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The Relationship between Core Rhizosphere Taxa and Peanut Nodulation Capacity under Different Cover Crop Amendments

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Abstract: Adequate exploitation of legume–rhizobia symbiosis for nitrogen fixation may help to alleviate the overuse of chemical nitrogen fertilizer and aid in sustainable agricultural development. However, controlling this beneficial interaction requires thorough characterization of the effects of soil rhizosphere microorganisms, especially core taxa, on the legume–rhizobia symbiosis. Here, we used Illumina sequencing to investigate the effects of cover crop (*Raphanus sativus* L. and *Lolium perenne* L.) residue on the rhizosphere soil microbial community and peanut nodulation ability. The results indicated that *Raphanus sativus* L. amendment (RS) significantly increased soil available phosphorus (AP) content and peanut nodulation ability, while the *Lolium perenne* L. amendment (LP) had no noticeable impact on peanut nodulation. LP and RS significantly elevated bacterial and rhizobial diversity, reduced fungal diversity, and shifted microbial community structure (bacteria, 14.7%, $p = 0.001$; rhizobia, 21.7%, $p = 0.001$; fungi, 25.5%, $p = 0.001$). Random forest analysis found that the core rhizosphere taxa, sharing similar ecological preferences, were the primary drivers of peanut nodulation. By least squares regression, soil AP content was found to be positively correlated with the relative abundance of key ecological clusters. Furthermore, RS was found to promote peanut nodulation by increasing the relative abundance of critical rhizosphere taxa. Overall, our findings emphasize that core microbial taxa might play an essential function in the modulation of legume nodulation and provide scientific evidence for the effective management of the plant microbiome.

Keywords: nodulation ability; cover crop; rhizosphere microorganisms; core taxa; sequencing

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1. Introduction

Across the globe, the consumption of chemical nitrogen fertilizer continues to increase sharply to meet the growing demand for food crop production [1,2]. However, the excessive application of chemical nitrogen fertilizers has led to adverse environmental outcomes, including the degradation of soil quality, the contamination of groundwater, and the increased emission of greenhouse gases [3]. One potential answer to the crisis of agricultural intensification is to maximize the nitrogen fixation capacity of legume crops [4]. Although numerous studies have been conducted on the symbiosis between legumes and rhizobia, these studies have neglected to take into account that rhizobia exist as part of a convoluted subsurface microbial community [5,6]. Rhizobia tend to thrive in harsh soil conditions, and often interact or compete with other microorganisms prior to forming symbiotic relationships with their host plants [7]. For example, Wang et al. (2021) [8] indicated that

arbuscular mycorrhizal symbiosis is considered essential for legume nodulation under natural conditions. Additionally, the presence of pathogenic microorganisms such as *Ralstonia solanacearum* has been found to inhibit *Medicago truncatula* nodulation [9].

However, the effects of soil indigenous microorganisms on legume–rhizobia symbiosis remain poorly characterized. Although naturally occurring soil microbial communities exhibit considerable taxonomic diversity, the functional potential of certain species tends to have a greater impact on ecosystem characteristics than all other species combined [10–12]. For example, the functional potential of microorganisms responsible for nitrification, denitrification, and biological nitrogen fixation may be related to the composition of the microbial community, especially pertaining to the abundant and ubiquitous core microbiota [13–15]. Therefore, we speculate that the core rhizosphere microbiota may exert important effects on the nitrogen fixation capacity of legume–rhizobia symbioses.

Previous studies have shown that the use of organic soil amendments results in increased bioavailability of mineral nutrients, thereby enhancing the nodulation and nitrogen fixation capacity of legume crops [16,17]. In temperate climates, the use of cover crops has been found to prevent nitrogen leaching, increase soil organic matter content, and reduce pest and disease pressure [18–21]. In peanuts, the incorporation of leguminous "green manure" into the soil has been shown to boost the beneficial effects of arbuscular mycorrhizal fungi, resulting in higher fertility and productivity [22]. Furthermore, both the amount and type of cover crop material returned to the field can significantly alter the productivity and quality of succeeding agricultural crops [23,24]. To date, there is no direct evidence that cover cropping affects the nodulation of legume crops. However, a recent study demonstrated that returning cover crop material to the field resulted in significant transformation of the rhizosphere microbial community [25]. This may explain why altering the composition of rhizosphere-associated microorganisms through the use of cover crops may result in changes to legume growth and productivity. However, the mechanism by which cover cropping may modulate nodulation ability through regulating rhizosphere microorganisms (especially the core rhizosphere microbiota) remains unclear.

