Germlasm Screening of Green Manure Rapeseed through the Effects of Short-Term Decomposition on Soil Nutrients and Microorganisms

Xiaodan Wang¹,², Hua Ma¹,², Chunyun Guan¹,²,³ and Mei Guan¹,²,³,*

¹ Hunan Branch of National Oilseed Crops Improvement Center, Changsha 410128, China; wxd314159@stu.hunau.edu.cn (X.W.); maxiao1016@stu.hunan.edu.cn (H.M.); guancy2001@hunau.edu.cn (C.G.)
² College of Agriculture, Hunan Agricultural University, Changsha 410128, China
³ Southern Regional Collaborative Innovation Center for Grain and Oil Crops in China, Changsha 410128, China
* Correspondence: gm7142005@hunau.edu.cn

Abstract: The rapidly emerging fertilizer rapeseed used as green manure has wide applications for use. However, there have been few studies on its decomposition and effects on soil nutrients and microorganisms after its decay. In this study, 12 rapeseed lines to be screened were decomposed through a randomized block field design with two green-manure-specific varieties as the controls. The contents of nitrogen, phosphorus, and potassium from the plants, soil nutrients, and microbial changes after degradation were measured. There were substantial differences in the rates of decomposition and cumulative release of nutrients among the different lines after 30 days of rolling. The contents of phosphorus and potassium in the soil were 1.23–2.03 and 3.93–6.32 times those before decomposition, respectively. In addition, there was a significant difference in the relative content of soil microorganisms at the phylum level after the decomposition of different species of rapeseeds. Most of the top 20 bacterial groups significantly correlated with the characteristics of plant decomposition and soil nutrient content, including Proteobacteria, Actinomycetes, Armatimonadetes, Rokubacteria, and Planctomycetes. A principal component analysis showed that the soil microorganisms and nutrients are the leading factors that enable the evaluation of the decomposing characteristics of green manure rapeseed. Numbers 5 (purple leaf mustard) and 8 (Xiafang self-seeding) were more effective than two controls, which can be used as excellent types of germplasm to promote the breeding of green manure rapeseed.

Keywords: green manure; rapeseed; decomposition; nutrient content; soil microorganisms

1. Introduction

In parallel with the rapid development of modern industry, the unreasonable application of chemical fertilizer has caused a series of environmental and safety problems [1–3], which deters the realization of the target for sustainable development [3]. Planting green fertilizer can reduce the use of chemical fertilizers and pesticides [4], and the use of green manure can increase the contents of soil nutrients and improve the soil fertility and structure of soil microbial community among others [5,6]. It is of substantial significance to cultivate high-quality green manure varieties and develop the green manure industry for the ecological environment and agricultural green sustainable development.

Green manure rapeseed, an organic fertilizer that has rapidly emerged in recent years, can be planted over a wide range of areas and is highly adaptable [7]. In addition to improving the contents of soil nutrients and increasing the number of beneficial bacterial communities in the soil [8,9], it results in the accumulation of large amounts of dry matter, which is twice or more than that of milk vetch (Astragalus sinicus L.) [10]. Moreover,
previous studies have shown that the C/N ratio of rapeseed is relatively moderate, which renders it more easily decomposed by soil microorganisms than milk vetch, and the nutrients are easily absorbed by the subsequent crops [11]. Glucosinolate hydrolysate in rapeseed can inhibit weeds, soil pathogens, and soil nematodes [12–14], which is one of the important characteristics that renders it different from other green fertilizer crops. In addition, green manure rapeseed can be combined with various farming systems, such as the “rice–rice–rapeseed” [15] and “cotton–rapeseed” crop rotations in the Yangtze River Basin [16], with particular application value and broad development prospects.

The efficiency of decomposed green manure to serve as fertilizer is one of the main goals of the evaluation of green manure germplasm resources. The accumulation of soil organic matter is affected by the type of green manure, time and quantity in which it is returned, and environmental factors, among others [17–19]. Studies have shown that the decomposition rate can reach more than 50% within one month by returning rapeseed at the blooming stage [20]. However, the rates of decomposition and patterns of nutrient release of various rapeseed varieties differ [21]. In addition, there are few studies on the effects of rapeseed decomposition on soil microorganisms, and there is a lack of excellent green manure-specific varieties. Based on this, the decomposition regularities of 12 types of rapeseed and their impacts on soil nutrients and microorganisms were studied to screen high-quality green manure germplasm resources and promote the breeding of green manure rapeseed.

2. Materials and Methods

2.1. Overview of the Experimental Field

The experimental site was located in the Yunyuan Base of Hunan Agricultural University, Changsha, Hunan, China (113°07' E, 28°18' N) (Figure S1). It is located in a humid subtropical monsoon climate. During the decomposition period (20 March 2019 to 19 April 2019), the cumulative rainfall was 157.8 mm, and the average temperature was 16.5 °C. The experimental field implemented a “rice–oilseed” rotation. The tested soil was yellow-brown loam. Before the treatment of returning green manure to the soil, the soil pH was 5.70, and the content of organic matter, total nitrogen, available phosphorus, and available potassium were 21.60 g kg⁻¹, 1.29 g Kg⁻¹, 10.23 mg kg⁻¹, and 137.76 mg kg⁻¹, respectively.

