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Rotation with green manure increased rice yield and soil carbon in paddies from Yangtze River valley, China

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ABSTRACT

The addition of organic matter via green manure rotations in paddy rice has previously been proposed as a smart agricultural practice to maintain soil productivity and support environmental sustainability. However, to date, only a few studies have assessed quantitatively the impact of green manure application on the interactions between agronomic management practices, soil fertility, and crop production. In this study, 800 pairs of data from 108 studies, conducted in the Yangtze River agricultural region of China were assessed, and a meta-analysis was carried out to evaluate and quantify the effect of green manure application on rice yield and soil properties. Firstly, compared to a winter fallow system, amendment with green manure significantly increased paddy rice yield and soil organic carbon (SOC) by 8.1% and 8.4%, respectively. Secondly, analysis using a Random Forest model revealed that paddy rice type, green manure application rate and duration, mineral and organic N fertilizer rate, and initial SOC and pH were identified as the main drivers for paddy rice yield and SOC change. Finally, Marginal Benefit Analysis (MBA) revealed that green manure application rates for production in early rice of double cropping rice (ER) and single rice (SR) were approximately of 20 and 26 t ha⁻¹ respectively. and further, for carbon sequestration, that the optimal rate was ~25 t ha⁻¹. It should, however, be noted that green manure application to soils with already high SOC level might

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result in the soil becoming a net C source. The study supported the scientific and quantitative indicators for achieving the best benefits of paddy rice yield and SOC increase after green manure application.

Key Words: green manure, paddy rice, random forest model, soil C sequestration, soil quality, Yangtze River

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INTRODUCTION

China accounts for about ~18.5% of global paddy rice planting acreage, and produces the approximately ~28.1% of global rice production (FAOSTAT, 2019). The neighborhood of the Yangtze River is the principal paddy rice production area in China, with over ~50.8% of national planting area and ~51.6% of total production in 2018 (SBS, 2019). However, current national rice yields are only 72% (7.4 t ha⁻¹ for single-rice) and 66% (5.9 t ha⁻¹ for double-rice per season) of the potential yields, while the Yangtze River region holds the greatest potential for yield improvement in both single- and double- rice cropping systems (Deng *et al.*, 2019). At the same time, soil disturbance and high intensity land use risks degradation (and loss of productivity) through mineralization of soil organic matter (Stockmann *et al.*, 2013). The maintenance of soil health is therefore vitally important to sustain or even increase production of rice-cropping systems (Galloway *et al.*, 2008; FAO, 2013).

Paddy-upland rotation is the most important intensive cropping system for food security in China. Although paddy rice yield has increased over recent decades due to the supply of mineral nitrogen (N) through fertilizers, N-use efficiency has decreased due to excessive application rates. This also has had an environmental cost through nitrate loss, soil acidification and increasing greenhouse gas emissions over time (Melero *et al.*, 2006; Ju *et al.*, 2009; Xie *et al.*, 2016).

Previous studies have proposed paddy rice-green manure rotation as an effective way for organic additions to maintain soil productivity and achieve environmental sustainability, reducing greenhouse gas emissions compared to conventional paddy rice production systems involving winter fallow (Yadvinder-Singh *et al.*, 2005; Cao *et al.*, 2017; Tribouillois *et al.*, 2018). When green manure is planted in winter instead of a bare fallow, it is incorporated into soil before rice is sown. If leguminous green manures were to be employed, they might not only fix atmospheric N *via* the rhizobia (Hargrove *et al.*, 1986) and activate insoluble nutrients in the soil for subsequent crops by secreting organic acids from plant roots (Lin *et al.*, 2014), but also directly reduce the requirements for mineral fertilizers and pesticides (Yadav *et al.*, 2000). After incorporation into soil, green manure

biomass accelerates the decomposition of soil substrate by optimizing the C/N ratio of substrates and thereby stimulating the activities involved microbial communities. This further activates soil nutrient bioavailability, and consequently improves rice yield and quality (Mishra *et al.*, 2001; Yang *et al.*, 2019). For example, Morris *et al.* (1986) demonstrated that a fast-growing tropical legume could accumulate about 80 kg N ha⁻¹ and that rice yield responses exceeding 2 t ha⁻¹ were possible from green manure incorporation. Likewise, Bai *et al.* (2019) showed that green manure (cover crop) incorporation increased soil organic carbon (SOC) content by 6% on average. The use of green manure provided additional above- and below-ground biomass (Blanco-Canqui *et al.*, 2011), which contains carbon (C) and other key elemental (*e.g.*, N, P, K) inputs. This, in turn, enhances the biodiversity of agroecosystems (Lal, 2004a; Yu *et al.*, 2017), and may therefore be defined as a “climate-smart” practices, supporting crop productivity and soil health (Paustian *et al.*, 2016; Ma *et al.*, 2021).

The benefits, in terms of yield and soil properties, resulting from the introduction of green manures in a paddy rice-green manure rotation system can vary with green manure biomass, underlying soil conditions, and other agronomic management practices (Yadvinder-Singh *et al.*, 1992; Yadav *et al.*, 2000; Cherr *et al.*, 2006; Thorup-Kristensen *et al.*, 2012; Yang *et al.*, 2019). Studies have reported that input of green manure increases grain yield in some cases (Abdalla *et al.*, 2019; Yang *et al.*, 2019), while excessive green manure use shows no additional benefit or might even negatively impact rice yield and soil health (Dawe *et al.*, 2003; Paustian *et al.*, 2016; Xie *et al.*, 2016; Lu *et al.*, 2017). It is therefore of key importance to define the optimum green manure application rate for sustainable production. Some peer studies have reported that organic matter and microbial biomass in fine-textured soils are more responsive to green manure amendment (Cherr *et al.* 2006), and that green manure and conservation tillage in combination, markedly increase SOC compared to their application in isolation (Duval *et al.*, 2016; Bai *et al.*, 2019). Hence, the combined effects of paddy rice cropping systems through fertilization and soil improvement should be significant in retaining soil productivity and sustainability instead of simply focusing on a single effect (Paustian *et al.*, 2016; Abdalla *et al.*, 2019; Bai *et al.*, 2019), which has not been well addressed. Therefore, the objectives of this study were (i) to evaluate quantitatively the effects of paddy rice-green manure rotation on paddy rice yield and soil properties; (ii) to determine the main drivers that influence the benefits for paddy rice-green manure rotation system; and (iii) to optimize values for main drivers of paddy rice yield and SOC in the context of climate smart agriculture.

