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RESEARCH ARTICLE

Changes in rhizosphere soil bacterial, fungal, and protistan communities during tomato (*Solanum lycopersicum*) growth after reductive soil disinfestation

Yuxin Zhao · Hongkai Liao · Taishan Ran · Hua Yang

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Abstract

Background Reductive soil disinfestation (RSD) is an effective agricultural practice to improve the soil microbial community. However, most RSD research has focused on single bacteria or fungi, little is known about the combined influence on the entire microbiome, particularly impacts on protists and the relationships of these groups linked to plant biomass in rhizosphere soil.

Methods In this study, four treatments, i.e., untreated control (CK), RSD with 1% corn straw (CS), 1% miscanthus (MS), and 1% arundo donax (Ad) were performed.

Results RSD treatment decreased bacterial and fungal community diversity, but increased the diversity of protistan community, along with the relative abundances of Cercozoa and Amoebozoa belonging to phagotrophic protists. The bacterial community diversity rapidly increased with plant growth in the RSD treatment, and we observed that the bacterial community diversity and structure were the major predictors of plant biomass. The RSD treatment had significantly lower relative abundances of potential pathogenic fungi (e.g., Fusarium and Cladosporium) compared to the CK treatment, and the CK treatment showed a dramatic decrease in fungal community diversity. Additionally, RSD treatment increased both bacteria-bacteria and bacteria-protist connections, as reflected by co-occurrence network analysis. The Mantel test demonstrated that soil pH and NO_3^--N contents were intensively correlated with bacterial and protistan community diversity, respectively. Moreover, the Ad treatment had notably higher soil LOC and NO_3^--N contents compared to the CK treatment after 90 days of plant growth.

Conclusion RSD treatment promoted plant biomass by increasing soil nutrient turnover and inhibiting pathogen persistence through affecting more connections among soil microbial communities within the rhizosphere.

Keywords Reductive soil disinfestation · Rhizosphere microbiome · Soil protist community · Plant growth

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Y. Zhao · H. Liao (✉) · T. Ran
Key Laboratory for Information System of Mountainous Areas and Protection of Ecological Environment of Guizhou Province, Guizhou Normal University, Guiyang 550001, People's Republic of China
e-mail: liaohongkai66@163.com

H. Yang (✉)
Department of Geography and Environmental Science, Guizhou Normal University, Guiyang 550025, People's Republic of China
e-mail: huayang113@163.com

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Introduction

Due to limited arable lands and increasing market demands, long-term continuous cropping patterns are frequently adopted in the production of economic crops (Runia and Molendijk 2010; Tilman et al. 2002). Unfortunately, these patterns often result in soil ecological degradation and serious soil-borne diseases (Song et al. 2022; Chen et al. 2020), ultimately leading to stunted plant growth and reduced yields. Tomatoes, a highly efficient and globally consumed crop, are often grown in a continuous mono-cropping pattern for economic benefits and market demands (Fu et al. 2017). Many growers rely on such patterns, prioritizing profits over environmental concerns. Under such circumstances, there is an urgent need for effective and eco-friendly practices to address the issues of continuous cropping obstacles caused by intensive mono-cropping systems. Reductive soil disinfestation (RSD), developed in the Netherlands and Japan (Blok et al. 2000; Shinmura 2000), involves the incorporation of easily decomposable organic amendments in an anaerobic environment. The soil is then saturated with water, covered with impermeable plastic film, and cultured at high temperatures for a shorter period (Momma et al. 2013). RSD has proven successful in growing a variety of crops (Di Gioia et al. 2017; Liao et al. 2021; Liu et al. 2018).

The benefits of RSD can be attributed to the production of antagonists like organic acids and metal ions during the degradation of organic materials (Momma et al. 2006, 2011). These compounds are harmful to pathogenic microorganisms (Cai et al. 2015; Huang et al. 2016), making RSD a superior alternative to ecologically destructive traditional chemical fumigation (Butler et al. 2014; Mowlick et al. 2013; Shennan et al. 2018). In contrast, RSD may have a sustainable suppressive effect on soil-borne pathogens without causing significant ecological damage (Huang et al. 2019a; 2019b). Moreover, RSD has shown promising results in improving soil acidification and secondary salinization (Zhu et al. 2014), selectively recruiting beneficial microbes, and enhancing the metabolic activity and functional diversity of the soil microbial community (Zhao et al. 2020; Huang et al. 2019b). These outcomes contribute to restoring soil health and creating a favorable environment for subsequent plant growth, which is crucial for replanting performance. However, there is a lack of understanding about interactions of soil microbiomes in the rhizosphere of replanted plants after RSD treatment.

As a unique interface connecting plants and soil, the rhizosphere is a dynamic region for complex and diverse interactions within the plant-soil-microbial system (Gkarmiri et al. 2017; Liu et al. 2019). The rhizosphere microbiome is the functional core of the rhizosphere and can carry out active material transformation, which directly affects plant growth and nutrient absorption. Wei et al. (2019) revealed that the composition and function of soil microbiome that host plants initially establish can predict future plant health and disease status. Rhizosphere bacteria and fungi, which are extensively studied microbiomes, are strongly influenced by environmental conditions following RSD treatment (Meng et al. 2019; Zhao et al. 2018). Protists, the most diverse eukaryotic group and an essential component of the rhizosphere microbiome (Geisen et al. 2018; Gao et al. 2019), occupy an important part of microbial food webs as active consumers of bacteria, fungi, and other small eukaryotes, therefore playing a crucial role in nutrient cycling within the rhizosphere (Clarholm 1985; Rønn et al. 2002). Previous studies have revealed that rhizosphere protists are key determinants of plant health and important indicators of plant performance (Xiong et al. 2020; Guo et al. 2021). Therefore, protists are likely non-negligible in the improvement of degraded soil by RSD and in the rhizosphere environment after planting. However, target research on the changing characteristics of the entire microbiome, including bacteria, fungi, and protists during tomato growth, particularly in the case of higher plant biomass after RSD treatment, remains surprisingly scarce.

Various organic materials can affect plant performance after RSD treatment. In this study, we utilized locally available and cost-effective organic waste as the carbon source for RSD. We aimed to (1) understand the dynamic characteristics of the composition, diversity, and structure of rhizosphere bacterial, fungal, and protistan communities of replanted plants after RSD treatment, with a particular focus on the relationships among the three microbial taxa; and (2) investigate the factors that influence enhanced plant biomass resulting from RSD treatment in the rhizosphere environment, including soil microbial communities and physicochemical properties. We hypothesized that RSD treatment before planting would

have a significant positive impact on the rhizosphere, improving the three microbial communities and promoting plant biomass by strengthening the interactions among microorganisms and the associations between biotic and abiotic environments.

