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Integration of Growing Milk Vetch in Winter and Reducing Nitrogen Fertilizer Application Can Improve Rice Yield in Double-Rice Cropping System

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Abstract: To study whether integrative fertilization [growing milk vetch in winter and reducing the dose of chemical nitrogen (N) fertilizer] can improve rice yield, and to reveal the underlying regulatory mechanisms for integrative fertilization, a three-year field trial including two treatments, milk vetch-rice-rice (MRR) and winter fallow-rice-rice (FRR), was conducted in 2010, 2011 and 2012. Our results demonstrated that the MRR treatment could significantly improve rice yield compared with the FRR treatment, especially when the application ratio of milk vetch and chemical fertilizer was 1:2. MRR treatment increased the effective panicle number and the spikelet number per panicle. In addition, a higher tillering number, leaf area index, photosynthetic-potential and photosynthetic-potential to grain ratio were observed in MRR treatment, which could provide enough dry matter for yield formation. Moreover, in MRR treatment, we discovered a higher transportation ratio and transformation ratio of dry matter in culm and leaves, and a stronger total sink capacity and spikelet-root bleeding intensity at the heading stage and 15 d after heading. Furthermore, the MRR treatment showed higher total N, phosphorus and potassium uptakes than FRR treatment, which was associated with the higher root dry weight in each soil layers. These results suggest that growing milk vetch in winter can improve rice yield under less chemical N fertilizer application, which is due to the improvement of soil nutrient status and the increased of rice root growth and development.

Key words: milk vetch; double-rice cropping system; dry matter; sink-source circulation; yield; nitrogen; rice

Rice is the most important cereal crop for a large portion of world population (Zhang, 2007). In China, rice planting area is approximately 2.84 × 10^7 hm^2, accounting for about 30% of the total food crop planting area. Besides, rice yield contributes to more than 40% of the total cereal yield (Huang et al, 2013). However, rice production is confronted with the great challenges from rapid increase of population and decrease of cultivated land and economics (Godfray et al, 2010). Therefore, it is quite necessary to further improve rice yield.

Besides genetic improvement, advanced cultivation is also a very important strategy to improve rice yield. Rational fertilization plays a crucial role in advanced rice cultivation. Adequate nitrogen (N) supply throughout the rice growth cycle is a main strategy to improve grain yield (Fageria and Baligar, 2001; Deng

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et al, 2012). However, to maximize the grain yield, farmers often apply excessive N fertilizer (Lemaire and Gastal, 1997). The chemical N fertilizer application in China is approximately 75% higher than the average one of the world (Peng et al, 2002). The over-use of chemical N fertilizer has become widespread (Miao et al, 2011), while the agronomic N use efficiency has significantly decreased in China (Peng et al, 2006). However, high N fertilizer level can lead to soil acidification (Guo et al, 2010) and environmental problems (Tilman et al, 2001).

The double-rice cropping (early and late rice) system is the dominated cropping pattern in China (Cassman and Pingali, 1995), and there is an approximately six-month from mid-October to late-April winter fallow after the harvest of previous late rice. Recently, the increase of winter fallow-rice-rice area (Huang et al, 2013) has destroyed soil nutrient status and the physical properties of reddish paddy soil (Yang et al, 2012). In addition, the convenience of applying chemical fertilizer can result in poor soil organic matter (Sharma et al, 1999). Soil fertility plays a key role in maintaining or improving plant growth and productivity, and can be effectively improved by applying organic matter. Many studies have focused on developing cultivation systems with organic materials (Ashraf et al, 2004; Asagi and Ueno, 2009).

Green manure is a type of organic material and planted in winter fallow field. It plays an important role in improving soil fertility and providing some essential nutrients to crops (Fageria et al, 2005). The cultivation pattern using green manure has several advantages, including improved biological nitrogen fixation, decreased NO_3^--N leaching loss and soil erosion (Hartwig and Ammon, 2002). To protect soil and ecological environment, many countries have focused on establishing a green manure crop pattern (Gabriel and Quemada, 2011). In addition, green manures can improve soil properties and increase crop yields (Budhar and Palaniappan, 1996; Cherr et al, 2006; Latt et al, 2009; Yang et al, 2012). In China, leguminous green manure crops, such as Chinese milk vetch (*Astragalus sinicus* L.), are important N sources for wetland rice (Singh et al, 1991). However, it remains unclear whether growing milk vetch as a cover crop in winter can improve rice yield.

Therefore, in this study, we investigated whether the integration of growing milk vetch in winter and reducing the dose of chemical N fertilizer can improve rice yield. We also dissected the potential regulatory basis of this integrative fertilization.

MATERIALS AND METHODS

Plant materials

A three-year field trial was conducted in alluvial paddy fields at Henghu Farm in Henghu County, Nanchang City, Jiangxi Province of China (29.00° N and 28.83° E) with an annual mean temperature of 17.3 °C and annual mean rainfall of 1 610 mm. The physical and chemical properties of experimental paddy soil were as follow: pH 5.1, organic matter 13.6 g/kg, total N 1.4 g/kg, total P 1.0 g/kg, total K 16.5 g/kg, Alkali-N 95.5 mg/kg, Olsen-P 32.5 mg/kg and available K 108.4 mg/kg.

The rice varieties Ganxin 203 and Wufengyou T025 were used as early and late rice, respectively. Chinese milk vetch Yujiangdaye was served as the green manure crop.

Field experiment design

Two treatments milk vetch-rice-rice (MRR) and winter fallow-rice-rice (FRR) in 2010, 2011 and 2012 were employed. Each treatment contained four plots which were all randomly ranged with each plot of 100 m^2 in size. Each plot was spaced with wide ridges and a thin fabric layer to prevent the mixing of water and fertilizer from another treatment. Besides, each plot was independently irrigated and drained. All rice straws were grinded and returned to field. A total of 30 kg/hm^2 milk vetch was seeded before the harvest of the previous late rice, and the fields were plowed at 5 d before the transplanting of early rice. Shallow field water was used for decomposing milk vetch. Milk vetch was sampled to measure N content and the biomass. No chemical fertilizer was applied during milk vetch planting.

