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Green manure incorporation with reductions in chemical fertilizer inputs improves rice yield and soil organic matter accumulation

Zengqiang Li[1] • Xian Zhang[1] • Jing Xu[1] • Kai Cao[1] • Jianhong Wang[1] • Changxu Xu[2] • Weidong Cao[3]

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Abstract

Purpose A 6-year (2011–2016) field experiment was performed to explore the effects of partial substitution of chemical fertilizer by green manure on rice yield, sustainability yield index, and the building-up of different fractions of soil organic carbon, soil nitrogen, and phosphorus.

Materials and methods The experiment included no fertilization (NF), chemical fertilizer only (CF_{100}), and Chinese milk vetch (*Astragalus sinicus* L.) incorporation with 80%, 60%, 40%, 20%, and 0% of total N, P, and K supplied from chemical fertilizer ($MVCF_{80}$, $MVCF_{60}$, $MVCF_{40}$, $MVCF_{20}$, and $MVCF_0$, respectively) treatments. The soil organic carbon fractions, soil nitrogen, and phosphorus fraction contents were measured over 6 years.

Results and discussion In comparison with CF_{100} treatment, the $MVCF_{80}$, $MVCF_{60}$, and $MVCF_{40}$ treatments significantly increased rice yield between 2013 and 2016, thus improving sustainability yield index. The soil organic carbon fractions increased 15–58%, 16–61%, 14–50%, and 12–33% in the $MVCF_{80}$, $MVCF_{60}$, $MVCF_{40}$, and $MVCF_{20}$ treatments, respectively, compared with the CF_{100} treatment ($p < 0.05$). The easily oxidizable nitrogen, acid hydrolysable pool II nitrogen, total nitrogen, NaOH extractable phosphorus, HCl extractable phosphorus, and total phosphorus contents in the $MVCF_{40}$ treatment were 17%, 28%, 9%, 12%, 15%, and 8% higher than those in the CF_{100} treatment ($p < 0.05$). The $MVCF_{60}$ and $MVCF_{80}$ treatments further increased the contents of these nitrogen and phosphorus fractions compared with the CF_{100} treatment. Stepwise multiple linear regression analysis showed that the average yield was positively influenced by the contents of total phosphorus, easily oxidizable nitrogen, and dissolved organic nitrogen, and that the sustainability yield index was positively influenced by the contents of easily oxidizable carbon and total organic carbon.

Conclusions Chinese milk vetch incorporation with a 20–40% reduction in chemical fertilizer inputs may be a potential fertilization practice for improving rice productivity and sustainability.

Keywords Green manure · Reduction of chemical fertilizer input · Rice yield · Soil organic carbon fractions · Soil nitrogen fractions · Soil phosphorus fractions

1 Introduction

China is one of the most important rice-producing countries in the world (Xie et al. 2016). To pursue high rice yield, excessively high rates of chemical fertilizers have been applied into rice field since the early 1980s (Ju et al. 2009). However, the substantial inputs of chemical fertilizers do not reliably produce the expected yields, and the growth rate of rice yield has noticeably decelerated and even stagnated in some areas of China over recent years (Chen et al. 2014). In addition, over-fertilization has caused a large number of adverse impacts on the environment (e.g., greenhouse gas emissions, and nutrient leaching and runoff) (Ju et al. 2009; Zhao et al. 2015). It has also led to soil structure deterioration, soil

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✉ Jianhong Wang
jianhong1203@sina.com

[1] Institute of Environment and Resource & Soil Fertilizer, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, Zhejiang, China

[2] Institute of Soil & Fertilizer and Resource & Environment, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, Jiangxi, China

[3] Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China

acidification, and a decrease in microbial activities (Bronick and Lal 2005; Guo et al. 2010), which have influenced the sustainability of the rice cropping system. Therefore, the development of productive and sustainable rice fertilization practices is necessary to meet growing food demand and simultaneously decrease environmental pollution.

One possible practice to reduce chemical fertilizer inputs while stabilizing or increasing rice yield is the use of green manure as a substitute (Yadav et al. 2000; Liu et al. 2009; Xie et al. 2016). Green manure, especially leguminous green manure, is widely known to be a promising nutrient source that can provide nutrients for crop growth and improve soil nutrient accumulation (Zhu et al. 2014; Li et al. 2015). Furthermore, the incorporation of green manure has been shown to stimulate microbial biomass and enzyme activities associated with nutrient transformation (Liu et al. 2009; Zhang et al. 2017; Fang et al. 2019), which can optimize the soil microbial-driven internal cycling of nutrients, leading to a reduction in chemical fertilizer inputs. Several studies have investigated the effects of substitution of chemical fertilizer by green manure on rice yield and soil properties and shown negative effect of excessive or sole application of green manure on rice yields (Thorup-Kristensen et al. 2012; Xie et al. 2016; Yang et al. 2019). They suggested that the reduction rates of chemical fertilizer inputs could not be as high as possible after green manure incorporation (Xie et al. 2016) and that the optimum substitution rate of green manure for chemical fertilizers depended on soil fertility, cropping system, and crop species (Yadav et al. 2000). In fact, most studies have mainly focused on reducing chemical fertilizer N inputs after green manure incorporation, but did not reduce chemical fertilizer P and K input rates (Yadav et al. 2000; Bi et al. 2009; Xie et al. 2016). This suggests that the green manure plus chemical fertilizer treatments actually received higher total P and K nutrient inputs compared with the conventional chemical fertilizer treatments. Zhang et al. (2003) suggested that high P nutrient inputs enhanced P losses through soil erosion and/or leaching in rice fields. Therefore, considering the abundant contents of P and K nutrients in green manure, it is achievable and necessary to decrease chemical fertilizer P and K inputs when the chemical fertilizer N input is reduced after green manure incorporation. However, it remains unclear the effects of green manure combined with reduced chemical fertilizer N, P, and K on rice yield and sustainability.

