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Yield sustainability, soil organic carbon sequestration and nutrients balance under long-term combined application of manure and inorganic fertilizers in acidic paddy soil

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ABSTRACT

Organic and inorganic fertilization management in intensive cropping system is important to achieve long-term high crop yield sustainability. We quantitatively investigated crop yield sustainability through soil fertility and nutrients balance in 34-years long-term experiment under double rice cropping system in acidic paddy soil. Seven treatments were studied: CK (no fertilization); NPK (Chemical nitrogen, phosphorus and potassium fertilizer); NPM (Chemical N, P and manure); NKM (Chemical N, K and manure); PKM (Chemical P, K and manure); NPKM (Chemical N, P, K and manure) and M (Manure). Manure was applied at the rate of 45,000 kg ha^{-1}. Results showed that crop yield and sustainability yield index under combined application of manure and chemical fertilizers was significantly higher than chemical fertilization and highest crop yield was under NPKM treatment. Long-term combined manure and chemical fertilization improved soil fertility as compared to CK and NPK treatments. Soil organic C sequestration rates under NPM, NKM, PKM and NPKM treatments were increased, while decreased under CK and NPK over the fertilization years. The uptake of N, P and K was increased over the fertilization years in all the treatments that received manure, compared with CK and NPK. Apparent K balance was negative in all the treatments. N balance (except CK and NPKM) and P balance (except CK) was positive in all fertilization treatments. P balance was exceeded the environmental risk threshold under combined application of chemical P fertilizer and manure. Boosted regression tree indicated that soil available N (AN), orgnaic carbon (OC) and total N (TN) were the most influencing factors of crop yield, accounted 36.5 %, 17.8 %, 13.4 % of variations of relative yield, respectively. Path analysis showed that long-term fertilizer inputs increased soil nutrient contents and C input directly affected soil OC. C input and soil pH indirectly influenced the relative crop yield. This study concluded that long-term combined application of manure and inorganic fertilizers increased crop yield sustainability, organic carbon sequestration rate compared to the inorganic fertilization. But long-term combined application of manure and inorganic phosphorus fertilizer increased the P balance. Therefore, rate of P inputs should be reduced under combined application of manure and inorganic P fertilizers in acidic paddy soil.

1. Introduction

Sustainable crop production in China as well as in the world is needed to feed the ever-growing world population. Improvement in the field managing technologies has accounted a significant contribution to increase the crop productivity (Deryng et al., 2011; Doltra et al., 2019). In China, during 1970–1990 the annual rice production was increased by 3.37 % (FAO, 2008), mostly due to the cultivation of high yielding varieties and high consumption of chemical fertilizers (Tong et al., 2003; Yuan, 1996). However, yield growth rate was dropped by 0.6 %

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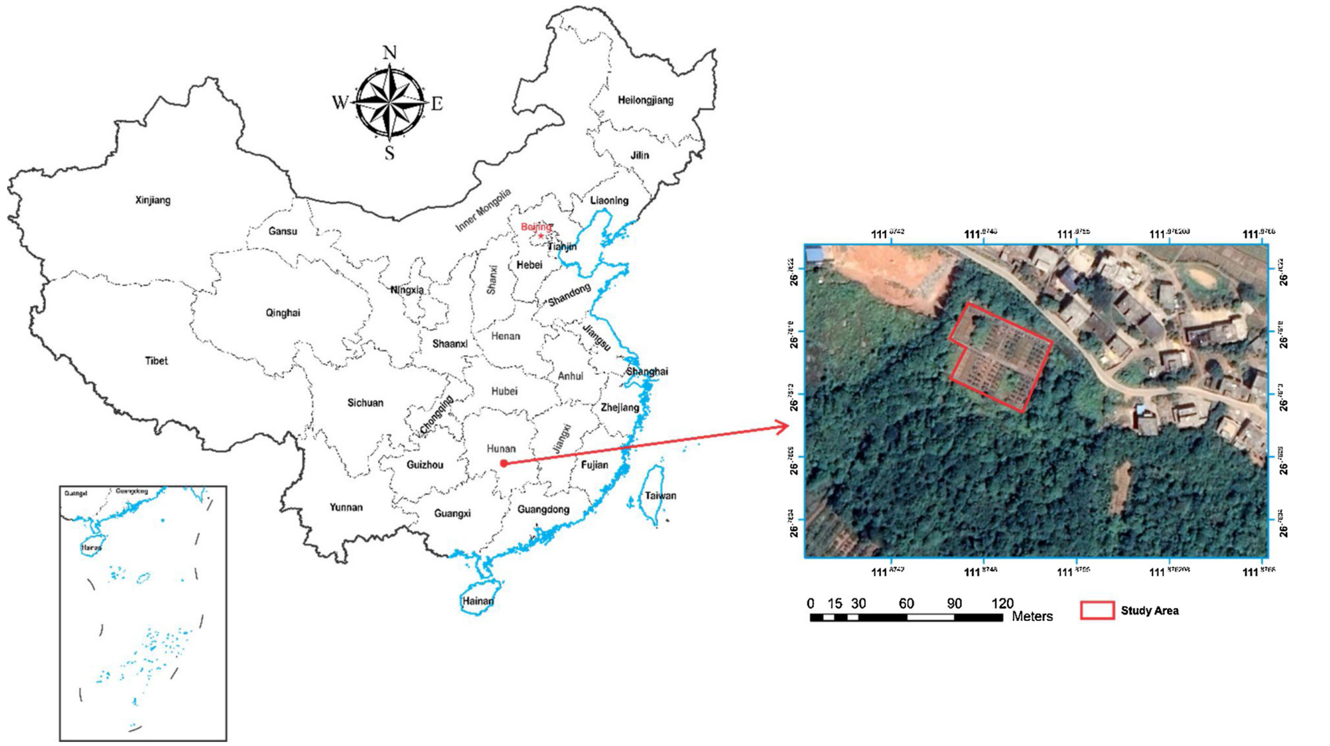

Fig. 1. Map representing an experimental location in Southern China, made by GIS using coordinates for this location.

per year from 1990 to 2006 (FAO, 2008). This might be further challenged by changes in global climate (Lobell and Asner, 2003; Peng et al., 2004) and scarcity of water resources (Van Nguyen and Ferrero, 2006). The fertilization approach is one of the best field management practices to achieve high crop yield. Chen et al. (2014) from 153 field studies found that manure addition increased crop yield by 8.5 to14.2 Mg ha^{-1} without increasing N input. In another study, based on 20 different field trials, Hijbeek et al. (2017) found that inorganic fertilization increased the yield of crop by 2.0 Mg ha^{-1} and yield did not increase significantly by manure application. It is clear that the mechanism of the yield response to different types of fertilization is not consistent. Therefore, to achieve sustainable and high yield of crop, it is essential to know the influence of long-term fertilization on crop productivity. Fertilizers application effects crop yield by changing soil chemical properties, such as soil pH and nutrient contents and C inputs.

