



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

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26 Short Summary

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

32 **Abstract:** To find one suitable vegetation restoration type as a good means of restoring newly
33 reclaimed croplands in subtropical of China. This study investigated the effect of vegetables,
34 corn, and peach in soil properties, bacterial communities, and metabolites of newly reclaimed
35 lands after three years restoration. Results from this study indicated that soil physicochemical
36 properties were differentially affected by vegetation restoration of three different plants, while
37 the effect depends on both the vegetation types and the kind of soil parameters. Indeed, the pH,
38 soil bulk density (SBD), soil organic matter (SOM) and total nitrogen (TN) were generally
39 unaffected except a significant reduction in SBD (13.97%) and SOM (35.41%) by vegetable and
40 peach, respectively. However, three different plants significantly increased the available
41 phosphorus (AP) (75.03–143.02%), available potassium (AK) (154.90% and 103.93%) and
42 microbial biomass carbon (MBC) (37.71–144.93%), with the greatest increase by vegetable
43 relative to the control except a significant reduction in the AK (41.73%) by peach. Furthermore,
44 the analysis of 16S rRNA gene high-throughput sequencing revealed that the vegetation of three
45 plants increased the relative abundances (RAs) of soil bacterial phyla and genera with 6.21–
46 10.54% increase in operational taxonomic units (OTUs), 6.22–10.53% increase in Chao1 and
47 2.30–3.11% increase in Shannon indices, while redundancy discriminant analysis (RDA)
48 revealed that the change of soil properties were highly related to the variation in bacterial
49 community composition. In addition, 130 significantly differential metabolites (SDMs) that
50 belong to organic acid, amino acid, heterocyclic compounds between vegetable and the control
51 were identified based on liquid chromatography-mass spectrometry (LC-MS) analysis, while the
52 top 20 SDMs were highly correlated with the 7 enriched bacterial genera. Overall, the results
53 showed that the vegetation of three plants, in particular vegetable can ameliorate soil quality of
54 newly reclaimed croplands by improving soil chemical properties, and increasing the richness



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

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

59



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).



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

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

118 **2. Materials and Methods**

119 **2.1. Experimental design and sample collection**



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

126 The experiment consisted of four different treatments ~~through the~~ application of vegetable,
127 corn, peach vegetation restoration, and the control without any plants in the newly reclaimed
128 cropland (Figure 1a–c). For each treatment, there were five plots, and each plot had an area of
129 about 125 m² (25 m × 5 m). The planting density of vegetable and corn was 25 cm × 25 cm and
130 30 cm × 50 cm, respectively, while sheep manure (1.50 kg/m²) and chemical compound fertilizer
131 (0.075 kg/m²) was applied for both treatments at early spring and autumn (twice per year).
132 Furthermore, the planting density of peach was 4 m × 5 m, while sheep manure (3.00 kg/m²) and
133 chemical compound fertilizer (0.15 kg/m²) was applied at early winter (once per year). In
134 addition, no fertilizer was used for the control.

135 In September 2024 (three years later), about 1.0 kg of fresh soil for each plot was sampled
136 from the root zones of the plants (5–20 cm, the top soil layer for the control) and then packed in
137 a sterile bag using a shovel. Meanwhile, the soil of each plot was also collected using a stainless-
138 steel cylinder (5 cm in height and 100 cm³ in volume). Then, all soil samples were quickly
139 transported to the laboratory in an ice box for further analysis.

140 **2.2. Soil physiochemical properties measurement**

141 To study the soil properties, about 1.0 kg fresh soil of each plot was sampled, air-dried and
142 passed through a 0.45-mm sieve to measure the soil pH, and soil bulk density (SBD), as well as
143 SOM, TN, AP, and AK. In detail, soil pH was measured at a soil suspension (soil: water = 1 g: 5
144 mL) using a pH meter (FE28, MettlerToledo, Zurich, Switzerland), while the SOM, TN, AP, and
145 AK contents were determined using the K₂Cr₂O₇ oxidation method, Kjeldahl distillation-titration
146 method, molybdenum-based colorimetric method, flame photometer method, respectively
147 (Brookes et al., 1985). Furthermore, the SBD was calculated using the oven drying method,
148 while microbial biomass carbon (MBC) was determined on fresh soil samples using the
149 fumigation-extraction method (Vance et al., 1987; McLaren and Cameron, 1990).



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.ATM 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 ddH₂O (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°C for 3 min, 25 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and finally an extension at 72°C for 5 min. After purification using Hieff NGSTM DNA selection beads (Yeasten, 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: H₂O = 7: 3), vortexed at 35 HZ for 3 min, sonicated for 10 min in an ice-water bath, and incubated at -20°C for 30 min. After centrifugation at 12,000 rpm for 10 min (4°C) 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