The peanut (*Arachis hypogaea* L.) is one of the major oilseeds and cash crops in the red soil region of southern China. Given the considerable financial rewards and scarcity of acreage, peanuts were continuously grown [26]. However, the continuous monoculture led to a notable deterioration in the productivity and quality of peanuts and gradually depleted soil nutrient conditions [27,28]. As a typical legume, peanuts can form a symbiotic nitrogen fixation system with rhizobia to fix atmospheric nitrogen, which is of great significance for peanut growth. However, peanut nodulation capacity has been severely degraded in the acidic and infertile red soil drylands [29]. Although inoculation with rhizobia is considered a common practice to improve the nodulation ability of peanuts, it is not fully accepted by farmers due to the extra investment and the unstable performance in the cultivation process. We believe that restoring and improving the nodulation and nitrogen fixation ability of peanut is a long-term effective solution. However, the majority of studies have concentrated on the effects of soil fertility and peanut yield under different cropping regimes, while the exploration of rhizosphere microbial communities and nodulation ability in peanuts was easily neglected.

Here, we hypothesized that: (1) the use and return of different cover crops will have different effects on peanut nodulation ability; and (2) changes in the core rhizosphere microbiota resulting from the return of cover crop material to the field will result in changes to peanut nodulation ability. To validate these hypotheses, we selected two common cover crop species (*Lolium perenne* L. and *Raphanus sativus* L.). The soils were collected from long-term peanut cultivation for pot experiments to examine the effects of different cover crops on the core rhizosphere microbiota and on the nodulation ability of peanuts.

2. Materials and Methods

2.1. Experimental Material Collection

The experimental soil was collected from red soil for long-term peanut cultivation at the Jiangxi Institute of Red Soil in Jinxian County, Jiangxi Province, China (116°26′11″ E, 28°37′59″ N; 30 m elevation). This region has a subtropical climate with an average annual temperature of 18 °C and average annual precipitation of 1730 mm. The soil is classified as Quaternary red clay, and as an Ultisol according to the U.S. Department of Agriculture (USDA) soil classification. The basic physicochemical properties of the experimental soil were as follows: 4.54 pH (water:soil ratio of 2.5:1); 12.58 g kg^{-1} soil organic matter (SOM); 0.87 g kg^{-1} total nitrogen (TN); 76.64 mg kg^{-1} available nitrogen (AN); 20.42 mg kg^{-1} available phosphorus (AP); and 77.10 mg kg^{-1} available potassium (AK). The experimental soil was sampled in mid-March 2021. The soil was mixed evenly, cleared of residual roots and stones, passed through a 5-mm sieve, and air-dried and stored for use in the pot experiment. Cover crop (*Lolium perenne* L. and *Raphanus sativus* L.) samples were obtained in mid-April 2021 (7–15 days before peanut sowing), from the same site used for soil collection. The fresh cover crop sample was divided into two parts: one prepared for the pot experiment; one dried in an electric thermostatic oven at 70 °C for moisture content determination of cover crops and a plant chemical properties determination experiment.

2.2. Pot Experiment and Sampling

The pot experiment was conducted in the greenhouse of the Jiangxi Institute of Red Soil in April 2021. Cover crops were blended with soil at a ratio of 1 g of dried plant material per 1 kg of dried soil, according to local cover crops application conventions. The cover crop-amended soil was transferred to plastic pots with an inner diameter of 25 cm and a height of 30 cm, with each pot containing 5 kg (dry-weight) of test soil. The same amount of basal chemical fertilizer was applied to each pot, with each pot containing 0.64 g pot^{-1} of nitrogen (urea, 46% N), 1.2 g pot^{-1} phosphorus (calcium magnesium phosphate, 12% P_2O_5), and 0.57 g pot^{-1} potassium (potassium chloride, 50% K_2O). Each pot was sown with three peanut (cv. Ganhua 1) seeds. After germination, only the healthiest seedling was left in each pot. Soil moisture was maintained at about 60% of the field water-holding capacity. Each pot was replenished every 3–5 days by a weighing method according to the amount of water loss. The pot experiment consisted of three treatments, with sixteen replicates (eight replicates were used to collect rhizosphere soil samples at the pod setting stage, and the other eight replicates were used to determine peanut biomass at maturity) per treatment: (1) CK (control, without cover crop application); (2) LP (application of *Lolium perenne* L.); and (3) RS (application of *Raphanus sativus* L.).

The rhizosphere soil samples were collected at the peanut pod setting stage (16 July 2021). After completely removing the peanut plant, the roots were gently shaken to collect the soil. A sterile brush was used to gently remove any soil left adhering to the root surface [30]. Soil samples were quickly transported to the laboratory, cleared of residual roots and stones, and passed through a 2 mm sieve. The sieved rhizosphere soil samples were divided into two subsamples: one air-dried and stored for analysis of soil chemical properties, and one frozen at −80 °C and stored for DNA extraction. Peanut samples were collected at maturity (19 August 2021) and dried at 70 °C to constant weight for biomass determination.