Soil organic carbon (OC) content is one of the main indexes of soil fertility (Hijbeek et al., 2017). The sequestration of soil OC could be increased by the application of different fertilizers which provides high carbon inputs, such as manure application or incorporation of crop residue (Cai et al., 2015). Benbi and Chand (2007) have shown that each Mg of OC in the soil layer (0 − 15 cm) increased wheat productivity by 15 − 33 kg ha^{-1} under long-term organic fertilization. There is thus a strong need to increase OC density for sustainable crop productivity. In another study, compared with the winter fallow, the green manure rotation under double cropping system increased the OC stock up to 24 % (Yao et al., 2017). McDaniel et al. (2014) in their study, found that cover crops significantly increased rate of OC sequestration as compared to no cover crops. Chemical fertilizers also influence the OC sequestration by returning residues of crop to field such as roots and stubbles residues. For example, Zhang et al. (2012) found that inorganic fertilizers increased carbon inputs by 2.5 to 5.0 Mg ha^{-1} in southern China. Only the few studies have investigated the effect of combined organic and inorganic fertilization on organic C sequestration and sustainable crop production. For example, Wang et al. (2019) has observed the high crop yield and organic C accumulation in soil under combined application of inorganic fertilizer and mnaure, compared to the alone application of orgnaic or inorganic fertilizer. However, the mechanism by which different fertilizers application influence the crop yield by changing soil OC is not clear. Soil nutrients are main factors limiting crop yield, therefore, high crop yield mainly depends on fertilizer (organic and/or inorganic fertilizers) application rates. Though, lower than half amount of total inputs from chemical fertilizers efficiently used and did not show surplus nutrient accumulation in soil (Galloway et al., 2008). Meanwhile, excessive use of chemical fertilizers not only increase the air and water pollution (Lu and Tian, 2013; Peñuelas et al., 2012), but also degrade the soil quality such as acidification (Lin et al., 2014).

Acidification of paddy soils has received significant attention due to its negative effects on soil fertility and crop production (Cai et al., 2015; Guo et al., 2010). One of the main reasons of soil acidity is the excessive rates of chemical fertilizer application (Zhu et al., 2018). However, China has achieved huge progress in attaining higher crop production, but most of the agricultural lands are still being affected by substantial acidification since 1980s (Guo et al., 2010). Nitrification process in soil can produce protons which may decrease soil pH (Xu et al., 2006). Addition of manure to the agricultural soil can improve not only soil OC content but also can provide essential nutrients for plant uptake. Application of manure to the cropland can reduce the soil acidity by improving soil pH due to alkalinity of manure (Mi et al., 2018; Rukshana et al., 2013). Furthermore, after many years of manure application, its residual effects were observable, leading to availability of different nutrients for uptake by crop (Demelash et al., 2014).

The subtropical region of China is dominant with paddy soil under rice cultivation, playing a major role in national grain production (Huimin et al., 2009). However, soil acidification is a major problem that limit the soil fertility and sustainable crop production in that region (Cai et al., 2015). Therefore, we hypothesized that combined application of manure and inorganic fertilizer could be more effective fertilization practice to increase the yield sustainability and to reduce surplus nutrient accumulation in soil, compared to the inorganic fertilization alone in acidic paddy soil. The specific objectives of this study were to investigate the effects of different combination of organic and inorganic

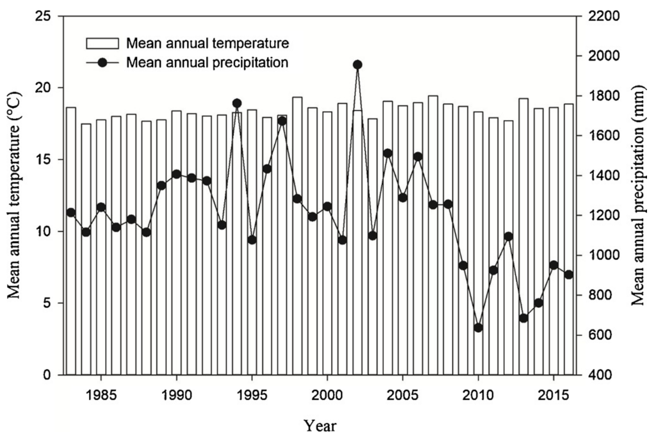

Fig. 2. Mean annual temperature (MAT) and mean annual precipitation (MAP) during long-term experimental period.

fertilization on sustainable crop production and nutrient balance under double rice cropping system in the acidic paddy soil.

2. Materials and methods

2.1. Site description

A long-term field experiment was established at the national observation and research station of farmland ecosystem (26°45′42″N, 111°52′32″E) in south of China (Fig. 1). This region obtains annual rainfall of 1290 mm and average temperature 17.8 °C. The rain fall duration is from April to end of June. The mean annual temperature (MAT) and mean annual precipitation (MAP) during the experiment period is given in Fig. 2 The climate data (i.e., MAT and MAP) were collected from county weather station, which was situated at less than 8 km away from the experiental site, where weather data of dry- and wet-bulb temperature, minimum and maximum air temperature, and precipitation were recorded daily at 0800 following the National Standard of Specifications for Surface Meteorological Observations (1979). The soil type is Ferralic cambisol according to World Reference Base for soil resources (WRB) (FAO, 2014) and also known as red soil according to Chinese soil classification (Baxter, 2007). The initial properties of topsoil were as follow: soil pH of 5.2; total N (TN) of 1.1 g kg^{-1}; total P (TP) of 0.47 g kg^{-1}; total K (TK) of 17 g kg^{-1}; available N (AN) of 82 mg kg^{-1}; available P (AP) of 4.7 mg kg^{-1}; available K (AK) of 55 mg kg^{-1} and soil organic carbon (OC) of 12.0 g kg^{-1}.

2.2. Experimental design

The study was designed with different organic and inorganic fertilization treatments. We selected seven treatments in this study (Table 1): (1) CK (no fertilizer); (2) NPK (inorganic nitrogen, phosphorus and potassium fertilizer); (3) NPM (inorganic NP fertilizer and manure); (4) NKM (inorganic NK fertilizer and manure); (5) PKM (inorganic PK fertilizer and manure); (6) NPKM (inorganic NPK fertilizer and manure) and (7) M (manure). The CK treatment was started from 2001 while all the other treatments were randomly designed in 1982. Rate of manure application was same in all treatments receiving manure (Table 1). Each plot (1.8 m × 15.0 m) had three replications, which were separated from adjacent plot by 60-cm cemented barriers. Inorganic fertilizers were applied in the form of urea for N, calcium superphosphate for P and potassium chloride for K. Manure was fresh cattle manure contained water content about 20 %. The average nutrient contents of manure were; 400 g kg^{-1} of carbon, 3.2 g kg^{-1} of N, 1.09 g kg^{-1} of P and1.24 g kg^{-1} of K. All the inorganic fertilizers and manure were applied as basal application, before rice transplantation. Annual input rates of manure and inorganic fertilizers are given in Table 1.

2.3. Crop management and soil sampling

The cropping system was rice-rice rotation system. The experimental field was disposed of for three years to confirm the same soil properties. Extensive locally used rice varieties were selected for cultivation with transplanting 200,000 seedlings per hectare. The early rice was transplanted in end of April and harvested in July, while late rice was transplanted in end of July and harvested in October. Conventional management practices such as ploughing and raking field at the depth of 15 − 20 cm, intermittent irrigation and use of pesticide for pest control were carried out following the farmer practices. The early rice was kept flooding, and field was drained at the ripening stage of late rice (Yang et al., 2012; Zhang et al., 2017). Crop was manually harvested; stubbles and roots were remained in the field. After the crop harvest, the straw and grain yields were air-dried and weighted. Soil samples from the topsoil (0 − 20 cm) were taken at five different points of each replicated plot every year once within first week after the late rice crop was harvested. Then, samples from each plot were thoroughly mixed to make homogeneous composite sample of each replication, air-dried and transferred to laboratory for analysis in clean polythene bags. Soil bulk density was estimated once in 2013 in undisturbed soil samples, that were collected with cutting ring of 50.46-mm inner diameter, 100 cm^3 volume and 50-mm depth of soil sampling (Lu, 2000).

