Substitution of fertilizer-N by green manure improves the sustainability of yield in double-rice cropping system in south China

Zhijian Xie^{a,b,c}, Shuxin Tu^{a,c*}, Farooq Shah^d, Changxu Xu^{b*}, Jingrui Chen^b, Dan Han^a, Guangrong Liu^b, Hailan Li^a, Imtiaz Muhammad^a, Weidong Cao^e

^aCollege of Resources and Environment, Microelement Research Center, Huazhong Agricultural University, Wuhan 430070, China

^bInstitute of Soil & Fertilizer and Resources & Environment, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, China

^cHubei Collaborative Innovation Center for Grain Industry, Jingzhou 434023, China

^dDepartment of Agriculture, Abdul Wali Khan University Mardan, 23200, Khyber Pakhtunkhwa, Pakistan

^eInstitute of Agricultural Resources and Regional Planning, CAAS, Beijing 100081, China

* Corresponding authors at:

E-mail addresses: stu@mail.hzau.edu.cn (S. Tu) and changxux@sina.com (C. Xu)

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Abstract

Rice-rice rotation is the most important intensive cropping system for food security in China. So far, few studies have examined sustainability of double-rice cropping system using partial substitution of fertilizer N (FN) by green manure (GM). The effects of 100% FN (N_{100}) and different substitution rates of FN by GM (80%, 60%, 40% and 20% FN plus 20%, 40%, 60% and 80% N through GM, and represented respectively by $N_{80}M_{20}$, $N_{60}M_{40}$, $N_{40}M_{60}$ and $N_{20}M_{80}$) on the rice productivity and N-supplying capacity of paddy soil were evaluated in double-rice system from 2008 to 2013. Soil organic matter and total N content in the 0-15 cm layer and rice grain yield of early and late rice annually increased in $N_{80}M_{20}$ and $N_{60}M_{40}$ plots, but decreased in N_{100} , $N_{40}M_{60}$ and $N_{20}M_{80}$ plots. Compared with N_{100} plots, the NH_4^+ -N content and agronomic efficiency of applied N significantly increased in $N_{80}M_{20}$ and $N_{60}M_{40}$ plots. The grain yield and sustainable yield index of rice crops were improved in $N_{80}M_{20}$ and $N_{60}M_{40}$ plots, while declined in $N_{40}M_{60}$ and $N_{20}M_{80}$. Soil NO_3^- -N content decreased significantly under partial substitutions of GM for FN. It can be concluded that the appropriate substitution of GM for FN (e.g. 20%-40%) is beneficial for improving the productivity and sustainability of paddy field under double-rice cropping system.

Keywords: Double-rice cropping system; Green manure; N fertilizer; N-supplying capacity; sustainability indices

1. Introduction

China is one of the largest rice producers in the world, which occupied 18.7% of global rice growing area and contributed 8.7% of production in 2012 (FAOSTAT, 2013). As its population grows, China will need to produce ~20% more rice by 2030 in order to meet the domestic need, if rice consumption per capita stays at the current level (Peng et al., 2009). Although rice yield has been significantly increased during the past decades due to injudicious use of fertilizer N (FN), N-use efficiency has decreased which also led to greater soil NO₃⁻-N content over time. This over-fertilization of FN has not only resulted in wastage of a valuable chemical input but has also led to deteriorated soil, water and atmospheric quality (Melero et al., 2006; Liu et al., 2013a). The degraded soil quality due to increased soil acidification and structural damage, and decreased water table level, has thus already started negative effects on rice yield (Guo et al., 2010; Liu et al., 2013b).

Legumes as GMs can fix atmospheric N with rhizobia (Hargrove, 1986). Studies have shown that GM can effectively reduce the species, density and population of weeds in early season rice fields (Krishnan et al., 1998; Norsworthy et al., 2005). Likewise, GM improves soil physical and biological properties (e.g., urease activity and microbial biomass), enhances soil carbon and N cycling (Tejada et al., 2008; Lee et al., 2010; Piotrowska et al., 2012). Growing legume as GM crop such as Chinese milk vetch (*Astragalus sinicus* L.) in paddy fields can fully exploit the natural resources (e.g., light, water and heat) during the winter period and also improve rice yield at a minimum environmental and economic cost (Crews et al., 2004; Voisin et al., 2014).