We used urea (46% N), super phosphate (12% P_2O_5) and potassium chloride (60% K_2O) as chemical fertilizers. In both MRR and FRR treatments, the total P_2O_5 (72 kg/hm^2) and K_2O (180 kg/m^2) applied in early and late rice fields were consistent. Shoot biomass and N content of milk vetch were measured to determine the amount of chemical N fertilizer in MRR treatment. The amount of chemical N fertilizer applied on early rice of MRR treatment (Table 1) was calculated according to the formula: N (MRR) = 180 – N content of milk retch (%) × biomass of milk retch (kg/m^2). Two types of N fertilizer applications with

Table 1. Chemical fertilizer application of different treatments on early rice. kg/hm^2

Treatment	2010			2011			2012		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
MRR	122.7	72	180	86.7	72	180	119.6	72	180
FRR	180.0	72	180	180.0	72	180	180.0	72	180

MRR, Milk vetch-rice-rice; FRR, Winter fallow-rice-rice.

different ratios of green manure to chemical fertilizer (1:1 in 2011 and 1:2 in 2010 and 2012) were applied. N fertilizer was applied as base fertilizer, tillering fertilizer and panicle fertilizer with the ratio of 5:2:3. P_2O_5 and K_2O fertilizers were all applied as base fertilizer. Base fertilizers were applied at 1 d before transplanting, and tillering fertilizers were applied at 7 d after transplanting. Field managements were all based on farmers' practice. Early and late rice sowing was carried out by means of water-raised seedling. The sowing and transplanting time is presented in Table 2. In early rice, the seedlings were transplanted with the spacing of 13.3 cm × 23.3 cm, and each hill contained three seedlings. In late rice, the seedlings were transplanted with spacing of 13.3 cm × 26.7 cm, and each hill contained two seedlings.

Measurement of N, P and K contents

During the mid-tillering, panicle initiation, full heading and maturity stages, three hills in each plot of each treatment were sampled and the samples were, divided into culm, leaves and panicles, then dried and weighed. Tissue samples were digested with H_2SO_4-H_2O_2 and filtered. N content was measured using the Kjeldahl digestion nitrogen determination semimicro-distillation method (Bremner and Mulvaney, 1982). P content was measured using the vanadium molybdate yellow colorimetric method, and K content was detected by a flame photometry (Walinga et al, 1995).

Field investigation and grain yield measurements

From 5 d after transplanting to heading stage, 20 hills from four plots of each treatment were selected to count tillering number in 5 d interval.

At the maturity stage, a 30-m^2 subplot was marked out in the middle of each plot. The plants in each subplot were harvested to determine grain yield. Fifteen individuals along the diagonal of each subplot were selected to investigate the effective panicle number, spikelet number per panicle, seed-setting rate and 1000-grain weight.

Table 2. Sowing and transplanting time of early and late rice.

Year	Season	Sowing date (Month-Day)	Transplanting date (Month-Day)
2010	Early rice	03-27	04-27
	Late rice	06-26	07-25
2011	Early rice	03-28	04-28
	Late rice	06-29	07-30
2012	Early rice	03-25	04-26
	Late rice	06-25	07-26

Determination of leaf area index (LAI) and dry weight

During the mid-tillering, panicle initiation, full heading and maturity stages, the leaf area of three representative individual plants from each plot of each treatment were measured using an automatic leaf area meter (LI-3100, LI-COR, Lincoln, Nebraska, USA), and LAI was calculated by the following formula: LAI = Average leaf area × Number of leaves per shoot × Number of shoots per hill × Number of hills per unit of ground area. Then, plants were dried and weighed after being divided into culm, leaves and panicles.

Distribution of roots in different soil layers

During the mid-tillering, panicle initiation, full heading and maturity stages, the plant roots of five hills in each treatment were sampled for measuring the ratio of root to shoot and the dry root weight per hill. The root dry weight in different soil layers (0–5 cm, 5–10 cm and > 10 cm) were only measured at the maturity stage.

Investigation of bleeding rate

At 5:00–6:00 pm at the heading stage and 15 d (late rice, 20 d) after heading, 10 consistent single culm from each plot of each treatment were selected, cut at 5–10 cm over ground and covered with the plastic bags containing absorbent cotton. Bleeding sap was collected till 6:00–7:00 am next day, and the bleeding intensities were calculated by the formula: Bleeding intensity = (Weight of bag after collection – Weight of bag before collection) / Interval time.

Investigation of flower numbers

Three hills of each treatment were sampled when 2/3 of spikelets headed at the panicle initiation stage. The numbers of degenerated and formed flowers per spikelet were counted, and the total differentiated number of flowers was calculated. The degenerated numbers of the primary branch, the secondary branch and flowers per spikelet were measured using magnifier. The formed numbers of the primary branch, the secondary branch and flowers per spikelet were investigated through naked-eye observation.

Grain filling rate

Eighty spikelets with consistent flowering time of each treatment were marked, 10 marked spikelets were sampled every 5 d, oven-dried at 105 °C for 30 min, and dried in bags at 75 °C until their weight remained constant. The dry weights of culm, leaves and 100 grains were measured. Grain filling rate was calculated according to the formula: Grain filling rate = (Later 100-grain weight – Previous 100-grain weight) / Interval time.

Calculation and statistical analysis

Apparent transportation ratio of dry matter in culm and leaf (%) = (Culm and leaf dry weight at the full heading stage – Culm and leaf dry weight at the maturity stage) / Culm and leaf dry weight at the full heading stage × 100 (Yang et al, 1997).