2.4. Laboratory analysis

After the crop harvest and air-dried, grain and straw samples were oven-dried for 30 min at 105 °C then heated at 70 °C to a constant weight for determination of dry matter and nutrient contents. Oven-dried straw and grains were ground and digested with H_2SO_4-H_2O_2 at 260–270 °C. The plant N and P contents were measured according to semi micro Kjeldahl digestion and the vanadomolybdate yellow method, respectively (Jackson, 1969; Nelson and Sommers, 1982). While K content in the plant was determined using flame photometer. Nutrients uptake in plant were estimated by multiplying plant nutrient content with yield. To determine the chemical properties of soil, a part

Table 1
Annual input rates of inorganic nitrogen (In-N), phosphorus (In-P), potassium (In-K) fertilizer, and organic nitrogen (Or-N), phosphorus (Or-P) and potassium (Or-K) added in various treatments in the form of cattle manure.

Treatments	Manure (Kg ha^{-1})	In-N (Kg ha^{-1})	In-P (Kg ha^{-1})	In-K (Kg ha^{-1})	Or-N (Kg ha^{-1})	Or-P (Kg ha^{-1})	Or-K (Kg ha^{-1})
CK	0	0	0	0	0	0	0
NPK	0	145	49	56	0	0	0
NPM	45000	145	49	0	145	49	56
NKM	45000	145	0	56	145	49	56
PKM	45000	0	49	56	145	49	56
NPKM	45000	145	49	56	145	49	56
M	45000	0	0	0	145	49	56

from composite samples, subset of soil samples were crushed to pass through 0.25-mm sieve. Soil OC was measured by oxidation method using vitriol acid potassium dichromate oxidation (Pages et al., 1982). Soil total N, P and K contents were determined by procedures described by Black (1965); Murphy and Riley (1964) and Knudsen et al. (1982) respectively. Soil available N, P and K were measured in accordance with Lu et al., (2000), Olsen (1954) and Page et al., (1982), respectively.

2.5. Calculations

Sustainable yield index (SYI) is quantitative measure to assess the sustainability of agricultural practices (Singh et al., 1990). Yield sustainability index was estimated using following equation (Singh et al., 1990):

$$\text{Sustainable yield index } (SYI) = \frac{Ymean - \sigma}{Ymax}$$

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

Apparent nutrient balance is defined as the difference between nutrient inputs entering the farming system and nutrient output leaving the farming system (OECD, 2013). Apparent nutrient balance (AB) (Kg ha^{-1} $year^{-1}$) was measured using the following equation (Ouyang et al., 2017):

$$Apparent\ nutrient\ balance = \sum N_{input} - \sum N_{uptake}$$

where, N_{input} is annual N, P or K input (kg ha^{-1} $year^{-1}$) to the field through organic and inorganic fertilization. N_{uptake} is N, P or K uptake (kg ha^{-1}) by crop in above-ground biomass. The positive value of AB indicates the nutrient surplus and negative value of AB (nutrient deficit) indicates declining soil fertility (OECD, 2013).

The amount of total C input included returned manure plus plant residues. The annual C input (C input, t ha^{-1}) was measured from the C content of belowground biomass (C_{root}, t ha^{-1}), incorporated stubble ($C_{stubble}$, t ha^{-1}) and the amount of applied manure (C_{manure}, t ha^{-1}).

$$C\ input = C_{bellowground} + C_{stubble} + C_{manure}$$

$$C_{bellowground} = Rr \times CR \times 10^{-3}$$

$$C_{stubble} = SR \times Cs \times 10^{-3}$$

Rr Residue of roots (t ha^{-1} $year^{-1}$) estimated as 30 % of above-ground rice biomass (Kundu et al., 2007). CR is carbon content in rice plant (418 g kg^{-1}). SR is stubble quantity of rice field (t ha^{-1} $year^{-1}$) estimated as 5.6 % of rice straw yield (Jing et al., 2015). Cs is carbon content of rice straw (444 g kg^{-1}).

We estimated the annual organic carbon stocks (t ha^{-1} $year^{-1}$) using following equation:

$$SOC = C \times BD \times H \times 10^{-1}$$

where, SOC is stock of OC (t ha^{-1} $year^{-1}$), C is soil C content (g kg^{-1}), BD is soil bulk density (g cm^{-3}) and H is soil depth (cm).

Soil organic carbon sequestration rate (CSR, t ha^{-1} $year^{-1}$) was calculated by following equation (Zhang et al., 2012):

$$CSR = (SOC_T - SOC_0)/t$$

Where, SOC_t and SOC_0 is stock of OC (t ha^{-1}) at the time t and in the initial year (1982),respectively. t was duration of experiment (34 years).

The relative yield (RY) was calculated for use in yield prediction through boosted regression tree model (BRT). Relative yield allow the yield data from individual treatment to be more comparable. The relative yield (RY) was estimated as follow:

$$RY = Y_{treatment} - Y_{control}$$

Where, $Y_{treatment}$ is the yield of fertilizer treatment (t ha^{-1}) in a specific year and $Y_{control}$ is the yield (t ha^{-1}) of control treatment in same year.

2.6. Statistical analysis

The differences among fertilization treatments for different parameters were analyzed (1982–2000; 2001–2010 and 2011–2016) by one-way ANOVA followed by Tukey's HSD test at P = 0.05 level of significance using the software SPSS 19.0. The trends of change in soil OC content under different treatments were examined by simple linear regression. Boosted regression tree (BRT) model was conducted using the recommended indexes (Elith et al., 2008) to predict the relative crop yield by relative influence of soil properties. The BRT was constructed using gbm package in R version 3.3.3 (Elith et al., 2008). The structural equation modeling (SEM) was used to analyze the direct and indirect relationship between relative yield and soil fertility parameters using Amos package. In SEM analysis, we used annual estimated C input, annual N,P and K inputs as exogenous variables. While, soil OC, pH, AN, AP, AK and relative yield used as endogenous variables. The long-term data from 1982 to 2016 was used for BRT and SEM analysis.

3. Results

3.1. Crop yields and sustainability yield index

Long-term different fertilization significantly influenced the crop yield (Fig. 3 and Table 2), which decreased in CK and NPK and increased in NPM, NKM, PKM, NPKM and M by increasing the fertilization periods (Table 2). Among all the treatments, annual grain yield during different fertilization periods (1982–2000. 2001–2010 and 2011–2016) was highest in NPKM and was lowest in CK treatment (Table 2). Compared with 1982–2000 period, the increase in annual grain yield during period of 2011–2016 in NPM, NKM, PKM and NPKM was by 7.2 %, 9.6 %, 7.3 %, 7.5 % and 2.4 %, respectively and

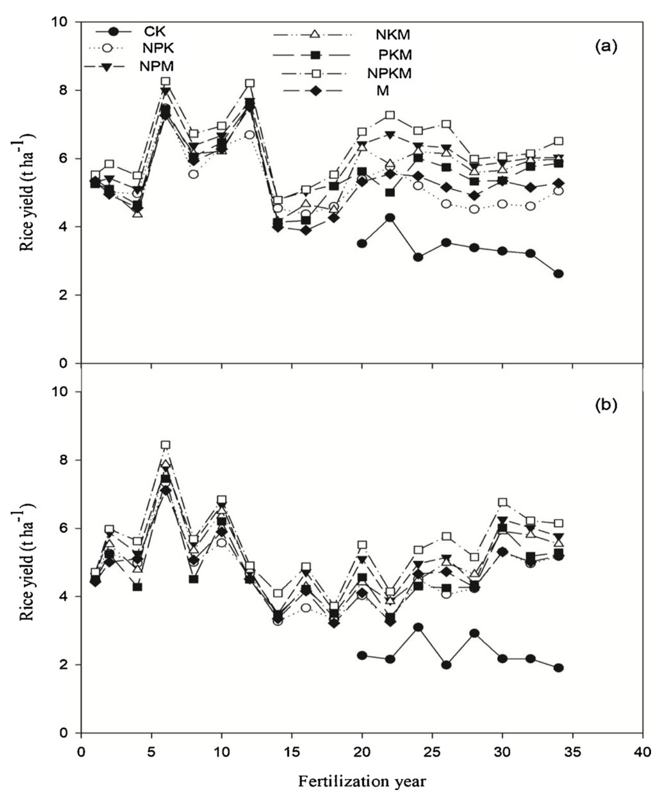

Fig. 3. Early rice (a) and late rice (b) yield under various fertilization treatments of a long-term experiment in double rice cropping system.