181 μm , 2.1 mm $\times$ 100 mm); mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1%
182 formic acid in acetonitrile; gradient program: 5 to 20% in 2 min, increased to 60% in the
183 following 3 min increased to 99% in 1 min and held for 1.5 min, then come back to 5% B within
184 0.1 min and held for 2.4 min; column temperature: 40°C, flow rate: 0.4 ml/min, and injection
185 volume: 4 μl . The ESI source conditions of Q Exactive HF-X were set as follows: ion spray
186 voltage, 3.5 KV (positive) or 3.2 KV (negative), respectively; sheath gas, 30 Arb; aux gas, 5 Arb;
187 ion transfer tube temperature, 320°C; vaporizer temperature, 300°C; scan range, 75–1,000 Da;
188 resolution, 35,000; collision energy, 30, 40, 50 V; signal intensity threshold, 1 $\times$ 10⁶ cps; top N vs
189 top speed, 10; exclusion duration, 3 s. The repeatability and reliability of the entire analysis
190 process were evaluated by inserting one quality control sample, which was prepared by pooling
191 and mixing 10 μl of each sample. All treatments had five replicates. The data obtained in this
192 study were converted into the mzXML format using ProteoWizard, processed with XCMS
193 program, and then compared with the in-house database (HMDB, <http://hmdb.ca/>) and KEGG,
194 <https://www.kegg.jp/>) for metabolite annotation.

195 **2.5. Data analysis**

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



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



233 After original data of all soil samples from four different treatments were quality-controlled,
234 a total of 1,124,416 high-quality 16S rRNA gene sequences were obtained by high-throughput
235 amplicon sequencing, while the distribution of 59,893 bacterial OTUs that identified in this study
236 across all four treatments was shown in Figure 1d. The average number of bacterial OTUs was
237 3001.80 (2907 to 3079), 3026.60 (2887 to 3100), 3124.00 (3054 to 3191), and 2826.20 (2765 to
238 2876) in vegetable, corn, peach, and the control, respectively. Furthermore, bacterial species
239 richness and diversity among all soil samples were evaluated by using the alpha diversity indices
240 including Chao1 and Shannon. Indeed, the average Chao1 index was 3003.47 (2847.84 to
241 3180.84), 3028.25 (2902.80 to 3175.97), 3125.40 (3065.14 to 3161.56), and 2827.60 (2765.36 to
242 2877.20), while the Shannon index was 10.46 (10.41 to 10.56), 10.38 (10.30 to 10.50), 10.44
243 (10.18 to 10.56), and 10.15 (9.83 to 10.33) in vegetable, corn, peach, and the control,
244 respectively (Figure 1e,f). Obviously, vegetation of the three plants significantly affected the
245 bacterial richness and diversity during soil restoration with increases in the number of OTUs
246 (6.21–10.54%), the Chao1 (6.22–10.53%) and Shannon indices (2.84–3.11%) relative to the
247 control.

248 The PCA results in this study revealed the OTUs abundance from 20 soil samples of
249 vegetable, corn, peach, and the control formed four different groups, while peach was well
250 separated from all the other three treatments. However, there was noticeable overlap among
251 vegetable, corn, and the control. Furthermore, the results of PCA1 and PCA2 revealed that the
252 bacterial community account for 43.99% and 8.86% of the variability (Figure 2), respectively,
253 while the result of permutational multivariate ANOVA (PERMANOVA) indicated that different
254 vegetation types explained 66.2% of the variation ($p = 0.001$). In addition, the soil bacterial
255 community structure was differentially changed by three different vegetation types during soil
256 restoration. In general, the number of phyla, class, order, family and genus were unaffected by
257 the three plants except that the number of family was significantly increased by corn (7.48%),
258 while the number of genera were significantly increased by vegetable (10.14%), and corn
259 (10.14%) compared to the control (Table 2).

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



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



types of *uncultured_Firmicutes_bacterium*, *Rokubacterales*, *Methyloirabilia*, and *Methyloirabilota* (Figure 4b).

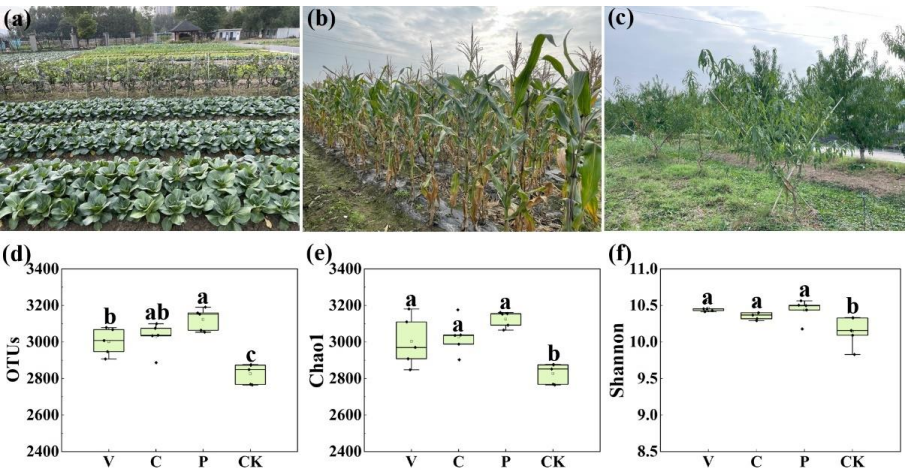


Figure 1. The situation of different vegetation restoration types in the field (a, vegetable; b, corn; c, peach), and the distribution of OTUs (d), the Chao1 (e), and Shannon (f) indices of bacterial communities among four different treatments during soil restoration. Statistical differences ($p < 0.05$)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

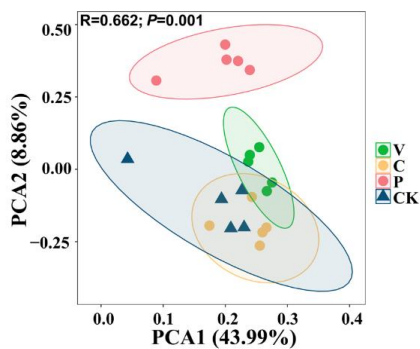


Figure 2. Principal component analysis (PCA) of the soil bacterial communities performed at the OTU level. Ellipses are included in the plot, indicating the 0.95 confidence limit. V, vegetable; C, corn; P, peach; CK, control.