A combination of FN and GM can not only increase soil organic matter (SOM) and total N contents (Bedada et al., 2014), but also improves the activity of arbuscular mycorrhizal fungi, the population and activity of extra-radical hyphae, and the structure, population and activity of soil microbial communities in the rhizosphere (Zhang et al., 2012; Bedini et al.,

2013). Additionally, the proportion of water-stable macro-aggregates goes up in the topsoil, while the ability of soil microbes to mineralize organic N, thus can increase the N-supplying capacity and productivity of soil (Liu et al., 2013c; Mohanty et al., 2013; Kumar et al., 2014). However, it does not imply that the substitution rate of GM for FN could be as high as possible. Excessive or sole application of GM exhibits a negative impact on rice yield (Dawe et al., 2003; Thorup-Kristensen et al., 2012), and the optimum substitution rate of GM for FN varies depending on crop species, soil type and soil fertility (Yadav et al., 2000a).

Using GM as an alternative to FN can reduce rice production cost and environmental degradation (Tejada et al., 2008). Previous studies have mainly investigated the effects of GM on rice production in monoculture or over a short term (Ashraf et al., 2004; Zhao et al., 2015) however; the influence of GM on the sustainability of rice productivity is not fully understood. Especially, no continuous studies have evaluated the sustainability of N-supplying capacity and productivity of paddy soil in double-rice cropping system with partial substitution of GM for FN.

The present study was aimed at: 1) evaluating the effect of different substitution rates of GM for FN on the continuous status of soil N-supplying capacity in double-rice cropping system, and 2) exploring the sustainability of double-rice productivity under different substitution rates of GM for FN over six consecutive years. The results are of guiding significance in maintaining sustainability of soil productivity in double-rice cropping systems in south China, and the study also provides a reference for reducing N loss and the associated environmental risks in paddy fields.

2. Materials and Methods

2.1. Experimental Site, Soil and Climate

The study was conducted at an experimental station managed by National Engineering and Technology Research Center for Red Soil Improvement in Fengcheng, Jiangxi Province, China (N 28°07', E 115°56' and altitude 25.4 m). The experimental site has a subtropical monsoon climate, characterized by heavy rain from April to June and seasonal drought from September to December. The average annual temperature is 17.7°C and the accumulated temperature greater than 10°C is 5581.9°C. The average annual rainfall is 1552.1 mm. The annual sunshine is 1935.7 h, with an average total radiation of 4637.9 MJ m⁻². The average frost-free period lasts for 274 d. The average monthly temperature (°C) and total monthly precipitation (mm) at the experimental site from 2008 to 2013 are shown in Fig.1.

The soil is silt-clay derived from Quaternary red soil. The initial soil chemical properties of 0-15 cm soil layer are as follows: soil organic matter (SOM) 29.4 g kg⁻¹, total N (TN) 3.06 g kg⁻¹, total P 0.36 g kg⁻¹, total K 35.2 g kg⁻¹, available N 178.2 mg kg⁻¹, available P 5.4 mg kg⁻¹, available K 62.0 mg kg⁻¹, and pH 5.02 (1:1 soil/water).

2.2. Crop Management and Experimental Design

The experiment was conducted for six consecutive years (2008-2013). Early rice (cv. Zhuliangyou 35 bred by China National Rice Research Institute) was transplanted in the first week of May and harvested in the mid-July. Late rice (cv. Ilyou 305 bred by Jiangxi Seed Company, Longping High Tech.) was transplanted in the last week of July and harvested in the last week of October. Rice seedlings (25-d-old for early rice and 30-d-old for late rice) were transplanted at the spacing of 16.5 cm × 20.0 cm (early rice) or 22.0 cm × 15.5 cm (late rice).

The experiment included six treatments arranged in a randomized complete block design with three replications: control (no FN or GM); 100% FN (N₁₀₀); 80% FN plus 20% GM (N₈₀M₂₀); 60% FN plus 40% GM (N₆₀M₄₀); 40% FN plus 60% GM (N₄₀M₆₀); 20% FN plus 80% GM (N₂₀M₈₀). Experimental plots with an area of 20 m² (4 m×5 m) each were separated

1 by a fastened ridge (0.5 m wide and 15 cm aboveground) to prevent the movement of water
2 and nutrients between plots.