Table 2
Annual nutrients uptake, balance and crop yield in acidic paddy soil for the period of 1982–2000, 2001–2010, and 2011–2016 under different fertilization of long-term experiment in double rice cropping system.

Year	Treatments	NU (Kg ha^{-1})	PU (Kg ha^{-1})	KU (Kg ha^{-1})	N balance (Kg ha^{-1} $year^{-1}$)	P balance (Kg ha^{-1} $year^{-1}$)	K balance (Kg ha^{-1} $year^{-1}$)	Grain Yield (t ha^{-1})
1982–2000	NPK	98 ± 1.7 e	33 ± 0.7 d	107 ± 3.6 e	47 ± 1.7 c	16 ± 0.7 d	−51 ± 3.6 a	10.3 ± 0.06 e
	NPM	124 ± 0.8 b	41 ± 0.9 b	133 ± 2.7 d	166 ± 0.8 b	57 ± 0.9 b	−77 ± 2.7 c	11.2 ± 0.07 b
	NKM	116 ± 1.1 c	34 ± 0.8 d	173 ± 2.6 b	174 ± 1.1 a	15 ± 0.8 d	−61 ± 2.6 b	10.6 ± 0.02 c
	PKM	104 ± 2.0 d	37 ± 0.8 c	163 ± 1.6 c	41 ± 2.0 d	61 ± 0.8 a	−51 ± 1.6 a	10.4 ± 0.08 d
	NPKM	358 ± 4.0 a	46 ± 0.7 a	219 ± 3.7 a	−68 ± 4.0 e	52 ± 0.7 c	−107 ± 3.7 d	11.7 ± 0.03 a
	M	99 ± 0.3 de	33 ± 0.2 d	125 ± 2.4 d	46 ± 0.3 cd	16 ± 0.2 d	−69 ± 2.4 c	10 .2 ± 0.03 e
2001–2010	CK	42 ± 0.7 f	12 ± 0.1 f	40 ± 4.7 f	−42 ± 0.7 e	−12 ± 0.1 g	−40 ± 4.7 a	6.1 ± 0.11 g
	NPK	78 ± 0.2 e	25 ± 0.8 e	129 ± 2.6 e	67 ± 0.2 c	24 ± 0.8 d	−73 ± 2.6 b	9.1 ± 0.07 f
	NPM	123 ± 1.4 b	37 ± 0.3 b	207 ± 5.5 c	167 ± 1.4 b	61 ± 0.3 b	−151 ± 5.5 e	11.0 ± 0.06 b
	NKM	116 ± 2.6 c	33 ± 0.6 c	236 ± 3.7 b	174 ± 2.6 a	16 ± 0.6 f	−124 ± 3.7 d	10.5 ± 0.02 c
	PKM	96 ± 0.3 d	32 ± 0.8 c	213 ± 9.5 c	49 ± 0.3 d	66 ± 0.8 a	−101 ± 9.5 c	9.7 ± 0.06 d
	NPKM	384 ± 5.2 a	45 ± 0.4 a	302 ± 9.8 a	−94 ± 5.2 f	53 ± 0.4 c	−190 ± 9.8 f	12 .0 ± 0.03 a
	M	93 ± 0.7 d	30 ± 0.4 d	177 ± 0.8 d	52 ± 0.7 d	19 ± 0.4 e	−121 ± 0.8 d	9.5 ± 0.03 e
2011–2016	CK	32 ± 0.6 f	10 ± 0.1 f	36 ± 6.6 f	−32 ± 0.6 e	−10 ± 0.1 f	−36 ± 6.6 a	5.1 ± 0.04 g
	NPK	81 ± 2.2 e	23 ± 1.2 e	177 ± 9.9 e	64 ± 2.2 c	26 ± 1.2 d	−121 ± 9.9 b	9.9 ± 0.26 f
	NPM	134 ± 2.6 b	41 ± 0.3 b	281 ± 7.4 c	156 ± 2.6 b	57 ± 0.3 b	−225 ± 7.4 d	12.0 ± 0.07 b
	NKM	122 ± 2.0 c	35 ± 0.3 d	314 ± 4.5 b	168 ± 2.0 a	14 ± 0.3 e	−202 ± 4.5 cd	11.6 ± 0.02 c
	PKM	107 ± 3.0 d	38 ± 1.0 c	295 ± 11 bc	38 ± 3.0 d	60 ± 1.0 a	−183 ± 11.4 c	11.2 ± 0.17 d
	NPKM	408 ± 9.0 a	48 ± 0.2 a	412 ± 11.9 a	−118 ± 9.0 f	50 ± 0.2 c	−300 ± 11.9 e	12.6 ± 0.04 a
	M	105 ± 2.2 d	34 ± 0.7 d	252 ± 15.1 d	40 ± 2.2 d	15 ± 0.7 e	−196 ± 15.1 c	10.4 ± 0.12 e

Note: Values are means (n = 3) ± standard deviations. Means followed by different letters are significantly ($P \leq 0.05$) different from each other according to Tukey's HSD test.
Abbreviations: NUN uptake; PUP uptake; KUK uptake; nnumber of replications.

Table 3
Sustainability yield index (SYI) of early and late rice under different fertilization of long-term experiment in double rice cropping system.

Treatments	SYI of (Early rice)	SYI (Late rice)
CK	0.68 ± 0.019 a	0.61 ± 0.013 a
NPK	0.59 ± 0.007 de	0.49 ± 0.005 c
NPM	0.65 ± 0.006 b	0.53 ± 0.014 b
NKM	0.63 ± 0.009 bc	0.49 ± 0.009 c
PKM	0.62 ± 0.003 cd	0.50 ± 0.006 c
NPKM	0.65 ± 0.009 b	0.52 ± 0.005 b
M	0.58 ± 0.007 e	0.53 ± 0.011 b

Values are means (n = 3) ± standard deviations. Means followed by different letters are significantly ($P \leq 0.05$) different from each other according to Tukey's HSD test. Number of replications (n) = 3.

decreased in NPK by 3.2 %. Compared with 1982–2000, the annual grain yield during the period of 2001–2010 was decreased in NPK and M treatment and was significantly increased in NPKM treatment. Compared with chemical fertilization, combined application of manure and chemical fertilizers increased sustainability yield index (SYI) for both early and late rice (except NKM in late rice) (Table 3). For both early and late rice SYI in CK was highest among all the fertilization treatments. SYI in early rice increased with NPM, NKM, PKM and NPKM by 10 %, 6.9 %, 4.3 % and 10 %, respectively and decreased by1.2 % with M treatment compared with NPK. SYI in late rice increased with NPM, PKM, NPKM and M by 7.3 %, 0.6 %, 6.1 % and 6.6 %, respectively and decreased with NKM by 0.01 % compared with NPK. Overall, the early rice yield was more sustainable compared with late rice crop.

