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Co-Incorporating Chinese Milk Vetch and Rice Straw Increases Rice Yield by Improving Nutrient Uptake during Rice Growth

Tingting Ma [1,2], Guopeng Zhou [2], Jia Liu [3], Xiaofen Chen [3], Guilong Li [3], Wenjing Qin [3], Danna Chang [2,]* and Xingjia Xiang [1,]*

[1] Anhui Province Key Laboratory of Wetland Ecosystem Protection and Restoration, School of Resources and Environmental Engineering, Anhui University, Hefei 230601, China; x21301102@stu.ahu.edu.cn
[2] State Key Laboratory of Efficient Utilization of Arid and Semi-Arid Arable Land in Northern China, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China; zhouguopeng@caas.cn
[3] Soil and Fertilizer & Resources and Environment Institute, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, China; liujia@jxaas.cn (J.L.); xfchen@jxaas.cn (X.C.); glli@jxaas.cn (G.L.); qinwenjing033@jxaas.cn (W.Q.)
* Correspondence: changdanna@caas.cn (D.C.); xjxiang@ahu.edu.cn (X.X.)

Abstract: In the past ten years, in paddy rice systems in southern China, the co-incorporation of Chinese milk vetch (MV) and rice straw (RS) has become a new and effective practice in which the advantages of the two species are combined to improve rice yields. However, more studies are needed to better understand the mechanisms by which rice productivity is improved through this practice. In this study, a pot experiment was performed to investigate the effects of different residue management treatments on rice productivity and soil properties. Five treatments were tested: (i) CK (no residue and no chemical fertilizer); (ii) CF (chemical fertilizer); (iii) FM (CF with MV returning); (iv) FR (CF with RS returning); and (v) FMR (CF with a mixture of MV and RS returning). The results showed that the application of MV and/or RS returning improved grain yields by between 13.7% and 31.5%, compared with CF treatment alone. In addition, the application of MV significantly improved rice yield relative to RS returning. However, co-incorporation of MV and RS resulted in the highest yield productivity of all. FMR treatment significantly increased shoot biomass and shoot N, P, and K uptake, compared with FR treatment, at all three growth stages, and compared with FM treatment at the jointing and maturity stages. Moreover, FMR treatment significantly improved grain N, P, and K uptake, relative to FM and FR treatments. These results clearly demonstrated that co-incorporation management promotes nitrogen and phosphorus nutrient uptake at jointing and maturity stages of the rice growth process, compared to application of single residues alone, resulting in higher rice yields. Because incorporation of MV and/or RS increases the available nutrients in the soil and enhances nutrient uptake by the crop, wide-scale adoption of the co-incorporation of residues would significantly increase rice yields and improve soil fertility.

Keywords: co-incorporation; Chinese milk vetch; rice straw; rice yield; nutrient uptake

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1. Introduction

China is one of the largest rice producers in the world and its annual production is about 206 million metric tons (MMT) which represents 28% of the global rice supply [1,2]. Because of the potential impact of even small reductions in this rice production area [3], China faces the great challenge of pursuing self-sufficiency by increasing yields on existing rice areas with minimal environmental and economic cost.

Nutrient uptake characteristics differ with respect to rice cultivars, fertilizer and soil types, fertilization technology, and environmental factors [4,5]. Nutrient uptake amounts also vary across the different stages of the rice growth process. Absorption is low at the seedling stage, then peaks at the jointing stage, and finally decreases as root activity declines [6]. Green-manure cultivation can help to maintain stable soil productivity and high rice yields without increasing inputs of inorganic fertilizer [7–10]. Cultivation of Chinese milk vetch (*Astragalus sinicus* L.) has been widely recommended as an environmentally friendly practice for rice production in paddy fields, due to its ability to fully exploit the natural resources of light, water, and heat during the winter fallow period [8]. Chinese milk vetch is a common green-manure legume plant which can fix atmospheric nitrogen with rhizobia and thereby increase the soil supply of N to subsequently grown rice crops. Previous studies have demonstrated that application of Chinese milk vetch can increase crop yields and the enhance the effectiveness of nitrogen fertilizer [11–14]. Legume green manure decomposes rapidly due to its low C/N ratio and high N concentration, leading to accumulation of soil mineral N, which reaches a peak approximately 2–4 weeks after incorporation [10]. Farmers always transplant rice seedlings two weeks after incorporating green manure. However, because low amounts of available N are needed at the rice seedling stage, most of the N may be lost by leaching or denitrification. Straw incorporation has been widely recommended to improve soil fertility and increase crop yields [15,16]. Rice straw has a high C/N ratio and contains high levels of lignin and polyphenolic content, resulting in a slow decomposition [17]. Moreover, due to low N concentration, amounts of soil-available N may be immobilized by microbes after rice-straw incorporation, resulting in a decrease in plant-available N for subsequently grown crops [14,18] with negative effects on rice yields in the short-term period [19].

Legume–non-legume mixtures have been proposed as a strategy to augment services from cover crops in Europe and the United States [20–22]. Legume–non-legume mixtures improve N management in crop production systems by combining the N-scavenging ability of non-legumes with the biological N_2 fixation ability of legumes [23,24]. This has been shown to increase biomass production and N accumulation in cover-crop mixtures, and to modulate the C/N ratio of the cover crop itself [25,26]. Previous studies have also demonstrated that a mixture of legumes and non-legumes enables a modulated supply and release of N for subsequently grown crops [27–29]. However, less research has been carried out on dynamic nutrient uptake in such crops, especially rice.

