

# Maize growth responses to soil microbes and soil properties after fertilization with different green manures

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**Abstract** The use of green manures in agriculture can provide nutrients, affect soil microbial communities, and be a more sustainable management practice. The activities of soil microbes can effect crop growth, but the extent of this effect on yield remains unclear. We investigated soil bacterial communities and soil properties under four different green manure fertilization regimes (*Vicia villosa*, common vetch, milk vetch, and radish) and determined the effects of these regimes on maize growth. Milk vetch showed the greatest potential for improving crop productivity and increased maize yield by 31.3 %. This change might be related to changes in soil microbes and soil properties. The entire soil bacterial community and physicochemical properties differed significantly among treatments, and there were significant correlations between soil bacteria, soil properties, and maize yield. In particular, abundance of the phyla

*Acidobacteria* and *Verrucomicrobia* was positively correlated with maize yield, while *Proteobacteria* and *Chloroflexi* were negatively correlated with yield. These data suggest that the variation of maize yield was related to differences in soil bacteria. The results also indicate that soil pH, alkali solution nitrogen, and available potassium were the key environmental factors shaping soil bacterial communities and determining maize yields. Both soil properties and soil microbes might be useful as indicators of soil quality and potential crop yield.

**Keywords** Green manures · Bacterial communities · Soil physicochemical properties · Maize yield

## Introduction

The continued use of chemical fertilizers in agriculture has created several issues including environmental pollution, high product costs, and health effects. Many experts have promoted the use of renewable and sustainable farming methods (Aulakh and Pasricha 1998). Such agricultural methods include application of organic and animal manures (Cline and Silvernail 2002) (Haynes and Naidu 1998), farmyard manures (Webb et al. 2004), composts (Sikora and Enkiri 1999), and green manures (Aulakh 1994). Compared with chemical fertilizers, use of organic manures is a more sustainable way to increase crop productivity (Chang et al. 2007). Green manure is one of the cleanest organic manure resources, as it lacks heavy metals, antibiotics, hormones, and other residual hazards. Green manure is a crop used primarily as a nutrient source for subsequent crops and as a microbial soil amendment to improve crop quality and

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yields (Cherr et al. 2006). Application of green manures can reduce soil erosion (Dapaah and Vyn 1998), enhance soil nutrient-holding ability (Gaston et al. 2003), suppress weed reproduction (Burgos and Talbert 1996), and reduce crop pest populations (Caswell et al. 1991). Compared to chemical fertilizers, green manure provides more organic substrates and carbon resources for microbial growth, changes soil biomass (Esperschütz et al. 2007), and increases microbial activity and diversity (Jangid et al. 2008; van Diepeningen et al. 2006). However, the specific responses of microbes to green manure application are unclear.

Soil contains many living organisms clustered into complex and diverse communities. The diversity, community composition, and structure of soil microorganisms in agricultural soils are critical to the maintenance of soil health and quality (Shen et al. 2008). Significant interactions occur between the soil environment, soil microbes, and plant quality (Garbeva et al. 2004). Nutrients such as N, P, and K, together with energy resources, can be directly released from green manures into soil by microbial decomposition and subsequently used by both microorganisms and plants (Veen and Kuikman 1990). Slowly released N from green manures is better synchronized with plant uptake than inorganic N fertilizers, thereby increasing crop yield (Cline and Silvernail 2002). On the other hand, the whole soil microbiota might affect crop performance. Productivity of crops with indigenous microbiota was higher than crop productivity with exogenous microbiota (Verbruggen et al. 2012), indicating that soil microbiota might be an important factor in the regulation of plant productivity. Plant–microbe interactions could be used to predict plant growth and fitness, carbon sequestration, secretion of plant growth hormones (Ali et al. 2009), and nutrient cycling (Kulmatiski et al. 2008). Therefore, there may be a close link between soil microorganisms, fertilizer type, and crop growth.

Soil contains a diversity of microbes with unknown functions, and it is challenging to determine the impact of the microbial community on plant growth (Edwards et al. 2015; Peiffer et al. 2013). In this study, next-generation high-throughput sequencing was used to link the details of the microbial community with crop growth. There are many reports that soil microbes and soil fertility can alter plant morphology and affect plant growth, but the degree to which crop growth is affected by the microbiota and soil properties remains unclear. To explore the possible relationship between soil properties, soil microbes, and plant growth, a total of 40 soil samples with four different green manures (ten samples of each manure) and a control (ten samples) were collected. We analyzed soil physicochemical properties,

investigated soil bacterial communities by next-generation sequencing, and compared maize yield among the treatments. The study provides insight into the links between soil microbial communities, soil fertility, and maize yield and also provides indicators of the biological and physicochemical factors that regulate crop yield.

## Materials and methods

### Experimental site and design

The field experiment was conducted from October 2012 to September 2014 at Xiangxi Tobacco Test Base (109° 30' E, 28° 01' N, el. 452 m) in Fenghuang County, Hunan Province, China. This region has a subtropical continental monsoon climate. The annual mean temperature and precipitation are 15.2–15.5 °C and 1308.1 mm, respectively. The soil of the field experiment is paddy field loam (previous crop was rice). Planting system was 1-year maturity.

