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Effects of different green manure treatments on soil apparent N and P balance under a 34-year double-rice cropping system

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Abstract

Purpose Although green manure rotation is often used to promote soil fertility and crop yield, the effects of this management practice on the nitrogen or phosphorus balance and the relationship between nutrient balance and the increase in soil nutrients have not been systematically studied.

Materials and methods We investigated the apparent nitrogen and phosphorus balances and their associations with soil nitrogen and phosphorus increases, respectively, in a 34-year-old experimental site with various green manures and rice rotations using linear and logistic models. Cropping treatments included a rice-rice-winter fallow treatment as a control (RRW) and three green manure rotation treatments: rice-rice-ryegrass (RRR), rice-rice-oil rape (RRO), and rice-rice-Chinese milk vetch (RRC).

Results and discussion We found that apparent nitrogen and phosphorus balances of RRR, RRO, and RRC were 164, 162, and 149 kg hm⁻², which were all significantly lower than 200 kg hm^{-2} of RRW (P < 0.05). Moreover, the optimal fitted model of the relationship between cumulative nutrient balance and the increase in soil nutrients was different among treatments. Specifically, the correlation coefficients of cumulative nitrogen balance and soil nitrogen increase of RRR, RRO, and RRC in the linear model (0.49, 0.80, and 0.63) were all significantly lower than in the logistic model (0.81, 0.90, and 0.82). The correlation coefficients of cumulative phosphorus balance and increase in soil phosphorus of RRW in the linear model (0.81) were significantly lower than in the logistic model (0.91). Parameter analysis of the optimal fitted model revealed that RRC would increase the storage capacity of soil nitrogen and decrease the rate of soil phosphorus accumulation.

Conclusions Our results suggested that long-term rice-rice-green manure rotation could significantly change the apparent nitrogen and phosphorus balance and their association with soil nitrogen and phosphorus content, respectively. Our study highlights the importance of green manure rotation in an agro-ecological environment and soil fertility in a double rice cropping system in red paddy soil.

Keywords Long-term field experiment · Nitrogen and phosphorus balance · Red paddy soil · Rice-rice-green manure rotation · Soil nitrogen and phosphorus increases

Xi Hong and Chao Ma contributed equally to this work.

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1 Introduction

Nitrogen (N) and phosphorus (P) are two of the most important plant nutrients. Their balance—the difference between input and output of N and P—in agricultural soils is essential for crop maintenance and yield. Investigations into soil N and P balance are vital for optimizing soil fertility management and reducing agricultural pollution (Ekholm et al. 2005; Heming 2007; Ross et al. 2008; Sun et al. 2008; Bouwman et al. 2009; Shi et al. 2015). Regional soil N and P levels are primarily contingent upon climate, soil type, fertilizer composition, length of fertilization, and crop rotation methods (Ladha et al. 2000; Gasser et al. 2002; Sun et al. 2008; Constantin et al. 2010; Mitran and Mani 2017). Since environmental contamination is aggravated by the application of chemical fertilizers, many researchers are considering the use of organic fertilizers, especially green manures (Gao et al. 2015). Nevertheless, the effects of green manure on soil N and P balances, particularly during seasonal crop fallow periods, have seldom been investigated.

