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Nitrogen Reduction with Bio-Organic Fertilizer Altered Soil Microorganisms, Improved Yield and Quality of Non-Heading Chinese Cabbage (*Brassica campestris* ssp. *chinensis* Makino)

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Abstract: Excessively using fertilizers poses serious problems such as environmental pollution, soil degeneration, and quality and yield reduction of vegetables. This study aimed to illustrate the effect of different organic manure and inorganic fertilizers on the characteristics of soil, and the growth, yield, and quality of non-heading Chinese cabbage. There were 28 treatments in the first experiment: no fertilization (CK), conventional fertilization (100% nitrogen T1), 20% reduction of total nitrogen (T2), 30% reduction of total nitrogen (T3), and 20% or 30% reduction of total nitrogen with four kinds of fertilizers and three kinds of dosages (24 treatments). Six treatments, being selected from the first experiment based on growth of plants, were further applied to the second experiment. The results of the second experiment showed that the pH, nitrate nitrogen, and organic matter content of soil treated by N2 (20% reduction of total nitrogen with 1500 kg·ha^{-1} No.1: *Bacillus*-enriched bio-organic fertilizer) were significantly enhanced compared with T1 (100% nitrogen). The N2-treated plants showed an 11.66% increase in root activity, 9.24% enhancement in yield, 5.79% increase in vitamin C (VC), and 47.87% decrease in nitrate content compared with T1. Nitrogen reduction with bio-organic fertilizer significantly increased the dominant phyla of *Gemmatimonadetes* and *Chytridiomycota* and significantly decreased *Ascomycota*, and increased the dominant genera of *Gemmatimonas* and *Bacillus* and decreased *Fusarium*, indicating that this treatment altered the microbial community composition of soil. Redundancy analysis (RDA) showed that AP (available phosphorus), OM (organic matter), and UREA (urease activity) of the soil were significantly correlated with microbial community structure. Yield was significantly, positively correlated with *Rhodanobacter* and *Olpidium*. In conclusion, nitrogen reduction with bio-organic fertilizer benefited growth, yield, and quality of non-heading Chinese cabbage by improving the soil quality.

Keywords: bio-organic fertilizer; nitrate content; non-heading Chinese cabbage; yield; soil; microbial community

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1. Introduction

The mineral fertilizer used in vegetable production is 2.3 times more than that used on other crops in China [1]. Consumption of fertilizer in China is about 7.73 times more than the amount in Europe, and the utilization rate of nitrogen fertilizer is only 30–40% that of the Europeans [2]. The excessive application of mineral fertilizer leads to decreased soil quality and reduced food quality, and particularly, increased nitrate accumulation in vegetal products [3]. In addition, the excessive application of chemical fertilizer also has an adverse impact on the environment [4].

Rational fertilization is crucial to promote beneficial rhizosphere interactions for sustainable agricultural production [5]. Organic fertilizer replaces parts of mineral fertilizer and can enhance the healthy development of the vegetable industry [6]. Researchers suggest that biofertilizer with mineral fertilizer is a promising approach to maintaining the soil microbiota balance by ameliorating soil pH, TN (total nitrogen content), and organic matter statuses [7]. Furthermore, bio-organic fertilizers usually contain a unique microbial community, which can activate soil, increase soil biodiversity, and improve soil enzyme activities [8,9]. Soil microorganisms have crucial roles in nutrient cycling and the fertility maintenance of soil. The soil microbiome has both direct and indirect effects on the health of plants and animals in terrestrial ecosystems [10]. *Bacillus* and *Trichoderma* are reported as well-known PGPR (plant growth-promoting rhizobacteria) which could improve the growth of plants and the resistance to diverse environmental stress [11–14]. Research showed that bio-organic fertilizer could enhance tomato yield and quality more than PGPR or organic fertilizer solely [3]. Gao et al. [15] found that six years of continuous biochar and biochar-based fertilizer application could increase *Acidobacteria* in peanut soil. Fulvic acid organic fertilizer could increase the quality of tomatoes [16].

Non-heading Chinese cabbage accounts for 30–40% of the vegetable multiple cropping area in the south of China [17]. The cultivated area in China increased from 533,300 ha in 2005 to 1,333,300 ha in 2020 [18]. Excessive nitrogen fertilizer application is an extremely common problem during cabbage production. The aim is to study the effects of reduced nitrogen with different commercial bio-organic fertilizers on the microbial community structure and soil characteristics, as well as on plant growth, yield, and quality, of non-heading Chinese cabbage. Our study can provide a theoretical support for scientific fertilization during crop production.

2. Materials and Methods

2.1. Materials

The seeds of non-heading Chinese cabbage "Siyuebai" and "Jinpin28" were provided by the key Chinese Cabbage Breeding Laboratory of Nanjing Agricultural University. Field experiments were conducted 2018–2019 at the Agricultural Expo Garden, Jurong, Jiangsu, China (32° N, 119°12′ E). The soil texture (0–15 cm) had a pH of 4.83, an organic matter content of 29.18 $g \cdot kg^{-1}$, a total nitrogen content of 1.50 $g \cdot kg^{-1}$, and an available potassium content of 190.00 $mg \cdot kg^{-1}$.

Mineral fertilizers (46% urea, 12% superphosphate, and 52% anhydrous potassium sulfate) were provided by Yuntianhua Co., LTD, Kunming, China. A bio-organic fertilizer named "No.1" (Bacillus $2 \times 10^8 \cdot g^{-1}$ living bacteria count, 3-5-0.7 N-P-K) was bought from Lianye Biotechnology Co., LTD, Jiangyin, China. "Bamboo charcoal" (biochar-based fertilizer, 3-5-0.7 N-P-K) was bought from Shike Bamboo Charcoal Co., LTD, Shanghai, China. "Jiajiapei" (Trichoderma, etc., 2-2-1 N-P-K) was bought from Delong Biotechnology Co., LTD, Xi'an, China. "Fulvic acid" (3-3-1 N-P-K) was bought from Yimutian Biotechnology Co., LTD, Qingzhou, China.