The treatments were arranged in a randomized complete block design with three replications, and the plot size was 39 m<sup>2</sup>. The experiment compared five cropping systems, deriving from four green manure crops of *Vicia villosa* (Y1), common vetch (Y2), milk vetch (Y3), radish (Y4), and idle soil as control (CK). The four green manures were surface broadcast on October 26, 2012, and October 25, 2013, at a rate of 45 kg ha<sup>-1</sup> for Y3 and Y4 and 75 kg ha<sup>-1</sup> for Y1 and Y2. The green manure crops were harvested on May 5, 2013, and May 7, 2014. After being harvested in 2014, green manures were incorporated to a 25-cm depth by chisel plowing and disking with the amended amount of 33,570.00; 30,368.40; 20,930.40; and 22,500.00 kg/hm<sup>2</sup>, respectively. The maize test crop was seeded after green manure harvest with a row spacing of 0.485 m and was harvested on September 9, 2013, and September 7, 2014. No pest or disease controls were used during the whole experiment.

### Maize yields and soil physicochemical property measurement

During the growth period of the second year, maize agronomic characters were measured. At maturity, maize ears were hand harvested and shelled, air-dried grains were weighed, and moisture content was measured with a DICKEY-john Tri-Grain moisture meter (DICKEY-john, Auburn, IL, USA).

A total of 50 soil samples were collected from five experimental fields, and ten soil cores in each plot were collected

randomly to a depth of 0–20 cm at harvest. A 5-g amount of each soil sample was dissolved in 25 ml sterile water, and soil pH was measured by a pH measure (Portable ORP meter, BPH-220, Bell, China). A 5-g amount of each soil sample was oven dried for 24 h at 98 °C, and then, soil moisture content was determined. Geochemical properties of soil were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Ramsey and Thompson 1987).

### DNA extraction, PCR amplification, and MiSeq sequencing

Soil DNA from each sample was extracted with TIANamp Bacterial DNA Kit (MO BIO Laboratories, Inc., Carlsbad, CA) and was checked by 1 % agar gel electrophoresis. Pico Green with a FLUOstar OPTIMA fluorescence plate reader (BMG LABTECH, Jena, Germany) was used to quantify DNA. The V4 region of the 16S ribosomal RNA (rRNA) genes was amplified with the primer pair 515F (5'-GTGC CAGCMGCCGCGGTAA-3') and 806R (5'-GGAC TACHVGGGTWTCTAAT-3') combined with Illumina adapter sequences, a pad and a linker of two bases, and barcodes on the reverse primers (Caporaso et al. 2012). Sample libraries were generated from purified PCR products. The amplified volume was 25 µl including 12.5-µl PCR Mix (Vazyme Biotech, China), 1 µl of a 0.25-mM solution of forward/reverse primer, 1 µl template DNA, and dd H<sub>2</sub>O. Then, the amplicons were sequenced by the Illumina MiSeq platform (Illumina, San Diego, CA) with a 500-cycle kit (2 × 250-bp paired ends). Raw sequences with perfect matches to barcodes split to sample libraries and were trimmed using Btrim with threshold of QC higher than 20 over a 5-bp window size and the minimum length of 100 bp. The MiSeq sequencing raw data were deposited in the NCBI Sequence Read Archive (SRA) database, and the project ID is [SRP075935](https://www.ncbi.nlm.nih.gov/sra/SRP075935).

### Sequence preprocessing and statistic analysis

Operational taxonomic unit (OTU) clustering was done through UCLUST at a 97 % similarity level (Edgar 2010), and taxonomic assignment was through RDP classifier (Wang et al. 2007) with a minimal 50 % confidence estimate. Samples were rarefied at 19,600 sequences per sample, and these were classified into 15,706 OTUs. The previously mentioned steps were all performed through the Galaxy pipeline (<http://zhoulab5.rccc.ou.edu/>) developed by Qin et al. (unpublished). Dissimilarity analysis, detrended correspondence analysis (DCA), and microbial community diversity, calculated using Shannon–Weiner's index and Pielou evenness, were performed in R v. 2.11.1 with the packages vegan v. 1.11-3 (Team RC 2014) or online

(<http://ieg.ou.edu/>). All the graphs and charts were generated by Origin 8.0, and statistical differences between the microbial communities or the soil physicochemical properties in the soils amended with different green manures were determined by a one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test in SPSS 17.0 (Mitchell and Mitchell 1981).

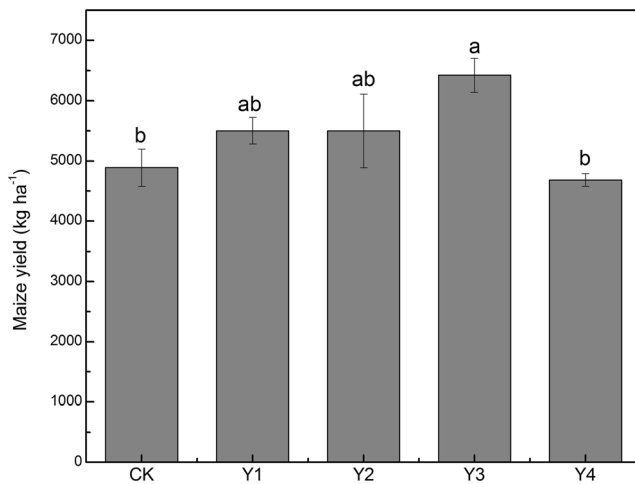
## Results

### Effect of different green manures on maize yield and quality

The crop yields of different treatments are shown in Fig. 1. Maize yield in the Y3 treatment was the highest at 6420.69 kg ha<sup>-1</sup>, and increased crop yield, compared to the control soil, was 31.34 %. This was followed by Y1 (5503.88 kg ha<sup>-1</sup>, 12.58 % increase) and Y2 (5500.03 kg ha<sup>-1</sup>, 12.51 % increase). Y4 did not increase maize yield. Agronomic characters are shown in Table S1. Plant height, strain number, and cob weight were highest in Y3 and significantly ( $p < 0.05$ ) increased by 2.7, 17.9, and 40.4 %, respectively, compared to the control soil. Straw weight and ear diameter and length were much higher in Y3. The results indicated that maize agronomic characters in the Y3 treatment were significantly better than the other four groups.