Materials and methods

Soil sample collection and organic material preparation

Soil samples were collected from a field in Xiuwen County, Guiyang City, Guizhou Province, southwest China (26°46′42″N, 106°34′55″E), where tomatoes are universally grown. The soil is yellow–brown loam with a history of rotating tomato-rapeseed cultivation for more than five years, and soil-borne diseases such as Fusarium wilt have begun to appear in the past two years. Soil samples were taken from the 0 to 20 cm topsoil layer, following the principle of random multi-point sampling. The collected soil was homogenized and purified by removing stones and plant debris for carbon, nitrogen, and phosphorus determination. The soil had the following properties: pH 6.94, soil organic matter (SOM) 36.78 g kg^{-1}, labile organic carbon (LOC) 1.54 g kg^{-1}, ammonium nitrogen (NH_4^+-N) 0.51 mg kg^{-1}, nitrate nitrogen (NO_3^--N) 15.23 mg kg^{-1}, total phosphorus (TP) 1.10 g kg^{-1}, and Olsen phosphorus (Olsen-P) 20.59 mg kg^{-1}.

The organic materials with different C/N used were agricultural residue corn (Zea mays L.) straw, naturally-grown miscanthus (Miscanthus), and typical waste arundo donax (Arundo donax L.), all collected in the field area around Guiyang City. The three materials were cut and passed through a 2 mm sieve.

Pot experiment design

The soil samples collected from the field were used in a pot experiment, which tomato plants were grown. To normalize the influence of environmental parameters (such as moisture regime and temperature) on the growth conditions of the plants, the experiment was carried out in the greenhouse at Guizhou Normal University, China. Four treatments were conducted for the pot experiment: CK, untreated soil, and in the RSD treatments, 1% (w/w) corn straw (CS, C/N 28.85), 1% (w/w) miscanthus (MS, C/N 56.57), 1% (w/w) arundo donax (Ad, C/N 42.18) were added respectively, and sterile water was irrigated to 100% water holding capacity and sealed well. Each treatment contained three replicates, and involved nine self-sealing bags considering phased sampling. A total of 36 identically self-sealing bags (20 × 30 cm) filled with 1.5 kg of soil, with the corresponding proportion of materials added and evenly mixed, were placed into a constant temperature incubator set at 35 ℃ for 2 weeks. After that, soil samples were collected for determination and then the remaining soil naturally dried for 4 days to about 30% water holding capacity. The soil in each self-sealing bag corresponded to a pot for later planting.

Tomato (Solanum lycopersicum) seeds (Hezuo 903, Changfeng Co., Ltd., Shanghai, China) were treated with a warm soup soaking method to kill pathogens present in the seeds and then placed in a constant temperature incubator (28 ± 1 ℃) for germination. The uniformly grown and germinated seeds were transplanted into ABS pots (12 × 16 × 16.5 cm) filled with treated soil in March 2023. Before planting, the soil was amended with urea and KH_2PO_4 at the rate of 100 mg total N kg^{-1} soil, 50 mg P_2O_5 kg^{-1} soil, and 50 mg K_2O kg^{-1} soil as base fertilizer (Liao et al. 2021). Five tomato seeds were sowed and one healthy seedling was kept per pot after 14 days of stable growth. The day and night periods and temperatures were set at 14 h and 10 h, 21 ℃ and 16 ℃, respectively (Cordovez et al. 2021).

Rhizosphere soil and plant samples were collected at 30, 60, and 90 days during tomato growth. Three pots were required for each sampling in the different treatments, and the sample collected from each pot served as a replicate. The rhizosphere soil was collected using the shaking method, in which soil closely adhering to the plant roots was gently brushed off with a sterile brush, and approximately 5 g was stored in a -80 ℃ refrigerator for determination of rhizosphere soil microorganisms.

Determination of soil physiochemical properties and plant growth indicators

Soil pH was measured in a 1:2.5 (w/v) soil/water ratio using a pH meter (PHS-3E, INASE Scientific Instrument Co., Ltd., Shanghai, China). SOM and LOC were analyzed by KCr_2O_4-$FeSO_4$ external heating method and detected using 0.2 M and 0.1 M $FeSO_4$

solution titration, respectively. Soil NH_4^+-N and NO_3^--N were extracted with 2 M and 1 M KCl at a soil-solution ratio of 1:5 (w/v) on a shaker at 220 rpm at 25 ℃ for 1 h, and an ultraviolet–visible spectrophotometer (Yoke Instrument Co., Ltd., Shanghai, China) was used for determination. TP and Olsen-P were quantified using H_2SO_4-$HClO_4$ digestion. TC and TN contents were analyzed using an elemental analyzer (vario MACRO cube, Element, Hanau, Germany). The LOC content was determined according to Zhou et al. (2019) and Bao (2000) guided the determination of other indicators.

Tomato plant height, root biomass, and shoot biomass were measured using plant samples collected at different stages. The tape measure was used to measure plant height. Plant root and shoot materials were first fixed at 105 ℃ for 30 min, and then dried to constant weight at 70 ℃ to determine the dry weight (plant biomass).

DNA extraction, PCR amplification and Illumina sequencing

Total genomic DNA was extracted from frozen soil samples (0.25 g) using the Power Soil DNA Isolation Kit (MoBio, San Diego, USA) according to the manufacturer's instructions. Genomic DNA concentration and purity were measured using a NanoDrop Spectrophotometer (ThermoScientific, Wilmington, USA). Soil bacteria, fungi and protists were characterized using the primer sets 515F and 907R (515F: 5'-GTG CCAGCMGCCGCGGTAA-3', 907R: 5'-CCGTCA ATTCCTTTGAGTTT-3'), ITS3-2024F and ITS4-2409R (ITS3-2024F: 5'-GCATCGATGAAGAAC GCAGC-3', ITS4-2409R: 5'-TCCTCCGCTTATTGA TATGC-3'), 528F and 706R (528F: 5'-GCGGTA ATTCCAGCTCCAA-3', 706R: 5'-AATCCRAGA ATTTCACCTCT-3') to amplify prokaryotic 16S rRNA gene V4-V5 regions (Zhou et al. 2011), fungal ITS2 region (White et al. 1990), and eukaryotic 18S rRNA gene V4 region (Elwood et al. 1985). Library construction and sequencing were conducted on the Illumina NovaSeq platform by Novogene Bioinformatics Technology Co. Ltd. (Beijing, China). We used the standard operating procedure to analyze the generated sequences in QIIME (Caporaso et al. 2010). Chimeras were checked and filtered using the USEARCH tool with the UCHIME algorithm (Edgar et al. 2011). Each samples were rarefied to a total of 24,359, 110,799, and 91,072 high-quality 16S, ITS, and 18S rRNA gene sequences to ensure an equivalent sequencing depth. The operational taxonomic units (OTUs) were clustered at 18,017 bacterial OTUs, 1650 fungal OTUs, and 2391 protistan OTUs at a 97% similarity threshold. Representative sequences clusters were annotated with the SILVA database, UNITE database, and Protist Ribosomal Reference (PR^2) database respectively. We removed sequences belonging to Rhodophyta, Streptophyta, Metazoa, and Fungi to generate the retained and conservative protistan OTU table for subsequent analysis.