Table 2. Impact of different vegetation types on soil bacterial community composition during 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

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

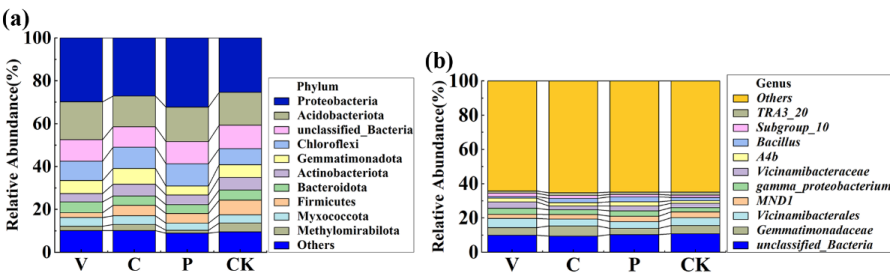


Figure 3. Relative abundances (RAs) of bacterial composition at the bacterial phylum (a) and genus (b) level, respectively, across different vegetation restoration types. V, vegetable; C, corn; P, peach; CK, control.

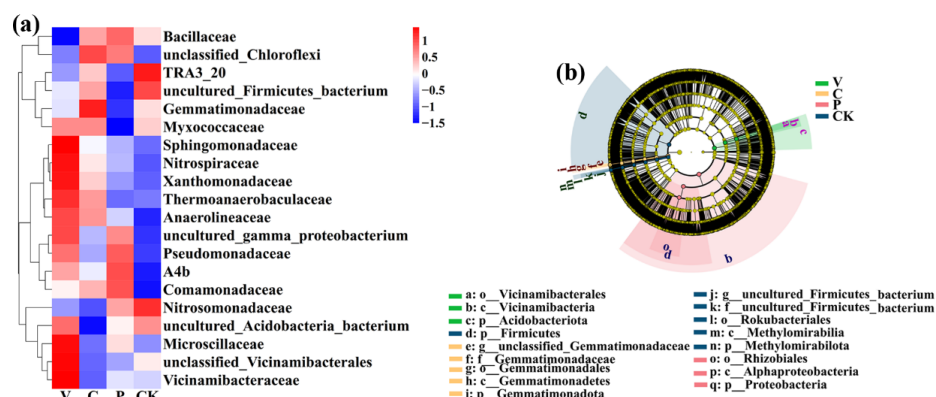


Figure 4. The effect size (LEfSe) of Liner discriminant analysis (LDA) on the bacterial taxa (a). Only bacterial taxa with LDA values > 4 ($p < 0.05$) are displayed. The heat map shows the abundance of dominant bacterial community abundance at the family level (b). V, vegetable; C, corn; P, peach; CK,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



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

353 All these SDMs belong to alcohol and amines, aldehyde, Ketones, Esters, amino acid and its
354 metabolites, benzene and substituted derivatives, bile acids, carbohydrates and its metabolites,
355 co-Enzyme and vitamins, FA, flavonoids, GP, heterocyclic compounds, hormones and hormone
356 related compounds, lignans and coumarins, nucleotide and its metabolites, organic acid and its
357 derivatives, and SP. Furthermore, enrichment analysis of the KEGG pathway was carried out
358 according to these SDMs, which showed that these SDMs between vegetable and the control are
359 mainly associated with metabolic pathways, biosynthesis of secondary metabolites, and
360 glycerophospholipid metabolism (Figure 6b); these SDMs between corn and the control are
361 mainly associated with biosynthesis of secondary metabolites, glycerophospholipid metabolism,
362 biosynthesis of cofactors, and biosynthesis of amino acids (Figure 6d); these SDMs between
363 peach and the control are mainly associated with glycerophospholipid metabolism, alpha-
364 linolenic acid metabolism, arachidonic acid metabolism, and linoleic acid metabolism (Figure
365 6f).

