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Dynamic alterations and ecological implications of rice rhizosphere bacterial communities induced by an insect-transmitted reovirus across space and time

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Abstract

Background Cereal diseases caused by insect-transmitted viruses are challenging to forecast and control because of their intermittent outbreak patterns, which are usually attributed to increased population densities of vector insects due to cereal crop rotations and indiscriminate use of pesticides, and lack of resistance in commercial varieties. Root microbiomes are known to significantly affect plant health, but there are significant knowledge gaps concerning epidemics of cereal virus diseases at the microbiome-wide scale under a variety of environmental and biological factors.

Results Here, we characterize the diversity and composition of rice (*Oryza sativa*) root-associated bacterial communities after infection by an insect-transmitted reovirus, rice black-streaked dwarf virus (RBSDV, genus *Fijivirus*, family Spinareoviridae), by sequencing the bacterial 16S rRNA gene amplified fragments from 1240 samples collected at a consecutive 3-year field experiment. The disease incidences gradually decreased from 2017 to 2019 in both Langfang (LF) and Kaifeng (KF). BRSDV infection significantly impacted the bacterial community in the rice rhizosphere, but this effect was highly susceptible to both the rice-intrinsic and external conditions. A greater correlation between the bacterial community in the rice rhizosphere and those in the root endosphere was found after virus infection, implying a potential relationship between the rice-intrinsic conditions and the rhizosphere bacterial community. The discrepant metabolites in rhizosphere soil were strongly and significantly correlated with the variation of rhizosphere bacterial communities. Glycerophosphates, amino acids, steroid esters, and triterpenoids were the metabolites most closely associated with the bacterial communities, and they mainly linked to the taxa of Proteobacteria, especially Rhodocyclaceae, Burkholderiaceae, and Xanthomonadales. In addition, the greenhouse pot experiments demonstrated that bulk soil microbiota significantly influenced the rhizosphere and endosphere communities and also regulated the RBSDV-mediated variation of rhizosphere bacterial communities.

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Conclusions Overall, this study reveals unprecedented spatiotemporal dynamics in rhizosphere bacterial communities triggered by RBSDV infection with potential implications for disease intermittent outbreaks. The finding has promising implications for future studies exploring virus-mediated plant-microbiome interactions.

Keywords Rice microbiome, Reovirus, Amplicon sequencing, Metabolomics, Spatiotemporal heterogeneity, Intermittent epidemics

Background

Cereal diseases caused by insect-transmitted viruses often occur intermittently or one after another in major rice-growing countries, and some of these diseases have worsened with the adoption of modern agricultural techniques [1, 2]. Like vector-borne diseases of humans and domestic animals, epidemics of rice viral diseases typically involves in multiple grains and variable environments, resulting in challenges to predict the start and duration of an outbreak [3, 4]. Rice black-streaked dwarf virus (RBSDV, genus *Fijivirus*, family Spinareoviridae), a reovirus transmitted by the small brown planthopper (SBPH, *Laodelphax striatellus* Fallén), naturally infects rice, maize, wheat, barley, and some gramineous weeds [5, 6]. Since its first major outbreak in Japan in maize in 1957, several outbreaks have been recorded in rice, maize, or wheat at various times in the mid-1960s, 1975–1976, 1991–2002, 2008–2009, and 2013–2016 [5–9]. The disease outbreaks have usually been attributed to a variety of environmental and biological factors including the indiscriminate use of pesticides and introduction of rice-wheat rotation system, which greatly increase the population density of the insect vectors and the probability of its overwintering, as well as lack of resistance in commercial varieties [1, 5, 6]. However, other important factors such as the plant microbiomes, which can significantly influence plant development, health, and productivity [10, 11], have been mostly ignored.

