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Long-Term P Fertilizer Application Reduced Methane Emissions from Paddies in a Double-Rice System

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Abstract: Rice is the main staple food worldwide, yet paddy fields are a primary source of artificial methane (CH_4) emissions. Phosphorus (P) is a key element in the growth of plants and microbes, and P fertilizer input is a conventional agricultural practice adopted to improve rice yield. However, the impact of long-term P fertilizer addition on CH_4 emissions in rice paddies is still unclear. To test this impact, a 36-yr field experiment with and without P fertilizer application treatments under a double-rice cropping system was used in this study to explore how continuous P application affects CH_4 emissions and related plant and soil properties. The cumulative CH_4 emissions were 21.2% and 28.6% higher without P fertilizer application treatment than with P fertilizer application treatment during the early and late season, respectively. Long-term P fertilizer application increased the rice aboveground biomass by 14.7–85.1% and increased grain yield by 24.5–138.7%. However, it reduced the ratio of root biomass to aboveground biomass. Long-term P fertilizer input reduced the soil NH_4^+ concentrations in both rice seasons but increased the soil DOC concentrations in the late season. The soil methanogenic abundance and CH_4 production potential were similar without and with P fertilizer application treatments; however, the methanotrophic abundance and soil CH_4 oxidation potential with P fertilizer application treatment were significantly higher than without P fertilizer application treatment. Our findings indicate that long-term P fertilizer input reduces CH_4 emissions in rice fields, mainly by improving CH_4 oxidation, which highlights the need for judicious P management to increase rice yield while reducing CH_4 emissions.

Keywords: CH_4 emissions; phosphorus; long-term; methanogens; methanotrophs; paddy soil

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Citation: Zhu, X.; Li, J.; Liang, X.; Chen, Y.; Chen, X.; Ji, J.; Xia, W.; Lan, X.; Peng, C.; Chen, J. Long-Term P Fertilizer Application Reduced Methane Emissions from Paddies in a Double-Rice System. *Agronomy* **2022**, *12*, 2166. https://doi.org/10.3390/agronomy12092166

Academic Editors: Zhenwei Song and Xiaogang Yin

Received: 12 August 2022
Accepted: 8 September 2022
Published: 12 September 2022

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1. Introduction

Methane (CH_4) is the second most important greenhouse gas after CO_2, and the atmospheric CH_4 concentrations increased by 156%, from 729.3 ppb to 1866.3 ppb, in 2019 [1]. Paddy fields are a major source of CH_4 emissions, accounting for 9% of human-caused CH_4 emissions [1]. Meanwhile, rice is a staple crop of importance to humans, and rice yields need to increase by ~28% from 2007 to 2050 to fulfill the growing global food demand [2]. However, due to soil fertility depletion (e.g., decreased nutrient availability and lower soil acidification) across 35% of global rice-growing areas, yields either collapse, or stagnate [3–5]. Therefore, it is urgent to increase soil fertility while reducing CH_4 emissions. Because phosphorus (P) is a key component for plants and a widespread lack of P occurs in paddy fields, the application of P fertilizer exerts an important role in the improvement of rice yields [6,7]. Yet, the impact of P fertilizer management on CH_4 emissions from paddy fields is still unclear.

CH_4 emissions from paddy soils were determined by the net impact of the CH_4 production and consumption processes [8]. CH_4 is mainly produced by methanogenic archaea in flooded paddy soil, and this procedure has been greatly influenced by the soil C supply [9,10]. In flooded rice fields, CH_4 was consumed mainly by aerobic methanotrophic bacteria, and the growth of methanotrophs was influenced by soil-available nutrients (e.g., N and P) [11–13]. Indeed, the soil-available P concentration can directly affect CH_4 production and oxidation [14]. Microcosm incubation studies have indicated that the negative correlation between CH_4 formation, oxidation and available P concentrations [15–17] is because a high concentration of available P can restrain CH_4 production via acetoclastic methanogenesis [18]. Moreover, P fertilizer application can indirectly affect CH_4 emissions through rice-plant growth and soil properties. For example, P fertilizer amendment often enhances rice-plant growth, which may provide more substrates for methanogens and more oxygen for methanotrophs [19,20]. The P fertilizer application can stimulate carbon mineralization that affects soil C availability [21]. Taken together, these findings suggest that P fertilizer application may largely affect CH_4 emission.