Statistical analysis

The analysis of variance (ANOVA) and T-test with SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) were used to analyze statistical significance of the differences in the obtained data between the RSD and CK rhizosphere soils. For alpha diversity, the filtered OTU table was calculated based on the "vegan" package and visualized with the "ggplot" package of R (version 4.2.0). For beta diversity, Permutational multivariate analysis of variance (PERMANOVA) was used for principal coordinate analysis (PCoA) based on the Bray–Curtis distance to test the differences in microbial community structure among treatments. The relationships of biotic factors (including soil bacterial, fungal, and protistan communities and their respective abundant taxa) and soil physicochemical properties as well as plant growth parameters were assessed by the Mantel test (based on the Bray–Curtis dissimilarity matrices). The co-occurrence network analysis among microbial taxa at the genus level with a total relative abundances greater than 0.5%, and the correlation with Spearman correlation coefficients $|r| \geq 0.7$ and $p \leq 0.05$ were retained, eventually visualized in Cytoscape software (version 3.9.1).

Results

Differences in soil physicochemical properties and plant growth parameters

The RSD treatment initially significantly increased soil pH, which later decreased to about 7 as the plants grew. Additionally, the RSD treatment resulted in a notable increase in SOM, LOC, and NH_4^+-N contents, while significantly reducing the NO_3^--N content

compared to the CK treatment. On day 90 of plant growth, the Ad treatment showed significantly higher levels of LOC and NO_3^--N contents compared to the CK treatment. Throughout each stage, the Olsen-P content was consistently lower in the RSD treatment than in the CK treatment. However, no significant difference was observed in the TP content, except for a decrease on the 90th day of plant growth Table 1.

Tomato plant growth progress was monitored by measuring the biomass and height of the plants at each stage. It was observed that the plants grown with the RSD treatment had a significantly higher biomass compared to those with the CK treatment after 30 days of growth. However, on day 90 of plant growth, the CK treatment showed the tallest plant height, and among the RSD treatments, the Ad treatment had the largest root, shoot, and total biomass, which was significantly greater than that of the CS and MS treatments (Fig. 1).

Composition of soil microbial communities at phylum and genus levels

Overall, the RSD treatment markedly modulated the soil microbial community compositions at the phylum and genus levels compared with the CK treatment. Proteobacteria consistently constituted the most abundant phylum throughout the growth stage. The relative abundances of Gemmatimonadota and Chloroflexi dramatically increased in both the CK and Ad treatments compared with the 30th and 90th day of plant growth, while the relative abundances of Bacteroidota and Firmicutes in the Ad treatment were higher than those in the CK treatment (Fig. 2a). Compared with the 30th day of plant growth, the relative abundance of Gemmatimonas in the Ad treatment increased by 73.85% on the 90th day of plant growth, correspondingly, the relative abundance of Flavisolibacter decreased in both treatments. The genera Sphingobium and Altererythrobacter had higher relative abundances in the Ad treatment. The relative abundances of UC_Vicinamibacteraceae, Sphingomonas, and UC_SC-I-84 were significantly lower in the Ad treatment compared to the CK treatment (Fig. 2d).

Regarding the fungal communities, Ascomycota was consistently the most abundant phylum. The relative abundance of Chytridiomycota significantly increased in the CK treatment but dramatically

Table 1 Effects of RSD compared with CK on soil physicochemical properties at different growth stages. Different lowercase letters in a column indicate significant differences among treatments ($P < 0.05$). Stage 0, after RSD treatment; stage 1, the plant grows for 30 days after transplantation; stage 2, the plant grows for 60 days after transplantation; stage 3, the plant grows for 90 days after transplantation

		pH	SOM (g kg^{-1})	LOC (g kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	TP (g kg^{-1})	Olsen-P (mg kg^{-1})
stage 0	CK	6.33 ± 0.07b	34.79 ± 1.24b	1.73 ± 0.03a	6.69 ± 4.58b	47.15 ± 19.87a	1.20 ± 0.01a	23.49 ± 2.07a
	CS	7.25 ± 0.02a	39.83 ± 1.21a	1.73 ± 0.12a	27.34 ± 1.67a	0.81 ± 0.22b	1.20 ± 0.04a	21.48 ± 0.89ab
	MS	7.20 ± 0.00a	37.31 ± 1.28ab	1.68 ± 0.04a	24.75 ± 3.09a	2.20 ± 0.55b	1.14 ± 0.02a	17.51 ± 0.85bc
	Ad	7.32 ± 0.02a	37.57 ± 0.50ab	1.74 ± 0.12a	27.01 ± 5.35a	3.22 ± 0.91b	1.17 ± 0.06a	15.43 ± 1.71c
stage 1	CK	7.34 ± 0.04c	34.18 ± 0.18b	1.63 ± 0.10a	2.10 ± 0.57b	63.51 ± 0.70a	1.17 ± 0.03a	22.05 ± 0.97a
	CS	7.64 ± 0.01a	37.63 ± 0.41a	1.91 ± 0.10a	2.79 ± 0.06ab	1.09 ± 0.25c	1.22 ± 0.01a	12.29 ± 1.25b
	MS	7.57 ± 0.04a	37.17 ± 1.06a	1.86 ± 0.08a	4.26 ± 1.00a	2.41 ± 0.95c	1.00 ± 0.01b	14.93 ± 2.09b
	Ad	7.46 ± 0.01b	37.44 ± 0.84a	1.83 ± 0.18a	2.04 ± 0.25b	29.72 ± 0.58b	1.21 ± 0.01a	13.05 ± 0.67b
stage 2	CK	7.43 ± 0.10a	36.62 ± 0.58b	1.73 ± 0.11a	0.85 ± 0.38a	28.43 ± 15.37a	1.20 ± 0.03a	17.37 ± 0.98a
	CS	7.41 ± 0.03a	39.66 ± 1.08a	1.88 ± 0.12a	1.05 ± 0.05a	4.66 ± 0.94a	1.18 ± 0.04a	10.47 ± 0.06b
	MS	7.27 ± 0.01ab	39.61 ± 0.49a	1.93 ± 0.07a	1.63 ± 0.43a	2.44 ± 0.47a	1.16 ± 0.03a	12.67 ± 0.27b
	Ad	7.21 ± 0.00b	38.83 ± 0.48ab	1.82 ± 0.07a	1.12 ± 0.29a	1.85 ± 0.24a	1.17 ± 0.06a	12.16 ± 0.99b
stage 3	CK	7.06 ± 0.01a	35.04 ± 0.41c	1.99 ± 0.07b	1.70 ± 0.28b	0.68 ± 0.13b	0.93 ± 0.02a	26.57 ± 3.62a
	CS	7.02 ± 0.02ab	39.42 ± 0.37a	2.23 ± 0.12ab	2.39 ± 0.16a	1.39 ± 0.28ab	0.87 ± 0.06a	15.11 ± 1.22b
	MS	7.00 ± 0.01b	39.08 ± 0.48a	2.47 ± 0.07a	1.49 ± 0.09b	1.17 ± 0.62ab	0.94 ± 0.00a	15.97 ± 1.27b
	Ad	7.00 ± 0.02b	36.55 ± 0.56b	2.34 ± 0.03a	1.62 ± 0.09b	2.00 ± 0.29a	0.92 ± 0.01a	12.56 ± 0.64b

Different lowercase letters indicate significant differences among treatments ($P < 0.05$)