Transformation ratio of dry matter in culm and leaf (%) = (Culm and leaf dry weight at the full heading stage – Culm and leaf dry weight at the maturity stage) / Dry weight of spikelets × 100 (Yang et al, 1997).

Photosynthetic potential = $(L_2 - L_1) \times (T_2 - T_1)$. Where T_1 and T_2 represent sampling times; and L_1 and L_2 are mean leaf area at T_1 and T_2.

Photosynthetic potential to grain ratio = Photosynthetic potential / Total number of differentiated flowers.

Grain to leaf area ratio = Total number of differentiated flowers / Leaf area.

Total sink capacity = Total number of differentiated flowers × 1000-grain weight.

Ratio of sink increase to source increase = $(W_2 - W_1) / (G_2 - G_1)$. Where W_1, W_2 represent adjacent dry weights of individual shoots (including leaf and culm) in 5 d. G_1 and G_2 represent the corresponding adjacent dry weights of spikelets in 5 d (Cao et al, 2002).

Microsoft Excel 2007 and SAS 8.1 (SAS Institute, Cary, NC, USA) were used to analyze the data. The analysis of variance and mean comparison were based on the least significant difference test with the significance level of 0.05.

RESULTS

Impacts of different treatments on rice grain yield and yield components

The grain yield in 2010 was much lower than those in 2011 and 2012 due to chilling injury at the growing prophase of early rice and a strong cold wave during growing of late rice (Table 3). Compared to the grain yield of FRR treatment, those of MRR treatment were increased by 1.5% to 15.6% in early rice and by 4.1% to 21.7% in late rice. In early rice, the grain yields of MRR treatment in 2010 and 2011 were higher than those of FRR treatment, without reaching statistical significance, but showed statistically significant in 2012. In late rice, the grain yields of MRR treatment were significantly higher than those of FRR treatment in 2010, 2011 and 2012.

The impacts of different treatments on the yield components are presented in Table 3. The differences in the number of effective panicles (PN) during the five of the six seasons and the spikelet number per panicle (SPP) during the three of the six seasons

Table 3. Rice yield and yield components of different treatments (Mean ± *SD*, *n* = 3).

Year	Season	Treatment	Yield (kg/hm^2)	PN (× 10^4/hm^2)	SPP	SSR (%)	TGW (g)
2010	Early rice	MRR	7 653 ± 104 a	397 ± 8.7 a	130 ± 3.2 a	77.0 ± 2.1 b	27.9 ± 0.4 a
		FRR	7 416 ± 119 a	342 ± 12.9 b	128 ± 4.9 a	87.0 ± 4.6 a	27.4 ± 2.8 a
	Late rice	MRR	7 970 ± 134 a	283 ± 9.1 a	151 ± 2.9 a	82.2 ± 1.3 a	22.2 ± 0.1 a
		FRR	7 654 ± 69 b	287 ± 5.3 a	147 ± 4.8 a	85.4 ± 2.8 a	20.4 ± 0.3 a
2011	Early rice	MRR	8 588 ± 159 a	464 ± 18.4 a	94 ± 1.7 a	93.0 ± 1.7 b	28.5 ± 0.9 a
		FRR	8 459 ± 102 a	430 ± 7.2 b	93 ± 1.8 a	96.1 ± 2.7 a	29.0 ± 3.6 a
	Late rice	MRR	9 783 ± 120 a	421 ± 3.3 a	165 ± 8.1 a	73.8 ± 0.2 b	23.5 ± 0.3 a
		FRR	9 353 ± 66 b	401 ± 5.3 b	153 ± 1.4 b	75.7 ± 1.5 a	23.6 ± 0.2 a
2012	Early rice	MRR	9 437 ± 102 a	391 ± 5.3 a	110 ± 2.1 a	84.5 ± 1.1 b	29.1 ± 0.1 a
		FRR	8 162 ± 78 b	337 ± 7.3 b	101 ± 2.1 b	88.2 ± 1.3 a	29.1 ± 0.1 a
	Late rice	MRR	10 079 ± 110 a	393 ± 4.2 a	114 ± 3.3 a	82.8 ± 2.4 b	22.9 ± 1.0 a
		FRR	8 281 ± 87 b	373 ± 8.2 b	102 ± 1.9 b	86.7 ± 1.7 a	22.4 ± 2.4 a

MRR, Milk vetch-rice-rice; FRR, Winter fallow-rice-rice; PN, Number of effective panicles; SPP, Spikelet number per panicle; SSR, Seed-setting rate; TGW, 1000-grain weight.

ANOVA was conducted among different treatments at the same growth stage. Different lowercase letters indicate significance at the 0.05 level.

between the two treatments were statistically significant, which might contribute to the difference in grain yield. PNs of MRR treatment were mostly higher than those of FRR treatment in 2010, 2011 and 2012, and a statistical significance was reached in early rice. SPP of MRR treatment was higher than that of FRR treatment during the six seasons of rice, and a statistical significance was reached in three of the six seasons.

Impacts of different treatments on tillering dynamics and leaf area index

The tillering dynamics of rice in 2010 and 2011 are shown in Fig. 1. The tillering rate and the maximum tillering number of both treatments in 2011 were higher than those in 2010. In early rice, the increase of tillering number at 16 d after transplanting to the top tillering number stage was: MRR treatment > FRR treatment. Overall, the maximum tillering number of MRR treatment was significantly higher than that of FRR treatment in early rice. In late rice, the same trend was observed but without statistical significance.

There was significant difference in LAI value at the full heading stage (FHS) of both treatments in early and late rice (Fig. 2). In 2010, the LAI of MRR treatment was higher than that of FRR treatment after booting stage, and was significantly higher at the full heading (both early and late rice) and maturity stages (late rice). The LAI of MRR treatment was higher than that of FRR treatment after full tillering stage, and was significantly higher at the full heading and maturity stages in 2011.