2.2. Experimental Design

Field simulation experiments were utilized with three replicates in this study, and the abridged general view is shown in Figure S1. The plants were collected at the blooming stage. The samples were cut into 2–3 cm segments and weighed until 300 g of each had been collected. They were then mixed evenly and packed in nylon mesh bags that were 25 × 35 cm with an aperture of 74 μm. The bags were buried 20 cm below the soil surface with the samples 30 cm apart from each other. The plant residues and soil samples were taken after 30 days. The plant residues were dried to determine their nutrient contents and rate of decomposition of each variety. One part of the soil samples was dried to determine the nutrient content, while the other part was quickly placed in liquid nitrogen and stored at −80 °C to determine their microbial diversity. The basic traits of rapeseed before decomposition are shown in Table 1.
Table 1. Basic characters of rapeseed before decomposition.

| Number | Name               | N     | P      | K     | Decomposition Quantity (Dry Weight) | Glucosinolate Content µmol g⁻¹ |
|--------|--------------------|-------|--------|-------|-------------------------------------|-------------------------------|
| 1      | 991                | 22.69 | 4.37   | 27.45 | 47.18 ± 0.59                       | 241.80 ± 0.55                 |
| 2      | A1                 | 16.21 | 3.64   | 38.31 | 44.33 ± 2.96                       | 704.91 ± 4.37                 |
| 3      | Xiangyou 1035      | 18.66 | 3.89   | 24.58 | 51.50 ± 1.18                       | 314.82 ± 2.33                 |
| 4      | Brassica Campestris | 21.95 | 4.46   | 24.11 | 40.72 ± 2.43                       | 499.42 ± 4.42                 |
| 5      | Purple leaf mustard| 28.90 | 3.88   | 32.20 | 65.75 ± 3.18                       | 182.43 ± 1.63                 |
| 6      | Green leaf mustard | 19.90 | 4.18   | 21.79 | 49.32 ± 1.83                       | 394.26 ± 4.47                 |
| 7      | Rhubarb mustard    | 20.67 | 3.68   | 22.07 | 48.70 ± 0.40                       | 394.81 ± 4.21                 |
| 8      | Xiafang self-seeding| 25.29 | 5.89   | 25.67 | 43.58 ± 3.13                       | 248.37 ± 6.24                 |
| 9      | 78                 | 21.25 | 5.01   | 26.63 | 37.52 ± 4.10                       | 427.33 ± 6.69                 |
| 10     | Chenxi Wax rapeseed| 20.05 | 3.92   | 31.95 | 56.77 ± 3.95                       | 496.50 ± 7.47                 |
| 11     | Xiangyou No. 4     | 17.88 | 4.13   | 33.67 | 41.25 ± 3.21                       | 512.43 ± 2.92                 |
| 12     | Huapingjing rapeseed| 17.46 | 3.79   | 25.90 | 40.90 ± 1.34                       | 773.94 ± 4.59                 |
| CK1    | Youfei No. 1       | 14.47 | 3.83   | 27.72 | 59.27 ± 0.82                       | 911.66 ± 2.21                 |
| CK2    | Youfei No. 2       | 28.29 | 6.37   | 33.49 | 46.09 ± 0.67                       | 477.26 ± 3.20                 |

Values are the means ± standard error (n = 3). The values with different superscript letters in a column are significantly different (p < 0.05).

2.3. Test Method

The nutrients of plant samples were determined as follows: The total nitrogen was determined using a SKALAR interval flow analyzer. The total phosphorus was determined by the Mo-Sb-Vc colorimetric method [22], and the total potassium was determined by atomic absorption spectrophotometry [23].

The soil samples were analyzed to determine their contents of nutrients: The total nitrogen was determined using the semi micro Kjeldahl method [24]. The available phosphorus was determined using the Olsen sodium bicarbonate method [25], and the available potassium was determined by NH₄OAc (1.0 mol·L⁻¹) extraction atomic absorption spectrophotometry [26].

The experiment was conducted as described by Wang Yingying [23]. Novogene Biotech Co., Ltd. (Beijing, China) was utilized to perform 16S (V3 + V4) regional amplicon sequencing by Single-End based on an IonS5™XL sequencing platform.

2.4. Data Analysis

Decomposition rate was calculated as follows:

Decomposition rate (%) = (M₀ - M₃₀)/M₀ × 100

Plant nutrient release rate was calculated as follows:

Plant nutrient release rate (%) = (C₀ - M₀ - C₃₀ × M₃₀)/C₀ × M₀ × 100

where M₀ is the initial dry matter weight of the test material; M₃₀ is the dry matter weight (residue) of green fertilizer for 30 days; C₀ is the initial nutrient concentration of the test material, and C₃₀ is the nutrient concentration of the test material for 30 days.

