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Impacts of vegetation restoration on soil physicochemical properties, bacterial communities, and metabolites in newly reclaimed croplands

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Short Summary

Vegetables, corn, and peach improved soil in reclaimed croplands of subtropical China. Vegetables lowered bulk density, peach reduced organic matter. All non-grain crops raised phosphorus, potassium, and microbial carbon, though peach lowered potassium. Bacterial diversity rose, and vegetables altered metabolites. Vegetables gave the most consistent gains in soil, microbes, and metabolites, making them the best option for restoration.

Abstract: To find one suitable vegetation restoration type as a good means of restoring newly reclaimed croplands in subtropical of China. This study investigated the effect of vegetables, corn, and peach in soil properties, bacterial communities, and metabolites of newly reclaimed lands after three years restoration. Results from this study indicated that soil physicochemical properties were differentially affected by vegetation restoration of three different plants, while the effect depends on both the vegetation types and the kind of soil parameters. Indeed, the pH, soil bulk density (SBD), soil organic matter (SOM) and total nitrogen (TN) were generally unaffected except a significant reduction in SBD (13.97%) and SOM (35.41%) by vegetable and peach, respectively. However, three different plants significantly increased the available phosphorus (AP) (75.03–143.02%), available potassium (AK) (154.90% and 103.93%) and microbial biomass carbon (MBC) (37.71–144.93%), with the greatest increase by vegetable relative to the control except a significant reduction in the AK (41.73%) by peach. Furthermore, the analysis of 16S rRNA gene high-throughput sequencing revealed that the vegetation of three plants increased the relative abundances (RAs) of soil bacterial phyla and genera with 6.21–10.54% increase in operational taxonomic units (OTUs), 6.22–10.53% increase in Chao1 and 2.30–3.11% increase in Shannon indices, while redundancy discriminant analysis (RDA) revealed that the change of soil properties were highly related to the variation in bacterial community composition. In addition, 130 significantly differential metabolites (SDMs) that belong to organic acid, amino acid, heterocyclic compounds between vegetable and the control were identified based on liquid chromatography-mass spectrometry (LC-MS) analysis, while the top 20 SDMs were highly correlated with the 7 enriched bacterial genera. Overall, the results showed that the vegetation of three plants, in particular vegetable can ameliorate soil quality of newly reclaimed croplands by improving soil chemical properties, and increasing the richness

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and complexity of bacterial community structure, as well as specific bacterial genus and metabolites.

Keywords: newly reclaimed croplands; restoration types; soil properties; soil bacterial communities; soil metabolites

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1. Introduction

With the ongoing rapid urbanization in China over the past several decades (Zhou et al., 2004; Bai et al., 2014), the total amount of croplands decreases sharply, which has become an important risk to ensure national food security and social stability in China (Jiang et al., 2013; Lai et al., 2020). To expand the available land supply and meet the demand for croplands, the wastelands and abandoned gravel lands of China are gradually being reclaimed for agricultural use (Wang et al., 2017; Yan et al., 2021). However, due to their poor soil properties (poor soil structure and low soil maturity), the newly reclaimed croplands were not suitable for cultivation in most situations relative to the occupied land (Jiang et al., 2013; Li et al., 2023). For example, Yan et al. (2009) reported that the production capacity of arable lands occupied by urbanization was 80% higher than that of the newly cultivated lands in some regions. Hu et al. (2014) also showed that the productivity of new reclamation land was 10–30% of the occupied cultivated land in Hangzhou, China. Therefore, it is extremely important to increase the productivity by improving soil quality of the newly reclaimed croplands (Liu et al., 2016).

A lot of work has been done to improve the soil quality of newly reclaimed croplands by field engineering measures, such as land leveling, irrigation and water conservancy construction (Li et al., 2014). Indeed, the addition of minerals such as phosphogypsum, fly ash, and soft rock had a positive impact on soil nutrients and crop yield by restoring the soil nutrient status (Sun and Han, 2018), while soil properties of newly reclaimed croplands could be modified by organic amendments such as livestock manure, wood residuals, biosolids, and crop residues (Larney and Angers, 2012). Furthermore, microbial-organic fertilizers could increase soil quality and crop yield by improving soil physiochemical properties, enriching organic matter and balancing nutrient levels (Li et al., 2023), while tillage was also beneficial to increase soil quality and crop yield in newly reclaimed croplands by improving soil water thermal properties, structural stability, and nutrients (Liu et al., 2021). Exploring further, combination of water depth and plant species could enhance soil quality in near-natural restoration of reclaimed wetland, especially affecting soil pH, organic carbon recovery rates, and labile organic carbon content (Yang et al., 2024). Additionally, Zhang et al. (2011) also showed that natural recovery was the best choice for soil revegetation of sloping croplands in the Loess Plateau (among six different vegetation types, including two shrublands, two grasslands, and two species from croplands abandoned for natural recovery).

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It has been well known that appropriate vegetation restoration modes are of great significance to remediate and improve the stability of soil ecosystem (Lu et al., 2022). Previous studies have revealed that soil microbes and plant species, always influence soil quality. For example, soil physicochemical properties (including soil pH, moisture, bulk density, and nutrient content) were differentially altered by different plant species, which shape litter input and root architecture during vegetation restoration (Augusto et al., 2000; Zheng et al., 2020). Plants absorb water and nutrients through their root, and also release various substances into the soils, while these root exudates selectively influence the composition of soil-specific microbes (Pang et al., 2024). Furthermore, Guo et al. (2018) reported that soil microbial communities and enzyme actives in land restored with trees were higher than that with grasses, while Lu et al. (2022) found that compared with natural enclosure, artificial vegetation restoration rapidly promoted community succession in Karst areas, in particular construction of deciduous broad-leaved forest improved soil nutrients, altered soil key microbial populations, and promoted ecosystem services. All these studies demonstrated that different plant species could improve soil quality to varying degrees in restored land, and soil microbes could be served as the connection between soil and vegetation during the process due to its importance to soil ecosystem. Indeed, soil microbes play a critical role in maintaining soil health and fertility by participating in the decomposition and mineralization of soil organic matter, regulating carbon storage and nutrient cycling, and determining the nutritional status and overall health of crops (Chen et al., 2024a). However, the effects of different plant species on the newly reclaimed croplands during soil restoration remain poorly understood.

The objective of this study was to find one suitable vegetation restoration type as a good means of restoring newly reclaimed croplands in subtropical of China, which was carried out by investigating the effects of three different plant species (vegetable, corn, and peach) on soil quality by measurement of soil physiochemical properties, as well as soil bacterial community structure and metabolites based on 16s rRNA high-throughput sequencing and liquid chromatography-mass spectrometry (LC-MS) analyses.