Soil organic matter (SOM) containing carbon (C), nitrogen (N), and phosphorus (P) elements is a continuum of heterogeneous substances and can be physically or chemically divided into various fractions (Haynes 2005; Kuo et al. 2005; Rovira et al. 2010). The increase of SOM contents could improve soil structure, enhance soil nutrient availability, and increase microbial biomass and diversity (Bronick and Lal 2005; Li et al. 2019). In addition, crop yield and sustainability are also significantly influenced by SOM contents (Manna et al. 2005; Yang et al. 2018). Fertilization is a widely accepted management practice to influence SOM contents but different SOM fractions have various responses to green manure and chemical fertilizers (Sun et al. 2013; Yang et al. 2018). In contrast to chemical fertilizers, green manure has different nutrient forms and release rates, which could lead to a different building-up process of nutrient pools. Moreover, the input of green manure C could also influence the turnover of soil N and P among the different fractions through changing soil microbial community composition and various environmental factors (Vinten et al. 2002; Giesler et al. 2005). In turn, green manure C and soil organic C turnover and accumulation processes were also significantly influenced by soil nutrient availability (Carreiro et al. 2000). Therefore, exploring the changes in the size of the SOM fractions is necessary to comprehensively evaluate the effects of green manure incorporation plus reduced chemical fertilizer and to broaden our understanding of SOM accumulation.

Chinese milk vetch (thereafter MV) is a leguminous green manure commonly planted in rice cropping systems across Southern China. We hypothesized that MV incorporation combined with appropriate reductions in chemical fertilizer inputs would enhance rice yield and promote the accumulation of SOM fractions. The objectives of this study were (1) to evaluate the effects of green manure incorporation plus different reductions in chemical fertilizer input rates on rice yield and sustainability; (2) to explore the responses of different soil organic carbon and nutrient fractions to green manure incorporation plus different reductions in chemical fertilizer input rates; and (3) to investigate the relationships between rice yield and the different soil organic carbon and nutrient fractions. The results of this study may provide a reference for maintaining rice yield and simultaneously decreasing chemical fertilizer inputs in Southern China.

2 Materials and methods

2.1 Field site and experiment descriptions

The experiment was performed in a paddy field in Jinhua City, Zhejiang Province, China (119° 32′ E, 29° 04′ N, 63 m a.s.l.). This area has a typical subtropical monsoon climate with a mean annual precipitation of 1500 mm and an average annual temperature of 18 °C. The paddy soil has a silt clay texture (40.9% clay), and local crop system consists of rice (June to October) followed by a fallow period (November to May). The soil in the plow layer (0–20 cm) before the experiment contained 12.5 g kg^{-1} soil organic carbon, 1.45 g kg^{-1} total nitrogen, 25 mg kg^{-1} available phosphorus, 34 mg kg^{-1} available potassium, and pH (H_2O) of 5.38.

The fertilization experiment was established in April, 2011, and was based on a randomized design including seven fertilization treatments with three replicate plots. Each plot was 20 m^2 (4 m × 5 m). The treatments included (1) NF, no fertilization; (2) CF_{100}, 100% chemical fertilizer N, P, and K; (3) $MVCF_{80}$, MV plus 80% chemical fertilizer N, P, and K; (4) $MVCF_{60}$, MV plus 60% chemical fertilizer N, P, and K; (5) $MVCF_{40}$, MV plus 40% chemical fertilizer N, P, and K; (6) $MVCF_{20}$, MV plus 20% chemical fertilizer N, P, and K; (7) $MVCF_0$, MV plus 0% chemical fertilizer N, P, and K. In the five MV treatments, fresh MV with 90% water content was cut from other fields at the full blooming stage and then plowed into the plot at 45,000 kg ha^{-1} as a basal fertilizer. Based on oven-dried base, the MV contained 38.2 g N kg^{-1}, 4.16 g P kg^{-1}, and 36.1 g K kg^{-1}. The total nutrients applied to the different fertilization treatments are shown in Table 1. For chemical fertilizer N application, 45% was applied in ammonium bicarbonate as a basal fertilizer, and 40% and 15%, in the form of urea, were top-dressed at the tillering stage and at the panicle initiation stage, respectively. The calcium phosphate was used as basal fertilizer, whereas 60% of the potassium chloride was used at the tillering stage and another 40% was applied at the panicle initiation stage. Rice seedlings (30-day-old) were transplanted in the first week of June and harvested in the last week of October. Rice variety and agronomic practices were identical to local farmers. The rice grains from the entire plot were weighed and recorded after air drying, and the rice straw was removed from the plots after harvest. The sustainable yield index (SYI) was used to evaluate rice production sustainability and was calculated as follows (Xie et al. 2016):

$$\mathrm{SYI} = \left(\overline{Y} - \sigma_{n-1}\right)/Y_{\max} \quad (1)$$

where $\overline{Y}$ is the mean yield, σ_{n-1} is the standard deviation of the yield for a specific treatment across years, and Y_{max} is the maximum yield obtained under that treatment through 2011–2016.