Since the 1990s, several studies have been performed to clarify the impact of P fertilizer addition on CH_4 emissions in rice fields. Adhya et al. (1998) [22] showed that P amendment decreased CH_4 emissions from paddy fields. Yang et al. (2010) [23] indicated that P addition had no influence on CH_4 emissions in rice fields. Datta et al. (2013) [24] found that P addition could result in higher CH_4 emissions during the wet season. The high variation of results across individual experiments suggests that more experiments are needed to investigate the influence of P fertilizer application under different conditions. Furthermore, because the effect of P fertilizer input on soil traits operates at different time scales [23,25], it is urgent to focus on the impact of long-term P fertilizer amendment on CH_4 emissions. However, the effect of long-term P fertilizer addition on CH_4 emissions in paddies is rarely evaluated. Furthermore, an exploration of how P fertilizer application affects CH_4 emissions through changing CH_4 production and CH_4 oxidation is needed.

China is the largest rice-producing country, contributing to 21.9% of global paddies' CH_4 emissions [26]. Rice yields from the double-rice system account for 33.3% of China's total rice production [27]. Furthermore, the double-rice cropping system has the highest area-scaled and yield-scaled CH_4 emissions among China's rice cropping systems [28]. Therefore, based on a 36-yr field experiment, we investigated the CH_4 emissions, plant growth, and soils microbes involved in CH_4 emissions without and with P fertilizer application treatments in a double-rice system. The purposes of this study were: (1) to determine the effect of continuous P fertilizer application on CH_4 emissions; and (2) to learn the underlying mechanisms of continuous P fertilizer addition effects on CH_4 emissions. We hypothesized that long-term P fertilizer application would affect soil P availability and soil microbes relative to CH_4 production and oxidation, and thus change CH_4 emissions in paddy field.

2. Materials and Methods

2.1. Site Description

The long-term field experiment began in 1984 at Nanchang (28°06′ N, 115°09′ E), Jiangxi province, China. The local climate is characterized by a subtropical monsoonal humid climate. Meteorological data, including the average daily temperature and rainfall during double-rice-growing periods in 2021, are shown in Figure 1. The early rice was transplanted on 30 April and harvested on 16 July; the late rice was transplanted on 26 July and harvested on 17 October in 2021. The soil of the experimental site is classified as red clay soil. The initial soil (0–20 cm) characteristics were pH (H_2O) 6.5, soil organic matter 25.6 g kg^{-1}, total nitrogen (N) 1.36 g kg^{-1}, available N 81.6 mg kg^{-1}, Olsen P 20.8 mg kg^{-1}, and exchangeable potassium (K) 35.0 mg kg^{-1}.

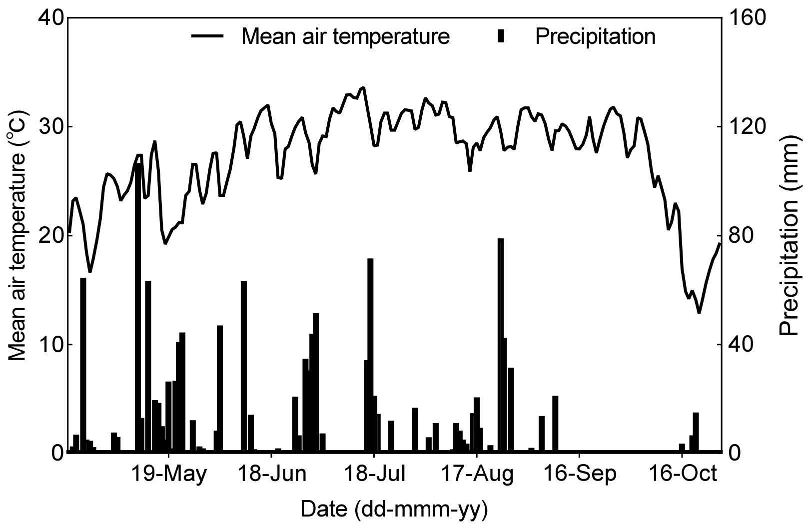

Figure 1. The average daily air temperature and precipitation in early and late rice seasons in 2021 at the experimental site.

2.2. Experiment Design

A randomized block design with three replicates was conducted, including two long-term P fertilizer treatments, unit plots of 33.3 m^2 size, and fertilizer treatments as follows: N, P, and K fertilizers applied simultaneously (NPK), and only N and K fertilizers applied (NK). In the NPK treatment, the N application rate was 150 kg N ha^{-1} in the early season and 180 kg N ha^{-1} in the late season: the same amount of P (60 kg P_2O_5 ha^{-1}) and K (150 kg K_2O ha^{-1}) fertilizers were applied in both the early and late rice seasons. The amount of N and K fertilizers were kept the same between the NPK and NK treatments. Urea (46.7% N) for N fertilizer, calcium superphosphate (12.5% P_2O_5) for P fertilizer, and potassium chloride (60.0% K_2O) for K fertilizer were used throughout the experiments. After rice transplanting, each plot was flooded with 3–5 cm water until midseason drainage at the jointing stage. Afterwards, all plots were flooded before the anthesis stage, and were then intermittently flooded until harvest. Herbicides and pesticides were applied according to standard commercial practice.