2. Materials and Methods

2.1. Experimental design and sample collection

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The experiment was conducted in September 2021 at the Zhijiang Base of Hangzhou Academy of Agricultural Sciences in Zhejiang province, China (30°9′12″N; 119°5′36″E). All sites had similar conditions with the top 0–20 cm soil layer belonging to sandy loam according to the USDA classification system, while the basic soil properties were pH 7.86, with 6.03 g/kg of soil organic matter (SOM), 0.42 g/kg of total nitrogen (TN), 12.30 mg/kg of available phosphorus (AP), and 378.70 mg/kg of available potassium (AK).

The experiment consisted of four different treatments through the application of vegetable, corn, peach vegetation restoration, and the control without any plants in the newly reclaimed cropland (Figure 1a–c). For each treatment, there were five plots, and each plot had an area of about 125 m^2 (25 m × 5 m). The planting density of vegetable and corn was 25 cm × 25 cm and 30 cm × 50 cm, respectively, while sheep manure (1.50 kg/m^2) and chemical compound fertilizer (0.075 kg/m^2) was applied for both treatments at early spring and autumn (twice per year). Furthermore, the planting density of peach was 4 m × 5 m, while sheep manure (3.00 kg/m^2) and chemical compound fertilizer (0.15 kg/m^2) was applied at early winter (once per year). In addition, no fertilizer was used for the control.

In September 2024 (three years later), about 1.0 kg of fresh soil for each plot was sampled from the root zones of the plants (5–20 cm, the top soil layer for the control) and then packed in a sterile bag using a shovel. Meanwhile, the soil of each plot was also collected using a stainless-steel cylinder (5 cm in height and 100 cm^3 in volume). Then, all soil samples were quickly transported to the laboratory in an ice box for further analysis.

2.2. Soil physiochemical properties measurement

To study the soil properties, about 1.0 kg fresh soil of each plot was sampled, air-dried and passed through a 0.45-mm sieve to measure the soil pH, and soil bulk density (SBD), as well as SOM, TN, AP, and AK. In detail, soil pH was measured at a soil suspension (soil: water = 1 g: 5 mL) using a pH meter (FE28, MettlerToledo, Zurich, Switzerland), while the SOM, TN, AP, and AK contents were determined using the $K_2Cr_2O_7$ oxidation method, Kjeldahl distillation-titration method, molybdenum-based colorimetric method, flame photometer method, respectively (Brookes et al., 1985). Furthermore, the SBD was calculated using the oven drying method, while microbial biomass carbon (MBC) was determined on fresh soil samples using the fumigation-extraction method (Vance et al., 1987; Mclaren and Cameron, 1990).

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2.3. Soil 16S rRNA high-throughput sequencing analysis

About 20 g fresh soil of each plot was sampled for 16S rRNA gene high-throughput sequencing. In detail, the PCR amplification for the V3-V4 region of bacterial 16S rRNA genes was performed using the universal primers 341F and 805R (5′–CCTACGGGNGGCWGCAG–3′; 5′–GACTACHVGGGTATCTAATCC–3′, respectively) (Wu et al., 2015), following the extraction of soil sample DNAs using the E.Z.N.A™ Mag-Bind Soil DNA Kit (OMEGA, Norcross, GA, USA), and assessment by a NanoDrop (ND-1000) spectrophotometer (ThermoFisher Scientific, United States). The PCR components included ddH_2O (12 μl), 2×Hieff® Robust PCR Master Mix (15 μl), 10 μM universal primer (1 μl of each primer), and DNA template (1 μl). The PCR thermal protocol consisted of an initial denaturation at 94℃ for 3 min, 25 cycles of denaturation at 94℃ for 30 s, annealing at 55℃ for 30 s, extension at 72℃ for 30 s, and finally an extension at 72℃ for 5 min. After purification using Hieff NGS™ DNA selection beads (Yeasen, Shanghai, China), the PCR product was sequenced using the pair-end (2 × 250 bp) sequencing on an Illumina MiSeq system (Tsingke Biotechnology Co., Ltd., Hangzhou, China).

The bioinformatics analysis of soil bacterial microbiome was performed by analyzing clean reads using USEARCH (v10) with a 97% similarity cutoff to generate operational taxonomic units (OTUs). To ensure data quality, the subreads were preprocessed using SMRT Link (v8.0) by removing low-quality reads (minPasses < 5, minPredictedAccuracy < 0.9), and then merged to obtain circular consensus sequences (CCS) that identified using lima (v1.7.0), while the CCS without primers or average length < 1200 bp (or > 1650 bp) were cutoff with cutadapt (v2.7). After selection of the representative read of each OTU using the VSEARCH (v2.4.3), all 16S rRNA representative reads were annotated by blasting against the Silva Release 138 Database using the RDP classifier (Edgar, 2013, 2016; Quast et al., 2012).

2.4. Soil liquid chromatography-mass spectrometry (LC-MS) analysis

About 10 g fresh soil of each plot was sampled for metabolomics assay. In detail, 250 mg of each soil sample was added into 500 μl extract solution (methanol: H_2O = 7: 3), vortexed at 35 HZ for 3 min, sonicated for 10 min in an ice-water bath, and incubated at -20℃ for 30 min. After centrifugation at 12,000 rpm for 10 min (4℃) and filtering using 0.22 μm PTFE filter, the supernatant was transferred into a brown glass vial for LC-MS analysis. The HPLC conditions were set as follows: chromatographic column: waters ACQUITY premier HSS T3 column (1.8

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µm, 2.1 mm × 100 mm); mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile; gradient program: 5 to 20% in 2 min, increased to 60% in the following 3 min increased to 99% in 1 min and held for 1.5 min, then come back to 5% B within 0.1 min and held for 2.4 min; column temperature: 40°C, flow rate: 0.4 ml/min, and injection volume: 4 µl. The ESI source conditions of Q Exactive HF-X were set as follows: ion spray voltage, 3.5 KV (positive) or 3.2 KV (negative), respectively; sheath gas, 30 Arb; aux gas, 5 Arb; ion transfer tube temperature, 320°C; vaporizer temperature, 300°C; scan range, 75–1,000 Da; resolution, 35,000; collision energy, 30, 40, 50 V; signal intensity threshold, 1*e6 cps; top N vs top speed, 10; exclusion duration, 3 s. The repeatability and reliability of the entire analysis process were evaluated by inserting one quality control sample, which was prepared by pooling and mixing 10 µl of each sample. All treatments had five replicates. The data obtained in this study were converted into the mzXML format using ProteoWizard, processed with XCMS program, and then compared with the in-house database (HMDB, http://hmdb.ca/) and KEGG, https://www.kegg.jp/) for metabolite annotation.