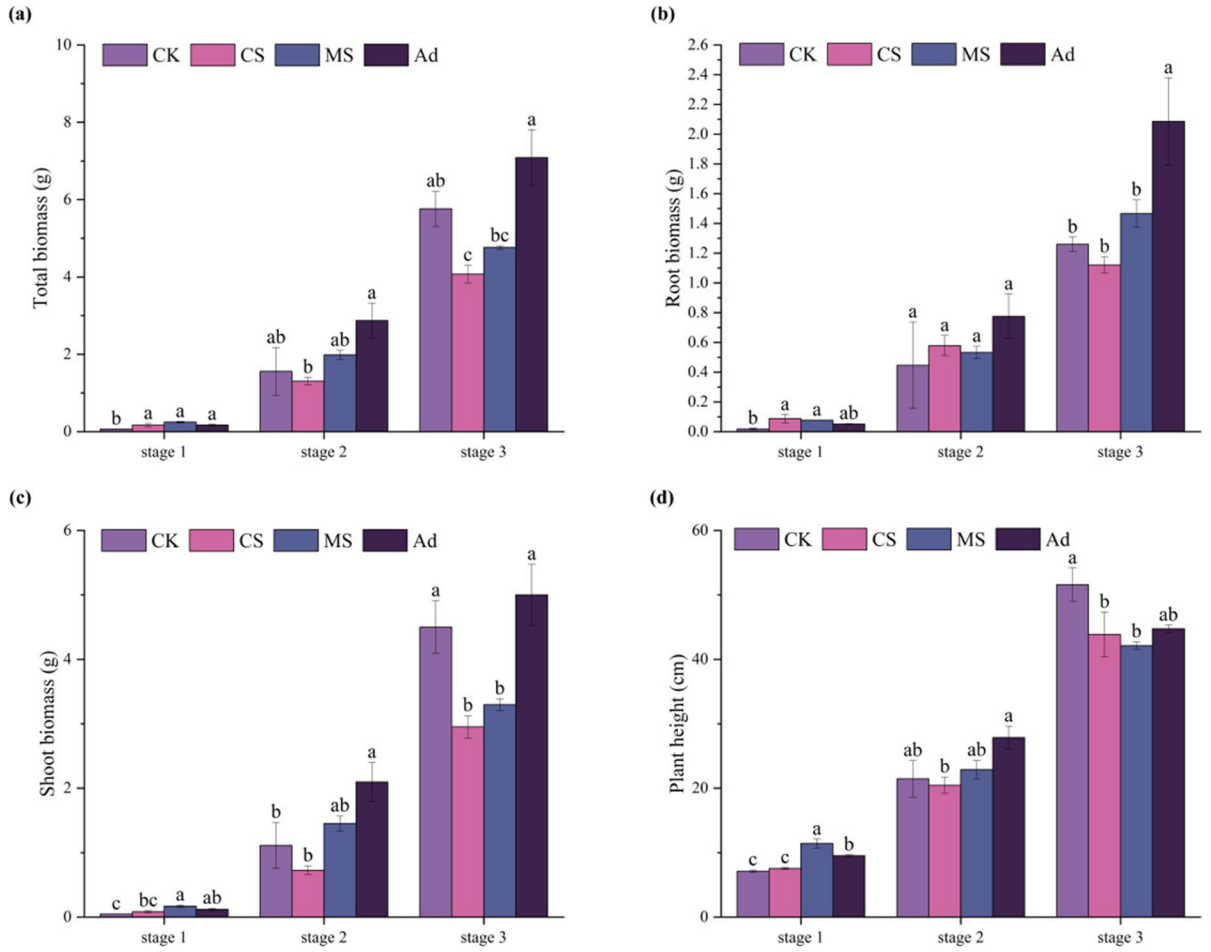

Fig. 1 Effects of RSD on shoot biomass (**a**), root biomass (**b**), total biomass (**c**), and plant height (**d**) compared with CK at different growth stages. Different lowercase letters indicate significant differences among treatments at the same stage at $P < 0.05$. Stage 0, after RSD treatment; stage 1, the plant grows for 30 days after transplantation; stage 2, the plant grows for 60 days after transplantation; stage 3, the plant grows for 90 days after transplantation

decreased in the Ad treatment with plant growth (Fig. 2b). The dominant genus in the Ad treatment was UC_Podosporaceae, which was significantly higher than in the CK treatment. The Ad treatment decreased the relative abundances of Fusarium and Cladosporium, which remained significantly lower than the CK treatment during the 90 days of plant growth. On the 90th day of plant growth, the relative abundances of Podospora and Schizothecium significantly increased by 18.53% and 10.29% respectively in the Ad treatment compared with the 30th day (Fig. 2e).

Within the protistan community, the relative abundances of Cercozoa, Ciliophora, and Amoebozoa increased by 57.84%, 107.45%, and 37.46%, respectively, while the relative abundances of Ochrophyta and Diatomea decreased by 39.26% and 85.25% on the 90th day compared with the 30th day of plant growth. The Ad treatment had higher relative abundances of Cercozoa, Amoebozoa, Ochrophyta, SAR, Gracilipodida, and Heterolobosea, while the relative abundances of Chlorophyta, Diatomea, and Apicomplexa were significantly higher in the CK treatment (Fig. 2c). At the genus level, the relative abundances of UC_Chlorophyceae and UC_Trebouxiophyceae in the CK treatment were significantly higher than that in the Ad treatment. The relative abundances of Heteromita, Vermamoeba, Colpoda, and Stylonychia