3 The recommended rates of chemical fertilizers to rice in Central China are: 150 kg ha⁻¹
4 N (urea 46.4% N), 75 kg ha⁻¹ P (super phosphate 12.0% P₂O₅) and 120 kg ha⁻¹ K (potassium
5 chloride 60% K₂O) for early rice; and 180 kg ha⁻¹ N, 75 kg ha⁻¹ P and 150 kg ha⁻¹ K for late
6 rice. For urea application, 40% urea was broadcasted as basal application, 30% top-dressed at
7 the tillering stage, and another 30% was top-dressed at the panicle initiation stage. Basal P
8 and K fertilizers were consistently applied at the recommended rates.

9 Chinese milk vetch (*Astragalus sinicus* L. var. Fengchengqinggan, CMV) which
10 contained 25.4 g N kg⁻¹ and was having a moisture content was 90.5%, was directly seeded
11 without tillage 20 days before the harvest of late rice from the experimental plots during the
12 winter season (except the control and N₁₀₀ treatments). Fresh vetch was harvested and
13 measured sequentially every year at the full-bloom stage. The fresh vetch straw was applied
14 at the rate of 12.4×10³, 24.9×10³, 37.3×10³ and 49.7×10³ kg ha⁻¹ as equal substitutions of
15 20%, 40%, 60% and 80% FN for the following early rice respectively, 5 d prior to early rice
16 transplanting, mixed mechanically within 15 cm depth of surface soil, and then flooded up to
17 5-7 cm depth. The applied amounts of CMV depended on the yields in each plot during every
18 year. All N treatments were fertilized with the same amount of N which was either from FN
19 alone or both from GM and FN. Rice straw was removed from the plots after harvesting a
20 quarter of the rice field.

21 2.3. Sampling and Chemical Analysis

22 Each year composite soil samples from five randomly selected locations in each plot
23 were annually collected from the upper horizon (0-15 cm) after late rice harvest. Samples
24 were obtained using a soil auger (3.8 cm in diameter), air dried, crushed to pass through a
25 0.25-mm sieve, labeled and then stored in plastic bags. SOM was determined by wet

digestion (120°C, 2 h) using potassium dichromate along with a mixture of H₂SO₄ and 85% H₃PO₄ (3:2, v/v) (Snyder et al., 1984). Pre-treatment of soil with 3 ml of 1 N HCl g⁻¹ was carried out to remove carbonate and bicarbonate. Mineral N (including ammonium, NH₄⁺ and nitrate, NO₃⁻) was extracted with 2 M KCl at a 5:1 ratio (KCl:soil, v/v) (Keeney et al., 1982). Filtrate concentrations of NH₄⁺ and NO₃⁻ were analyzed with a discrete auto analyzer (SmartChem TM200, USA). Total N was determined by sulfuric acid digestion and Kjeldahl distillation (Lu, 2000).

At physiological maturity, grains were separated from straw using a plot thresher for double rice crops in the whole plot every year. The grains were sun-dried and weighed separately to obtain the annual yield.

2.4. Statistical Analysis

The minimum guaranteed yield that could be obtained relative to the maximum observed yield over the years of double-rice cropping system was quantified through the sustainable yield index (*SYI*). The *SYI* was calculated as follows (Singh et al., 1990):

$$SYI = \frac{\bar{Y} - \sigma_{n-1}}{Y_{\max}} \quad (1)$$

where $\bar{Y}$ is the mean yield, σ_{n-1} is the standard deviation of yield for specific treatment across years, and $Y_{\max}$ is the maximum yield obtained under that treatment in any year.

Since σ_{n-1} measures the variation in yield caused by soil and climatic factors, low σ_{n-1} suggests sustainability of the system (Efthimiadou et al., 2010), while high σ_{n-1} indicates unsustainable management practice (Singh et al., 1990). The nearness of *SYI* to 1.0 implies the closeness to an ideal situation that can sustain the maximum crop yield over years, while the deviation from 1.0 indicates the loss to sustainability. Furthermore, agronomic efficiency of applied N (AE_N) was calculated as follows:

$$AE_N = \frac{Y_N - Y_0}{F_N} \quad (2)$$

where Y_N was the rice grain yield (kg ha^{-1}) of fertilized plot, Y_0 was the rice grain yield (kg ha^{-1}) without N application, F_N was the rate of applied N.

Linear regression analyses of soil chemical properties (SOM, TN, NH_4^+ and NO_3^-) across years were performed to determine their trends (slopes). The P values on the slope indicate the level of significance of the observed yield changes. For computing temporal changes in soil chemical properties, original data for the experimental period were subjected to simple regression analysis: $y = at + b$, where Y is the estimated value, b the constant, a the slope and t is the time in years.