In recent decades, as mechanized agricultural operations have become more widespread, farmers in rice-growing countries have increasingly harvested in winter, with high retention of leftover stubble [30]. In China, and in contrast to other countries' use of legume–non-legume mixtures, milk vetch has been incorporated with leftover rice straw at flowering in late April each year. In southern China, such co-incorporation practice has become more and more popular on account of its many benefits. Yang et al. (2019) [31] found that the co-incorporation of Chinese milk vetch and rice straw could increase rice yields by improving N uptake and the enhancing the efficiency of nitrogen fertilizer usage. Zhou et al. (2020) [32] showed that co-incorporation practices could combine the advantage of Chinese milk vetch in increasing soil total nitrogen and mineral N with the advantages of rice straw in increasing soil organic matter and available potassium, thus providing a well-balanced nutrient supply. Co-incorporation management has also been found to stimulate microbial growth, thereby altering the microbial community structure of the soil and increasing soil enzyme activity, enhancing the efficiency of nitrogen fertilizer usage.

For this reason, in this study, we investigated dynamic nutrient uptake during rice growth under different management treatments of Chinese milk vetch and rice straw residues. The objective of our study was to determine whether the co-incorporation of Chinese milk vetch and/or rice straw could promote nutrient uptake in rice during the critical growth period and thus increase rice yields.

2. Materials and Methods

2.1. Soil and Residues

Soil was sampled from a paddy field used for long-term rice cultivation at the Dengjiabu rice-cropping farm (28°15′ N, 116°55′ E, 37.8 m altitude) in Yujiang County, Jiangxi Province, China. The soil was a stagnic anthrosol (FAO classification) derived from river alluvium deposits, with a composition of 345 g kg^{-1} sand, 359 g kg^{-1} silt, and 296 g kg^{-1} clay. Soil samples were randomly collected from soil depths of 0 to 20 cm after the removal of large roots and stones; bulk soil samples were then homogenized, air-dried, sieved through a 2 mm mesh, and stored in a dry area for the pot experiment. The main properties of the soil were as follows: pH = 5.5; soil organic C = 16.5 g kg^{-1}; total N = 1.48 g kg^{-1}; total P = 0.30 g kg^{-1}; total K = 23.6 g kg^{-1}; mineral N = 87.0 mg kg^{-1}; available P = 21.6 mg kg^{-1}; and available K = 103.2 mg kg^{-1}.

The residues of green manure (Chinese milk vetch, *Astragalus sinicus* L., MV) and rice straw (*Oryza sativa* L., RS) were collected from the same location as the soil samples. The collected material was dried at 60 °C for 24 h and then cut to 2 cm lengths. The main properties of the MV residue were as follows: total C = 40.4%; total N= 2.5%; total P = 0.3%; and total K = 2.1%. The main properties of the RS residue were as follows: total C = 40.8%; total N = 0.6%; total P = 0.05%; and total K = 3.2%.

2.2. Experimental Design and Sampling

The outdoor pot experiment was conducted from 25 May to 20 September 2017 at the Chinese Academy of Agricultural Sciences, Beijing. The pot experiment was carried out with five different treatments: (1) CK (no residue and no chemical fertilizer); (2) CF (conventional farmers' practice, chemical fertilizer only); (3) FM (CF with MV returning); (4) FR (CF with RS returning); and (5) FMR (CF with a mixture of MV and RS returning). The amounts of chemical fertilizer and residues applied in each treatment are shown in Table 1. Urea, calcium superphosphate, and potassium chloride were used as N, P, and K fertilizers, respectively. One half of the urea was broadcast as a basal fertilizer; the other half was top-dressed at the jointing-booting stage. P and K fertilizers were both applied as basal fertilizer. Pots were arranged in a completely randomized design with three replications and placed under natural field conditions with 35 cm distance between them. The PCV pots used in the experiments were 30 cm in height with an internal diameter of 23 cm. The residues and chemical fertilizers were mixed into the soil and immediately hydrated with deionized water, leaving a 2 cm layer of water above the soil surface throughout the rice growing period. The rice plants were transplanted 15 days later at a rate of 4 seedlings (20-day-old) per pot.

Table 1. Amounts of residues, chemical fertilizer inputs, and nutrients applied under each treatment.

Treatments	Rice Straw (g pot^{-1})	Green Manure (g pot^{-1})	N–P–K Inputs via Residue (g pot^{-1})	N–P–K Inputs via Inorganic Fertilizer (g pot^{-1})	Total Exogenous Inputs of N–P–K (g pot^{-1})
CK	0	0	0	0	0
CF	0	0	0	1.34–0.29–0.88	1.34–0.29–0.88
FM	0	20	0.50–0.06–0.42	1.34–0.29–0.88	1.84–0.35–1.30
FR	60	0	0.36–0.03–1.92	1.34–0.29–0.88	1.70–0.32–2.80
FMR	60	20	0.86–0.09–2.34	1.34–0.29–0.88	2.20–0.38–3.22

CK, no fertilizer or residues; CF, conventional farmers' practice; FM, conventional farmers' practice and MV; FR, conventional farmers' practice and RS; FMR, conventional farmers' practice with MV and RS returning.