Cultivation of green manure has several effects on farmland ecology, including the following: (1) improving the supply of water and nutrients to crops, (2) increasing nutrient levels in the soil, (3) altering soil microorganism composition and activity, (4) changing soil nutrient speciation and proportions, and (5) promoting the formation of organic-inorganic complexes to improve soil structure (Yang et al. 2012; Zhang et al. 2013; Yang et al. 2014; Gao et al. 2015). Nutrient balances in the soil are the differences between input and output. For example, major N inputs include fertilizer application (both chemical and organic), atmospheric deposition, and biological fixation, while main outputs are grain harvesting, ammonia volatilization, leaching, and runoff (Bouwman et al. 2009). Green manure affects soil nutrient balance, but the magnitude of this influence depends on the type and application method of the green manure being used. For example, Kumar and Goh (2000) tested the impact of various Fabaceae on soils and found that white clover produced a more positive N balance than field pea. Ladha et al. (2000) stated that the addition of fresh azolla increases soil N balance more than sesbania. Managing the fallow periods also modifies the biochemical processes utilizing soil nutrients (Becker et al. 2007; Wells et al. 2014; Sainju et al. 2016). Becker et al. (2007) demonstrated that straw reuse or cover crop planting during the fallow period can efficiently reduce soil nitrogen losses. Wells et al. (2014) discovered that moisture content and straw management during the fallow period affect soil N balances. However, there are relatively few studies concerning the effects of using various green manures during the fallow period on soil N and P balances.

In farmland ecosystems, variations in soil nutrient levels are determined by their surpluses and deficits. The relationship between N balances and P balances and changes in soil N

and P concentrations have been extensively studied (Lu et al. 1996; Blake et al. 2000; Fodor et al. 2013; Zhan et al. 2015). However, there are very few recent investigations into the relationship between total soil N or P content and N or P balance, respectively. Variation in ecosystem nitrogen levels is difficult to determine because N conversion processes are complex. Moreover, obvious variations in total N and P content are seldom detected in short-term experiments. The first quantitative analysis of the relationship between P balance and soil available P content was conducted in Lausanne, Switzerland (Blake et al. 2000). Previous studies mainly employed linear models to evaluate the effects of nutrient balances on soil nutrient concentrations. Soil nutrient levels are limited by threshold values, and balance surpluses cannot be infinitely converted into soil fertility. For example, Lu et al. (1996) found that soil N levels do not increase in response to N balance surpluses. A nationwide investigation by Yang et al. (2015) in China revealed that Olsen-P increased by ~ 20.8 mg kg⁻¹ for every 100 mg kg⁻¹ increase in soil P content. This trend, however, decreased with time. Therefore, it is necessary to apply other mathematical models such as a logistic model to evaluate the effects of N and P balances on soil N and P content and also consider other parameters such as maximum soil N level. Regional climate, soil types, fertilizer types, and application methods significantly affect N and P balances. Therefore, the relationship between N and P balances and soil N and P levels is probably influenced by these factors. Zhan et al. (2015) showed that applying fertilizers with different N-P-K ratios to black soils significantly changes the total P content and the relative rate of increase in available P regulated by the cumulative P balance. The same authors also pointed out that available P varies in efficiency among the major soil types in China and ranges from 0.47-10.76 mg kg⁻¹. The average available P efficiency (mg kg⁻¹– 100 mg ha⁻¹) follows the order paddy soil > black soil > purple soil > brown soil > irrigated soil. It is, therefore, necessary to investigate the effects of regional factors, soil types, and fertilizer application on the relationship between nutrient balances and soil nutrient levels.

In this study, samples and data were collected from an experimental site established in 1982. A rice-rice-winter fallow control was used on red paddy soils planted with green manure species. Annual variations in apparent N and P balances, soil N and P contents, and their relationships under different crop rotation modes (including rice-rice-winter, rice-rice-ryegrass, rice-rice-oil rape, and rice-rice-Chinese milk vetch) were analyzed. Over the long term, irrigation water input is usually balanced by leaching output, and atmospheric deposition input is balanced by soil volatilization output. Biological fixation and runoff processes were already affected by the experimental design. Therefore, only fertilizer input and harvesting output were considered when calculating the apparent N and P balances. Both linear and logistic models



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were applied to quantify the soil N and P balances and levels. The objectives of the study were to (1) investigate the effects of growing various green manure species during the winter fallow periods on the apparent N and P balances, (2) determine how the relationship between the N and P balances and the soil N and P levels respond to the application of different green manures, and (3) characterize the relationship between the N and P balances and soil fertility.