Values obtained from different treatments were subjected to ANOVA tests. Separation of means was performed on significant ANOVA tests by Tukey HSD ($P < 0.05$) using the SAS (v.9.1) package (SAS Institute, 2009). Sigmaplot 10.0 and MS Excel were used to generate graphs and tables, respectively.

3. Results

3.1. Changes in soil productivity under different substitution rates of GM for FN

3.1.1. Rice grain yields

Analysis of variance for the six years period showed significant year $\times$ treatment interactions in both early and late rice as well as the double-rice system grain yields. However, all the fertilized treatments significantly ($P < 0.05$) improved rice yields relative to unfertilized control (Table 1).

The yield data obtained over the period 2008-2013 (Table 1) clearly demonstrate the superiority of $\text{N}_{80}\text{M}_{20}$ and $\text{N}_{60}\text{M}_{40}$ plots, which provided greater stability in crop production in comparison to N_{100} treatment. The beneficial effect of moderate substitution of legume GM

for FN (20%-40%) was more pronounced and effective in enhancing the productivity with the advancement of year of cultivation (Table 1 and Fig. 2).

The mean yields of early and late season rice observed in control plots suggest that the soil of our experimental field is capable of giving average grain yield of both early and late rice of about 2.78×10^3 and 2.86×10^3 kg ha⁻¹, respectively, without any external application of N (Table 1). Lower substitution rates of GM for FN (e.g. 20%) was remarkably able to maintain higher grain yield of early rice by 6.81% over the mean yield of N₁₀₀ plots, however, continuous higher substituting rates of GM for FN (60%-80%) dramatically decreased the grain yield of early rice by 6.01%-11.4% when compared with the mean yield of N₁₀₀ treatment (Table 1).

Furthermore, grain yields of early rice, late rice and double-rice system fluctuated over the years. The rice grain yields in the control, N₁₀₀, N₄₀M₆₀ and N₂₀M₈₀ plots decreased over time during the experimental years; however, lower substitution rates of GM for FN (20%-40%) reversed the trends (Fig. 2).

3.1.2. Agronomic Efficiency of Applied N (AE_N)

Sole application of FN as N₁₀₀ resulted in significantly lower AE_N (~ 15.4 %) of early rice when compared with N₈₀M₂₀ (Fig. 3). With the increase in substitution rate of GM for FN, the AE_N exhibited a decreasing trend, especially in N₂₀M₈₀ plots (~ 23.9%). In terms of annual data, the AE_N did not show significant differences in N₈₀M₂₀ or N₆₀M₄₀ plots, but it significantly decreased in N₄₀M₆₀ and N₂₀M₈₀ (~ 16.1% and 25.1% respectively) versus N₁₀₀ plots.

3.1.3. Sustainable Yield Index

The sustainable yield index (SYI) for early rice, late rice and early rice–late rice system reveals that it was greater for early rice than for late rice in all plots, indicating that early rice grain yields are more sustainable than those of late rice grain (Table 2). The SYI for early rice

and double-rice cropping system was highest in the N₈₀M₂₀ treatment, whereas the lowest *SYI* was in the N₂₀M₈₀ plot. The *SYI* of the double rice system in N₆₀M₄₀ was at par with that of N₈₀M₂₀. No distinct trend of *SYI* was observed under the GM-amended plots for late rice. Furthermore, the N-treated plots depicted significantly higher *SYI* than the unfertilized control during both seasons. Such results imply that GM substitution for FN at appropriate rates (e.g., 20%-40%) has the potential to maintain sustainable production for rice grain yield under the double-rice cropping system in south China.

3.2. Soil Chemical Properties

Analysis of variance over six years showed significant year $\times$ treatment interactions in soil organic matter (SOM), total N, NO₃⁻ and NH₄⁺ contents in 0-15 cm soil layer of paddy field after harvesting late rice (Table 4). In addition, the contents of SOM, total N and NH₄⁺ data obtained over the period 2008-2013 clearly demonstrate the superiority of N₈₀M₂₀ and N₆₀M₄₀ plots, which provided greater soil fertility and N-capacity in comparison to N₁₀₀ treatment (Table 4). The beneficial effect of lower substitution of legume GM for FN (20%-40%) was more pronounced and effective in enhancing the soil fertility with the advancement of year of cultivation (Table 4 and Fig. 4).