3.2. Nutrients uptake and apparent balances

Nutrients uptake and apparent nutrients balance are shown in Table 2. Increasing the nutrient inputs by combined application of manure and chemical fertilizers, significantly increased nutrients uptake compared with chemical fertilization. Among all the fertilization treatments, highest uptake of N, P and K was under NPKM and lowest uptake was in CK during different fertilization periods. Compared with fertilization period of 1982–2000, the uptake of N decreased in NPK, NPM, PKM and M treatment and increased in NPKM treatment during period of 2001–2010. P uptake decreased in all the treatments during period of 2001–2010, compared to period of 1982–2000 and the highest decrease was observed under NPK. K uptake during 2001–2010 increased in all the treatments compared with the period of 1982–2000. The uptake of N, P and K during fertilization period of 2010–2016 in all treatments was greater than period of 1982–2000.

During all three fertilization periods, the annual apparent N balance was ranged from −118 kg ha^{-1} $year^{-1}$ in NPKM to 174 kg ha^{-1} $year^{-1}$ in NKM and P balance was ranged from −12 kg ha^{-1} $year^{-1}$ in CK to 66 kg ha^{-1} $year^{-1}$ in PKM. K balance in all the treatments was negative during all the three cropping periods. K balance was ranged from −300 kg ha^{-1} $year^{-1}$ in NPKM to −36 kg ha^{-1} $year^{-1}$ in CK. N, P and K balance in CK treatment was increased with the increase in fertilization period. Compared with 1982–2000 period, N and P balance during 2001–2010increased in all treatments except N balance in NKM and NPKM. N balance in NKM did not change and decreased in NPKM during 2001–2010 compared with 1982–2000 period. During the period of 2010–2016, N balance in NPM, NKM, PKM, NPKM and M treatments and P balance in NKM, PKM, NPKM and M treatments decreased compared with 1982–2000 period. K balance in all the treatments except CK decreased during period of 2001–2010 and 2011–2016, compared to 1982–2000 period.

3.3. Changes in soil nutrients and organic carbon sequestration rate (CSR)

Long-term fertilization significantly influenced soil pH, nutrients and organic carbon sequestration rate (CSR). During all three fertilization periods, the soil pH ranged from 4.6 in NPK to 6.5 in PKM (Table 4). Soil pH decreased under NPK but increased in NKM, PKM and M treatments during the period of 1982–2000, compared to the initial soil pH (5.2). During the period of 2001–2010 and 2011–2016, soil pH in all the treatments receiving manure was higher than initial soil pH. Soil pH in all treatments except NPK during 2001–2010 and 2011–2016 was higher than soil pH during 1982–2000. Soil pH under NPK decreased by the increase of fertilization period. Soil TN, AN, TP, AP, TK and AK contents during all three fertilization periods were significantly higher than their initial values in all the treatments except CK (Table 4). Soil AN content was lower but TP and AP contents were higher in CK during periods of 2001–2010 and 2011–2016 compared with their

Table 4
Soil nutrient contents, annual carbon input and soil organic carbon sequestration rate (CSR) in acidic paddy soil for the period of 1982–2000, 2001–2010, and 2011–2016 under different fertilization of long-term experiment in double rice cropping system.

Year	Treatments	pH	TN ($g\ kg^{-1}$)	AN ($mg\ kg^{-1}$)	TP ($g\ kg^{-1}$)	AP ($mg\ kg^{-1}$)	TK ($g\ kg^{-1}$)	AK ($mg\ kg^{-1}$)	C input ($t\ ha^{-1}\ y^{-1}$)	CSR rate ($t\ ha^{-1}\ y^{-1}$)
Initial year		5.2	1.1	82	0.47	4.7	17.0	55	–	–
1982–2000	NPK	4.9 ± 0.11 b	1.8 ± 0.05 c	99 ± 1.72 d	0.70 ± 0.004 c	20 ± 0.21 c	21.4 ± 0.14 c	100 ± 1.34 b	2.30 ± 0.02 e	0.31 ± 0.06 b
	NPM	5.2 ± 0.14 a	2.0 ± 0.01 b	115 ± 0.62 b	0.78 ± 0.007 b	23 ± 0.50 b	21.8 ± 0.27 bc	102 ± 2.13 b	16.9 ± 0.02 b	0.58 ± 0.02 a
	NKM	5.3 ± 0.01 a	2.2 ± 0.06 a	113 ± 2.38 b	0.57 ± 0.005 e	11 ± 0.29 d	23.8 ± 0.05 a	154 ± 0.17 a	16.8 ± 0.01 c	0.60 ± 0.05 a
	PKM	5.3 ± 0.02 a	1.8 ± 0.01 c	106 ± 1.15 c	0.81 ± 0.005 a	23 ± 0.29 b	23.5 ± 0.19 a	102 ± 2.09 b	16.7 ± 0.02 c	0.57 ± 0.02 a
	NPKM	5.2 ± 0.03 a	2.2 ± 0.05 a	129 ± 1.37 a	0.82 ± 0.02 a	28 ± 0.44 a	23.8 ± 0.22 a	156 ± 4.06 a	17.1 ± 0.04 a	0.65 ± 0.05 a
	M	5.4 ± 0.10 a	2.1 ± 0.01 b	105 ± 1.19 c	0.61 ± 0.001 d	12 ± 0.47 d	22.0 ± 0.09 b	104 ± 3.78 b	16.7 ± 0.02 d	0.52 ± 0.08 a
2001–2010	CK	5.0 ± 0.04 d	1.5 ± 0.01 d	62 ± 2.46 f	0.58 ± 0.010 e	9 ± 0.57 e	16.4 ± 0.10 e	37 ± 0.46 f	1.3 ± 0.02 f	−0.132 ± 0.07 c
	NPK	4.8 ± 0.11 d	1.7 ± 0.02 c	139 ± 2.17 e	0.79 ± 0.008 d	34 ± 1.22 c	25.4 ± 0.21 d	100 ± 3.23 e	2.1 ± 0.20 e	0.271 ± 0.05 b
	NPM	5.9 ± 0.16 c	2.3 ± 0.03 a	172 ± 1.10 b	0.94 ± 0.018 c	47 ± 2.51 b	25.5 ± 0.14 cd	131 ± 2.42 d	16.7 ± 0.01 b	0.787 ± 0.09 a
	NKM	5.9 ± 0.05 bc	2.2 ± 0.03 b	168 ± 2.46 b	0.55 ± 0.003 e	13 ± 0.47 de	26.1 ± 0.20 bc	146 ± 3.21 b	16.6 ± 0.01 bc	0.815 ± 0.09 a
	PKM	6.2 ± 0.05 a	2.2 ± 0.01 b	158 ± 2.72 c	1.08 ± 0.035 b	48 ± 0.65 b	26.5 ± 0.24 ab	129 ± 0.42 d	16.5 ± 0.01 cd	0.791 ± 0.06 a
	NPKM	6.1 ± 0.01 ab	2.2 ± 0.01 a	181 ± 1.03 a	1.18 ± 0.017 a	51 ± 1.04 a	26.9 ± 0.01 a	160 ± 3.46 a	17.0 ± 0.01 a	0.901 ± 0.06 a
	M	6.0 ± 0.08 abc	2.2 ± 0.03 b	146 ± 3.15 d	0.58 ± 0.002 e	14 ± 0.34 d	25.4 ± 0.45 d	139 ± 0.75 c	16.4 ± 0.01 d	0.748 ± 0.08 a
2011–2016	CK	5.2 ± 0.02 d	1.0 ± 0.01 d	70 ± 1.51 d	0.50 ± 0.43 e	5 ± 0.04 d	14.5 ± 0.18 d	22 ± 1.19 e	1.1 ± 0.02 f	−0.25 ± 0.04 d
	NPK	4.6 ± 0.10 e	1.9 ± 0.17 c	134 ± 1.52 c	1.03 ± 0.012 d	37 ± 0.63 b	25.7 ± 0.46 c	93 ± 2.92 d	2.1 ± 0.04 e	0.29 ± 0.07 c
	NPM	6.1 ± 0.02 b	2.6 ± 0.02 b	184 ± 2.76 ab	1.19 ± 0.048 c	55 ± 1.57 a	26.3 ± 0.34 c	150 ± 3.72 c	16.9 ± 0.02 b	1.07 ± 0.02 ab
	NKM	6.0 ± 0.004 c	2.6 ± 0.03 b	173 ± 11.9 b	0.95 ± 0.002 d	17 ± 0.26 c	27.3 ± 0.15 ab	170 ± 2.06 b	16.8 ± 0.01 c	1.01 ± 0.06 ab
	PKM	6.5 ± 0.06 a	2.7 ± 0.01 b	170 ± 5.76 b	1.44 ± 0.094 b	55 ± 3.02 a	27.1 ± 0.20 b	147 ± 2.71 c	16.7 ± 0.03 c	1.10 ± 0.05 ab
	NPKM	6.1 ± 0.02 bc	3.0 ± 0.01 a	195 ± 2.65 a	1.84 ± 0.058 a	55 ± 1.45 a	27.9 ± 0.30 a	179 ± 3.85 a	17.1 ± 0.02 a	1.23 ± 0.08 a
	M	6.0 ± 0.01 bc	2.7 ± 0.01 b	170 ± 1.45 b	0.91 ± 0.006 d	18 ± 0.43 c	25.8 ± 0.19 c	146 ± 4.86 c	16.5 ± 0.02 d	0.93 ± 0.17 b

