

## Article

# Effects of Chinese Milk Vetch Returning on Soil Properties, Microbial Community, and Rice Yield in Paddy Soil

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**Abstract:** The application of green manure is considered a sustainable management approach to improve soil quality and crop yield in agricultural production. However, few studies have focused on the effects on soil properties and the microbial community when varied green manure rates are applied in rice cropping systems. We examined the soil and microbial properties in paddy soil and evaluated rice productivity and sustainable yield index in response to Chinese milk vetch (MV) application at various rates (0, 22.5, 30, 45, and 60 Mg ha<sup>-1</sup>, labeled MV0, MV22.5, MV30, MV45, and MV60, respectively) in a seven-year (2014–2020) field experiment. For all treatments, MV application significantly improved rice yield by 22.5–29.7% and sustainable yield index by 10.3–13.0% compared with no MV application, and the highest average yield across the 7 years of the study was found in MV60, followed by MV45, MV30, and MV22.5. Compared with MV0, MV45 and MV60 treatments significantly reduced soil bulk density (BD), increased soil organic carbon (SOC), total nitrogen (TN), total potassium (TK), and alkali solution nitrogen (AN), particularly in MV45. Moreover, compared with MV0, a marked decrease in the alpha diversity (Shannon index) of the bacterial community and a significant increase in the relative abundance of Firmicutes were observed under MV45. The alpha diversity of the fungal community did not vary across treatments, but MV45 significantly reduced the relative abundance of Ascomycota and Chytridiomycota compared with MV0. In addition, redundancy analysis indicated that the fungal community structure was significantly related to soil BD, and random forest classification analysis demonstrated that SOC and AN were the most important factors for rice yield in this study. Overall, these results indicated that MV application at 45 Mg ha<sup>-1</sup> should be recommended to simultaneously achieve the improvement of rice yield and soil quality in the South China.

**Keywords:** Chinese milk vetch (*Astragalus sinicus* L.); rice yield; soil physicochemical properties; microbial community

## 1. Introduction

Rice (*Oryza sativa* L.) is a staple food crop and feeds over half of the world's population. China is one of the largest rice producers in the world, contributing to 28.0% of global rice production in 18.5% of the global rice growing area [1]. With the growing population, global rice production will need to increase by an annual yield of 1.2–1.5% over the next few decades to satisfy expected food demands without rice-growing area expansion [2]. This will increase the consumption of chemical fertilizers [3]. However, the excessive use of chemical fertilizers has created negative impacts, including low resource utilization

efficiency, soil quality degradation, and environmental health threats [4]. Therefore, finding a sustainable and ecofriendly alternative strategy to increase rice production and satisfy food demands is critical.

Sustainable and renewable farming through the application of animal manure [5], farmyard manure [6], and compost [7] has experienced rapid growth in recent years. However, the application of organic manure may have some adverse effects, including the accumulation of heavy metals and antibiotics in soil and crop grains, and high economic risks [8,9]. Green manure, a clean organic manure resource, lacks heavy metals, antibiotics, and other residual hazards, and can effectively reduce these adverse effects [10]. As a winter-growing legume green manure, Chinese milk vetch (*Astragalus sinicus* L., MV) has been widely grown within rice production systems as an alternative to bare fallow in southern China [11]. Some studies have shown that planting MV can fully exploit and obtain natural resources (e.g., light, temperature, and water) in the winter fallow rice fields [12,13], improve the carbon flux into soil, and increase soil organic carbon sequestration [14]. Additionally, it can fix atmospheric nitrogen in root nodules with rhizobia and improve phosphorus and potassium availability by regulating soil enzymatic activities in the soil, resulting in nutrient accumulation in MV and the soil [15,16]. Therefore, returning MV to the paddy fields not only provides nutrients to meet the growth demand of succeeding crops (e.g., rice), but also improves soil quality, ultimately improving grain yield [17,18]. Previous reports have shown that the substitution of chemical fertilizers with MV can increase the soil organic carbon content and nutrient pool, thus improving rice yield [12]. However, research on the effects of different MV application rates on rice yield and soil properties, in particular, soil microbial community properties, is limited.

Soil microorganisms play critical roles in soil nutrient cycling, litter decomposition, soil fertility maintenance, and crop productivity, which maintain multiple agroecosystem functions and sustainability [19,20]. Several studies have indicated that soil microorganisms (e.g., *Proteobacteria* and *Acidobacteria*) promote the decomposition of green manure and release vital nutrients into the soil by secreting extracellular enzymes, which could subsequently be assimilated by crops and affect soil quality [21,22]. A 31-year long-term rotation of rice-rice-different green manure treatments shaped the rhizosphere microbial community and improved the relative abundance of beneficial bacteria (e.g., *Acinetobacter* and *Pseudomonas*) compared to rice-rice-winter fallow treatment [23]. Compared to no green manure-returning treatment, the average soil organic carbon (SOC), total nitrogen (TN) and available N levels under MV-returning treatment were increased, and the relative abundances of *Acidobacteria* and *Verrucomicrobia* were also increased, which was conducive to the gain in maize yield [10]. Although previous studies have demonstrated that adding different kinds of green manure improved soil nutrient concentrations and altered soil microbial communities [10,22], these effects under varied MV-returning rates are currently unclear.