2 Materials and methods

2.1 Study sites

The experiment was conducted at a red paddy soil experimental station in the Chinese Academy of Agricultural Science, Qiyang County, Hunan Province (26°45′42″N, 111°52′32″E). This area has a subtropical humid monsoon climate with a mean annual rainfall of 1290 mm. The rainy season occurs from April to June, during the early rice cultivation period. Seasonal drought occurs from August to October, which coincides with the late rice cultivation period. The annual average temperature is 17.8 °C and the average annual number of frost-free days is 293. The paddy soil is classified as a Ferralic Cambisol (FAO 2006) derived from Quaternary red clay. The initial pH, total organic carbon, total N, total P, total K, alkalihydrolyzed N (AN), available P (AP), and available K (AK) levels of the plowed soil layer (0-15 cm) were 6.5, 11.7 g kg^{-1} , 0.94 g kg^{-1} , 0.66 g kg^{-1} , 11.5 g kg^{-1} , 156.0 mg kg^{-1} , 18.0 mg kg^{-1} , and 176.0 mg kg^{-1} , respectively.

2.2 Experimental design

Four cropping treatments, including a rice-rice-winter treatment (RRW), a rice-rice-ryegrass treatment (RRR), a ricerice-oil rape treatment (RRO), and rice-rice-Chinese milk vetch treatment (RRC), were conducted. All treatments except for the control were replicated three times in a randomized complete block design. The control had only one replicate (Table 1). Each replicate had an area of 37.5 m² (2.5 m \times 15.0 m) with three small plots and was isolated from the adjacent replicates by a 60-cm cement barrier to prevent water and nutrient exchange. Total fertilizer (basal and top-dressing) was applied during the early and late rice cultivation periods (rainy and drought seasons, respectively) and consisted of urea, P2O5, and K2O (Table 1). Compounded fertilizer (600 kg hm⁻²; 14% each N, P₂O₅, and K₂O) was used as a basal fertilizer whereas urea (150 kg hm⁻²) and K₂O (45.0 kg hm⁻²) were used as top-dressing. The basal fertilizer was applied before rice transplantation, and the top-dressing was administered 6-10 days after the transplant. Green manure species were sown 10-15 days before the late rice was harvested and were sown again in the same plot 15 days before early rice transplantation. Stubble-free rice straw was removed after every harvest. No fertilizer was applied during winter to the green manure cultivation. Conventional farmland management practices (irrigation, pest control) were used (Yang et al. 2012; Zhang et al. 2017).

2.3 Sampling and measurements

Early and late rice grain and straw were manually harvested, weighed, oven-dried at 105 °C for 30 min, then heated at 70 °C to a constant weight for dry matter, total N, and P determination. Dried grain or straw was 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).

Soil samples were collected once every 2 years from the top 0–15-cm layer after the late rice harvest. Five soil cores were randomly sampled from the center area of each plot. Soils were then mixed, air-dried, and sieved for the determination of pH, TOC, TN, TP, AN, AP, and AK according to conventional methods.

All soil and crop data were collected from previous runs of the same experiment (Gao et al. 2013; Yang et al. 2014; Zhang et al. 2017) except for those collected in 2015, which were sampled and tested by the team participating in the present study.

2.4 Statistical analysis

The annual apparent N or P balance was calculated as the N or P fertilizer application rate minus the crop N or P offtake for each year. The cumulative N or P balance is defined as the sum of all annual balances over the course of the study years (Blake et al. 2003; Shi et al. 2015). The N or P fertilizer input is shown in Table 1. The crop N or P offtake (N_c or P_c , in kg hm⁻² a⁻¹) was calculated as follows:

$$N_{c} = Y_{g} \times N_{g} + Y_{s} \times N_{s} \tag{1}$$

$$P_c = Y_g \times P_g + Y_s \times P_s \tag{2}$$

where Y_g , Y_s , N_g , N_s , P_g , and P_s represent the grain yield, straw yield, grain N concentration, straw N concentration, grain P concentration, and straw P concentration, respectively.