366

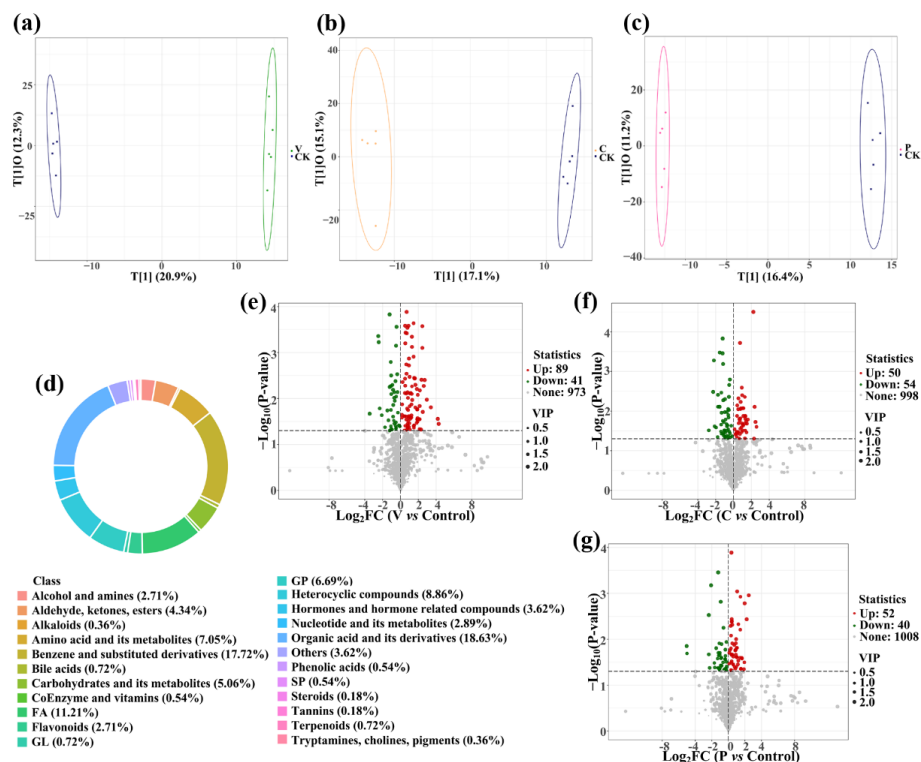


Figure 5. Orthogonal projections to latent structures-discriminant analysis (OPLS-DA) score map of vegetable (a), corn (b), and peach (c) treatments. Donut plot of metabolite classification and proportion (d). Volcano plot of differentially accumulated metabolites in V vs control (e), C vs control (f), 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.

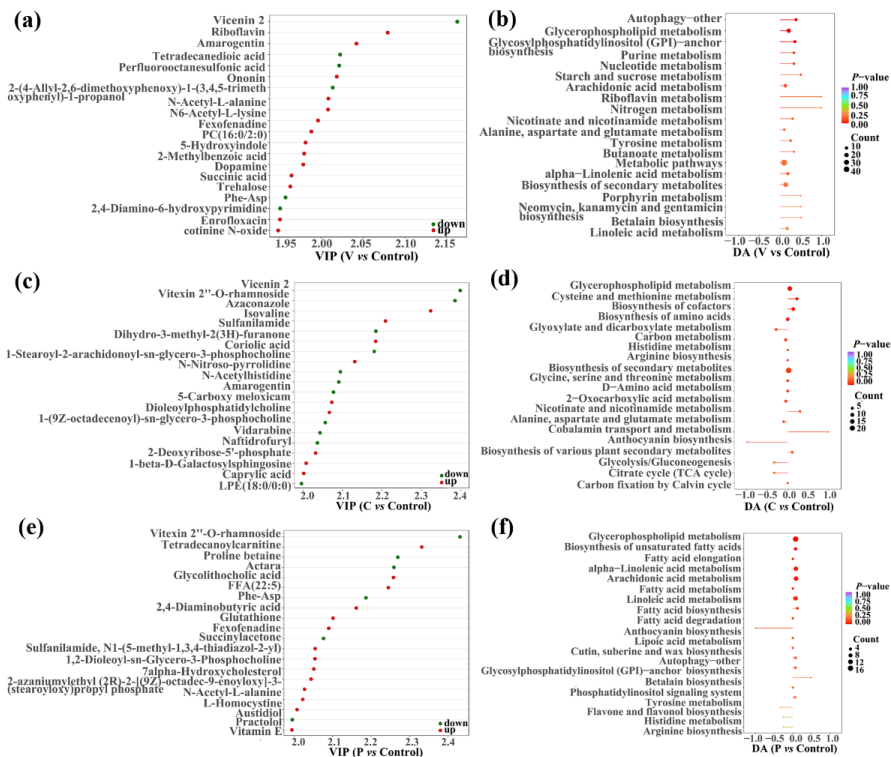


Figure 6. VIP value map of significantly differential metabolites (top 20 VIP value) in V vs control (a), C vs control (c), and P vs control (e). KEGG enrichment analysis of significantly differential metabolites in V vs control (b), C vs control (d), and P vs control (f). V, vegetable; C, corn; P, peach;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



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*, *proteobacterium*, *Subgroup_10*, *A4b*, *Vicinamibacterales*, *unclassified_Bacteria*, *MND1*, and *Bacillus*), while 9 metabolites negatively correlated with seven bacterial genera (*Subgroup_10*, *Vicinamibacteraceae*, *proteobacterium*, *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 *proteobacterium*). 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.