We postulated that: (1) varied long-term MV application rates could improve soil physicochemical properties and the soil microbial community, thus improving rice productivity in paddy soil; (2) there is a threshold rate of MV application, above which there will be no further increase on soil properties and rice productivity. Therefore, the objectives of this study were to verify suitable MV application rates in terms of assessing their ability to increase rice yield, improve soil physicochemical and microbial properties, and provide guidance for regional agricultural sustainable production.

## 2. Materials and Methods

### 2.1. Study Area and Site Characterization

The study site was located at the Xinyang Academy of Agricultural Sciences Experimental Park (32°07' N, 114°05' E) in the southern Henan Province, China. This area belongs to the transition zone from the subtropical to the warm temperate zone. The mean annual precipitation is 900–1400 mm. The field plot experiment began in 2014, and the tested soil was yellow–brown gley paddy soil. The basic chemical properties in the top 20-cm soil

profiles were as follows: 12.9 g kg<sup>-1</sup> soil organic carbon (SOC), 1.15 g kg<sup>-1</sup> total nitrogen (TN), 76.8 mg kg<sup>-1</sup> alkali solution nitrogen (AN), 5.4 mg kg<sup>-1</sup> available phosphorus (AP), 59.1 mg kg<sup>-1</sup> available potassium (AK), and pH 6.7.

## 2.2. Experiment Design and Field Management

The field experiment was conducted for 7 consecutive years (2014–2020), and included five treatments: (1) MV0, no addition of MV; (2) MV22.5, 22.5 Mg ha<sup>-1</sup> MV; (3) MV30, 30 Mg ha<sup>-1</sup> MV; (4) MV45, 45 Mg ha<sup>-1</sup> MV; and (5) MV60, 60 Mg ha<sup>-1</sup> MV. The area of each plot was 6.67 m<sup>2</sup> (3.33 m × 2.0 m), and all treatments were allocated in a randomized block design with four blocks. The rice cultivar used in this experiment was ‘Yang Liangyou 013’, and the sowing date was late April, with transplant in late May. The transplant density of rice was at a spacing of 16.7 cm × 20 cm with artificial seeding and 2 seedlings per hole. The final rice harvest of the experiment occurred in late September. At maturity, rice was harvested manually from the entire plot and divided into straw and grain by a plot-thresher. The rice straw was removed, and the grain was weighed after being sun-dried to obtain the annual yield. Chinese MV (*Astragalus sinicus* L., cv. ‘Xinzi 1’) was directly sown after the rice harvest during the winter season, and it was harvested every year at the full-bloom stage, measured, and applied sequentially to each plot based on the experimental design. The water content of fresh MV was 90%, and the N, P, and K concentrations of dried MV were 3.75, 0.34, and 3.5 g kg<sup>-1</sup>, respectively. Ridges were built between plots and covered with plastic film to prevent the movement of water and fertilizer. A 0.3-m-wide ditch was reserved between the blocks to facilitate water supply and drainage. Irrigation and control of diseases and insect pests were consistent with local agronomic practices.

## 2.3. Sampling and Laboratory Procedures

Soil samples (0–20 cm soil depth) were collected in September 2020 (after rice harvest). Five soil cores were collected from each plot, mixed into one composite sample, and dried naturally. The air-dried soil samples were ground to pass through a 2-mm sieve to determine the AN, AP, and AK concentrations. Then, the soil samples were ground to pass through a 0.25-mm sieve to determine the SOC, TN, total P (TP), and TK concentrations. Another five soil cores in each plot were collected randomly, combined into one sample, and preserved at –80 °C for microbial community analysis.

Soil organic carbon and total N concentrations were determined using a Vario MACRO elemental analyzer (Elementar, Langensfeld, Germany). The alkali nitrogen solution was measured by a 1 mol L<sup>-1</sup> NaOH alkaline hydrolysis diffusion method [23]. The total P was determined by H<sub>2</sub>SO<sub>4</sub>–HClO<sub>4</sub> digestion, followed by molybdenum antimony anti-colorimetry, and available P was extracted with 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub> [24]. The total K was determined by the NaOH melting method, and available K was extracted with 1 mol L<sup>-1</sup> ammonium molybdate and analyzed by flame photometry [25,26]. The BD was determined by collecting undisturbed soil samples with a cutting ring (50.46 mm inner diameter, 50 mm long) and oven-drying at 105 °C to a constant weight for each plot [15].

## 2.4. DNA Extraction, PCR Amplification, and MiSeq Sequencing of the Soil

Microbial community genomic DNA was extracted from 0.5 g of freeze-dried soil samples using the E.Z.N.A.<sup>®</sup> Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer’s instructions [27]. The DNA extract was checked on a 1% agarose gel, and DNA concentration and purity were determined with a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, OH, USA). The hypervariable region V3–V4 of the bacterial 16S rRNA gene and the ITS1 variable region of the fungal ITS gene were amplified with primer pairs 338F/806R (5’-ACTCCTACGGGAGGCAGCAG-3’/5’-GGACTACHVGGGTWTCTAAT-3’ and ITS1F/ITS2R (5’-CTTGGTCATTTAGAGGAAGTAA-3’/5’-GCTGCGTTCTTCATCGATGC-3’) by an ABI GeneAmp<sup>®</sup> 9700 PCR thermocycler (ABI, Los Angeles, CA, USA). PCR amplification was performed as follows: initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturing at 95 °C for 30 s,

annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, a single extension at 72 °C for 10 min, and ending at 4 °C. The PCR mixtures contained 4 µL 5 × TransStart FastPfu buffer, 2 µL 2.5 mM dNTPs, 0.8 µL forward primer (5 µM), 0.8 µL reverse primer (5 µM), 0.4 µL TransStart FastPfu DNA Polymerase, 10 ng template DNA, and enough ddH<sub>2</sub>O to reach a volume of 20 µL. PCR reactions were performed in triplicate. The PCR product was extracted from a 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified using a Quantus™ Fluorometer (Promega, Madison, WI, USA). Purified amplicons were pooled in equimolar concentrations and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, CA, USA) according to the standard protocols of Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw files were demultiplexed, quality-filtered using fastp (<https://github.com/OpenGene/fastp>, version 0.20.0, accessed on 20 September 2020), and merged using FLASH (<http://www.cbc.umd.edu/software/flash>, version 1.2.7, accessed on 20 September 2020). Operational taxonomic units with 97% similarity cut-off were clustered using UPARSE (<http://drive5.com/uparse/>, version 7.1, accessed on 20 September 2020), and chimeric sequences were identified and removed. The taxonomy of each operational taxonomic unit representative sequence was analyzed by the Ribosomal Database Project Classifier (<http://rdp.cme.msu.edu/>, version 2.2, accessed on 20 September 2020) against the 16S rRNA database (Silva v138) using a confidence threshold of 70% [28]. The MiSeq sequencing data were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (the accession number: PRJNA816666).

### 2.5. Statistical Analyses

Rice production sustainability was evaluated by the sustainable yield index, and the calculation formula is as follows:

$$SYI = (Y_{\text{mean}} - \sigma - 1) / Y_{\text{max}} \quad (1)$$

where SYI is the sustainable yield index,  $Y_{\text{mean}}$  is the mean yield,  $\sigma - 1$  is the standard deviation of yield for specific treatment across years, and  $Y_{\text{max}}$  is the maximum yield obtained under that treatment during 2014–2020.

The alpha diversities of the bacterial and fungal communities were estimated through the Chao richness index and Shannon diversity index, which were generated by Quantitative Insights into Microbial Ecology (QIME version 1.17).

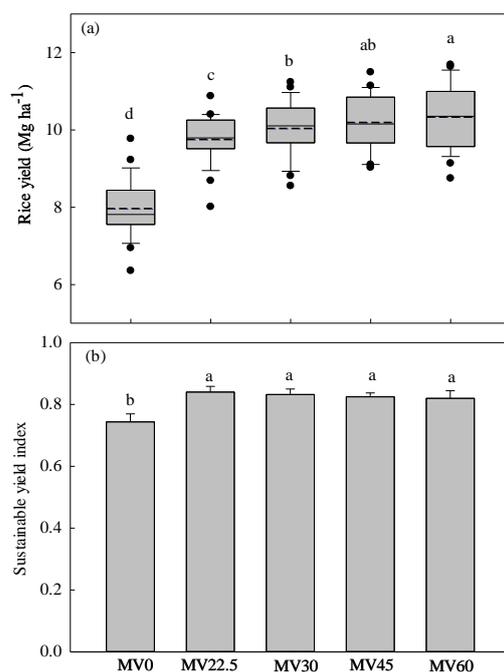
The effects of treatment and year on rice yield were evaluated using a two-way analysis of variance (ANOVA). The effects of treatment on soil physicochemical properties (BD, SOC, TN, AN, TP, AP, TK, and AK), alpha diversity (Chao and Shannon indexes), and the compositions of bacterial and fungal communities were analyzed using a one-way ANOVA. Multiple comparisons of treatment mean values were separated by Duncan's test to identify significant effects ( $p < 0.05$ ). Statistical analyses of all data were conducted using SAS software (SAS Institute; Cary, NC, USA). The relationships between microbial community structure, soil properties, and rice yield were estimated with Spearman correlation coefficients using SPSS software (version 20.0). The importance of certain soil properties in rice yield among treatments was analyzed by random forest classification analysis using the "randomforest" package in R (version 4.1.2). Redundancy analysis (RDA) was conducted to estimate the relationship between soil physicochemical properties and microbial community structure using Canoco software 5.0.

## 3. Results

### 3.1. Rice Yield and Sustainability Yield Index

Rice yield was markedly affected by treatment, year, and interactive effects (treatment × year; Table S1,  $p < 0.001$ ). Over the 7 years, the average rice yield under all treatments exhibited the following order: MV0 < MV22.5 < MV30 < MV45 < MV60. The

yield under the four MV treatments was markedly higher than that of the MV0 treatment by 22.5–29.7%, and there was a similar trend each year (Figures 1a and S1). In 2014 and 2015, the rice yield under MV30, MV45, and MV60 was markedly higher than that under MV22.5, and no significant difference was observed between MV45 and MV60. However, rice yields were not significantly different among the four MV treatments from 2016 to 2019. In 2020, the rice yield under MV45 was markedly higher than that under MV22.5, and no significant differences were observed between MV30, MV45, and MV60 (Figure S1). Across all treatments, the average rice yield in 2019 was 10.5 Mg ha<sup>-1</sup>, which was significantly higher than that in other years by 3.23–20.0%, and the lowest yield was observed in 2020. Compared with the MV0 treatment, the sustainability yield index markedly increased by 10.3–13.0% under the four MV treatments, and there was no significant difference among them (Figure 1b).