2.2. Experiment Design

There were 28 treatments in the first experiment ("Siyuebai"): no fertilization (CK), conventional fertilization (the average level of fertilization that farmers commonly use, (T1), 193.2 $kg \cdot ha^{-1}$ N, 135 $kg \cdot ha^{-1}$ P_2O_5, 135 $kg \cdot ha^{-1}$ K_2O), a 20% reduction of total nitrogen (T2), a 30% reduction of total nitrogen (T3), and a 20% or 30% reduction of total nitrogen with four bio-organic fertilizers and three kinds of dosages each. The fertilization information of each treatment is shown in Table S1. The first experiment was conducted from September to December 2018. Every treatment was set up with three repetitions in a random block arrangement. Each plot's area was 4 m × 1.5 m with 96 plants in each repetition. All the fertilizers were applied as base fertilizer.

According to the results of the first experiment, we screened out six treatments to further initiate the second experiment ("Jinpin28"). The treatments are shown in Table 1. The second experiment was carried out from March to May 2019.

Table 1. Fertilization situation in second experiment.

Treatment	Mineral Fertilizer			Bio-Organic Fertilizer		
	N kg·ha^{-1}	P_2O_5 kg·ha^{-1}	K_2O kg·ha^{-1}	No.1 kg·ha^{-1}	Seek kg·ha^{-1}	Jiajiapei kg·ha^{-1}
No fertilizer (CK)	-	-	-	-	-	-
Conventional fertilizer (T1)	193.2	135	135	-	-	-
−N20% (T2)	154.5	135	135	-	-	-
−N20% + No.1 (N2)	109.5	60	125	1500	-	-
−N20% + Seek (S2)	109.5	60	125	-	1500	-
−N20% + Jiajiapei (J2)	140.1	120.6	127.8	-	-	720

2.3. Determination Index and Method

2.3.1. Determination of Soil Characteristics

Soil samples were collected from 5 to 10 cm soil layers around the plant root and passed through a sterilized 2 mm sieve to remove rocks, roots, and organic residues. Fresh soil was stored at 4 °C for soil enzyme activity analyses and at −80 °C for DNA sequencing. The electrical conductivity (EC) and pH of soil were determined by mixing soil with deionized water at 1:2.5 and 1:5 (*w*/*v*), respectively. Nitrate nitrogen content of the soil was determined by using a continuous flow analyzer (BRAN + LUEBBE Auto Analyzer3, Hamburg, Germany) [19]. Soil organic matter and total nitrogen content were determined using an elemental analyzer (Vario EL elemental analyzer, Hanau, Germany) [20]. Available P and available K content in soil were detected using the methods of Bao [21]. The content of P, K, Ca, and Zn elements of soil and plant was determined using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7800, Santa Clara, CA, USA) [22]. Activity of soil urease and invertase was determined according to the method descried by Sun et al. [23]. Phosphatase and FDA (fluorescein diacetate) were measured following the methods of Guan [24] and Taylor et al. [25], respectively.

2.3.2. DNA Extraction and PCR Amplification

Soil subsamples for molecular analysis were kept in a freezer at −80 °C before use. Total DNA was extracted from 18 samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. The DNA extract was checked on 1% agarose gel, and DNA quantity and quality were determined by the NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, Wilmington, NC, USA). Three commonly used primer sets were used to study soil bacterial 16S rRNA genes and fungal 18S rRNA genes [26,27]. The PCR conditions for each primer set are shown in Table S2. The system included 0.4 µL of TransStart FastPfu DNA Polymerase and 10 ng of template DNA with ddH_2O up to 20 µL. PCR reactions were performed in triplicate according to the manufacturer's instructions and quantified by using the Quantus™ Fluorometer (Promega, Madison, WI, USA). The PCR product was extracted from 2% agarose gels and purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). Purified amplicons were pooled in equimolar and then sent for paired-end sequencing on an Illumina MiSeq PE 300 × 2 Sequencer (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China).

2.3.3. Growth, Yield, Quality, and Photosynthetic Parameter Indexes

Photosynthesis was determined with the Li-6400 (LI-COR Inc. Lincoln, NE, USA) from 9–11 a.m. Chlorophyll content was determined with the ethanol (95%) extraction-colorimetric method [28]. Root vitality, nitrate content, soluble protein content, VC (L-ascorbic acid, AsA) content, and total soluble sugar content were determined using TTC (triphenyltetrazolium chloride) [29], salicylic acid colorimetry [30], Coomassie Brilliant Blue G-250 colorimetry, o-phenanthroline colorimetry [31], and anthrone colorimetry [32], respectively.

2.4. Statistical Analysis

Alpha-diversity characteristics in each soil sample were calculated by QIIME 2. Bacterial database used was RDP version 11.5 rRNA (http://rdp.cme.msu.edu/) accessed on 2 February 2020. Fungal databases used were UNITE version 8 (https://unite.ut.ee//) accessed on 2 February 2020 and PR2 version 1 (https://github.com/vaulot/pr2_database) accessed on 2 February 2020. A Venn diagram was obtained by the Venny tool. The relationship between soil environmental factors and microbial communities was analyzed by performing Spearman's rank correlation analysis and redundancy analysis (RDA). Bonferroni correction was used for further *p*-value study. Significant difference analysis was performed by Duncan's test ($p < 0.05$) using SPSS (IBM, Chicago, IL, USA, version 22.0) and the relevant figures were made using Microsoft Excel 2010.