SPSS 24.0 (IBM, Inc., Armonk, NY, USA) was used for correlation and principal component analyses, and Origin 2019 (OriginLab, Northampton, MA, USA) was used for mapping. The LSD method was used to determine the difference significance test. The difference was considered to be significant at p < 0.05. The bioinformatics analysis of high-throughput data was conducted using Novo Magic (https://magic.novogene.com, accessed on 23 August 2021).

3. Results and Analysis

3.1. Decomposition of Rapeseed Plants and the Influence of Soil Nutrients

The analysis of the plants in Table 2 shows that the rate of decomposition and the cumulative release rate of nitrogen (N) and phosphorus (P) vary substantially among the
The analysis of soil nutrients of rapeseeds before and after decomposition is shown in Table 2. The contents of soil nitrogen (N) increased after the decomposition of most rapeseed lines, whereas the difference was not significant. The contents of soil phosphorus (P) increased significantly after the decomposition of rapeseed numbers 12, 2, and 3, which was higher than that of both controls. The content of soil potassium (K) increased significantly and was 3.93- to 6.32-fold higher than that before decomposition. With the exception of 6, 4, 1, and 2, the other strains were higher than both controls.

3.2. The Effects of Rapeseed on Soil Microbial Diversity and Community Structure after Decomposition

3.2.1. The Effect of Rapeseed on Soil Microbial Community Structure after Decomposition

On the decomposed and pre-decomposed soil samples of 14 types of rapeseeds, 16S sequencing was performed. A total of 3,588,932 raw reads were obtained, and each sample obtained an average of 79,754 ± 9571 sequences (Table S1). The sample-based species accumulation box plot in Figure 1a shows that the number of orthologous taxonomic units (OTUs) increased with the number of samples. As the number of OTUs became saturated, the curve was asymptotically stable, and the number of new OTUs added to each sample decreased, indicating that the sequencing depth was accurate enough to represent the diversity of soil bacteria. The dilution curve (Figure 1b) shows that with the increase in number of samples, the bacterial species reached the saturation stage, indicating that the microorganisms in the population captured most of the bacterial members of each strain. After the quality control of the sequences, OTU cluster analysis was conducted using 97% similarity (Figure 1c). The results showed that the common number of OTUs in each sample was 1166, comprising 39.27% of the total OTUs. This indicated that the populations of various varieties (lines) differed somewhat, with the number of specific OTUs of the CK3 totalizing 418, comprising 15.31% of the total OTUs. Alternatively, there were differences in the soil microorganisms before and after decomposition. Based on the annotation results of the SILVA database (Figure 1d), the top 10 species of soil bacteria at the phylum level were Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, Chloroflexi, Gemmatimonadetes,
Thaumarchaeota, Firmicutes, Verrucomicrobia, and unknown microorganisms. Among them, CK3 and the other strains clustered into two branches. The abundances of Acidobacteria and Bacteroides in the decomposed soil increased, while the abundances of Actinomyces and Chloroflexi decreased. In general, there was no significant difference in the composition of soil microorganisms at the phylum level after the decomposition of different varieties (lines), but the relative content differed somewhat.

![Figure 1](image1.png)

3.2.2. The Effects of Rapeseed on the Diversity and Function of Soil Microbial Communities after Decomposition

The α diversity of soil bacteria in Table 3 shows that the sequencing depth of samples was >96.50% (coverage index), and there was no significant difference between the original soil (CK3, Table 2) and decomposed soil of the rapeseed plants in the Chao1 value and OTU number, with the exception of number 12. The Shannon (except for number 9) and Simpson indices of the original soil were lower than those of the soil that contained the decomposed tissue. The former did not differ significantly among the decomposed soil of rapeseeds. Overall, the dominance of soil microbial community did not change significantly after the decomposition of rapeseeds. The soil samples of numbers 11 and 6 had a higher abundance and diversity of colonies.

![Figure 2](image2.png)
The functional analysis of each sample community obtained by the FAPROTAX software (Figure 2) showed that the first 15 types of functions were primarily types of nitrogen metabolism. Compared with the original soil, the abundance of genera related to nitrogen metabolism in the decomposed soil was higher, while the abundance of animal parasitic or symbiotic bacteria and animal human intestinal microflora was lower. This differed from the abundance of microbial functional categories for the soil in which the rapeseeds had decomposed. The original soil (CK3, Table 2) and the decomposed soil clustered into two branches, indicating that the main functional groups of soil microorganisms had clearly changed.