Rice plant and soil samples were collected at the tillering, jointing, and maturity stages. The above-ground biomass of the rice plant was considered as a whole at the tillering and jointing stages, and then divided into grain and straw at the maturity stage. The rice plant was washed with deionized water. Then, the plant samples were transferred to the oven operated at 105 °C and oven-dried at 70 °C until a constant weight was reached and then weighed [19]. Soil samples were collected by randomly drawing three cores from each pot using a soil auger (3 cm in diameter and 20 cm in depth). Large roots or belowground stems of rice were removed and soils were then thoroughly mixed to form composite samples; these were then stored at 4 °C for physicochemical determination.

2.3. Chemical Analysis

Soil and plant total organic C and total N were measured using a CHN elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Soil-available P and K and plant-total P and K were determined according to Chinese Soil Society Guidelines [33]. Microbial-biomass C and N were estimated by the chloroform fumigation–extraction method [34]. Concentrations of dissolved organic C and mineral N in soil solutions were determined as described by Zhou et al. (2019) [35]. Soil pH was measured with a compound electrode (LE438, Mettler-Toledo Instruments, Shanghai, China) at a soil-to-water ratio of 1:2.5.

2.4. Statistical Analysis

Statistical analysis was performed using SAS version 8.1 (SAS Institute Inc., Cary, NC, USA). Effects of crop residue management on soil and plant traits were analyzed using ANOVA at 0.05 levels. The LSD test was used to compare the differences among treatments. Pearson correlation coefficients were calculated for rice plant traits and soil properties, and then analyzed.

Partial least squares path modeling (PLS-PM) was used to explore relationships between residues, soil properties (soil organic matter, soil total nitrogen, mineral nitrogen, available phosphorous, available potassium, soil water-soluble organic matter, soil microbial carbon, and soil microbial nitrogen), rice plant nutrient uptake (N, P, and K), and rice grain yield [36]. Two treatments (RS; GM) were categorical variables with two levels: 1 (a particular treatment) and 0 (the remaining considered treatments). Modeling was carried out in R using the package plspm (1000 bootstraps). The direct effects were represented by path coefficients, indicating the direction and strength of the linear relationships between variables. Indirect effects were the sum of multiplied path coefficients between a predictor and a response variable except for the direct effect. The goodness-of-fit index (GOF > 0.75) and root-mean-square error of approximation (RMSEA < 0.05) were both measured to ensure the adequate fit of the model.

3. Results

3.1. Rice Growth and Grain Yields

Compared with the control (CK), chemical fertilizer or chemical fertilizer with MV and/or RS significantly ($p < 0.05$) increased the shoot biomass (dry weight) of rice at all three growth stages (Figure 1). At the tillering stage, shoot biomass was highest under FM treatment; at the jointing and maturity stages, shoot biomass was highest under FMR treatment. FMR treatment significantly increased shoot biomass in comparison with FM treatment at the jointing and maturity stages, while FMR and FM treatments significantly increased shoot biomass, compared with FR treatment at all three stages. Finally, RS returning significantly decreased shoot biomass, compared with CF treatment at the tillering and jointing stages, but significantly increased shoot biomass at the maturity stage.

The rice grain yields under different treatments ranked from high to low as follows: FMR > FM > FR > CF > CK (Figure 2). Compared with the control (CK), treatment with chemical fertilizer or chemical fertilizer with MV and/or RS significantly ($p < 0.05$) increased grain yields by between 63.0% and 74.7%. Co-incorporations of MV and/or RS (i.e., FMR, FM, and FR treatments) significantly ($p < 0.05$) improved grain yields by between 13.7% and 31.5%, compared with CF treatment. Moreover, the co-incorporation of MV and RS (FMR treatment) significantly ($p < 0.05$) increased grain yields by 11.5% and 20.6%, respectively, in comparison with applications of MV (FM treatment) and RS (FR treatment) alone. In addition, the application of MV significantly increased grain yields by 11.5%, compared with the application of RS.

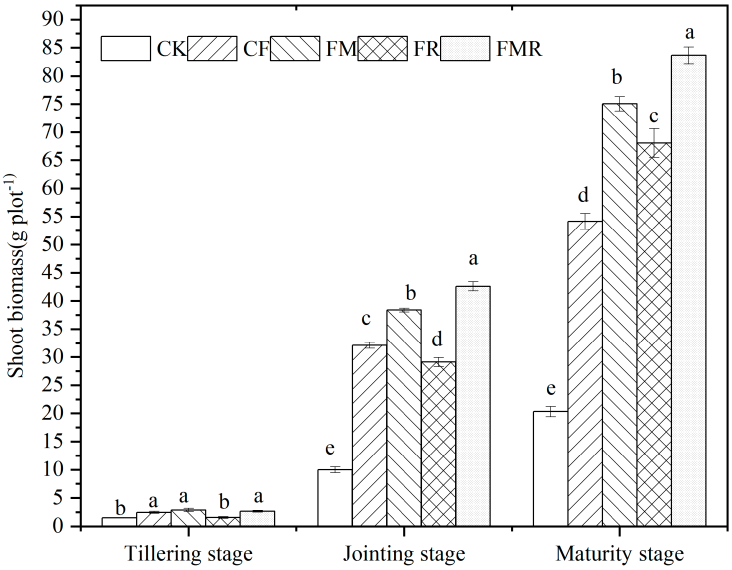

Figure 1. Shoot biomass of rice at different stages under different treatments, given as mean ± standard error (n = 3). Different letters above columns indicate statistical differences among treatments by least significant difference (LSD) test ($p < 0.05$). CK, no fertilizer or residues; CF, conventional farmers' practice; FM, conventional farmers' practice and MV; FR, conventional farmers' practice and RS; FMR, conventional farmers' practice with MV and RS returning.