**Figure 1.** Rice yield (a) and sustainable yield index (b) under application of varied Chinese milk vetch (MV) rates from 2014 to 2020. In the box-plots (a), the solid and dashed horizontal lines represent the median and mean values within each box. The box boundaries indicate the 25th and 75th percentile values, and whisker caps show the 5th and 95th percentile values, while the dots show outliers. Values represent data from the 7 years and four replicates ( $n = 28$ ) under each box. In bar graph (b), horizontal error bars denote the standard deviation of the means ( $n = 4$ ). Different letters indicate significant differences between application of varied MV rates at  $p < 0.05$ .

### 3.2. Soil Physicochemical Properties

Soil properties are important indicators for evaluating soil quality. After 7 years of MV application, marked effects of treatment on SOC ( $p < 0.001$ ), TN ( $p < 0.001$ ), total potassium (TK) ( $p < 0.05$ ), alkali solution nitrogen (AN) ( $p < 0.001$ ), and available potassium (AK) ( $p < 0.05$ ) were observed (Table 1). SOC was the highest in the MV45 treatment, followed by MV30, MV60, and MV22.5, compared with the MV0 treatment, with increases of 23.6%, 22.6%, 21.7%, and 13.2%, respectively. The TN in MV45 and MV60 was significantly elevated by 22.4% and 28.0%, respectively, compared to MV0, followed by MV30 with an increase of 10.3%, and did not differ markedly between MV22.5 and MV0. The TK in MV45 and MV60 was significantly higher than that in MV0 by 15.5% and 12.6%, respectively, and no obvious changes among the other treatments were observed. Compared with MV0, the AN content in MV45 and MV30 was significantly increased by 93.0% and 87.0%, respectively, and by 68.9% and 35.9% in MV60 and MV22.5, respectively. For AK content, MV60 had the highest value, and the AK content was significantly increased by 17.2%

compared with the MV0 treatment, with no obvious change among the other treatments (Table 1).

**Table 1.** Effect of seven years of application of varied Chinese milk vetch (MV) rates on soil properties.

	BD (g cm <sup>-3</sup> )	SOC (g kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	TP (g kg <sup>-1</sup> )	TK (g kg <sup>-1</sup> )	AN (mg kg <sup>-1</sup> )	AP (mg kg <sup>-1</sup> )	AK (mg kg <sup>-1</sup> )
MV0	1.24 ± 0.04 a	10.6 ± 0.25 c	1.07 ± 0.06 c	0.21 ± 0.03 a	7.04 ± 0.49 c	46.0 ± 5.13 c	6.23 ± 0.76 a	50.56 ± 2.14 b
MV22.5	1.22 ± 0.03 ab	12.0 ± 0.94 b	1.08 ± 0.08 c	0.23 ± 0.02 a	7.44 ± 0.52 bc	62.5 ± 9.06 b	6.74 ± 0.24 a	53.08 ± 2.99 b
MV30	1.20 ± 0.04 ab	13.0 ± 0.94 ab	1.18 ± 0.05 b	0.23 ± 0.03 a	7.36 ± 0.08 bc	86.0 ± 11.1 a	6.09 ± 0.64 a	50.98 ± 3.13 b
MV45	1.16 ± 0.04 b	13.1 ± 0.20 a	1.31 ± 0.07 a	0.25 ± 0.04 a	8.13 ± 0.59 a	88.8 ± 7.18 a	6.39 ± 0.81 a	52.82 ± 4.82 b
MV60	1.18 ± 0.05 b	12.9 ± 0.57 ab	1.37 ± 0.09 a	0.22 ± 0.04 a	7.93 ± 0.39 ab	77.7 ± 5.59 b	6.61 ± 0.39 a	59.28 ± 4.54 a
ANOVA	ns	***	***	ns	*	***	ns	*

\* $p < 0.05$ ; \*\*\*  $p < 0.001$ ; Values (mean ± standard deviation) were tested in Analysis of variance (ANOVA) with four replicates in each treatment. Means followed by different letters within a row are significantly different ( $p < 0.05$ ) from each other according to Duncan's multiple comparison test. Notes: MV, Chinese milk vetch; BD, bulk density; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, alkali solution nitrogen; AP, available phosphorus; AK, available potassium.

No marked effects of treatment on bulk density (BD), total phosphorus (TP), or available phosphorus (AP) were observed (Table 1). Compared with the MV0 treatment, BD in MV45 and MV60 was significantly reduced by 6.45% and 4.84%, respectively, but no obvious changes were observed among the other treatments. The TP and AP contents in the four MV treatments were slightly higher than those in the MV0 treatment, excluding AP in MV30, but there was no significant difference among treatments (Table 1).