MATERIALS AND METHODS

Database

Data were collected from published scientific papers both in Chinese and English *via* the China National Knowledge Infrastructure, and ISI-Web of Knowledge databases using the search terms “green manure”, “paddy rice”, “soil organic carbon”, and “yield”. The available data were required to meet the following criteria: (i) all the data were measured from field experiments; (ii) paired data

were available, considering paddy rice yield and soil properties under a leguminous green manure treatment and a non-green manure control; (iii) rice residues were always incorporated; (iv) agronomic management practices such as green manure application rate, experiment duration, and fertilizer application rate were provided. A dataset based on 108 studies with 800 paired measurements was compiled from 80 sites covering different crop types and management practices in the Yangtze River agricultural region of China. The reported measurements were located across latitudes of 23.9° to 32.1° and longitudes of 111.0° to 121.1°.

This study only focused on the changes in paddy rice yield and soil properties in the first paddy rice season after green manure incorporation, since most nutrients are released from green manure biomass in the following crop (Zhou *et al.*, 2019). Information in the dataset generally included geographic location (latitude and longitude); climate data (annual average temperature and annual average precipitation); green manure application rate (GAR) and green manure planting duration (GPD); rice types (early rice or double cropping rice (ER) and single rice (SR)); mineral N fertilizer rate (MNR) and organic N fertilizer rate (ONR); management practices--including straw return, irrigation, tillage; initial (before green manure planting) and final (after paddy rice harvest) soil properties--including bulk density, clay content, pH, SOC, nitrogen content, phosphorus content, and potassium content; and paddy rice grain yield. More information is shown in Table SI (see Supplementary Material for Table SI).

Meta-analysis

Meta-analysis was carried out to determine the changes in paddy rice yield and soil fertility (SOC, total nitrogen (TN), available phosphorus (AP), available potassium (AK), pH, and bulk density (BD)) following green manure amendment. The natural log-transformed response ratio (lnRR) was employed to reflect the effects of green manure on crop yield and soil properties, and was calculated using Equation (1) (Hedges *et al.*, 1999):

$$\ln RR = \ln \left(\frac{\bar{X}_t}{\bar{X}_c} \right) = \ln(\bar{X}_t) - \ln(\bar{X}_c) \quad (1)$$

where \bar{X}_t and \bar{X}_c are means of the treatment and control groups for variable X , respectively.

The weighting factor, weighted response ratio (RR₊₊), the standard error of RR₊₊, and 95% confidence interval (CI) of RR₊₊ were calculated following Curtis and Wang (1998) and Morgan *et al.* (2003). If the 95% CI of RR₊₊ for a given variable included zero, effects were not significant, while if the 95% CI was above or below zero, a significant effect was concluded. The relative rate of change was subsequently calculated as $(e^{\text{RR}^{++}} - 1) \times 100\%$ (Liu *et al.*, 2014).

Random Forest model

Measurement data were randomly split into two groups: 70% for Random Forest model

establishment, and 30% for model validation. A Random Forest model was used to fit relationships between crop yield, soil C sequestration and environmental variables. The Random Forest model is an ensemble of regression trees, which constructs a multitude of regression trees and then aggregates them to provide a final prediction. Details on the Random Forest model can be found in Chagas *et al.* (2016). Random Forest models were established for paddy rice yield and soil C sequestration. Soil C sequestration is the difference in the final and initial measurements of SOC. All candidate covariates were used in modeling and important covariates were selected with the P value < 0.05 . Finally, the best model was chosen based on R-squared values.

The importance of each variable was also determined with the Random Forest model. A variance analysis was carried out using the “varImpPlot” command in “randomForest” package to determine the variance explained by each of the significant factors relative to the others.

The performance accuracy of the Random Forest models was assessed based on the RMSE and R-squared values fitted on the 30% of the observations retained for evaluation (including 179 and 48 observations for yield and soil C sequestration models respectively). First, the RMSE (a low RMSE value often indicating a strong predictive power) were calculated using Equation (2) to assess the total difference between measured and predicted values (Smith *et al.*, 1997). Second, the coefficient of determination (R^2 , value closer to 1 indicating better model performance) was used to show the accuracy of the model simulation *via* linear regression fitting, while the t -statistic was used to test for a significant difference between the predicted and measured values by t -test.

$$RMSE = \sqrt{\{\sum_{i=1}^n (P_i - M_i)^2\}/n} \quad (2)$$

Where P_i and M_i represent the predicted and measured values, respectively; n is the number of measurements.

Marginal benefit analysis

Marginal Benefit Analysis (MBA) was carried out to identify which values of the model factors GAR, GPD, MNR, ONR, initial soil pH and SOC resulted in the optimum paddy rice yield and soil C sequestration rate. Firstly, this optimum GAR was determined in the above models, with all other covariates set to the average of those in the dataset. For example, the average values of GPD, MNR, ONR, pH, and SOC were set to be 1 year, 108 kg N ha⁻¹ for ER and 133 kg N ha⁻¹ for SR, 16 kg N ha⁻¹, 6.02, 16.4 g kg⁻¹ for paddy rice yield model, and 1 year, 112 kg N ha⁻¹ for ER and 150 kg N ha⁻¹ for SR, 21 kg N ha⁻¹, 5.87, 16.6 g kg⁻¹ for soil C sequestration model. Subsequently, the optimum for other variables with the green manure application rate was set to the optimum value from the first step, and other variables set to the average value.