2.2 Soil sampling and analysis

Soil samples were collected from the plow layer (0–20 cm depth) in each replicate plot after the rice harvest in October 2016. One composite soil sample per plot was consisted of eight soil cores that were randomly collected from each plot. Soil samples were transported to the laboratory on ice. The field-moist soil samples were then sieved (< 2 mm) and divided into two subsamples. One portion was stored at − 20 °C and remainder was air-dried.

Soil inorganic nitrogen was extracted using 2 mol L^{-1} KCl solution and determined by a segmented flow analyzer (Skalar, San Plus System, Breda, The Netherlands). The dissolved organic carbon (DOC) and nitrogen (DON) were determined according to Jones and Willett (2006). Microbial biomass carbon (MBC) and nitrogen (MBN) were measured by the fumigation-extraction method as described by Wu et al. (1990). The factors 0.45 and 0.54 were used to convert extracted C and N to MBC and MBN, respectively (Brookes et al. 1985; Wu et al. 1990).

Easily oxidizable organic carbon (EOC) and nitrogen (EON) were measured according to Blair et al. (1995) and Westerhof et al. (1998), respectively. Briefly, air-dried soil samples were mixed with 0.333 mol L^{-1} $KMnO_4$ solution and shaken overhead for 1 h at 25 °C. Then, the $KMnO_4$ solution was poured out through centrifuging. The $KMnO_4$ solution was diluted and detected the absorbance. The change in the $KMnO_4$ concentration was used to estimate the EOC content. The difference between total organic carbon and EOC was the non-oxidizable organic carbon (NOC). The residual soil was washed with distilled water, centrifuged to remove any excess $KMnO_4$, and then dried at 60 °C. The N content in the residual soil was measured by an elemental analyzer (Thermo Flash EA1112, Thermo Scientific, Waltham, USA) and was referred as non-oxidizable nitrogen (NON). The difference between total nitrogen and NON was the EON fraction (Westerhof et al. 1998).

Table 1 Average input amount of nutrients in seven treatments over 6 years

	Chinese milk vetch (kg ha^{-1})			Chemical fertilizer (kg ha^{-1})			Total (kg ha^{-1})		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
NF	0	0	0	0	0	0	0	0	0
CF_{100}	0	0	0	210	56.3	112.5	210	56.3	112.5
$MVCF_{80}$	171.9	42.9	195.8	168	44.8	90.0	339.9	87.7	285.8
$MVCF_{60}$	171.9	42.9	195.8	126	33.6	67.5	297.9	76.5	263.3
$MVCF_{40}$	171.9	42.9	195.8	84	22.4	45.0	255.9	65.3	240.8
$MVCF_{20}$	171.9	42.9	195.8	42	11.2	22.5	213.9	54.1	218.3
$MVCF_0$	171.9	42.9	195.8	0	0	0	171.9	42.9	195.8

Acid hydrolysable C and N were measured according to Rovira et al. (2010). Briefly, the soil samples were refluxed with 2.5 mol L^{-1} H_2SO_4 solution at 105 °C for 30 min. The hydrolysate was recovered by centrifugation and was named acid hydrolysable pool I (AHI). The residual soils were dried at 60 °C, and hydrolyzed again with 13 mol L^{-1} H_2SO_4 solution overnight at room temperature. Then, the acid was diluted to 1 mol L^{-1} and the mixture was refluxed for 3 h at 105 °C. The second hydrolysate was named acid hydrolysable pool II (AHII). The unhydrolyzed soil was dried and was referred to as the acid non-hydrolyzable pool (ANH). The C and N contents in the AHI and AHII were determined by a TOC analyzer (Multi N/C 3100 TOC/TN, Analytik Jena AG, Jena, Germany) and named AHIC, AHIN, AHIIC, and AHIIN, respectively. The C and N content in ANH was measured using an elemental analyzer (Thermo Flash EA1112) and named ANHC and ANHN, respectively.

Soil total organic carbon (TOC) and total nitrogen (TN) contents were also measured by the elemental analyzer (Thermo Flash EA1112).

The soil P fractions were determined using a sequential extraction method according to Tiessen and Moir (2008). Briefly, air-dried soil samples were extracted using various extractants in the following order: (1) distilled water and two resin strips (resin-P), (2) 0.5 mol L^{-1} $NaHCO_3$ ($NaHCO_3$-P), (3) 0.1 mol L^{-1} NaOH (NaOH-P), and (4) 1 mol L^{-1} HCl (HCl-P). The mixtures were oscillated for 16 h in each extraction process, and then centrifuged. Inorganic phosphorus (P_i) in the supernatant was determined by the ascorbic acid molybdenum blue method. Extracts made with $NaHCO_3$ and NaOH were digested with acidified ammonium persulfate to determine total P. Organic P (P_o) in these fractions was calculated as the difference in P between the digested and undigested samples. The remaining soil residue (residual-P) was digested by concentrated H_2SO_4/H_2O_2 and then determined colorimetrically as previously described. Therefore, the sequential extraction procedure results in seven specific P fractions: resin-P, $NaHCO_3$-P_i, $NaHCO_3$-P_o, NaOH-P_i, NaOH-P_o, HCl-P, and residual-P. Total P (TP) was referred to the sum of all seven P fractions (Tiessen and Moir 2008).