3. Results

3.1. Soil Chemical Properties, Yield, and Quality of the First Experiment

The P, K, Ca, and Zn contents of soil with 20% nitrogen reduction in four kinds of bio-organic fertilizers were different (Table S3). The P, K, and Ca contents of N2 and S1 were the highest. The *P*n of J2 was significantly higher than other treatments. (Table S4).

Nitrogen reduction of 20% in four different bio-organic fertilizers promoted the growth of non-heading Chinese cabbages. N1, N2, S1, S2, J1, and J2 had an advantageous effect on growth (Tables S5 and S6). Compared with T1, the yields of N2, S2, and J2 were increased by 20.9%, 6.5%, and 19.8%, respectively (Figure 1).

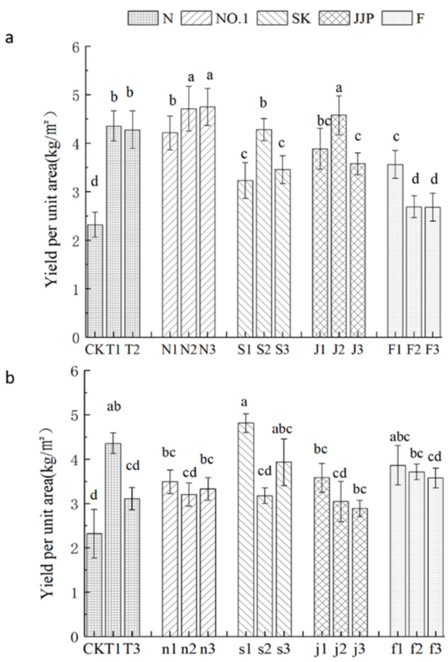

Figure 1. Effects of different fertilization treatments on the yield of cabbage. Note: (**a**) 20% reduction of total nitrogen with different bio-organic fertilizer, (**b**) 30% reduction of total nitrogen with different bio-organic fertilizer. Different small letters represent a significant difference at 0.05 level by Duncan's test.

3.2. Soil Chemical Properties, Soil Element Content, and Soil Enzyme Activities of the Second Experiment

The soil pHs of N2 and S2 were significantly higher than that of the conventional fertilization treatment (T1). On the contrary, the EC was lower than in T1. The available phosphorus and ammoniacal nitrogen of N2 were higher than that of T1. The nitrate nitrogen content of N2 and J2 was decreased compared with T1 (Table 2). The FAD enzyme activity of N2 was significantly higher than other treatments (Figure S1). Invertase activity of N2 and S2 showed no significant differences with T1.

Table 2. Effects of nitrogen reduction combined with biological organic fertilizer on soil's physical and chemical properties of non-heading Chinese cabbage.

Treatment	pH	EC $ms\cdot m^{-1}$	Organic Matter $g\cdot kg^{-1}$	Available Phosphorus $g\cdot kg^{-1}$	Total Nitrogen $g\cdot kg^{-1}$	Ammonium Nitrogen $g\cdot kg^{-1}$	Nitrate Nitrogen $g\cdot kg^{-1}$	Organic Carbon $g\cdot kg^{-1}$
CK	5.15 ± 0.04 bc	75.2 ± 7.15 e	13.47 ± 0.37 b	18.45 ± 1.14 b	1.80 ± 0.02 b	5.70 ± 1.63 ab	8.78 ± 0.19 d	8.06 ± 0.3 b
TI	5.14 ± 0.07 c	148.60 ± 6.03 a	16.44 ± 1.44 ab	31.45 ± 5.10 b	1.81 ± 0.04 b	4.50 ± 0.18 b	18.95 ± 0.52 a	9.54 ± 0.84 ab
T2	5.18 ± 0.03 bc	111.27 ± 5.46 ab	15.57 ± 0.39 ab	30.58 ± 2.5 b	1.85 ± 0.03 b	8.34 ± 1.04 ab	15.80 ± 0.47 b	9.03 ± 0.23 ab
N2	5.38 ± 0.04 a	92.83 ± 8.94 de	18.07 ± 0.31 a	64.93 ± 3.07 a	2.07 ± 0.02 a	12.57 ± 2.69 a	11.29 ± 0.57 c	10.59 ± 0.13 a
S2	5.32 ± 0.04 ab	95.23 ± 10.65 ed	17.08 ± 1 ab	22.80 ± 2.87 b	1.80 ± 0.01 b	5.68 ± 0.95 ab	11.18 ± 0.24 c	9.91 ± 0.58 a
J2	5.07 ± 0.03 c	134.67 ± 2.34 ab	14.88 ± 0.45 ab	21.82 ± 1.76 b	1.83 ± 0.04 b	7.18 ± 0.64 ab	9.40 ± 0.72 d	9.79 ± 0.55 a

Note: CK is no fertilization, T1 is 100% fertilization, T2 is 20% reduced nitrogen, N2 is 20% reduced nitrogen + 1500 $kg\cdot ha^{-1}$ "No.1", S2 is 20% reduced nitrogen +1500 $kg\cdot ha^{-1}$ "Seek", J2 is 20% reduced nitrogen + 720 $kg\cdot ha^{-1}$ "Jiajiapei". The different small letters represent a significant difference at 0.05 level by Duncan's test.

The P, K, Ca, and Zn contents of soil have no significant differences among different treatments. However, the K and Zn contents in the N2 plant, and the Ca contents in the J2 plant were significantly higher than in T1 (Tables S7 and S8).

3.3. Soil Microbiomes

The rarefaction curves were close to plateau (Figure S2), indicating that the sequencing depth met the requirements, and the results can truly reflect the sample condition. After quality filtering, 895,811 sequences were clustered into 3663 OTUs with the bacteria, and 1,069,019 sequences were clustered into 1393 OTUs with the fungi.