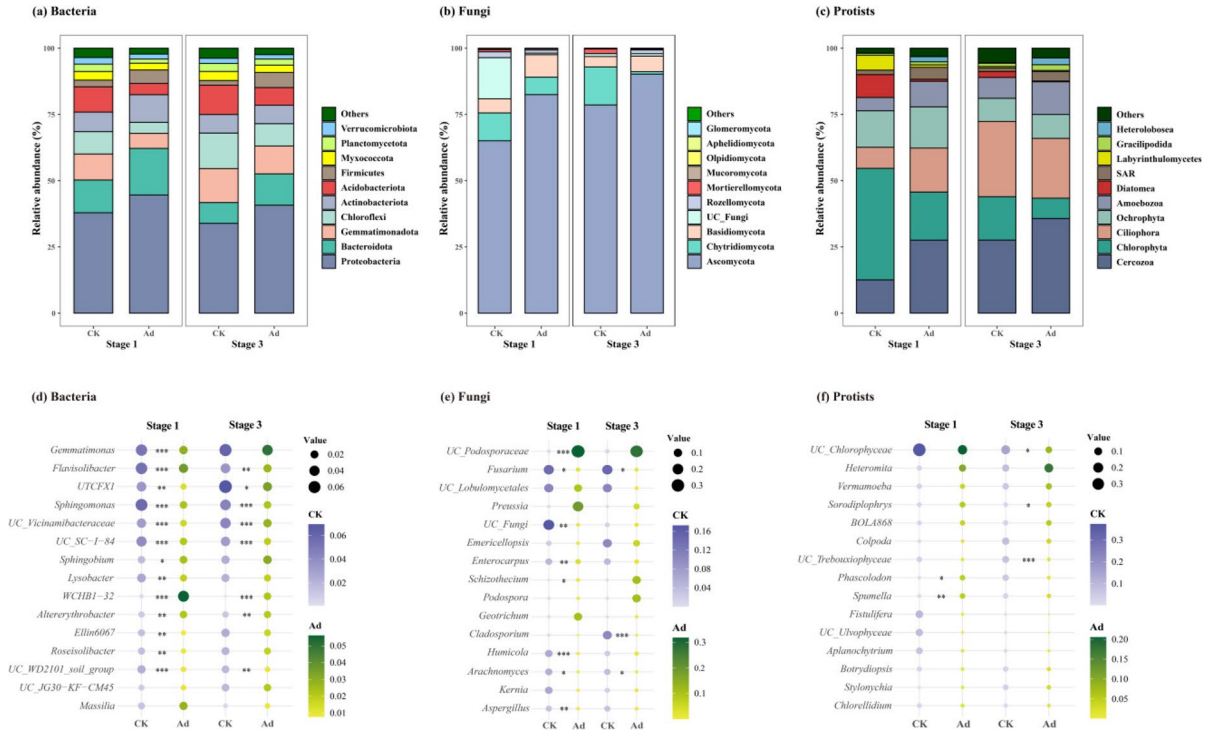

Fig. 2 Relative abundances of soil microbial communities at phylum (**a**-**c**) and genus (**d**-**f**) levels under the early and late stages between CK and Ad treatments. The significance level is *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

significantly increased in the Ad treatment. The relative abundances of Phascolodon and Spumella increased in the CK treatment, with no significant differences between both treatments (Fig. 2f).

Microbial diversity and community structure

The observed richness, Shannon diversity, and evenness of the bacterial community in the CK treatment were significantly higher than those in the Ad treatment. However, only these indices of the protistan community increased in the Ad treatment. With plant growth, the diversity and evenness indices of the bacterial community increased in both treatments and improved rapidly in the Ad treatment, but all indices of the fungal community notably decreased mainly in the CK treatment (Fig. 3).

There were differences in the bacterial, fungal, and protistan community structures between the CK and Ad treatments during tomato growth (Fig. 4a-c). The Ad treatment significantly affected the community structure of bacteria and protists (bacteria: $R^2=0.452$, $P=0.003$; protists: $R^2=0.170$, $P=0.027$; fungi: $R^2=0.152$, $P=0.059$) (Fig. 4d). Plant growth had significant effects on the community structure of the three microbial taxa (Fig. 4e; Table S2). The importance of diversity and structure of three groups on plant biomass were analyzed by multiple linear regression. The results showed that bacterial community diversity and structure were the major microbial parameters explaining plant biomass across all groups during plant growth (Fig. 4f; Table S3). In contrast, the diversity and structure of neither the fungal nor the protistan communities were significantly associated with biomass.

Correlation between microbial communities and physicochemical properties

The correlations among microbial communities, physicochemical factors, and plant growth parameters were analyzed by the Mantel test. Results showed that soil pH, TP, and NO_3^--N contents were negatively correlated with total biomass and plant height,

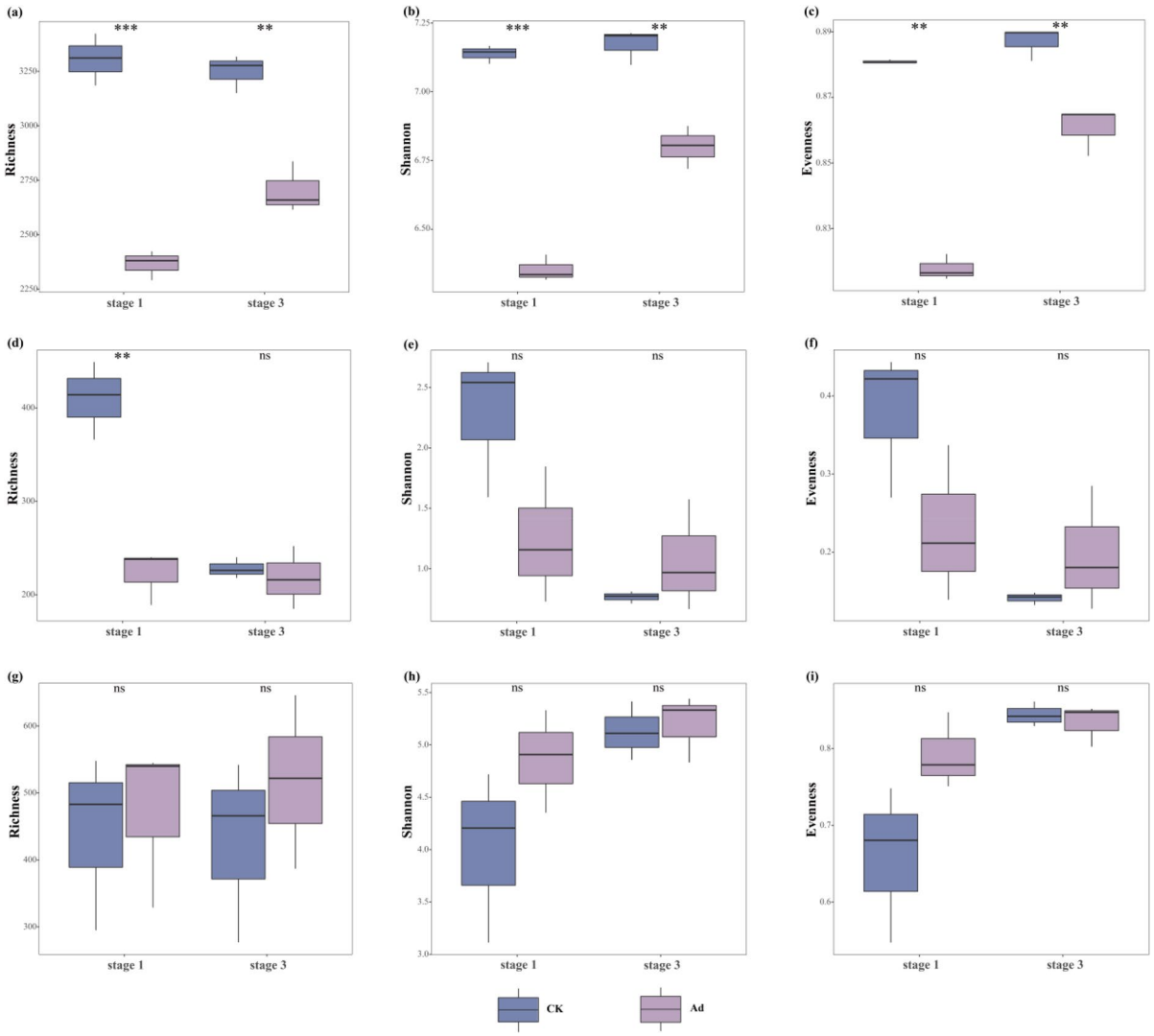

Fig. 3 Richness, diversity, and evenness of soil bacterial (**a**-**c**), fungal (**d**-**f**), and protistan (**g**-**i**) communities under the early and late stages between CK and Ad treatments

while LOC content was positively correlated with plant biomass. The diversity of protists and protistan-abundant taxa showed a significant correlation with the NO_3^--N content. The bacterial community diversity and structure had a significant positive correlation with soil pH, and the bacterial community structure was positively correlated with plant height and several physicochemical factors, such as pH, SOM, TP, and Olsen-P content. The relative abundance of potential pathogenic fungi Fusarium was negatively correlated with plant biomass (Fig. S1, S2)