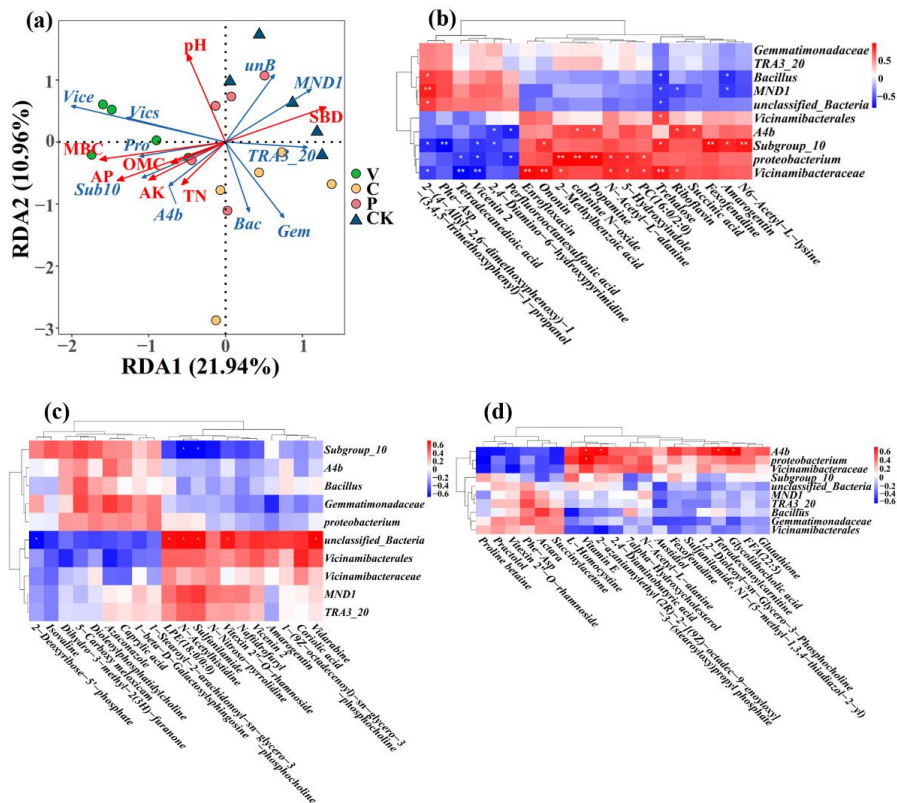


Figure 7. Redundancy discriminant analysis (RDA) of the soil bacterial community compositions at the genus level with soil physicochemical properties (a). Arrows indicate the direction and magnitude of soil physicochemical properties (pH, SBD, SOM, TN, AP, AK, and MBC) associated with the different bacterial 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



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,



451 while the RA at genera of *Gemmatimonadaceae*, *Vicinamibacterales*, *Vicinamibacteraceae*, and
452 *Bacillus* were differentially changed by the three vegetation types. In agreement with the result
453 of this study, previous studies also showed that soil microbial community composition varied
454 with different plants. For example, Li et al. (2024) reported significant differences in zone-soil
455 bacterial communities among different varieties of Sanyeqing. Shi et al. (2022) found that the
456 rhizosphere soil of bacterial wilt resistant mutant tobacco and susceptible tobacco caused
457 different bacterial community abundance when compared to the top 15 bacterial taxa. Zuo et al.
458 (2021) showed that *Dendrobium*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were the
459 dominant phyla of bacteria in the rhizosphere soil. Therefore, it was necessary to conduct further
460 research on these specific bacteria enriched by different vegetation types.

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

473 **4.2 Role of metabolites in improvement of soil quality**

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



482 acids and purines, and free fatty acids and lipids is key to organismal development, maintenance
483 of a diverse community, apoptotic events (Brown et al., 2024; Brown et al., 2022; Chen et al.,
484 2024c).

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

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

510 **4.3 Correlation among soil properties, microbiome, and metabolites**

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



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



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

558

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562 Ahmed: Software, Methodology, writing review and editing. Muhammad Shafiq Shahid,
563 Gabrijel Ondrasek and Branko Petrinc: Data curation, Visualization, Software, writing review
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574 **Declarations of Generative-AI and AI-assisted technologies in the writing process**

575 During the preparation of this work the author(s) used ChatGPT to improve language and
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578 **References**