Quantification of yield and soil C sequestration for paddy rice cultivation in Yangtze River region

To evaluate the potential benefits of paddy rice-green manure rotations using the Random Forest models in 2.3.1, all rice planting areas were theoretically set to adopt paddy rice-green manure rotation and the green manure application rate set to the optimum from the MBA. Using a $0.5^\circ \times 0.5^\circ$ grid of cultivated areas in Yangtze River region, a paddy rice field distribution map was obtained from EarthStat (Monfreda *et al.*, 2008). The data of for paddy rice planting area and N fertilizer application rates were obtained from the China Rural Statistical Yearbook (SBS, 2020). pH and SOC were obtained from the Harmonized World Soil Database (FAO/IIASA/ISRIC/ISS-CAS/JRC, 2012). It was assumed that there was no significant change in the spatial distribution of crop area and fertilizer application rates under green manure incorporation. The sum of crop area and fertilizer application rates in all the grid cells of each province were then matched to the statistical data from this province in 2019.

All the statistical analyses in this study were conducted in RStudio using the R packages: “randomForest” “ggplot2”, “gridExtra”, “Matrix”. The all codes were shown in R version 4.0.2 (R Core Team, 2020).

RESULTS

Response of rice yield and soil properties to paddy rice-green manure rotation

Generally, the use of green manure significantly increased paddy rice yield by 8.1% ($P < 0.05$) with a 95% CI of 7.6%--8.5% over all 185 comparisons (Fig. 1). Similarly, under green manure amendment, the SOC content was increased by 8.4% (95% CI of 7.7%--9.2%, $P < 0.05$), likely owing to the input of plant biomass. Soil nutrient concentrations also increased; soil TN by 22.4% (95% CI of 20.1%--24.7%, $P < 0.05$), AK by 18.0% (3.5%), and AP by 5.9% (1.9%). Independently of initial soil structure properties, green manure amendment decreased soil BD by 2.4%. Soil acidity, however, appeared to decrease slightly (by 1.1%).

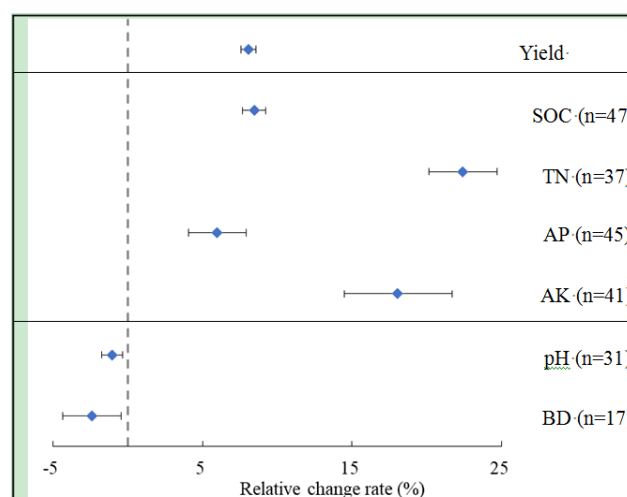


Fig. 1 Response of agricultural system variables to green manure amendment (n = measurement number).

Random Forest model for paddy rice yield and soil C sequestration

As shown in Fig. 2, the correlation of predicted *versus* observed values had R^2 values of 0.80 for rice yield and 0.63 for soil C sequestration, in which the RMSE was 0.81 t ha^{-1} and 2.49 g C kg^{-1} , respectively.

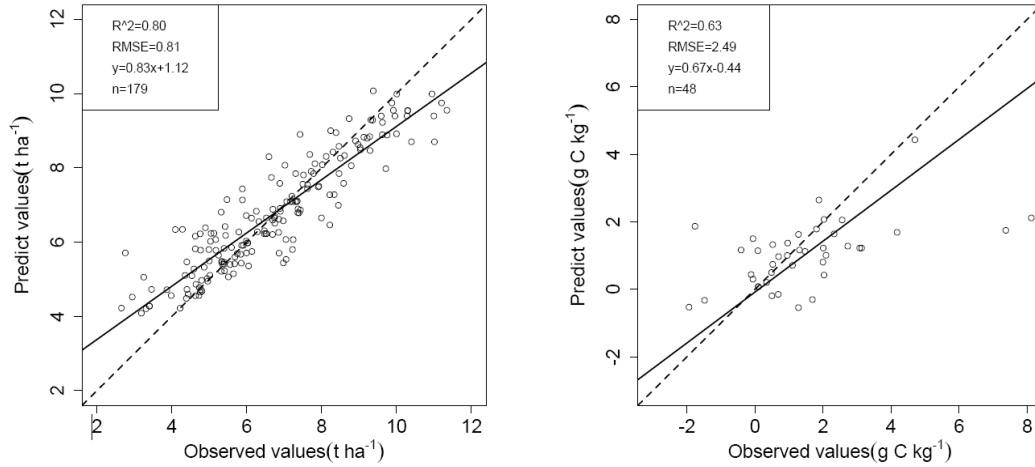


Fig. 2 Comparison of predicted and observed values for Random Forest methods for paddy rice yield (a) and soil C sequestration (b). Solid lines represent the regression lines, and dotted lines represent the 1:1 ($y = x$) line.

Significant variables in both of the emergent best models included crop type, green manure application practices (GAR and GPD), initial soil characteristics (SOC and pH), and fertilization management (MNR and ONR). The mean of squared residuals for the models were 0.63 t ha^{-1} for paddy rice yield and 4.7 g C kg^{-1} for soil C sequestration, explaining 81.7% and 82.5%, respectively, of the variance.