2.3. Determination of Soil and Plant Chemical Properties

Soil pH was determined using a pH meter (FE30; Mettler-Toledo, Zurich, Switzerland) in a 2.5:1 (v/w) water-soil suspension. The potassium dichromate oxidation method was used to determine soil organic carbon (SOC). Soil TN and AN were determined according to the Kjeldahl method. Soil total phosphorus (TP) and total potassium (TK) were determined using the HF-$HClO_4$ digestion method. Soil AP and AK were determined using the sodium bicarbonate extraction method and flame photometric method, respectively [31]. For plant samples, both total carbon (TC) and TN were determined as described above. Cover crop TP was determined according to the vanadium-molybdenum yellow colorimetric method.

Active root nodules were excised from peanut plants using an aseptic surgical knife, and the number of nodules was used as a measure of nodulation capacity.

2.4. DNA Extraction, Sequencing and Bioinformatics Analysis

Total DNA was extracted from a 500 mg sample of fresh rhizosphere soil using the FastDNA SPIN Kit (MP Biomedicals, Santa Ana, CA, USA), according to the manufacturer's instructions. The extracted total DNA was then purified using the PowerClean DNA Clean-up Kit (MOBIO Laboratories, Carlsbad, CA, USA). The extracted DNA concentration and quality (OD260/OD280) were determined using a NanoDrop ND-1000 spectrophotometer. Bacterial 16S rRNA gene fragments were amplified using the 515F/907R universal primers [32]. The Rhizobium *rpoB* gene was amplified using the *rpoB*1479F/*rpoB*1831R primers [33]. The fungal ITS1 region was amplified using the ITS1F/ITS2R universal primers [34]. Please refer to Table S1 for specific primer information. Paired-end sequencing was carried out on an Illumina MiSeq sequencing platform (Biozeron Biotechnology Co., Ltd., Shanghai, China).

Original sequences were analyzed downstream using the QIIME (1.9.1) platform [35]. Sequences with lengths less than 200 bp and average quality scores below 25 were excluded. For both bacteria and fungi, chimeric sequences were removed using usearch (V10) [36]. Operational taxonomic units (OTUs) were clustered at a 97% similarity level, and the representative bacterial and fungal sequences under each OTU were selected for species annotation using the RDP and UNITE databases, respectively. The singletons were deleted. For Rhizobium, only sequences belonging to the Alphaproteobacteria were retained. OTUs were clustered at a 97.7% similarity level [37,38], and the longest sequence under each OTU was selected as the representative sequence for comparison against the *rpoB* gene reference database [39]. The obtained OTU data were resampled to consistent sequencing depths based on the minimum number of sequences in the sample. Finally, we obtained 9677 OTUs for the bacteria, 1110 OTUs for the rhizobia, and 1421 OTUs for the fungi, respectively.

2.5. Statistical Analysis

Statistical analysis was conducted with R 4.2.1 version. Unless otherwise noted, statistically significant differences were determined by the one-way analysis of variance (ANOVA) and Tukey's HSD post hoc test ($p < 0.05$), using the function *TukeyHSD* in the "*stats*" package. Partial canonical analysis of principal coordinates (CAP), based on the Bray–Curtis distance and permutation test, was carried out using the *capscale* function from the "*vegan*" package for R [40]. Analysis of indicator species was carried out using the indicspecies package for R [41]. The core microbiota of the bacterial, rhizobial, and fungal communities were identified according to the following criteria: (1) high-abundance OTUs in the top 10% of all samples with the highest relative abundance, and (2) OTUs present in at least 90% of the soil samples [42]. The primary drivers of peanut nodulation were identified by applying a random forest analysis. Random forests provide predictors using decision trees based on bootstrap samples of the dataset [43]. The portion of the dataset that was drawn is referred to as the *in-bag* data, while the data that was not drawn is referred to as the *out-of-bag* data [44]. Decision trees are grown completely to predict *out-of-bag* data. The importance of the predictor variable is estimated by randomly permuting the values of this variable for out-of-bag data and calculating the increment percentage of the mean squared error (MSE), which was predicted by 999 iterations of the algorithm. Random forest analysis was carried out using the rfPermute package for R [45]. The ordinary least squares linear regressions using the *lm* function in the "*stats*" package.

For the core soil microbiota, the weighted gene co-expression network analysis (WGCNA) was carried out using the WGCNA package for R [46]. The soft threshold power was determined with a signed scale-free topological R^2 of 0.9. The adjacency matrix was constructed according to the most appropriate soft threshold power of 10, which was then transformed into a topological overlap matrix (TOM). The TOM-based dissimilarity and dynamic branch cutting were used to identify ecological clusters. The relative

abundances of OTUs included in the clusters were normalized and averaged to obtain the relative abundance of the cluster [47].