Impacts of different treatments on rice root growth

The ratio of root to shoot and dry root weight per hill in 2010 were measured to investigate the root growth at different stages under different treatments (Table 4). At the full heading stage, the ratio of root to shoot of MRR treatment was significantly increased compared to that of FRR treatment, suggesting that the root vitality of MRR treatment was significantly stronger than that of FRR treatment during the late stages of reproduction. The same trend was also observed for the root dry weight at the full heading stage.

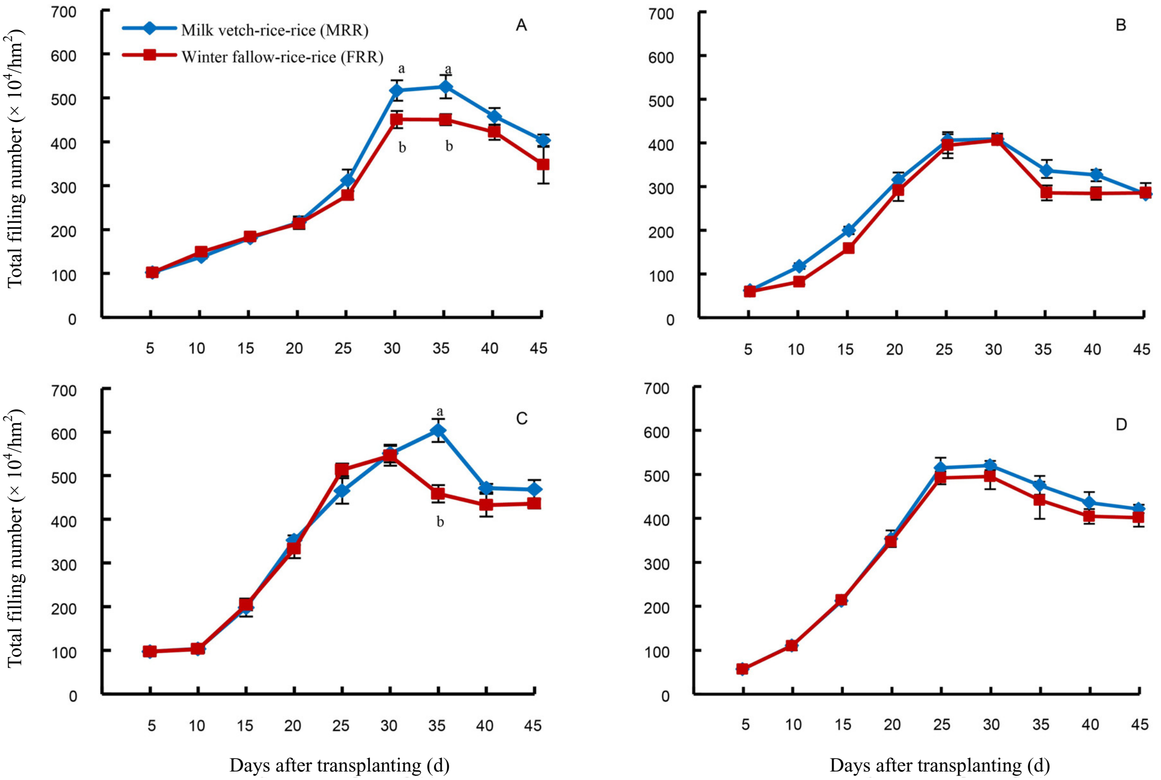

Fig. 1. Total tillering number of rice.

A, Early rice in 2010; B, Late rice in 2010; C, Early rice in 2011; D, Late rice in 2011.

ANOVA was conducted among different treatments at the same growth stage. Vertical error bars are the standard error of means (n = 4). Different lowercase letters indicate significance at the 0.05 level.

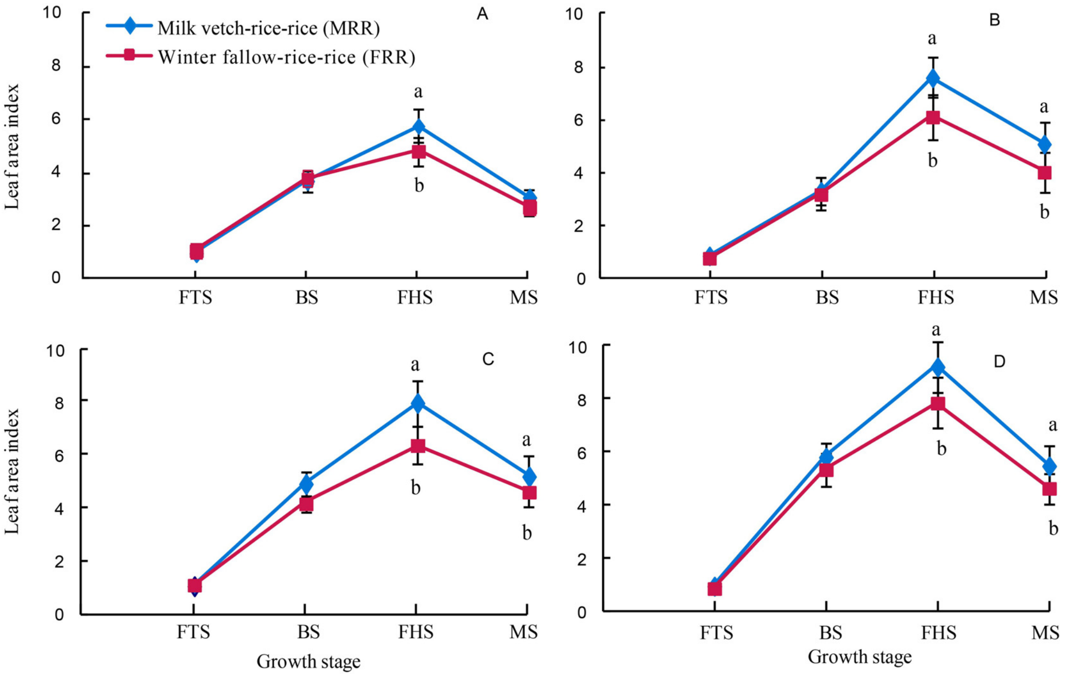

Fig. 2. Dynamics of rice leaf area index.