Generally, plant roots comingle with diverse microbial mutualists, pathogens, and commensals [12–14]. The spatial resolution of the study distinguished two root-associated compartments, the endosphere (root interior) and rhizosphere (soil close to the root surface), each of which was found to harbor a distinct microbiome [15, 16]. During root development, numerous biological and external environmental factors, such as the crop species and cultivar (e.g., host genotype), cropping rotation system, climate, and soil type, influence the abundance and composition of microbial communities in different niches [17–19]. In irrigated rice, root microbial communities are affected by geographical location, soil source, host genotype, and cultivation practice [15]. Moreover, the temporal population dynamics of microbial communities associated with rice roots is considerably conserved, despite undergoing an initial rapid turnover during early vegetative stages of the plant, which subsequently transitions into a relative stabilization of the communities as the host progresses into its reproductive stage [20, 21]. Evidence is accumulating that plant infected by various soil-borne fungal, bacterial, and viral pathogens can alter the abundance, composition, and function of the soil and root microbes compared to healthy plants [22–24]. However, there are significant knowledge gaps concerning the dynamic alterations of root microbiomes under various environmental and biological factors.

The root microbiota plays a significant role in promoting plant growth and enhancing resistance against both biotic and abiotic stresses [25, 26]. Consequently, the constitution of the associated microbial community exerts a discernible influence on the overall plant phenotype [27]. Some disease-resistant tomato and common bean varieties are associated with an enriched set of bacterial species in the rhizosphere to suppress pathogen infection [28, 29]. Infection by soil-borne pathogenic bacteria and fungi can also activate a "cry for help" strategy in a plant by inducing the emission of volatile organic compounds or modifying the synthesis and secretion of particularly root exudates, thus recruiting specific beneficial soil microbes that contribute to plant defense [10, 26, 30, 31]. These beneficial microbes might also accumulate to generate disease-suppressive soils and a "soil-borne legacy" that enhances survival rates of plant offspring [32, 33]. Beneficial keystone microbes in the rhizosphere and plant microbiome can also contribute to plant disease suppression by excreting antibiotic compounds, competing with the pathogen for resources and priming the plant immune system based on the plant's recognition of microbe-associated molecular patterns [34–36]. Plant root exudates and metabolites in rhizosphere soil also stimulate, enrich, and support soil microorganisms as the first line of defense against soil-borne pathogens [37]. Selected rhizosphere metabolites have been demonstrated as prebiotics to reduce the effect of the plant pathogen *Ralstonia* [38]. Interestingly, l-malic acid is secreted by the roots after infection by the foliar pathogen *Pseudomonas syringae* pv. tomato and recruits the beneficial *Bacillus subtilis* FB17, resulting in a stronger immune response to the pathogen [39]. However, most

of our knowledge in this area is from the studies of soil-borne fungal and bacterial pathogens [40, 41]. The impact of plant diseases caused by insect-transmitted viruses on root microbiomes in complex environmental conditions has been largely overlooked.

Given that RBSDV infection initiates aboveground and subsequently induces systemic infection, leading to diverse molecular, physiological, and morphological changes in rice plants [6, 42, 43], we hypothesized that these changes alter the rhizosphere microbiome, which in turn would affect plant growth, resistance, and disease epidemics. Here, we comprehensively characterized the dynamics of rice rhizosphere bacterial communities in response to RBSDV infection during 3 consecutive years under various environmental and biotic factors to answer the following questions: Does systemic viral infection from aboveground plant parts alter the diversity and composition of microbiota in the rice rhizosphere under complex environmental and biotic factors? If so, what is the main driver for the changes? Is there a correlation between changes in the soil and root bacterial community dynamics and disease occurrence? The answers to these questions will provide new insights into the reasons that contribute to outbreaks of insect-transmitted virus diseases.