The cumulative N or P balance in this study is defined as the sum of the annual apparent N or P balances over the course of the study. It is based on the soil surface (lower plow) balance but does not incorporate the potential losses incurred from runoff or soil erosion (Sheldrick et al. 2002).

The increase of the soil N or P content ($^{\triangle}TN$ or $^{\triangle}TP$) was calculated as follows:



Table 1 Descriptions of the treatments used in this study

Treatment	Chemical fertilizers input and green manure plant as well as yields				
	Early rice (kg hm ⁻²)	Late rice (kg hm ⁻²)	Winter (kg hm ⁻²)		
RRR	153N, 84 P ₂ O ₅ , 129 K ₂ O	153N, 84 P ₂ O ₅ , 129 K ₂ O	Ryegrass, 15.0		
RRO	153N, 84 P ₂ O ₅ , 129 K ₂ O	153N, 84 P ₂ O ₅ , 129 K ₂ O	oilseed rape, 7.5		
RRC	153N, 84 P ₂ O ₅ , 129 K ₂ O	153N, 84 P ₂ O ₅ , 129 K ₂ O	Chinese milk vetch, 37.5		
RRW	153N, 84 P ₂ O ₅ , 129 K ₂ O	153N, 84 P ₂ O ₅ , 129 K ₂ O	Fallow		

RRR, RRO, RRC represent rice-rice-ryegrass, rice-rice-oilseed rape, and rice-rice-Chinese milk vetch, respectively. Cultivation was established at 1982. RRW means rice-rice-winter fallow. Data for it were first recorded in 1990

$$\Delta TN = N_i - N_0 \tag{3}$$

$$\triangle TP = P_i - P_0 \tag{4}$$

where N_i and P_i represent the soil total N and P concentrations (g kg⁻¹) at year i, respectively, while N_0 and P_0 are the initial concentrations of soil total N and P (g kg⁻¹), respectively.

We used a one-way ANOVA followed by Duncan's test to identify differences in the average N and P balances among treatments. The relationships between the increase of soil total N and the cumulative N balance and between the soil total P increase and the cumulative P balance were fitted with logistic and linear models using Origin v. 9.0 (Microcal Software, Northampton, MA, USA). Statistical analyses were run with SPSS v. 19.0 (IBM Inc., Chicago, IL, USA).

3 Results

3.1 Annual apparent N or P balance in red paddy soils under various rice-rice-green manure rotation systems

In terms of N balance, the four treatments led to surplus N and their variation was volatile as the experiment progressed (Fig. 1a). There was a significant difference in soil N balance between the RRW control and the other three rice-rice-green manure rotation system (P < 0.05; Fig. 1b). The soil N balance of the RRC treatment also significantly differed from that of the RRR and RRO treatments (P < 0.05; Fig. 1b). The soil P balances of RRR, RRO, RRC, and RRW were also positive and fluctuating; they increased over the first few years then decreased thereafter (Fig. 1c). Nevertheless, the only significant difference in soil P balance was found between the RRW control and the three rice-rice-green manure treatments (P < 0.05; Fig. 1d). In short, the three rice-rice-green manure rotation systems reduced the N and P balances and the effect for reducing N balance by RRC was better than the other green manure treatments.



3.2 Response of soil total N or P increase to cumulative N or P balance under different rotation systems

The changes in soil total N concentrations in response to the cumulative N balance of the RRW control were significant compared to those for the other three treatments (Fig. 2a). Using linear and logistic models, we fit the relationship between the soil total N increase and the cumulative N balances at P < 0.05 for both models and the correlation coefficients of all treatments. On the other hand, the relationship for the linear model of RRW was significantly lower than those obtained using a logistic model (Table 2; Fig. 2b). Nevertheless, the RRC could more rapidly promote the soil total N content to a steady state than the other two rice-rice-green manure rotation systems. We compared green manure treatment parameters using the logistic model and found that the orders of their r and K values were RRR > RRC > RRO and RRC > RRO > RRR, respectively. Therefore, planting Chinese milk vetch could increase the soil total N carrying capacity without delaying the attainment of a steady state (Table 2, Fig. 2b).