3. Results

3.1. Cover Crop Nutrient Contents, Rhizosphere Soil Chemical Properties, and Peanut Nodulation Ability

Raphanus sativus L. contained significantly ($p < 0.05$) more phosphorus and significantly ($p < 0.05$) less nitrogen than *Lolium perenne* L. (Figure S1). There was no significant difference in carbon content between the two cover crops. Compared with the CK, RS treatment significantly ($p < 0.05$) increased soil AP content, which elevated AP by 18.68% (Figure 1b). However, RS treatment resulted in significantly ($p < 0.05$) lower soil TN content than both the CK and LP treatments (Figure 1c). There were no significant differences in other nutrient contents between the different treatments. Compared with the CK, RS treatment significantly ($p < 0.05$) enhanced peanut nodulation ability of peanuts, while there was no significant alteration in peanut nodulation ability under LP treatment (Figure 1e). The number of nodules in RS treatment was 81.61% and 59.78% higher than CK and LP, respectively. In addition, the nitrogen uptake of peanut under RS treatment increased by 25.91% and 11.84% compared to CK and LP, respectively (Table S2).

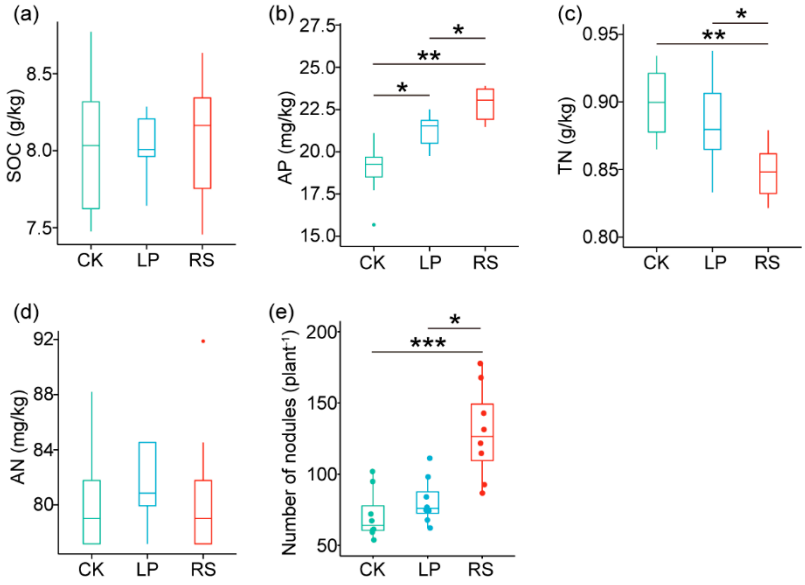

Figure 1. The effects of cover crops on soil properties: (**a**) soil organic carbon (SOC), (**b**) available phosphorus (AP), (**c**) total nitrogen (TN), (**d**), available nitrogen (AN), and (**e**) peanut nodulation (CK, no cover crop; LP, application of *Lolium perenne* L.; RS, application of *Raphanus sativus* L.; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

3.2. Rhizosphere Soil Microbial Community Diversity and Structure

Cover crop treatment (LP, RS) significantly ($p < 0.05$) increased the diversity of bacteria and rhizobia, but decreased the diversity of fungi (Figure S2). Notably, the fungal diversity index was significantly ($p < 0.05$) reduced under LP treatment (Figure S2c). CAP analysis indicated that cover cropping altered the structure of the microbial community (bacteria, 14.7%, $p = 0.001$; rhizobia, 21.7%, $p = 0.001$; fungi, 25.5%, $p = 0.001$) (Figure 2a–c).

We carried out indicator species analysis to identify rhizosphere soil taxa whose abundances varied between the different treatments. For bacteria, there were 6 OTUs enriched in CK, 16 OTUs enriched in LP, and 32 OTUs enriched in RS (Figure 2d). Among the OTUs enriched in CK, the most dominant orders were *Xanthomonadales*, *Sphingomonadales*, *Chitinophagales*, and *Burkholderiales* (Figure S3a). In LP, the most dominant orders were *Rhodospirillales*, *Micrococcales*, and *Catenulisporales*. In RS, the most dominant phyla were Firmicutes and Chloroflexi. For rhizobia, the greatest number (six) of OTUs were observed

in the RS treatment, whereas two OTUs, belonging to *Bradyrhizobium diazoefficiens*, were significantly ($p < 0.05$) enriched in the LP treatment (Figure S3b). For fungi, 17 taxonomically diverse OTUs were enriched in the CK, whereas only 1 OTU was enriched in the LP treatment and 7 OTUs were enriched in the RS treatment (Figure 2f).