2.3 Statistical analysis

Statistical analyses were carried out by using SPSS statistical software (version 17.0). The differences in the data attributed to treatments were analyzed using one-way ANOVA followed by least significant difference (LSD) test. The differences were considered to be significant at the 5% probability level. Stepwise multiple linear regression was used to analyze the effects of soil organic carbon and nutrient fractions on average yield and the SYI.

3 Results

3.1 Rice yield and sustainability yield index

Rice yield showed a dynamic with increasing experiment time (Fig. 1). All the fertilized treatments significantly increased rice yield compared with the NF treatment in each year. The CF_{100} treatment had a significantly higher rice yield than the $MVCF_0$ treatment except for the year 2013. From 2013 to 2016, rice yield increased by 4–17% and 6–17% in the $MVCF_{80}$ and $MVCF_{60}$ treatments, respectively, compared with the CF_{100} treatment ($p < 0.05$). With the exception of 2012, rice yield in the $MVCF_{40}$ treatment was significantly higher than in the CF_{100} treatment. The $MVCF_{20}$ treatment had a significantly lower rice yield than the CF_{100} treatment in 2011, but higher rice yield in 2014 and 2016.

The average rice yield and the sustainability yield index (SYI) significantly increased in all the fertilized treatments compared with the NF treatment, except for the SYI in the $MVCF_0$ treatment (Fig. 2). The CF_{100} treatment showed significantly higher average rice yield than the $MVCF_0$ treatment, but had a lower SYI than the $MVCF_{20}$ treatment. Average rice yield and SYI in the CF_{100} treatment were significantly lower than those in the $MVCF_{80}$, $MVCF_{60}$ and $MVCF_{40}$ treatments.

3.2 Soil organic carbon fractions

The levels of the different soil organic carbon fractions significantly increased in all the fertilized treatments compared with the NF treatment (Table 2). The DOC content increased 30%, 37%, 28%, 23%, and 12% in the $MVCF_{80}$, $MVCF_{60}$, $MVCF_{40}$, $MVCF_{20}$, and $MVCF_0$ treatments, respectively, compared with the CF_{100} treatment ($p < 0.05$). The trends for EOC and AHIC contents were similar to the DOC content trend. In addition, the MBC and AHIIC contents were higher in the $MVCF_{60}$ treatment (25% and 61%), followed by the $MVCF_{80}$ (20% and 58%), $MVCF_{40}$ (16% and 50%), and $MVCF_{20}$ (12% and 33%) treatments compared with the CF_{100} ($p < 0.05$). Milk vetch incorporation combined with 20–80% reductions in chemical fertilizer input treatments had similar NOC and TOC contents, and they were all significantly higher than the CF_{100} treatment.

3.3 Soil nitrogen fractions

The contents of all the nitrogen fractions significantly increased in every fertilized treatment compared with the NF treatment except for ANHN content (Table 3). Inorganic nitrogen content in the CF_{100} treatment was significantly higher than in the $MVMF_0$ treatment. Inorganic nitrogen, DON,

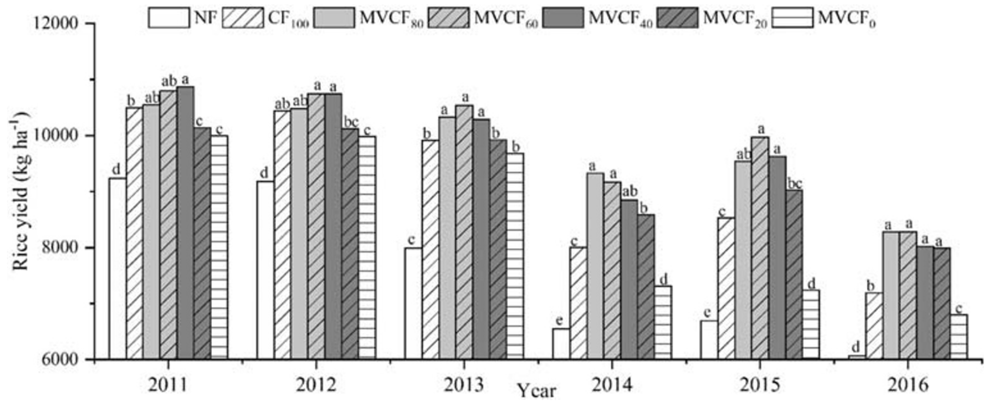

Fig. 1 Temporal changes in rice yield in different fertilization treatments from the years 2011 to 2016. Different letters indicate significant differences between fertilization treatments within the same year at $p < 0.05$

MBN, EON, NON, AHIN, AHIIN, and TN contents in the $MVCF_{80}$ treatment increased by 11%, 10%, 16%, 22%, 8%, 33%, 49%, and 13%, respectively, compared with the CF_{100} treatment ($p < 0.05$). These N fractions also showed a similar trend in the $MVCF_{60}$ treatment. In addition, EON, AHIIN, and TN contents in the $MVCF_{40}$ treatment were 17%, 28%, and 9% higher than those in the CF_{100} treatment, respectively ($p < 0.05$).