A, Early rice in 2010; B, Late rice in 2010; C, Early rice in 2011; D, Late rice in 2011.

FTS, Full tillering stage; BS, Booting stage; FHS, Full heading stage; MS, Maturity stage.

ANOVA was conducted among different treatments at the same growth stage. Vertical error bars are the standard error of means (*n* = 4). Different lowercase letters indicate significance at the 0.05 level.

The variation of rice roots at the maturity stage of early and late rice in 2010 and 2011 were investigated (Table 5). The total root dry weight of MRR treatment was significantly higher than that of FRR treatment during all the four rice seasons. In 2010, the root dry weight of MRR treatment was significantly higher than that of FRR treatment in all the three soil layers during the two rice season. Compared with that of

Table 4. Root/shoot ratio and dry weight of single hill rice root in different treatments in 2010 (Mean ± *SD*, *n* = 3).

Season	Treatment	FTS		BS		FHS	
		R/S	RDW (g/hill)	R/S	RDW (g/hill)	R/S	RDW (g/hill)
Early rice	MRR	0.26 ± 0.02 a	0.58 ± 0.06 b	0.19 ± 0.03 a	2.36 ± 0.25 a	0.27 ± 0.01 a	5.58 ± 0.82 a
	FRR	0.30 ± 0.03 a	0.72 ± 0.04 a	0.20 ± 0.01 a	2.22 ± 0.29 a	0.15 ± 0.01 b	4.49 ± 0.28 b
Late rice	MRR	0.19 ± 0.02 a	1.34 ± 0.13 a	0.15 ± 0.02 a	2.56 ± 0.23 a	0.23 ± 0.01 a	5.63 ± 0.35 a
	FRR	0.19 ± 0.01 a	1.25 ± 0.08 a	0.14 ± 0.01 a	2.25 ± 0.18 a	0.12 ± 0.02 b	4.89 ± 0.34 b

MRR, Milk vetch-rice-rice; FRR, Winter fallow-rice-rice; FTS, Full tillering stage; BS, Booting stage; FHS, Full heading stage; R/S, Ratio of root to shoot; RDW, Root dry weight.

ANOVA was conducted among different treatments at the same growth stage. Different lowercase letters indicate significance at the 0.05 level.

Table 5. Root dry weight of rice at the maturity stage in various soil layers of different treatments in 2010 and 2011 (Mean ± *SD*, *n* = 3).

Year	Season	Treatment	Root dry weight in different soil layer depth (g/hill)			Total root dry weight (g/hill)
			0–5 cm	5–10 cm	10–20 cm	
2010	Early rice	MRR	3.72 ± 0.04 a	3.35 ± 0.04 a	0.59 ± 0.03 a	7.66 ± 0.05 a
		FRR	2.82 ± 0.05 b	2.58 ± 0.03 b	0.19 ± 0.08 b	5.59 ± 0.03 b
	Late rice	MRR	3.63 ± 0.04 a	3.64 ± 0.07 a	0.54 ± 0.05 a	7.81 ± 0.10 a
		FRR	2.83 ± 0.21 b	2.84 ± 0.08 b	0.24 ± 0.08 b	5.91 ± 0.26 b
2011	Early rice	MRR	3.88 ± 0.09 a	3.93 ± 0.06 a	0.52 ± 0.01 a	8.33 ± 0.03 a
		FRR	3.05 ± 0.06 b	2.96 ± 0.18 b	0.33 ± 0.04 b	6.34 ± 0.14 b
	Late rice	MRR	3.91 ± 0.07 a	4.02 ± 0.04 a	0.61 ± 0.03 a	8.54 ± 0.25 a
		FRR	3.08 ± 0.04 b	3.11 ± 0.04 b	0.37 ± 0.04 b	6.56 ± 0.07 b

MRR, Milk vetch-rice-rice; FRR, Winter fallow-rice-rice.

ANOVA was conducted among different treatments at the same growth stage; Different lowercase letters indicate significance at the 0.05 level.

FRR treatment, the total root dry weight of MRR treatment was increased by 37.0% and 32.1% in early and late rice, respectively. In 2011, the same trend was also observed. In all of the three soil layers, the root dry weight of MRR treatment was significantly higher than that of FRR treatment. Compared with that of FRR treatment, the total root dry weight of MRR treatment was increased by 31.4% and 30.2% in early and late rice, respectively. The above results suggest that the root system of MRR treatment is stronger than that of FRR treatment.

Impacts of different treatments on dry matter transport in rice

Because culm and leaves can transport photosynthetic products and the transformation ratio of dry matter contributes to grain formation, we measured the apparent transportation ratio and the transformation ratio of dry matter in 2010 and 2011 (Table 6). Overall, the apparent transportation ratio of MRR treatment was significantly higher than that of FRR treatment in three of the four rice seasons (except the early rice season in 2010), and the transformation ratio of MRR treatment was significantly higher than that of FRR treatment in all the four rice seasons. Compared with that of FRR treatment, the apparent transportation ratio of MRR treatment was increased by 0.5% to 2.2% and 2.4% to 5.1% in early and late rice, respectively. In addition, the transformation ratio of MRR treatment was increased by 1.1% to 6.0% and 1.4% to 6.7% in early and late rice, respectively (Table 6). Significant positive correlations between grain yield and apparent transportation were observed in 2010 and 2011 rice seasons, with the correlation coefficients of 0.9652 and 0.9322, respectively. Additionally, significant positive correlations between grain yield and transformation ratio were also observed in 2010 and 2011 rice seasons, with the correlation coefficients of 0.8562 and 0.9945, respectively.