2.5. Data analysis

One-way analysis of variance (ANOVA) was performed using the SPSS software (v16.0, SPSS Inc., Chicago, IL, USA) to analyze the statistical significance among four different treatments. Chao1 and Shannon indices were calculated using Origin (v2022, Hampton, MA, USA) based on OTU data to analyze abundance and alpha diversity in soil bacterial communities. Principal component analysis (PCA) was conducted using Bray-Curtis metrics to assess structural variation in soil bacterial communities (Ramette, 2007). Relative abundances (RAs) (at the phylum and genus level, respectively) and heat map (at the family level) of the dominant bacteria were calculated using Origin. Linear discriminant analysis (LDA) effect size (LEfSe) was carried out by using default parameters to discover the differentially abundant taxa between groups (Segata et al., 2011). Furthermore, to investigate the effect of different plants on metabolites, MetaboAnalyst 4.0 was used to platform, orthogonal projections to latent structures-discriminant analysis (OPLS-DA), volcano plots, variable importance in the projection (VIP) value maps, and KEGG enrichment analysis. To investigate the impact of different environmental factors (such as pH, SBD, SOM, TN, AP, AK, and MBC) on soil bacterial community structure, redundancy discriminant analysis (RDA) was also performed using Origin. In addition, to investigate the association between significantly different metabolites (SDMs) and

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differential bacteria in different treatments, clustering heat maps were used to measure the high RA of soil bacteria and SDMs, while the screening thresholds for top 20 SDMs were set as VIP > 1, $p < 0.05$ (Paulson, 2009).

3. Results

3.1. Impact on soil physicochemical properties

Results from this study indicated that soil physicochemical properties were differentially affected by three years vegetation restoration of three different plants, while the effect depends on both the vegetation types and the kind of soil parameters. Indeed, the pH, SBD, SOM and TN were generally unaffected by three different plants except a significant reduction in SBD (13.97%) and SOM (35.41%) by vegetable and peach, respectively. However, three different plants significantly ($p < 0.05$) increased the AP (75.03–143.02%), AK (154.90% and 103.93%) and MBC (37.71–144.93%), with the greatest increase in vegetable treatment relative to the control except a significant ($p < 0.05$) reduction in the AK (41.73%) by peach (Table 1). These results suggest that the three plants in particular vegetable may be a good means of restoring newly reclaimed croplands in subtropical of China.

Table 1. Impacts of different vegetation types on soil physicochemical properties during soil restoration.

Parameters	Vegetable	Corn	Peach	Control
pH	8.21 ± 0.16 a	7.90 ± 0.21 b	8.14 ± 0.13 ab	8.07 ± 0.20 ab
SBD (g/cm^3)	1.17 ± 0.06 b	1.31 ± 0.07 a	1.27 ± 0.12 a	1.36 ± 0.06 a
SOM (%)	10.63 ± 0.92 a	9.04 ± 2.23 a	5.71 ± 1.59 b	8.84 ± 1.56 a
TN (g/kg)	0.86 ± 0.08 a	0.87 ± 0.26 a	0.57 ± 0.14 b	0.75 ± 0.10 ab
AP (mg/kg)	67.34 ± 4.50 a	53.16 ± 2.03 b	48.50 ± 3.54 b	27.71 ± 2.93 c
AK (mg/kg)	436.24 ± 12.40 a	349.01 ± 7.60 b	99.73 ± 8.42 d	171.14 ± 13.79 c
MBC (mg/kg)	104.51 ± 12.23 a	65.29 ± 6.77 b	58.76 ± 3.68 b	42.67 ± 5.07 c

Values that are separated by distinct lowercase letters within the same line indicate a significant difference at $p < 0.05$. SBD, soil bulk density; SOM, soil organic matter; TN, total nitrogen; AP, available phosphorus; AK, available potassium; MBC, microbial biomass carbon.

3.2. Impact on soil microbial community diversity and structure

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After original data of all soil samples from four different treatments were quality-controlled, a total of 1,124,416 high-quality 16S rRNA gene sequences were obtained by high-throughput amplicon sequencing, while the distribution of 59,893 bacterial OTUs that identified in this study across all four treatments was shown in Figure 1d. The average number of bacterial OTUs was 3001.80 (2907 to 3079), 3026.60 (2887 to 3100), 3124.00 (3054 to 3191), and 2826.20 (2765 to 2876) in vegetable, corn, peach, and the control, respectively. Furthermore, bacterial species richness and diversity among all soil samples were evaluated by using the alpha diversity indices including Chao1 and Shannon. Indeed, the average Chao1 index was 3003.47 (2847.84 to 3180.84), 3028.25 (2902.80 to 3175.97), 3125.40 (3065.14 to 3161.56), and 2827.60 (2765.36 to 2877.20), while the Shannon index was 10.46 (10.41 to 10.56), 10.38 (10.30 to 10.50), 10.44 (10.18 to 10.56), and 10.15 (9.83 to 10.33) in vegetable, corn, peach, and the control, respectively (Figure 1e,f). Obviously, vegetation of the three plants significantly affected the bacterial richness and diversity during soil restoration with increases in the number of OTUs (6.21–10.54%), the Chao1 (6.22–10.53%) and Shannon indices (2.84–3.11%) relative to the control.

The PCA results in this study revealed the OTUs abundance from 20 soil samples of vegetable, corn, peach, and the control formed four different groups, while peach was well separated from all the other three treatments. However, there was noticeable overlap among vegetable, corn, and the control. Furthermore, the results of PCA1 and PCA2 revealed that the bacterial community account for 43.99% and 8.86% of the variability (Figure 2), respectively, while the result of permutational multivariate ANOVA (PERMANOVA) indicated that different vegetation types explained 66.2% of the variation (p = 0.001). In addition, the soil bacterial community structure was differentially changed by three different vegetation types during soil restoration. In general, the number of phyla, class, order, family and genus were unaffected by the three plants except that the number of family was significantly increased by corn (7.48%), while the number of genera were significantly increased by vegetable (10.14%), and corn (10.14%) compared to the control (Table 2).

Based on a histogram of RAs at the top 10 phylum (Figure 3a) and genus (Figure 3b) levels, a discrepancy was found in soil bacterial community structure between different plant treatments and the control. In detail, similar trends for the three plants were observed in the 5 main bacterial phyla with the increase in the RAs of Proteobacteria and Chloroflexi, and the decrease in the RA

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of Actinobacteriota compared to the control. However, differential effect was observed for the three plants in the 7 main bacterial genus, while the RA of *Gemmatimonadaceae* was decreased by vegetable (7.47%) and peach (24.88%), but increased by corn (23.78%); the RA of *Vicinamibacterales* was increased by vegetable (17.92%), but decreased by corn (11.39%) and peach (12.23%); the RA of MND1 was decreased by vegetable (27.34%), corn (23.90%), and peach (8.09%); the RA of *gamma_proteobacterium* were increased by vegetable (39.40%), corn (9.86%), and peach (26.97%); the RA of *A4b* were increased by vegetable (51.81%), corn (27.11%), and peach (7.84%); the RA of *Vicinamibacteraceae* was increased by vegetable (44.14%) and peach (12.02%), but decreased by corn (10.68%); the RA of *Bacillus* was decreased by vegetable (53.27%), but increased by corn (33.68%) and peach (57.02%) compared with the control.