Materials and methods

Field experiment (2017–2019)

The experiment was carried out simultaneously in a field in Xiaogang Village, Henan province, China (114.51°E,

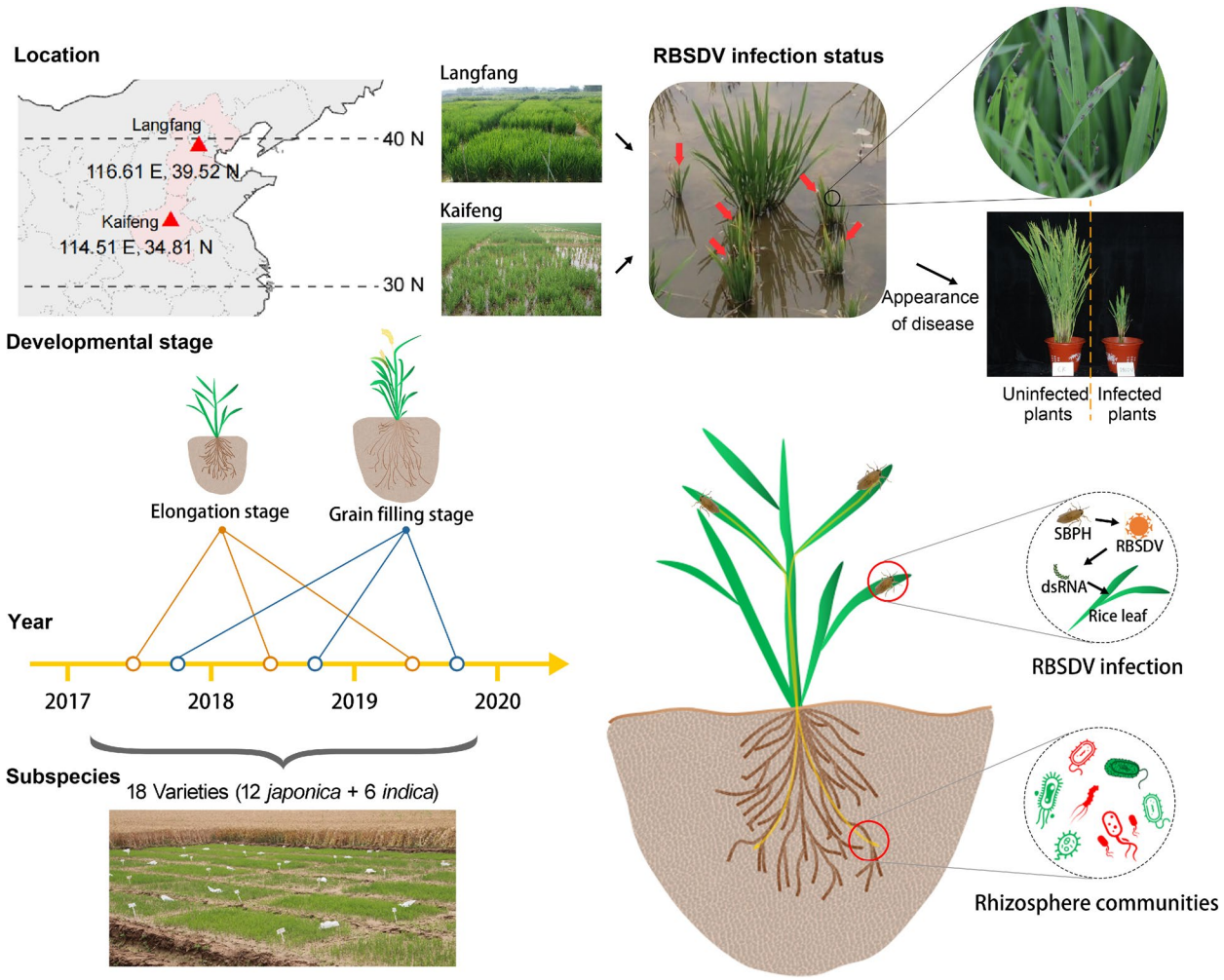

Fig. 1 Overview of field experiments in two locations during 2017 to 2019. A total of 18 varieties were selected, including 12 *japonica* rice and 6 *indica* rice. Each year consisted of two sampling stages, the elongation stage, and the grain filling stage. According to the appearance of the plants, the health status of rice plants was initially determined. RBSDV-infected and -uninfected plants were collected, and rhizosphere soil was collected for microbial community detection. RT-qPCR was used to confirm the presence of RBSDV in rice plants in the laboratory to verify the infection status