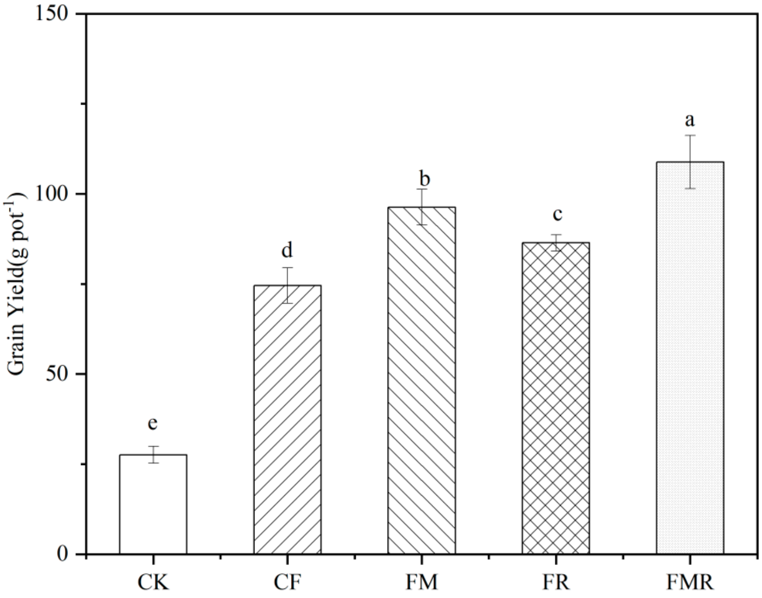

Figure 2. Rice grain yields under different treatments, given as mean ± standard error (n = 3). Different letters above columns indicate statistical differences among treatments by least significant difference (LSD) test ($p < 0.05$). CK, no fertilizer or residues; CF, conventional farmers' practice; FM, conventional farmers' practice and MV; FR, conventional farmers' practice and RS; FMR, conventional farmers' practice with MV and RS returning.

3.2. Uptake of Nitrogen, Phosphorus, and Potassium in Rice Plants

Application of chemical fertilizer or chemical fertilizer with MV and/or RS significantly ($p < 0.05$) increased shoot N, P, and K uptake in comparison with CK at all three stages, with the exception of shoot P uptake under FR treatment at the tillering stage (Figure 3a–c). Moreover, either application of chemical fertilizer alone or with MV and/or RS returning significantly improved grain N, P, and K uptake relative to CK (Figure 3d). FMR treatment significantly promoted shoot N and P uptake in comparison with FR treatment at all three stages, and in comparison with FM treatment at the jointing and maturity stages. FM treatment significantly increased shoot N uptake at all three stages relative to FR treatment, and also increased shoot P uptake at the tillering and jointing stages. Compared with CF treatment, FR treatment significantly decreased shoot N and P uptake at the tillering and jointing stages, but significantly increased shoot N, P, and K uptake at the maturity stage. FMR treatment significantly increased shoot K uptake relative to FM treatment at the jointing and maturity stages, and also improved shoot K uptake relative to FR treatment at all three stages. Shoot K uptake was significantly lower under FR treatment at the tillering stage, in comparison with FM treatment, but significantly higher at the maturity stage. FMR treatment significantly improved grain N, P, and K uptake, in comparison with both FM and FR treatments. Finally, grain N and K uptake was significantly higher under FM treatment, compared with FR treatment, but there was no significant difference between FM and FR treatments with respect to grain P uptake.