- 579 Ahmed, T., Luo, J., Noman, M., Ijaz, M., Wang, X., Masood, H. A., Manzoor, N., Wang, Y., and Li, B.: Microbe-
580 mediated nanoparticle intervention for the management of plant diseases, *Crop Health*, 1, 3, 2023.
- 581 Augusto, L., Ranger, J., Ponette, Q., and Rapp, M.: Relationships between forest tree species, stand production and
582 stand nutrient amount, *Annals of Forest Science*, 57, 313-324, 2000.
- 583 Bai, X., Shi, P., and Liu, Y.: Society: Realizing China's urban dream, *Nature*, 509, 158-160, 2014.
- 584 Bi, B., Yuan, Y., Zhang, H., Wu, Z., Wang, Y., and Han, F.: Rhizosphere soil metabolites mediated microbial
585 community changes of *Pinus sylvestris* var. *mongolica* across stand ages in the Mu Us Desert, *Applied Soil Ecology*,
586 169, 104222, 2022.
- 587 Brookes, P., Landman, A., Pruden, G., and Jenkinson, D.: Chloroform fumigation and the release of soil nitrogen: a
588 rapid direct extraction method to measure microbial biomass nitrogen in soil, *Soil biology and biochemistry*, 17, 837-
589 842, 1985.
- 590 Brown, R. W., Chadwick, D. R., Zang, H., and Jones, D. L.: Use of metabolomics to quantify changes in soil microbial
591 function in response to fertiliser nitrogen supply and extreme drought, *Soil Biology and Biochemistry*, 160, 108351,
592 2021.
- 593 Brown, R. W., Reay, M. K., Centler, F., Chadwick, D. R., Bull, I. D., McDonald, J. E., Evershed, R. P., and Jones, D. L.:
594 Soil metabolomics-current challenges and future perspectives, *Soil Biology and Biochemistry*, 193, 109382, 2024.
- 595 Brown, R. W., Chadwick, D. R., Bending, G. D., Collins, C. D., Whelton, H. L., Daulton, E., Covington, J. A., Bull, I. D.,
596 and Jones, D. L.: Nutrient (C, N and P) enrichment induces significant changes in the soil metabolite profile and
597 microbial carbon partitioning, *Soil Biology and Biochemistry*, 172, 108779, 2022.
- 598 Cao, X., Ma, Q., Zhong, C., Yang, X., Zhu, L., Zhang, J., Jin, Q., and Wu, L.: Elevational variation in soil amino acid
599 and inorganic nitrogen concentrations in Taibai Mountain, China, *PLoS One*, 11, e0157979, 2016.
- 600 Chen, F., Tan, M., Yang, Y., Ma, J., Zhang, S., and Li, G.: The diversity changes of soil microbial communities
601 stimulated by climate, soil type and vegetation type analyzed via a functional gene array, *World Journal of*
602 *Microbiology and Biotechnology*, 31, 1755-1763, 2015.
- 603 Chen, Q., Song, Y., An, Y., Lu, Y., and Zhong, G.: Soil microorganisms: Their role in enhancing crop nutrition and
604 health, *Diversity*, 16, 734, 2024a.
- 605 Chen, Q., Liu, C., Qi, H., Wu, N., Liu, Z., Tian, Q., Zhu, X.-X., Li, X., Chen, Y., and Ma, Z.: A novel highly antifungal
606 compound ZJS-178 targeting myosin I inhibits the endocytosis and mycotoxin biosynthesis of *Fusarium*
607 *graminearum*, *Crop Health*, 2, 14, 2024b.



- 608 Chen, Y., Wang, Z., Gao, T., Huang, Y., Li, T., Jiang, X., Liu, Y., Gao, L., and Xia, T.: Deep learning and targeted
609 metabolomics - based monitoring of chewing insects in tea plants and screening defense compounds, *Plant, Cell &*
610 *Environment*, 47, 698-713, 2024c.
- 611 Cheng, R., Ke, T., Gui, F., Li, J., Zhang, X., Vilchez, J. I., and Matsumoto, H.: drSMALL: Database for disease
612 resistance-shaping small molecules derived from the plant microbiome, *Crop Health*, 3, 2, 2025.
- 613 Edgar, R. C.: UPARSE: highly accurate OTU sequences from microbial amplicon reads, *Nature methods*, 10, 996-998,
614 2013.
- 615 Edgar, R. C.: SINTAX: a simple non-Bayesian taxonomy classifier for 16S and ITS sequences, *bioRxiv*, 074161, 2016.
- 616 Guo, S., Han, X., Li, H., Wang, T., Tong, X., Ren, G., Feng, Y., and Yang, G.: Evaluation of soil quality along two
617 revegetation chronosequences on the Loess Hilly Region of China, *Science of the Total Environment*, 633, 808-815,
618 2018.
- 619 Hu, G., Chen, D., Huang, X., Zhou, C., Yu, G., and Pan, J.: The research about balance system of farm land in
620 occupation and supplement: A demonstration from Lin'ancity, *J. Agric*, 4, 112-115, 2014.
- 621 Huang, X.-f., Li, S.-q., Li, S.-y., Ye, G.-y., Lu, L.-j., Zhang, L., Yang, L.-y., Qian, X., and Liu, J.: The effects of biochar
622 and dredged sediments on soil structure and fertility promote the growth, photosynthetic and rhizosphere microbial
623 diversity of *Phragmites communis* (Cav.) Trin. ex Steud, *Science of The Total Environment*, 697, 134073, 2019.
- 624 Islam, Z. F., Cordero, P. R., Feng, J., Chen, Y.-J., Bay, S. K., Jirapanjawat, T., Gleadow, R. M., Carere, C. R., Stott, M. B.,
625 and Chiri, E.: Two Chloroflexi classes independently evolved the ability to persist on atmospheric hydrogen and
626 carbon monoxide, *The ISME journal*, 13, 1801-1813, 2019.
- 627 Jiang, L., Deng, X., and Seto, K. C.: The impact of urban expansion on agricultural land use intensity in China, *Land*
628 *use policy*, 35, 33-39, 2013.
- 629 Jiang, S., Song, M., Du, H., Wang, F., Song, T., Chen, H., Zeng, F., and Peng, W.: Soil Properties Regulate Soil
630 Microbial Communities During Forest Succession in a Karst Region of Southwest China, *Microorganisms*, 12, 2136,
631 2024.
- 632 Kersters, K., De Vos, P., Gillis, M., Swings, J., Vandamme, P., and Stackebrandt, E.: Introduction to the Proteobacteria,
633 in: *The prokaryotes: a handbook on the biology of bacteria*, Springer, 3-37, 2006.
- 634 Lai, Z., Chen, M., and Liu, T.: Changes in and prospects for cultivated land use since the reform and opening up in
635 China, *Land Use Policy*, 97, 104781, 2020.
- 636 Larney, F. J. and Angers, D. A.: The role of organic amendments in soil reclamation: A review, *Canadian Journal of*
637 *Soil Science*, 92, 19-38, 2012.
- 638 Li, X., Li, D., Jiang, Y., Xu, J., Ren, X., Zhang, Y., Wang, H., Lu, Q., Yan, J., and Ahmed, T.: The effects of microbial
639 fertilizer based *Aspergillus brunneoviolaceus* HZ23 on pakchoi growth, soil properties, rhizosphere bacterial
640 community structure, and metabolites in newly reclaimed land, *Frontiers in Microbiology*, 14, 1091380, 2023.