For the yield model, crop type and MNR were the most important factors accounting for 27% and 20% of the variation, respectively (Fig. 3a). The variables pH and SOC explained 18%, and 15% of the variation, respectively. For the soil C sequestration model, initial SOC and pH accounted for 51% and 28% of the variation, respectively, whereas rice type, GPD, and MNR explained 6%, 5%, and 4% (Fig. 3b).

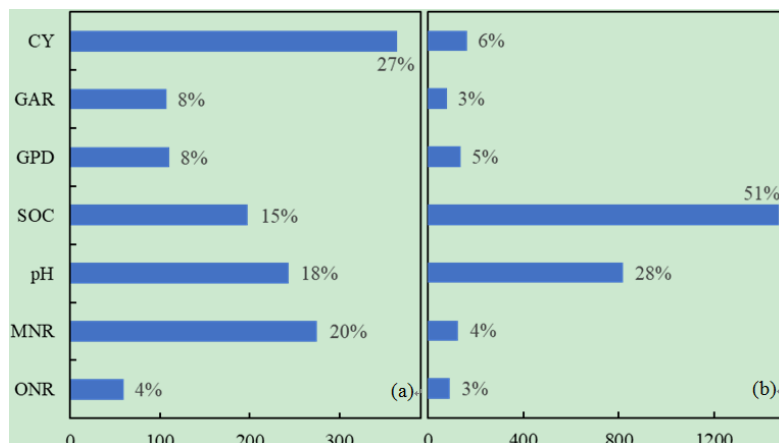


Fig. 3 Relative importance of variables in Random Forest models for paddy rice yield (a) and soil C

sequestration (b).

Optimum driving factors via Marginal effect analysis

Data plotted in Fig. 4 show that the optimum green manure application rates for double rice and single rice production systems were ~ 20 and 26 t ha^{-1} (fresh weight), respectively. Across different years, rice yield changed slightly but achieved $7.5\text{--}8.0$ and $5.5\text{--}6.5 \text{ t ha}^{-1}$ for SR and ER, respectively. No obvious relationship between paddy rice yield and organic fertilizer rate was observed due to the limitation of available data. A maximum rice yield occurred at the soil pH between 6.5 and 7.5 .

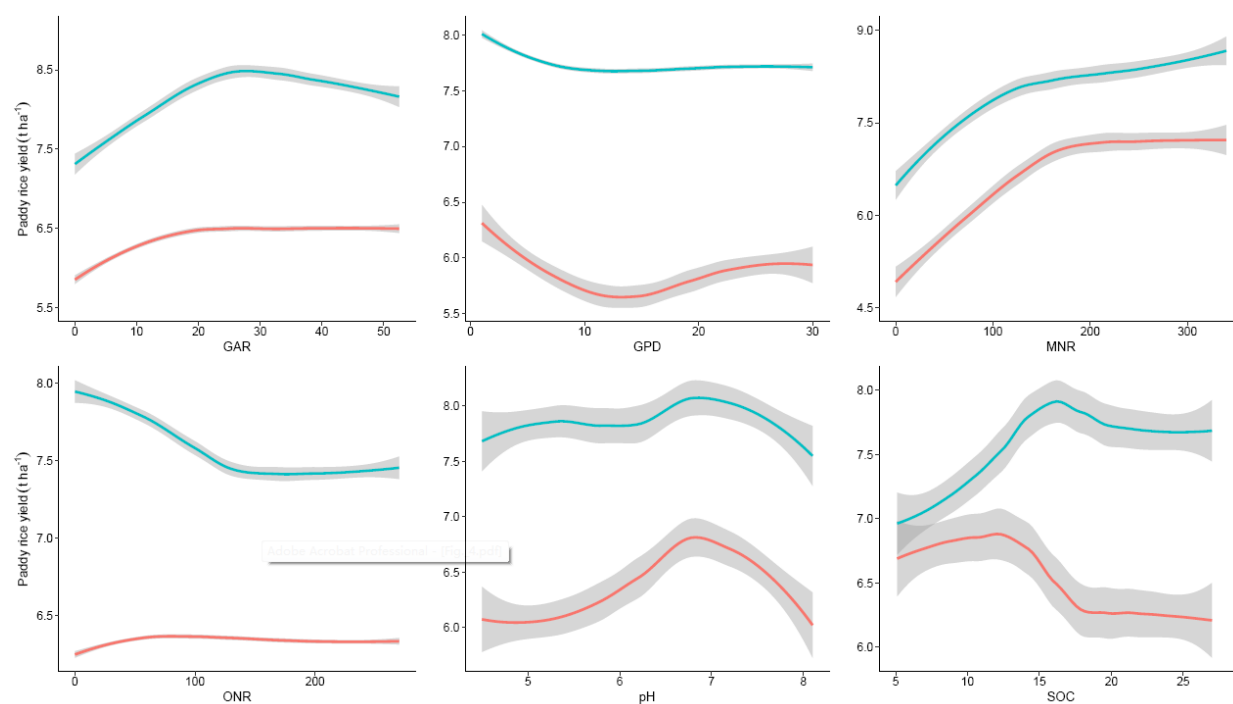


Fig. 4 Marginal benefit analysis for the paddy rice yield model. Red and blue lines represent the regression curves for ER and SR, respectively; and grey lines represent the $\pm 95\%$ CI.

The maximum soil C sequestration of double- and single- cropping rice was 0.5 g C kg^{-1} and 1.2 g C kg^{-1} , respectively, both occurring at green manure applications rate of $\sim 25 \text{ t ha}^{-1}$ (Fig. 5). In addition, soil C sequestration achieved a maximum in the first year after green manure addition, and subsequently decreased to equilibrium. There was a significant negative correlation between soil C sequestration and initial SOC content. This indicated that SOC turnover is equal to soil C sequestration at higher values of initial SOC, and indeed that the soil might be a C source once a threshold of SOC has been exceeded. Similarly, soil C sequestration reached an optimal level for pH between 6.5 and 7.5 .