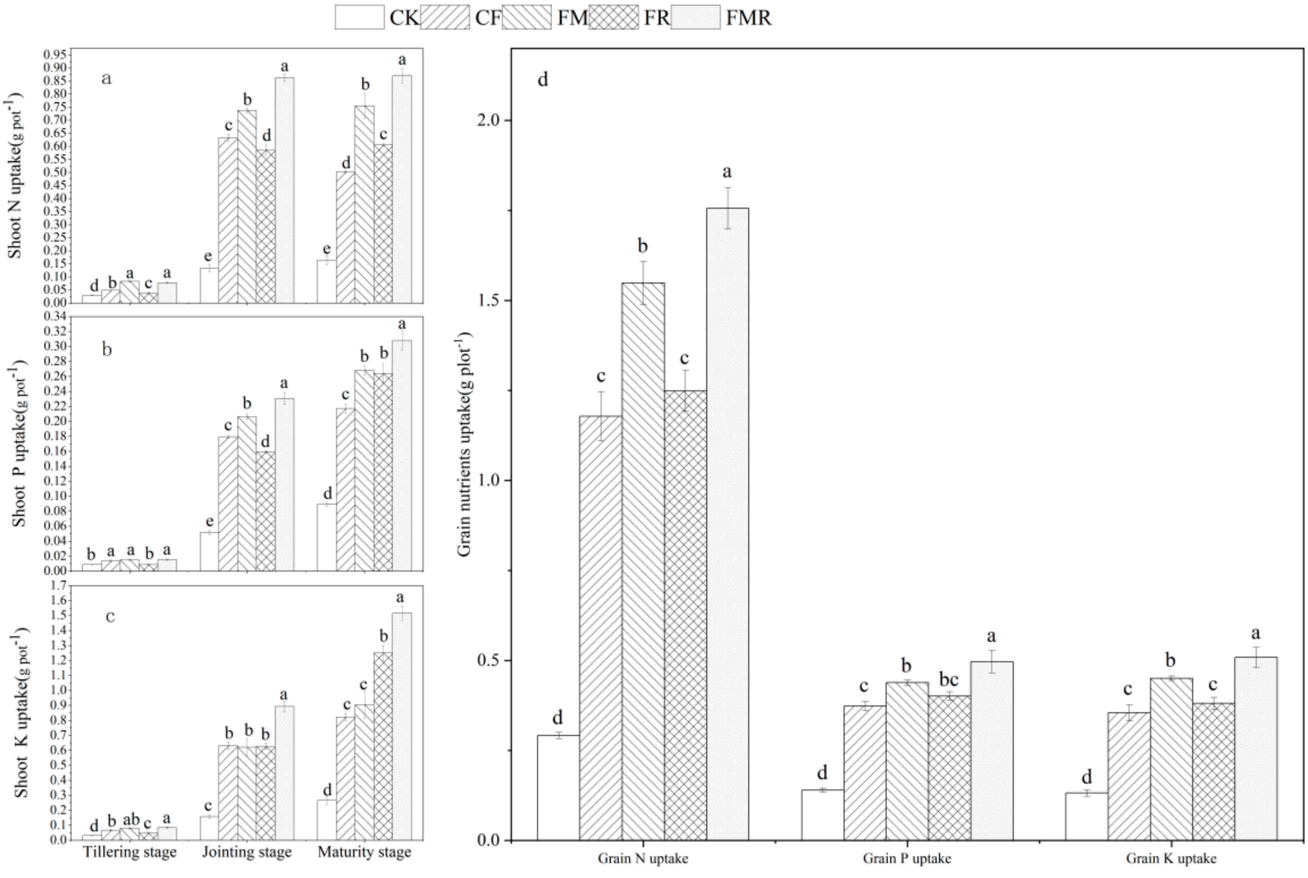

Figure 3. Shoot N uptake (**a**); shoot P uptake (**b**); shoot K uptake (**c**); and grain N, P, and K uptake (**d**) at different growth stages under different treatments, given as mean ± standard error (n = 3). CK, no fertilizer or residues; CF, conventional farmers' practice; FM, conventional farmers' practice and MV; FR, conventional farmers' practice and RS; FMR, conventional farmers' practice with MV and RS returning. Different letters above bars represent differences from Duncan's HSD comparisons ($p < 0.05$).

3.3. Soil Properties

At all three stages, FM, FR, and FMR treatments significantly increased soil mineral N (N_{min}), soil microbial carbon (SMBC), soil microbial nitrogen (SMBN), and dissolved organic carbon (DOC), compared with CF treatment. In addition, FM and FMR treatments significantly increased total nitrogen (TN), and FM, FR, and FMR treatments increased soil organic carbon (SOC) at the maturity stage, in comparison with CF treatment. At all three stages, N_{min} was significantly higher under FMR treatment, compared with FM and FR treatments. N_{min} was significantly higher under FM treatment in comparison with FR treatment at the jointing stage, but significantly lower in comparison with FR treatment at the maturity stage. FMR treatment significantly increased soil-available phosphorus (AP) and soil-available potassium (AK) in comparison with FM and FR treatments at all three stages, with the exception of AK under FR treatment at the tillering stage. Furthermore, at all three stages, FR treatment significantly improved AP and AK, compared with FM treatment. FR and FMR treatments significantly improved SMBC and SMBN in comparison with FM treatment at all three stages. Finally, FMR treatment significantly increased DOC in comparison with FM treatment at the tillering and maturity stages, and as well as improving DOC in comparison with FR treatment at tillering and jointing stages (Table 2).

Table 2. Soil nutrients at different stages under different treatments.