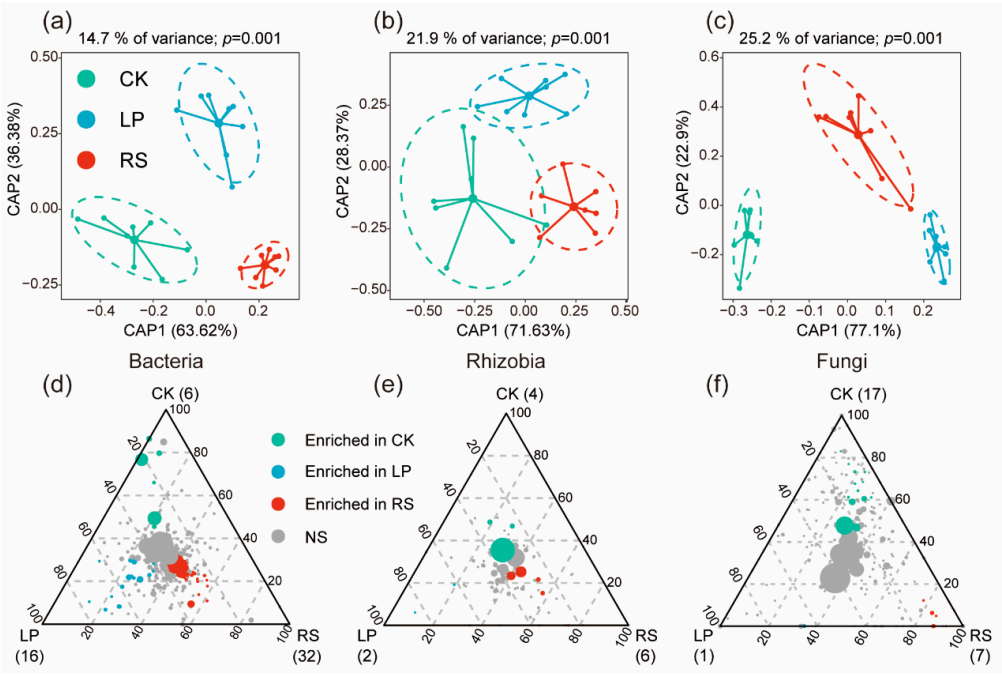

Figure 2. The effect of cover crops on the rhizosphere microbial community structure. Constrained principal coordinates analysis (CAP) plot showing bacterial (**a**), rhizobial (**b**), and fungal (**c**) community structure across treatments. Ternary plots show indicator operational taxonomic units (OTUs) of bacterial (**d**), rhizobial (**e**), and fungal (**f**) communities across treatments. Each dot indicates an OTU. The size of the dot indicates the average relative abundance of OTUs. The location of the dot indicates its relative abundance with respect to individual treatments. Green, blue, and red dots indicate OTUs notably enriched in CK, LP, and RS, respectively. The number in parentheses indicates the number of enriched OTUs.

3.3. Correlation between Core Microbiota and Peanut Nodulation Ability

To determine the core microbiota, OTUs with the highest relative abundance and prevalence among all samples were selected for further analysis. A total of 210 OTUs were denoted as the core taxa, with 175 bacterial taxa, 19 rhizobial taxa, and 16 fungal taxa (Table S3). These OTUs included 82 species of Proteobacteria, 39 species of Actinobacteria, 37 species of Chloroflexi, 17 species of Firmicutes, 15 species of Ascomycota, 10 species of Acidobacteria, and a few representatives of Bacteroidetes and Chytridiomycota (Figure 3a). In addition, the random forest model was applied to evaluate the contribution of individual species to peanut nodulation ability. The results indicated that the core microbiota, rather than other non-core taxa, were the primary drivers of peanut nodulation ability (Figure 3b).