Note: Values are means (n = 3) ± standard deviations. Means followed by different letters are significantly ($P \leq 0.05$) different from each other according to Tukey's HSD test.
Abbreviations: TN, total N; AN, available N; TP, total P; AP, available P; TK, total K; AK, available K; CSR, soil organic carbon sequestration rate; n, number of replications.

initial values. TK and AK contents under CK treatment were also lower during periods of 2001–2010 and 2011–2016. Combined application of chemical fertilizers and manure significantly increased soil total and available nutrient contents compared with chemical fertilization. Among all treatments, AN, TP, AP, TK, AK contents were highest under NPKM treatment compared with all other fertilization treatments during different fertilization periods. TN during 2001–2010 was highest under NPM treatment compared with all other treatments. Soil TN did not show significant differences between NPM and M treatment, and between NKM and NPKM treatment during 1982–2000. NKM, PKM and M during 2001–2010 and NPM, NKM, NPM and M during period of 2011–216 did not show significant differences for TN. Similarly, during all three periods of fertilization, NPM and NKM did not showsignificant differences for AN. During 1982–2000, PKM and NPKM for TP content, NKM, PKM and NPKM for TK content and NPK, NPM, PKM and M for AK content also did not show significant differences.

Among different fertilization periods, the annual C input in CK and NPK was very low, compared to NPM, NKM, PKM, NPKM and M treatment. Among all fertilization treatments, C input was highest under NPKM treatment during different fertilization treatments. Soil organic carbon (OC) content showed increasing trends over the long-term fertilization for all fertilization treatments except CK (Fig. 4). Soil

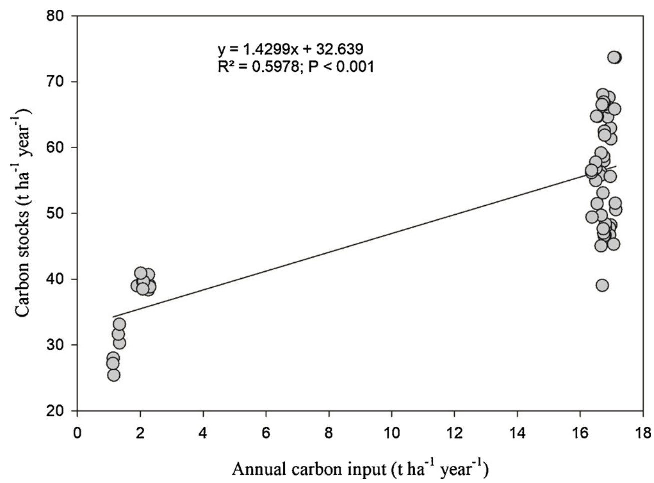

Fig. 5. Relationship between annual carbon inputs and carbon stock in long-term experiment of acidic paddy soil under double rice cropping system.

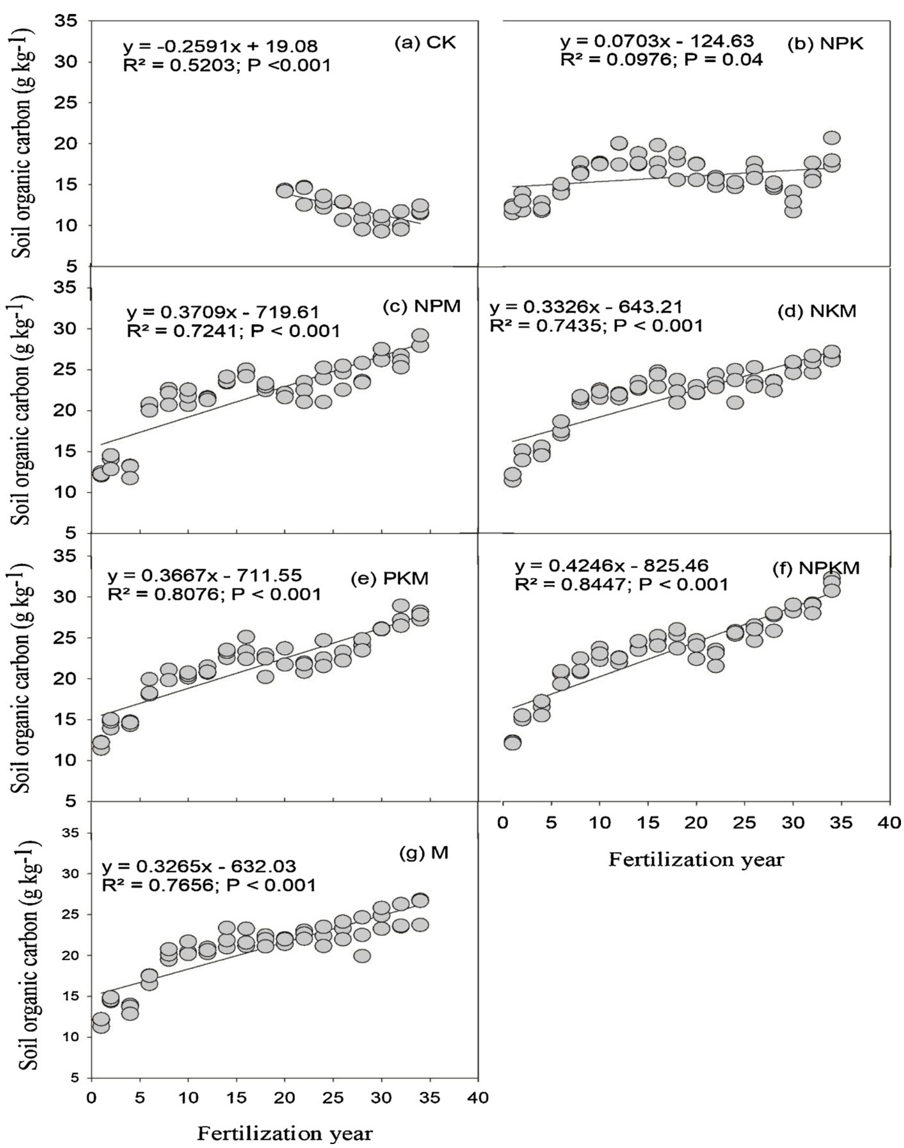

Fig. 4. Changes in soil organic carbon (OC) content under various fertilization treatments of long-term experiment in double rice cropping system.