The difference in RA composition of soil bacterial community among all four different treatments was further visualized by heat map at family level (Figure 4a). In detail, the vegetable treatment was enriched with *Vicinamibacteraceae*, *Sphingomonadaceae*, *unclassified_Vicinamibacterales*, *Microscillaceae*, *Nitrospiraceae*, *Xanthomonadaceae*, *Thermoanaerobaculaceae*, *Anaerolineaceae*, and *uncultured_gamma_proteobacterium*, but was significantly reduced with *Bacillaceae* ($p < 0.05$); the corn treatment was enriched with *Gemmatimonadaceae* and unclassified_Chloroflexi, but was significantly reduced with uncultured_*Acidobacteria*_bacterium and *Nitrosomonadaceae* ($p < 0.05$); the peach treatment was enriched with *A4b* and *Comamonadaceae*, but was significantly reduced with *Myxococcaceae*, *uncultured_Firmicutes_bacterium*, and *Gemmatimonadaceae* ($p < 0.05$).

LEfSe analysis was carried out in this study to further identify specific bacterial biomarkers, which may play an important role in reshaping soil bacterial communities during soil restoration. Indeed, this result showed that 17 bacterial biomarkers were found in vegetable, corn, and peach and the control, which were able to be used to distinguish the soil bacterial communities among all four different treatments. In detail, the vegetable treatment was enriched with *Vicinamibacterales*, *Vicinamibacteria*, and *Acidobacteriota*; the corn treatment was enriched with *unclassified_Gemmatimonadaceae*, *Gemmatimonadaceae*, *Gemmatimonadales*, *Gemmatimonadetes*, and *Gemmatimonadota*; the peach treatment was enriched with *Rhizobiales*, *Alphaproteobacteria*, and *Proteobacteria*; and the control was enriched with *Firmicutes*, two

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types of *uncultured_Firmicutes_bacterium*, *Rokubacteriales*, *Methylomirabilia*, and *Methylomirabilota* (Figure 4b).

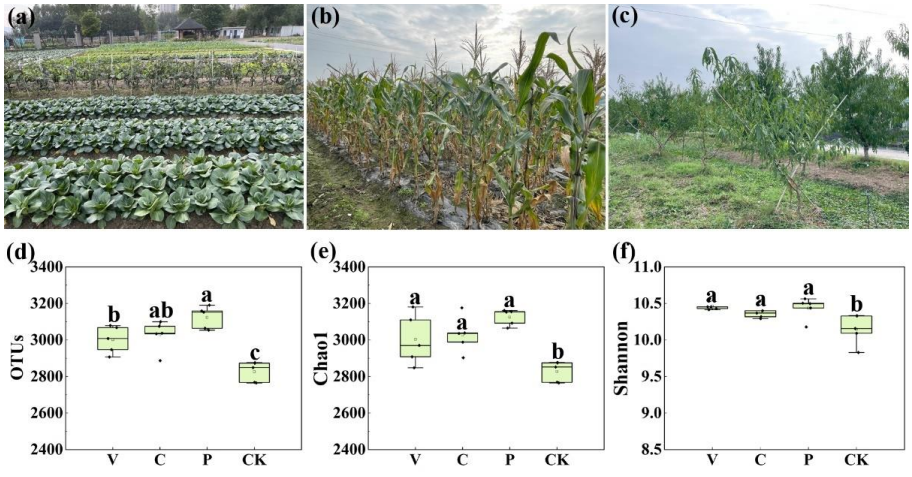

Figure 1. The situation of different vegetation restoration types in the field (a, vegetable; b, corn; c, peach), and the distribution of OTUs (d), the Chao1 (e), and Shannon (f) indices of bacterial communities among four different treatments during soil restoration. Statistical differences ($p < 0.05$) are indicated by different lowercase letters above the columns. V, vegetables; C, corn; P, peach; CK, control.

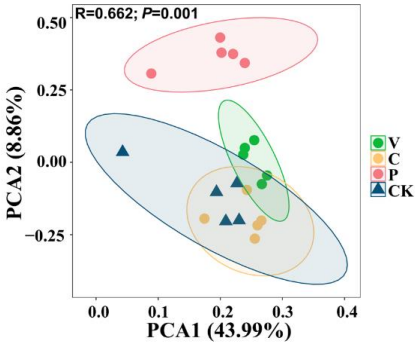

Figure 2. Principal component analysis (PCA) of the soil bacterial communities performed at the OTU level. Ellipses are included in the plot, indicating the 0.95 confidence limit. V, vegetable; C, corn; P, peach; CK, control.

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309 **Table 2.** Impact of different vegetation types on soil bacterial community composition during
310 soil restoration.

Parameter	Vegetable	Corn	Peach	Control
Phylum	33.2 ± 1.10 a	32.0 ± 0.71 ab	30.6 ± 1.34 b	32.4 ± 1.52 ab
Class	86.6 ± 5.13 ab	88.8 ± 4.09 a	81.6 ± 2.41 b	84.6 ± 2.70 ab
Order	231.2 ± 7.46 ab	241.4 ± 6.47 a	223.0 ± 7.48 b	228.8 ± 13.86 ab
Family	405.8 ± 12.97 ab	411.0 ± 7.81 a	385.8 ± 18.35 abc	382.4 ± 26.67 bc
Genus	632.0 ± 31.53 a	632.0 ± 33.28 a	603.2 ± 30.26 ab	573.8 ± 37.78 b

311 Values that are separated by distinct lowercase letters within the same line indicate a significant
312 difference at $p < 0.05$.

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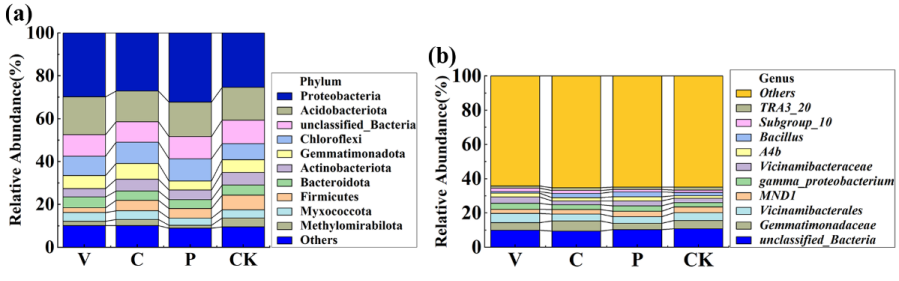

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317 **Figure 3.** Relative abundances (RAs) of bacterial composition at the bacterial phylum (a) and
318 genus (b) level, respectively, across different vegetation restoration types. V, vegetable; C, corn;
319 P, peach; CK, control.