- 641 Li, X., Lu, Q., Hafeez, R., Ogunyemi, S. O., Ibrahim, E., Ren, X., Tian, Z., Ruan, S., Mohany, M., and Al-Rejaie, S. S.:
642 The response of root-zone soil bacterial community, metabolites, and soil properties of Sanyeqing medicinal plant
643 varieties to anthracnose disease in reclaimed land, China, *Heliyon*, 10, 2024.
- 644 Li, Y., Liu, Y., Long, H., and Cui, W.: Community-based rural residential land consolidation and allocation can help
645 to revitalize hollowed villages in traditional agricultural areas of China: Evidence from Dancheng County, Henan
646 Province, *Land Use Policy*, 39, 188-198, 2014.
- 647 Liu, Y., Long, H., Chen, Y., Wang, J., Li, Y., Li, Y., Yang, Y., and Zhou, Y.: Progress of research on urban-rural
648 transformation and rural development in China in the past decade and future prospects, *Journal of Geographical
649 Sciences*, 26, 1117-1132, 2016.
- 650 Liu, Z., Cao, S., Sun, Z., Wang, H., Qu, S., Lei, N., He, J., and Dong, Q.: Tillage effects on soil properties and crop yield
651 after land reclamation, *Scientific Reports*, 11, 4611, 2021.
- 652 Lu, Z.-X., Wang, P., Ou, H.-B., Wei, S.-X., Wu, L.-C., Jiang, Y., Wang, R.-J., Liu, X.-S., Wang, Z.-H., and Chen, L.-J.:
653 Effects of different vegetation restoration on soil nutrients, enzyme activities, and microbial communities in degraded
654 karst landscapes in southwest China, *Forest Ecology and Management*, 508, 120002, 2022.
- 655 Mahapatra, S., Yadav, R., and Ramakrishna, W.: *Bacillus subtilis* impact on plant growth, soil health and
656 environment: Dr. Jekyll and Mr. Hyde, *Journal of applied microbiology*, 132, 3543-3562, 2022.
- 657 Maron, P.-A., Sarr, A., Kaisermann, A., Lévêque, J., Mathieu, O., Guigue, J., Karimi, B., Bernard, L., Dequiedt, S., and
658 Terrat, S.: High microbial diversity promotes soil ecosystem functioning, *Applied and Environmental Microbiology*,
659 84, e02738-02717, 2018.
- 660 McLaren, R. G. and Cameron, D. K. C.: *Soil science: an introduction to the properties and management of New
661 Zealand soils*, Oxford University Press Auckland 1990.
- 662 Minhas, B., Katoch, P., Singha, A., Minhas, N., and Kaushik, N.: Microbial Metabolites and Their Role to Improve Soil
663 Health, in: *Advancements in Microbial Biotechnology for Soil Health*, Springer, 67-95, 2024.
- 664 Pang, X., Chen, M., Miao, P., Cheng, W., Zhou, Z., Zhang, Y., Zhang, Q., Ye, J., Jia, X., and Wang, H.: Effects of
665 different planting years on soil physicochemical indexes, microbial functional diversity and fruit quality of pear trees,
666 *Agriculture*, 14, 226, 2024.
- 667 Paulson, D. S.: *Nonparametric statistical methods*, 2009.
- 668 Pedrinho, A., Mendes, L. W., de Araujo Pereira, A. P., Araujo, A. S. F., Vaishnav, A., Karpouzas, D. G., and Singh, B.
669 K.: Soil microbial diversity plays an important role in resisting and restoring degraded ecosystems, *Plant and Soil*, 1-
670 25, 2024.
- 671 Qadir, T., Amin, A., Sharma, P. K., Jeelani, I., and Abe, H.: A review on medicinally important heterocyclic
672 compounds, *The Open Medicinal Chemistry Journal*, 16, 2022.
- 673 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F. O.: The SILVA
674 ribosomal RNA gene database project: improved data processing and web-based tools, *Nucleic acids research*, 41,
675 D590-D596, 2012.