**Table 3. α-Diversity of the bacterial community in different types of rapeseed cultivars.**

| Number | Bacteria Abundance | Bacterial Diversity | Sequencing Depth Index |
|--------|--------------------|---------------------|------------------------|
|        | Observed_Species (OTUs) | Simpson | Shannon | Goods_Coverage |
| 1 | 4058.6072 ab | 2734.6667 abc | 0.9891 ab | 9.2763 a | 0.9711 |
| 2 | 4413.1777 ab | 2412.0000 abc | 0.9956 a | 9.2743 a | 0.9729 |
| 3 | 3804.7922 ab | 2499.6667 abc | 0.9950 a | 9.3046 a | 0.9732 |
| 4 | 3830.2588 ab | 2733.0000 abc | 0.9954 a | 9.6060 a | 0.9737 |
| 5 | 3973.0098 ab | 2640.0000 abc | 0.9917 ab | 9.0900 a | 0.9710 |
| 6 | 4582.5752 ab | 2850.0000 a | 0.9966 a | 9.6172 a | 0.9681 |
| 7 | 4302.5791 ab | 2815.3333 ab | 0.9943 ab | 9.3451 a | 0.9692 |
| 8 | 4028.1704 ab | 2733.6667 abc | 0.9958 a | 9.4356 a | 0.9714 |
| 9 | 3459.6882 ab | 2301.0000 c | 0.9928 ab | 8.9602 a | 0.9762 |
| 10 | 3894.9641 ab | 2484.6667 abc | 0.9929 ab | 9.0594 a | 0.9722 |
| 11 | 4744.3932 a | 2874.0000 a | 0.9919 ab | 9.3011 a | 0.9664 |
| 12 | 3435.0007 b | 2354.6667 bc | 0.9939 ab | 9.0739 a | 0.9755 |
| CK1 | 3805.9963 ab | 2519.0000 abc | 0.9949 a | 9.2522 a | 0.9726 |
| CK2 | 3801.0806 ab | 2503.3333 abc | 0.9942 ab | 9.2416 a | 0.9743 |
| CK3 | 4670.4347 ab | 2869.0000 a | 0.9815 b | 8.9931 a | 0.9669 |

The values with different superscript letters in a column are significantly different (p < 0.05).

Figure 2. Cluster diagram of soil microbial community function in different varieties.
3.3. Correlation Analysis and Principal Component Analysis of Environmental Factors, Plant Nutrients, Soil Nutrients, and Microorganisms

3.3.1. Correlation Analysis of Environmental Factors, Plant Nutrients, Soil Nutrients, and Microorganisms

A Spearman analysis was used to analyze the correlation between plant properties before and after decomposition and soil nutrients and microbial properties after decomposition (Figure 3). There were significant positive correlations between the plant cumulative decomposition rates of N and P, whereas there was a significant positive correlation between the dry matter weight and the soil N and P. Most of the top 20 bacterial groups significantly correlated with the decomposition characters of the plants, and the soil nutrient content, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Rokubacteria*, and *Planctomycetes*, extremely significantly correlated with the traits of plants before and after decomposition and soil nutrients in particular.

**Figure 3.** Correlation analysis between environmental factors and soil nutrients and microorganisms. DW: dry weight; GLU: glucosinolate content; AD: accumulative decomposition rate; NRR, PRR, and KRR: the cumulative release rate of nitrogen, phosphorus, and potassium, respectively; TN, TP, and TK: the contents of soil nitrogen, phosphorus, and potassium, respectively. Asterisks denote statistical significance at the \( p < 0.05 \) (*) and \( p < 0.01 \) (**) level.

3.3.2. Environmental Factors and Principal Component Analysis of Soil Microbial Community

Based on the analysis of Section 3.3.1, there were positive or negative correlations among the plant nutrient content, soil nutrients, and microorganisms. The nutrient content of plant samples before and after decomposition, the corresponding soil nutrient content after decomposition, and the five types of bacteria with significant differences described above were standardized for a principal component analysis. Table 4 lists the first six principal components, which can represent 87.08% of the nutrient information of 14 types of rapeseed plants. The first principal component contributed the most, comprising 29.86%, and the soil microorganisms and plant nutrients comprised the larger positive load. The second principal component comprised 16.71%, and the contents of soil nutrients comprised a large positive load. In the third principal component, the rate of plant decomposition and nutrient release comprised a large positive load. In the fourth principal component, the content of soil nitrogen and rate of plant decomposition comprised a large positive load. The fifth and sixth principal components that comprised the larger positive load were the plant trait factors before decomposition. The normalized values of plant and soil nutrients and microbial characteristics were multiplied by the eigenvectors of the first six principal components and weighted to obtain the scores and ranking of each principal component (Table 5). Compared with the two controls, numbers 5, 9, 8, and 10 had higher characteristics of soil microbes and nutrients, and numbers 8, 6, 2, and 5 had...
higher characteristics of plant nutrients and decomposition. The overall analysis indicated that numbers 5 and 8 were more effective than those of CK1 and CK2.