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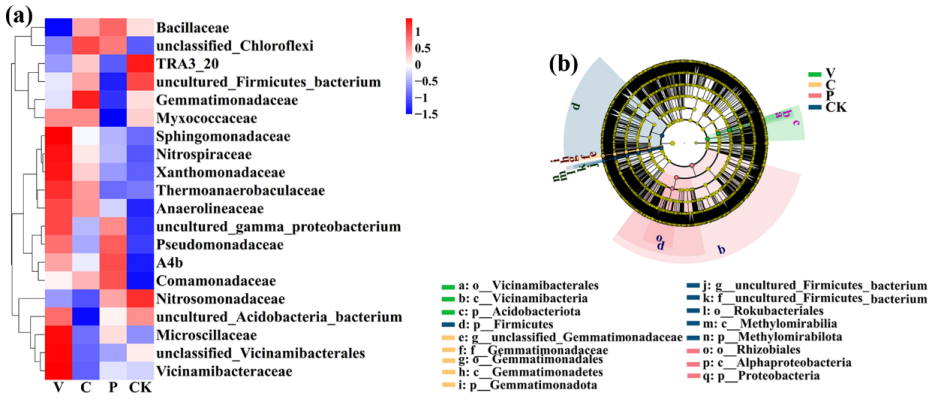

Figure 4. The effect size (LEfSe) of Liner discriminant analysis (LDA) on the bacterial taxa (a). Only bacterial taxa with LDA values > 4 ($p < 0.05$) are displayed. The heat map shows the abundance of dominant bacterial community abundance at the family level (b). V, vegetable; C, corn; P, peach; CK, control.

3.3. Impact on soil metabolomics

Results showed that the distribution of different treatments could be effectively separated between the three plants and the control following the identification of a total of 1,429 metabolites from all four different treatments based on LC-MS analysis and construction of a score map of metabolites by OPLS-DA (Figure 5a-c). Indeed, Figure 5a presents the sampling distributions of vegetable and the control in the positive and negative areas of t[1], respectively, while the model values of vegetable and the control were R^2X (cum) = 0.332, R^2Y (cum) = 1.000, Q^2 (cum) = 0.777. Similarly, Figure 5b presents the sampling distributions of corn and the control in the positive and negative areas of t[1], respectively, while the model values of corn and the control were R^2X (cum) = 0.322, R^2Y (cum) = 0.998, Q^2 (cum) = 0.636. Furthermore, Figure 5c presents the sampling distributions of peach and the control in the positive and negative areas of t[1], respectively, while the model values of peach and the control were R^2X (cum) = 0.276, R^2Y (cum) = 0.999, Q^2 (cum) = 0.574. In addition, the three groups of models were reliable due to that the Q^2 (cum) were greater than 0.5. Therefore, it could be inferred that the metabolites in control were significantly changed by different vegetation types.

The 1,429 identified metabolites mainly refer to organic acid and its derivatives (18.63%), benzene and substituted derivatives (17.72%), FA (11.21%), heterocyclic compounds (8.86%), amino acid and its metabolites (7.05%), GP (6.69%), carbohydrates and its metabolites (5.06%),

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and so on (Figure 5d). Among them, 326 SDMs with VIP > 1 and $p < 0.05$ were found between three plants and the control (Figure 5e-g), while there were 130 SDMs (89 upregulated and 41 downregulated) between vegetable and the control, 104 SDMs (50 upregulated and 54 downregulated) between corn and the control, 92 SDMs (52 upregulated and 40 downregulated) between peach and the control. Furthermore, the top 20 SDMs with the largest VIP value were showed in each group (Figure 6 a, c, e), while there were 6 downregulated and 14 upregulated SDMs between vegetable and the control (Figure 6a), 11 downregulated and 9 upregulated SDMs between corn and the control (Figure 6c), and 6 downregulated and 14 upregulated SDMs between peach and the control (Figure 6e).

All these SDMs belong to alcohol and amines, aldehyde, Ketones, Esters, amino acid and its metabolites, benzene and substituted derivatives, bile acids, carbohydrates and its metabolites, co-Enzyme and vitamins, FA, flavonoids, GP, heterocyclic compounds, hormones and hormone related compounds, lignans and coumarins, nucleotide and its metabolites, organic acid and its derivatives, and SP. Furthermore, enrichment analysis of the KEGG pathway was carried out according to these SDMs, which showed that these SDMs between vegetable and the control are mainly associated with metabolic pathways, biosynthesis of secondary metabolites, and glycerophospholipid metabolism (Figure 6b); these SDMs between corn and the control are mainly associated with biosynthesis of secondary metabolites, glycerophospholipid metabolism, biosynthesis of cofactors, and biosynthesis of amino acids (Figure 6d); these SDMs between peach and the control are mainly associated with glycerophospholipid metabolism, alpha-linolenic acid metabolism, arachidonic acid metabolism, and linoleic acid metabolism (Figure 6f).

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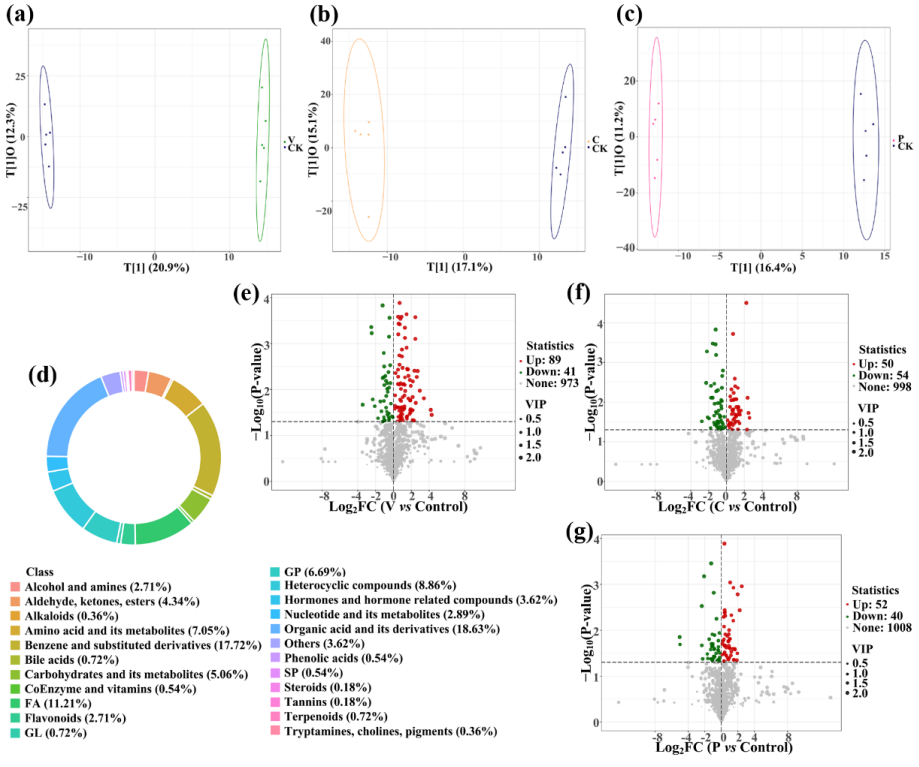