Impacts of different treatments on sink-source circulation

Because photosynthetic potential indicates the quantity of photosynthetic products after heading, photosynthetic potential was compared between the two treatments. As shown in Table 6, we discovered that the photosynthetic-potential of MRR treatment was significantly higher than that of FRR treatment from full heading to maturity stage in 2010 and 2011. Significant positive correlation was detected between photosynthetic potential and grain yield, with the correlation coefficient ranging from 0.6732 to 0.9962.

Total spikelet number and 1000-grain weight contribute to grain yield potential. We investigated the total sink capacity to explain these two parameters. The total sink capacities of MRR treatment in 2010 and 2011 were significantly higher than those of FRR treatment (except the early rice in 2011) (Table 6). Significant positive correlations between the total sink capacity and grain yield were observed in 2010 and 2011, with the correlation coefficient ranging from 0.6752 to 0.9968.

Because photosynthetic potential to grain ratio indicates the capacity and development of sink and source from heading stage to maturity stage, the photosynthetic-potential to grain ratios were investigated. The photosynthetic potential to grain ratios of MRR treatment was higher than those of FRR treatment in all the four rice seasons, and a statistical significance was reached in 2010 late rice and 2011 early rice (Table 6). A significant positive correlation between grain yield and photosynthetic potential to grain ratio was observed in 2010 and 2011.

Grain to leaf area ratio is the ratio between grain

Table 6. Transformation ratio of dry matter in clum and leaves and sink-source characteristics under different treatments from full heading to maturity stage of rice in 2010 and 2011 (Mean ± *SD*, *n* = 3).

Year	Season	Treatment	Transportation ratio of dry matter (%)	Transformation ratio of dry matter (%)	Photosynthetic potential ($\times 10^4 \cdot m^2 \cdot d$)	Photosynthetic potential/grain ratio ($m^2 \cdot d$/grain)	Grain/leaf area ratio (grain/cm^2)	Total sink capacity (t/hm^2)
2010	Early rice	MRR	33.5 ± 2.9 a	36.6 ± 0.3 a	7.19 ± 1.92 a	20.98 ± 2.33 a	0.77 ± 0.04 b	14.36 ± 0.99 a
		FRR	33.0 ± 1.8 a	35.5 ± 0.6 b	6.06 ± 1.06 b	20.72 ± 1.77 a	0.91 ± 0.01 a	12.03 ± 0.65 b
	Late rice	MRR	32.7 ± 2.3 a	37.7 ± 1.0 a	10.12 ± 1.25 a	35.50 ± 1.01 a	0.57 ± 0.01 b	9.51 ± 0.76 a
		FRR	30.3 ± 1.2 b	36.3 ± 0.8 b	8.42 ± 0.90 b	29.94 ± 0.78 b	0.68 ± 0.02 a	8.59 ± 0.65 b
2011	Early rice	MRR	39.0 ± 1.4 a	41.0 ± 2.7 a	9.44 ± 0.56 a	32.46 ± 0.25 a	0.61 ± 0.00 a	12.44 ± 0.19 a
		FRR	36.8 ± 2.1 b	35.0 ± 2.4 b	8.26 ± 1.03 b	31.10 ± 0.29 b	0.64 ± 0.01 a	11.56 ± 0.20 a
	Late rice	MRR	34.2 ± 2.6 a	39.4 ± 4.2 a	11.98 ± 1.16 a	25.80 ± 2.34 a	0.71 ± 0.03 a	16.33 ± 1.44 a
		FRR	29.1 ± 3.1 b	32.7 ± 4.9 b	10.48 ± 1.26 b	25.63 ± 1.18 a	0.75 ± 0.05 a	14.46 ± 0.98 b

MRR, Milk vetch-rice-rice; FRR, Winter fallow-rice-rice.

ANOVA was conducted among different treatments at the same growth stage. Different lowercase letters indicate significance at the 0.05 level.

number and the maximum leaf area index, which can suggest the balance between sink and source. Overall, the grain/leaf area ratios of FRR treatment were significantly higher than those of MRR treatment in 2010 (Table 6). A significant negative correlation between grain to leaf area ratio and grain yield was observed in 2010.

The bleeding intensities at heading and 15 d after heading were investigated (Table 7). No significant advantage of spikelet-root bleeding intensity at the heading stage was observed in the MRR treatment compared to that of FRR treatment in 2010 and 2011. However, the spikelet-root bleeding intensity at 15 d after heading of MRR treatment was significantly higher than that of FRR treatment in 2010 and 2011. In addition, significant positive correlations between grain yield and bleeding intensity at 15 d after heading were observed.

Impacts of different treatments on nutrient uptake in rice

The total N, P and K uptakes are shown in Table 7. The total N accumulation of MRR treatment was significantly higher than that of FRR treatment in all the four rice seasons of 2010 and 2011. The MRR treatment resulted in a higher total P uptake than FRR treatment in both early and late rice in 2010 and 2011, and the total K uptakes of MRR treatment were significantly higher than those of FRR treatment in 2010 and 2011. Finally, rice plants of MRR treatment have significantly stronger ability than those of FRR treatment on absorbing nutrients, including N, P and K.

DISCUSSION

Our results demonstrated that growing milk vetch (cover crop) in winter is beneficial to double-rice production compared with keeping fallow in winter. Cropping milk vetch in winter can significantly increase rice yield, which is associated with improved soil nutrient status, rice root growth and development, and yield components traits. This is consistent with previous reports (Fageria, 2007; Yang et al, 2012).