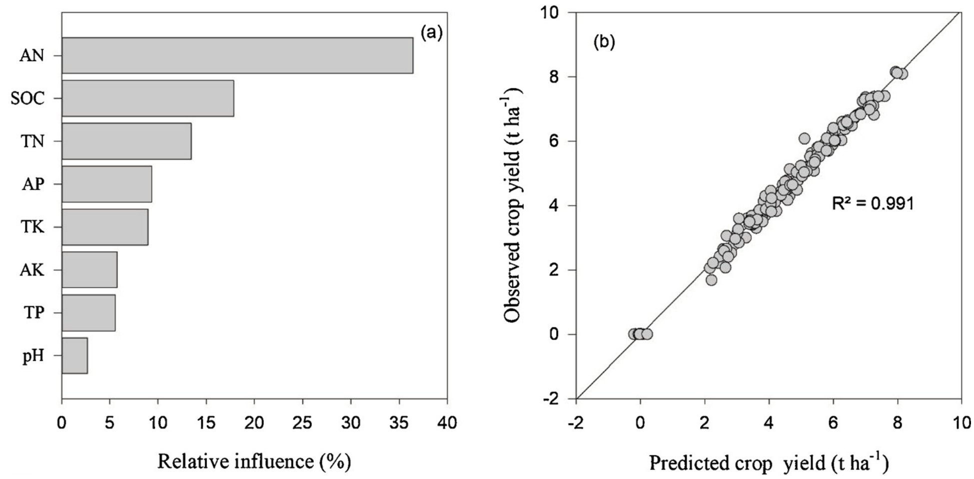

Fig. 6. The relative contribution (%) of predictor variables for the boosted regression tree model of relative yield (a). Observed and predicted relative crop yield by the boosted regression tree model using predictors shown in Fig. 5b.

OC content in CK treatment decreased over the time. During different fertilization periods, OC content was highest in NPKM and lowest in CK treatment. Annual soil carbon stock showed positive relationship with annual carbon input (Fig. 5). Combined application of manure and chemical fertilization significantly increased CSR compared with NPK and CK. The treatments NPM, NKM, PKM NPKM and M during 1982–2000 and 2001–2010 and the treatments NPM, NKM, PKM and NPKM during 2011–2016 did not show significant differences for CSR.

3.4. Mechanisms of relative yield in response to long-term fertilization

The results of BRT analysis suggested that for acidic paddy soils, AN, OC and TN contents could be the most influencing factors for relative yield of the crop among eight different variables (Fig. 6). The path analysis indicated that N, P and K inputs directly affected the AN, AP and AK,respectively (Fig. 7). Soil C input indirectly affected the relative yield by directly affecting OC content. N input showed significant direct effect on soil pH and soil pH indirectly affected the relative yield by directly affecting soil P availability. AN also had a direct effect on AP and AK and RY. While, AK had indirect effect on RY. The path analysis explained 86 % of variance of relative yield (R^2 = 0.86).

4. Discussion

In our results, during the long-term experiment, grain yield decreased in NPK treatment from 1982 to 2000 to 2001–2010 and 2011–2016. Grain yield increased in NPM, NKM, PKM, NPKM and M treatments from 1982 to 2000 to 2011–2016 period, while grain yield of CK treatment was decreased over the years (Table 2 and Fig. 3). The long-term sustainability of grain yield under manure treatments could

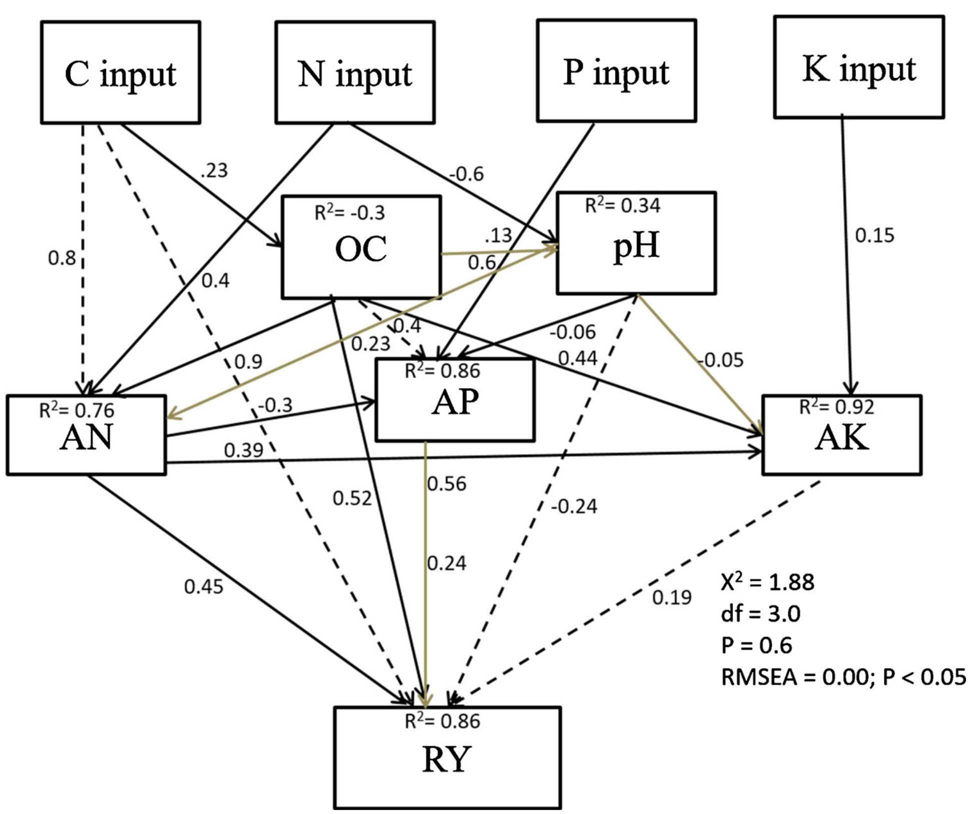

Fig. 7. Path analyses showing the direct and indirect effects of nutrient inputs on relative yield. Numbers next on the endogenous variables are the explained variance. Numbers next to the arrows are the standardized path coefficients. A solid-line path indicates that the effect is significantly positive; dashed-lines path indicate that the effect is significantly negative and grey lines indicate the non-significant effects. Notes: OC: soil organic carbon; AN: available nitrogen; AP: available phosphorus; AK: available potassium; RY: relative yield. X^2 = Chi-square; df = Degrees of freedom; P = probability level; RMSEA = Root mean square error of approximation.