The changes in soil total P concentrations responded to the cumulative P balance in a similar manner for all four treatments (Fig. 3a). After fitting the relationship between soil total P increases and cumulative P balances with linear and logistic models, we found that the results of both models were significantly similar, but the linear model was a better fit for RRW (Table 3; Fig. 3b). Nevertheless, a rice-rice-green manure rotation system would delay the soil total P content from reaching a steady state. The orders of the slope and the r values for the various green manure treatments were RRR > RRO > RRC and RRC > RRO > RRR, respectively. Therefore, planting Chinese milk vetch increased the growth rates only at certain stages but it would decrease them overall for the entire development process (Table 3; Fig. 3b).

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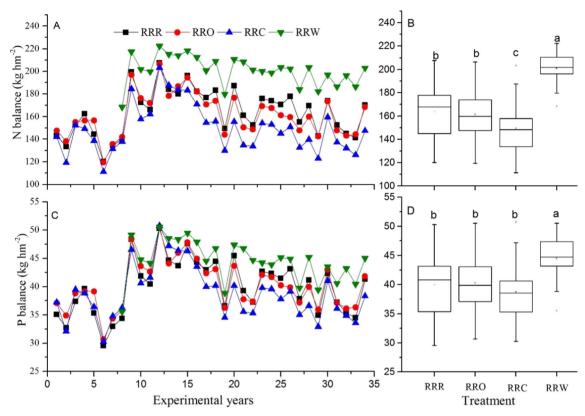


Fig. 1 Variation and distribution of apparent soil N (**a**, **b**) and P (**c**, **d**) balances under different treatments. Apparent soil N or P balances are the differences between the N or P fertilizer application rate and the crop N or P offtake for each year. RRR, RRO, RRC, and RRW are rice-rice-ryegrass

(black square), rice-rice-oilseed rape (red circle), rice-rice-Chinese milk vetch (blue triangle), and rice-rice-winter fallow (green triangle) rotation systems, respectively. Different lower case letters between bars indicate statistically significant differences (p < 0.05)

4 Discussion

4.1 Mediating N and P balance by green manure treatments

Green manures mediate the soil N and P balances. Results of this study showed that the N balance significantly decreased after green manures were grown during the winter fallow periods of a rice-rice-green manure system. In particular, the maximum decrease in N balance occurred when Chinese milk vetch is planted as the source of green manure. Green manure reduces N balance because planting green manure promotes crop growth which, in turn, extracts more nitrogen from the soil at harvest. This fact was verified by testing fields under different treatments (Fig. S1, Electronic Supplementary Material). Our result that green manures decreased N balance was consistent with that of Balik et al. (2003) but differed from that reported by Ladha et al. (2000), possibly because crop yields in the study by Ladha et al. (2000) did not significantly change after green manure planting relative to those obtained by applying other fertilizers. In the present study, Chinese milk vetch more significantly decreased the N balance than the other two green manures probably because of the way Chinese milk vetch increased crop yields and the N absorbed by crops (Zhang et al. 2013; Gabriel et al. 2012; Zhang et al. 2017). In addition, the P balance also significantly decreased after growing green manures in winter fallow periods in this experiment, but the magnitude of this reduction was less than that of the N balance. This finding corroborates that of a study conducted in Tianjin (Yang et al. 2015). However, the P balance was not significantly different among green manure treatments in this study. Blake et al. (2000) reported that soil P balance is mediated by abiotic or biological factors, such as soil organisms, soil properties, and climates, but that the effect of green manure species is minimal, which was similar with our results. Furthermore, the responses of the P balances to different crop rotation systems were weak compared to those for the N balances. One explanation of this result is that the P levels in early/lateripening rice and straw were significantly lower than the N content (Shi et al. 2015). Moreover, the changes in the P output per unit yield increase were substantially lower than those for the N output (Table S1, Electronic Supplementary Material).