2.3. Sampling and Measurement Methods

The CH_4 flux was measured once every 5–7 days using the static closed chamber gas chromatography method during both rice-growing periods in 2021. The PVC chamber with a size of 50 × 50 × 15 cm was fixed into each plot before rice transplanting. The gas samples were taken at five minute intervals between 09:00 and 11:00 AM and measured by gas chromatography (Agilent 7890B, Santa Clara, CA, USA). The CH_4 fluxes (F) were calculated by the following equation:

$$F = \Delta C/\Delta T \times V/A \tag{1}$$

where $\Delta C/\Delta T$ is the increase rate of CH_4 concentration (mg m^{-3} h^{-1}) in the chamber, V is the volume of the chamber (m^3), and A indicates the enclosed area of the chamber (m^2). Only measurements for which $R^2 > 0.90$ were used to calculate $\Delta C/\Delta T$. We discarded approximately 4% of the measurements. Accumulative CH_4 emissions during rice-growing seasons were calculated by linear interpolation.

Topsoil (0–15 cm) samples were randomly taken from each plot before early rice transplanting in 2021. Soil organic carbon (SOC) content was measured following the method of vitriol acid potassium dichromate oxidation [29]. Soil total N and P were determined following Black (1965) [30] and Murphy and Riley (1962) [31], respectively.

We also investigated the concentrations of available N [30], available P [32], and available K [33].

Fresh soil was also collected at the jointing stage in the early and late rice seasons, when CH_4 flux reached a peak during the rice-growing season. CH_4 potential production and oxidation rates were determined following the method of Zhu et al. (2016) [34]. Briefly, about 15 g soils (dry weight) and 15 mL of deionized sterile water were transferred into a 150 mL serum bottle. Then, some bottles were purged with N_2 gas for 10 min to clear O_2 and cultured in the dark at 25 °C for two days. Other bottles were cultured with 10,000 ppm_v CH_4 at 25 °C for 24 h and 120 r.p.m shaking condition. Gas samples in headspace were collected every 2 h (five time points in total). CH_4 concentration in headspace was determined by gas chromatography. Potential CH_4 production and oxidation rates of each soil sample were calculated as the slope of the changes in CH_4 concentration during the incubation time. A continuous-flow injection analyzer (SKALAR SansPlus Systems, The Netherlands) was used to measure the concentrations of soil dissolved ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) concentrations. Additionally, soil dissolved organic carbon (DOC) concentration was analyzed using an organic carbon analyzer (multi N/C UV, Analytik Jena AG, JenaGermany). A real-time quantitative polymerase chain reaction (PCR) was used to quantify the copies of the *mcrA* gene (abundances of methanogens) and *pmoA* gene (abundances of methanotrophs). Soil DNA was extracted using Power soil DNA Isolation Kit (MoBio, Vancouver, BC, Canada). The detailed information of primer pairs used for PCR amplification of the *mcrA* genes and *pmoA* genes was shown in Holmes et al. (1995) [35] and Luton et al. (2002) [36], respectively.

At the maturity stage, we determined rice yield by aboveground and root biomass. All hills in each chamber were manually weighed and corrected to a moisture of 14%. At the same time, root samples were collected from 0–20 cm soil depth using a square frame (20 × 15 × 20 cm). The aboveground and root samples were placed in an oven at a constant temperature of 70 °C for 72 h until they reached a constant weight. The root/shoot ratio was calculated by aboveground biomass to root biomass.

2.4. Statistical Analysis

A two-way ANOVA (the P fertilizer management and growing season) was performed to analyze plant traits, the seasonal CH_4 emissions, and soil microbes. If ANOVA showed interactive effects at a significance level of p <0.05, the method of multiple comparisons using the least significant difference (LSD) test was performed. We also used an independent sample *t*-test to examine the influence of long-term P fertilizer amendment on soil chemical properties. We used the statistical package SPSS 19.0 to perform all analyses.

3. Results

3.1. CH_4 Emissions

The NPK and NK treatments showed similar dynamics of CH_4 fluxes in early and late rice seasons (Figure 2). The CH_4 fluxes peak appeared, approximately, at the first 30–40 days (jointing stage) during both seasons. The cumulative CH_4 emissions were significantly influenced by the seasons and P fertilizer management, but there was no interaction between the season and P fertilizer management. The cumulative CH_4 emissions of the early season were 28.1% lower than that of the late season. Compared to NK, NPK reduced cumulative CH_4 emissions by 21.2% during the early season (Figure 3a) and 28.6% during the late season (Figure 3b). The yield-scaled cumulative CH_4 emissions during the early season were 46.1% higher than that during the late season. We also found an interaction effect of season and P fertilizer management on yield-scaled CH_4 emissions. The NPK treatment decreased yield-scaled CH_4 emissions strongly during the early season (−67.8%), in comparison to the late season (−42.3%).