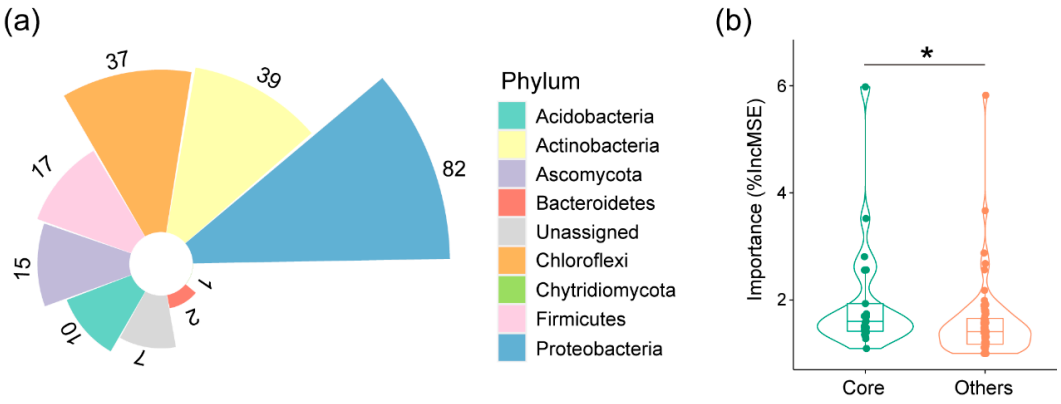

Figure 3. Correlation between core microbiota and nodulation ability of peanut. (**a**) Circular bar chart shows the compositions of core microbiota at the phylum level. (**b**) Mean contribution (% of increased mean square error) of microbial taxa to peanut nodulation ability based on random forest modeling. The magnitude of the MSE% value implies the importance of the predictors (* $p < 0.05$).

A co-occurring network of core rhizosphere taxa was constructed using WGCNA. Based on their strong association patterns, the core taxa were conglomerated into four ecological clusters (Figure 4a). Further regression analysis revealed that Cluster 1 was positively associated with the number of peanut nodules (Figure 4b), although no significant associations were found between the other clusters and peanut nodulation. Cluster 1 contained primarily Chloroflexi, Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria, and Ascomycota (Figure 4c, Table S4). Different cover crop treatments resulted in alterations to the relative abundance of critical ecological clusters. For example, the relative abundance of Cluster 1 under RS treatment was significantly ($p < 0.05$) higher than under CK or LP treatment, with no significant difference between CK and LP (Figure 4d).

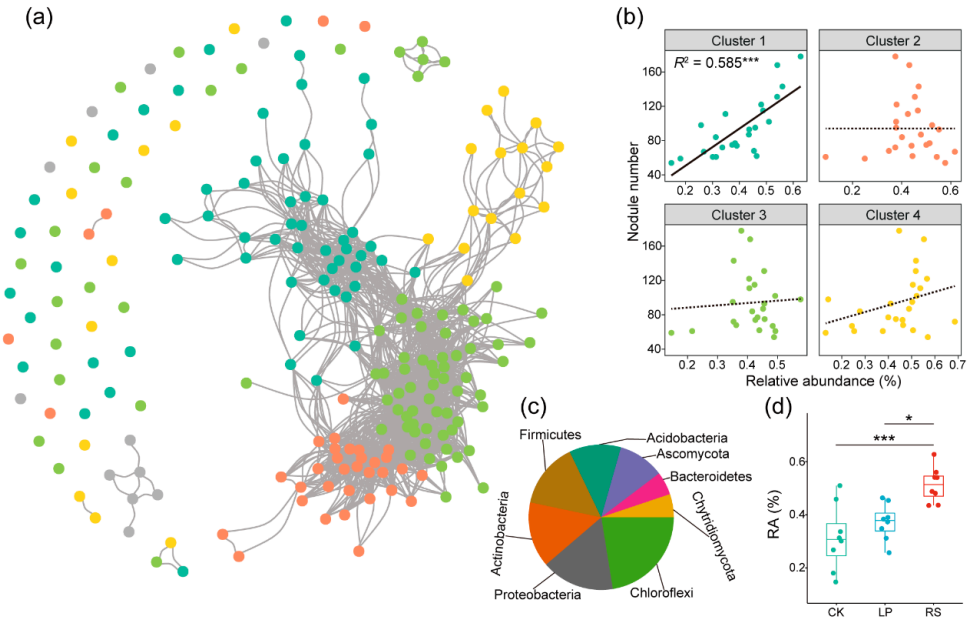

Figure 4. Correlation between key ecological clusters and nodulation ability of peanut. (**a**) The weighted gene correlation network analysis (WGCNA) of core microbiota. The color of the nodes indicates various ecological clusters. Each node in the figure represents an OTU. (**b**) The regressions between peanut nodule number and microbial eigentaxa weighted correlation network modules (statistically significant differences using ordinary least squares regression). (**c**) Composition of core Cluster 1 microbiota at the phylum level. (**d**) Relative abundance of Cluster 1 in different treatments (* $p < 0.05$; *** $p < 0.001$).

Taking soil properties into account, random forest models were used to detect the principal drivers of peanut nodulation. The results indicated that the relative abundance of Cluster 1 was the foremost driver (Figure 5a). Soil AP was also a strong driver of peanut nodulation, with increased soil AP resulting in greater peanut nodulation (Figure 5b). A significant positive correlation was also found between soil AP and the relative abundance of Cluster 1 (Figure 5c).