Growing milk vetch in winter can improve uptake and utilization of N, P and K nutrients

Green manure is a plant material which can be incorporated into the soil. It represents an important alternative source of mineral fertilizers. Milk vetch is commonly used as a green manure (Clement et al, 1998; Fageria, 2007).

N is the most important limiting nutrient in irrigated rice systems (Deng et al, 2012). In this study, different N fertilization patterns, MRR and FRR, were used to investigate the impacts of integrating green manure with reduced dose of chemical N fertilizer on N, P and K uptakes in rice. The total N accumulation in MRR treatment was obviously higher than that in FRR treatment in 2010 and 2011 (Table 7). The total N application of MRR treatment was considered to be strictly consistent with that of FRR treatment when reducing the total fixed N by milk vetch root nodules in the roots and shoots. But the total N application of MRR treatment actually decreased the N fixed by milk vetch root nodules and soil N in shoot in our experiments. Thus, according to Yuan et al (2011), the actual N application on MRR treatment should be less than that on FRR treatment because the amount of N fixed by root nodules was less than that of the soil N in milk vetch shoot. These results indicate that the integrative fertilization of green manure N from milk vetch and less chemical N fertilizer can facilitate N absorption in rice plants. Therefore, the integration of chemical N and milk vetch can improve soil N status

Table 7. Spikelet-root bleeding intensity under different treatments in 2010 and 2011 (Mean ± *SD*, *n* = 3).

Year	Season	Treatment	SRBHS (mg/h)	SRBDAH (mg/h)	Nutrient uptake		
					N	P_2O_5	K_2O
2010	Early rice	MRR	1.28 ± 0.04 a	0.67 ± 0.05 a	167 ± 5.5 a	50 ± 1.7 a	196 ± 5.8 a
		FRR	1.22 ± 0.01 a	0.58 ± 0.03 b	138 ± 3.1 b	44 ± 1.9 b	172 ± 4.8 b
	Late rice	MRR	1.02 ± 0.05 a	0.57 ± 0.03 a	177 ± 3.9 a	51 ± 1.3 a	227 ± 7.2 a
		FRR	0.96 ± 0.06 a	0.47 ± 0.04 b	158 ± 2.1 b	46 ± 2.1 b	207 ± 4.9 b
2011	Early rice	MRR	1.88 ± 0.10 a	1.29 ± 0.12 a	237 ± 6.5 a	57 ± 2.6 a	288 ± 8.1 a
		FRR	1.73 ± 0.20 a	1.02 ± 0.13 b	211 ± 4.9 b	51 ± 1.5 b	258 ± 4.9 b
	Late rice	MRR	1.18 ± 0.03 a	0.80 ± 0.01 a	218 ± 4.3 a	57 ± 1.8 a	278 ± 5.1 a
		FRR	1.14 ± 0.03 a	0.73 ± 0.02 b	184 ± 3.4 b	52 ± 1.2 b	233 ± 6.8 b

MRR, Milk vetch-rice-rice; FRR, Winter fallow-rice-rice; SRBHS, Spikelet-root bleeding intensity at the heading stage; SRBDAH, Spikelet-root bleeding intensity at 15 d after heading.

ANOVA was conducted among different treatments at the same growth stage. Different lowercase letters indicate significance at the 0.05 level.

and reduce the required chemical N fertilization.

Similar to milk vetch, Fageria (2007) has reported that graybean can incorporate other essential nutrients besides N, such as P. However, little is known about the impacts of covering green manure (such as milk vetch) in winter on rice P uptake. In this study, it is discovered that growing milk vetch cover crop in winter can improve total P accumulation compared to keeping fallow in winter. The increased P accumulation is probably due to the advanced ability of mobilizing soil P (Nziguheba et al, 2002; Phiri et al, 2003). In addition, growing milk vetch in winter can also improve total K accumulation compared to keeping fallow in winter. These results suggest that cropping milk vetch in winter can improve nutrient including N, P and K status, which is congruent with the results of applying *Sesbania rostrata* in rice cultivation (Latt et al, 2009). Chang et al (2010) reported that the application of more than 20 t/hm^2 (fresh weight) of *Sesbania rostrata* can significantly increase the uptake of N, P and K in rice. Similarly, in maize, the amounts of available N, P and K after the application of green manure are significantly increased (Sangakkara et al, 2004).

We speculate that the higher amounts of available N, P and K in rice after the application of the cover crop milk vetch in winter result from two important factors. Firstly, cropping milk vetch in winter can improve rice root system which will facilitate nutrient absorption from the soil. Sangakkara et al (2004) reported that the application of green manure can significantly increase root dry weight and the root weight per unit length in maize, and can stimulate the partitioning of dry matter to later roots which play an essential role in acquiring nutrients. In this study, growing milk vetch in winter can increase root vitality and rice root dry weight in each soil layer, and total rice root dry weight. It can also promote rice root growth toward deep layer, which is beneficial for absorbing more nutrients. These results indicate that the stronger root system of MRR treatment can result in the absorption of more N, P and K, which may largely contribute to the high grain yield. Secondly, growing milk vetch in winter may significantly improve the physical properties of paddy soil compared to fallow in winter. Some studies have reported that application of green manure can enable a greater water holding capacity, soil organic carbon content, soil fertility, cation exchange capacity and bulk density (Sangakkara et al, 2004; Fageria et al, 2005; Yang et al, 2012). In this study, after growing milk vetch in winter and two seasons of rice in 2010 and 2011, the soil of MRR treatment has lower bulk density and higher total porosity, capillary porosity, aeration porosity and water holding capacity than that of FRR treatment after harvesting (Supplemental Table 1). The advanced soil fertility can then provide a beneficial soil environment for rice root growth.