### 3.3. Soil Microbial Community Structure

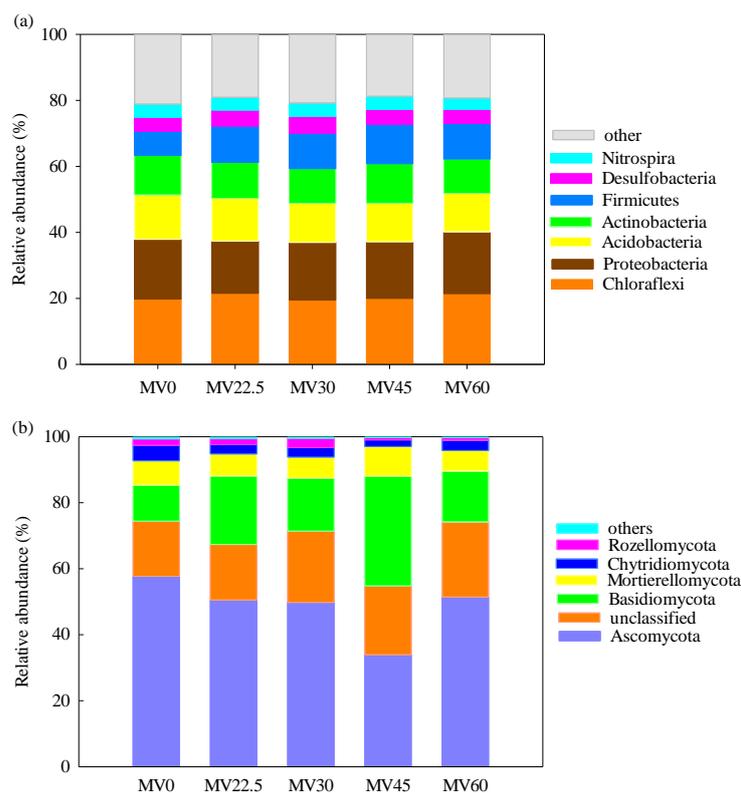
Chao richness and Shannon diversity indexes were used to reflect the alpha diversity of the bacterial and fungal communities. No marked effects of treatment on the Chao richness index or Shannon diversity index of the bacterial and fungal communities at the phylum level were observed (Table 2). Compared with the MV0 treatment, the Chao richness indexes of the bacterial and fungal communities under the four MV treatments were lower but showed no significant difference among treatments. The Shannon diversity index of the bacterial community in MV22.5, MV30, MV45, and MV60 was reduced, and the reduction in MV45 was statistically significant compared with the MV0 treatment (Table 2).

**Table 2.** Effect of seven years of application of varied Chinese milk vetch (MV) rates on richness and diversity of bacterial and fungal communities.

Treatment	Bacteria		Fungi	
	Chao Richness	Shannon Diversity	Chao Richness	Shannon Diversity
MV0	4518.9 ± 318.3 a	7.02 ± 0.09 a	1257.7 ± 192.8 a	4.53 ± 0.62 a
MV22.5	4329.6 ± 96.8 a	6.93 ± 0.02 ab	1236.6 ± 141.7 a	4.42 ± 0.67 a
MV30	4149.3 ± 316.8 a	6.85 ± 0.13 ab	1236.0 ± 419.1 a	4.54 ± 0.41 a
MV45	4125.7 ± 338.5 a	6.80 ± 0.16 b	1206.3 ± 124.2 a	4.19 ± 0.80 a
MV60	4170.8 ± 361.6 a	6.86 ± 0.09 ab	1030.5 ± 180.8 a	4.13 ± 0.43 a
ANOVA	ns	ns	ns	ns

Values (mean ± standard deviation) were tested in Analysis of variance (ANOVA) with four replicates in each treatment. Means followed by different letters within a row are significant differences ( $p < 0.05$ ) from each other according to Duncan's multiple comparison test. Notes: MV, Chinese milk vetch.

The dominant phyla of bacteria and fungi were identified by high-quality sequencing in all treatments (Figure 2). *Chloroflexi* (19.5–21.6%), *Proteobacteria* (15.8–18.7%), *Acidobacteriota* (11.7–13.5%), *Actinobacteriota* (10.4–12.0%), and *Firmicutes* (7.36–11.9%) were the five most abundant bacterial phyla across all treatments (Figure 2a, Table S2). The relative abundance of *Firmicutes* in the four MV treatments increased compared with the MV0 treatment, and the increases in MV22.5 and MV45 were statistically significant. The relative abundance of *Desulfobacterota* and *Nitrospirotae* showed no marked difference among all treatments.



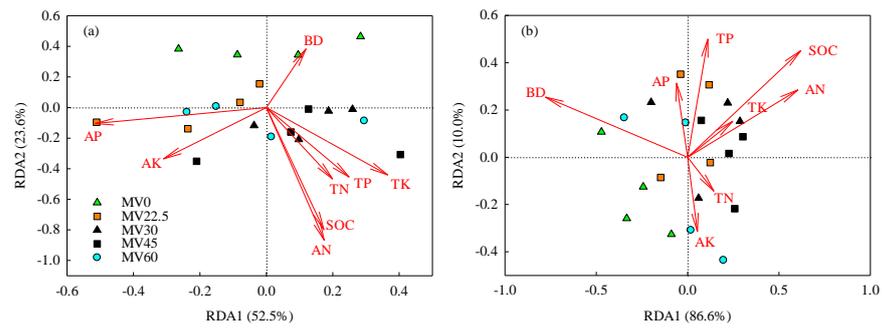
**Figure 2.** Relative abundance of the dominant phyla of bacteria (a) and fungi (b) after seven years of application of varied Chinese milk vetch (MV) rates. Phyla of bacteria with relative abundance <5% and phyla of bacteria with relative abundance <1% are collectively called “others”.

*Ascomycota* (34.0–57.7%) was the most abundant fungal phyla among all treatments (Figure 2b, Table S3). The relative abundance of *Ascomycota* in the four MV treatments was lower than that in MV0, and was markedly reduced in MV45. Compared with the MV0 treatment, the relative abundance of *Chytridiomycota* in MV22.5, MV30, and MV45 was significantly lower, but no obvious differences between MV60 and MV0 were observed.