Co-occurrence networks of microbial communities

Co-occurrence patterns of bacterial, fungal, and protistan taxa at the genus level were explored based on strong and significant relationships. We observed 2613 bacteria-bacteria connections and 1352 (67.16%) cross-group connections between bacterial and protistan taxa, 473 (23.50%) bacteria-fungi connections, and 188 (9.34%) protist-fungi connections in the Ad treatment. There were 1305 (57.79%) bacteria-protist connections and 2480 bacteria-bacteria

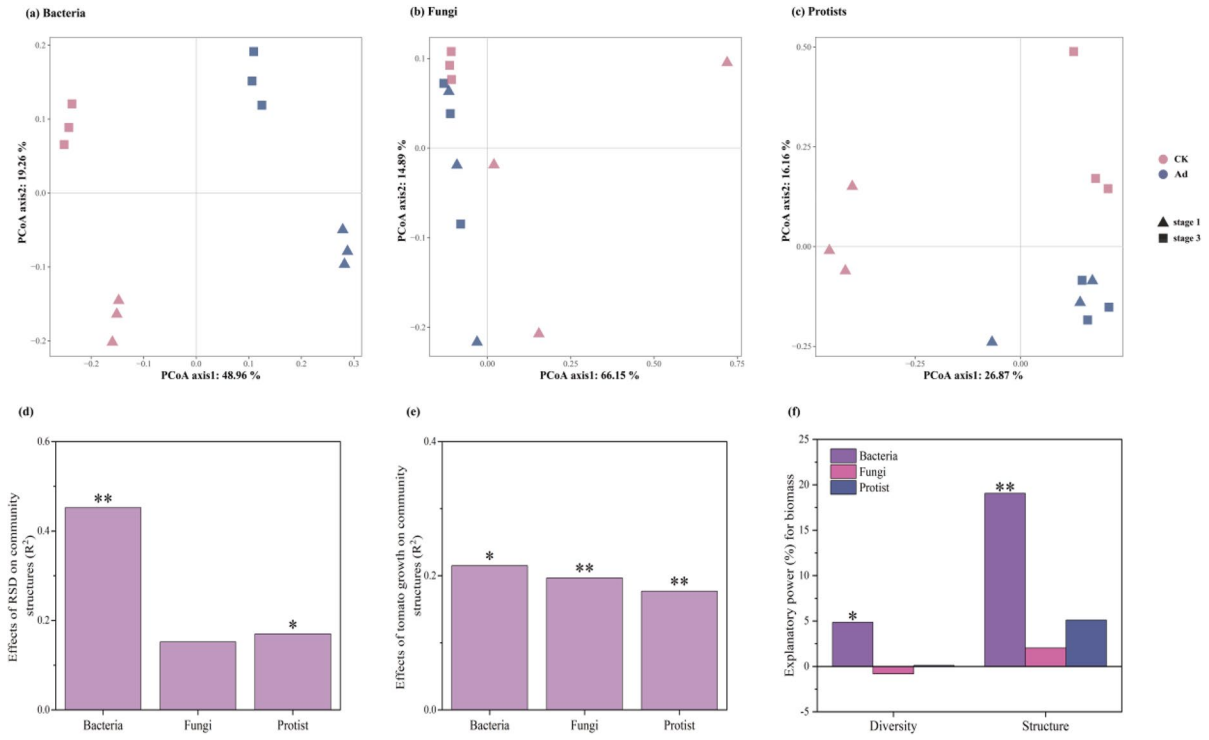

Fig. 4 The PCoA plot of soil bacterial (**a**), fungal (**b**), and protistan (**c**) communities under the early and late stages based on bray distance. The overall effects of RSD (**d**) and plant growth (**e**) on distinct microbial groups based on PERMANOVA analysis. The relative importance of bacterial, fungal, and protistan diversity and community structure for plant biomass (**f**). In panel (**f**), statistical significance for explanatory power is calculated by multiple regression using linear models

connections observed in the CK treatment, which were less than in the Ad treatment (Fig. 5).

Discussion

In this study, we examined the physicochemical properties and microbial characteristics of the tomato rhizosphere soil in the RSD treatment at the 30th and 90th days, and observed distinct changes compared to the CK treatment. After 30 days of plant growth, the bacterial and fungal communities in the RSD treatment exhibited low levels of richness, diversity, and evenness, presumably due to the degradability and carbon composition of organic materials. The addition of refractory organic materials to the RSD treatment appeared to stimulate only a handful of bacterial and fungal taxa, resulting in the inability to maintain the high α-diversity of these communities (Huang et al. 2019b; Zhao et al. 2018). Meanwhile, the soil disinfestation process is mainly mediated by anaerobic microorganisms in the RSD method, and the enrichment of characteristic microorganisms may also have contributed to the reduction of microbial community diversity (Meng et al. 2019). In detail, Firmicutes with higher relative abundance in the RSD treatment can inhibit the growth of soil pathogenic microorganisms by producing organic acids (Tan et al. 2019) and antibiotics (Xiong et al. 2015) during the degradation of organic materials. The root system did not seem to play a significant role in the planting period of a month, as suggested by lower root biomass and underdeveloped structure. Thus, it is reasonable to conclude that the observed alteration in microbial community diversity could be the result of pre-planting RSD treatment.

The RSD method had a significant impact on the soil bacterial community, mainly manifested as an initial decrease but a rapid increase in established bacterial populations in plant rhizosphere soil of 90 days. These results indicated that although the