Table 4. Principal component analysis of plant and soil nutrient characteristics.

|                      | Y1     | Y2     | Y3     | Y4     | Y5     | Y6     | Rank |
|----------------------|--------|--------|--------|--------|--------|--------|------|
| Plant characters     |        |        |        |        |        |        |      |
| before decomposition |        |        |        |        |        |        |      |
| N                    | 0.752  | −0.017 | 0.565  | −0.009 | −0.247 | 0.040  | 2    |
| P                    | 0.544  | −0.328 | 0.526  | −0.012 | 0.418  | 0.246  | 8    |
| K                    | −0.181 | 0.640  | 0.352  | −0.296 | 0.140  | 0.514  | 12   |
| Decomposition        |        |        |        |        |        |        |      |
| quantity (dry weight)| 0.051  | 0.615  | 0.006  | 0.581  | −0.475 | −0.048 | 13   |
| Glucosinolate content| −0.734 | 0.041  | −0.212 | 0.237  | 0.408  | 0.266  | 4    |
| Decomposition rate   | 0.509  | −0.398 | 0.170  | 0.442  | 0.146  | 0.101  | 10   |
| nutrient release rate|        |        |        |        |        |        |      |
| rate of plants       | 0.309  | 0.010  | 0.598  | −0.339 | −0.445 | −0.310 | 15   |
| YN                   | 0.714  | −0.403 | −0.014 | 0.129  | −0.052 | 0.370  | 5    |
| YP                   | 0.197  | 0.398  | 0.475  | 0.000  | 0.473  | −0.371 | 16   |
| Soil nutrient content|        |        |        |        |        |        |      |
| TN                   | 0.531  | 0.164  | −0.013 | 0.723  | 0.074  | −0.199 | 17   |
| TP                   | −0.195 | 0.897  | 0.277  | 0.202  | 0.041  | 0.073  | 11   |
| TK                   | 0.314  | 0.320  | −0.662 | −0.306 | 0.181  | −0.325 | 14   |
| Soil microorganism   |        |        |        |        |        |        |      |
| Proteobacteria       | 0.664  | 0.307  | −0.522 | 0.080  | −0.117 | 0.303  | 6    |
| Actinobacteria       | −0.650 | −0.461 | 0.058  | 0.519  | −0.038 | −0.149 | 7    |
| Bacteroidetes        | 0.804  | −0.121 | 0.408  | 0.027  | 0.314  | −0.118 | 1    |
| Rokubacteria         | 0.738  | −0.160 | −0.232 | −0.105 | 0.413  | −0.214 | 3    |
| Planctomycetes       | −0.514 | −0.426 | 0.013  | −0.128 | −0.442 | 0.065  | 9    |
| Characteristic value | 5.076  | 2.840  | 2.389  | 1.774  | 1.613  | 1.109  |      |
| Contribution rate %  | 29.861 | 16.709 | 14.055 | 10.436 | 9.489  | 6.524  |      |
| Cumulative contribution rate % | 29.861 | 46.570 | 60.625 | 71.062 | 80.551 | 87.075 |      |

Table 5. Comprehensive comparison of nutrient characteristics via a principal component analysis.

| Number | y1   | y2   | y3   | y4   | y5   | y6   | y    | Rank |
|--------|------|------|------|------|------|------|------|------|
| 1      | 1.66 | −0.52| −0.26| −0.79| −1.75| 0.79 | 0.18 | 5    |
| 2      | −4.41| 1.39 | 1.11 | −1.62| 0.70 | 0.31 | −1.01| 13   |
| 3      | −1.08| 0.15 | 0.60 | −0.32| −0.90| −1.26| −0.58| 11   |
| 4      | −1.23| −3.91| −0.18| −0.43| −1.67| 0.97 | −1.19| 14   |
| 5      | 2.94 | 2.62 | 1.02 | 1.21 | 2.06 | −0.21| 1.38 | 1    |
| 6      | −1.47| −1.52| 1.75 | 1.31 | −0.06| 1.93 | −0.44| 10   |
| 7      | 0.49 | 0.45 | −1.30| −0.40| −0.21| −1.51| −0.12| 8    |
| 8      | 3.87 | −1.62| −0.17| 0.49 | 1.33 | 0.88 | 1.09 | 2    |
| 9      | 2.67 | −0.39| −1.14| −1.01| 2.19 | −1.17| 0.60 | 4    |
| 10     | −0.08| 2.19 | −1.16| −0.86| −0.61| 0.44 | 0.06 | 6    |
| 11     | −0.27| 0.99 | −0.55| −1.31| 0.80 | 1.19 | 0.02 | 7    |
| 12     | −1.61| −0.90| −0.95| 0.07 | 0.34 | −0.13| −0.73| 12   |
| CK1    | −2.19| 0.79 | −1.68| 3.53 | 0.90 | 1.01 | −0.24| 9    |
| CK2    | 0.71 | 0.27 | 4.11 | 0.12 | 1.01 | 0.60 | 0.98 | 3    |

4. Discussion

4.1. Decomposition of Rapeseed

The most effective fertilization can be achieved by returning rapeseed at the blooming stage [27]. Therefore, the materials at this stage were selected for decomposition in this experiment, and the decomposition rate after 30 days was between 37.12% and 76.13%. Except for numbers 2, 9, 10, and 11, the rate of decomposition of other strains exceeded 50%, which was consistent with the findings of previous studies [20]. The decomposition and
cumulative rates of release of N and P among the different rapeseeds are highly variable, which may be related to their own characteristics. The high rate of decomposition of number 8 may be owing to its low contents of lignin and cellulose (Table S2).