		$\mathbf{N_{min}}$	**AP**	**AK**	**SMBC**	**SMBN**	**DOC**	**TN**	**SOM**
Stage	Treatment	$mg\ kg^{-1}$							
Tillering stage	CK	11.64 ± 0.77^{b}	35.76 ± 0.68^{b}	29.70 ± 1.43^{d}	848.98 ± 14.39^{c}	62.83 ± 2.33^{c}	24.31 ± 2.85^{d}		
	CF	8.92 ± 0.66^{c}	30.19 ± 1.89^{d}	46.47 ± 3.72^{c}	841.14 ± 14.27^{c}	55.51 ± 3.53^{d}	28.31 ± 2.36^{d}		
	FM	11.96 ± 0.44^{b}	33.28 ± 0.10^{c}	35.32 ± 2.53^{d}	940.63 ± 10.87^{b}	75.17 ± 4.32^{b}	53.34 ± 3.40^{b}		
	FR	12.74 ± 0.79^{b}	39.33 ± 1.51^{b}	87.46 ± 5.80^{a}	997.01 ± 11.75^{a}	85.75 ± 4.37^{a}	41.78 ± 1.55^{c}		
	FMR	14.70 ± 0.80^{a}	43.80 ± 2.54^{a}	63.25 ± 5.26^{b}	993.34 ± 9.96^{a}	83.65 ± 3.22^{a}	66.54 ± 4.03^{a}		
Jointing stage	CK	12.45 ± 0.87^{c}	13.27 ± 0.84^{d}	30.46 ± 0.88^{c}	677.47 ± 18.25^{b}	19.77 ± 3.51^{c}	57.21 ± 3.55^{c}		
	CF	10.76 ± 0.64^{d}	30.53 ± 1.47^{c}	25.71 ± 0.65^{d}	645.96 ± 17.79^{c}	14.13 ± 1.55^{d}	48.75 ± 4.10^{d}		
	FM	17.65 ± 0.95^{b}	28.97 ± 1.55^{c}	26.19 ± 0.80^{d}	688.57 ± 5.08^{b}	22.95 ± 1.67^{b}	93.71 ± 5.66^{a}		
	FR	10.05 ± 0.25^{d}	33.29 ± 1.77^{b}	38.12 ± 2.26^{b}	812.49 ± 7.17^{a}	29.87 ± 1.27^{a}	87.59 ± 4.11^{b}		
	FMR	21.80 ± 0.73^{a}	57.60 ± 1.88^{a}	42.36 ± 2.00^{a}	821.97 ± 11.31^{a}	32.21 ± 1.61^{a}	96.45 ± 5.51^{a}		
Maturity stage	CK	12.50 ± 0.95^{d}	12.85 ± 0.90^{d}	35.76 ± 0.68^{b}	746.94 ± 21.29^{e}	43.31 ± 4.15^{ab}	76.10 ± 5.39^{c}	1.44 ± 0.01^{c}	16.50 ± 0.06^{d}
	CF	14.43 ± 1.29^{d}	18.87 ± 2.80^{c}	30.19 ± 1.89^{d}	814.46 ± 31.25^{d}	32.41 ± 4.12^{c}	55.68 ± 3.32^{d}	1.46 ± 0.01^{c}	16.13 ± 0.14^{e}
	FM	20.23 ± 0.90^{c}	18.47 ± 0.88^{c}	33.28 ± 0.10^{c}	890.66 ± 10.93^{c}	40.47 ± 4.75^{b}	84.56 ± 4.44^{b}	1.54 ± 0.01^{b}	17.12 ± 0.12^{c}
	FR	26.54 ± 2.07^{b}	30.81 ± 2.36^{b}	42.33 ± 1.51^{a}	938.62 ± 56.13^{b}	48.67 ± 3.18^{a}	90.32 ± 2.21^{a}	1.48 ± 0.02^{c}	17.81 ± 0.13^{b}
	FMR	33.16 ± 1.28^{a}	51.55 ± 0.61^{a}	43.80 ± 2.54^{a}	1072.22 ± 17.82^{a}	47.01 ± 3.56^{a}	92.04 ± 1.40^{a}	1.59 ± 0.03^{a}	18.41 ± 0.09^{a}

N_{min}, mineral N; AP, available phosphorus; AK, available potassium; SMBC, soil microbial carbon; SMBN, soil microbial nitrogen; WSOC, water-soluble organic carbon. Values (mean ± S.D.) in the same column followed by different letters are significantly different from Duncan's HSD comparisons ($p < 0.05$). CK, no fertilizer or residues; CF, conventional farmers' practice; FM, conventional farmers' practice and MV; FR, conventional farmers' practice and RS; FMR, conventional farmers' practice with MV and RS returning.

3.4. Interaction between Soil Properties and Rice Traits

The Pearson correlation analysis showed significantly positive correlations of rice grain yields, shoot N and P uptake, and grain N and P uptake with most soil properties, namely N_{min}, AP, SMBC, DOC, soil total nitrogen (TN) and soil organic matter (SOM); correlations with AK and SMBN were not significant. However, soil AK was significantly and positively correlated with straw yield and straw K uptake (Figure S1).

The relationships among those variables were further explored using PLS-PM (Figure 4). Although most soil properties exhibited significant positive correlations with grain N, P, and K uptake, the direct effects (−0.0038) of soil on grain N, P, and K uptake were negative; in contrast, larger indirect effects (0.74) on soil and grain N, P, and K uptake were identified in the PLS-PM model (Table S1). Moreover, the direct effects (0.80) of soil on straw N, P, and K uptake were significantly positive and an obvious correlation (0.92) was found between straw and grain N, P, and K uptake. Those results may demonstrate that soil indirectly influenced grain nutrient uptake through nutrients transferred from straw to grain. Green manure and rice straw both had direct effects on soil properties, of 0.59 and 0.70, respectively, as well as indirect effects on rice yield, of 0.46 and 0.54, respectively, implying that both green manure and rice-straw incorporation improved rice yield by affecting soil nutrients. The loading scores of soil properties suggested that soil N_{min} was the most direct indicator of soil-available nutrients and that SMBN was a better indirect indicator than other soil nutrients, based on Pearson correlation analysis (Figure S1).