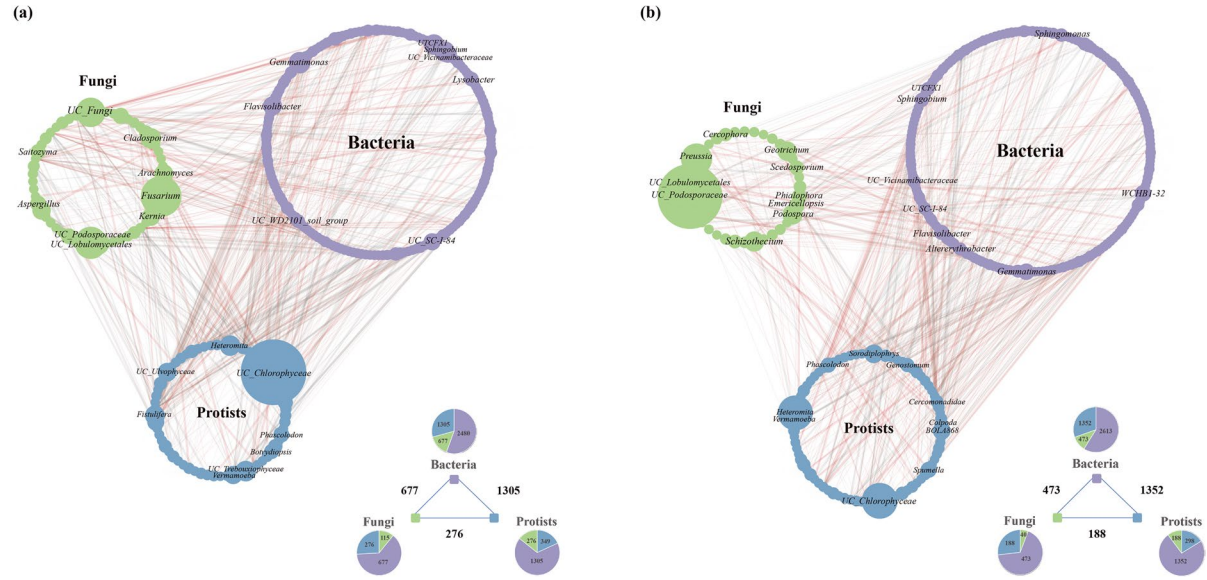

Fig. 5 Co-occurrence networks of bacteria, fungi, and protists in the rhizosphere environment of CK (**a**) and Ad (**b**). Only cross-group interactions among microbial groups are shown. A connection indicates a strong and significant Spearman correlation, divided into positive (red) or negative (grey) edges. Each node represents the selected groups whose relative abundance is ≥ 0.005 at the genus level. The size of the nodes is a reflection of the relative abundance of the genus. The thickness of the edge is a reflection of Spearman correlation coefficients

anaerobic environment and the degradation of organic materials associated with RSD treatment led to a decrease in bacterial community diversity after incubation, this decrease would gradually recover after planting to a certain extent. In addition to the role of RSD treatment, plants also have an important effect on increasing bacterial community diversity. Our findings suggest that bacterial community succession was powerfully driven by the cultivation of tomato plants, which is in line with previous studies (Huang et al. 2019b; Meng et al. 2019; Liu et al. 2018). Generally, plants are capable of filtering certain microbial groups by releasing specific root exudates to shape the microbial seed banks, which promote the growth of beneficial microorganisms, thus enhancing nutrient cycling and inhibiting pathogen survival (Chaparro et al. 2013; Haichar et al. 2008; Liu et al. 2018). Previous studies have shown that rhizosphere bacteria have the ability to colonize developing root systems and compete with other soil microbial populations, upon reintroduction to the vegetative parts of plants (Kloepper et al. 1999). This could explain the increasing diversity of the bacterial community we observed as plants grew.

In addition to the direct disturbance of the soil bacterial community by the inherent characteristics of the RSD method and plant rhizosphere effects, it is also related to improvements in soil physicochemical properties, indicated by the Mantel test. Soil environmental factors disturbed by RSD treatment, which potentially exert a significant influence on nutrient availability and create a suitable niche for microorganisms, are capable of regulating the rhizosphere microbial community, either directly or indirectly by manipulating the host plant (Barriuso et al. 2008; Prashar et al. 2014). The anaerobic environment of RSD treatment significantly increased soil pH, which was later reduced probably because of the effect of tomato rhizosphere acid secretions (Tan et al. 2013). The result that the diversity and structure of the bacterial community were positively correlated with soil pH, as revealed by the Mantel test, could be due to the alteration of the soil's acid–base chemistry caused by the degradation of organic materials added to the RSD treatment. Generally, soil pH is considered a primary factor influencing soil bacterial composition and microbial community structure (Fierer and Jackson 2006; Lauber et al. 2009). The alteration of soil pH influences nutrient availability and

can interfere with nutrient uptake (Li et al. 2016a, b; Huang et al. 2018). It also affects microbial community activity, ultimately impacting overall ecosystem function. Bacteria are typically more sensitive to pH (Rousk et al. 2009) and there is higher bacterial community diversity in neutral soil (Fierer and Jackson 2006; Lauber et al. 2009). Therefore, the neutral pH bias in our study could have contributed to increased bacterial community diversity.

The changes in bacterial community in response to RSD treatment were the strongest and most noteworthy in the microbiome. Our results showed that bacterial community diversity and structure contributed greatly to plant biomass. Remarkable enhancement of rhizosphere soil bacterial community diversity in the RSD treatment, which might have the duration of the legacy effect on potentially benefit the development of soil microorganisms. The bacterial community is influenced by the external environment, in turn, it is also linked to a multitude of crucial functions of agroecosystems underlying carbon fixation, mineralization of soil organic matter, and maintenance of plant hormone balance (Berendsen et al. 2012). All these effects are beneficial to plant growth, eventually contributing to higher biomass and better performance of the plant. It is unlikely that a single role of a separate species in the rhizosphere microbiome displays multiple functions to meet the demands of plant growth, while most bacterial aggregations can possess traits that benefit plants such as pathogen inhibition and plant growth promotion (Agaras et al. 2015). Interestingly, studies have reported that bacterial strains may cover up to 15% of the root surface (Van 2006). Hence bacteria are the most abundant microorganisms in the rhizosphere, they are bound to influence the plant in a significant manner. We observed that the relative abundance of potentially beneficial genus Gemmatimonas, which considerably increased after three months of planting in the RSD treatment, could be significant for promoting plant growth and enhancing nutrient absorption (Chee-Sanford et al. 2019). The co-occurrence network indicated that the number of connections among bacteria taxa increased in the RSD treatment. This phenomenon reflected that RSD has the ability to promote the formation of more intensive and stable bacterial community networks with plant growth. There may be an overall correlation between the changes in RSD treatment environment, bacterial community, and plant biomass in a large system.

Soil microbial communities rapidly respond to changes in the RSD environment due to the imposition of anoxia and the input of readily available carbon source. We observed a significant increase in the relative abundance of characteristic (e.g., anaerobic, material degrading) bacterial taxa, such as Proteobacteria, Bacteroidota, Actinobacteriota, and Firmicutes. Among these taxa, Bacteroidota is involved in cellulose degradation in carbon-rich RSD-treated soil, affecting the metabolism of nitrogen, phosphorus, and protein and playing a vital role in regulating plant growth (Yang et al. 2020). Proteobacteria are fast-growing symbiotic bacteria that thrive in carbon-rich environments (Fierer et al. 2007), making them more likely to exploit the abundant carbon sources present in RSD. The increased LOC content may be related to the role of these taxa in carbon source degradation. The LOC, as a highly effective part of soil organic carbon, is easily decomposed and utilized by soil microorganisms (Liao et al. 2012). Li et al. (2016a, b) demonstrated that the decomposition of carbon from straw application contributes to the active organic carbon pool. The observed significant positive correlation may prove that LOC is beneficial for enhancing plant biomass. Proteobacteria and Firmicutes play an essential role in nitrogen fixation, included in the nitrogen cycle process (Ren et al. 2018), and these taxa with higher relative abundance in the RSD treatment may be important for higher nitrogen availability. Members of the genus Sphingobium, belonging to the phylum Bacteroidota, are commonly known as rhizosphere microorganisms with positive effects on plants. Sphingobium participates in the nitrogen cycle and some Sphingobium species can fix nitrogen and convert it into ammonia or nitrite available to plants, which helps to provide the nitrogen source needed by plants (Zou et al. 2023). The increase in the relative abundance of these beneficial bacterial taxa may contribute to the higher NO_3^--N content of the Ad treatment with the addition of higher C/N arundo donax material at 90 days of plant growth.