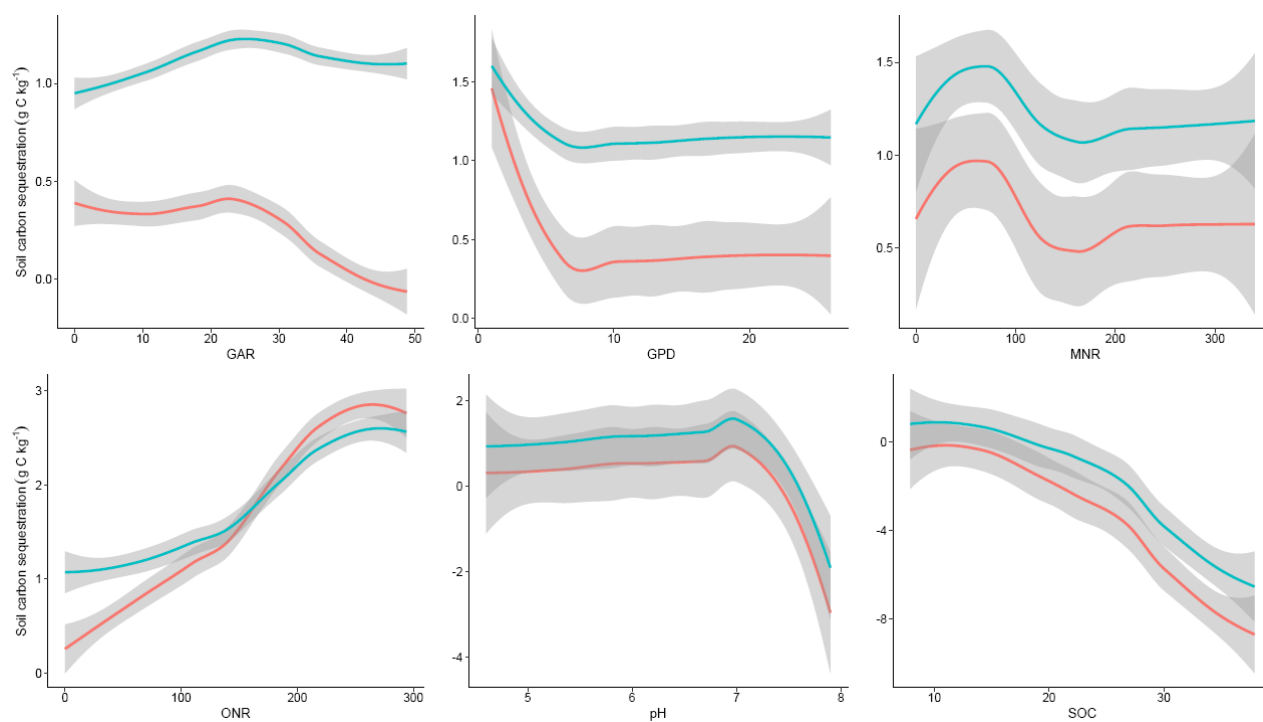


Fig. 5 Marginal benefit analysis curve for the soil C sequestration model. Red and blue lines represent the regression curves for ER and SR, respectively; and grey lines represent the $\pm 95\%$ CI.

Paddy rice yield and soil C sequestration potential for Yangtze River region

Using the optimum green manure application rate and Random Forest models, paddy rice yield and soil C sequestration potential was estimated in the year 2019 for Yangtze River region. The production potential showed notable spatial variability (Fig. S1, see Supplementary Material for Fig. S1), with a potential of 3.9 Tg for yield and 5.3 Tg for soil C sequestration (Table I). With respect to rice types, SR showed a high yield increase of 2.1 Tg, as well as a great C sequestration potential with a value of 4.0 Tg C.

TABLE I

Paddy rice yield and soil C sequestration potential under green manure application in the Yangtze River region

Cropping system	Planting area	Average application rate	N	Optimum green manure application rate	Yield increase	Soil C sequestration
	$\times 10^6$ ha	kg N ha ⁻¹		$\times 10^3$ kg ha ⁻¹ (fresh)	Tg	Tg C
ER	5.6	173	20		1.8	1.3
			25		/	/
SR	4.2	262	26		2.1	4.0
			25		/	/

DISCUSSION

Drivers of paddy rice-green manure rotation benefits

Large spatial heterogeneity was found in the effect of paddy rice-green manure rotation on rice yield and SOC in the present study. On average, paddy rice-green manure rotation significantly increased paddy rice yield by 8.1% with a confidence interval of ~4% for the Yangtze River agricultural region based on our meta-analysis. This increase could result from the positive effect of green manure amendment on soil fertility, pore structure and pH value. Comparable studies from Abdalla *et al.* (2019) reported that the dry-land crop yield might be increased by up to 13% through deployment of cover crops at the national scale in China, and Ma *et al.* (2021) stated that maize yield was significantly increased under green manure application by 11% in northern China. This paper serves to support the findings, which has direct agronomic relevance for the Yangtze River agricultural region of China.