Alternatively, returning rapeseed to the field plays an important role in improving the soil and farmland systems [7–10]. In this experiment, the contents of soil P and K increased significantly, and the content of K, in particular, increased by 4–6-fold after the return of rapeseeds, which showed that one month’s decomposition time can effectively meet the growth of the next crops. In addition, although the cumulative rate of release of N was between 17.88% and 54.59%, there was no apparent increase in the soil N. This could be caused by the action of soil denitrifying bacteria, the alkaline soil pH, high content of soil CaCO3, or high temperature [17], which could make a profound study. Although the increase in soil nitrogen is not significant, it can also reduce the use of nitrogen fertilizer. Xiao Xiaojun et al. [28] showed that under the condition of returning rapeseed, only a small amount of nitrogen fertilizer can meet the growth of the subsequent rice crop. In general, utilizing soil nutrients as screening conditions, except for CK1 and CK2, numbers 2 and 3 are suitable for use in soil that is slightly deficient in nitrogen. Numbers 9, 5, 7, and 8 are suitable for phosphorus deficient soil, while all of the strains are suitable for potassium deficient soil.

4.2. Effects of Decomposition on Soil Bacterial Diversity and Community Structure

In addition to increasing the content of soil nutrients and improving the soil physical and chemical properties, the impact on soil microorganisms is a critical question. Based on the annotation results of the SILVA database, there was no significant difference in the composition of soil microorganisms at the phylum level after the decomposition of different rapeseeds, and the Chao index and OTUs of the original soil (CK3) were relatively high, indicating that the abundance and composition of the soil bacterial community in natural soil field were relatively stable in a certain environment, which suggests that the decomposition of rapeseed does not destroy the original microbial structure. After decomposition, the Simpson and Shannon indices increased (with the exception of number 9), indicating that sugars, proteins, amino acids, and other substances may provide nutrition for microorganisms or form part of the soil humus during the decomposition process of rapeseed, which increases the diversity of some soil bacteria [29,30].

Furthermore, a cluster analysis showed that the microbial groups of CK3 and the soil after rapeseed decomposition are divided into two branches, and the abundances of Acidobacteria and Bacteroidetes increased significantly after decomposition. Acidobacteria, known as “ecosystem engineer”, participate in the metabolism and transportation of carbon, nitrogen, and sulfur and are the core community of nitrogen cycle [31]. Simultaneously, it also regulates the biosynthesis of secondary metabolites and the stress and hunger response. In addition, overturning and covering the green manure or adding organic fertilizer are conducive to the growth of Bacteroidetes [32,33]. It is hypothesized that Acidobacteria and Bacteroidetes play important roles in the decomposition of rapeseed. Alternatively, the decrease in the abundance of Actinobacteria and Chloroflexi in the soil after rapeseed decomposition is completely consistent with the research of Li Lina et al. [34]. Although Chloroflexi is the dominant population in soil, there is little understanding of the function of such microorganisms because most of them are not culturable [35]. Some previous studies have shown that some of the members of Chloroflexi, such as GP6, GP16, and GP4, significantly negatively correlate with the content of organic carbon in soil [36], which may be the reason for the relatively high content of Chloroflexi without returning rapeseed in this experiment. Similarly, Actinobacteria are less competitive than other microbial groups in soils that contain a higher level of nutrients [32]. However, although the increase in Acidobacteria and Bacteroidetes and the decrease in Actinobacteria and Chloroflexi can indicate that the populations of soil microorganisms have changed after decomposition, it is not an effective method to evaluate the difference in green manure rapeseed because its changes are similar in all strains.
4.3. Correlation between Soil Nutrients, Microbial Composition, and the Plant Decomposition Index