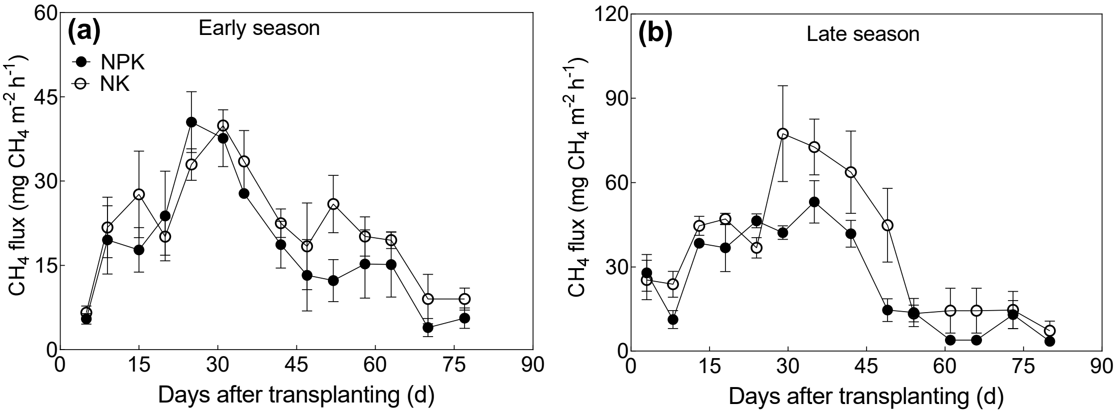

Figure 2. CH_4 fluxes from paddy fields under different long-term P addition treatments during early (**a**) and late (**b**) rice seasons. NPK: long-term N, P, and K fertilizers application; NK: long-term N and K fertilizers application. Error bars represent S.E. (n = 3).

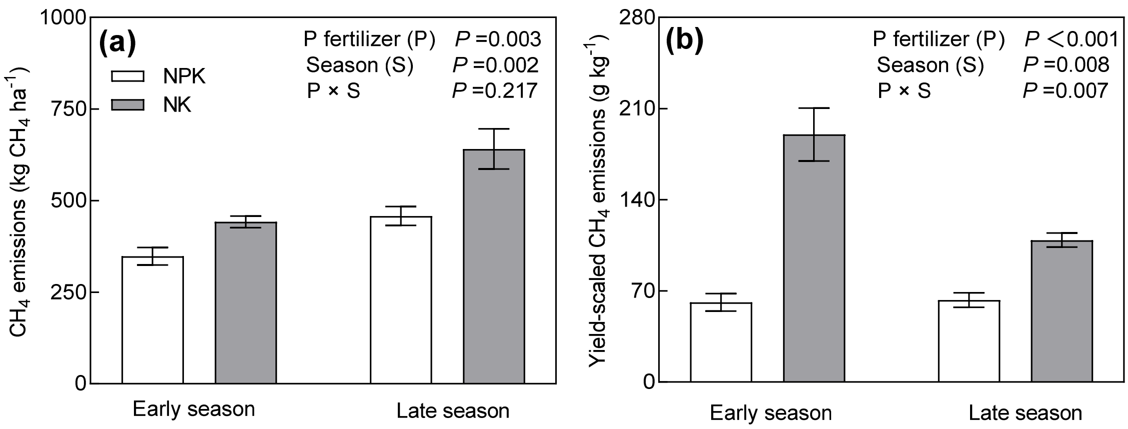

Figure 3. Cumulative CH_4 emissions (**a**) and yield-scaled CH_4 emissions (**b**) under different long-term P addition treatments during early and late rice seasons. NPK: long-term N, P, and K fertilizers application; NK: long-term N and K fertilizers application. Error bars represent S.E. (n = 3).

3.2. Soil Properties

Total phosphorus, Olsen phosphorus, available potassium, and pH were significantly influenced by P fertilizer application (Table 1). The NPK treatment increased total phosphorus and Olsen phosphorus concentrations by 139.3% and 1177.8% relative to NK, respectively, but decreased available potassium concentration and pH by 28.7% and 4.1%, respectively. Soil organic C, total N, and available N concentrations were similar between the two P fertilizer treatments.

Table 1. Influences of long-term P fertilizer amendment on soil properties.