be due to continuous supply of reserve nutrients by addition of manure to the soil (Cai et al., 2019). Therefore, combined application of manure and chemical fertilizers significantly increased sustainability yield index (SYI) for both early and late rice, compared with chemical fertilization alone (Table 3). These results were consistent with the finding of Choudhary et al. (2018) for wheat-soybean rotation in the Indian mid-Himalayas, who observed that long-term chemical fertilization decreased the crop yield and SYI by due to soil acidity. In another study, Xie et al. (2016) observed that replacing chemical N with green manure in paddy soil increased the SYI under double rice cropping system. The crop yield could be sustained for many years after manure application ceases, due to residual effect of manure (Demelash et al., 2014). In previous studies positive residual effects on crop yield under different long-term experiments (Cai et al., 2018; Manna et al., 2005; Tirol-Padre et al., 2007; Xu et al., 2008) were also observed. One of the main reasons for decreased crop yield under CK and NPK could be the soil acidification, because continuous application of chemical fertilizers assists to degrade the soil quality by lowering soil pH (Sarkar et al., 2018). In previous studies a high reduction of crop yield was observed under chemical fertilization in paddy soils over the time (Zhu et al., 2018). Cai et al. (2019), also found that there was no yield increase due to decreasing in soil pH after 12 years of chemical N fertilization. The yield reduction in CK and NPK could also be attributed to low nutrients uptake under CK and NPK compared with combined application of manure and chemical fertilizers (Table 2). Imbalanced fertilization might have influenced the nutrients uptake from soil, which may directly affect the crop yield (Zhang et al., 2010). In our results, K deficiency was observed in all treatments in the form of negative K balance. Our results were consistent with Zhang et al. (2010), who observed negative K balance under long-term chemical and manure fertilization in rice-wheat and rice-rice based cropping systems. In the present study, K input was lower, compared with K uptake (Table 1 and 2), which resulted in negative K balance. In fact in paddy soils AK could be fixed with 2:1 type clay mineral which may create negative K balance. Imbalanced fertilization not only affects the crop yield but also could be risk of nutrient losses to the soil, water and air environment (Das et al., 2014; Katzenberger et al., 2009; Sims et al., 2013). In our results, combined application of chemical fertilizer and manure increased N, P and K uptake compared with NPK and CK (Table 2), which were consistent with previous studies (Hui-min et al., 2009). But due to excessive fertilization of N and P under combined application of manure and fertilizers, we observed positive N balance in all treatments except CK and NPKM and positive P balance in all the treatments except CK under different fertilization periods. The negative N balance under NPKM treatment might be due to high crop yield which increased the N uptake, compared with N inputs. We applied chemical fertilizer N, P and K with manure which increased N uptake, it could be attributed to mutual nutrients interaction, which enhanced N uptake and created negative N balance (Ågren, 2008; Prasad, 2009). Nutrients interaction and their stoichiometry in soil affect the nutrient cycling in soil plant system (Delgado-baquerizo et al., 2013), which may influence their uptake. In contrast, the positive N balance in NPK, NPM, NKM, and M was due to lower uptake of N. The main reason could be imbalanced fertilization, which might decrease the N use efficiency and decreased the N uptake. Different studies reported that inappropriate fertilization decreased the nutrient use efficiency (NUE) and increased nutrient balance and nutrient loses (Wang et al., 2009). Similarly, P balance in all the treatments except CK was positive, and we observed that under most of the treatments under combined application of manure and chemical fertilizers increased P balance during different cropping periods above the threshold level of 35 kg ha^{-1} $year^{-1}$ P (Bai et al., 2013), therefore, risk of P pollution appears in this paddy field. In previous studies, (Bashkini et al. (2002) in the Republic of Korea, estimated the N budget for 1994–1997 and observed that excessive nitrogen inputs from both organic and inorganic fertilization caused surplus N accumulation in landfills and ground water. In another study, Hong et al. (2019) also found positive P balance under chemical fertilization in acidic paddy soil under double rice cropping system, but P balance in his study was not exceeding the risk of threshold level of P losses, reason could be particularly lower P inputs rate compared to our study.

Soil total and available nutrient contents and C input rates, under combined application of chemical fertilizers and manure were much higher than NPK (Table 4), which increased the organc C sequestration rates and soil OC contents over the time (Fig. 4). Tian et al. (2015) in a meta-analysis study reported that continuous manure application increased the soil OC content and sequestration rates by increasing crop yield and organic matter (OM) return from stubbles and roots. Majumder et al. (2007) observed the C input of 1.95 to 4.1 t ha^{-1}, in the current study C input ranged 1.1 to 17.3 t ha^{-1} $year^{-1}$ (Table 4), this high C inputs increased organic C sequestration rate and nutrient availability over the time under combined application of manure and chemical fertilizers. Hijbeek et al. (2017) and Cai et al. (2019) reported that C input increased the nutrient availability by improving soil physical and chemical properties such as soil aeration, porosity and soil pH.

Chemical fertilization increased soil acidification by decreasing soil pH. While, addition of manure combined with chemical fertilizers prevented soil acidification by improving soil pH (Table 4). Many studies reported the soil acidification by long-term chemical fertilization (Lin et al., 2014; Zhu et al., 2018). Plant generally release the net H^+ ions; on the other hand, when uptake of anions exceeds the cations uptake, they release net excess of OH^- or HCO_3- (Tang et al., 2011). Chemical N fertilization decreases the base cations in soil, which reduces the soil pH. Moreover, in previous studies, it was observed that chemical N application shifted the soil in to Al^{3+} buffering stage. Al released in soil solution by hydrolysis of Al-hydroxides on the surface of clay minerals at the soil pH below 5, which decrease the saturation of base cations, therefore increases the soil acidification (Stevens et al., 2009). Alkalinity of manure neutralizes the protons in acidic soil and improves the soil pH (Rukshana et al., 2013). The alkaline nature of manure is mainly due to decarboxylation of organic anions and ammonification of organic N. However, nitrification process can produce the protons to some degree, which can reduce soil pH (Xu et al., 2006). As manure significantly influenced the crop yield (Fig. 6),the main role in improving plant growth, by regulating the soil nutrient inputs, improving soil physical and biochemical properties (Fließbach et al., 2007). Additionally, long-term manure application can also increase the soil microbial biomass which provides better soil conditions for crop growth (Peacock et al., 2001). Path analysis also indicated fertilization inputs directly influenced nutrients availability. Long-term C inputs indirectly affected the AN and RY by directly affecting OC. While, N input had direct effect on soil pH. Soil pH indirectly influenced relative yield by directly affecting AP (Fig. 7). These results indicated that in acidic paddy soil, nutrient availability is regulated by soil OC and soil pH, which may limit or promote the crop growth. Moreover, the combined application of manure and chemical fertilizers could be a better fertilization management for regulating crop yield, but the rates of N and P inputs should be reduced to prevent the nutrient losses in to soil, air and water environment.

5. Conclusion

Combined application of manure and chemical fertilizers significantly increased crop yield, SYI, soil nutrients availability and organic carbon sequestration rate,compared with chemical fertilization. Soil available N, OC and total N were the main driving factors of crop yield under long-term combined application of manure and inorganic fertilization. Soil pH, which was directly affected by N input, showed direct effect on AP and indirect effect on relative crop yield. While, long-term C input indirectly influenced relative crop yield by direct affecting soil OC content.

Furthermore, apparent P balance under combined application of manure and chemical fertilizer exceeded the environmental risk threshold level, which can pose the serious threat to the aquatic environment. Therefore, duing the long-term combined application of manure and inorganic fertilizer in acidic paddy soil, the rate of P input should be reduced to avoid surplus P accumulation in soil. While, negative K balance indicates K deficiency of acidic paddy soil Therefore, combined application of inorganic and organic fertilizer is a better approach to enhance the crop yield sustainability but rate of P input under combined manure and inorganic fertilization should be reduced to save the fertilizer resources as well as to reduce the environmental P losses.

Declaration of Competing Interest

All authors confirmed that there is no conflict of interest for this manuscript.

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