In addition to the prevalence of microbial taxa involved in organic materials degradation and the cycle of carbon and nitrogen nutrients, RSD treatment increased the relative abundance of microbial groups that could effectively inhibit pathogens and decreased the enrichment of potential pathogens. This includes the phylum Firmicutes, previously mentioned as potential disease-suppressive bacteria, and

Altererythrobacter (which belongs to Proteobacteria), a potential novel biocontrol agent against various fungal pathogens. Its abundance negatively correlated with the incidence of tomato Fusarium wilt (Tang et al. 2023). Podospora, a beneficial fungal genus that helps in plant growth, was observed to control Verticillium wilt infections in tomatoes as demonstrated in studies by Xu et al. (2012) and Dutta (1981). The higher relative abundance of Podospora in the RSD treatment may have an inhibitory effect on pathogens during tomato growth, while Fusarium, a potentially harmful fungal pathogen, was significantly less abundant in the RSD treatment. This may be due to anaerobic degradation of organic carbon sources, which produces organic acids and reducing compounds (e.g., NH_3, H_2S) that inhibit pathogen survival (Butler et al. 2012; Huang et al. 2019b; Momma et al. 2013). Importantly, the relative abundance of Fusarium have a significant negative correlation with biomass in the Mantel test. As a member of the genus Cladosporium, Cladosporium fulvum, the causal agent of tomato leaf mold, is a destructive fungal disease that poses a serious threat to tomato yield and quality, causing significant economic losses, especially in protected cultivation (Griffiths et al. 2018). In our study, Cladosporium showed a remarkably higher relative abundance in the CK treatment, causing characteristic symptoms such as curled and withered leaves of tomato plants. The RSD treatment mitigated the serious threat of these potential pathogens to tomato performance.

Geisen et al. (2018) classified protists into four functional groups: phototrophy, phagotrophy, symbiosis, and saprotrophy. It was found that the relative abundances of phagotrophic protists, such as Cercozoa and Amoebozoa, increased with plant growth, and these predatory groups were mostly present in the RSD treatment. Additionally, Chlorophyta, Diatomea, and Apicomplexa, which are photosynthetic protists and parasitic protists, were more prevalent in the CK treatment than in the RSD treatment. Cercozoa, one of the most abundant soil protistan groups globally (Oliverio et al. 2020; Dumack et al. 2020), reduces the abundance of pathogenic bacteria by preying on them (Sleigh 1991), and also promotes plant growth through interaction with other microorganisms (Guo et al. 2021). RSD anaerobic conditions are formed by flooding, where the soil is maintained at its maximum water-holding capacity. Protistan populations in soil are mainly regulated by the availability of water and food (Geisen et al. 2014). As protists essentially depend on the water layer connecting soil pores to move, feed, and multiply, the habitat size of these organisms will increase or shrink with changing soil water content (Ritz and Young 2011). Therefore, the higher α-diversity indices of the protistan community were observed in the RSD treatment with higher habitat space and connectivity. Previous studies demonstrated that the protistan community was more shaped by long-term fertilization than water management (Murase et al. 2014), and protists served as the most sensitive bio-indicators of soil nitrogen fertilizer application (Zhao et al. 2019). The significant changes in nitrogen content affected by RSD treatment may have impacted the protistan community diversity with more complex factors described in the Mantel test for the correlation between protistan community diversity and nitrate nitrogen content.

Another reason for the increased protistan community diversity after RSD treatment could be related to their predatory effect on bacterial taxa. Previous studies have shown that plants or their associated rhizosphere bacterial communities are responsible for shaping the formation of protists, rather than protistan grazing pressure affecting bacterial communities (Jousset and Bonkowski 2010; Saleem et al. 2012). Most protists feed on bacteria rather than nutrition for a living (Geisen et al. 2016), with abundant phagotrophic protists such as Cercozoa and Amoebozoa are crucial to reducing bacterial community diversity and inhibiting pathogens in the RSD treatment. These taxa are likely to facilitate microbial activities by preying on soil microorganisms and releasing nutrients from microbial biomass which acting as a source of plant nutrients, thereby contributing to nutrient flow (Bonkowski 2004). Xiong et al. (2020) have confirmed that phagotrophic protists differ between later healthy and diseased plants, and predator–prey interactions may have a negative impact on pathogens and reduce their reproduction. Therefore, predatory protists which are plentiful in the RSD treatment may regulate the biomass, activity, and structure of other microbial communities, indicating their potentially powerful impact on microbial-driven ecosystem functions (Li et al. 2021), such as suppressing pathogen reproduction and managing plant biomass, ultimately affecting plant performance.

Our study further revealed an interesting trend from the co-occurrence network in the RSD treatment, where the most abundant association across trophic levels was observed between bacteria and protists. Such findings highlight the considerable top-down effect of protists on the bacterial community, as bacteria are the most prevalent and dominant prey for the protistan community (Geisen et al. 2016). Moreover, bacterivorous protists engage in symbiotic relationships with bacteria in terms of nutrient cycling and environmental adaptation (Gast et al. 2009; Nguyen et al. 2023). It is therefore plausible that more of the connections may be caused by predatory protists, which could shape an environment that benefits plants by preying on harmful microorganisms and promoting plant-beneficial microbes through species-specific protistan feeding differences. The interactions between rhizosphere soil bacteria and protists were strengthened by plant growth after RSD treatment, which might produce a combination of effects including enhancing nutrient turnover and manipulating plant hormone balance (Jousset 2017; Guo et al. 2021), and these interactions may have potentially positive effects on the presence of higher plant biomass in the RSD treatment.

Conclusions

In this study, we present the first comprehensive characterization of bacterial, fungal, and protistan community dynamics in the RSD-treated rhizosphere soil of plants as they undergo the tomato growing process in a greenhouse. Our results showed that the pre-planting RSD treatment enhanced plant performance compared to the CK treatment and different outcomes were observed over time. The bacterial diversity was significantly affected by RSD treatment, initially decreased but rapidly increased with plant growth. The protistan diversity showed a consistent increase throughout the study. Moreover, we found that RSD treatment increased the connections within bacterial communities and between bacteria-protist communities. Bacterial community diversity and structure were important microbial parameters in explaining plant biomass. Additionally, phagotrophic protists which has the function of preying on bacteria may also potentially affect plant biomass. The RSD treatment significantly reduced the relative abundances of some potentially pathogenic fungi compared to the CK treatment. The dynamic characteristics of rhizosphere bacterial, fungal, and protistan communities played an essential role in increasing plant biomass in the RSD treatment. Further research should focus on related functions of the entire microbiome linked to plant productivity.

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Data availability The datasets generated during and analyzed during the current study are available in the NCBI Sequence Read Archive (SRA) database (accession number: PRJNA1004424).

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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