34.81°N) and a field at the Langfang experimental farm, Hebei province, China (116.61°E, 39.52°N) (Fig. 1). Physicochemical properties of the bulk soil at the two fields are described in Table S1. Five environmental and biotic factors were assessed in the field experiment: location (Kaifeng/Langfang), year (2017/2018/2019), and developmental stage (elongation stage/grain filling stage), subspecies (*indica*/*japonica*), and RBSDV infection status of rice plants. Six varieties of *O. sativa* subsp. *indica* and 12 of subsp. *japonica* were used in the field experiment (Table S2). Each variety was evaluated in the same field in Kaifeng in 2014 for incidence of rice black-streaked dwarf disease (RBSDD) (from 13.0 to 93.7% depending on the variety) [44]. The cropping system in the Kaifeng field is rice-wheat rotation but rice monoculture in the Langfang field. The field was divided 2×3 m plots, and one rice variety was randomly selected for transplanting into three plots with one seedling in each hole to facilitate disease assessment and sampling. Each hole is spaced 15 cm apart, and meanwhile, each plot is spaced 20 cm apart. One plot was left fallow in each field each year as the blank soil control. During the experimental period, the fields were irrigated once a week; fertilizer and herbicides were not applied, but weeds were pulled by hand at regular intervals.

Prior to sowing, the seeds of each variety were soaked in 10% v/v sodium hypochlorite for 30 min and washed with sterilized water at least 3 times and then directly sown into the nursery in Xiaogang Village in mid-May. Because of frequent RBSDD occurrences at Kaifeng since 2013, seedlings in the nursery are expected to be naturally infected by RBSDV during feeding by viruliferous SBPHs that are abundant in the surrounding wheat fields [44]. About 1 month later, seedlings were transplanted to the fields in Kaifeng and in Langfang. The whole field was designed and divided into many plots according to the size of 2 m×3 m, and one variety rice was planted into three plots. Two-hundred rice plants in the elongation stage were randomly selected from plots of each variety, and the incidence of RBSDD was determined by individually identifying and recording symptomatic plants in the field. The typical symptoms used for identification included severe stunting and dark green leaf coloration of plant. Diseased plants were verified by RT-PCR to further detect the presence of RBSDV. The calculation method for disease incidence involved counting the total number of plants in a specific field plot at first and then determining the proportion of infected plants to the total plant count. For RT-PCR, total RNA of rice leaves was extracted using the Total RNA Extraction Reagent (Vazyme, Nanjing, China) as instructed. First-strand cDNA was then generated using the M-MLV kit (Promega, Madison, WI, USA) for RBSDV-S10 fragment amplification and the 2×M5 HiPer plus Taq HiFi PCR mix (Mei5bio, Beijing, China) as described by Tian et al. [45]. From 2017 to 2019, the disease incidence in both Kaifeng and Langfang gradually decreased (Figure S1). Entire rice plants and the attached soil were collected from the fields at the elongation stage (early August, ~50 days after transplanting) and grain filling stage (mid to late September, ~90 days after transplanting) each year from 2017 to 2019. First, a circle with a radius of ~20 cm was marked around the plant as the center point, and then a spade was pushed 30 cm vertically downward around the circumference of circle to avoid damaging the roots. The entire plant and attached soil were lifted from the soil and placed in a plastic ice bag and transported as soon as possible to the laboratory for RBSDV detection and collecting the rhizosphere soil and roots.

Pot experiment in greenhouse (2020)