### Phylogenetic composition and structure of soil microbial communities

A total of 19,600 rarefied 16S rRNA gene sequences were obtained per sample. These were clustered into 15,706 OTUs at a 97 % similarity threshold. Rarefaction curves, which show the observed OUT richness as a function of sequencing effort, indicated that the sequencing depth was insufficient to capture all of the diversity present (Fig. S1). The number of OTUs in CK was relatively high, followed by Y2, Y1, Y4, and Y3. In addition to observed OTU richness, Shannon–Wiener diversity, Pielou evenness, and the Chao estimator of total species richness were calculated and are presented in Table 1. All of the three diversity parameters were lower in green manure-amended soils than in the CK, and the difference between Y3 and CK was significant ( $p < 0.05$ ). The results of dissimilarity test indicated different structures of microbial communities among the five groups (Table 2). Except Y2, microbial communities were significantly ( $p < 0.05$ ) different among the other four soils; Y2 was only significantly different from Y3 ( $p = 0.02$ ). Similar



**Fig. 1** Maize biological and economic yields at harvest in soils amended with different green manures. Abbreviations of CK, Y1, Y2, Y3, and Y4 represent the control group and soils amended with four green manures of *Vicia villosa*, common vetch, milk vetch, and radish, respectively. Different small letters indicated significant differences at the level of 0.05

results were also observed in detrended correspondence analysis (DCA) of the 16S rRNA gene sequences (Fig. S2).

For all hierarchical ranks of the taxonomic system, from phylum to genus, the same units were detected from the five soils, but their abundances had different rankings. Overall, 27 phyla, 70 classes, 87 orders, 210 families, and 557 genera were detected. Analysis of 16S rRNA gene sequences showed that the bacterial community composition of the five soils was apparently different at different levels (Fig. S3). The most abundant phylum was *Proteobacteria* in CK and *Acidobacteria* in green manure-amended soils, with a proportion of 27.47 and 23.44–34.20 % of the sequences, respectively (Table S2a). The most abundant classes in all treatments were *Acidobacteria\_Gp6*, *Alphaproteobacteria*, *Betaproteobacteria*, and *Deltaproteobacteria*, which together comprised 25.7–38.49 % of all bacterial

**Table 1** Alpha diversity of 16S rDNA sequencing data

Treatment	Shannon–Wiener index (H)	Pielou evenness index (J)	Chao value
CK	7.09169860 a	0.8670280220 a	11,882 a
Y1	6.9753226 bc	0.8593130220 b	11,747 ab
Y2	7.0347326 ab	0.864459711 ab	11,781 ab
Y3	6.91132420 c	0.8556596780 b	11,054 b
Y4	6.9534216 bc	0.8571371890 b	11,656 ab

Abbreviations of CK, Y1, Y2, Y3, and Y4 represent the control group and soils amended with four green manures of *Vicia villosa*, common vetch, milk vetch, and radish, respectively. Different small letters after the numbers in the same column under the different densities indicated significant differences at the level of 0.05

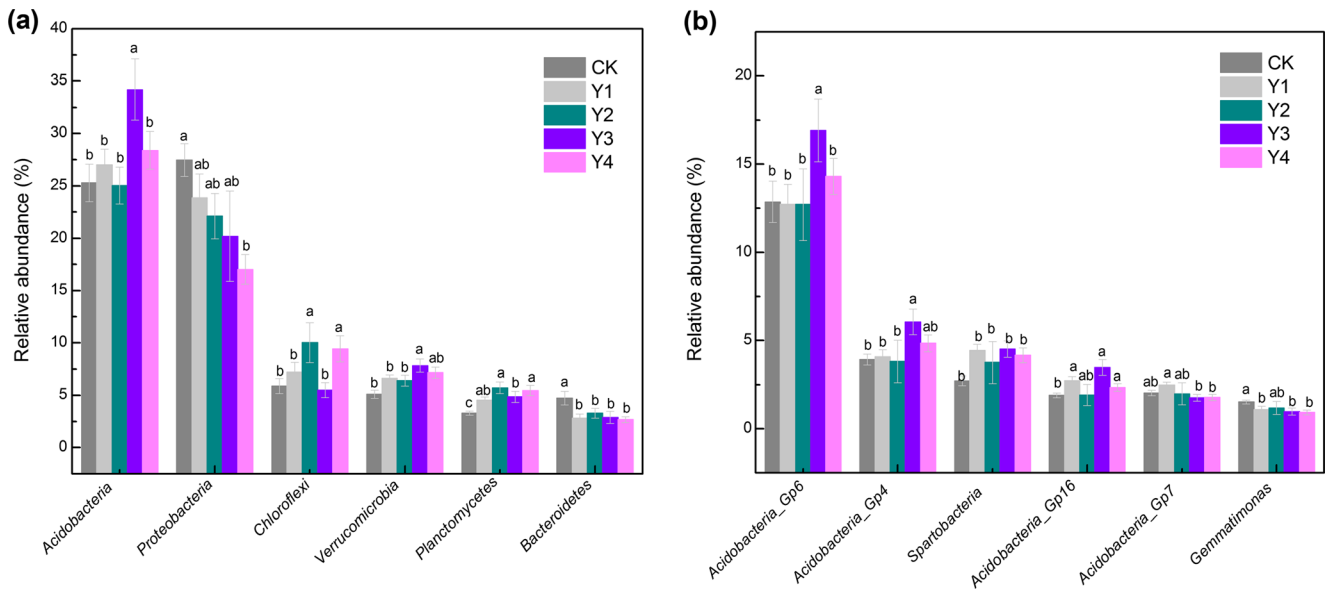
**Table 2** Dissimilarity test of 16S rRNA gene sequencing data among five groups