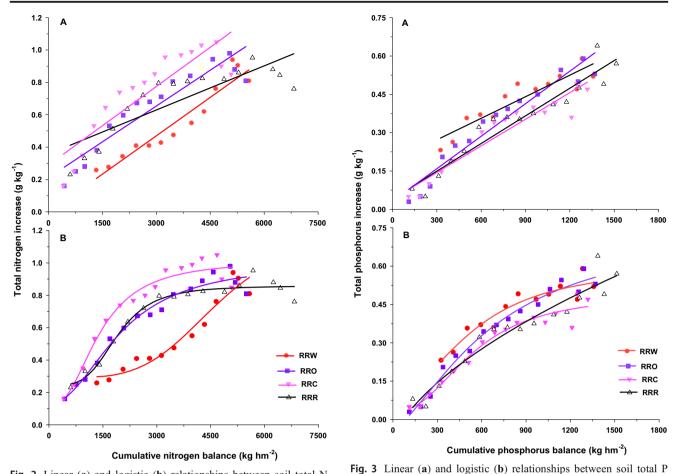


Fig. 2 Linear (a) and logistic (b) relationships between soil total N increase and cumulative N balance under different treatments. Cumulative N balance is the cumulative amount of apparent N balance from the beginning of the trial to the year of monitoring. RRR, RRO, RRC, and RRW are rice-rice-ryegrass (black square), rice-rice-oilseed rape (red circle), rice-rice-Chinese milk vetch (blue triangle), and rice-rice-winter fallow (green triangle) rotation systems, respectively

4.2 Relationships between N or P balance and soil N or P content regulated by green manures

The relationships between nutrient balance and soil nutrient content are complex, especially for nitrogen. The nitrogen cycle is intricate and there are few reliable methods for

Cumulative P balance is the cumulative amount of apparent P balance from the beginning of the trial to the year of monitoring. RRR, RRO, RRC, and RRW are rice-rice-ryegrass (black square), rice-rice-oilseed rape (red circle), rice-rice-Chinese milk vetch (blue triangle), and rice-rice-winter fallow (green triangle) rotation systems, respectively

increase and cumulative P balance under different treatments.

measuring nitrogen sources and fluxes, so there are few quantitative models available except for that reported by Shepherd and Bhogal (1998), who focused on the apparent N balance and simplified its calculation. The relationship between N balance and soil N content under various crop rotation treatments is quantitatively determined.

Table 2 Models analyzing the effects of cumulative N balance on soil total N increase in different treatments

Treatment	Linear model				Logistic model		
	Slope	R ²	P	r	K	R^2	P
RRR	5.86049E-5	0.49233	0.01425	0.00179	0.85238	0.81252	9.37031E-9
RRO	1.11522E-4	0.79573	3.21618E-4	0.00105	0.91739	0.89533	2.83957E-8
RRC	9.57099E-5	0.63137	0.00368	0.0013	0.96561	0.8129	3.57906E-8
RRW	1.36443E-4	0.92981	4.25162E-6	4.49793E-4	1.35911	0.93264	2.57511E-7

The slope is the average increase of total nitrogen. R is the coefficient of the correlation between the cumulative apparent nitrogen balance and the soil nitrogen increase. The value r is the instantaneous increase of total nitrogen when it reaches K/2. K is the maximum value of the cumulative nitrogen balance for the soil total nitrogen increase (g kg⁻¹)



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Table 3 Models analyzing the effects of cumulative P balance on soil total P increase in different treatments

