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Long-Term Green Manure Rotations Improve Soil Biochemical Properties, Yield Sustainability and Nutrient Balances in Acidic Paddy Soil under a Rice-Based Cropping System

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Abstract: Cultivation of green manure (GM) crops in intensive cropping systems is important for enhancing crop productivity through soil quality improvement. We investigated yield sustainability, nutrient stocks, nutrient balances and enzyme activities affected by different long-term (1982–2016) green manure rotations in acidic paddy soil in a double-rice cropping system. We selected four treatments from a long-term experiment, including (1) rice-rice-winter fallow as a control treatment (R-R-F), (2) rice-rice-milkvetch (R-R-M), (3) rice-rice-rapeseed (R-R-R), and (4) rice-rice-ryegrass (R-R-G). The results showed that different GM rotations increased grain yield and the sustainable yield index compared with those of the R-R-F treatment. Compared with those of R-R-F, the average grain yield of early rice in R-R-M, R-R-R, and R-R-G increased by 45%, 29%, and 27%, respectively and that of late rice increased by 46%, 28%, and 26%, respectively. Over the years, grain yield increased in all treatments except R-R-F. Green manure also improved the soil chemical properties (SOM and total and available N and P), except soil pH, compared to those of the control treatment. During the 1983–1990 cultivation period, the soil pH of the R-R-M treatment was lower than that of the R-R-F treatment. The addition of green manure did not mitigate the soil acidification caused by the use of inorganic fertilizers. The soil organic matter (SOM), total nitrogen (TN) and total phosphorus (TP) contents and stocks of C, N and P increased over the years. Furthermore, GM significantly increased phosphatase and urease activities and decreased the apparent N and P balances compared with those in the winter fallow treatment. Variance partitioning analysis revealed that soil properties, cropping systems, and climatic factors significantly influenced annual grain yield. Aggregated boosted tree (ABT) analysis quantified the relative influences of the different soil properties on annual grain yield and showed that the relative influences of TN content, SOM, pH, and TP content on annual crop yield were 27.8%, 25.7%, 22.9%, and 20.7%, respectively. In conclusion, GM rotation is beneficial for

sustaining high crop yields by improving soil biochemical properties and reducing N and P balances in acidic soil under double- rice cropping systems.

Keywords: Acidic soil; crop rotation; enzyme activities; green manure; sustainable yield index; nutrient balance

1. Introduction

Long-term fertility experiments have been important resources for exploring nutrient cycling and performing overall assessments of soil fertility [1]. Long-term experiments provide a platform for investigating crop yield trends, productivity, soil quality, and other factors that contribute to agricultural sustainability [2]. Worldwide, irrigation and fertilization are the two most important factors for obtaining higher agricultural productivity and sustainability [3]. However, other factors, such as climate, pests, cultivars, soil types, and fertilization patterns, also contribute to changing yield trends and sustainability in long-term cropping systems [4].

During the past decades, due to the continuous and unwise use of fertilizers, rice (*Oryza sativa*) yield has increased significantly, but nutrient use efficiencies have decreased. This over fertilization has not only resulted in economic losses to farmers due to the misuse of expensive fertilizers but has also led to degradation of the soil and affected air, and water quality [5,6]. The negative effects of degraded soil quality, such as acidification and poor soil structure, on rice yield have been found in many studies [7,8]. Crop rotation with legumes such as green manure (GM) not only improves the soil quality and soil water content by increasing soil organic matter but also increases nutrient availability through atmospheric N fixation with rhizobia [9]. Studies have found that GM decreased the population, species, and density of unwanted weeds in rice fields [10]. Similarly, GM not only improves the soil biological (microbial activities) and physical properties [11,12] but also increases the carbon (C) and N cycling [13–15]. Furthermore, GM increases the quantity of water-stable macroaggregates in the topsoil and increases the capacity of microbes to mineralize organic N; consequently, it can enhance the N supply capacity and crop productivity [16,17]. Cultivation of GMs such as Chinese milkvetch (*Astragalus sinicus* L.) in paddy soils can exploit their natural qualities and improve rice productivity with minimal economic and environmental losses [18,19].

Previous studies of different long-term experiments at different locations have indicated decreasing yield trends after a few years of continuous cropping under rice-wheat (*Triticum aestivum*) rotations [20]. The cultivation of input-responsive high-yielding cultivars has been increased, compelling farmers to apply high doses of chemical fertilizers. Excessive use of inorganic fertilizers is a common practice to meet the nutrient need of high-yield varieties. Different long-term experiments have indicated that long-term continuous and excessive chemical fertilization degraded the soil quality and increased acidification, which resulted in poor crop sustainability [21,22].

The sustainable yield index (SYI) represents the actual yields over a long period. A higher sustainable yield index indicates that an area will produce an acceptable yield over the years through better cultivation practices [20–22]. SYI is a quantitative measure with which to assess the sustainability of an agricultural practice [23]. Long-term experiments provide one of the means of measuring the sustainability of an agricultural system [2]. Data records from long-term experiments can be used as early warning systems for the future [24]. In China, many long-term fertility experiments have indicated wide variability in crop yields that has been attributed to the continued reduction of soil fertility [6,25]. To monitor the long-term fertility and yield response of crops to fertilization, the first step in analyzing long-term experiments is to identify the sustainability of crop productivity. Previous studies have mainly focused on the effects of GM on rice production in short-term experiments or in a single cropping system [26,27]. However, little is known about the benefits of different GM rotations for sustainable rice productivity and nutrient balance, especially in paddy soils under long-term

rice-based cropping systems [28–30]. The aims of this study were to investigate the effect of different green manure rotations on SYI, crop yield, apparent nutrient balance, and enzyme activities in an acidic paddy soil.