Treatment	Y2	Y3	Y4	CK
Y1	0.22	<i>0.0099</i>	<i>0.037</i>	<i>0.036</i>
Y2		<i>0.02</i>	0.909	0.0639
Y3			<i>0.011</i>	<i>0.003</i>
Y4				<i>0.006</i>

Numbers in table represent *p* values of the degree of dissimilarity. Significant differences ( $P < 0.05$ ) are indicated in italics. Abbreviations of CK, Y1, Y2, Y3, and Y4 represent the control group and soils amended with four green manures of *Vicia villosa*, common vetch, milk vetch, and radish, respectively

amplicons (Table S2b). The most abundant genera *Gp6* (*Acidobacteria*) accounted for 11.58–16.92 % of the 557 genera (Table S2c).

The proportion of the bacterial sequences that could be assigned to the different taxonomic ranks declined with increasing discriminatory taxonomic resolution. For example, 84.71 % of the bacterial sequences could be clustered into phyla, while only 56.9 % of the sequences were associated with distinct genera. Given the high levels of OTU richness and limited sequencing depth, only the most abundant OTUs and those of the highest taxonomic ranks could be quantified with a level of precision sufficient for comparison. Comparisons of the differences in key bacterial phyla and genera among five soils are shown in Fig. 2. At the phylum level, the most abundant phylum in the control soil was *Proteobacteria*, followed by *Acidobacteria*, *Chloroflexi*, *Verrucomicrobia*, *Actinobacteria*, and *Planctomycetes*, while *Acidobacteria* accounted for the largest proportion, and *Proteobacteria* ranked second in the other four green manure-amended soils, followed by different phyla in different soils, i.e., *Chloroflexi*, *Verrucomicrobi*, *Planctomycetes*, and *Actinobacteria* in Y1; *Chloroflexi*, *Verrucomicrobia*, *Planctomycetes*, and *Actinobacteria* in Y2; *Verrucomicrobia*, *Chloroflexi*, *Planctomycetes*, and *Actinobacteria* in Y3; and *Chloroflexi*, *Verrucomicrobia*, *Planctomycetes*, and *Crenarchaeota* in Y4. Furthermore, comparison of key taxonomic groups among the five soils was conducted to reveal response of specific microbial populations to green manures. The microbial communities in Y3 and the CK showed the most significant differences. Compared to CK, *Acidobacteria*, *Verrucomicrobia*, and *Planctomycetes* were significantly ( $p < 0.05$ ) more abundant and *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* were significantly ( $p < 0.05$ ) less abundant in Y3. The microbial communities in the five soils were also different at the genus level. For example, except for the genus *Acidobacteria\_Gp7*, the other five microbial genera



**Fig. 2** Differences in the relative abundance of main bacterial phyla (a) and genera (b) in soils amended with different green manures. Abbreviations of CK, Y1, Y2, Y3, and Y4 represent the control group

and soils amended with four green manures of *Vicia villosa*, common vetch, milk vetch, and radish, respectively. Different small letters indicated significant differences at the level of 0.05

were significantly ( $p < 0.05$ ) different between Y3 and CK.

**Soil physicochemical properties**

Soil properties are important indicators of soil fertility. Both physical and chemical properties have significant effects on crop growth. The physical properties of the soil samples are shown in Table 3. Bulk density and pH of the soils decreased in the plots amended with green manures, and the decrease level depended on the green manure type. The CK had the highest values of the two parameters. Compared to CK, bulk density decreased 10.3 % in Y2, followed by Y1, Y4, and Y3 with reductions of 5.13, 5.13, and 2.56 %. The pH decreased 6.77, 6.62, 6.17, and 5.56 %, respectively, in Y1, Y2, Y3, and Y4, compared to CK, and statistical analyses showed that these reductions were significantly different

( $p < 0.05$ ). Compared to the CK, water content in Y1, Y2, and Y4 increased, and the increase in Y3 was statistically significant.