Under green manure application, the extent of paddy rice yield increase and soil C sequestration was closely related to soil properties, fertilization management and green manure application rate and duration (Yadvinder-Singh *et al.*, 1992; Yadvinder-Singh *et al.*, 2005; Bai *et al.*, 2019). According to our model, the key drivers which had significant effect on yield, in order of their relative contributions, were crop type, MNR, pH, SOC, GAR, GPD, and ONR. It had been previously established that the yield of double- and single- cropped rice systems were strongly related to sowing date and growth period, which were originally determined by species genes (Liu *et al.*, 2021). Therefore, the yield potential varied highly with crop type. As indicated by Dong and Lin (2020) and Zhao *et al.* (2018), N application markedly benefited the paddy rice growth processes (*e.g.*, through protein, chlorophyll, and enzyme synthesis, as well as cell division), and significantly contributed to SOC sequestration. Therefore, the grain yield of paddy rice was limited by MNR up to a certain threshold, beyond which further N application did not increase yield. Indeed, with further N applications, the rate of SOC change and the yield even appear to decrease. It has previously been observed that excessive mineral N inputs can constrain SOC sequestration in soils through the stimulation of decomposition of cellulose-dominant crop residues (Fogg *et al.*, 1988), reduce C retention efficiency by favoring bacteria over fungi (Six *et al.*, 2006), and even limit root growth by stimulating soil acidification (Guo *et al.*, 2010). Additionally, Anjana and Iqbal (2007) observed that if the plant is supplied excess N, protein synthesis and carbohydrate consumption may increase while yield decreases. Ecological benefits were therefore only observed for judicious application rates of chemical N fertilizer. Most prior research has demonstrated the most obvious benefits at the first year following green manure application whereas Yang *et al.* (2019) found negative cumulative effects were observed for soil organic matter, total N, and available N after 5 years of rotation. On the other hand, Zhang *et al.* (2017) showed that long-term paddy rice-green manure rotation enriched the beneficial bacteria in the rice rhizosphere and enhanced the nutrient uptake and grain yield in a 31-year, long-term experiment. This discrepancy is difficult to reconcile based on the current study, but is perhaps was due to the substantial inter-annual change in climate and soil texture across the farming regions.

The initial properties of soil pH and organic C content also played an important role in terms of both yield and soil C storage (Yadvinder-Singh *et al.*, 2005). This study showed that an optimum yield occurred at soil pH between 6.5 and 7.5, consistent with the findings from Buckman and Brady (1960), which reported that most essential nutrients for crop growth were most active in this pH range. For example, the elements of P, copper (Cu), boron (B) availability were highest at pH of 6.0--7.0. These elements may promote chlorophyll synthesis and the development of crop reproductive organs, thereby improving photosynthesis, and root development, as well as increase production (Pichu *et al.*, 2016). Further, there was a significant negative correlation between soil C sequestration and initial SOC content (Fig. 5).

Sustainability of paddy rice-green manure rotations

The reasons for the beneficial effect of paddy rice-green manure on yield were likely that green manure amendment improved SOC, TN, AP and AK content, reduced soil pH and BD (Chen *et al.*, 2012; Bi *et al.*, 2015; Li *et al.*, 2020), and provided macro- and micro- nutrients to support rice growth (Yadvinder-Singh *et al.*, 1992; Sainju *et al.*, 2003; Piotrowska and Wilczewski, 2012). Previous studies have documented a positive correlation between crop yield and SOC (Lal, 2004b; Qiu *et al.*, 2009). In particular, Jackson *et al.* (2017) showed that plant (green manure) C inputs via shoots, roots, and the associated mycorrhizal fungi were important drivers of SOC stock and turnover, while Huang *et al.* (2021) determined that these plant C inputs contributed 44%, 28% and 28% to new SOC formation respectively. In this study, SOC enhancement through green manure applications for the Yangtze River region might achieve 8.4%, higher than the global estimate of 6% from Bai *et al.* (2019), but slightly lower than that of 10% in Mediterranean cropping systems (Aguilera *et al.*, 2013). Variation in benefits was dependent on many factors including local climate, fertigation, irrigation, tillage and soil type (Yadvinder-Singh *et al.*, 2005; Bai *et al.*, 2019; Huang *et al.*, 2021). In addition, it was further calculated that a 1% increase in soil C in paddy rice cropland soils might increase rice yield by 1% in the Yangtze River region. This phenomenon was comparable to the result of Xu *et al.* (2015), which highlighted that a 0.1% increase in soil C in paddy rice fields of southern China was correlated to 0.8 t ha⁻¹ of grain production capacity, but that the stability of grain production could be increased by up to 10%, when soil organic matter increased by 0.1% at the national level. As shown in Section 3.4, a potential 3.9 Tg for paddy rice yield might be realized in the best case scenario. This potential was equivalent to the provincial paddy rice yield of Fujian in the year 2020. Scientific guidance on green manure application in paddy rice systems is therefore important for stabilizing or increasing yield if the rice planting area declines further. Many field experiments (Chang *et al.*, 2015; Yang *et al.*, 2019) have indicated that green manure application could sustain or improve rice productivity through a 10%--30% reduction in N application. Otherwise, Chen *et al.* (2022) observed that protein content (and consequently grain "quality") might decline directly due to the increase of

grain N absorption if N rates increased.

Incorporation of large amounts of fresh green manure might enrich soil carbon and substrates required for microbial activity, and also optimize the C/N ratio of substrates, accelerating decomposition and stimulating the activities of involved microbial communities (Dong *et al.*, 2014). This would in turn activate soil nutrient bioavailability, and improve crop quantity and quality (Mishra *et al.*, 2001; Yang *et al.*, 2019). Green manure rotation in paddy rice has been seen to influence the microbial community in the rice rhizosphere; in particular, of some beneficial bacteria, *Acinetobacter* and *Pseudomonas* (Zhang *et al.*, 2017). Green manure incorporation might also stimulate crop residue decomposition by retaining a relatively low C/N ratio, enhancing Gram-negative bacterial abundance and hydrolase activities (Zhou *et al.*, 2021). With the exception of bioavailable C pools such as lower cellulose, hemicellulose, and acid soluble lignin, and inorganic N, green manure biomass contains much P, K, sulfur, as well as various trace elements such as iron, zinc, manganese (Johnson *et al.*, 2007; Yu *et al.*, 2017; Veronika *et al.*, 2021), which enrich the soil nutrient pool and enhance soil biological stability. Meanwhile, green manure plants also have the ability to fix N and activate soil nutrients. For instance, leguminous green manure (Chinese milk vetch) was shown to biologically fix N at a rate of about 33 kg ha⁻¹ per season (Yuan *et al.*, 2011), and stimulate the mechanism of phosphorus mobilization by secreting organic acids from plant roots during its growing season (Lin *et al.*, 2014). Further, it might be possible to improve the genetics of green manure species in order to provide deeper rooting crops, which have higher N use efficiency due to better nitrate scavenging abilities and lower N leaching potential (Yadav *et al.*, 2000; Abdalla *et al.*, 2019).