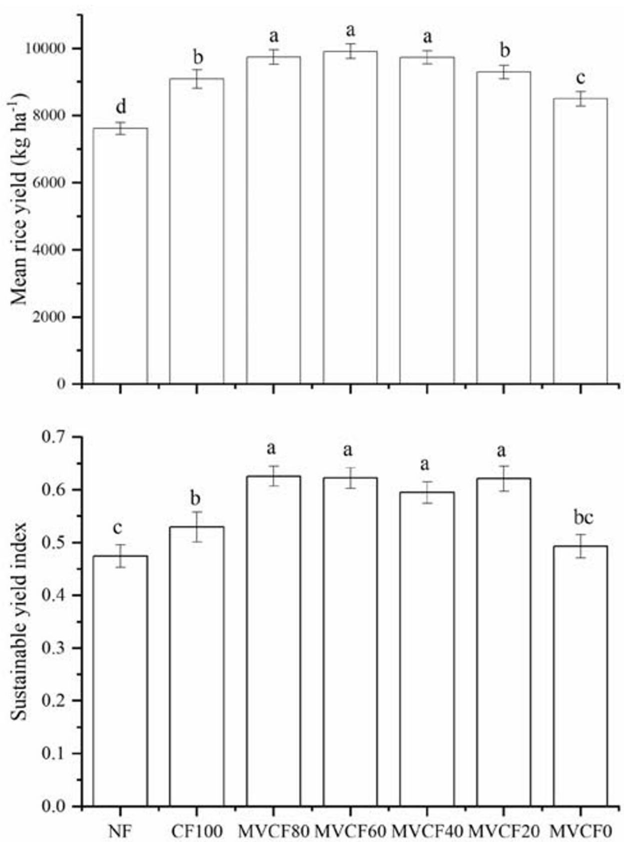

Fig. 2 Average rice yield for 6 years (a) and sustainable yield index (b) in different fertilization treatments. Vertical bars denote the standard deviation of the means. Different letters above the bars indicate significant differences between fertilization treatments at $p < 0.05$

3.4 Soil phosphorus fractions

Except for HCl-P and residual-P contents, the contents of the other P fractions in the NF treatment were significantly lower than in all the fertilized treatments (Table 4). Resin-P and $NaHCO_3$-P_i contents in the CF_{100} treatment were significantly higher than in the $MVMF_0$ treatment. In comparison with the CF_{100} treatment, $NaHCO_3$-P_o, NaOH-P_i, NaOH-P_o, HCl-P, and total P contents in the $MVCF_{80}$ and $MVCF_{60}$ treatments increased 33% and 23%, 17% and 15%, 34% and 26%, 27% and 21%, and 18% and 14%, respectively ($p < 0.05$). Furthermore, the $MVCF_{40}$ treatment had 9%, 28% 15%, and 8% higher NaOH-P_i, NaOH-P_o, HCl-P, and total P contents than the CF_{100} treatment, respectively ($p < 0.05$).

3.5 Relationships between average yield and SYI, and the soil organic matter fractions

Stepwise multiple linear regression analysis showed that the explained variance of the limited models was 0.955 and 0.804 for the average yield and SYI, respectively (Table 5). The TP, EON, and DON contents had significant, positive impacts on average yield, whereas $NaHCO_3$-P_o content was the negative impact factor. The SYI was positively influenced by the EOC and TOC contents.

4 Discussion

4.1 Rice yield and sustainability

Our results showed that higher rice yield was achieved in the $MVCF_{80}$, $MVCF_{60}$, and $MVCF_{40}$ treatments compared with the CF_{100} treatment, which indicated that MV incorporation coupled with an appropriate reduction in chemical fertilizer inputs improved rice productivity. Similar results were also reported by Xie et al. (2016), who showed that 20–40% substitution of MV for chemical fertilizers was benefit for improving rice yield in a 6-year double-rice cropping system.

Table 2 Changes in the contents of different organic carbon fractions under seven fertilization treatments

	DOC (mg kg^{-1})	MBC (mg kg^{-1})	EOC (g kg^{-1})	NOC (g kg^{-1})	AHIC (mg kg^{-1})	AHIIC (mg kg^{-1})	ANHC (g kg^{-1})	TOC (g kg^{-1})
NF	34 ± 1 e	571 ± 23 e	2.62 ± 0.03 f	9.82 ± 0.08 c	2143 ± 121 f	2994 ± 74 d	7.30 ± 0.16 b	12.44 ± 0.10 c
CF_{100}	43 ± 2 d	639 ± 6 d	2.86 ± 0.00 e	11.27 ± 0.71 b	2500 ± 60 e	3652 ± 160 c	7.98 ± 0.62 ab	14.13 ± 0.71 b
$MVCF_{80}$	56 ± 4ab	764 ± 19 b	3.80 ± 0.01 a	12.92 ± 0.99 a	3428 ± 168 ab	4602 ± 287 a	8.69 ± 0.85 a	16.73 ± 0.98 a
$MVCF_{60}$	59 ± 4a	796 ± 29 a	3.81 ± 0.03 a	13.11 ± 0.16 a	3574 ± 147 a	4664 ± 289 a	8.68 ± 0.08 a	16.92 ± 0.15 a
$MVCF_{40}$	55 ± 2 ab	741 ± 17 bc	3.63 ± 0.05 b	12.80 ± 0.27 a	3372 ± 36 b	4477 ± 192 ab	8.58 ± 0.41 a	16.43 ± 0.28 a
$MVCF_{20}$	53 ± 1 b	714 ± 27 c	3.43 ± 0.03 c	12.68 ± 0.52 a	3063 ± 161 c	4204 ± 120 b	8.65 ± 0.31 a	16.11 ± 0.50 a
$MVCF_{0}$	48 ± 2 c	661 ± 13 d	3.04 ± 0.02 d	11.41 ± 0.50 b	2764 ± 68 d	3719 ± 231 c	7.96 ± 0.43 ab	14.45 ± 0.48 b