The SOM in plow layer (0-15 cm) at the beginning of the study was 29.4 g kg⁻¹. At the end of 6 years, application of FN showed significantly higher SOM (30.0 g kg⁻¹) versus the control (28.3 g kg⁻¹). Substituting GM for FN resulted in accumulation of SOM in the 0-15 soil layer (Fig. 4). Soil in plots receiving lower substitution rates of GM for FN (N₈₀M₂₀ and N₆₀M₄₀) contained higher SOM by 9.52%-13.4% in the 0-15 cm soil layer than the FN plots (Fig. 4).

The total N in the tested soil layer at the beginning of the experiment was 3.06 g kg⁻¹. At the end of 6 years, total N content in soil increased in all the plots except the control (Fig. 4). As shown for SOM accumulations, the accumulations of N in soils under lower substitution

1 rates (20%-40%) of GM for FN were higher and the maximum N content was observed in the
2 0-15 cm soil profile under N₈₀M₂₀ and N₆₀M₄₀ treatments (3.53 and 3.43 g kg⁻¹ respectively).

3 During the experimental period, the plots with lower substitution rates of GM for FN
4 (20%-40%) increased the NH₄⁺-N contents by 3.58%-5.88% in the plow layer when
5 compared with N₁₀₀ plot, however, higher substitution rates of GM for FN (60%-80%)
6 significantly decreased the NH₄⁺-N content by 5.81%-8.40% versus N₁₀₀ plots. Furthermore,
7 the substitution of GM for FN dramatically decreased the NO₃⁻-N contents in paddy soil by
8 8.13%-22.0% in comparison to N₁₀₀ plots (Table 3). These results revealed that replacement
9 of 20%-40% FN by GM might not only lower the environmental risks related to N loss (e.g.
10 infiltration of NO₃⁻-N) but also increase the immobilization of NH₄⁺-N in the flooded paddy
11 soil.

12 The SOM, total N and NH₄⁺-N contents exhibited decreasing trends, but an increasing
13 trend was observed over years in N₁₀₀ plots (Table 3 and Fig. 4). Furthermore, SOM and total
14 N contents in GM-treated plots exhibited significant increasing trends over years as well as
15 the NH₄⁺-N contents in N₈₀M₂₀ and N₆₀M₄₀ plots, while a reverse trends were observed in the
16 N₄₀M₆₀ and N₂₀M₈₀ plots over years. In addition, the NO₃⁻-N contents in GM-treated plots
17 exhibited a stable decreasing trend over years in paddy soil of double-rice cropping system in
18 south China (Table 3 and Fig. 4). It can be concluded that lower substitution rates of GM
19 (20%-40%) for FN was not only beneficial for improving soil fertility, but also decreasing
20 environmental risks related to N loss and increasing the possibility of NH₄⁺ fixation in paddy
21 soil.

22 4. Discussion

23 Soil fertility and crop production are two major indicators for measuring the
24 sustainability of cropping systems (Tirol-Padre et al., 2007). The results revealed that SOM
25

and total N contents in control and N₁₀₀ plots of double-rice cropping soil decreased over years (Fig. 4 and Table 3), which could be the deciding factors responsible for the declining trends of grain yields in double-rice system over years (Fig. 2). It has been shown that the continuous application of FN as the sole N source declines soil pH (Lin et al., 2014) and the associated soil hardening and damage to structure can negatively affect soil quality and N-supplying capacity (Liu et al., 2013a). Decreasing soil nutrient-supplying capacity for SOM and N is the major factor causing reduction in crop production (Yadav et al., 2000b; Tirol-Padre et al., 2007). Such results point towards the unsuitability of no application of external N or continuous application of FN as the sole N source for sustainable soil fertility and productivity in double-rice cropping system of south China.