Paddy rice-green manure rotation has potential to be employed as a so-called climate smart practice. The SOC changes were mainly controlled by the quantity and quality of C inputs and their retention in the soil, rather than by C outputs (Eclesia *et al.*, 2016). In the present study, it was revealed that the optimum green manure application rate to optimize yield was ~23 t ha⁻¹, and to sequester soil C was ~25 t ha⁻¹. Similarly, Wang *et al.* (2021) showed that green manure additions of 15--23 t ha⁻¹ had beneficial effects on the stability of paddy rice yield as well as the fertilizer efficiency for double rice cropping system in East China. However, both yield and soil C sequestration response curves tended to flatten for higher green manure application rates, and above a certain point yield and soil C sequestration even decreased with a further green manure amendment (Figs. 4 and 5). For example, Liu *et al.* (2007) and Xie *et al.* (2016) both found that yield declines for green manure application rates over 30 t ha⁻¹. On the contrary, the soil organic matter gradually decreased, and it might be expected that soil productivity would eventually decline when organic material inputs were insufficient. Hence, it is important to apply green manure judiciously. The use of green manures might cause the soil to become a C source when initial SOC stock was high (Soong *et al.*, 2020). Huang *et al.* (2021) previously reported that plant C inputs led to net SOC losses in soils with higher organic matter or high fertility. And Phillips *et al.* (2009) and Tanvir *et al.* (2018) noted the vulnerability of soil organic matter to increased decomposition with increased plant inputs that alleviated microbial C limitation-indicating that deep soil C might be vulnerable to decomposition if

elevated CO₂ and N enrichment were to change root exudation by plants. Therefore, the benefit in terms of paddy rice yield and soil C storage are likely to be greater for weak acid and neutral soils with low organic C content. Paddy rice-green manure rotation also significantly decreased the need for N fertilizer, which increased N use efficiency. Generally, excess green manure biomass could encourage methanogenesis, resulting in increased methane emission immediately after application to the field (Cai *et al.*, 1997; Zhang *et al.*, 2013 Qian *et al.*, 2021). Taking crop rotation as a climate smart practice should consider both positive and negative impacts on climate change, therefore delivering the triple win of enhancing SOC sequestration and reducing greenhouse gas emissions while ensuring crop productivity.

Limitations of the study

Although the R² of the Random Forest models were high in this study, some limitations existed in the analysis and modeling work. It is important to consider the amount of data used for analysis and modeling. As shown in Fig. 1, 185 measurements of paddy rice yield were used for meta-analysis, but fewer than 50 measurements for soil properties were obtained in the study. For the development of the models 692 measurements data of mineral N fertilizer were available compared to 127 measurements data of organic N fertilizer with an application rate ranging from 20--300 kg N ha⁻¹. The model performance of paddy rice yield under different organic N fertilizer was poor for different paddy rice types (Fig. 4).

In addition to those from above- and below-ground green manure biomass, nutrients (*e.g.*, C, N, trace elements) from paddy rice and organic fertilizer might also affect the benefits of paddy rice-green manure rotation (Bai *et al.*, 2019; Yang *et al.*, 2019). Yevdokimov *et al.* (2013) revealed that the "new" C proved to be a preferable substrate for microbial growth than the "old" C. Therefore, C sources from crop residue or organic fertilizer could alter the soil conditions such as the C/N ratio of substrates, and in turn influence the microbial population and activity in soil and subsequent nutrient transformations. For example, Bai *et al.* (2019) and Yang *et al.* (2019) found that green manure only improved SOC when straw residues were returned, otherwise it had no effect and might even reduce the SOC content if straw was removed. Co-incorporation of straw residue and green manure was documented as an efficient collaboration technology to benefit crop production and soil property (Bai *et al.*, 2019). But previous studies have mainly investigated the effects of green manure on rice production with or without crop residue returns, whereas few continuous studies have revealed the effect of C inputs from crop residue and other organic matters. Otherwise, such information was mostly absent from existing reports or published literature.

Finally, while climate information (temperature and precipitation), and soil types, and management practices (*e.g.*, tillage, irrigation) might affect rotation benefits, these factors were not taken into account in this study. Firstly, air temperature and precipitation directly determine the soil temperature and moisture, thus affecting soil microbial activities. Waldrop and Firestone (2004)

documented that microorganism altering utilization patterns of young and old soil C varied not only with N additions but also with changes in temperature. Secondly, Ladd *et al.* (1996) pointed out that clay minerals might stabilize SOC against microbial attack through absorption of organic molecules. SOC mineralization rate probably diminishes as clay concentrations increase (Sainju *et al.*, 2002). Likewise, nutrient accumulation was usually the greatest on loamy soils due to their relatively high inherent fertility, nutrient and water retention capacity, and microbial biomass. Finally, some studies have found that conservation tillage reduced soil disturbance and soil organic matter decomposition rate, and promoted fungal and earthworm biomass, thereby improving SOC stabilization (Briones and Schmidt, 2017; Bai *et al.*, 2019; Man *et al.*, 2021). More data on such effects would improve the robustness and accuracy of our findings.

CONCLUSIONS

(1) Green manure application significantly increased paddy rice yield by 8.1% and SOC content by 8.4%. The Random Forest models were developed to evaluate paddy rice yield and soil C sequestration under green manure amendment in the Yangtze River Valley of China, explaining 81.7% and 82.5% of the variance, respectively.