Soil chemical properties are listed in Table 3. Soil organic matter (SOM), alkali solution nitrogen (N), available phosphorus (P), and available potassium (K) contents were much higher in green manure-amended soils, compared to the CK. Specifically, SOM was highest in Y1, followed by Y4, Y2, and Y3, with increases of 22.87, 22.15, 21.24, and 20.47 %, respectively, compared to the CK. The increases were statistically significant ( $p < 0.05$ ). The contents of alkali solution nitrogen, available phosphorus, and available potassium were highest in Y3, Y4, and Y3, respectively. The contents of both alkali solution nitrogen and available potassium in Y3 were significantly different ( $p < 0.05$ ) compared to the other treatments. The content of total N in soils amended with green manures was higher than that in

**Table 3** Some selected physical and chemical properties of soils at harvest

Treatment	Bulk density	pH	Water content (%)	SOM (mg/kg)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)
CK	0.39 a	6.65 a	32.2 b	22.08 b	131.67 c	31.07 c	150.33 d	1.39 b	0.72 a	30.99 ab
Y1	0.37 ab	6.20 b	32.28 b	27.13 a	138 b	26 d	161.08 c	1.44 ab	0.69 b	30.49 b
Y2	0.35 b	6.21 b	32.16 b	26.77 a	137.67 b	34.57 bc	184.51 b	1.43 ab	0.68 b	31.19 a
Y3	0.38 a	6.24 b	33.56 a	26.6 a	145.33 a	35.43 b	202.19 a	1.47 a	0.67 b	31.32 a
Y4	0.37 ab	6.28 b	32.35 ab	26.97 a	134 bc	43.50 a	169.36 c	1.42 b	0.7 ab	30.24 b

Abbreviations of CK, Y1, Y2, Y3, and Y4 represent the control group and soils amended with four green manures of *Vicia villosa*, common vetch, milk vetch, and radish, respectively. SOM, N, P, and K represent soil organic matter, alkali solution nitrogen, available phosphorus, and available potassium, respectively. Different small letters after the numbers in the same column under the different densities indicated significant differences at the level of 0.05

CK but contrasted to the content of total P. These results indicated that both soil physical and chemical properties in Y3 were significantly different from CK and the other green manure-amended soils.

### Links between soil bacteria, soil physicochemical properties, and maize yield

Correlation analysis between soil properties and maize yield (Table 4) indicated a significant positive correlation between maize yield and N and K levels ( $p < 0.01$ ) but a significant negative correlation between maize yields and soil pH ( $p < 0.05$ ). These results show that maize yield might be improved by an increase of N and K and a reduction of soil pH. Soil pH had a significant negative correlation ( $p < 0.01$ ) with organic matter and the content of N, K, and total N.

Correlations between soil bacterial phyla and genera (relative abundance  $>0.5\%$ ), soil properties, and maize yield are shown in Tables 5 and S3, respectively. The results clearly show that there were four phyla and nine genera closely correlated to maize yield. At the phylum level (Table 5), *Acidobacteria* and *Verrucomicrobia* had a significant positive correlation ( $p < 0.01$ ) with maize yield, while *Proteobacteria* and *Chloroflexi* had a significant negative correlation ( $p < 0.01$ ) with maize yield. At the genus level (Table S3), seven bacterial genera such as *Acidobacteria\_Gp6*, *Acidobacteria\_Gp16*, and *Spartobacteria* were significantly ( $p < 0.01$ ) and positively correlated to maize yield, while *Sphingosinicella* and *Pseudolabrys* had a significantly ( $p < 0.05$ ) negative correlation with maize yield.

Tables 5 and S3 show relationships between soil properties and bacteria. Soil pH, N, and K levels were the key factors linked to soil bacteria. Bulk density only correlated to the

phylum of *Nitrospira* and the genus of *Spartobacteria*; SOM only correlated to the phylum of *Verrucomicrobia* and *TM7*. Soil pH was significantly ( $p < 0.05$ ) related to the abundance of six phyla (e.g., *Proteobacteria*, *Acidobacteria*, and *Planctomycetes*) and seven genera (e.g., *Spartobacteria*, *Acidobacteria\_Gp16*, and *Sphingomonas*). N content had a significant ( $p < 0.05$ ) relationship with the abundance patterns of six phyla (e.g., *Proteobacteria*, *Acidobacteria*, and *Chloroflexi*) and nine genera (e.g., *Acidobacteria\_Gp6*, *Acidobacteria\_Gp4*, and *Acidobacteria\_Gp16*); K content had a significant ( $p < 0.05$ ) relationship with the abundance patterns of seven phyla (e.g., *Proteobacteria*, *Acidobacteria*, and *Planctomycetes*) and nine genera (e.g., *Acidobacteria\_Gp4*, *Acidobacteria\_Gp16*, and *Subdivision3*).

In addition to correlation analysis, the relative importance of the association of maize growth with soil abiotic properties and microbial properties was analyzed using the partial least square path model (PLSPM) (Fig. 3). Abundance of *Acidobacteria* and *Verrucomicrobia* had a significantly direct effect (path coefficient = 0.77,  $p < 0.001$ ) on maize growth. Indirect effects of microbial diversity and the whole community structure on maize growth were positive and negative, respectively, but not significant. Soil nutrients including N and K contents had a significant positive effect on maize yield (path coefficient = 0.59,  $p < 0.01$ ) and microbial abundance 1 (path coefficient = 0.73,  $p < 0.001$ ), but a negative effect on microbial abundance 2 (path coefficient =  $-0.40$ ,  $p < 0.001$ ). Soil pH had a significant positive effect on microbial diversity (path coefficient = 0.29,  $p < 0.05$ ), but a significant negative effect on maize yield (path coefficient =  $-0.31$ ,  $p < 0.05$ ), the content of N and K (path coefficient =  $-0.58$ ,  $p < 0.01$ ), and microbial abundance 1 (path coefficient =  $-0.42$ ,  $p < 0.01$ ).