Chao, Shannon, and Simpson indexes were computed based on the OTUs. For the Simpson index, the larger the value, the lower the community diversity; however, the Shannon index was just the opposite. In this experiment, the Shannon values of N2 of the bacteria decreased compared with conventional fertilization treatment. On the contrary, the Simpson value of fungi increased significantly, indicating that whole chemical fertilizers decreased species' richness and diversity (Table S9). The Venn diagram revealed the overlapped and unique OTUs with all of the samples. The OTUs shared by all of the samples were 1593, 325, and 132 for the bacteria and fungi (Figures 2a and 3a). There were also 144 and 99 unique OTUs in N2 and 102 and 111 in T1 for the bacteria and fungi, respectively, indicating that fertilizers changed the communities in the soil of non-heading Chinese cabbage. N2 had a lager influence on the bacterial communities than the application of nitrogen fertilizer solely. On the contrary, nitrogen fertilizer had a larger influence on the fungal communities. Bacterial and fungal diversity of the N2 soil was increased compared with T1.

At the phylum level (Figure 2b), the dominant bacterial phyla in all of the samples were Proteobacteria, Actinobacteria, Chloroflex, Acidobacteria, Bacteroidetes, Firmicute, Planctomycetes, Cyanobacteria, and Gemmatimonadetes. Proteobacteria was the most abundant phylum, followed by Acidobacteria and Chloroflex. The Proteobacteria (32.23%) and Bacteroidetes (7.66%) contents of S2 in soil were both increased compared with CK. The Firmicutes of CK (5.33%) was decreased compared with other treatments. In addition, the Gemmatimonadetes content of N2 increased by 47.86% compared with T1. The dominant fungal phyla of all samples were Ascomycota, Mortierellomycota, Basidiomycota, and Olpidiomycota. Among them, Ascomycota was the most abundant. With fertilizers, Ascomycota decreased compared with the non-fertilization treatment. In addition, the Ascomycota content of N2 decreased by 45.47% compared with T1 (Figure 3b).