Nitrogen is the mineral element required in greatest amounts by plants and it is most readily available from the soil as NH₄⁺ and NO₃⁻ (Haynes et al., 1978; Taylor et al., 1998). Rice is one of the crops that prefer to absorb more NH₄⁺-N than NO₃⁻-N (Kronzucker et al., 1998). Our findings suggest that SOM, total N, total mineral N and NH₄⁺-N increased in the plow layer while NO₃⁻-N decreased when 20% or 40% of FN was substituted by GM (N₈₀M₂₀ and N₆₀M₄₀) versus zero substitution (N₁₀₀) (Fig. 4 and Table 3). These changes provide explanation for the significant increase in rice grain yields (Table 1) as the N from GM and FN is equally important for rice (Ladha et al., 1997). Legume GM stubbles can be easily degraded in the soil and the released nutrients (especially N) can be absorbed by rice plants. This degradation process also helps the mineralization of organic N in the soil, thereby improving soil N-supplying capacity and N availability (Mohanty et al., 2013; Bedada et al., 2014). Over time, rice grain yields increased in N₈₀M₂₀ and N₆₀M₄₀ versus N₁₀₀ plots (Fig. 2), which contributed to the highest AE_N (Fig. 3).

In previous studies (Srinivasarao et al., 2014; Kumar et al., 2014), substituting GM for partial FN reduced soil bulk density and the consumption rate of soil organic C in paddy

fields. This practice improved soil porosity and the proportion of macro-aggregates, as well as the level of aggregation and the stability of aggregates. It also improved soil enzyme's such as urease activity, enhanced the resistance of soil microorganism to environmental stresses and facilitated their recovery for activity. Essentially, partial substitution of GM for FN improved the growth environment of rice roots, resulting in increased root density and biomass, as well as higher root activity and nutrient absorption; factors extremely vital for rice plants to absorb N (Boparai et al., 1992; Anjali et al., 2009). However, when the proportion of GM-N in total external N exceeded (e.g. 60% or higher) than the optimum level, SOM and total N decreased in N₄₀M₆₀ and N₂₀M₈₀ versus N₁₀₀ plots (Table 3). These changes might cause N deficiency in the early and middle growth stages of rice plant, thus negatively affecting the tillering and productive panicles number per unit area (Huang et al., 2013). Additionally, they also reduced AE_N (Fig. 3), resulting in rice yield reduction over time (Fig. 2 and Table 1). Especially, when 80% of external N was derived from legume GM (N₂₀M₈₀), rice grain yield declined faster than that under FN as the sole N source (N₁₀₀) (Fig. 2). Hence, it may be suggested that legume GM may better serve as a complement to the recommended dose of FN, rather than the primary N source in the double-rice cropping system (Dawe et al., 2003).

In mono rice-based cropping system, planting and utilizing legume GM as a winter crop can efficiently reduce N loss through surface runoff, NH₃ volatilization and N₂O emission (Zhao et al., 2015). In the double-rice cropping system, soil NH₄⁺-N content increased while NO₃⁻-N content decreased in case of 20% or 40% substitution of GM for FN (N₈₀M₂₀ and N₆₀M₄₀) versus zero substitution (N₁₀₀) (Fig. 4 and Table 3). Soil NO₃⁻-N and NH₄⁺-N are closely related to N losses through surface runoff, NH₃ volatilization and N₂O emission in flooded paddy fields (Zhalnina et al., 2012; Rochette et al., 2013; Li et al., 2014). One might speculate that using GM as a complement to FN could reduce the environmental risks from N

runoff in double-rice cropping systems, although this point still needs to be further proved. In addition, how the nitrification and denitrification of N and NH₃ volatilization are affected by the substitution of GM for FN in flooded paddy fields need to be studied in the future.

The sustainability of crop production is a major part of agricultural sustainability (Chaudhury et al., 2005). Similarly, the *SYI* is considered as an important indicator for the sustainability of nutrient management systems and soil productivity (Manna et al., 2005; Tirol-Padre et al., 2006). The higher the *SYI*, the more sustainable the system is (Yadav et al., 2000b). In this experiment, N application improved the *SYI* of rice grain compared with the control. The *SYI* of early rice grain increased under FN as the major N source (N₈₀M₂₀ and N₆₀M₄₀), while that of early grain decreased under GM as the major N source (N₄₀M₆₀ and N₂₀M₈₀) versus FN as the sole N source (N₁₀₀). Overall, *SYI* of rice grain yields of double-rice system increased to varying degrees, indicating a positive effect of substituting GM for FN on the sustainability of annual productivity in the double-rice cropping system (Table 2). Presumably, substituting GM for FN at an appropriate rate improved the quality and quantity of SOM over years and positively affected the combination of hydrolytic N (e.g., NH₄⁺-N and other unknown forms) with aggregates, as well as the NH₃-sugar N content in soil (Manna et al., 2005). In this way, soil N-supplying capacity was improved, which helped in enhancing the sustainability of rice production. Increases in the *SYI* of late rice grain regardless of the substitution rate could be mainly attributed to the residual effects from GM on the succeeding crop (Yadav et al., 2000b).