Growing milk vetch in winter and obtaining high rice grain yield

Rice is one of the most important cereal crops in the world, and it is the essential production target to obtain high grain yield. Advanced breeding and efficient cultivation management are the two key strategies for increasing rice grain yield (Dobermann et al, 2002; Zhang, 2007). In this study, we investigate the impacts of integrating the application of milk vetch in winter and reduction of chemical fertilizer dose on succeeding rice growth and yield. Our three-year field trial demonstrates that growing milk vetch in winter can significantly increase rice yield compared to the FRR treatment (Table 3). These results suggest that the combinational application of milk vetch and chemical fertilizer can improve rice yield, indicating that a higher rice yield can be obtained under the reduced application of chemical fertilizer.

Compared with FRR treatment, growing cover crop milk vetch in winter can more significantly increase grain yield in late rice than that in early rice. This method induces the maximum mineralization rate in late rice period, which can be more beneficial for late rice yield (Table 3). This is consistent with the report of Gao et al (2010). In contrast, Li S L et al (2012) report a lower yield increase in late rice than that in early rice when decreasing 20% and 40% of chemical fertilizer application. These results indicate that different ratios of milk vetch to chemical fertilizer (or different application amounts of milk vetch) have different impacts on the yields of early and late rice, and an appropriate amount of milk vetch can have stronger impacts on late rice yield compared to early rice yield. Although there are few reports about the impacts of green manure on the second crop rice (late rice), the underlying mechanism is still unknown.

The application of green manure alone cannot provide sufficient nutrients for obtaining the maximum economic yield (Budhar and Palaniappan, 1996; Fageria, 2007; Siavoshi et al, 2011). Therefore, it is critical to choose an adequate ratio of green manure to chemical fertilizer in accordance with the N amount for the application of milk vetch and chemical fertilizer with the ratio of generating the maximum grain yield. In this study, the application of milk vetch and chemical fertilizer with the ratio of 1:2 is applied in 2010 and 2012, and 1:1 is applied in 2011. Our results demonstrate that the ratio of 1:2 in 2012 results in a larger yield improvement compared to the ratio of 1:1 in 2011, which indicates that the application ratio of 1:2 between milk vetch and chemical fertilizer as calculated by N amount is a more beneficial fertilization strategy (as rice is suffered from continuous raining at the heading stage in 2010, only the grain yield improvements in 2011 and 2012 are used for comparative analysis).

The increase of grain yield in MRR treatment was mainly due to the improvement of the number of effective panicles per unit area and the spikelet number per panicle. However, Budhar and Palaniappan (1996) reported that the yield increase under the integrative application of green manure (*Sesbania rostrata*) and chemical fertilizer results in the changes of panicle weight, seed-setting rate and grain weight as well as the number of effective panicles and spikelet number per panicle compared to single-chemical fertilizer. Latt et al (2009) reported that plant height and tiller number (associated with the panicle number per unit area) can both contribute to yield increase. Comprehensively, it can be concluded that the integrative fertilization of milk vetch and chemical fertilizer at an appropriate ratio can steadily improve grain yield by increasing panicle number which can contribute to the efficient cultivation of rice.

Tillering and LAI are associated with high grain yield

Rice tillering plays an important role in improving grain yield, and is a key determinant of the panicle number. Many studies have been conducted to investigate the relationship between rice tillering and grain yield under different environments (Shahidullah et al, 2009; Yan et al, 2010; Zeng et al, 2012). Hasanuzzaman et al (2010) reported the impacts of different fertilizer patterns on the number of tillers, and concluded that the number of tillers per plant is influenced by available nutrients. In this study, we investigated the tillering dynamics of rice under different treatments, and discovered that the total tiller number of MRR treatment is significantly higher than that of FRR treatment according to a two-year field trial. It indicates that growing milk vetch in the winter can improve rice tillering compared to winter fallow, which is probably due to that milk vetch can provide enough and balanced nutrients, such as necessary micronutrients (Belefant-Miller, 2007).

Rice LAI is an important physiological parameter, and can significantly influence grain yield. There are many studies focused on improving grain yield by optimizing LAI (Peng et al, 2007; Xu et al, 2008; Li D Q et al, 2012) LAI is influenced by both organic and inorganic nutrients, fertilization rate and method, and planting density (Zeng et al, 2012). In this study, we investigate the impacts of different treatments on LAI dynamics. At the full heading stage, significantly higher LAI values are observed in MRR treatment than in FRR treatment when only applying chemical fertilizers (Fig. 2). And more tillering and higher LAI can contribute to the higher grain yield of MRR.

Optimized sink-source circulation and grain yield when growing milk vetch in winter

Rice yield is closely associated with sink-source circulation. The sink size and source strength are important for growth rate and yield formation (Sheehy et al, 2001). Our results show that a significantly higher photosynthetic potential, total sink capacity and photosynthetic potential to grain ratio, and a lower grain to leaf ratio are observed in MRR treatment compared to FRR treatment, which may contribute to high yield. In addition, our results indicate that MRR treatment has more efficient transportation and transformation of photosynthetic product from source to sink organ, and more efficient transport of dry matter. The photosynthetic products stored in culm and leaves contribute to 20%–30% of the grain yield in rice (Wang et al, 2005), and can be transported to the spikelets and grains of rice after heading. Therefore, the apparent transportation ratio and the transformation ratio of dry matter play vital roles in grain yield formation. These results indicate that rice plants of MRR treatment have higher apparent transportation ratio and transformation ratio than those of FRR treatment.

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SUPPLEMENTAL DATA

The following material is available in the online version of this article at http://www.sciencedirect.com/science/journal/16726308; http://www.ricescience.org.

Supplemental Table 1. Physic properties of cultivated paddy soil of different treatments in 2010 and 2011.

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