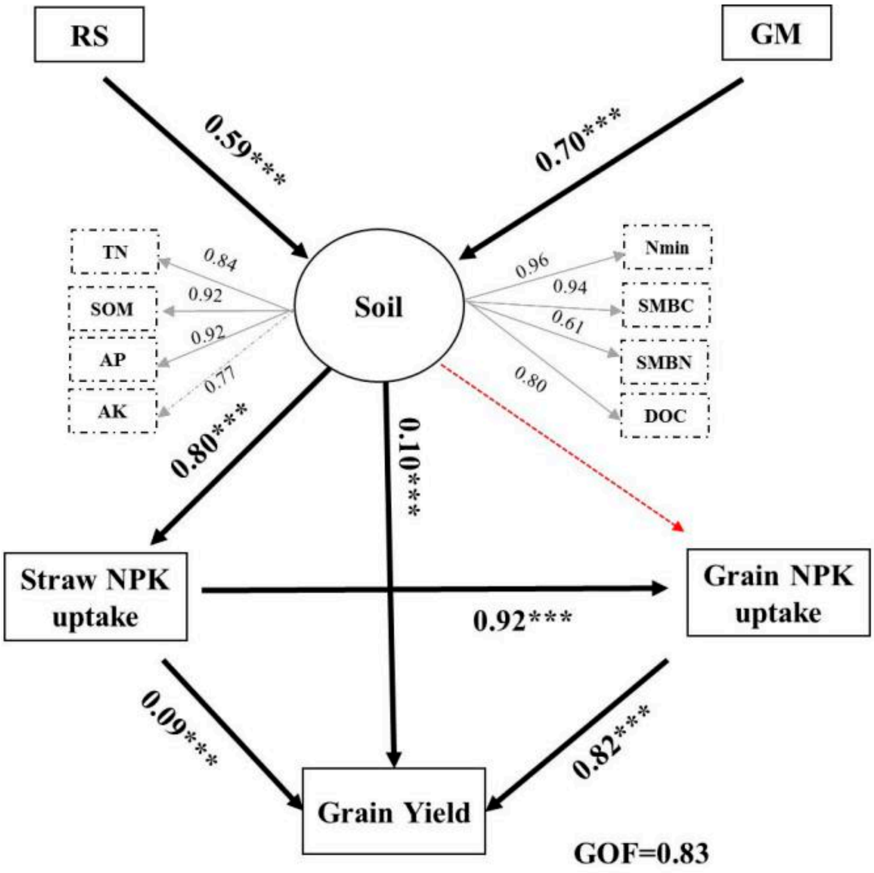

Figure 4. Partial least squares path model (PLS-PM) based on the effects of soil nutrient uptake and rice nutrient uptake on the rice yield (*** $p < 0.001$).

4. Discussion

Cultivations of green manure and straw returning are both effective ways to improve soil fertility and increase crop yields, and they offer a great potential opportunity to reduce the use of inorganic fertilizer [12,15,16,37]. Our results showed that the application of MV and/or RS returning significantly increased grain yield by between 13.7% and 31.5% in comparison with CF treatment. The application of MV also significantly improved grain yield in comparison with RS returning; however, co-incorporating MV and RS resulted in the highest yield of all. Similar results have been reported in previous studies when combinations of legume and non-legume (gramineous) residues have been applied [37–39]. These studies have also revealed possible reasons for such improvements in grain yield, including the following: (1) co-incorporation management prolongs the availability of soil nutrients, and synchronizes the release of nutrients with the demands of rice, thereby increasing growth and yield in the resulting crop; (2) the decomposition of rice straw may be accelerated by addition of legume residues as a result of a decreased C/N ratio in the mixture, thus mitigating N immobilization and increasing plant-available N for the subsequent rice crop; (3) combined residue management results in a greater accumulation of more balanced soil nutrients from the mixture than use of a single residue alone, leading to higher plant nutrient availability for rice growth. However, few studies have investigated how co-incorporation management affects the dynamics of growth and nutrient uptake in the subsequent rice crop.

Crop residue return plays an essential role in soil nutrient cycling, thereby affecting plant growth and nutrient uptake [40]. In our study, the application of MV significantly increased shoot biomass and shoot N at all three stages, and also improved shoot P uptake at the tillering and jointing stages in comparison with RS returning. In addition,, the application of MV significantly improved grain N, P, and K uptake relative to RS returning.

Finally, the application of MV also increased rice growth and N and P uptake in comparison with RS returning. This may be due to the fact that MV has a low C/N ratio of 16, resulting in rapid decomposition and high N and P release, but rice straw has a high C/N ratio of 68, resulting in slow decomposition and low N and P release [37,39]. RS returning significantly decreased shoot biomass as well as shoot N and P uptake at the tillering and jointing stages in comparison with CF treatment. This result shows that rice straw fixes applied N in soil, resulting in a decrease in plant-available N for rice and leading to negative effects on rice growth over short-term periods [14,18,19]. However, compared with CF treatment, RS returning significantly increased shoot biomass as well as shoot N, P, and K uptake at the maturity stage. This might be because of remineralization from the immobilized microbial biomass N in RS, promoting further decomposition [38]. Moreover, the addition of ear-differentiation-fertilizer N could mitigate N immobilization and accelerate RS decomposition.