**Table 4** Pearson's correlation coefficient ( $r$ ) between soil physical properties, chemical properties, and maize yield

Factors	Bulk density A1	pH A2	Water content A3	Organic matter A4	N A5	P A6	K A7	Total N A8	Total P A9	Total K A10	Yield B
A1	1.00										
A2	0.24	1.00									
A3	0.15	-0.10	1.00								
A4	-0.06	-0.526 (<0.001)	0.04	1.00							
A5	-0.18	-0.419 (0.001)	-0.12	0.23	1.00						
A6	0.02	-0.15	-0.01	0.04	-0.04	1.00					
A7	-0.16	-0.558 (<0.001)	0.335 (0.001)	0.258 (0.049)	0.438 (0.001)	0.21	1.00				
A8	0.02	-0.322 (0.012)	0.07	0.19	0.20	-0.08	0.316 (0.015)	1.00			
A9	0.14	0.326 (0.012)	0.03	-0.22	-0.24	-0.05	-0.21	-0.17	1.00		
A10	-0.24	-0.12	0.00	0.21	0.280 (0.030)	-0.01	0.15	-0.09	-0.24	1.00	
B	-0.21	-0.291 (0.022)	0.06	0.11	0.764 (<0.001)	-0.06	0.510 (<0.001)	0.21	-0.21	0.21	1

N, P, and K represent alkali solution nitrogen, available phosphorus, and available potassium, respectively

**Table 5** Pearson's correlation coefficient (*r*) between soil bacteria phyla, soil physicochemical properties, and maize yield

Bacteria phyla	Bulk density	pH	Water content	Organic matter	N	P	K	Total N	Total P	Total K	Yield
<i>Proteobacteria</i>	0.15	0.355 (0.006)	-0.16	-0.03	-0.347 (0.007)	-0.17	-0.474 (<0.001)	-0.16	0.03	-0.15	-0.392 (0.001)
<i>Acidobacteria</i>	-0.19	-0.345 (0.007)	0.05	0.13	0.757 (<0.001)	0.14	0.555 (<0.001)	0.20	-0.290 (0.022)	0.260	0.864 (<0.001)
<i>Verrucomicrobia</i>	-0.25	-0.513 (<0.001)	0.17	0.275 (0.033)	0.567 (<0.001)	0.267 (0.039)	0.537 (<0.001)	0.21	-0.20	0.17	0.652 (<0.001)
<i>Chloroflexi</i>	0.03	-0.05	0.07	-0.08	-0.329 (0.011)	-0.06	-0.03	-0.05	0.298 (0.013)	-0.06	-0.437 (<0.001)
<i>Planctomycetes</i>	-0.17	-0.409 (0.002)	0.18	0.20	0.05	0.17	0.382 (0.003)	0.25	-0.21	0.17	0.03
<i>Firmicutes</i>	-0.02	-0.16	-0.04	-0.20	0.10	0.00	-0.11	0.06	-0.11	-0.386 (0.002)	0.10
<i>Actinobacteria</i>	0.15	0.370 (0.004)	-0.25	-0.15	0.15	-0.15	-0.13	-0.11	-0.04	-0.22	-0.16
<i>Bacteroidetes</i>	0.23	0.401 (0.002)	-0.08	-0.07	-0.12	-0.07	-0.325 (0.012)	-0.10	-0.04	-0.11	-0.09
<i>Crenarchaeota</i>	0.12	0.03	0.04	-0.12	-0.23	0.350 (0.006)	-0.20	-0.04	0.15	-0.23	-0.14
WS3	-0.13	-0.16	0.14	0.04	0.19	0.14	0.400 (0.002)	-0.01	-0.04	0.16	0.24
<i>Gemmatimonadetes</i>	0.11	0.18	-0.23	-0.14	0.316 (0.015)	0.01	-0.21	-0.23	-0.09	-0.03	-0.21
<i>Nitrospira</i>	0.269 (0.040)	0.18	-0.17	-0.20	-0.350 (0.006)	-0.06	-0.19	-0.22	0.17	-0.22	-0.16
<i>Armatimonadetes</i>	-0.12	0.06	-0.05	-0.17	-0.01	-0.02	-0.07	-0.18	-0.06	0.308 (0.017)	0.13
TM7	0.12	0.20	0.04	-0.287 (0.025)	-0.18	0.07	-0.417 (0.001)	-0.270 (0.035)	-0.03	-0.277 (0.032)	-0.10
<i>Euryarchaeota</i>	0.09	0.13	0.03	-0.08	-0.15	-0.14	-0.16	-0.02	0.266 (0.042)	-0.14	-0.13