Treatment	Soil Organic C ($g\ kg^{-1}$)	Total N ($g\ N\ kg^{-1}$)	Total P ($g\ P\ kg^{-1}$)	Available N ($mg\ N\ kg^{-1}$)	Available P ($mg\ P\ kg^{-1}$)	Available K ($mg\ K\ kg^{-1}$)	pH
NPK	22.9 ± 1.4	2.2 ± 0.1	1.1 ± 0.0	210.0 ± 9.0	63.4 ± 5.0	88.9 ± 6.5 *	6.1 ± 0.1 *
NK	21.3 ± 0.9	2.0 ± 0.1	0.5 ± 0.0 *	192.3 ± 12.7	5.0 ± 0.4 *	124.8 ± 11.1	6.4 ± 0.0

Notes. Mean ± SD (n = 3). NPK: long-term N, P, and K fertilizers application; NK: long-term N and K fertilizers application. * represents significant differences within P fertilizer treatments ($p < 0.05$).

The soil DOC concentrations during the early season were lower than that during the late season (Table 2). We found that the interaction effect of season and P fertilizer management on soil DOC concentrations by NPK increased soil DOC concentration by 34.0% in the late season but did not have an effect in the early season. The soil NH_4^+ concentration was not affected by season or the interaction of season and P fertilizer management (Table 2). Compared to NK, NPK increased soil NH_4^+ concentration by 33.3% during the early season and by 39.3% during the late season. The soil NO_3^- concentration during the early season was higher than that during the late season (Table 2). We also detected the interaction effect of season and P fertilizer management on soil NO_3^- concentrations by the NPK-stimulated soil NO_3^- concentrations by 165.5% during the early season, but this had no influence during the late season.

Table 2. The effect of long-term P addition on soil DOC, NH_4^+, and NO_3^- concentrations during early and late rice seasons.

	Early Season		Late Season		ANOVA (*p* Value)		
	NPK	**NK**	**NPK**	**NK**	**P Fertilizer (P)**	**Season (S)**	**P × S**
Dissolved organic C (mg L^{-1})	32.4 ± 0.8	34.6 ± 2.8	76.8 ± 4.2	54.9 ± 5.1 *	0.025	<0.001	0.01
Ammonium nitrogen (NH_4^+, mg kg^{-1})	12.0 ± 0.4	16.6 ± 0.4	12.8 ± 1.3	21.0 ± 2.4	0.001	0.06	0.36
Nitrate nitrogen (NO_3^-, mg kg^{-1})	2.9 ± 0.6 *	7.7 ± 1.1	1.8 ± 0.1	2.0 ± 0.1	0.001	0.004	0.008

Notes. Mean ± S.E. (*n* = 3). NPK: long-term N, P, and K fertilizers amendment; NK: long-term N and K fertilizers amendment. * represents significant differences within P fertilizer treatments ($p < 0.05$).

The CH_4 production and oxidation potential during the early season were lower than that of the late season (Figure 4). CH_4 production potential was not affected by P fertilizer management (Figure 4a). Yet, CH_4 oxidation potential was significantly affected by P fertilizer management (Figure 4b). Compared to NK, NPK increased CH_4 oxidation potential by 19.5% during the early season and 32.7% during the late season. The interaction effect of season and P fertilizer management on CH_4 production and oxidation potential was not significant.

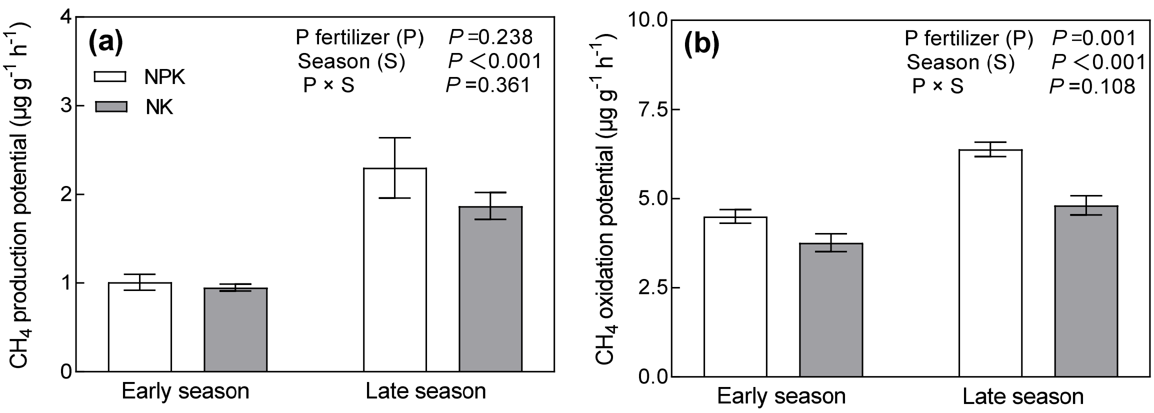

Figure 4. The CH_4 production potential (**a**) and oxidation potential (**b**) under different long-term P addition treatments during early and late rice seasons. NPK: long-term N, P, and K fertilizers application; NK: long-term N and K fertilizers application. Error bars represent S.E. (*n* = 3).