Figure 5. Orthogonal projections to latent structures-discriminant analysis (OPLS-DA) score map of vegetable (a), corn (b), and peach (c) treatments. Donut plot of metabolite classification and proportion (d). Volcano plot of differentially accumulated metabolites in V *vs* control (e), C *vs* control (f), and P *vs* control (g). Each point represents a metabolite with VIP > 1 and $p < 0.05$. V, vegetable; C, corn; P, peach; CK, control.

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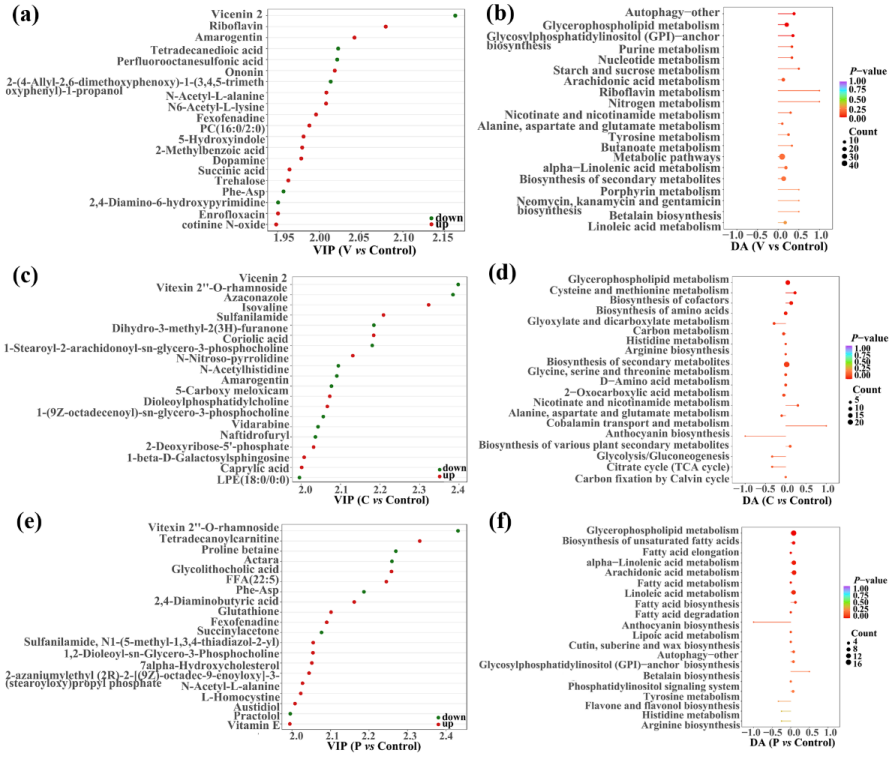

Figure 6. VIP value map of significantly differential metabolites (top 20 VIP value) in V *vs* control (a), C *vs* control (c), and P *vs* control (e). KEGG enrichment analysis of significantly differential metabolites in V *vs* control (b), C *vs* control (d), and P *vs* control (f). V, vegetable; C, corn; P, peach; CK, control.

3.4. Correlation among soil physiochemical properties, soil bacteria, and metabolites under different vegetation restoration types

Results of RDA revealed the correlation between soil physiochemical properties and bacterial communities, while the soil physiochemical properties explained 32.90% of the total variance in the bacterial community composition at the genus level (Figure 7a). In detail, the contributions of the 7 variables were MBC (F = 73.06%, p = 0.0005), AP (F = 67.85%, p = 0.0005), SBD (F = 56.69%, p = 0.0005), OMC (F = 51.06%, p = 0.0005), TN (F = 43.12%, p = 0.0030), AK (F = 36.51%, p = 0.0065), and pH (F = 1.95%, p = 0.8061). These results showed that there was a complex relationship between bacterial growth and soil physiochemical

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properties, while MBC, AP, SBD, SOM, and TN were main factors influencing the bacterial communities.

The correlation of bacteria with metabolites was determined by drawing the clustering heat maps following the normalization of the top 20 SDMs (Figure 7b-d). In detail, results of vegetable *vs* control showed that 15 metabolites were positively correlated with eight bacteria (*Vicinamibacteraceae*, *protebacterium*, *Subgroup_10*, *A4b*, *Vicinamibacterales*, *unclassified_Bacteria*, *MND1*, and *Bacillus*), while 9 metabolites negatively correlated with seven bacterial genera (*Subgroup_10*, *Vicinamibacteraceae*, *protebacterium*, *MND1*, *A4b*, *Bacillus*, and *unclassified_Bacteria*). Results of corn *vs* control showed that 5 metabolites were positively correlated with one bacterial genus (*unclassified_Bacteria*), while 3 metabolites were negatively correlated with two bacterial genera (*Subgroup_10* and *unclassified_Bacteria*). Results of peach *vs* control showed that 4 metabolites were positively correlated with two bacterial genera (*A4b* and *protebacterium*). Therefore, it can be inferred the change of the soil bacterial communities by the three plants especially vegetable may be mainly attribute to the SDMs, such as amino acid derivatives, pyridine derivatives, small peptide, and so on.