2. Materials and Methods

2.1. Site Description

A long-term (1982–2016) field experiment was conducted at the red soil experiment station of the Chinese Academy of Agricultural Sciences in Qiyang County (26°45′42″ N, 111°52′32″ E), Hunan Province, China. The climate in this region is a humid subtropical monsoon with a mean annual rainfall of 1290 mm. The main rainfall period is from April to the end of June, during the period of early-season rice cultivation. The drought period is from August to October during the late-season rice cultivation period, and the mean annual temperature is 17.8 °C. The mean annual temperature (MAT) and mean annual precipitation (MAP) for the experimental period are shown in Figure S1. The paddy soil of this region is categorized as a Ferralic Cambisol [31], which was made by Quaternary red clay. The initial soil properties were pH 6.1, soil organic matter (SOM) 20.4 g kg⁻¹, total N 0.94 g kg⁻¹, total P 0.65 g kg⁻¹, total K 10.6 g kg⁻¹, alkali-hydrolyzed N 148 mg kg⁻¹, Olsen-P 17.6 mg kg⁻¹ and available K 175 mg kg⁻¹ in the 0–20 cm soil layer.

2.2. Experimental Design

The four cropping treatments were rice-rice-winter fallow (R-R-F), rice-rice-milkvetch (*Astragalus sinicus* L.) (R-R-M), rice-rice-rapeseed (*Brassica napus* L.) (R-R-R) and rice-rice-ryegrass (*Lolium multiflorum*) (R-R-G). All treatments had three replicates arranged in a randomized complete block design. The area of each replicate was 37.5 m² (2.5 m × 15.0 m). Each replicate plot was separated from the adjacent replicates by a 60-cm cement barrier to prevent water and nutrient contamination from the nearby plot. The total fertilizer application rates for each rice growing season were 153 kg ha⁻¹ N, 84 kg ha⁻¹ P₂O₅, and 129 kg ha⁻¹ K₂O. The compound fertilizer (600 kg ha⁻¹) containing 14% each of N, P₂O₅, and K₂O was applied as basal application 1 day before rice transplantation, while urea and potassium chloride for N (69 kg ha⁻¹) and K₂O (45 kg ha⁻¹), respectively, were topdressed 6 to 10 days after rice transplantation. Green manure crops were sown in the middle of October after the late rice was harvested and were plowed into the soil as a whole crop before the early rice transplantation (in the first week of April) every year. The seeding rates of milkvetch, rapeseed, and ryegrass were 37.5, 7.5, and 15.0 kg ha⁻¹, respectively. Early and late rice seedlings were transplanted with spacing of 20 cm × 20 cm and 20 cm × 25 cm, respectively, and each hill contained three seedlings. All rice straw (except the rice stubble) was removed from the plots after each seasonal rice harvest. No fertilizer was applied to the green manure crop in winter. The fresh biomass of the incorporated ryegrass, milkvetch, and rapeseed was 5700 kg ha⁻¹, 6000 kg ha⁻¹, and 6200 kg ha⁻¹, while the dry matter was 1126 kg ha⁻¹, 1276 kg ha⁻¹, and 1249 kg ha⁻¹, respectively. Milkvetch contained 43.7% C and 3.9% N contents, ryegrass contained 41.5% C and 1.8% N contents, rapeseed contained 42.3% C and 1.76% N contents in samples collected in 2017. Conventional routine management practices were used for pest management and irrigation. The rice varieties used locally were selected and were replaced every 3–5 years (Table S1). Flooding was maintained throughout the early rice season, and the field was drained at the late rice ripening stage [32,33].

2.3. Sampling and Analysis

Early and late rice grain and straw samples were manually harvested at six randomly selected points from each plot and weighed. Grain and straw samples were oven-dried at 105 °C for 30 min and then heated at 70 °C to a constant weight for dry matter and total N and P determination. Dried grain and straw samples were ground and digested with H₂SO₄-H₂O₂ at 260–270 °C. The total plant N and P were determined using the semimicro Kjeldahl digestion method and the vanadomolybdate yellow

method, respectively (Jackson 1969; Nelson and Sommers 1980). The soil samples were collected every year from the starting date of the experiment (1983) to 2016. Soil samples were collected at a depth of 0–20 cm from five randomly selected points in each plot after late rice harvest, and plant materials and stones were removed from the soil. Soil samples were then mixed, air-dried, and sieved (0.2 mm) for the determination of soil chemical properties. Soil organic carbon (SOC) was determined with the potassium dichromate oxidation method [34], and total N (TN) was determined with the Kjeldahl method [35]. Soil total P (TP) was determined according to Murphy and Riley [36]. Alkali-hydrolyzable mineral N (AN) was determined according to Lu [37]. Available P (AP) was extracted using 0.5 mol L⁻¹ NaHCO₃, and the measurement of AP was performed by the Olsen method [38].

For enzyme activity determination, soil samples were collected in 2017 after the late rice harvest. Enzyme activities were determined in field-moist soil samples. A portion of the subsamples was used to determine the soil moisture content before the analysis of enzyme activities by oven drying at 105 °C for 24 h. Acid phosphomonoesterase (AcP) and phosphodiesterase (DP) activities were determined following Tabatabai [39]. Briefly, 1 g of soil was incubated at 37 °C for 1 h after adding *p*-nitrophenyl phosphate as a substrate solution with a buffer solution of pH 6.5 for AcP. DP was assayed using a substrate solution of bis-*p*-nitrophenyl phosphate with a buffer solution of pH 8. Urease activity was determined following the colorimetric method [40]. Briefly, 5 g of moist soil was incubated at 37 °C for 2 h after adding urea solution as the substrate. A modified Berthelot reaction was followed to extract the released ammonium by potassium chloride solution. The activities of AcP and DP were expressed as µg NP g⁻¹ soil h⁻¹, and urease activity was expressed as µg N g⁻¹ soil 2h⁻¹.

Stocks (S) of SOC, total N and total P were estimated as follows:

$$S = SOC \times BD \times H \times 10^{-1} \quad (1)$$

where, S is the stock (ton ha⁻¹) of soil organic carbon (SOC), total N or total P. The concentration of soil organic carbon (g kg⁻¹), total N (g kg⁻¹) or total P (g kg⁻¹) is used in the equation. BD is the soil bulk density (g cm⁻³), and H is the depth of soil sampling (cm). BD is the soil bulk density (g cm⁻³), and H is the depth of soil sampling (cm). In the present study, the soil bulk density was not measured directly from the field and was calculated using the following equation in ref. [41].

$$BD = -0.0048 \times SOC + 1.377 \quad (2)$$

The apparent nutrient balance (AB) (kg ha⁻¹ year⁻¹) is the difference in nutrient inputs to the field through fertilization and nutrient outputs from the field in harvested biomass [42]. The apparent nutrient balance is based on the soil surface (lower plow) balance but does not incorporate the potential losses incurred from runoff or soil erosion. A positive value of the apparent nutrient balance indicates a nutrient surplus, and a negative value of the apparent nutrient balance (nutrient deficit) indicates declining soil fertility [42]. The environmental risk threshold values are 45 kg ha⁻¹ year⁻¹ for the P balance and 180 kg ha⁻¹ year⁻¹ for the N balance [43,44]. The apparent nutrient balance was estimated using the following equation [44]:

$$\text{Apparent nutrient balance} = F_{\text{input}} - N_{\text{output}} \quad (3)$$

where F_{input} is the nutrient input from fertilizers and N_{output} is nutrient offtake by the crop in the harvested biomass (nutrients assimilated into straw and grains of rice).