Co-incorporation with MV and RS significantly increased shoot biomass and shoot N, P, and K uptake relative to FR treatment at all three stages, and relative to FM treatment at the jointing and maturity stages. Moreover, co-incorporation with MV and RS significantly improved grain N, P, and K uptake in comparison with FM and FR treatments. This demonstrated that co-incorporation increased growth and nutrient uptake in the subsequent rice crop, and also promoted nutrient transfer to grain, compared with single-application treatments. Previously, Kaewpradit et al. (2009) [38] and Yang et al. (2019) [31] found that co-incorporation management increased shoot N uptake. In addition, Kaewpradit et al. (2009) [38] revealed that co-incorporation management could delay N release in leguminous residue, leading to improved synchrony in N demand/supply and increased growth in subsequent rice crops. This was confirmed by our finding that co-incorporation management increased rice growth and nutrient uptake in the later stages (jointing and maturity), compared with the application of MV alone. Yang et al. (2019) [31] also considered that MV accelerated the decomposition of rice straw by decreasing the C/N ratio of the mixture, thus mitigating N immobilization. Furthermore, previous studies have also demonstrated that co-incorporation management may promote microbial growth, change the structure of the soil microbial community, and increase soil enzyme activity, further promoting the release of N, P, and K nutrients in soil, and increasing shoot biomass and nutrient uptake [32]. This explains why, in our study, co-incorporation management increased rice growth and N uptake at all three stages, in comparison with the application of RS returning alone.

Our results showed that, compared with single applications, co-incorporation management produced higher accumulations of soil-available nutrients (N_{min}, AP, AK, and DOC), even after higher rice N, P, and K uptake, with the exception of AK under FR treatment. N_{min} was significantly lower under FM treatment, compared with FR treatment, at the maturity stage. In addition, AP and AK were significantly lower under FM treatment, compared with FR treatment, at all three stages. These results imply that co-incorporation management could prolong nutrient availability, in comparison with the application of MV alone.

The PLS-PM model showed that both green manure and rice-straw incorporation improved rice yield through affecting soil nutrients; among these, soil mineral N was the most powerful indicator. Previously, Kaewpradit et al. (2009) [38] found a close correlation of soil mineral N with grain yield and also with N uptake. The C/N ratios of residues have also been shown to influence soil N availability for subsequent crops [41]. In general, residues with C/N ratios <25 result in net N mineralization, while residues with C/N ratios >25 result in net N immobilization [42]. Previous studies have demonstrated that combinations of legume and non-legume residues changed the N mineralization of a single residue by manipulating the C/N ratios of residues. N mineralization and release from legume residues are thereby delayed thus reducing N leaching losses, and prolonging nutrient availability [43–47]. The dynamic nutrient uptake in our study showed that rice was able to effectively take advantage of the delayed N release i.e., co-incorporation management accumulated the highest soil-available nutrients at all three stages and increased nutrient uptake of rice at jointing and maturity stages relative to the application of MV alone.

In recent decades, a number of practical problems have been reported in southern China, such as waste of light and heat resources, as well as difficulties in the utilization of rice straw and loss of soil fertility in some areas. In addition, the practice of farmers burning straw after rice harvest for the sake of saving time and labor has continued to occur. This practice not only wastes resources, but also pollutes the environment and seriously affects the sustainable development of agriculture [48]. Intensive cropping with no return of crop residues or other organic inputs to the soil results in the loss of soil organic matter and nutrient supply; as a practice, it is assumed to be non-sustainable [49]. Sound agronomic practices are needed to maintain the carbon balance in an agro-ecosystem in order to sustain soil fertility. Previous studies of various cropping systems have shown that Chinese milk vetch or rice straw can increase soil C and N stocks, improve soil nutrient storage capacity, and thus enhance soil health and sustainability [31,50]. The results of this study show that co-incorporation management is able to increase growth and nutrient uptake in subsequently grown rice crops, and lead to the highest accumulation of soil-available nutrients. Although the results showed that co-incorporation with Chinese milk vetch and rice straw greatly improved rice yield and nutrient uptake, our current experiment was limited to a pot experiment, where the condition for rice growth was slightly different from the actual farmland, so further research needs to be conducted to confirm our findings under field conditions. Co-incorporation with Chinese milk vetch and rice straw also improves rice yields by affecting soil nutrients and manipulating the C/N ratios of residues. Overall, this technique may be recommended as a productive and sustainable practice for rice production in southern China.

5. Conclusions

Application of MV and/or RS returning evidently improved grain yields in comparison with CF treatment. Moreover, the application of MV significantly improved grain yield relative to RS returning, and co-incorporation of MV and RS produced the highest yields of all. Compared with single applications, co-incorporation management promoted nitrogen and phosphorus nutrient uptake at jointing and maturity stages of rice growth, resulting in higher yields. In addition, compared with single applications, co-incorporation management led to higher accumulations of soil nutrients in most cases. Overall, this study shows that the co-incorporation of MV and RS can be seen as a feasible means of addressing the problem of rice-straw resource utilization, and it provides a theoretical basis for achieving higher crop yields and more sustainable agricultural development.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su151612183/s1. Figure S1: Pearson correlation analysis between properties and rice traits; Table S1: Path analysis results of indirect effects on rice yield and nutrient uptake.

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