Data are shown as mean with standard deviation. Different letters within a column indicate significant differences at $p < 0.05$

DOC, dissolved organic carbon; *MBC*, microbial biomass carbon; *EOC*, easily oxidizable organic carbon; *NOC*, non-oxidizable organic carbon; *AHIC*, acid hydrolysable pool I organic carbon; *AHIIC*, acid hydrolysable pool II organic carbon; *ANHC*, acid non-hydrolyzable pool carbon; *TOC*, total organic carbon

One possible reason for this improvement could be that the soil N and P nutrient-supplying capacity was enhanced because their overall N and P levels had increased after the application of the green manure (Tables 3 and 4). Stepwise multiple linear regression further certificated the positive influences of nutrient availability on rice yield (Table 5). Yadav et al. (2000) suggested that a reduced soil nutrient-supplying capacity is the major factor causing decline in crop production. A sufficient supply of soil nutrients can significantly increase the grains per panicle, the panicle number per unit area, and the 1000-grain weight (Huang et al. 2013), which can contribute to the increase in the final rice yield. Furthermore, apart from the N, P, and K macro-nutrients, MV plants also contain some medium- and micro-nutrients (Ca, Mg, Fe, Zn, etc.) (Chen and Zhao 2009), which promote and ensure a balanced nutrient supply in paddy soil. The balanced supply of nutrients is important when attempting to improve plant growth and increase yields because they facilitate the translocation of nutrients to the economic part of the crop (Yang et al. 2004). On the other hand, the non-nutrient benefits of MV incorporation may also contribute to increases in rice yield. Efthimiadou et al. (2010) found that green manure incorporation enhanced the photosynthetic rate and stomatal conductance of rice plants, which led to greater carbon accumulation and better stabilized the dry matter in rice grain. In addition, a combination of chemical fertilizer and green manure can improve soil structure, increase root biomass and activity, and enhance soil microbial activity and diversity, which is also important for improving crop yield (Almagro et al. 2017; Zhang et al. 2017).

The SYI has been widely used as an important indicator for evaluating the sustainability of fertilization practices and soil productivity (Yadav et al. 2000; Xie et al. 2016). Yadav et al. (2000) suggested that the higher the SYI, the more sustainable the system is. This study also showed that only the 20–60% reductions in chemical fertilizer inputs after MV incorporation could significantly increase the SYI compared with the CF_{100} treatment. In reality, crop yield sustainability is the result of the interactions between soil factors (e.g., nutrient availability, soil structure, and microbes) and environmental factors (e.g., temperature). Stepwise multiple linear regression showed that the SYI was influenced by organic carbon availability (Table 5). Similar results were also reported by Manna et al. (2005), who showed that the content of soil organic carbon

Table 3 Changes in the contents of different nitrogen fractions under seven fertilization treatments

	Inorganic N (mg kg^{-1})	DON (mg kg^{-1})	MBN (mg kg^{-1})	EON (g kg^{-1})	NON (g kg^{-1})	AHIN (mg kg^{-1})	ANIIN (mg kg^{-1})	ANHN (g kg^{-1})	TN (g kg^{-1})
NF	36 ± 1 e	2.85 ± 0.09 d	52 ± 3 d	0.41 ± 0.06 d	0.94 ± 0.02 d	236 ± 15 d	270 ± 18 d	0.73 ± 0.02 a	1.24 ± 0.01 d
CF_{100}	71 ± 2 c	4.24 ± 0.03 c	70 ± 5 c	0.60 ± 0.02 bc	1.05 ± 0.03 bc	469 ± 18 bc	401 ± 46 c	0.76 ± 0.05 a	1.63 ± 0.03 c
$MVCF_{80}$	79 ± 3 a	4.67 ± 0.30 ab	81 ± 6 ab	0.73 ± 0.02 a	1.13 ± 0.03 a	625 ± 54 a	500 ± 47 a	0.85 ± 0.11 a	1.98 ± 0.11 a
$MVCF_{60}$	77 ± 7 ab	4.86 ± 0.39 a	86 ± 4 a	0.72 ± 0.04 a	1.10 ± 0.04 ab	584 ± 54 a	485 ± 46 ab	0.83 ± 0.05 a	1.90 ± 0.08 ab
$MVCF_{40}$	72 ± 3 bc	4.43 ± 0.18 bc	78 ± 7 abc	0.70 ± 0.08 a	1.10 ± 0.06 ab	509 ± 12 b	457 ± 36 abc	0.84 ± 0.11 a	1.80 ± 0.13 b
$MVCF_{20}$	69 ± 2 c	4.30 ± 0.27 bc	75 ± 7 bc	0.68 ± 0.06 ab	1.06 ± 0.02 bc	481 ± 38 bc	426 ± 23 bc	0.74 ± 0.12 a	1.65 ± 0.10 c
$MVCF_{0}$	62 ± 1 d	4.14 ± 0.21 c	72 ± 1 c	0.57 ± 0.03 c	1.03 ± 0.01 c	436 ± 17 c	390 ± 54 c	0.72 ± 0.02 a	1.55 ± 0.06 c