2.4. Sustainable Yield Index (SYI)

SYI is a quantitative measure with which to assess the sustainability of an agricultural practice [23]. The sustainable yield index (SYI) was estimated using the following equation [45]:

$$\text{Sustainable Yield Index (SYI)} = \frac{Y_{\text{mean}} - \sigma}{Y_{\text{max}}} \quad (4)$$

where Y_{mean} is the mean yield of a treatment, σ is the treatment standard deviation, and Y_{max} is the maximum yield in the experiment over the years for each treatment.

2.5. Statistical Analysis

We evaluated the significant differences among treatments by one-way ANOVA, and interactive effects of treatments and cultivation years were evaluated by two-way ANOVA followed by least significant difference (LSD) test at the $p = 0.05$ level significance [46] using SPSS 16.0 software (SPSS, Chicago, IL, USA). Variance partitioning analysis (VPA) was performed to evaluate the contributions of soil factors (soil pH, SOM, total N, and total P), cropping systems, climatic factors (MAT and MAP), and their interactions in changing the annual rice yield over the cropping period (1984–2016) using CANOCO for Windows (version 5.0). Aggregated boosted tree (ABT) analysis was performed to investigate the relative influence of the important soil factors on annual crop yield using the ‘gbmplus’ package in R software (version 3.3.3) [47].

3. Results

3.1. Crop Yield and Sustainability Index

The results showed that long-term rotations of different green manure (GM) types in the double-rice cropping system significantly ($p \leq 0.05$) increased grain yield and sustainable yield index (SYI) compared to those in the winter fallow system (Table 1 and Figure 1). The highest increase in the average grain yield and SYI over the period of early and late season cropping was under the milkvetch rotation (R-R-M). Compared to R-R-F, the average increases in grain yield in R-R-M, R-R-R, and R-R-G were 45%, 29%, and 27%, respectively, in early rice and 46%, 28%, and 26%, respectively, in late rice. Compared to those in the winter fallow system, crop yields of both early and late rice were increased over the year, but the highest increase was under the R-R-M treatment (Figure 1). The increases in SYI in R-R-M, R-R-R, and R-R-G compared to R-R-F were 36%, 21%, and 23%, respectively, in early rice, 24%, 20.3%, and 10%, respectively, in late rice and 19%, 16%, and 9.5%, respectively, in double-rice cropping systems overall.

Table 1. Effect of long-term green manure rotations on the sustainability yield index (SYI) of double-rice cropping systems in acidic paddy soil.

Treatments	Average Grain Yield (kg ha ⁻¹)		Yield Sustainability Index		
	Early Rice Yield	Late Rice Yield	Early Rice	Late Rice	Double Rice Cropping
R-R-F	4532 ± 812 c	4022 ± 780 c	0.53 c	0.59 c	0.63 c
R-R-M	6559 ± 819 a	5859 ± 741 a	0.72 a	0.73 a	0.75 a
R-R-R	5853 ± 917 b	5135 ± 623 b	0.64 b	0.71 a	0.73 ab
R-R-G	5769 ± 930 b	5076 ± 728 b	0.65 b	0.65 b	0.69 b

Average grain yield is based on mean of grain yield of all cultivation years (1982–2016). Means followed by different letters are significantly ($p \leq 0.05$) different from each other according to Tukey’s LSD test.