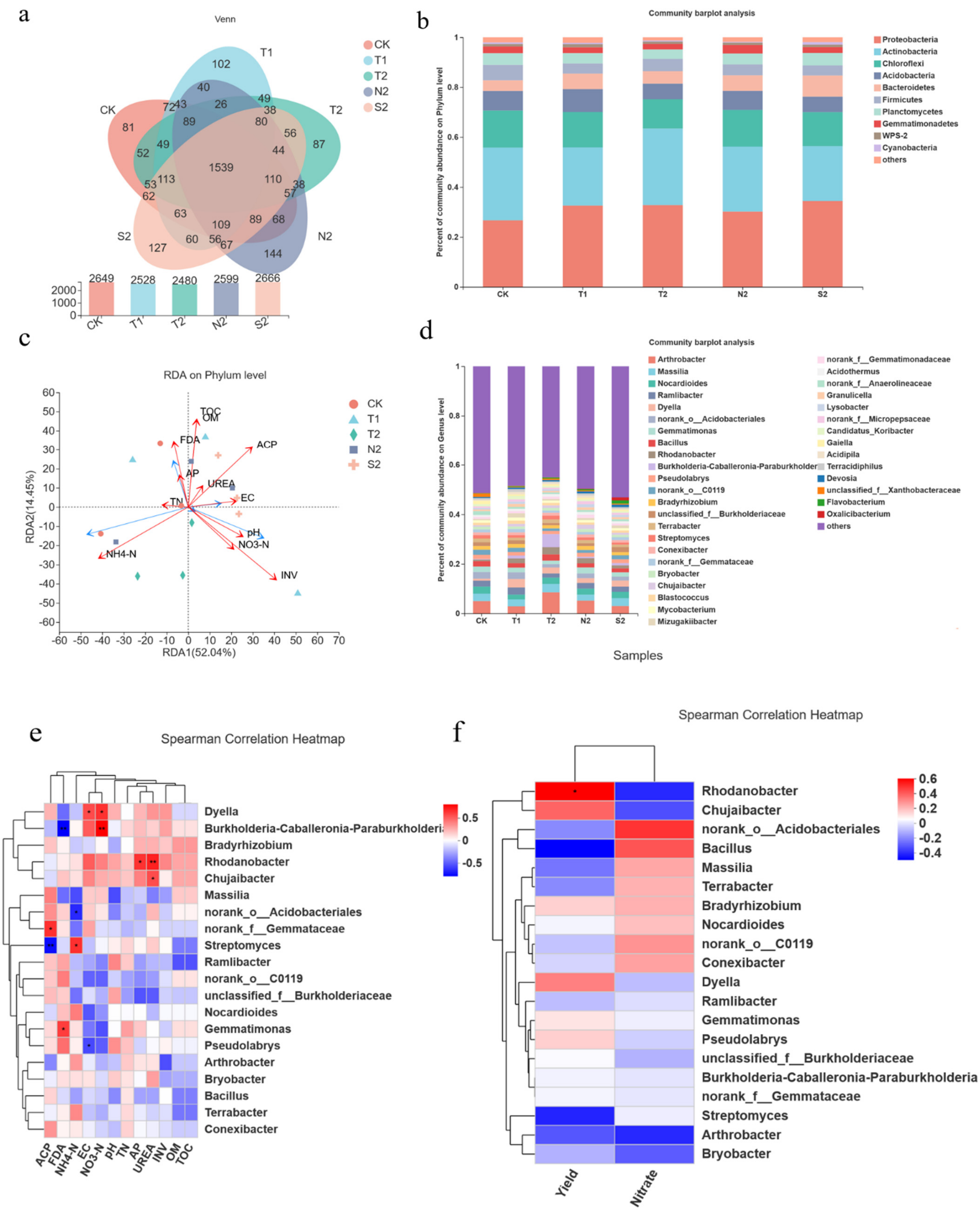

Figure 2. Number of common and unique OTUs based on bacteria (**a**) Venn analysis; relative abundances of bacteria (**b**) at the phylum level; redundancy analysis of the five (blue line) dominant bacteria (**c**) associated with soil properties; relative abundances of bacteria (**d**) at the genus level; the heatmap of the correlation between the top 20 dominant bacterial (**e**) genera associated with soil properties; the heatmap of the correlation between the top 20 dominant bacterial (**f**) genera associated with yield and nitrate content. Note: * at the 0.05 level (double-tailed), the correlation between them was significant; ** at 0.01 level (double-tailed), the correlation between them was significant. TN: soil total nitrogen content, NO_3^-: soil nitrate nitrogen content, OM: soil organic matter, ACP: acid phosphatase activity, AP: available phosphorus, TOC: total C, NH_4–N: ammonium nitrogen, UREA: urease activity of soil, INV: invertase activity, FDA: fluorescein diacetate activity.

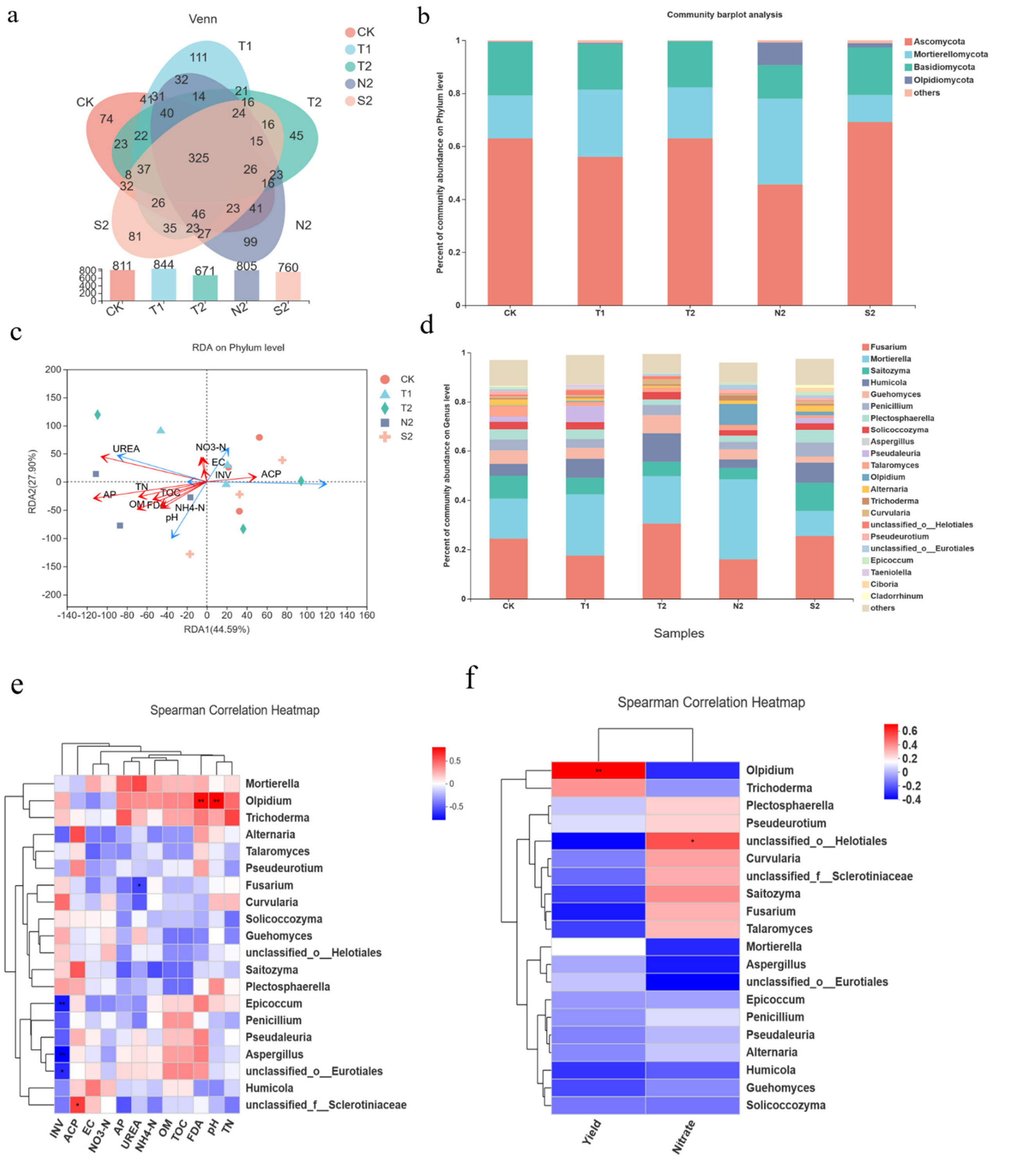

Figure 3. Number of common and unique OTUs based on fungal (**a**) Venn analysis; relative abundances of fungi (**b**) at the phylum level; redundancy analysis of the five (blue line) dominant fungi (**c**) associated with soil properties; relative abundances of fungi (**d**) at the genus level; the heatmap of the correlation with the top 20 dominant fungal (**e**) genera associated with soil properties; the heatmap of the correlation with the top 20 dominant fungal (**f**) genera associated with yield and nitrate content. Note: * at the 0.05 level (double-tailed), the correlation between them was significant; ** at 0.01 level (double-tailed).