Both season and P fertilizer treatment had no significant influence on the abundance of methanogens (Figure 5a). Methanotrophic abundance was unaffected by seasons, but it was significantly increased by P fertilizer amendment (Figure 5b). Compared to NK, NPK increased methanotrophic abundance by 144.2% during the early season and 82.9% during the late season. Methanogenic activity of the early season was lower than that of the late season (Figure 5c), but it was not affected by P fertilizer management. Methanotrophic activity was not affected by seasons, but it was significantly decreased by P fertilizer application (Figure 5d). Compared to NK, NPK reduced methanotrophic activity by 58.6% during the early season and 52.1% during the late season. The interaction effect of season and P fertilizer treatment on the abundance and activity of methanogens and methanotrophs was not significant.

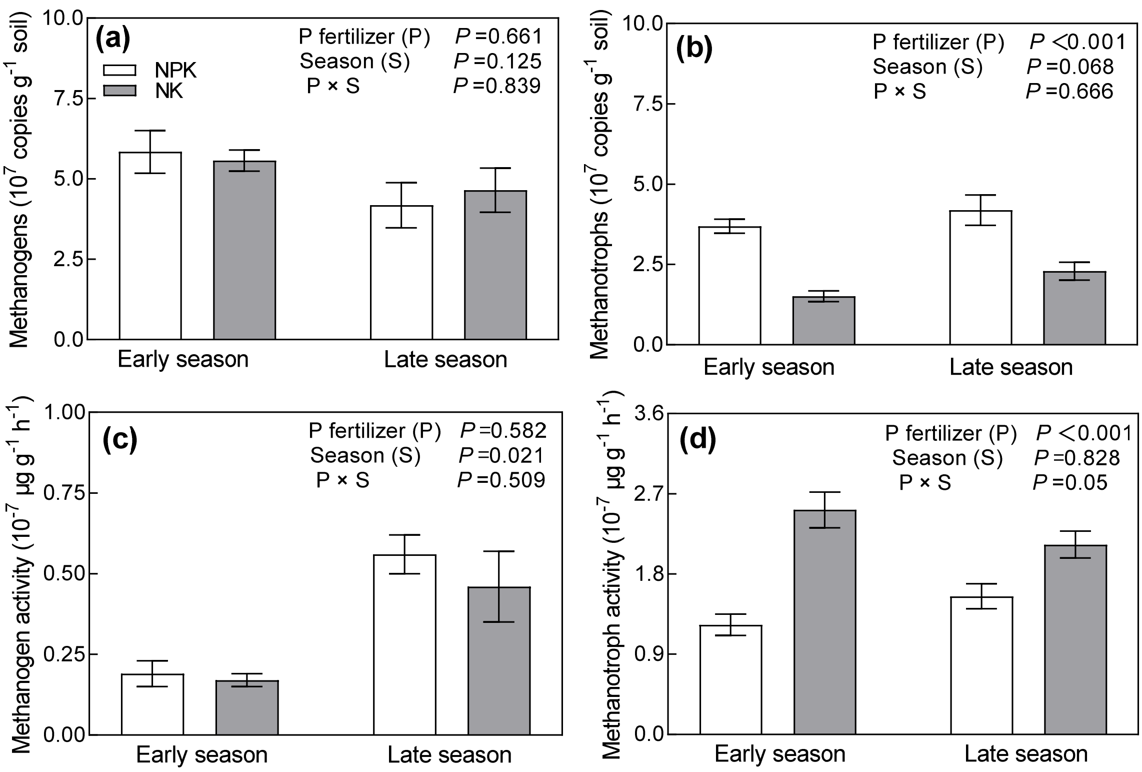

Figure 5. The abundances (**a**,**b**) and activities (**c**,**d**) of methanogens and methanotrophs under different long-term P addition treatments during early and late rice seasons. NPK: long-term N, P, and K fertilizers application; NK: long-term N and K fertilizers application. Error bars represent S.E. (n = 3).

3.3. Plant Traits

Rice grain yield of the early season averaged 38.2% lower than that of the late season (Figure 6a). We observed a season × P fertilizer application interaction, whereby NPK improved grain yield strongly during the early season (138.7%) in comparison to the late season (24.5%). The effects of season and P fertilizer management on aboveground biomass were similar to those on grain yield (Figure 6b). The root biomass of the early season was lower than that of the late season, but the root biomass was unaffected by P fertilizer amendment (Figure 6c). The root–shoot ratio was not affected by the seasons (Figure 6d). However, we found the interaction effect of season and P fertilizer management on the root–shoot ratio. NPK decreased the root–shoot ratio more strongly during the early season (37.4%) than during late season (9.9%).