Soil collected from the same field in Langfang was used in the greenhouse pot experiment at our institute in Beijing. The soil was passed through a 20-mesh sieve and then thoroughly mixed. Half of the soil was autoclaved three times at 121 °C for 15 min and used for the sterile soil treatment; the other half was used in its natural state for the natural soil treatment. Two consecutive batches were grown in pots filled with same soil type but in two different conditions: sterilized and non-sterilized. Before starting the second batch, the sterile soil was autoclaved again, but the natural soil was left untreated. Husked seeds of a commercial *japonica* rice cv. Zhendao 99 were sown. SBPHs were raised in our lab for more than 10 years in chamber (16-h light/8-h dark photoperiod at temperatures of 28 °C during the day and 25 °C at night) and let them acquire RBSDV from RBSDV-infected rice raised in greenhouse. After feeding on RBSDV-infected rice for 2 days, then we transferred SBPH to healthy rice seedlings for at least 15 days to complete the latent period to become viruliferous. Then, the rice seedlings (10 days) for inoculation were exposed to one viruliferous SBPH in a device that prevents the SBPH from escaping in greenhouse maintained at 30 °C during the day and 28 °C at night, with a relative humidity of 60%, and finally were observed by symptoms and tested by RT-PCR for RBSDV as described above after 45 days. Then, 10 pots with either infected or uninfected plants were inverted to remove the entire plant and all soil. Any soil loosely clinging to the roots was carefully removed with a gently shake and sterilized tweezers to avoid damaging roots. The rhizosphere soil was collected, and DNA was extracted as described below.

Sampling and DNA extraction

After detection of RBSDV infection status by RT-PCR, the rhizosphere soil and root endosphere of each plant were collected with minor modifications following Edwards et al. [15]. At least three replicate plants at each developmental stage were collected for each variety in each treatment at each field for further 16S rRNA amplicon sequencing. Briefly, the excess soil was manually shaken from the roots, leaving approximately 1 mm of soil attached to the roots. Then, the roots with rhizosphere soil were placed in a sterile flask with 50 mL of 0.01M sterile PBS solution and shaken on a horizontal shaker at 180 rpm for 15 min to thoroughly remove the attached rhizosphere soil from roots. The suspension was poured into a 50-mL Falcon tube and then centrifuged at 7000 rpm for 5 min, after that the supernatant was discarded and pellet soil was stored as the rhizosphere soil at 4 °C until DNA extraction the same day. DNA was extracted from 0.25 g of the precipitated rhizosphere soil using the DNeasy PowerSoil Pro Kit (QIAGEN, Dusseldorf, Germany) and dissolved in 100-μL ddH_2O for further 16S rRNA sequencing or stored at −80 °C. After removing the rhizosphere soil, the rice roots were gently brushed with a sterilized brush to eliminate any remaining soil. These roots served as the sample for extracting endophytic bacteria. The root was homogenized before the DNA extraction by bead beating for 1 min in FastPrep-24™ Classic bead beating grinder and lysis system (MP, CA, USA). The DNA for each sample was then extracted using the DNeasy PowerSoil Pro Kit and eluted in 100 μL of elution buffer.

16S rRNA amplicon sequencing and data preprocessing

16S rRNA amplicons were sequenced using paired-end (2×250 bp) sequencing and the HiSeq 2500 of Illumina platform (Illumina, San Diego, CA., USA) by Biomarker Technologies (Beijing, China). Primers 335F (5′-CAD ACT CCT ACG GGA GGC-3′) and 769R (5′-ATC CTG TTT GMT MCC CVC RC-3′) were used to amplify a fragment of the 16S rRNA gene V3–V4 region from the rice rhizosphere and endosphere bacterial DNA samples. The 50-μL reaction mixture contained 10-μL buffer, 0.2-μL Q5 high-fidelity DNA polymerase, 10-μL high GC enhancer, 1-μL dNTP, 10 μM of each primer, and 60-ng DNA. Thermal cycling conditions were initial denaturation at 95 °C for 5 min; 15 cycles at 95 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min; and final extension at 72 °C for 7 min. The PCR products were purified using VAHTS™ DNA Clean Beads (Vazyme). For the second-round PCR, the 40-μL reaction volume contained 20 μL 2×Phusion HF MM (NEB), 8-μL ddH_2O, 1 μL and 10 μM of each primer, and 10-μL PCR products from the first step. Thermal cycling conditions were initial denaturation at 98 °C for 30 s; 10 cycles at 98 °C for 10 s, 65 °C for 30 s, and 72 °C for 30 s; and final extension at 72 °C for 5 min. All PCR products were quantified using Quant-iT™ dsDNA HS reagent (Thermo Fisher Scientific, Waltham, MA, USA) and pooled together. The preprocessing of raw data was performed using the QIIME2 platform based on the DADA2 method, including quality control, paired-end read merging, denoising, ASVs (amplicon sequence variants) generation, and taxonomic annotation [46–51]. Ultimately, sequence counts were normalized to a uniform depth of 11,000 for all samples, reducing bias due to varying sequencing depths.