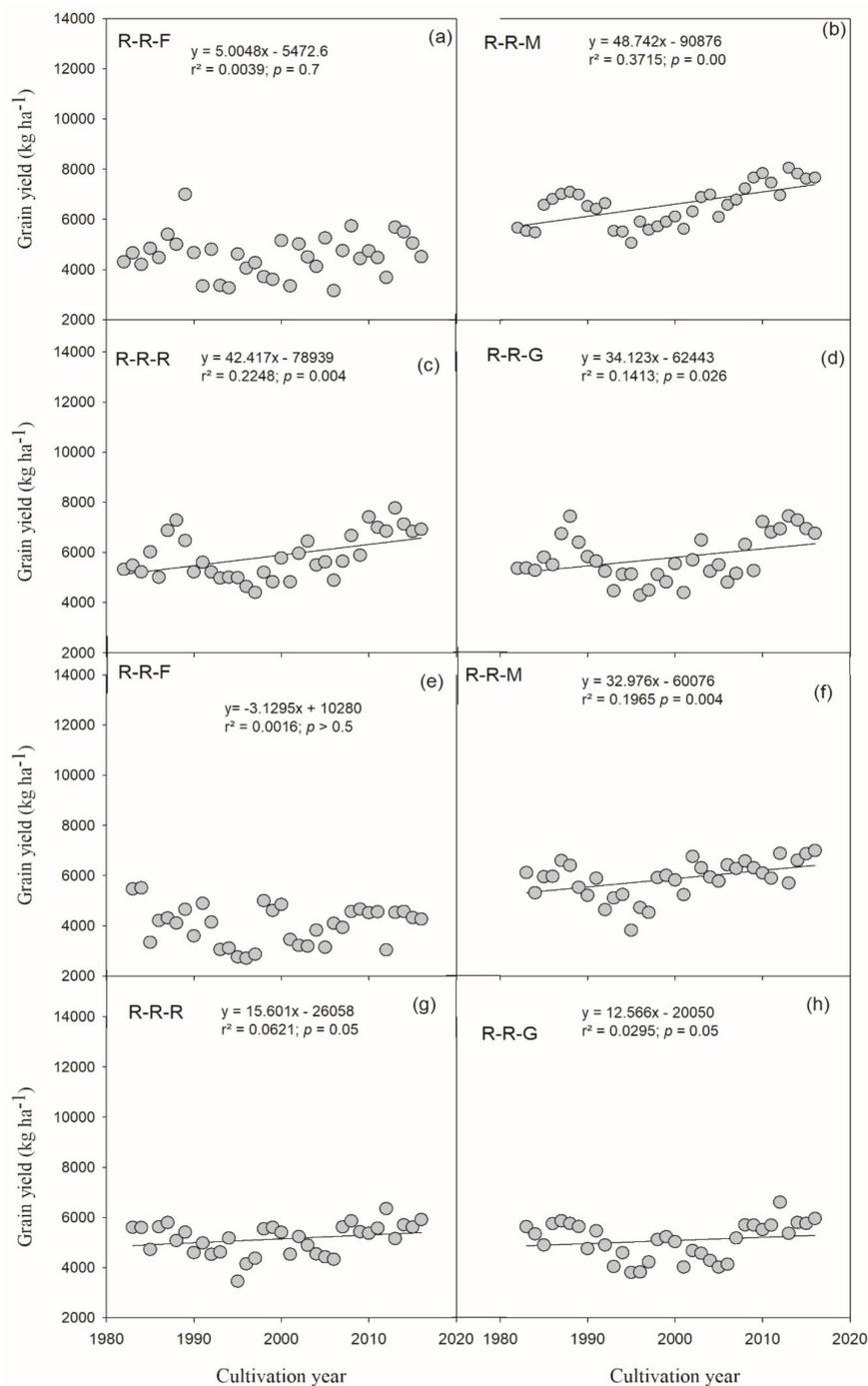


Figure 1. Linear regression showing trends of rice grain yields over the years (1982–2016) under green manure rotations in the rice-based cropping system.

3.2. Soil Nutrient Content, Nutrient Stocks, and Apparent Nutrient Balance

The treatment \times year interaction significantly affected soil properties (Table 2). During the fertilization period of 1983–1990, soil pH was not significantly different between the R-R-G and R-R-R treatments. Among all treatments, during 1983–1990, the soil pH was lowest under the R-R-M treatment. The soil pH under the R-R-F and R-R-M treatments during 1983–1990 was decreased by 0.98% and 2% compared to the initial soil pH. During the period of 1991–2000, the soil pH among all treatments was decreased compared to the soil pH during 1983–1990, and it was highest under the R-R-R treatment. In all treatments, over the years, the SOM content was higher than the initial SOM

content. Compared to the initial SOM content, the increases in SOM content under the R-R-F, R-R-M, R-R-G, and R-R-R treatments were 7.3%, 23%, 12.2%, and 12.7%, respectively, during 1983–1990 and 11.3%, 20%, 15.6%, and 16.6%, respectively, during 1991–2000. The respective increases in SOM content during 2001–2016 under the R-R-F, R-R-M, R-R-G, and R-R-R treatments were 33.8%, 40.6%, 36.7%, and 41.2%, respectively, compared to the initial SOM content. The soil TN content in all treatments was higher in all fertilization periods compared to the initial soil TN content. Compared to that in the R-R-F treatment, the increase in the soil TN content under the R-R-M, R-R-G, and R-R-R treatments was 15.9%, 14%, and 7.5%, respectively, during the period 1983–1990, by 23%, 7.0%, and 16%, respectively, during 1991–2000, and by 9.8%, 4.6%, and 3.9%, respectively, during 2001–2016. Over the years, the soil TP content increased in all treatments compared with the initial soil TP content. The soil TP content in the R-R-F, R-R-M, R-R-G, and R-R-R treatments increased by 6%, 7.7%, 12%, and 7.7%, respectively, during 1983–1990. During 1991–2000, the TP content was highest under the R-R-R treatment and did not show a significant difference among the R-R-F, R-R-M, and R-R-G treatments. During 2001–2016, the soil TP content also did not show a significant difference between the R-R-F and R-R-M treatments or between the R-R-G and R-R-R treatments. After 34 years of cropping, the increases in AN under R-R-M, R-R-R and R-R-G compared to R-R-F were 22%, 6.4%, and 16.5%, respectively, in early rice and 11%, 6%, and 5.6%, respectively, in late rice (Figure 2). Similarly, compared to R-R-F, the increases in Olsen-P under R-R-M, R-R-R, and R-R-G were 10.5%, 25.7%, and 22%, respectively, in early rice and 11.7%, 19.3%, and 25.5%, respectively, in late rice. Green manure increased stocks of C, N, and P compared to those in the winter fallow treatment (Figure 3). On average, across the years, the soil C and N stocks were not different between the R-R-R and R-R-G treatments. Over the years, soil C, N, and P stocks increased in all treatments, and the lowest increase was under the R-R-F treatment.