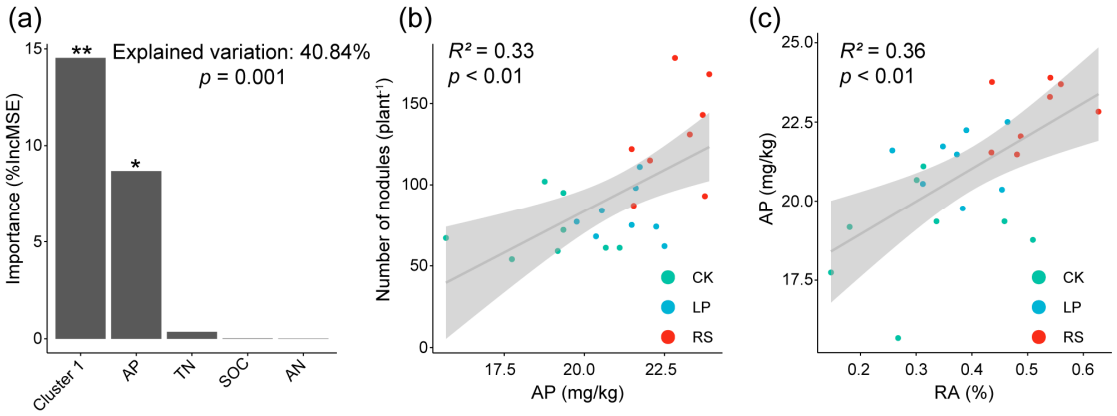

Figure 5. Drivers of peanut nodulation. (**a**) Mean contribution (% of increased mean square error) of relevant variables to peanut nodulation ability based on random forest modeling. The magnitude of the MSE% value implies the importance of the predictors (* $p < 0.05$; ** $p < 0.01$). (**b**) The regressions between peanut nodule number and soil AP. (**c**) The regressions between the relative abundance of Cluster 1 and soil AP. Statistical analysis was performed using ordinary least squares linear regressions.

4. Discussion

There is still a lack of information regarding the extent to which amending soil with cover crop residue benefits the soil, including whether and how cover crops act to establish interactions among soil phylotypes and interactions between crops and microbes. In this study, we discovered that peanut nodulation was notably enhanced under RS treatment and that the core rhizosphere taxa were the primary drivers of peanut nodulation. In particular, the core microbiota with similar ecological preferences plays a prominent role in peanut nodulation. These findings were further supported by random forest models and association analysis, while RS treatment was found to indirectly promote peanut nodulation by increasing the relative abundance of Cluster 1 microbes.

The type of cover crop utilized is known to affect the performance of subsequent crops [25]. In particular, the decomposability and elemental composition of cover crop residues appear to be the primary drivers of the growth performance of subsequent crops [48]. The results of our pot trial demonstrated that the addition of *Raphanus sativus* L. greatly increased the number of peanut nodules, while the addition of *Lolium perenne* L. did not affect peanut nodulation (Figure 1e). We found that the incorporation of *Raphanus sativus* L. residue resulted in increased soil AP and decreased soil TN, which may be due to the high P and low N contents of *Raphanus sativus* L. itself (Figure 1b,c). Previous studies have found that the use of *Raphanus sativus* L., belonging to the *Brassicaceae* family, as a cover crop is an effective method of raising soil AP [49].

Rhizobia-legume symbiosis is partially regulated by the rhizosphere microbiota [8,50]. We found that the core rhizosphere taxa were the primary drivers of peanut nodulation, compared to the effects of non-core taxa. These results may be attributed to the prevalence of core microbial communities in different habitats. These abundant and ubiquitous taxa occupy distinct niches, competitively exploit resources, and efficiently adapt to a variety of environmental conditions [42,51]. These taxa tend to also have genetic resources, which are vital to rhizobia-legume symbiosis (e.g., degradation of organic matter and mobilization