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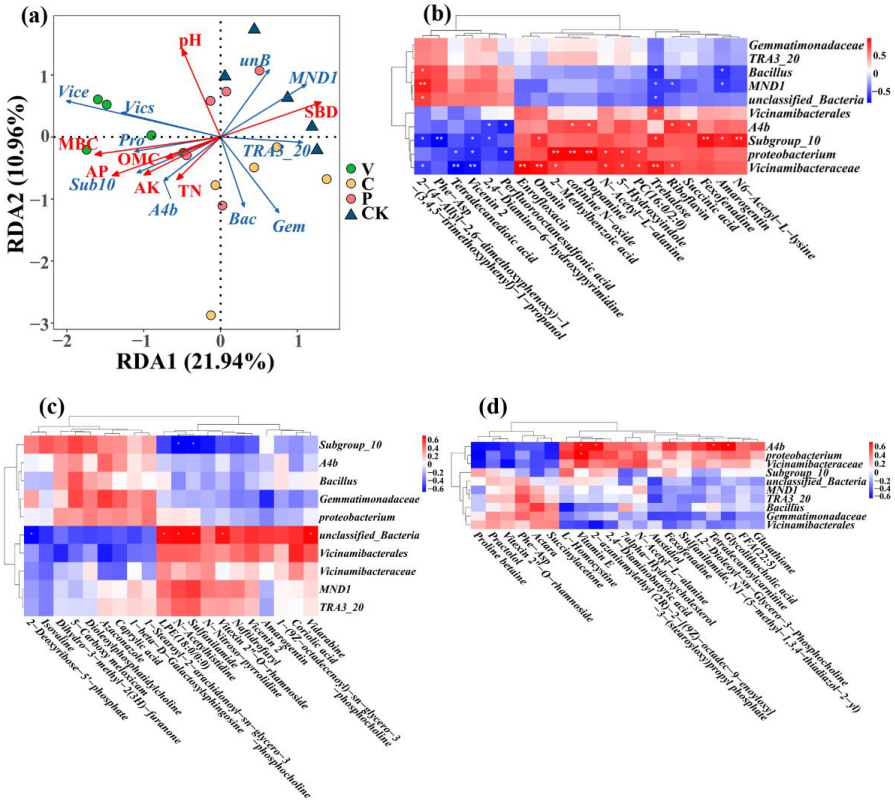

Figure 7. Redundancy discriminant analysis (RDA) of the soil bacterial community compositions at the genus level with soil physicochemical properties (a). Arrows indicate the direction and magnitude of soil physicochemical properties (pH, SBD, SOM, TN, AP, AK, and MBC) associated with the different bacterial genus. Ellipses are included in the plot, indicating the 0.95 confidence limit. *Sub10*: *Subgroup_10*; *Vice*: *Vicinamibacteraceae*; *Vics*: *Vicinamibacterales*; *Pro*: *proteobacterium*; *Bac*: *Bacillus*; *Gem*: *Gemmatimonadaceae*; *unB*: *unclassified_Bacteria*; SBD: soil bulk density; SOM: soil organic matter; TN: total nitrogen; AP: available phosphorus; AK: available potassium; MBC: microbial biomass carbon. V, vegetable; C, corn; P, peach; CK, control. Correlation heat map between top 20 SDMs and top 10 bacterial genera in vegetable *vs* the control (b), corn *vs* the control (c), and peach *vs* the control (d). *indicated a significant correlation at $p < 0.05$, **indicated a significant correlation at $p < 0.01$.

4. Discussion

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4.1 Role of microbes in improvement of soil quality

Results from this study indicated that the restoration of vegetable, corn, and peach caused a clear improvement in the microbial soil environment, in particular soil bacterial OTUs and the alpha diversity in newly reclaimed croplands compared to the control by measuring the community structure and diversity of soil bacteria under four different vegetation types using 16S rRNA gene high-throughput sequencing. In agreement with the findings of this study, previous studies had demonstrated a direct relationship between soil bacteria and vegetation. For example, Chen et al. (2015) reported that gene number in samples with cropping was higher than those without cropping. Zheng et al. (2020) showed that vegetation restorations increased OTUs, Chao1, and Shannon indices, while the greater was observed during the initial seven years. Zheng et al. (2022) revealed that different vegetation restoration including grassland, cropland, and plantation forest of degraded land significantly increased OTUs, ACE, and Chao1 indices, resulting in shifts in bacterial phyla favoring *Actinobacteria, Proteobacteria* and *Acidobacteria*.

It has been well known that microbes play an important role in improvement of soil quality although some of them have been reported to be the pathogen of various plants (Ahmed et al., 2023; Chen et al., 2024b; Pedrinho et al., 2024). Indeed, the diversity of microbes is important indicator of soil quality, while high microbial diversity can promote soil ecosystem function (Shen et al., 2015; Maron et al., 2018). The improvement of microbes in soil quality, may at least partially, be due to their beneficial function. For example, specific fungi or bacteria were able to form and stabilize soil aggregates, fix atmospheric N, unlock P and K in the soil, which enhance the resistance of plant to various biotic and abiotic stresses by providing essential nutrients for plant growth (Wei et al., 2024). Interestingly, this result revealed that the vegetation restoration of three plants had an impact on the abundances of beneficial bacteria. Therefore, further study should be carried out to elucidate the role of these specific bacteria during restoration of newly reclaimed croplands by different vegetation types.

PCA analysis showed that compared to the control, the community composition of soil bacteria was changed by the three vegetation types, which explained 66.2% (p = 0.001) of the variation. Following a histogram of RAs at phylum and genus, this results indicated that the three vegetation types resulted in the higher RA at phylum of *Proteobacteria* and *Chloroflexi*, and the lower RA at phylum of *Actinobacteriota* as well as the higher RA at genera of *gamma_proteobacterium* and *A4b*, and the lower at genus of *MND1* compared to the control,

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while the RA at genera of *Gemmatimonadaceae*, *Vicinamibacterales*, *Vicinamibacteraceae*, and *Bacillus* were differentially changed by the three vegetation types. In agreement with the result of this study, previous studies also showed that soil microbial community composition varied with different plants. For example, Li et al. (2024) reported significant differences in zone-soil bacterial communities among different varieties of Sanyeqing. Shi et al. (2022) found that the rhizosphere soil of bacterial wilt resistant mutant tobacco and susceptible tobacco caused different bacterial community abundance when compared to the top 15 bacterial taxa. Zuo et al. (2021) showed that *Dendrobium*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were the dominant phyla of bacteria in the rhizosphere soil. Therefore, it was necessary to conduct further research on these specific bacteria enriched by different vegetation types.

The improvement of microbes in soil quality may be also closely related to a total of 17 bacterial biomarkers, which were found in all soil samples. Indeed, more attention should be paid to the following bacteria due to their beneficial function in soil and plants. For instance, bacteria from *Proteobacteria* are of great importance to global carbon, N and sulfur cycling (Kersters et al., 2006). Bacteria from *Chloroflexi* have independently evolved the ability to persist on atmospheric hydrogen and carbon monoxide (Islam et al., 2019). Bacteria from *Actinobacteriota* can produce various kinds of antibiotics to improve disease resistance (Sanguin et al., 2009). Bacteria from *Gemmatimonadaceae* are able to contribute to N cycling and soil respiration in the soil ecosystem (Huang et al., 2019). Bacteria from *Bacillus* can play a key role in conferring biotic and abiotic stress tolerance to plants by inducing systemic resistance, biofilm formation and lipopeptide production (Mahapatra et al., 2022). Therefore, those bacteria may have great potential in plant growth promotion by improving soil structure and biological activity.