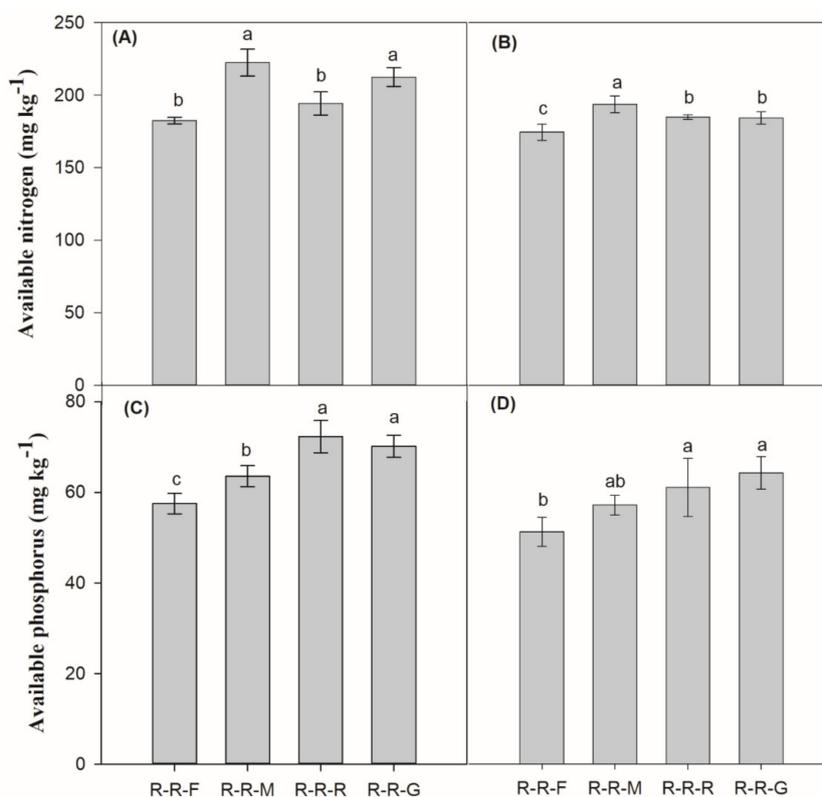


Figure 2. Effect of different long-term green manure rotations on soil available N after early rice harvest (A) and after late rice harvest (B), and available P after early rice harvest (C) and after late rice harvest (D). Error bars represent \pm standard deviations; different letters over the bars indicate significant ($p \leq 0.05$) differences according to Tukey's LSD test.

Table 2. Effect of long-term green manure rotations on soil pH, soil organic matter (SOM), total nitrogen, and total phosphorus content in acidic paddy soil.

Year	Treatment	pH	SOM (g kg ⁻¹)	TN (g kg ⁻¹)	TP (g kg ⁻¹)
Initial values		6.1	20.4	0.94	0.65
1983–1990	R-R-F	6.04 ± 0.06 bc	21.9 ± 0.34 c	1.07 ± 0.03 c	0.69 ± 0.01 b
	R-R-M	5.98 ± 0.07 c	25.1 ± 0.29 a	1.24 ± 0.03 a	0.70 ± 0.02 b
	R-R-G	6.07 ± 0.07 ab	22.9 ± 0.84 b	1.22 ± 0.02 a	0.74 ± 0.02 a
	R-R-R	6.13 ± 0.01 a	23.0 ± 0.57 b	1.15 ± 0.01 b	0.70 ± 0.02 b
1991–2000	R-R-F	5.45 ± 0.03 c	22.7 ± 0.45 c	1.13 ± 1.01 d	0.90 ± 0.02 b
	R-R-M	5.70 ± 0.13 a	24.5 ± 0.44 a	1.39 ± 0.02 a	0.91 ± 0.02 b
	R-R-G	5.57 ± 0.04 b	23.6 ± 0.51 b	1.21 ± 0.02 c	0.89 ± 0.04 b
	R-R-R	5.80 ± 0.04 a	23.8 ± 0.35 b	1.31 ± 0.02 b	0.96 ± 0.04 a
2001–2016	R-R-F	5.78 ± 0.05 ns	27.3 ± 0.52 c	1.67 ± 0.03 c	1.11 ± 0.03 b
	R-R-M	5.83 ± 0.08	28.7 ± 0.34 a	1.84 ± 0.09 a	1.13 ± 0.08 b
	R-R-G	5.77 ± 0.04	27.9 ± 0.50 a	1.75 ± 0.03 b	1.26 ± 0.08 a
	R-R-R	5.87 ± 0.08	28.8 ± 0.43 b	1.74 ± 0.04 b	1.22 ± 0.07 a
Two-way ANOVA	Treatment (T)	***	***	***	***
	Year (Y)	***	***	***	***
	T × Y	***	***	***	*

Values are means ± standard deviations. Means followed by different letters are significantly ($p \leq 0.05$) different from each other according to Tukey's LSD test. * Significant ($p \leq 0.05$). *** Very highly significant ($p \leq 0.001$). ns: nonsignificant ($p > 0.05$). Note: We divided the long-term (1983–2016) data into three groups by taking the average of years 1983–1990, 1991–2000, and 2001–2016 to better describe the long-term changes in the results.