(2) In the rice yield model, yield was determined by many factors in the rice-green manure rotation system, among which rice type, MNR, initial soil pH and SOC were the four main factors affecting rice yield, accounting for 27%, 20%, 18%, and 15% of the variation, respectively, while green manure amendment rate only accounted for 8% of the variation.

(3) For the soil C sequestration model, initial SOC content and pH were two important factors affecting SOC accumulation in the rice-green manure system, which accounted for 51% and 28% of the variation. Green manure planting duration was more important than green manure application rate for soil C sequestration.

(4) The optimal green manure application rates appear to be in a range of 20--26 t ha⁻¹ for optimal rice yield and soil C sequestration. And the highest potential of 3.9 Tg for paddy rice yield and 5.3 Tg for soil C sequestration might be achieved *via* optimum green manure application rate for paddy fields in the Yangtze River region.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version.

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Supplementary Information

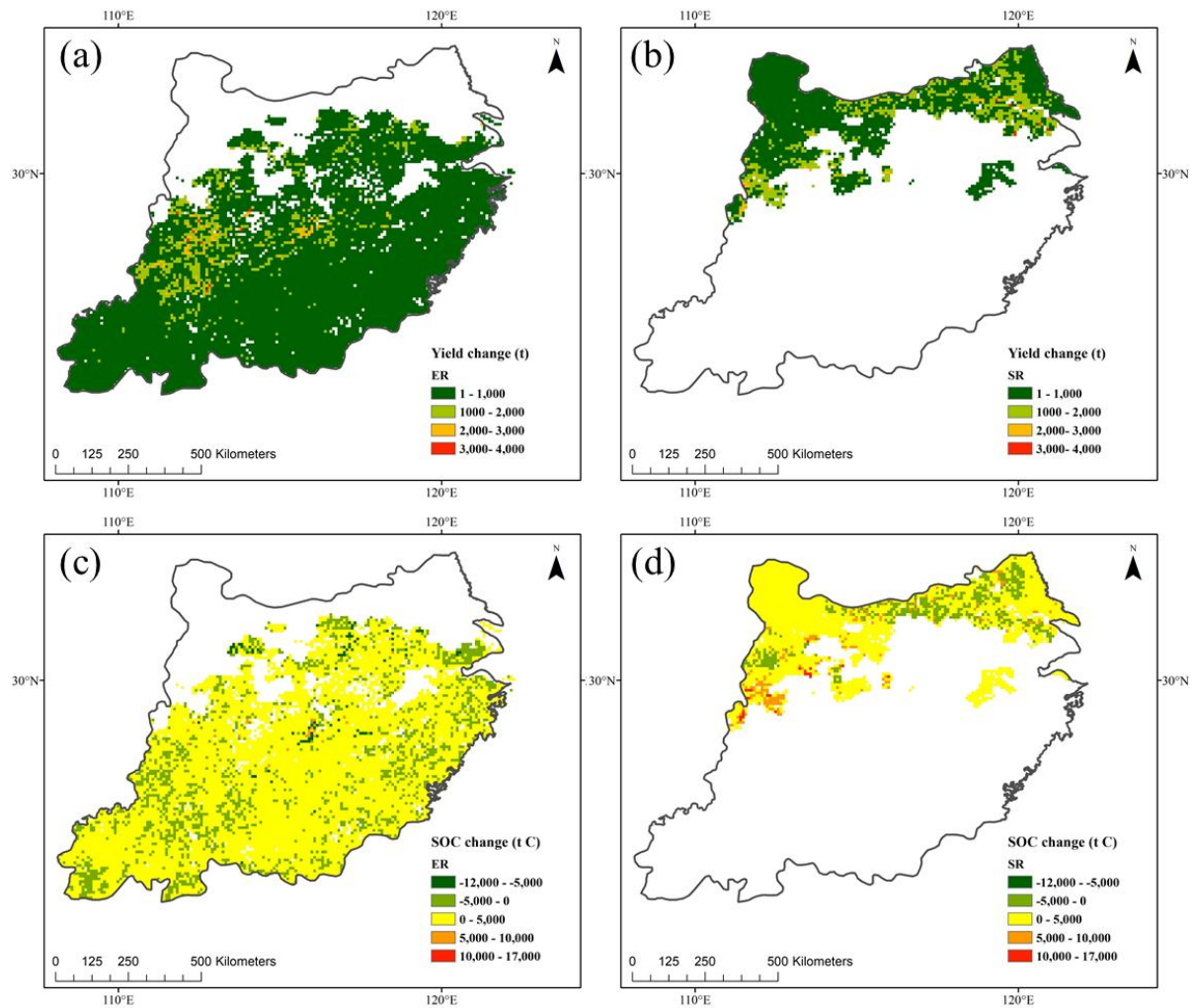


Figure S1 Geographical distribution of yield change and SOC change in PR cultivation of Yangtze River agricultural region in China

Models performance:

(a) PR yield Model

```
Call:
  randomForest(formula = yield ~ crop + GM + Duration + MinN +
    OrgN + soilpH + soilsoc, data = yield, mtry = 3, ntree = 1000,
    na.action = na.omit)
      Type of random forest: regression
      Number of trees: 1000
No. of variables tried at each split: 3

      Mean of squared residuals: 0.6348566
      % Var explained: 81.66
```

(b) SRSC Model

```
Call:
  randomForest(formula = soc ~ crop + GM + Duration + MinN + OrgN +
    soilpH + soilsoc, data = soc, mtry = 3, ntree = 1000, na.act
    ion = na.omit)
      Type of random forest: regression
      Number of trees: 1000
No. of variables tried at each split: 3

      Mean of squared residuals: 4.72531
      % Var explained: 82.46
```

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