The decomposition of green manure will affect the soil nutrients, and the latter will affect the structural diversity and abundance of the soil microbial community [5,6,18]. Microorganisms are the main driving force of the soil nutrient material cycle, and the microbial communities in the soil are vulnerable to the influence of the external environment; thus, they react to the soil environment [37,38]. In this study, most of the top 20 bacterial groups significantly correlated with the characteristics of plant decomposition and soil nutrient content, including Proteobacteria, Actinobacteria, Armatimonadetes, Rokubacteria, and Planctomycetes that extremely significantly correlated with soil nutrients and plant properties before and after decomposition. Proteobacteria play an important role in nitrogen fixation and degradation [39,40]. Similar to the Actinobacteria, their abundance reflects their ability to grow in different environments, which can regulate the amount of soil fertilization [41]. Armatimonadetes constitutes a moderately abundant and phylogenetically diverse bacterial phylum [42], which is known to be capable of the metabolism of ammonium [43]. Interestingly, similar to Actinobacteria, it has a significant positive correlation with the contents of plant glucosinolates and soil phosphorus. The establishment of a model to predict their content would help to elucidate their functions. Rokubacteria are known as the “genome giant in uncultured bacteria”, because they have the potential of a large genome (6–8 Mbps), a high GC content (66–71%) [44], and multifunctional and mixed-nutrient metabolism [45,46]. Planctomycetes are widely distributed and abundant components of the soil microbial community. They participate in sugar degradation and are highly significant to the global nitrogen cycle. The changes in soil organic matter, Ca\(^{2+}\) content, and pH will affect their community composition [47,48]. Buckley et al. [47] showed that the abundance of Planctomycetes was related to the spatial heterogeneity of nitrate, which can be used to judge the historical conditions of soil. Therefore, it seems likely that the suitable utilization of these bacteria can promote the fertilization of crops and healthy operation of the agroecosystem. More importantly, taking the relationship between these five bacteria and soil nutrients and the plant decomposition index as the evaluation factor for screening green manure rapeseed is of substantial significance for the development of green manure rapeseed breeding, which merits further study.

5. Conclusions

In summary, compared with the original soil, the decomposition of rapeseed significantly increased the contents of soil nutrients and bacterial community structure and diversity. Proteobacteria, Actinobacteria, Armatimonadetes, Rokubacteria, and Planctomycetes in the soil significantly correlated with soil nutrients and plant properties before and after decomposition. This indicates that they may play an important role in the decomposition of rapeseed. A principal component analysis showed that soil microorganisms and nutrients were the leading factors in the evaluation of the characteristics of decomposition of green manure rapeseed. Compared with the CK1 and CK2, numbers 5 (purple leaf mustard) and 8 (Xiafang self-seeding) performed outstandingly, which renders them valuable for use as high-quality green manure rapeseed materials for further research.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11121219/s1, Figure S1: Schematic diagram of experimental site, Table S1: Data preprocessing statistics and quality control, Table S2: Basic characters of oilseed rape before decomposition.