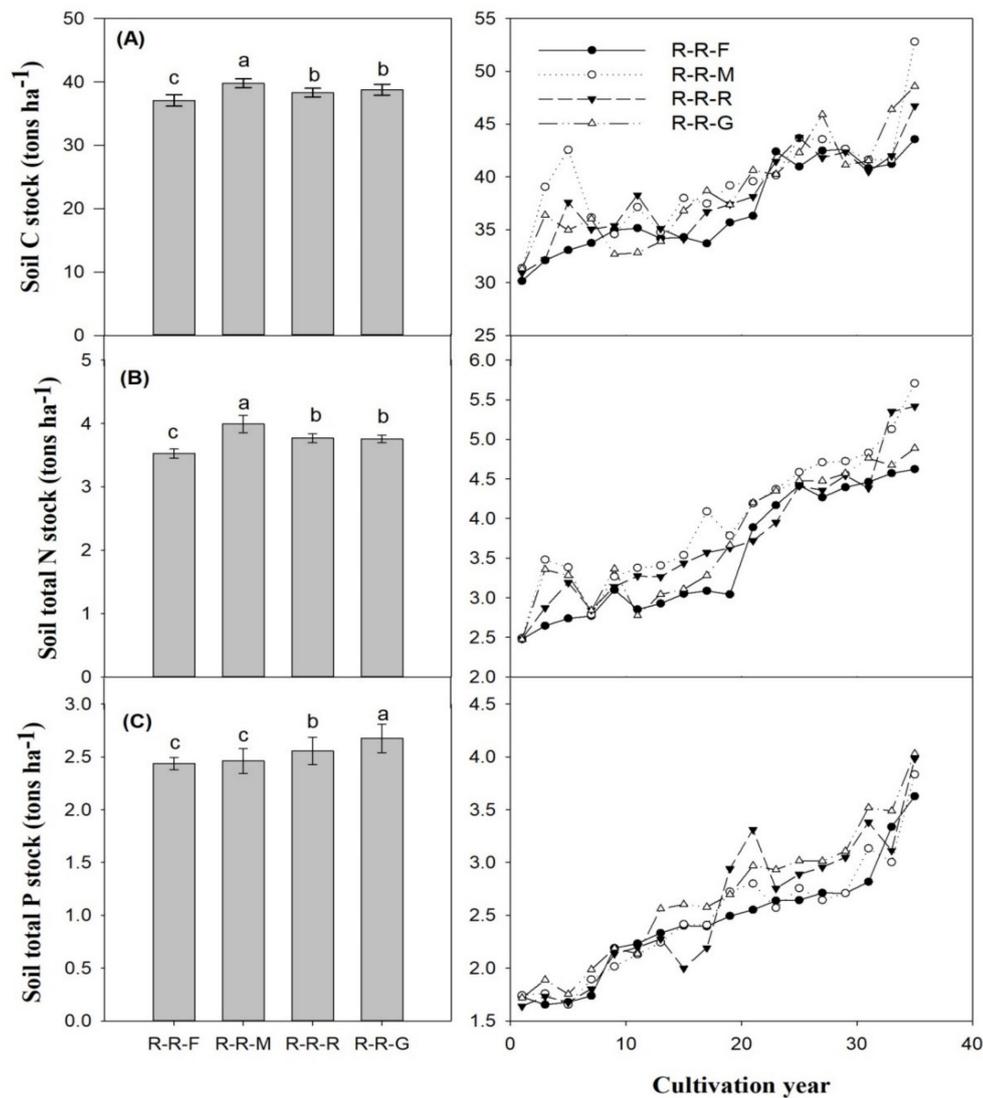


Figure 3. Effect of different long-term green manure rotations on stocks of soil organic carbon (A), total N (B) and total P (C) in acidic paddy soil. Error bars represent \pm standard deviations; different letters over the bars indicate significant ($p \leq 0.05$) differences according to Tukey's LSD test.

GM rotations significantly ($p \leq 0.05$) influenced the annual apparent N and P balance in the long-term experiment on the double-rice cropping system (Figure 4). In the control treatment (R-R-F), the apparent N and P balances were highest compared with those in all other GM treatments. The annual N balance in the R-R-M, R-R-R and R-R-R treatments was decreased by 38%, 21%, and 24%, respectively, and the annual P balance was decreased by 36%, 27%, and 34%, respectively, compared with those of R-R-F in the double-rice cropping system.

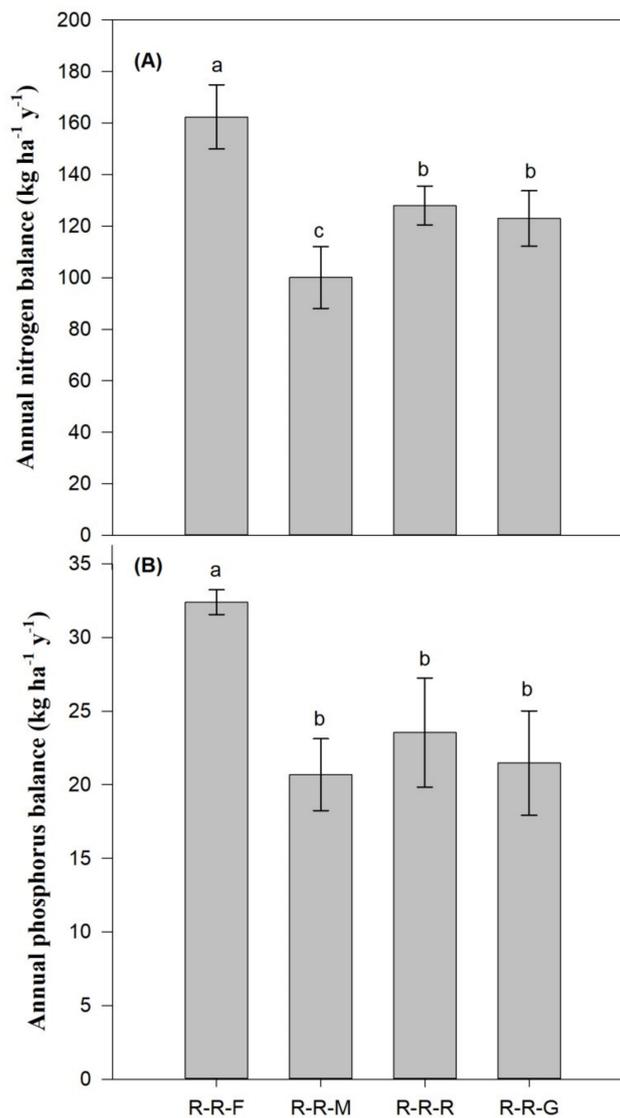


Figure 4. Effect of different long-term green manure rotations on annual apparent (A) N and (B) P balances in acidic paddy soil. Error bars represent \pm standard deviations; different letters over the bars indicate significant ($p \leq 0.05$) differences according to Tukey's LSD test.

3.3. Enzyme Activities

Different long-term GM rotations significantly influenced phosphatase and urease activities (Figure 5). Acid phosphomonoesterase (AcP), phosphodiesterase (DP), and urease activities were highest in the R-R-M treatment and lowest in the R-R-F treatment. Compared with those in the control, the R-R-M, R-R-R, and R-R-G treatments increased AcP activity by 68%, 52%, and 50%, DP activity by 83%, 70%, and 65% and urease activity by 72%, 20%, and 25%, respectively. Available N and P significantly positively affected AcP and DP activities (Figure S2). Available N also showed significant positive linear relationship with urease activities.