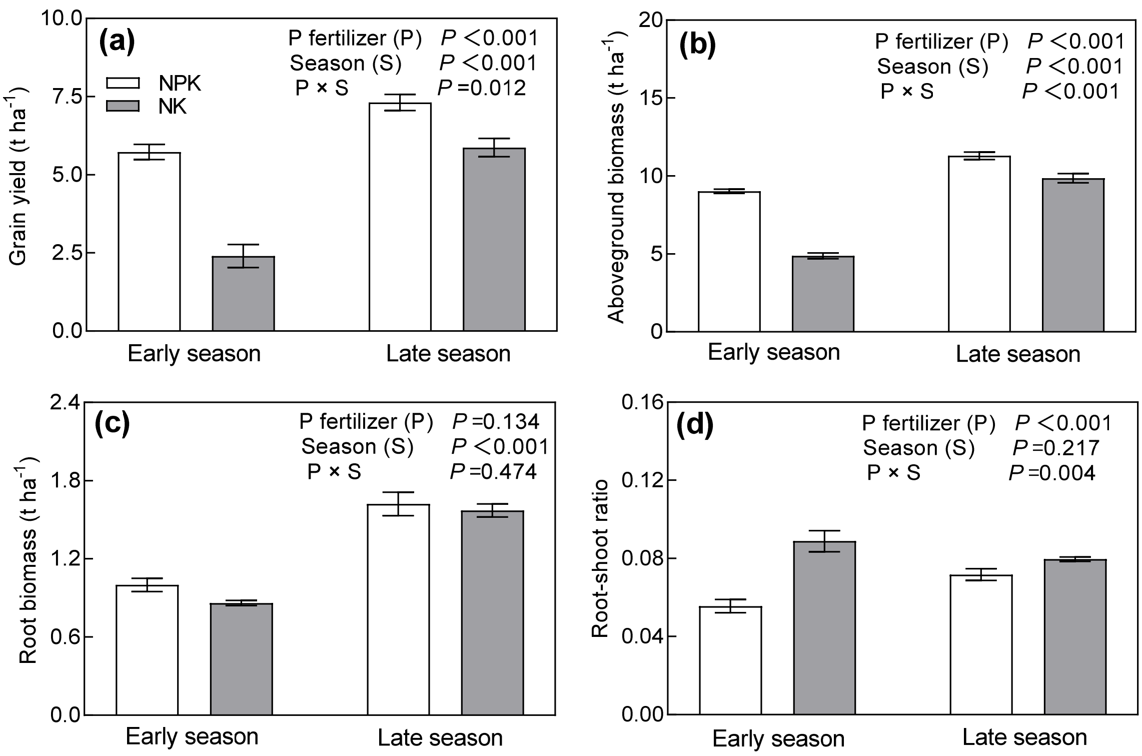

Figure 6. Grain yield (**a**), aboveground biomass (**b**), root biomass (**c**) and root–shoot ratio (**d**) under different long-term P addition treatments during early and late rice seasons. NPK: long-term N, P, and K fertilizers application; NK: long-term N and K fertilizers application. Error bars represent S.E. (n = 3).

4. Discussion

Our results showed that long-term P addition reduced CH_4 emissions in rice fields during both the early and late rice seasons, agreeing with previous studies [24,37]. On the contrary, Shang et al. (2011) [25] and Sheng et al. (2016) [38] found that long-term P addition did not affect or even increase CH_4 emissions. This can be interpreted by the variations in soil pH and the water regime between our study and those studies. Soil pH is the key factor of methanogenic and methanotrophic growth [39], but the soil pH curves in CH_4 production and CH_4 oxidation are varied [40,41]. The optimum pH for methanogens is less than that of methanotrophs [42]. The low soil pH (i.e., 5.1–5.3) may have limited the growth of methanotrophs in those studies [41]. Furthermore, the water regime (i.e., continuous flooding) in those studies was different from our study (intermittently irrigation), and the positive influence of P application on methanotrophic growth may be limited by low O_2 availability in soil under continuous flooding [43].