Author Contributions: C.G. and M.G. designed the experiment. H.M. conducted the experiment, and X.W. processed and analyzed data and wrote the first draft. C.G. and M.G. revised and edited. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Rapeseed Industrial Technology System (CARS-13) and the Natural Science Foundation of China (grant no. 31130040).
Data Availability Statement: All the data included in this study are available upon request by contact with the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Wang, Q.; Awasthi, M.K.; Zhang, Z.; Wong, J.W.C. Sustainable composting and its environmental implications. In Sustainable Resource Recovery and Zero Waste Approaches, 1st ed.; Taherzadeh, M.J., Bolton, K., Wong, J., Pandey, A., Eds.; Elsevier: Amsterdam, The Netherlands, 2019; Volume 7, pp. 115–132. [CrossRef]
2. Ren, F.L.; Zhang, X.B.; Liu, J.; Sun, N.; Wu, L.H.; Xu, M.G. A synthetic analysis of greenhouse gas emissions from manure amended agricultural soils in China. Sci. Rep. 2017, 7, 8123. [CrossRef]
3. Aktar, M.W.; Sengupta, D.; Chowdhury, A. Impact of Pesticides Use in Agriculture: Their Benefits and Hazards. Interdiscip. Toxicol. 2009, 2, 1–12. [CrossRef]
4. Lin, W.; Lin, M.; Zhou, H.; Wu, H.; Li, Z.; Lin, W. The effects of chemical and organic fertilizer usage on rhizosphere soil in tea orchards. PLoS ONE 2014, 9, e0217108. [CrossRef]
5. Dong, N.G.; Hu, G.L.; Zhang, Y.Q.; Qi, J.X.; Chen, Y.H.; Hao, Y.B. Effects of green-manure and tillage management on soil microbial community composition, nutrients and tree growth in a walnut orchard. Sci. Rep. 2021, 11, 16882. [CrossRef]
6. Costerousse, B.; Quattinri, J.; Grüter, R.; Frossard, E.; Thonar, C. Green manure effect on the ability of native and inoculated soil bacteria to mobilize zinc for wheat uptake (Triticum aestivum L.). Plant Soil 2021, 467, 287–309. [CrossRef]
7. Dong-Hui, F.U.; Jiang, L.Y.; Mason, A.S.; Xiao, M.L.; Zhu, L.R.; Li-Zhi, Li; Zhou, Q.H.; Shen, C.J.; Huang, C.H. Research progress and strategies for multifunctional rapeseed: A case study of china. J. Integr. Agric. 2016, 15, 1673–1684. [CrossRef]
8. Huang, M.; Zhou, X.F.; Xie, X.B.; Chen, J.N.; Zou, Y.B. Increased soil fertility in a long-term rice-oilseed rape cropping system and its potential roles in reducing nitrogen inputs and environmental impacts. In Cropping Systems: Applications, Management and Impact; Hodgds, J.G., Ed.; Nova Science Publishers: New York, NY, USA, 2017; pp. 103–113, ISBN 978-63485-888-5.
9. Li, W.G.; Yang, X.X.; Huang, C.G.; Xue, N.W.; Xia, Q.; Liu, X.L.; Zhang, X.Q.; Yang, S.; Yang, Z.P.; Gao, Z.Q. Effects of rapeseed green manure on soil fertility and bacterial community in dryland wheat field. Agric. Sci. China 2019, 52, 2664–2677. [CrossRef]
10. Gu, C.M.; Li, Y.S.; Xie, L.H.; Hu, X.J.; Liao, X.; Qin, L. Analysis on application advantages of rapeseed as green manure. Soil Fertil. Sci. China 2019, 01, 180–183. [CrossRef]
11. Wang, D.Y.; Peng, J.; Xu, C.M.; Zhao, F.; Zhang, X.F. Effects of rape straw manuring on soil fertility and rice growth. Chin. J. Rice Sci. 2011, 26, 85–91. [CrossRef]
12. Haramoto, E.; Gallandt, E. Brassica cover cropping for weed management: A review. Renew. Agric. Food. Syst. 2004, 19, 187–198. [CrossRef]
13. Poveda, J.; Eugui, D.; Velasco, P. Natural control of plant pathogens through glucosinolates: An effective strategy against fungi and oomycetes. Phytochem. Rev. 2020, 19, 1045–1059. [CrossRef]
14. Johnson, A.W.; Golden, A.M.; Auld, D.L.; Sumner, D.R. Effects of rapeseed and vetch as green manure crops and fallow on nematodes and soil-borne pathogens. J. Nematol. 1992, 24, 117–126. [CrossRef]
15. Huang, M.; Tian, A.L.; Zhou, X.; Gao, W.; Li, Z.B.; Chen, G.; Li, A.H.; Chen, Y.M.; Liu, I.S.; Yin, X.H.; et al. Yield performance of machine-transplanted double-season rice grown following oilseed rape. Sci. Rep. 2019, 9, 6818. [CrossRef]
16. Ren, T.; Li, H.; Lu, J.W.; Bu, R.Y.; Li, X.K.; Cong, R.H.; Lu, M.X. Crop rotation-dependent yield responses to fertilization in winter oilseed rape (Brassica napus L.). Crop J. 2015, 3, 396–404. [CrossRef]
17. Edmeades, D.C. The long-term effects of manures and fertilisers on soil productivity and quality: A review. Nutr. Cycl. Agroecosyst. 2003, 66, 165–180. [CrossRef]
18. Sharma, P.; Laor, Y.; Raviv, M.; Medina, S.; Saadi, I.; Krasnovsky, A.; Vager, M.; Levy, G.J.; Bar-Tal, A.; Borisover, M. Green manure as part of organic management cycle: Effects on changes in organic matter characteristics across the soil profile. Geoderma 2017, 305, 197–207. [CrossRef]
19. Adebayo, A.O.; Agbede, T.M.; Aboyeji, C.M.; Dunsin, O.; Ugbe, J.O. Green manures and npk fertilizer effects on soil properties, growth, yield, mineral and vitamin c composition of okra (abelmoschus esculentus (L.) moench). J. Saudi Soc. Agric. Sci. 2019, 18, 218–233. [CrossRef]
20. Liu, X.H.; Zhou, X.; Deng, L.C.; Fan, L.Y.; Qu, L.; Li, M. Decomposition characteristics of rapeseed green manure and effect of nutrient release on soil fertility. Hunan Agric. Sci. 2020, 416, 39–44. [CrossRef]
21. Kriaučiūnienė, Z.; Cepuliene, R.; Velička, R.; Marcinkevičienė, A.; Lekavičienė, K.; Šarasauskis, E. Oilseed Rape Crop Residues: Decomposition, Properties and Allelopathic Effects. In Sustainable Agriculture Reviews; Springer: Cham, Switzerland, 2018; Volume 32. [CrossRef]
22. Yuan, Y.Y.; Liu, Q.M.; Zhong, Y.S.; Liao, F.P.; Lin, J.R. Mechanism of CP7 antibacterial protein against Aeromonas hydrophila. Microbiol. Chin. 2012, 39, 949–957.
23. Wang, Y.Y. Evaluation of Different Varieties of Mung Bean Used as Green Manure. Master’s Thesis, Chinese Academy of Agricultural Sciences, Beijing, China, June 2011.
24. Fawcett, J.K. The semi-micro Kjeldahl method for the determination of nitrogen. J. Med. Lab. Technol. 1954, 12, 1–22.
25. Olsen, S.R.; Cole, C.V.; Watanabe, F.S.; Dean, L.A. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate; U.S. Department of Agriculture: Washington, DC, USA, 1954.