Extraction and profiling of the metabolites in rhizosphere soil

Gas chromatography-mass spectrometer (GC-MS) and liquid chromatography-mass spectrometer (LC-MS) were used to identify the compounds in the metabolites in rice rhizosphere soil in this study [52, 53]. Rice plants (cv. Zhengdao 99) were collected at the elongation stage in 2018 from the Langfang field, including three biological replicates each of infected and uninfected plants. The rhizosphere soil was collected from each plant using a small sterile brush. For GC–MS, 1 g of soil was placed in a 1.5-mL Eppendorf tube, to which 20 μL of internal standard matter (2-chloro-D-phenylalanine, 0.3 mg/mL dissolved in methanol) was combined with 1-mL 50% aqueous methanol. The mixture was frozen and ground and then freeze-dried and vacuum-concentrated and dried. Methoxylamine hydrochloride (80 μL) in pyridine (15 mg/mL) was then added, and the mixture was vortexed 2 min and incubated at 37 °C for 90 min. After the addition of 80 μL of *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) (with 1% trimethylchlorosilane) and 20 μL of *n*-hexane, the mixture was vortexed for 2 min, heated at 70 °C for 60 min, and then cooled at room temperature for 30 min before GC-MS analysis [52]. The derivatized samples were analyzed using an Agilent 7890B gas chromatograph system coupled to an Agilent 5977A MSD mass detector (Agilent Technologies Inc., Palo Alto, CA, USA). For the LC-MS, 1 g of the rhizosphere soil samples was ground, homogenized, extracted, and derivatized using the methods of the previous study [54]. The ACQUITY UPLC I-Class System (Waters Corp., Milford, MA, USA) coupled with the Xevo G2-XS QTOF mass spectrometer was equipped with heated electrospray ionization (ESI) source (Thermo Fisher Scientific) and ACQUITY UPLC BEH C18 column (1.7 μm, 2.1×100 mm), which were employed in both positive and negative modes to analyze the metabolic profile. The flow rate was 0.4 mL/min, and column temperature was 45 °C. Distilled water and acetonitrile–methanol (2:3 v/v), both containing 0.1% v/v formic acid, were used respectively

as mobile phases A and B. The injection volume was 2 µL. The resulting text files were exported to the data server with absolute spectra intensities and further processed with a filtering algorithm in the metabolomics BinBase database [54]. All our entries in BinBase were then matched to spectra in the Fiehn mass spectral library (https://fiehnlab.ucdavis.edu/). Data were normalized as described by Fiehn et al. using "total metabolite content" [55].

Statistical analyses and data visualization

The Shannon and Chao1 indices were calculated using mothur v1.30 software (https://mothur.org/wiki/download_mothur/) for the bacterial communities of the rhizosphere and root endosphere samples collected from all locations and years based on their ASVs profiles [56], then the R packages (version 3.6.1) ggplot2 (version 3.3.5) and ggsignif (version 0.6.3) [57] were used to determine statistically significant differences ($p < 0.05$) with the options "test = 'wilcox.test'" under the influence of location, year, development stage, subspecies, and RBSDV infection.

Using QIIME software [58], we compared the dissimilarity of bacterial communities in samples from different factors described above. The R package vegan (version 2.5–6, https://github.com/vegandevs/vegan) for a principal coordinate analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA or Adonis) based on the Bray-Curtis matrix of bacterial communities at the ASVs level were used to explain the contribution of different factors to variations in rhizosphere and endosphere communities in samples from the 3 years of field and 2 batches of greenhouse experiments. The R^2 value is the total amount of variation in the response (here, microbial community dissimilarity) explained by the factor being tested [59]. Statistical significance for the variation between combinations was determined by considering a p-value less than 0.05.