### 3.4. Relationships between Soil Properties, Microbial Community, and Rice Yield

The relationships between soil microbial community structure and soil physicochemical properties were analyzed by redundancy analysis (RDA) (Figure 3). The soil physicochemical property indexes explained 76.1% and 96.6% of the variation in the bacterial and fungal communities, respectively, with the first two RDA axes. BD was markedly ( $p < 0.05$ ) correlated with the soil fungal community composition, but there was no marked correlation between soil physicochemical property indexes and soil bacterial community composition.

The relationships between soil properties and rice yield were analyzed by Spearman correlation analysis (Table 3). Rice yield was positively correlated with SOC ( $p < 0.01$ ), TN ( $p < 0.01$ ), TK ( $p < 0.01$ ), AN ( $p < 0.01$ ), and AK ( $p < 0.05$ ), whereas it was negatively correlated with BD and the Shannon diversity index of the bacterial community ( $p < 0.05$ ). The importance of these indexes was analyzed by random forest classification analysis (Figure 4). SOC and AN were the most important indexes of rice yield according to the mean percentage decrease in accuracy, followed by TN, BD, Shannon diversity index of bacterial community, AK, and TK. In addition, SOC had a positive correlation with AN ( $p < 0.01$ ) and a negative correlation with BD ( $p < 0.01$ ). TN was positively correlated with TK ( $p < 0.01$ ) and AN ( $p < 0.05$ ), whereas it was negatively correlated with the Shannon diversity index of the bacterial community ( $p < 0.05$ ). Chao richness and Shannon diversity indexes of the bacterial community had a positive correlation with BD ( $p < 0.05$ ) and a negative correlation with TK ( $p < 0.05$  and  $p < 0.01$ , respectively).

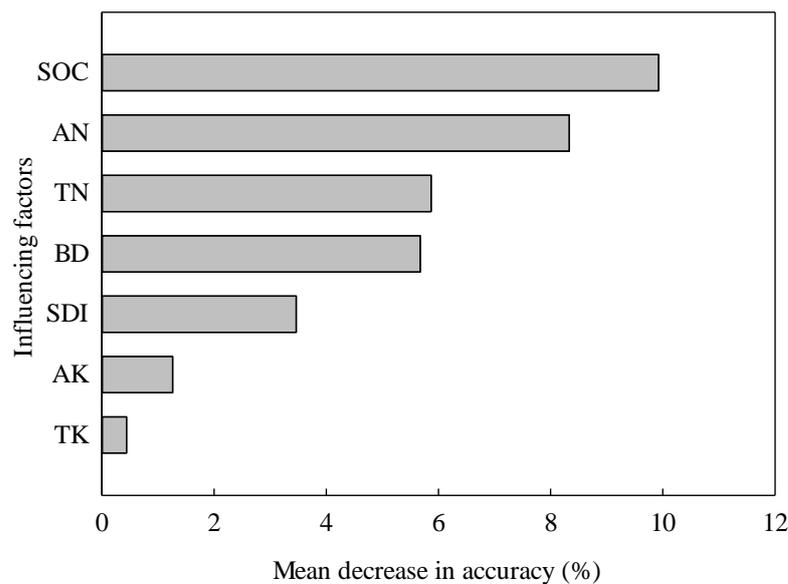


**Figure 3.** Redundancy analysis (RDA) of bacterial (a) and fungal (b) community structure on phyla level by soil properties.

**Table 3.** Spearman correlation coefficients for relationships among grain yield and soil properties ( $n = 20$ ).

	Yield	SOC	TN	TP	TK	AN	AP	AK	BD	Chao (B)	Shannon (B)	Chao (F)
SOC	0.69 **											
TN	0.75 **	0.39										
TP	0.29	0.16	0.12									
TK	0.57 **	0.32	0.62 **	0.04								
AN	0.71 **	0.62 **	0.51 *	0.23	0.39							
AP	−0.02	0.20	0.21	0.02	0.22	−0.02						
AK	0.47 *	0.10	0.30	−0.07	0.08	0.18	−0.25					
BD	−0.47 *	−0.61 **	−0.44	−0.08	−0.34	−0.31	−0.18	−0.02				
Chao (B)	−0.32	−0.33	−0.44	0.15	−0.51 *	−0.28	−0.04	0.19	0.46 *			
Shannon (B)	−0.50 *	−0.44	−0.48 *	0.03	−0.58 **	−0.41	−0.01	0.14	0.52 *	0.87 **		
Chao (F)	−0.17	−0.04	−0.37	0.20	−0.27	0.10	−0.25	−0.02	0.12	0.35	0.29	
Shannon (F)	−0.30	−0.31	−0.43	0.18	−0.16	−0.03	−0.31	−0.11	0.10	0.15	0.08	0.56 *

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; Chao, Chao richness index; Shannon, Shannon diversity index; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, alkali solution nitrogen; AP, available phosphorus (Olsen-P); AK, available potassium; BD, bulk density; (B), (bacteria); (F), (fungi).



**Figure 4.** Importance of soil properties in rice yield between different MV-dosage treatments by random forest classification analysis, expressed as variable importance (mean percentage decrease in accuracy). SOC, soil organic carbon; AN, alkali solution nitrogen; TN, total nitrogen; BD, bulk density; SDI, Shannon diversity index of bacterial community; AK, available potassium; TK, total potassium.