5. Conclusion

In double-rice cropping system, soil fertility (e.g. SOM and total N) of the 0-15 cm layer and the yields of early and late rice decreased over time when fertilizer N or legume GM were added as the sole and main N sources, respectively. However, when 20% of the external

N was derived from legume GM and 80% of it was derived from FN, both soil fertility and rice yields increased over time. Similar improvements were observed when 40% of the external N was derived from legume GM and 60% of it derived from FN, though at lower rates. Such results imply that partial substitution of legume GM for FN is beneficial for the N-supplying capacity and production sustainability of paddy soil in double-rice cropping system. Moreover, partial substitution of legume GM for FN significantly reduced NO_3^- -N in flooded rice soil, thus minimizing the environmental risks related to N filtration. Unrevealing its potential impacts on N_2O emission and NH_3 volatilization are worth further investigation to further determine sustainability of double-rice production systems.

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Figure captions

Figure 1 Mean monthly temperature (a) and total monthly precipitation (b) at experimental site from 2008-2013.

Figure 2 Sequential changes of rice grain yields under various substitution rates of GM for FN from 2008 to 2013

Figure 3 The mean agronomic efficiency of the applied N (AE_N) in double-rice cropping system over 6 years under various substitution rates of GM for FN

Figure 4 Sequential changes of soil organic matter (a), total N (b), NO_3^- -N (c) and NH_4^+ -N (d) contents under various substitution rates of GM for FN from 2008 to 2013

Tables

Table 1: Mean grain yields and the ANOVA analysis of variance for early and late rice as well as the double-rice system yields as influenced by various substitution rates of GM for FN over six years in the double-rice cropping system, south of China (2008-2013)

Treatments	Early rice		Late rice		Double-rice system	
Yield (× 10 ³ kg ha ⁻¹)						
CK	2.78 d		2.86 c		5.63 d	
N ₁₀₀	4.99 a		5.53 ab		10.5 ab	
N ₈₀ M ₂₀	5.33 a		5.69 a		11.0 a	
N ₆₀ M ₄₀	4.96 ab		5.43 b		10.4 b	
N ₄₀ M ₆₀	4.69 c		5.39 b		10.1 bc	
N ₂₀ M ₈₀	4.42 c		5.44 b		9.86 c	
Analysis of variance						
Source of variance	Mean square	F value	Mean square	F value	Mean square	F value
Year	0.69	10.3 ***	0.67	7.20 ***	2.22	14.7 ***
Treatment	20.6	308.4 ***	26.3	285.2 ***	93.4	617.9 ***
Yyear×Treatment	0.16	2.33 **	0.17	1.81 *	0.53	3.51 ***

*, ** and *** Significant at $P \leq 0.05$, 0.01 and 0.001 respectively.

Within each column, means followed by the same letter are not significantly different according to Tukey's test at 0.05 level of probability.

Table 2: Sustainable yield index (*SYI*) for early and late rice as well as double-rice system as influenced by various substitution rates of GM for FN over six years in the double-rice cropping system, south of China (2008-2013)

Treatment	Early rice	Late rice	Double-rice system
CK	0.392 d	0.356 c	0.380 d
N ₁₀₀	0.640 bc	0.586 b	0.622 c
N ₈₀ M ₂₀	0.679 a	0.604 ab	0.692 a
N ₆₀ M ₄₀	0.658 ab	0.593 ab	0.685 a
N ₄₀ M ₆₀	0.622 c	0.622 a	0.676 ab
N ₂₀ M ₈₀	0.623 c	0.599 ab	0.651 bc
HSD ($P \leq 0.05$)	0.024	0.039	0.012

Within each column, means followed by the same letter are not significantly different according to Tukey's test at 0.05 level of probability.