of phosphorus). The host-specificity of the rhizobia–legume symbiosis system is also an important driver of peanut nodulation. In this study, all rhizobia in the core microbiota belonged to *Rhizobiales* (Table S3) and were mutually interacting with leguminous plants to perform symbiotic nitrogen fixation, which is of great significance for the terrestrial nitrogen cycle [52]. In particular, the abundance of *Rhizobiales* in the inter-rhizosphere of legumes plays a crucial role in the formation of nodules [5]. Similarly, variation in the abundance of *Catenulispora* of Actinomycetes reshaped the rhizosphere microbial network structure of peanuts, accelerated rhizosphere organic nitrogen and phosphorus mineralization, and provided available nutrients to the underground portion of peanut for growth [53]. Moreover, we found that the majority of core rhizobial taxa belonged to *Bradyrhizobium*, the primary genus responsible for symbiotic nitrogen fixation in peanuts [54]. We also found that ecological Cluster 1 was significantly positively correlated with and was the primary driver of peanut nodulation (Figures 4b and 5a). This demonstrated that core taxa with similar ecological preferences might be pivotal for facilitating nodulation in peanuts. For instance, species from Actinobacteria and Firmicutes can antagonize phytopathogenic microorganisms and alleviate the inhibitory action of biotic stress on nodulation and nitrogen fixation in leguminous crops [55]. Meanwhile, the core taxa *Chitinophagaceae*, which belongs to Bacteroidetes, can mineralize complex organic compounds and enhance soil AP [56].Taken together, these results indicate that core taxa play vital roles in sustaining crop growth performance [57].

Variations in soil nutrient availability under organic management practices can impact crop growth performance by altering soil microbial communities [58]. Here, we found that amendment with *Raphanus sativus* L. resulted in higher soil AP, compared to CK or amendment with *Lolium perenne* L., and that soil AP was positively correlated with the relative abundance of Cluster 1 (Figures 1b and 5c). Recent research has found that soil AP can modulate the ecological interactions between soil microbiotas [59,60]. There are also studies that have demonstrated that the variability of soil nutrients can be partly explained by ecological interactions among soil microbes [61,62], which supported our finding. We also found that soil AP was positively correlated with peanut nodulation. This is due to the fact that phosphorus is an indispensable nutrient for the growth of legumes and diazotrophs and is also used to generate ATP to provide energy for symbiotic nitrogen fixation [63]. In addition, investigation revealed that the increase of soil AP raised the abundance of soil diazotroph, enhanced nitrogen-fixing enzyme activity, and up-regulated the expression of nitrogen cycle-related genes [64,65]. These results suggest that alterations in soil AP after cover crop residue incorporation could impact peanut nodulation by modifying the relative abundance of key ecological taxa. The *Raphanus sativus* L. amendment resulted in a significant increase in the relative abundance of Cluster 1, which presumably accounted for the improved peanut nodulation ability of peanuts.

The role of organic amendments on soil properties and crop performance, including nutrient availability and symbiotic nitrogen fixation, has been extensively investigated [19,66]. Improved nodulation of leguminous plants has primarily been attributed to organic amendments, which raise the soil pH and enhance the availability of nodulation-relevant trace elements [67,68]. Here, we found that the core microbial taxa were the primary drivers of peanut nodulation ability. Soil microbes are likely to be critical components of sustainable agricultural systems due to their ability to produce plant growth-regulating hormones, activate and mobilize nutrients, and increase plant stress resistance [69]. The pronounced effects of cover crop identity on the core microbial composition of peanut rhizosphere soils demonstrate the feasibility of conditioning beneficial microbial populations through rational agronomic practices. However, a number of obstacles must be overcome before solid recommendations on the utilization of cover crops to regulate rhizosphere microbial communities can be offered. In particular, the core rhizosphere taxa of the target crop should be identified and assessed to determine the optimal cover crop application level. Additionally, the type and frequency of cover crop application should also be taken into account.

5. Conclusions

Our study revealed the importance of core rhizosphere microbes on rhizobia–peanut symbiosis under cover crop incorporation. We identified specific core rhizosphere taxa with similar ecological preferences as the primary drivers of peanut nodulation. The *Raphanus sativus* L. amendment increased soil AP, which in turn promoted peanut nodulation by regulating the relative abundance of pivotal ecological cluster. In brief, our work highlighted the potential contribution of soil core taxa in enhancing the nodulation ability of legume crops. These results will aid in the creation of science-based cover-cropping systems for sustainable agricultural production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13020311/s1. Figure S1: Nutrient contents of the cover crops; Figure S2: Diversity indices of peanut rhizosphere soil microbes as affected by cover crops; Figure S3: Composition of bacterial, rhizobial, and fungal indicator species; Table S1: PCR information; Table S2: Effect of different cover crops on peanut biomass, nitrogen content, and nitrogen uptake; Table S3: Core rhizosphere taxa; Table S4: Ecological cluster 1.

Author Contributions: J.L. and X.X. designed the experiments. K.L. (Ke Leng), P.W., P.Z. and M.S. completed the field sampling. G.L., J.Z. and T.M. performed the data analysis and prepared the figures. K.L. (Ke Leng) and G.L. wrote the manuscript. J.L., K.L. (Kailou Liu) and X.X. contributed to the revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All the bacteria, fungi, and rhizobia sequences data obtained in this study are available in the NCBI database under the BioProject accession number SRP408950: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA901876/ (accessed on 15 November 2022).

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