- 676 Ramette, A.: Multivariate analyses in microbial ecology, *FEMS microbiology ecology*, 62, 142-160, 2007.
- 677 Ratnayake, R., Seneviratne, G., and Kulasooriya, S.: Effect of soil carbohydrates on nutrient availability in natural
678 forests and cultivated lands in Sri Lanka, *Eurasian Soil Science*, 46, 579-586, 2013.
- 679 Sanguin, H., Sarniguet, A., Gazengel, K., Moëne - Loccoz, Y., and Grundmann, G.: Rhizosphere bacterial
680 communities associated with disease suppressiveness stages of take - all decline in wheat monoculture, *New*
681 *Phytologist*, 184, 694-707, 2009.
- 682 Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., and Huttenhower, C.: Metagenomic
683 biomarker discovery and explanation, *Genome biology*, 12, 1-18, 2011.
- 684 Shen, C., Ni, Y., Liang, W., Wang, J., and Chu, H.: Distinct soil bacterial communities along a small-scale elevational
685 gradient in alpine tundra, *Frontiers in microbiology*, 6, 582, 2015.
- 686 Shi, H., Xu, P., Wu, S., Yu, W., Cheng, Y., Chen, Z., Yang, X., Yu, X., Li, B., and Ding, A.: Analysis of rhizosphere
687 bacterial communities of tobacco resistant and non-resistant to bacterial wilt in different regions, *Scientific Reports*,
688 12, 18309, 2022.
- 689 Sindhu, S. S., Sehrawat, A., and Glick, B. R.: The involvement of organic acids in soil fertility, plant health and
690 environment sustainability, *Archives of Microbiology*, 204, 720, 2022.
- 691 Song, Y., Li, X., Yao, S., Yang, X., and Jiang, X.: Correlations between soil metabolomics and bacterial community
692 structures in the pepper rhizosphere under plastic greenhouse cultivation, *Science of the Total Environment*, 728,
693 138439, 2020.
- 694 Sun, Z. and Han, J.: Effect of soft rock amendment on soil hydraulic parameters and crop performance in Mu Us
695 Sandy Land, China, *Field Crops Research*, 222, 85-93, 2018.
- 696 Vance, E. D., Brookes, P. C., and Jenkinson, D. S.: An extraction method for measuring soil microbial biomass C, *Soil*
697 *biology and Biochemistry*, 19, 703-707, 1987.
- 698 Wang, Z., Shen, Q., Shen, J., Zhu, J., Ma, W., and Yang, Y.: Investigation on soil physical and chemical properties of
699 new cultivated land in Yuhang District and improvement measures, *Zhejiang Agric. Sci*, 58, 1467-1470, 2017.
- 700 Wei, X., Xie, B., Wan, C., Song, R., Zhong, W., Xin, S., and Song, K.: Enhancing soil health and plant growth through
701 microbial fertilizers: Mechanisms, benefits, and sustainable agricultural practices, *Agronomy*, 14, 609, 2024.
- 702 Wu, L., Wen, C., Qin, Y., Yin, H., Tu, Q., Van Nostrand, J. D., Yuan, T., Yuan, M., Deng, Y., and Zhou, J.: Phasing
703 amplicon sequencing on Illumina Miseq for robust environmental microbial community analysis, *BMC microbiology*,
704 15, 1-12, 2015.
- 705 Xue, P.-P., Carrillo, Y., Pino, V., Minasny, B., and McBratney, A. B.: Soil properties drive microbial community
706 structure in a large scale transect in South Eastern Australia, *Scientific reports*, 8, 11725, 2018.
- 707 Yan, H., Liu, J., Huang, H. Q., Tao, B., and Cao, M.: Assessing the consequence of land use change on agricultural
708 productivity in China, *Global and planetary change*, 67, 13-19, 2009.
- 709 Yan, J., Zhang, M., and Wang, D.: Different amendments: Effect on soil physical properties of newly reclaimed land
710 in low hilly region, *Chin. Agric. Sci. Bull.*, 37, 67-73, 2021.



- 711 Yang, T., Jiang, J., Shi, F., Cai, R., Jiang, H., Sheng, L., and He C.: Combination of plant species and water depth
712 enhance soil quality in near-natural restoration of reclaimed wetland, *Ecological Engineering*, 208, 107376, 2024
- 713 Zhang, C., Xue, S., Liu, GB., and Song, Z.: A comparison of soil qualities of different revegetation types in the Loess
714 Plateau, China, *Plant Soil* 347, 163–178, 2011.
- 715 Zhang, X., Yang, Y., Yuan, W., and Ruess, L.: The response of microbial communities to environmental factors in
716 bank soil and river sediment: A case study along the mainstream of the Yangtze River, *Environmental Research*,
717 120903, 2025.
- 718 Zheng, X., Lin, C., Guo, B., Yu, J., Ding, H., Peng, S., Sveen, T. R., and Zhang, Y.: Effects of re-vegetation restoration
719 on soil bacterial community structure in degraded land in subtropical China, *European journal of soil biology*, 98,
720 103184, 2020.
- 721 Zheng, X., Yan, M., Lin, C., Guo, B., Ding, H., Yu, J., Peng, S., Sveen, T. R., and Zhang, Y.: Vegetation restoration types
722 affect soil bacterial community composition and diversity in degraded lands in subtropical of China, *Restoration*
723 *Ecology*, 30, e13494, 2022.
- 724 Zhou, L., Dickinson, R. E., Tian, Y., Fang, J., Li, Q., Kaufmann, R. K., Tucker, C. J., and Myneni, R. B.: Evidence for a
725 significant urbanization effect on climate in China, *Proceedings of the National Academy of Sciences*, 101, 9540-9544,
726 2004.
- 727 Zuo, J., Zu, M., Liu, L., Song, X., and Yuan, Y.: Composition and diversity of bacterial communities in the rhizosphere
728 of the Chinese medicinal herb *Dendrobium*, *BMC Plant Biology*, 21, 1-13, 2021.
- 729