Linear discriminant analysis (LDA) effect size (LEfSe) analysis was applied to identify biomarkers that differed in abundance among the samples from infected and uninfected plants [60]. Using the LEfSe function in the online software Galaxy/Hutlab (http://huttenhower.sph.harvard.edu/galaxy/), we set the LDA threshold value to 2 and p-value to 0.05 to indicate significant biomarkers. To clarify the effect of RBSDV infection on the relative abundance of abundant and rare bacteria, we counted abundant genera (relative abundance > 1%) and rare genera (relative abundance < 0.1%) in all 1075 rhizosphere samples according to previous methods [61].

To assess the influence of RBSDV infection status, planting location, year, developmental stage, and rice subspecies on rhizosphere bacterial communities, we employed a linear-mixed model (LMM). This statistical approach allowed us to calculate the significance of the relationship between the α-diversity indexes or compositional characteristics and the five factors mentioned above. For this analysis, we utilized the function lmer from the R package lme4 (version 1.1–34) with the definition option [62].

To investigate the correlation between the rice rhizosphere bacterial communities, as determined by ASVs profiles, and the rhizosphere metabolites, based on metabolite abundance profiles, the Mantel function was also used for studies on correlation between results for rhizosphere and root endosphere [62]. A permutation test was performed using the Adonis function in the same R package to determine the statistical significance ($p < 0.05$) of the correlation [63]. This analysis allowed us to assess whether there was a significant relationship between the composition of the bacterial communities in the rhizosphere and the composition of root exudates.

An orthogonal partial least-squares discrimination analysis (OPLS-DA) was used to discriminate the overall difference of the metabolites in rhizosphere between samples from infected and uninfected plants based on the metabolite abundance profile in the R package mixOmics (version 6.8.5). Intergroup variation was distinguished from the first axis, and intragroup variation was shown by the orthogonal principal components [64]. To identify differential metabolites between infected and uninfected samples, we combined single-dimensional and multidimensional analyses based on the metabolite profile. The variable importance in projection (VIP) from the OPLS-DA and the p-value from a t-test comparing samples from infected and uninfected plants were used to determine any significant differences in the differential metabolites between the samples from infected and uninfected plants ($VIP > 1$ and $p < 0.05$). The main differential metabolites between samples from infected and uninfected plants were visualized as a heatmap using the R package pheatmap (version 1.0.12) [65]. All known compounds in the GC-MS and LC-MS profiles were modularized using weighted correlation network analysis (WGCNA) [66]. The analysis was performed using the R package WGCNA (version 1.70–3) with the option "min Module Size = 50," resulting in 3236 compounds divided into 20 modules.

Results

In total, 1075 samples of rhizosphere soil, and 165 samples of root endosphere, were collected from the two experimental field plots. Likewise, 148 samples of both rhizosphere soil and root endosphere were obtained from the greenhouse experiment. The rhizosphere soil or root endosphere of uninfected and infected samples was used

for amplicon sequencing to study bacterial communities. The disease incidence exhibited a consistent decline across three consecutive years of field experiments (Figure S1).

Multiple factors contributed to variations in rice rhizosphere bacterial communities

Rhizosphere samples were categorized by location (Kaifeng/Langfang), year (2017/2018/2019), subspecies (*indica*/*japonica*), developmental stage (elongation stage/grain filling stage), and health condition (RBSDV-infected/-uninfected plants) (Fig. 1). The influences of the factors on the Shannon diversity of bacterial communities were measured and found that location (Kaifeng > Langfang; difference test $p < 0.05$, below), year (2017 > 2018 > 2019), subspecies (*indica* > *japonica*), and developmental stage (elongation stage > grain filling stage) all affected the α-diversity (Fig. 2A; Table S3). The impact was evaluated through a linear mixed model (LMM) analysis. Among the five factors considered, the analysis revealed that location ($p < 2.20e\text{-}16$) and year ($p < 2.20e\text{-}16$) exhibited the most pronounced influence on Shannon diversity, followed by developmental stage ($p = 2.59e\text{-}10$), health condition ($p = 3.06e\text{-}06$), and subspecies ($p = 1.35e\text{-}03$) (Table S4). In addition, the effects of the five factors on the composition dissimilarity of the rhizosphere bacterial community was analyzed using PCoA, PerMANOVA, and LMM based on a Bray–Curtis matrix. All factors exhibited significant effects on the composition of the rhizosphere bacterial communities, ranked from the greatest to the least contribution as above (Fig. 2B; Tables S5, S6). These results imply that it is crucial to consider a variety of environmental and biotic factors for a more accurate understanding of the interaction between RBSDV infection and the rhizosphere bacterial community.