## 4. Discussion

The application of MV from 2014 to 2020 significantly improved rice yield by 22.5–29.7% and sustainability yield index by 10.3–13.0%, relative to no addition of MV (Figure 1). These gains were substantial compared to the results of Chen et al. [29] and Li et al. [18]. Both high rice yield and sustainability with the application of different MV rates in paddy soil may be attributed to (i) the improvement of soil physicochemical properties due to the decomposition of MV and release of nutrients, especially the improvement of SOC and AN concentrations, which are important influencing factors on rice yield; and (ii) the changes in the soil microbial community structure, especially the specific functional microbial community, which are conducive to improving the soil microenvironment and crop production.

### 4.1. Effect of Varied MV Rates on Soil Physicochemical Properties

MV application improved soil physicochemical properties by reducing soil BD and increasing SOC, TN, AN, TK, and AK concentrations (Table 1). Previous studies have shown that a high BD might disturb the transport of water and nutrients, thereby affecting root growth and grain yield [30,31]. We found that adding MV improved the soil structure by reducing soil BD, particularly in the MV45 and MV60 treatments (Table 1), similar to the reports of Zhou et al. [13] and Adekiya et al. [32]. These effects might be attributable to an increase in soil total porosity due to occupying a large space by continuously adding MV into the soil, and the improvement of the proportion of macro-aggregates and SOC content caused by the mineralization and decomposition of crop residues [33,34]. In our study, SOC concentrations increased significantly after MV application, particularly at an MV rate of 45 Mg ha<sup>-1</sup>. Many studies have also demonstrated that the input of exogenous organic material is an important factor for the improvement of soil organic carbon [35,36]. MV itself contained rich N resources due to the nitrogen-fixation effect of leguminous green manure [15]. We also found marked increases in TN and AN with 30–60 Mg ha<sup>-1</sup> MV, which was attributed to the high organic N-input rates resulting from MV decomposed in soil [12]. This was similar to the results of Bedada et al. [37] and Yang et al. [38], who reported that adding organic materials (e.g., green manure) could increase total N inputs and improve soil N nutrient supply capacity and N availability. However, soil AN under MV60 treatment was markedly lower than that in the MV45 treatment, which was likely because excessive N-input rates increased stay-green, delayed crop senescence, and improved N uptake during the later stages of crop growth, thus resulting in the reduction of soil AN [39,40]. In addition, leguminous green manure might increase soil K availability and improve K uptake and accumulation due to the extensive root system by secreting organic acids [16]; thereby, green manure residues returned to soil improved soil K pools [10]. Our results also showed that different MV rates increased soil TK concentration, especially MV45 treatment. In summary, our results confirmed that the application of MV was beneficial to the improvement of soil physicochemical properties in paddy fields, particularly when applied at 45 Mg ha<sup>-1</sup>.

### 4.2. Effect of Varied MV Rates on Soil Microbial Community Structure

Soil microbial community can be a pivotal indicator for estimating soil quality [27]. Some studies have reported that different fertilization management practices could affect the richness or diversity of soil microbial communities [41,42]. The application of MV decreased bacterial alpha diversity (Shannon index), particularly when applied at 45 Mg ha<sup>-1</sup> (Table 2), which was similar to the results of Tao et al. [10], who contended that the application of different green manures reduced bacterial diversity parameters. This might be attributed to improvements in soil properties (e.g., BD, TN, and TK), which were vital factors influencing microbial community diversity [43,44]. These effects were also confirmed in the present study, in which we found that the bacterial Shannon diversity index was significantly correlated with BD, TN, and TK (Table 3). However, the alpha diversity (Chao richness index and Shannon diversity index) of soil fungal microbial communities

had no significant effect among the varied MV rates (Table 3), which is consistent with the results of Ai et al. [45], who found that the input of organic manure changed the bacterial alpha diversity but not the fungal alpha diversity. This might have occurred because the bacterial community was more sensitive to the input of organic material and alteration of nutrient availability than the fungal community [46,47].

Compared with MV0 treatment, the application of MV improved the relative abundance of *Firmicutes* (Figure 2a and Table S2), which was analogous to the reports of Caban et al. [48] and Gao et al. [49]. *Firmicutes* play important roles in maintaining the stability of agroecosystems and in withstanding extreme environmental conditions through producing spores [22,50]. Additionally, *Firmicutes* could effectively promote soil carbon and nitrogen cycling by secreting enzymes to degrade organic materials and plant-derived polysaccharides, and participating in various nitrogen metabolism functions [51,52], which might be conducive to improving soil quality. Among fungi, the dominant phylum associated with MV addition was *Ascomycota* in our study (Figure 2b and Table S3), which was similar to the results of previous studies on maize fields and an apple orchard in the Loess Plateau of China [47,53]. These results indicate that *Ascomycota* is a ubiquitous fungal phylum with important roles in agroecosystems [46]. Our results showed that the relative abundance of *Ascomycota* first reduced, and then increased, with increased MV rates. The results might be attributed to increases in soil nutrient levels from MV0 to MV45 and the reduction of soil nutrient levels in MV60. Kong et al. [52] also indicated that the preferred conditions for *Ascomycota* to grow successfully might be low nutrient levels; thus, the relatively high nutrient levels might decrease the relative abundance of *Ascomycota* [54,55]. However, excessive input of MV decreased soil nutrient levels, particularly soil AN content, and then slightly increased the relative abundance of *Ascomycota*. The application of MV reduced the relative abundance of *Chytridiomycota* (Figure 2b and Table S3), which might be due to changes in some soil physicochemical properties (e.g., SOC and TN). This was in accordance with the reports of Liang et al. [46] and Wang et al. [56], who confirmed that the relative abundance of *Chytridiomycota* was negatively correlated with most soil characteristics, including SOC and TN.