The RDA results of the bacterial and fungal communities were showed in Figures 2 and 3 (Figures 2c and 3c). They suggested that the first two RDA components explain 52.04 and 14.45% of the total variance in the bacterial community (RDA1 and RDA2) (Figure 2c), and 44.59 and 27.90% in the fungal community (RDA1 and RDA2) (Figure 3c). Furthermore, we calculated the *p*-values to investigate the significance effects of soil environmental factors on microbial community composition. Among the factors, AP ($p = 0.007$) and UREA ($p = 0.02$) had significant influence on the fungal community structure. OM ($p = 0.022$) indicated that microbial community compositions of soil were strongly affected by AP, UREA, and OM after fertilizers were applied.

At the genus level (Figure 2d), the top nine of the dominant bacterial genera of all samples were Arthrobacter, Massilia, Nocardioides, Ramlibacter, Dyella, norank_o_Acidobacteriales, Gemmatimonas, Bacillus, and Rhodanobacter. Additionally, Arthrobacter, Gemmatimonas, and Bacillus of N2 were increased compared with CK. Burkholderia-Caballeronia-Parabulkholderia of T2 was increased compared with CK. The top nine dominant fungal genera of all samples were Fusarium, Mortierella, Saitozyma, Humicola, Guehomyces, Penicillium, Plectosphaerella, Solicoccozyma, and Aspergillus. In addition, Fusarium, Saitozyma, and Humicola of N2 were decreased compared with other treatments. On the contrary, Mortierella and Pseudouralinia increased (Figure 3d).

Moreover, the Spearman's correlation analysis results revealed the relationships between the genus level and the environmental factors (Figures 2e and 3e). In the bacterial community, ACP had extremely significant negative correlation with *Streptomyces* ($p_{unadj} = 0.00414$), FDA had extremely significant negative correlation with *Burkholderia-Caballeronia-Paraburkholderia* ($p_{unadj} = 0.00207$), NO_3–N had extremely significant positive correlation with *Burkholderia-Caballeronia-Paraburkholderia* ($p_{unadj} = 0.00108$), and UREA had significant positive correlation with *Rhodanobacter* ($p_{unadj} = 0.00691$). In the fungal community, INV had extremely significant negative correlation with *Aspergillus* ($p_{unadj} = 0.00277$) and *Epicoccum* ($p_{unadj} = 0.00871$). FDA and pH had significant positive correlation with *Olpidium* ($p_{unadj} = 0.00625$, $p_{unadj} = 0.00372$). The results of Spearman's correlation analysis revealed the relationships between genus level and the yield and nitrate content (Figures 2f and 3f). In the bacterial community, yield had significant positive correlation with *Rhodanobacter* ($p_{unadj} = 0.02606$). In the fungal community, yield had significant positive correlation with *Olpidium* ($p_{unadj} = 0.00536$).

3.4. Vitality of the Root, Photosynthetic Characteristics, Quality, Growth, and Yield of the Second Experiment

The root vitality of N2 and S2 were higher than T1 (Figure S3). Pn, Gs, and Tr of N2 were significantly higher than T1 (Table S10). The soluble sugar content and cellulose of CK were higher than other treatments (Table 3). The VC content of N2 was increased; however, on the contrary, the nitrate content was decreased and significantly lower than T1. The soluble protein and nitrate content of J2 was the highest and significantly higher than T1. Nitrogen reduction with bio-organic fertilizer significantly increased cabbage growth compared with chemical fertilizer treatment solely. The plant height and petiole length of N2 and S2 were significantly higher than T1 (Table S11). The yield of N2 and S2 increased by 9.24% and 6.93%, respectively, compared with T1 (Figure S4). The data of the yield showed the same trend with the third and fourth experiment we conducted (Qi. et al. 2021). Correlation analysis illustrated that the yield was positively correlated with root vitality ($p < 0.05$), total chlorophyll ($p < 0.01$), soil pH ($p < 0.05$), and soil OM ($p < 0.05$), and was negatively correlated with soluble sugar ($p < 0.01$) (Table 4).

Table 3. Effects of bio-organic fertilizer with 20% nitrogen reduction on quality of non-heading Chinese cabbage.

Treatments	Soluble Sugar ($mg \cdot g^{-1}$)	Cellulose %	Soluble Protein ($mg \cdot g^{-1}$)	Vitamin C ($mg \cdot 100\ g^{-1}$)	Nitrate ($mg \cdot kg^{-1}$)
CK	1.07 ± 0.03 a	27.31 ± 1.36 a	10.51 ± 0.34 d	68.13 ± 2.45 cd	551.83 ± 24.66 bc
T1	0.53 ± 0.06 bc	18.87 ± 0.47 bc	11.15 ± 0.38 c	69.74 ± 1.38 ab	564.04 ± 25.27 b
T2	0.40 ± 0.05 c	16.94 ± 0.40 c	11.62 ± 0.12 ab	69.61 ± 3.55 ab	507.03 ± 42.90 bc
N2	0.63 ± 0.02 b	18.42 ± 1.23 c	11.20 ± 0.48 ab	73.23 ± 2.36 a	446.15 ± 29.04 c
S2	0.52 ± 0.01 bc	19.76 ± 0.42 b	10.56 ± 0.27 c	72.62 ± 2.77 ab	475.03 ± 21.91 bc
J2	0.56 ± 0.84 bc	18.13 ± 1.72 c	11.87 ± 0.29 a	68.90 ± 2.77 bcd	1309.40 ± 52.77 a

Note: CK is no fertilization, T1 is 100% fertilization, T2 is 20% reduced nitrogen, N2 is 20% reduced nitrogen + 1500 $kg \cdot ha^{-1}$ "No.1", S2 is 20% reduced nitrogen +1500 $kg \cdot ha^{-1}$ "Seek", J2 is 20% reduced nitrogen + 720 $kg \cdot ha^{-1}$. The different small letters represent a significant difference at 0.05 level by Duncan's test.