Table 3: Mean soil organic matter (SOM), total N (TN), NO₃⁻-N and NH₄⁺-N contents in the 0-15 cm soil layer after harvesting late rice as well as its trends as influenced by various substitution rates of GM for FN over six years in the double-rice cropping system, south of China (2008-2013)

Treatment	Means over 6 years				Changes with time (a)			
	SOM	TN	NO ₃ ⁻ -N	NH ₄ ⁺ -N	SOM	TN	NO ₃ ⁻ -N	NH ₄ ⁺ -N
	(g kg ⁻¹)		(mg kg ⁻¹)		(g kg ⁻¹ year ⁻¹)		(mg kg ⁻¹ year ⁻¹)	
CK	28.08 c	2.94 c	0.63 d	6.90 d	-0.102 (0.07ns)	-0.004 (0.03ns)	-0.043 (0.93**)	-0.238 (0.70**)
N ₁₀₀	30.01 b	3.15 b	0.96 a	9.82 b	-0.036 (0.05ns)	-0.011 (0.04ns)	0.031 (0.69**)	-0.078 (0.53**)
N ₈₀ M ₂₀	32.28 a	3.39 a	0.88 b	10.4 a	0.914 (0.90**)	0.074 (0.95**)	-0.017 (0.05ns)	0.041 (0.14*)
N ₆₀ M ₄₀	31.46 ab	3.30 a	0.84 b	10.1 ab	0.632 (0.85**)	0.055 (0.97**)	-0.020 (0.10ns)	0.049 (0.14*)
N ₄₀ M ₆₀	29.96 b	3.12 b	0.78 c	9.25 c	0.224 (0.39*)	0.017 (0.70**)	-0.012 (0.10ns)	-0.132 (0.54*)
N ₂₀ M ₈₀	29.66 b	3.10 b	0.75 c	9.00 c	0.198 (0.29*)	0.007 (0.41*)	-0.011 (0.08ns)	-0.044 (0.14*)

Within each column, means followed by the same letter are not significantly different according to Tukey's test at 0.05 level of probability. Data in parentheses indicate R² values. * and ** show significant differences at $P \leq 0.05$ and 0.01, respectively, while "ns" represents non-significant difference at 0.05 level of probability.

1 **Table 4: Analysis of variance (ANOVA)** of soil organic matter, total N, NO₃⁻-N and NH₄⁺-N contents in
2 0-15 cm soil layer after harvesting late rice as influenced by various substitution rates of GM for FN over
3 six years in the double-rice cropping system, south of China (2008-2013)

Source of variance	soil organic matter		total N		NO ₃ ⁻ -N		NH ₄ ⁺ -N	
	Mean square	F value	Mean square	F value	Mean square	F value	Mean square	F value
Year	6.76	135.9**	0.045	171.1**	0.011	694.7**	0.342	221.4**
Treatment	38.9	11.61 *	0.449	10.42 *	0.686	21.80 *	110.4	10.83 *
Year×Treatment	2.34	11.05 *	0.014	10.41 *	0.007	23.40 *	0.353	10.39 *

4 * and ** indicate significant differences at $P \leq 0.05$ and 0.01, respectively.

Appendix table

Appendix table: Above ground fresh biomass of Chinese milk vetch (CMV) at flowering stage across six years ($\times 10^3$ kg ha⁻¹)

Treatment	2008	2009	2010	2011	2012	2013	Average
N ₈₀ M ₂₀	30.6 a	31.6 a	31.8 a	32.5 a	33.2 a	32.9 a	32.1 a
N ₆₀ M ₄₀	30.1 a	31.1 a	30.9 a	31.8 a	32.9 a	32.1 ab	31.5 ab
N ₄₀ M ₆₀	29.7 a	29.5 ab	29.6 ab	29.8 b	30.3 b	30.9 b	30.0 bc
N ₂₀ M ₈₀	29.1 a	28.7 b	29.0 b	28.9 b	30.1 b	30.5 b	29.4 c

Within each column, means followed by the same letter are not significantly different according to Tukey's test at 0.05 level of probability.

1 **Figure 1**

Fig.1 The mean monthly temperature (a) and total monthly precipitation (b) at experimental site during the experimental years (2008-2013)

1 **Figure 2**

2
3 **Fig. 2 Sequential changes of rice grain yields under various substitution rates of GM for FN from**
4 **2008 to 2013**
5

Figure 3

Fig. 3 Mean agronomic efficiency of applied N (AE_N) in double-rice cropping system over 6 years under various substitution rates of GM for FN

Within each column in the same pattern, means followed by the same letter are not significantly different according to Tukey's test at 0.05 level of probability.

1 **Figure 4**

2 **Fig. 4 Sequential changes of soil organic matter (a), total N (b), NO₃⁻-N (c) and NH₄⁺-N (d) contents**
3 **under various substitution rates of GM for FN from 2008 to 2013**
4