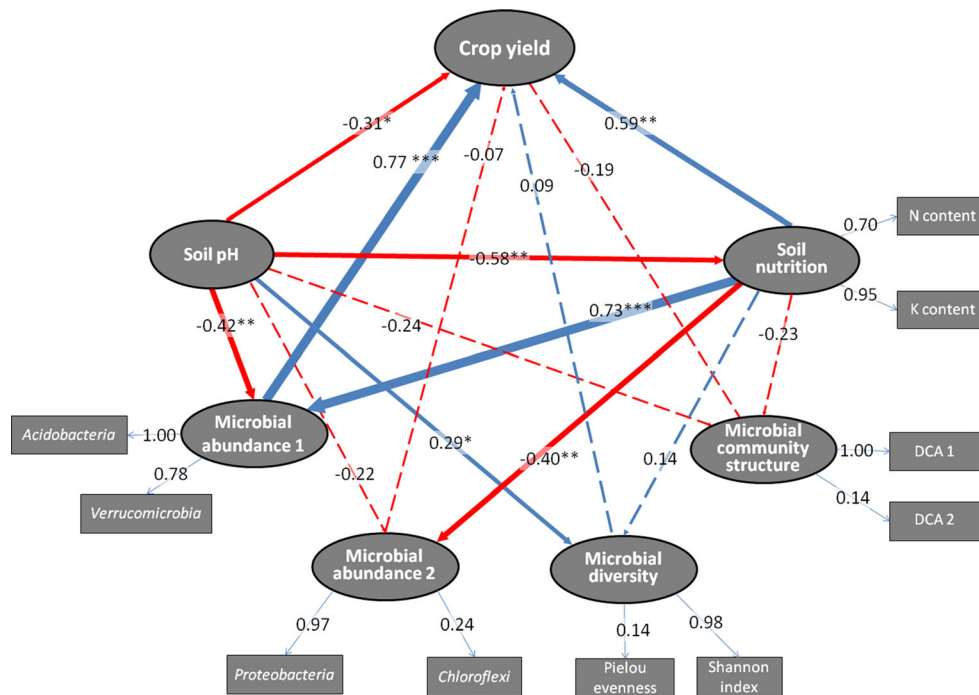
N, P, and K represent alkali solution nitrogen, available phosphorus, and available potassium, respectively

## Discussion

The use of green manure, as a clean organic fertilizer, represents a more sustainable agricultural practice. The relationship between soil microbial communities, soil physicochemical properties, and crop productivity is important in understanding the processes by which organic green manures benefit crops. We found that green manure application can significantly increase maize yields. This is consistent with previous studies demonstrating that application of green manures can significantly increase crop production (Cherr et al. 2006). Compared with the control field (without green manure application), milk vetch showed the greatest potential to promote crop productivity and increase maize yield by 31.3 % (Fig. 1), followed by *V. villosa*, common vetch, radish, and ryegrass. Milk vetch was clearly the most effective manure crop in promoting maize yield. The influence of green manures on maize might also be achieved through affecting soil microbial communities as well as soil properties. Because (i) soil physicochemical properties changed significantly under green manure application and (ii) soil bacterial communities were highly responsive to green manures, the responses differed among phyla or genera. Major phyla, such as *Acidobacteria*, *Verrucomicrobia*, and *Planctomycetes*, showed differences in abundance between treatments. Meanwhile, *Acido bacteria\_Gp6* was the most abundant group in milk vetch-amended soil so did *Pseudolabrys* in *V. villosa*-amended soil and *Sphingosinicella* and *Sphingomonas* in the control group (Table S2). Our findings suggest that organic crops might be selective to microbial populations, which is consistent with results from a previous study (Hartmann et al. 2008).

### Relationship between bacterial communities and maize yield

Regarding the entire microbial communities, the five soils in this study had different soil microbial community structures and compositions. Unlike previous reports that found that Shannon diversity under rotation cropping was significantly higher than that in continuous cropping (Fang et al. 2011; Horvath et al. 2006), all three diversity parameters (Shannon–Wiener diversity, Pielou evenness, and Chao estimator) in the experimental soils were lower than in the control soil. Milk vetch treatment soil had the lowest Shannon diversity but the highest maize yield. Previous studies have shown that plant growth response to microbiota shifts can be affected by plant genotype (Bainard and Klironomos 2013). For example, crops of *Beta vulgaris* L. and *Brassica oleracea* L. responded negatively to soil microbial diversity (Hol et al. 2010), but *Brassica rapa* L. showed a positive response to



**Fig. 3** Partial least square path modeling of the association of maize yield with soil and microbial properties across the green manure-amended soils. The model was constructed using the microbial data after growing maize. Goodness of fit of the modeling is 0.5258. Blue and red arrows

indicate positive and negative path coefficients, respectively, while solid and dashed lines indicate significant and nonsignificant path coefficients, respectively. \*, \*\*, and \*\*\* indicate significance at the level of  $P < 0.05$ , 0.01, and 0.001, respectively (Color figure online)

the diversity of whole microbiota (Lau and Lennon 2011). In the present study, microbial communities in the milk vetch-amended soil were significantly ( $p < 0.05$ ) different from control soils. In addition, microbial composition within each treatment showed great differences at the various taxonomic levels.

Soil microbiota have a significant effect on plant diversity and productivity in natural ecosystems (Wagg et al. 2014), and soil micronutrients are significant factors in determining crop quality and yield (Li et al. 2007). Our results revealed a close linkage between soil microbes and maize yield. Both bacterial phyla (e.g., *Proteobacteria*, *Acidobacteria*, and *Verrucomicrobia*) and genera (e.g., *Acidobacteria\_Gp6*, *Acidobacteria\_Gp16*, *Sphingosinicella*, and *Pseudolabrys*) were closely associated with maize yield. *Acidobacteria*, *Proteobacteria*, *Verrucomicrobia*, and *Chloroflexi* were the dominant phyla and made up nearly 70 % of the microbial community in milk vetch-amended soil, which produced the highest maize yield. *Acidobacteria* phylum and its five genera also had a significant positive effect on maize yield. OTUs belonging to *Acidobacteria\_Gp6*, *Acidobacteria\_Gp16*, *Acidobacteria\_Gp4*, *Acidobacteria\_Gp17*, and *Acidobacteria\_Gp25* were positively correlated to the maize yield and accounted for 83.45 % of the total reads of *Acidobacteria* in this study. *Acidobacteria* is an acidophilic, chemoorganotrophic bacterium (Sabree et al. 2006), and many gene sequences involved in nitrogen fixation and carbon cycling are associated with *Acidobacteria* (Jiménez et al. 2012).