Treatment	Linear model				Logistic model		
	Slope	R^2	P	r	K	R^2	P
RRR	3.51646E-4	0.87832	3.91537E-5	0.00224	0.67531	0.85589	1.62303E-6
RRO	3.43502E-4	0.93518	3.08595E-6	0.00348	0.538	0.95529	1.73297E-8
RRC	2.95707E-5	0.85564	7.82743E-5	0.0052	0.39719	0.89305	5.52788E-7
RRW	2.56404E-4	0.81591	2.10374E-4	0.00458	0.48505	0.91245	4.96087E-8

The slope is the average increase of total phosphorus. R is the coefficient of correlation between the cumulative apparent phosphorus balance and the soil total phosphorus increase. The value r is the instantaneous increase of soil total phosphorus when it reaches K/2. K is the maximum value of the cumulative phosphorus balance for the soil total phosphorus increase (g kg⁻¹)

Quantitative analyses with different mathematical models were used in a previous study to more accurately demonstrate the complexity of the relationship between nutrient balances and soil nutrient levels (Shi et al. 2015). Shi et al. (2015) applied both linear and logistic models to quantify the effects of N balances on soil N content and showed that the logistic model more aptly describes the relationship between the cumulative N balances and soil N content increases after growing green manures. The two models do not significantly differ in terms of depicting this relationship in the untreated control. We used both models to fit the relationship between the soil total N or P increases and the cumulative N or P balances and found that the soil total N and P increases were significantly correlated with the cumulative N and P balance, respectively, whether or not green manure was planted in winter. Specifically, soil N content first increased then stabilized with increasing cumulative N balance when green manures were planted. In the control, however, soil N content increased steadily. This phenomenon was not caused by the accumulation of soil nutrients in response to green manure cultivation. Rather, it was the result of decreasing soil N threshold values (Table S2, Electronic Supplementary Material). The soil N cycle is simplified by planting green manures because they favor the growth of only certain soil microorganism species and the decrease of soil biodiversity (Zhang et al. 2017). The soil N threshold values measured after planting Chinese milk vetch were probably higher than those obtained with ryegrass and oilseed rape. Most likely, the Rhizobium associated with Chinese milk vetch facilitates the growth of various microorganisms. Compared to the soil N cycle, the soil P cycle is simple, and numerous studies have been conducted on P cycles and soil P content increases. However, most of these studies were based on linear models (Heming 2007; Nagumo et al. 2013; Zhan et al. 2015). The present study showed that, unlike the relationship between cumulative N balances and soil N content increases after growing green manures, the relationship between cumulative P balance and soil P level increase was not dependent on the model. Treatments during winter fallow periods, however, were more accurately described by the logistic model, indicating that soil P content continued to increase after green manures were planted, whereas that for the control reached a threshold value. This difference is explained by the fact that the maximum total soil P capacity increased after growing green manure (Table S1). Relative to N, the conversion of P in the soil is simple and more closely associated with soil aggregates than soil microorganisms. Our previous studies demonstrated that the number of soil microaggregates (particle diameter range: 0.25-5.00 mm) significantly increases after green manure treatment (Yang et al. 2012). Variations in soil pH and organic carbon may account for the differences in the effects of various green manures on the unit P balance and soil P conversion efficiency (Blake et al. 2000; Zhan et al. 2015).

5 Conclusions

Winter fallow periods significantly decreased both N and P balances regardless of the type of green manure used. Planting Chinese milk vetch inhibited N and P balance, but the N balance was more strongly inhibited than the other green manure species tested. The correlations between cumulative N balance and soil N content increase and between cumulative P balance and soil P content increase were derived from both linear and logistic models. Nevertheless, the best fit models varied depending on the type of green manure sown in the winter fallow periods. The logistic model is the best fit for the relationship between cumulative N and P balance and soil N and P content in the treatment with green manure planting. Our study indicated that neither crop yield nor soil fertility was affected when nitrogen fertilizers were optimally reduced and rice-rice-green manure rotation was used on red paddy soil or when phosphorus fertilizer inputs were decreased on uncultivated farmland.



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