Table 4. Correlation with soil nutrients, yield, and quality of non-heading Chinese cabbage.

	Yield	VC	Nitrate	Soluble Sugar	Total Chloro-phyll	Root Activity	pH	OM	TN	TP	TK	TCa	TZn	
Yield	1													
VC	−0.098	1												
Nitrate	0.062	0.392	1											1
Soluble sugar	−0.688 **	0.097	−0.078	1										0.5
Total chlorophyll	0.804 **	−0.339	0.09	−0.602 **	1									0
Root activity	0.487 *	0.196	−0.245	−0.387	0.289	1								−0.5
pH	0.588 *	−0.36	−0.624 **	−0.291	0.407	0.434	1							−1
OM	0.565 *	0.274	0.071	−0.014	0.338	0.311	0.328	1						
TN	0.066	−0.073	0.156	0.023	0.048	0.201	−0.006	−0.293	1					
TP	0.007	−0.422	−0.336	0.171	0.224	−0.079	0.363	−0.115	−0.223	1				
TK	−0.311	−0.096	−0.408	−0.046	−0.399	−0.045	−0.014	−0.249	−0.366	0.051	1			
TCa	−0.203	−0.422	−0.346	0.264	−0.026	−0.282	0.217	−0.326	−0.375	0.856 **	0.323	1		
TZn	−0.578 *	−0.56 *	−0.553 *	0.532 *	−0.38	−0.301	0.127	−0.273	−0.167	0.557 *	0.434	0.692 **	1	

Note: * at the 0.05 level, the correlation between them was significant; ** at 0.01 level, the correlation between them was significant.

4. Discussion

Our results showed not only the soil properties and soil community composition, but also the quality and the yield of non-heading Chinese cabbage that were significantly influenced by nitrogen reduction with different bio-organic fertilizers, especially with the No.1 bio-organic fertilizer (Figures 2 and 3, Table 3).

4.1. Effects of Nitrogen Reduction with Bio-Organic Fertilizer on Soil Chemical Properties

Many studies indicated that a favorable environment was the prerequisite for high yield and outstanding quality of crops, and different nitrogen sources had enormous effects on soil character [33–35]. In this experiment, 20% nitrogen reduction with bio-organic fertilizers increased the pH, organic matter, available phosphorus, and total nitrogen content. Appropriate soil pH is beneficial for agricultural production, and organic farming could increase soil pH when compared to conventional farming in acidic soils [36], whereas the application of mineral fertilizer decreased soil acidity by acidification and nitrification [37]. In our study, a reduced mineral fertilizer combined with bio-organic fertilizers increased soil pH in acidic soil (Table 2). In the second experiment, in the case of the same total nitrogen application, the total soil nitrogen content, available phosphorus, and soil organic matter content of N2 (bio-organic fertilizer with *Bacillus*) were significantly higher than in other treatments (Table 2). We found that the organic matter and available phosphorus content of N2 soil increased by 11.95% and 106.45%, respectively, compared with T1 (all chemical fertilization). Ye et al. [3] also found that *Trichoderma*-enriched bio-organic fertilizer significantly enriched soil fertility, as the soil organic matter, total N, total P, and total K all increased. Research suggests that organic matter as an overall indicator is essential for soil quality, which can bring many benefits to soil and plants [38]. The application of organic fertilizer not only improves soil enzyme activity [39], but also improves the available macronutrient content and increases soil micronutrient availability [40]. In this study, nitrogen reduction with bio-organic fertilizer improved the invertase and FDA soil activities (N2 and S2) (Figure S1), as well as K and Zn contents (N2) compared with conventional fertilization. In chili and cotton, application of Bacillus also increased soil invertase and FDA activities [41,42].

4.2. Effects of Nitrogen Reduction with Bio-Organic Fertilizer on the Soil Microbial Community

Fertilization had an essential impact on the composition of the soil microbe community of cabbage (Figure 2). In general, bio-organic fertilizer increased the proportion of beneficial microorganisms and decreased the proportion of harmful microorganisms, which in turn improved soil biofertility. Previous research showed that fertilizers could change the dominant bacterial and fungal phyla of soil [43,44]. In this experiment, the relative proportions of *Proteobacteria* and *Bacteroidetes* were all increased, and especially the relative proportion of *Gemmatimonadetes* of N2 increased compared with T1. *Proteobacteria* is known as halophilic bacteria with hydrolase activities [45,46]. Some chemical fertilizer-associated rhizobacteria were related to the degradation of organic substances such as *Bacteroidetes* [3]. *Gemmatimonadetes* and *Bacteroidetes* are essential for soil carbon cycling, which could improve the soil C/N [47,48]. *Ascomycota* decreased significantly after applying fertilizers, which was consistent with the results of the studies by Feng et al. [49]. *Dyella* spp. was involved in the Se biogeochemical cycle [50]. *Bradyrhizobium* was a kind of nitrogen-fixing bacteria related to nitrogen fixation [51]. *Mortierella* dissolved insoluble phosphorus by releasing a variety of organic acids in soil and promoted phosphorus cycling [52]. *Mortierella* promoted plant growth by improving soil phosphatase activity [53]. *Humicola* had a strong decomposition effect on organic matter [54]. *Fusarium* caused a variety of plant diseases, such as root rot, and decreased the yield and quality of crops [55].

Microbial community composition of the soil was correlated with AP, UREA, OM, and INV after applying bio-organic fertilizers (Figures 2e and 3e). Abundances of several microbial phyla (e.g., *Proteobacteria*, *Bacteroidetes*, and *Gemmatimonadetes*) were mainly influenced by the soil's available nutrients [56,57]. The application of fertilizer increases soil organic matter and alters microbial community structure and keystone taxa [58]. In the experiment, ACP had extremely significant negative correlation with *Streptomyces*, and FDA had extremely significant negative correlation with *Burkholderia-Caballeronia-Paraburkholderia* and *Olpidium*. Organic amendment and mineral fertilizer affected the soil invertase activity and microbial functional diversity [59]. At the genus level, soil pH is maybe the most important factor that has notable influences on the structure of soil bacterial communities [10]. In this experiment, pH had significant positive correlation with *Olpidium*.