Data are shown as mean with standard deviation. Different letters within a column indicate significant differences at $p < 0.05$

DON, dissolved organic nitrogen; *MBN*, microbial biomass nitrogen; *EON*, easily oxidizable nitrogen; *NON*, non-oxidizable nitrogen; *AHIN*, acid hydrolysable pool I nitrogen; *AHIIN*, acid hydrolysable pool II nitrogen; *ANHN*, acid non-hydrolyzable pool nitrogen; *TN*, total nitrogen

Table 4 Changes in the contents of different phosphorus fractions under seven fertilization treatments

	Resin-P mg kg^{-1}	NaHCO$_3$-P$_i$	NaHCO$_3$-P$_o$	NaOH-P$_i$	NaOH-P$_o$	HCl-P	Residual-P	Total P
NF	7.2 ± 1.4 d	45 ± 1 e	31 ± 5 d	321 ± 13 e	61 ± 6 c	35.6 ± 2.5 d	127 ± 4 a	628 ± 20 e
CF$_{100}$	17.5 ± 2.2 b	68 ± 7 bc	43 ± 7 c	386 ± 14 d	87 ± 14 b	40.5 ± 5.0 cd	128 ± 12 a	771 ± 24 d
MVCF$_{80}$	21.4 ± 1.9a	79 ± 6 a	57 ± 5 a	453 ± 14 a	117 ± 10 a	51.5 ± 4.5 a	130 ± 10 a	909 ± 9 a
MVCF$_{60}$	20.6 ± 1.7ab	75 ± 4 ab	53 ± 4 ab	442 ± 11 ab	110 ± 16 a	49.1 ± 4.1 ab	131 ± 8 a	881 ± 18 a
MVCF$_{40}$	18.6 ± 2.0 ab	72 ± 5 abc	50 ± 4 abc	419 ± 20 bc	111 ± 2 a	46.7 ± 3.8 ab	131 ± 7 a	848 ± 13 b
MVCF$_{20}$	17.4 ± 2.1 b	64 ± 6 c	48 ± 2 bc	400 ± 24 cd	97 ± 14 ab	44.1 ± 6.1 abc	130 ± 6 a	801 ± 15 c
MVCF$_0$	13.5 ± 1.4 c	55 ± 2 d	47 ± 2 bc	378 ± 12 d	81 ± 12 b	42.0 ± 0.3 bcd	129 ± 6 a	745 ± 7 d

Data are shown as mean with standard deviation. Different letters within a column indicate significant differences at $p < 0.05$

significantly correlated with SYI in three long-term experiments. The increase in organic carbon contents (Table 2) indicated that there had been an improvement in soil structure because there were positive correlations between organic carbon content and soil macroaggregate amounts (Bronick and Lal 2005). Therefore, high SYI may be ascribed to the improvement of soil physical properties, which may have promoted better rooting, higher water and nutrient uptake, and transpiration efficiency by crops (Bronick and Lal 2005; Peoples et al. 2009).

4.2 Soil organic carbon fractions

Soil organic carbon consists of a complex set of pools and is considered to have an important influence on the physiochemical and biological properties of soils (Haynes 2005). The amount of C sequestered at a site reflects the balance between the C input and loss processes. The results obtained in this study showed a significant increase in the contents of labile organic carbon fractions (DOC, EOC, and AHIC) in the five MV treatments compared with the CF$_{100}$ treatment, which suggested that the input of exogenous organic materials had a significantly positive impact on labile organic carbon. Many studies have also shown that the incorporation of green manure or other organic materials is one of the most important factors contributing to labile organic carbon improvements (Liu et al. 2009; Sun et al. 2013; Li et al. 2017). Soil labile organic carbon fractions are mainly derived from organic materials, and the soil organic matter decomposition process (Haynes 2005) and MV decomposition may have contributed to the increase in the labile organic carbon contents. In addition, the MBC, AHIIC, and NOC contents in the CF$_{100}$ treatment were significantly lower than in the MVCF$_{20}$, MVCF$_{40}$, MVCF$_{60}$, and MVCF$_{80}$ treatments and they gradually increased with increasing chemical fertilizer input rates. Besides the contribution of MV-derived C to soil organic carbon sequestration, the results may also be attributed to the input of nutrients, which is also essential for soil organic carbon sequestration. The results were consistent with Bradford (2008), who suggested that soil organic carbon stocks in experimental mesocosms depend on the rates of nitrogen and phosphorus inputs to soils. A large nutrient supply can decrease the decomposition of recalcitrant organic carbon fractions by reducing the nutrient demand from the microorganisms, resulting a net increase in soil organic carbon contents (Liu et al. 2018). In addition, the increase in rice yield with increasing nutrient (Fig. 1) indicated more root and rhizodeposits inputs, which may increase the labile and non-labile organic carbon contents (Ghosh et al. 2012). Sokol et al. (2019) showed that living root inputs are essential to form both slow cycling, recalcitrant organic carbon fractions and fast cycling labile organic carbon pools.