The balance between CH_4 generation and oxidation determines CH_4 emissions in paddy soil. Yet, previous studies have rarely evaluated the response of CH_4 production and oxidation to P fertilizer application and its relation to CH_4 emissions in a long-term field experiment. Our results indicate that long-term P fertilizer application had no impact on the methanogenic abundance and activity in a double-rice system, which was similar to several previous observations [38,44]. However, Gao et al. (2020) [45] showed that P fertilizer addition could stimulate methanogenic growth in P deficient soil, because P fertilizer application stimulates rice-plant growth that produces more C substrate for methanogens. In our study, long-term P application increased soil DOC in the late season because it stimulated the rice aboveground biomass in the early season. Indeed, aside from C substrate, soil N availability and P availability affect CH_4 production. Higher NH_4^+ concentrations without P addition treatments may stimulate the straw decomposition and provide more substrates for methanogens [46]. Moreover, lower P concentrations can stimulate CH_4 production via acetoclastic methanogenesis [18]. Thus, the effect of NH_4^+ and P concentrations may offset the effect of C. In addition, P fertilizer application can affect the composition of root exudates, and then exert an influence on CH_4 production [47].

This study shows that long-term P fertilizer amendment stimulated the methanotrophic abundance, which agrees with [13,45]. There are several reasons for this phenomenon. First, P is the essential element for microbes, so soil P deficiency can limit the growth of methanotrophs. Moreover, P deficiency can decrease the activity of roots and thus lower radial oxygen loss from roots to the rhizosphere [47,48], which may restrict the aerobic methanotrophic growth. Furthermore, soil P deficiency increased soil NH_4^+ concentration in our study, which may limit CH_4 consumption by fighting for CH_4 monooxygenase [49]. In contrast, Sheng et al. (2016) [38] showed that P amendment decreased the abundance of methanotrophs, probably because the responses of diverse species of methanotrophs exist in different experimental soils to P deficiency are different [50]. In addition, our findings indicate that long-term P fertilizer amendment reduced methanotrophic activity, which may be due to the change in the community of methanotrophs. Recent research has shown that long-term P addition caused changes in the community of methanotrophs in paddy soils, resulting in an increase in type I methanotrophic abundance and a reduction in type II methanotrophic abundance [13]. Indeed, the CH_4 oxidation capacity of type I methanotrophs was lower than type II methanotrophs [51].

As a key element for rice-plant growth, P deficiency is widespread around the world and P fertilizer application is widely used to improve rice production [6,7]. This study also showed that long-term P addition promotes rice grain yield in both the early and late rice seasons. Yet, a higher increase was observed in the early season than in late season, which may be due to higher temperatures during the reproductive stage in the early season (30.1 °C) than in the late season (27.5 °C). The average air temperature of 30.1 °C may cause heat damage during the reproductive stage [52]. The P addition can increase rice resistance to heat stress, resulting in a higher rice yield [53]. Our findings indicate that the continuous P fertilizer amendment could play a more important role in rice yields in the global warming world.

We found that long-term P amendment reduced yield-scaled CH_4 emissions, indicating that P fertilizer addition can promote win–win outcomes for both food security and greenhouse gas reduction. However, long-term P amendment can affect soil N availability and ammonia oxidizing bacteria, which can directly affect N_2O emissions [54]. P addition also affects arbuscular mycorrhizal fungi [55], which may affect the decomposition of soil organic matter [56,57] and soil organic C storage. Therefore, we suggest that future study is needed to extend the impact of continuous P fertilizer amendment on net greenhouse gas emissions.

5. Conclusions

We found that long-term P fertilizer amendment improved aboveground dry matter accumulation and grain yield, but that it had no influence on root dry matter accumulation and also decreased the root–shoot ratio. Continuous P fertilizer amendment decreased soil NH_4^+ concentrations during both rice seasons, and increased soil DOC concentrations only in the late season. Long-term P fertilizer application increased methanotrophic abundance and CH_4 oxidation potential, without affecting methanogenic abundance and CH_4 production potential. Continuous P fertilizer addition reduced CH_4 emissions through stimulating CH_4 oxidation, without affecting CH_4 production. These findings suggest that long-term P fertilizer application can achieve higher rice productivity while mitigating CH_4 emissions under a double-rice cropping system.

Author Contributions: Conceptualization, J.C. and X.Z.; investigation, X.C., J.J., Y.C., X.L. (Xihuan Liang) and X.L. (Xianjin Lan); Data analysis, X.Z.; writing—original draft preparation, J.L. and X.Z.; writing—review and editing, J.C. and C.P.; funding acquisition, J.L., W.X. and J.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Natural Science Foundation of China (32060431), Collaborative Innovation Special Project of Jiangxi Modern Agricultural Research (JXXTCXQN 201904, JXXTCXQN202210), Science and Technology Project of Jiangxi Provincial Education Department (GJJ191138), Doctoral Research Fund of Nanchang Normal University (090170003312), Central Guided Local Science and Technology Development Fund (20221ZDH04057), and opening project of National Engineering and Technology Research Center for Red Soil Improvement (2020NETRCRSI-8).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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