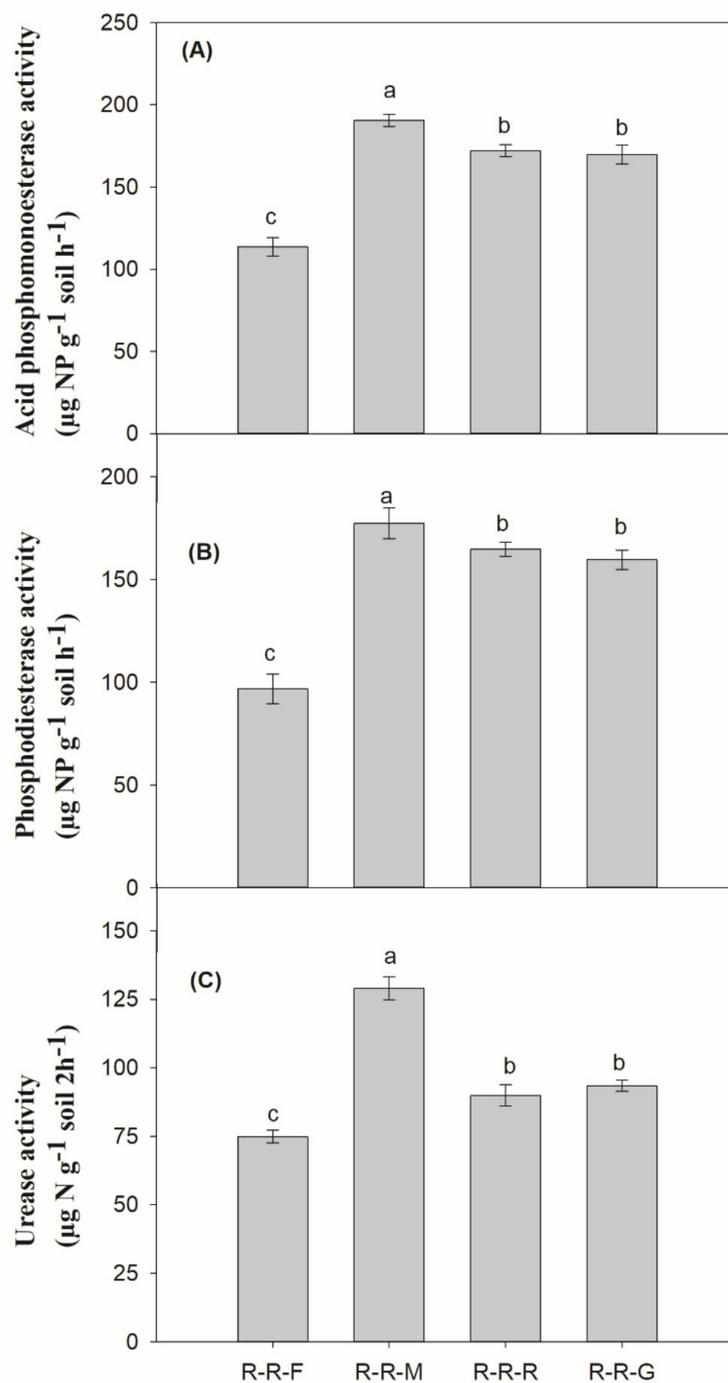


Figure 5. Effect of different long-term green manure rotations on enzyme activities in acidic paddy soil. Error bars represent \pm standard deviations; different letters over the bars indicate significant ($p \leq 0.05$) differences according to Tukey's LSD test.

3.4. Factors Influencing Crop Yield

We investigated the contributions of different factors influencing crop yield by variance partitioning analysis and aggregated boosted tree (ABT) analysis. The changes in early and late rice yield over the long period of cropping were due to variations in different soil and climatic factors in the long-term double-cropping system. Variance partitioning analysis showed that soil properties (pH, SOM, total N, and total P) accounted for 11.3% of the variation, the cropping system accounted for 8.8%, and climatic factors (MAT and MAP) accounted for 4.6% of the variation in annual grain yield. The total variance

explained by VPA was 36%, and the unexplained proportion was 64% for annual grain yield (Figure 6). Aggregated boosted tree (ABT) analysis indicated that soil TN, SOM, soil pH, and soil TP contents were the most influential factors on crop yield (Figure 7).

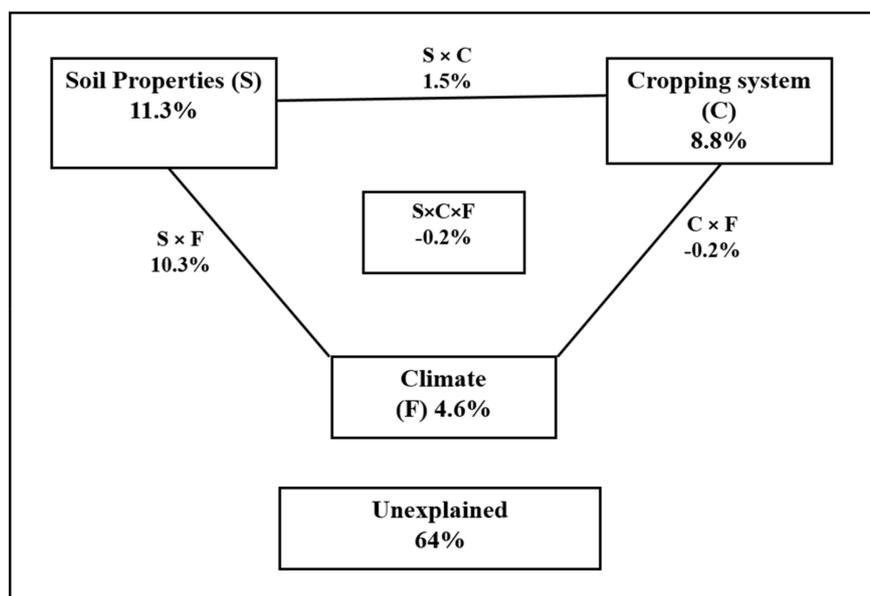


Figure 6. Variance partitioning analysis (VPA) indicating the effects of soil properties, cropping systems, and climatic factors on annual crop yield under long-term green manure rotations in rice-based cropping systems.

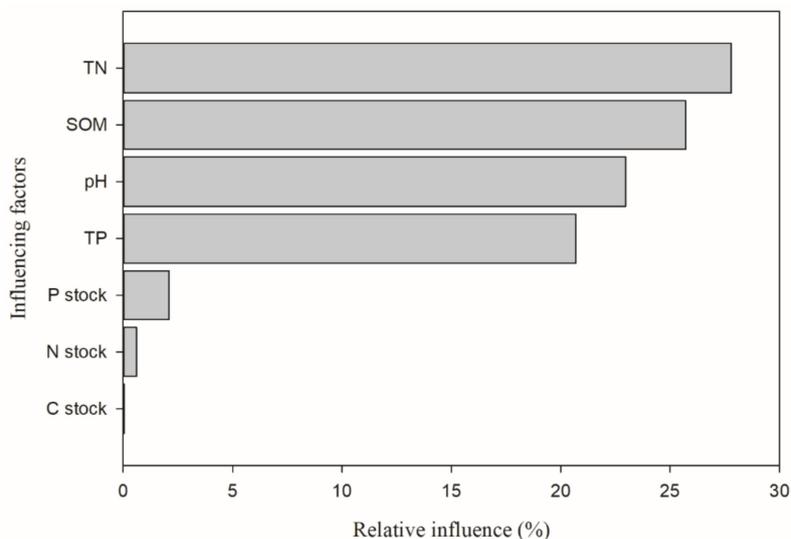


Figure 7. Relative influence (%) of different predictors on annual crop yield by aggregated boosted tree (ABT) analysis. Long-term data (1983–2016) were used for ABT analysis.

