W	0.119	0.074	**0.003**	0.982	**0.012**	**0.043**

Significant values are shown in bold (P<0.05)