4.2 Role of metabolites in improvement of soil quality

In addition to soil microbes, more and more attentions have also been paid on the role of metabolites during soil restoration and plant growth (Cheng et al., 2025), which can be elucidated by the application of metabolomics, and has been found to have large potential on soil science (Brown et al., 2024). Indeed, soil microbial community metabolomics analysis is a key aspect of understanding the complex interactions between microorganisms and their environment. Furthermore, metabolomics has evolved in assessing functional metabolome change with substrate addition or environmental change (Brown et al., 2021; Brown et al., 2022). In addition, it has been reported that sugars and carbohydrates, amines and peptides, organic

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acids and purines, and free fatty acids and lipids is key to organismal development, maintenance of a diverse community, apoptotic events (Brown et al., 2024; Brown et al., 2022; Chen et al., 2024c).

By studying the metabolites present in the soil, researchers can achieve a better understanding of the biochemical processes occurring within the microbial community. Results from this study indicated that a total of 1,429 metabolites were identified from all soil samples based on LC-MS analysis, while the soil metabolites were significantly changed by the restoration of three plants compared to the control based on the OPLS-DA of the metabolite profiles. Indeed, 130 SDMs (89 upregulation and 41 downregulation) were identified in vegetable vs control, 104 SDMs (50 upregulation and 54 downregulation) were identified in corn vs the control, 92 SDMs were identified in peach vs the control (52 upregulation and 40 downregulation). The analysis of these SDMs that were changed by different vegetation types can provide valuable insights into the functional diversity and metabolic potential of soil microbial communities.

These differential metabolites that identified in the soil treated with three different vegetation types were able to be grouped into seven main categories, which include organic acid, benzene, FA, heterocyclic compound, amino acid, GP, and carbohydrates. Organic acids have been reported to play significant roles in different biological processes, affect the physicochemical properties of soil, and contribute to improving plant growth and biomass (Sindhu et al., 2022). Benzene, as a representative volatile organic contaminant, has high toxicity, solubility, mobility and strong volatility. Heterocyclic compounds are extremely important with wide array of synthetic, pharmaceutical and industrial application, involving in a wide range of use in the field of agriculture and medicine (Qadir et al., 2022). Amino acids have been known to be important sources of soil organic N, which is essential for plant nutrition (Cao et al., 2016). Carbohydrates supply carbon sources for microbial activities that contribute to mineral nutrient production in soil (Ratnayake et al., 2013). Taken all together, these SDMs maybe play a vital role in the improvement of soil quality by involving into the metabolism of living cells.

4.3 Correlation among soil properties, microbiome, and metabolites

Results of RDA from this study showed that MBC, AP, SBD, SOM, and TN were the main variables of bacterial communities in all soil samples, indicating that soil properties influenced

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the soil bacterial communities. In agreement with the finding in this study, previous studies have shown that various environmental pressures were able to influence the microbial communities, which are crucial for the function and sustainability of soil ecosystem (Zhang et al., 2025). For example, Xue et al. (2018) reported that the nutrients, especially total carbon, total N, total P, and cation exchange capacity, were highly related with the microbial distribution in soil. Jiang et al. (2024) found that soil pH, soil organic carbon, total N, moisture, and AK, and soil microbial biomass were the drivers of soil bacterial community dynamics.

On the other hand, the relationship between different metabolites and bacteria under different vegetation types has been revealed based on the heat maps. Results from this study indicated that seven bacterial genus including *A4b*, *Bacillus*, *MND1*, *protebacterium*, *Subgroup_10*, *Vicinamibacteraceae*, and *Vicinamibacterales* exhibited significant connection with the metabolites such as amino acid derivatives, benzene and substituted derivatives, bile acids, CAR, coEnzyme and vitamins, FFA, flavonoid, heterocyclic compounds, indole and its derivatives, isoflavones, Lignans, nucleotide and its metabolites, organic acid and its derivatives, PE, phenolic acids, polyamines, pyridine and pyridine derivatives, small peptide, and sugars. In addition, these metabolites have been found to be mainly associated with kinds of microbial metabolisms and biosynthesis, alpha-linolenic acid metabolism, arachidonic acid metabolism, glycerophospholipid metabolism, such as linoleic acid metabolism, metabolic pathways, amino acids biosynthesis, cofactors biosynthesis, and secondary metabolites biosynthesis.

In agreement with the result of this study, a lot of studies have revealed that some metabolites were highly correlated with microbial abundance (Song et al., 2020). For example, Bi et al. (2022) reported that soil metabolites can strongly affect the structure and function of soil microbial community. Furthermore, it has been well known that microbial growth is normally dependent on the microbial primary metabolites, which include amino acids, alcohol, enzymes, nucleotides, organic acids, and vitamins, while the secondary metabolites including antitumor agents, pigments, antibiotics, and growth hormones have been found to play an important part in series of microbial metabolic processes (Minhas et al., 2024). Thus, it can be inferred that the improvement of soil quality may be, at least partially, attributed to certain metabolic pathways, which were mediated by soil microbial communities based on the relationships among soil properties, bacterial communities, and metabolites.

5. Conclusions

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This study indicated that three plants in particular vegetable could significantly improve the soil quality of newly reclaimed croplands compared with the control by investigating the effect of three different vegetation restoration types in the soil physicochemical properties, bacterial community, and metabolic diversity on newly reclaimed croplands after three years experiment. The soil improvement by vegetable may be mainly due to a significant increase in the richness, complexity and structure of the bacterial community in the newly reclaimed croplands, while the bacterial genus significantly enriched were *Vicinamibacterales*, *gamma_proteobacterium*, *Vicinamibacteraceae*, and *A4b*. Furthermore, a total of 1,103 SDMs that belong to organic acid, amino acid, heterocyclic compounds were identified in vegetable *vs* control, while the correlation heat map revealed a significant correlation between the top 20 SDMs and the enriched bacterial genus. In addition, RDA showed that soil properties (especially MBC, AP, SBD, SOM, and TN) were related to variations in bacterial community composition. Overall, this study indicated that vegetable can be used as a good vegetation restoration type in soil amelioration of newly reclaimed lands.

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Data Availability Statement: The data presented in this study can be found in online repositories. The names of the repositories are NCBI and CNGBdb, with accession numbers PRJNA1218658 and CNP0006854, respectively.

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Declarations of Generative-AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT to improve language and readability. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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