4.3 Soil nitrogen fractions

Soil nitrogen availability is one of the most important growth-limiting factors in agroecosystems. With the exception of ANHN, the MVCF$_{60}$ and MVCF$_{80}$ treatments significantly increased the contents of the other N fractions, whereas the MVCF$_{40}$ treatment only significantly increased EON, AHIIN, and TN contents compared with the CF$_{100}$ treatment, indicating that MV incorporation well compensated for soil N reserve

Table 5 Stepwise multiple regression analysis of the relationships of average yield and SYI with soil organic matter fractions

Models	R^2	p value
Average yield = 0.863 TP − 0.440 NaHCO$_3$-P$_o$ + 0.269 EON + 0.256 DON	0.955	< 0.01
SYI = 0.593 EOC + 0.273 TOC	0.804	< 0.01

TP, total P; *NaHCO$_3$-P$_o$*, NaHCO$_3$ extractable organic P; *EON*, easily oxidizable nitrogen; *DON*, dissolved organic nitrogen; *SYI*, sustainable yield index; *EOC*, easily oxidizable organic carbon; *TOC*, total organic carbon losses when 20–60% of the chemical fertilizer N input was removed. Similar results were also reported by Yang et al. (2018), who found that incorporating *Orychophragmus violaceus* as a green manure with a 15–30% reduction in chemical fertilizer inputs increased organic N and total N contents in a 4-year field experiment. Different N fractions differ widely from source to chemical composition, which leads to various responses to different fertilization practices (Yang et al. 2018; Wu et al. 2019). Generally, the dynamic patterns and the magnitude of change in the N pools are determined by the N input (e.g., fertilizers input) and output (e.g., crop uptake and leaching). One explanation for our results may be the high total N (green manure N plus fertilizer N) input rates (Table 1). Several studies have reported that increasing the input of chemical fertilizers or organic materials leads to an increase in the size of soil N pools (Yang et al. 2018; Wu et al. 2019). In addition, the increase in soil organic carbon contents (Table 2) may also contribute to the accumulation of soil N fractions because there was a simultaneous increase in C and N accumulation. High organic C availability can stimulate the growth and proliferation of heterotrophic microorganisms, which facilitate microbial N immobilization and transformation. In a meta-analysis, Cheng et al. (2017) found that soil microbial NO_3^--N immobilization was enhanced with the elevation of C availability. On the other hand, in comparison with chemical fertilizers, slower mineralization and release of N is expected in milk vetch, which may lead to less N loss (Zhu et al. 2014; Li et al. 2015). Sekhon et al. (2011) have also suggested that the application of organic materials had more efficient soil N pool building.

4.4 Soil phosphorus fractions

As different P pools have different crop availabilities and responses to fertilization, dividing the soil P into different fractions supplies a more effective means for detecting the P changes compared with the analysis of soil total P content (Song et al. 2011). We found significantly lower resin-P and $NaHCO_3$-P_i contents in the $MVCF_0$ treatment than in the CF_{100} treatment. These two P fractions are considered as labile P fractions and their changes are largely influenced by exogenous P inputs (Kuo et al. 2005; Wang et al. 2016). The different P input rates (Table 1) may contribute to the differences in these two fractions. Furthermore, this study also showed that the $MVCF_{40}$ and $MVCF_{60}$ treatments significantly increased the NaOH-P (NaOH-P_i and NaOH-P_o), HCl-P, and total P contents compared with the CF_{100} treatment, whereas the $MVCF_{80}$ treatment significantly increased all the P fraction contents (except for residual-P). The NaOH-P fraction represents P that is strongly adsorbed on the Fe and Al oxide surfaces in the soil, and the HCl-P fraction is considered as restrict lability for representing dissolved acid-soluble P in the form of calcium phosphates (Tiessen and Moir 2008). The increase in the NaOH-P and HCl-P contents indicated a stronger P sorption. This may be due to paddy soil treated by MV had greater amorphous Fe and Al oxyhydroxide contents that had a vigorous capacity of stabilizing NaOH-P (Yan et al. 2017). In addition, the increase in P sorption may attribute to the improvement of soil organic matter contents (Kang et al. 2009; Yan et al. 2017). The increases in organic matter (Table 2) may inhibit the crystallization of Al and Fe by forming stable complexes with them, which in turn can increase P sorption as noncrystalline Al and Fe increases (Kang et al. 2009). Moreover, the increase in soil organic matter could also lead to the formation of metal-OM complexes that can further provide reactive sites for P sorption (Giesler et al. 2005). The increased HCl-P content may be due to the addition of Ca via single super phosphate and MV inputs. Our findings are in agreement with those of Verma et al. (2005), who have reported that Ca addition via phosphatic fertilizers precipitated applied P as calcium phosphate and increased the transfer of P from the NaOH-P to HCl-P.

5 Conclusions

In a mono rice-based cropping system, milk vetch incorporation (45,000 kg ha^{-1}, fresh weight) along with 20–60% reductions in chemical fertilizer inputs led to increases in rice yield and sustainability. Milk vetch incorporation also increased the labile organic carbon contents. Furthermore, the 20–40% reduction in chemical fertilizer inputs after milk vetch incorporation significantly increased N and P fraction contents, whereas only part of N and P fractions increased when the chemical fertilizer reduction percentage increased to 60%. Therefore, considering rice yield and the building-up of soil organic carbon and nutrient pools, milk vetch incorporation with a 20–40% reduction of chemical fertilizer N, P, and K inputs may be a substitute practice for current fertilization practices that rely on large chemical fertilizer inputs during rice production. Unraveling its potential influence on greenhouse gas emission (e.g., N_2O and CH_4) and nutrient leaching is worth further investigation to further determine sustainability of mono rice production systems.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies involving human participants and/or animals performed by any of the authors.

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