4. Discussion

The sustainability yield index (SYI) is considered a major indicator of agricultural sustainability for crop production and soil fertility management [48,49]. A high SYI indicates a more sustainable cropping system [29]. In this study, different long-term (34-year) GM rotations significantly ($p \leq 0.05$) increased grain yield and sustainability yield index (SYI) values compared to those of a winter fallow system in acidic soil under a double-rice cropping system (Table 1 and Figure 1). The highest increases in grain yield and SYI for both early and late rice were under the milkvetch rotation because milkvetch

is a leguminous species that provides a large quantity of biologically fixed N for better plant growth and production [33]. GM rotation improves the soil properties and nutrient supply capacity [28]. Yang et al. [32] found that GM increased both the quality and quantity of SOM and had a positive impact on soil nutrient availability, which helped to increase the sustainability of rice yield. However, in previous studies, long-term experiments showed declining trends in grain yield under rice-rice and rice-wheat cropping systems [20,50]. In our results, the R-R-F treatment decreased the crop yield over the years (Figure 1). It has been shown that in double-cropping systems, long-term inorganic fertilization may decrease the crop yield over the years due to decreasing soil quality, including through increasing soil acidification [29,51,52]. In long-term experiments, different factors such as climate and soil properties influence productivity over the long cultivation period. In our study, soil properties, cropping systems, and climate significantly influenced crop yield. These results are consistent with previous studies, indicating the influence of soil properties and climate (mean annual temperature and mean annual precipitation) on rice yield [53,54]. However, in our VPA analysis results, the unexplained proportion was 64%, which was higher than the explained proportion. This might be due to the same rates of inorganic fertilization being applied in all cropping treatments because fertilizer is one of the main influencing factors on crop yield in long-term fertility experiments [55,56].

Soil fertility is one of the main indicators for measuring the sustainability of cropping systems [28,57]. Our results showed that GM increased nutrient contents and stocks compared to those under the winter fallow treatment, and the highest increase in available N content was under the R-R-M treatment (Figure 2). Non-leguminous cover crops decrease nutrient leaching and enhance nutrient availability [58]. Leguminous cover crops enhance N availability and therefore increase crop yield by improving N use efficiency [27]. The ability of legumes to fix atmospheric molecular dinitrogen into mineral N for plant uptake makes legumes the most efficient GM [59]. The decreasing trend in soil pH could have been due to long-term synthetic fertilization [28]. Lin et al. [51] also observed a declining trend in soil pH in paddy soil under long-term fertilization. Many studies observed soil acidification caused by long-term inorganic fertilization [51,60]. Moreover, plants release net H^+ ions; on the other hand, when the uptake of anions exceeds the cation uptake, they release a net excess of OH^- or HCO_3^- [61]. It was also observed in previous studies that N fertilization shifted the soil to the Al^{3+} buffering stage. Al is released in soil solution from the surface of clay minerals during the hydrolysis process of Al hydroxides at a relatively low soil pH, which may also decrease the amount of base cations and increase the soil acidity [62]. However, the alkaline nature of GM neutralizes the protons in acidic soil and helps to improve soil pH [63]. GM can also help to improve soil physical and chemical properties by improving soil porosity [17,64] and structure, reducing leaching [65] and increasing water holding capacity [32,66], thereby providing suitable conditions for proper plant growth to sustain long-term crop yield.

Nutrient release in soil from decaying SOM under long-term GM application increases the soil enzyme activities [67]. Leguminous cover crops change the microbial community in rhizosphere soil and increase the enzyme activities [33]. Moreover, available N and P showed significant positive relationship with enzyme activities (Figure S2), which indicate that higher enzyme activities enhance the nutrient availability and their uptake by crop, that may reduce the nutrient balance in the present study. The decreases in the annual N and P balances were consistent with the results of Balík et al., [68] but different from the results found by Ladha et al., [69] probably because the crop yield in the study by Ladha et al. [69] was not significantly different after GM rotation compared with the yields obtained by chemical fertilization. In this study, the lowest annual N balance was under the Chinese milkvetch rotation, possibly because the Chinese milkvetch increased crop yield and N uptake more than the ryegrass and rapeseed rotations [33,70,71]. In addition, the magnitude of the annual P balance reduction was less than that of the annual N balance (Figure 4). This finding was supported by a previous study conducted in Tianjin [72]. However, the annual P balances among all three GM treatments were not significantly different. The annual P balance is mainly controlled by soil abiotic

factors (soil properties and climate) or biological factors (microorganisms) and is less affected by the GM species [73], which was consistent with our findings.

5. Conclusions

We have concluded that green manure rotation in a rice-based cropping system significantly increased crop yield sustainability by increasing soil nutrient availability and enzyme activities in acidic paddy soil. The highest increase in crop yield was under the rice-rice-milkvetch rotation system. However, green manure crops did not mitigate soil acidity due to the long-term addition of inorganic fertilizer during the early and late rice seasons. Green manure also decreased the apparent N and P balances by increasing the N and P uptake compared to those in winter fallow in rice-based cropping systems. The soil properties, cropping system, and climatic conditions significantly contributed to changing the annual crop yield over the experimental period. Among the soil properties, TN, SOM, pH, and TP were the most influential factors on crop yield. Therefore, the cultivation of green manure in rice-based cropping systems could be helpful for enhancing crop yield sustainability but might not be very effective for mitigating soil acidity under long-term inorganic fertilization in acidic paddy soil. Moreover, GM crops could be helpful for minimizing the environmental losses of N and P by decreasing the apparent N and P balances.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4395/9/12/780/s1>, Figure S1: Long-term (1983–2016) mean annual temperature and precipitation during the period of experiment of GM rotation in double rice cropping system, Figure S2: Linear regression analysis indicating relationships of enzyme activities with available nitrogen and available Phosphorus under long-term green manure rotation in rice based cropping system, Table S1: The name of cultivars of rice sown in this experiment from 2009 to 2018.

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