#### 4.3. Relationships among Soil Properties, Microbial Community, and Rice Yield

The application of varied MV rates altered soil properties, such as soil BD, macronutrients, and microbial community characteristics, thereby increasing rice yield, similar to the results of previous studies on paddy soil in southern China [18,57]. We observed significantly positive relationships between soil chemical properties (e.g., SOC, TN, AN, TK, and AK) and rice yield (Table 3). These effects indicated that the increase in rice yield might be due to the improvement of soil fertility and nutrient supplies, which promoted nutrient uptake and crop growth, thereby achieving the gain in rice yield and sustainable yield index [38]. Furthermore, SOC and AN were the main soil environmental factors affecting rice yield (Figure 4), in accordance with previous results [15]. In addition, markedly negative relationships among soil BD, bacterial Shannon diversity index, and rice yield were found in our study; previous studies also had similar reports [44,58].

The changes in soil environmental factors caused by organic material can influence the soil microbial community structure [59,60]. In our study, the RDA results showed that only soil BD was profoundly associated with the fungal community (Figure 3b), which indicated that soil BD was the main environmental factor influencing the fungal community structure. Previous studies also consistently reported that soil BD significantly governs the dissimilarities in fungal community structure [61], which could occur because the reduction of soil BD affects soil porosity, promotes crop root growth, and increases the release of root exudates, thereby regulating fungal community structure [62,63]. Furthermore, soil BD was significantly positively correlated with the relative abundance of *Ascomycota* (Table S4), which was consistent with the results of Wan et al. [20]. However, previous studies indicated that the relative abundance of *Chytridiomycota* showed negative correlation trends with most soil chemical properties [55,56], which was confirmed in our study

(Table S4). These outcomes resulted in a notably negative correlation between the relative abundance of *Chytridiomycota* and rice yield (Table S4).

#### 4.4. Management of MV in Rice Production

In the rice cropping system, the application of green manure (e.g., MV) is a promising method to improve crop productivity by providing nutrient resources and improving soil quality in South China [49,64]. In this study, the increase in rice yield was remarkable when 30–60 Mg ha<sup>-1</sup> MV was applied. This might be explained by the reduction of soil BD, the improvement of soil nutrient levels, and the alteration of the soil microbial community structure in the current study, which was analogous to previous research [13,16,23]. However, the excessive application of MV might cause a potential risk of soil nutrient loss, thus reducing soil nutrient levels. For example, compared with MV45 treatment, we found slight decreases in SOC and TK concentrations and an obvious decrease in AN concentration when 60 Mg ha<sup>-1</sup> of MV was applied. Therefore, a synergistic improvement of rice yield and soil quality can be achieved by returning a relatively high amount of MV to the soil; in this study, the recommended amount of MV was 45 Mg ha<sup>-1</sup>.

MV, a leguminous green manure, plays a crucial role in sustainable agroecosystems due to its ability to develop natural resources and improve soil quality [10,29]. In the future, optimizing the application of green manure (e.g., mechanization and introducing soil conditioner) will be vital to improve the convenience of technical operation, to fully exploit the functional potential of green manure, and to further improve rice yield and soil quality. Furthermore, we encourage farmers to adopt this technology with government support (e.g., technical adoption subsidies). We also believe the gains in crop yield and soil quality through the application of this technology can be achieved in many crop types and regions to better boost sustainable agricultural green production.

## 5. Conclusions

In this study, MV application significantly increased rice yield by 22.5–29.7% and sustainable yield index by 10.3–13.0%, particularly when applied at 45 Mg ha<sup>-1</sup> MV. These gains were explained partly by decreased soil BD, increased SOC, AN, TN, and TK, and improved soil microbial community. Moreover, when 45 Mg ha<sup>-1</sup> MV was applied, the relative abundance of *Firmicutes* was significantly increased, and the relative abundance of *Chytridiomycota* was significantly reduced, relative to no addition of MV. Therefore, 45 Mg ha<sup>-1</sup> MV is strongly recommended in the rice cropping system of South China. These results can guide farmers and policymakers in achieving higher crop production and better soil quality, thereby promoting regional sustainable agricultural green development.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su142316065/s1>, Figure S1: Rice yield under application of varied Chinese milk vetch (MV) rates from 2014 to 2020.; Table S1: Rice yield as affected by the treatments, year and interaction between treatments and year. ANOVA for all data comes from Figure S1 in the supplemental material.; Table S2: Relative abundance of the dominant phyla of bacteria after seven years of application of varied Chinese milk vetch (MV) rates; Table S3: Relative abundance of the dominant phyla of fungi after seven years of application of varied Chinese milk vetch (MV) rates; Table S4: Spearman correlation coefficients for relationships among bacteria phyla, soil properties and grain yield ( $n = 20$ ).

**Author Contributions:** C.L. and Y.Z. designed the research and supervised the project. J.Z., C.Z. (Chunfeng Zheng), L.Z., C.Z. (Chenglan Zhang), X.Z. and B.L. collected and analyzed the data. Y.L., L.N., M.L. and W.C. provided a platform and technical service for field experiments. J.Z. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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