4.3. Effects of Nitrogen Reduction with Bio-Organic Fertilizer on the Growth, Yield, and Quality of Non-Heading Chinese Cabbage

The experiment indicated that nitrogen reduction with "No.1" was beneficial to improve the growth, yield, and quality of the cabbage. Efficiency of crop photosynthesis was improved by suitable nutrient supply [60]. We found that the *P*n of J2 and S2 was increased compared with T1 in the first experiment (Table S4), and *P*n, *G*s, and Tr of N2 were increased in the second experiment (Table S10). The results also illustrated that total chlorophyll content was positively correlated with the yield (Table 4). The P, K, and Ca contents of N2 were the highest and significantly higher than in CK. The accumulation and formation of plant dry matter was closely related to nitrogen, and nitrogen was closely related to the growth, development, and yield of vegetables [61]. Research also illustrated that organic fertilizer with *Bacillus* content could reshape the soil community structure and benefit the growth of cabbage [44]. *Bacillus* is a kind of probiotic, which can secrete plant hormones and promote plant growth [62]. In addition, nitrogen reduction with bio-organic fertilizer (N2, S2) can improve root vitality (Figure S4). We found that root vitality was positively correlated with yield (Table 4). Such well-developed roots could give a foundation for the development of aboveground parts and accumulation of biomass by providing favorable conditions and improve nutrient and water absorption for non-heading Chinese cabbage [63]. The application of bio-organic fertilizer may also stimulate enzyme secretion by the roots [9]. Therefore, in this study, compared with T1 (100% chemical fertilizer), the yield of N2 (total N reduced 20% with No.1) increased by 10.20%, 20.90%, and 9.24% in the two experiments by different non-heading Chinese cabbage cultivars (Figure 1 and Figure S4). In general, the yield was positively correlated with soil pH, organic matter, root vitality, and total chlorophyll (Table 4). Moreover, the yield had significant positive correlation with *Rhodanobacter* and *Olpidium*. *Rhodanobacter* is an essential clade involved in denitrification of acidic soils [64].

The soluble sugar and VC content in non-heading Chinese cabbage of S2 and J2 were also significantly higher than in T1 in the first experiment (Figure S1). On the contrary, the nitrate content of N2 was decreased and significantly lower than T1. Similar results were reported by Wang [65] and Qi [66]. Feng et al. [67] also found that Bacillus megaterium enriched bio-organic fertilizer, significantly increased the vitamin C content, and decreased the nitrite content in Chinese flowering cabbage. Nitrogen was the key factor which could influence the nitrate content in vegetables. In addition, nitrate was a precursor for the synthesis of nitrosamines, which damages people's health with excessive intake [68]. A total of 85–90% of an adult's dietary intake of NO_3^- is from vegetables, as the vegetables tend to have a concentration of NO_3^- due to the accumulation of nitrogen fertilizer in the soil [69,70]. Therefore, replacing nitrogen with bio-organic fertilizer can reduce the accumulation of nitrogen in vegetables and, in turn, can lower the risks of some nitrate-related health problems.

5. Conclusions

The results illustrated that the application of *Bacillus* enriched bio-organic fertilizer No.1 succeeded in avoiding the overuse of mineral fertilizers to a significant extent without compromising the yield. More importantly, 20% reduced nitrogen with No.1 led to the improvement of soil pH, organic matter, soil microbial environment, and associated factors, as well as plant photosynthesis and root activity. This in turn promoted the yield and VC contents and declined the nitrate content of non-heading Chinese cabbage. For the soil microbial environment, the dominant phyla of *Gemmatimonadetes* and *Chytridiomycota* increased significantly, whereas *Ascomycota* decreased. The dominant genera *Gemmatimonas* and *Bacillus* increased, whereas Fusarium decreased. Redundancy analysis showed that the AP, OM, UREA, and INV of the soil were significantly correlated with the microbial community structure.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12061437/s1, Figure S1: Soil-enzyme activities at the root soil of cabbage under different fertilization treatments; Figure S2: Dilution curve of (a,d) bacteria, (b,e) fungi species abundance Alpha diversity index of soil samples; Figure S3: Effects of bio-organic fertilizer with nitrogen reduction on root vitality; Figure S4: Effects of bio-organic fertilizer with nitrogen reduction on yield of non-heading Chinese cabbage; Figure S5: Field growth status of non-nodular Chinese cabbage; Table S1: Fertilization situation in first experiment; Table S2: Information of primers used in this study; Table S3: Effect of nitrogen reduction by 20% combined with bio-organic fertilizer on five elements in soil of cabbage; Table S4: Effects of different fertilization with nitrogen reduced 20% treatments on photosynthetic characteristics of cabbage; Table S5: Effects of different fertilization with nitrogen reduced 20% treatments on growth of cabbage; Table S6: Effects of different fertilization with nitrogen reduced 30% treatments on growth of cabbage; Table S7: Effects of bio-organic fertilizer with nitrogen reduction on soil element content of non-heading Chinese cabbage; Table S8: Effects of bio-organic fertilizer with nitrogen reduction on element content of the plant; Table S9: Effect of different fertilizer treatments on bacterial, fungi, protist Alpha diversity indexes of soil; Table S10: Effects of bio-organic fertilizer with nitrogen reduction on photosynthetic characteristics of the cabbage; Table S11: Effects of bio-organic fertilizer with nitrogen reduction on growth of the cabbage.

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