This suggests that *Acidobacteria* plays an important role in the carbon and nitrogen cycles of soil. *Acidobacteria* also has genes that encode polyketide synthase and nonribosomal peptide synthase enzymes, which catalyze the synthesis of siderophores and other natural products such as antibiotics, antifungals, antivirals, antitumor agents, and antineoplastic agents (Ward et al. 2009). Excretion of siderophores by some soil bacteria can stimulate plant growth by improving iron (Fe) nutrition or by inhibiting infection of plant pathogens and other harmful microorganisms. Therefore, its antifungal nature might make *Acidobacteria* a beneficial bacterium for improving maize growth and controlling disease. In contrast to *Acidobacteria*, the phyla *Proteobacteria* and *Chloroflexi* had a negative effect on maize growth. *Proteobacteria* are copiotrophic soil bacteria that are usually selectively enriched by root-derived carbon; thus, they might be a nutrient competitor of plants (Ai et al. 2015). *Chloroflexi* is a green nonsulfur bacterium without nitrogen fixation capability (Kragelund et al. 2007); it may compete with crops for nitrogen resources. In conclusion, the biological properties of phyla *Proteobacteria* and *Chloroflexi* might explain their negative effects on maize growth.

### Relationship between soil properties and maize yield, soil properties, and bacterial communities

After application of green manures, soil characteristics such as pH, organic matter, and macronutrients were significantly



changed, which was in agreement with other long-term experiments (Coolon et al. 2013; Mäder et al. 2002). Furthermore, a positive correlation between soil properties (e.g., soil alkali solution N and available K) and maize yield indicated that enhanced maize yield might result from the increase of soil nutrient elements. This is consistent with the finding that crop yield is related to the amount of nitrogen fertilizer added to soil (Cerrato and Blackmer 1990). In the present study, milk vetch had the greatest potential to promote maize productivity. Milk vetch is a leguminous green manure with nitrogen fixation capability, and it can provide N nutrients for plant growth (Chen et al. 2014). In addition, potassium plays a role in increasing plant resistance to environmental stress factors (Marschner and Rimmington 1988), so higher potassium content in the green manures added to fields might help protect maize from environmental stresses.

The interactions between soil microbial communities, soil physicochemical properties, and maize yield generate questions about the mechanisms by which green manure modifies crop growth. Diversity, composition, and structure of the rhizosphere microbial communities can be strongly affected by soil nutrient availability, which restrict nutrition assimilation by soil microbes directly or change root exudation indirectly (Rengel and Marschner 2005). Root exudates have selective and beneficial effects on specific microbial populations (Hartmann et al. 2008). We showed that pH and the levels of N and K in soil were the key factors shaping the soil microbial communities. The lower pH of the green manure-amended soils may partly explain the higher abundance of members of *Acidobacteria*. Soil microorganisms also have an impact on nutrition release, transformations, and acquisitions of plants (Richardson et al. 2009). The decomposition of organic matter from plant residues in soils depends on microbial activities and environmental factors such as temperature, moisture, pH, and soil fertility (García-Fraile et al. 2016). Through microbial activities, nutrient cycling is driven and nutrients become available for plants (Robertson and Groffman 2007). This point agrees with our finding that pH value was significantly ( $p < 0.05$ ) related to the abundance patterns of seven phyla and nine genera. Meanwhile, pH is the most important environmental factor affecting the abundance of *Acidobacteria* (Jones et al. 2009), which were positively correlated to maize yield. In addition, abundance of the bacteria beneficial to maize yield had a significantly positive correlation with N and K contents. Therefore, we believe that soil bacteria played an important role in decomposing green manures to release nutrients and produce a more favorable environment for crop growth. Soil pH, alkali solution nitrogen, and available potassium in soil were key factors affecting the soil microbial community composition and key nutrition assimilation favoring maize growth.

Increasing food demand and shortages of land and resources will make it more difficult for future agriculture.

This problem cannot be resolved by the current agricultural soil management based solely on physical and nutrient management. Physicochemical properties exactly have a great effect on microbial communities and crop performance. But the microorganisms in soil contribute to plant residue decomposition, nutrition release, transformations, and acquisitions. In this study, certain bacterial phyla (e.g., *Acidobacteria* and *Verrucomicrobia*) and genera (e.g., *Acidobacteria\_Gp6*, *Acidobacteria\_Gp16*, and *Spartobacteria*) were positively correlated with maize yield. Through green manure decomposition and nutrition release, these results might reveal the relationships between soil microbes, soil physicochemical properties, and crop yield. The results highlight the importance of soil microbes in affecting soil fertility and demonstrate a potential indicator for increasing crop yield. This new information can be used to improve current management systems or invent novel agricultural practices for more productive and sustainable agriculture.

To conclude, we demonstrated that milk vetch fertilizer application had the greatest effect on maize growth and it increased maize yield by 31.3 %. Abundance of bacteria in the phyla *Acidobacteria* and *Verrucomicrobia* was positively correlated with maize yield, and soil physicochemical properties, such as pH, alkali solution nitrogen, and available potassium, played important roles in determining maize yield and modifying soil microbial communities.

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**Conflict of interest** The authors declare that they have no conflicts of interest.

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