RBSDV infection triggered high temporal and locational heterogeneity in the alterations of the rhizosphere bacterial communities

The LMM analysis showed that the influence of RBSDV infection on α-diversity (Shannon index) remained significant despite the inclusion of multiple confounding factors, including location and year. However, establishing a consistent pattern of alterations in rhizosphere bacterial communities after RBSDV infection proves to be challenging because of the diverse responses exhibited by the communities following RBSDV infection across different times and locations (Figs. 2C, D, E, F and S2; Table S8). PCoA and PerMANOVA showed that RBSDV infection had a highly significant effect ($p = 0.001$) on the composition of bacterial communities in the rice rhizosphere in all combinations of factors (rhizosphere samples were divided into 12 combinations, i.e., 2 locations × 3 years × 2 developmental stages), and samples were categorized as two clusters in each combination based on the presence or absence of RBSDV infection (Figs. 2G, H and S3). Thus, RBSDV infection affected the composition of the rhizosphere bacterial community across the locations, years, and developmental stages investigated, but the degree of influence varied with time and location. For example, the impacts of RBSDV infection on the rhizosphere community decreased with rice growth and development because R^2 was always less for the grain filling stage than for the elongation stage (Fig. 2G, H). Remarkably, changes in the α-diversity of rhizosphere samples from RBSDV-infected plants were highly heterogeneous from 2017 to 2019, even at different developmental stages in the same year, as indicated by the Shannon index (Figs. 2C, D, E, F and S2). Seven of the eight combinations (i.e., 2 locations × 2 developmental stages × 2 subspecies) showed interannual changes in the Shannon indices after RBSDV infection (Table S8). For example, the infected *japonica* samples at the elongation stage from Kaifeng had a significantly higher Shannon index than the uninfected samples in 2017 (Fig. 2C1), but not in 2018 (Fig. 2C2), while the index was significantly higher for the uninfected samples in 2019 (Fig. 2C3). Similarly, changes in α-diversity of communities following RBSDV infection also varied among locations. For example, the Shannon indices were not significantly different between samples from infected and uninfected *japonica* plants at the elongation stage at Kaifeng in 2018 (Fig. 2C2), but the index was significantly higher in samples from the

(See figure on next page.)

Fig. 2 RBSDV infection caused heterogeneous effects on rice rhizosphere communities among various environmental and biotic factors. **A** Results of *t*-test to compare Shannon indices for significant differences among communities by A1 RBSDV infection (infected and uninfected), A2 location (Kaifeng and Langfang), A3 year (2017, 2018, 2019), A4 developmental stage (elongation and grain filling), and A5 subspecies (*indica* and *japonica*). **B** Principal coordinate analysis (PCoA) with Bray-Curtis distance showing the differences in the rhizosphere bacterial communities under the five factors. Significant differences ($p < 0.05$) among communities by factor were determined using the permutational multivariate analysis of variance (PERMANOVA). **C**, **D**, **E**, **F** *T*-test was used to compare Shannon indices of rhizosphere bacterial communities for significant differences between RBSDV-infected and -uninfected *japonica* and *indica* rice plants at elongation stage by location and year. **G**, **H** PERMANOVA results based on Bray-Curtis distance matrices demonstrate the effects of RBSDV infection on bacterial communities in the rice rhizosphere, highlighting changes across different planting years and developmental stages. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS $p > 0.05$

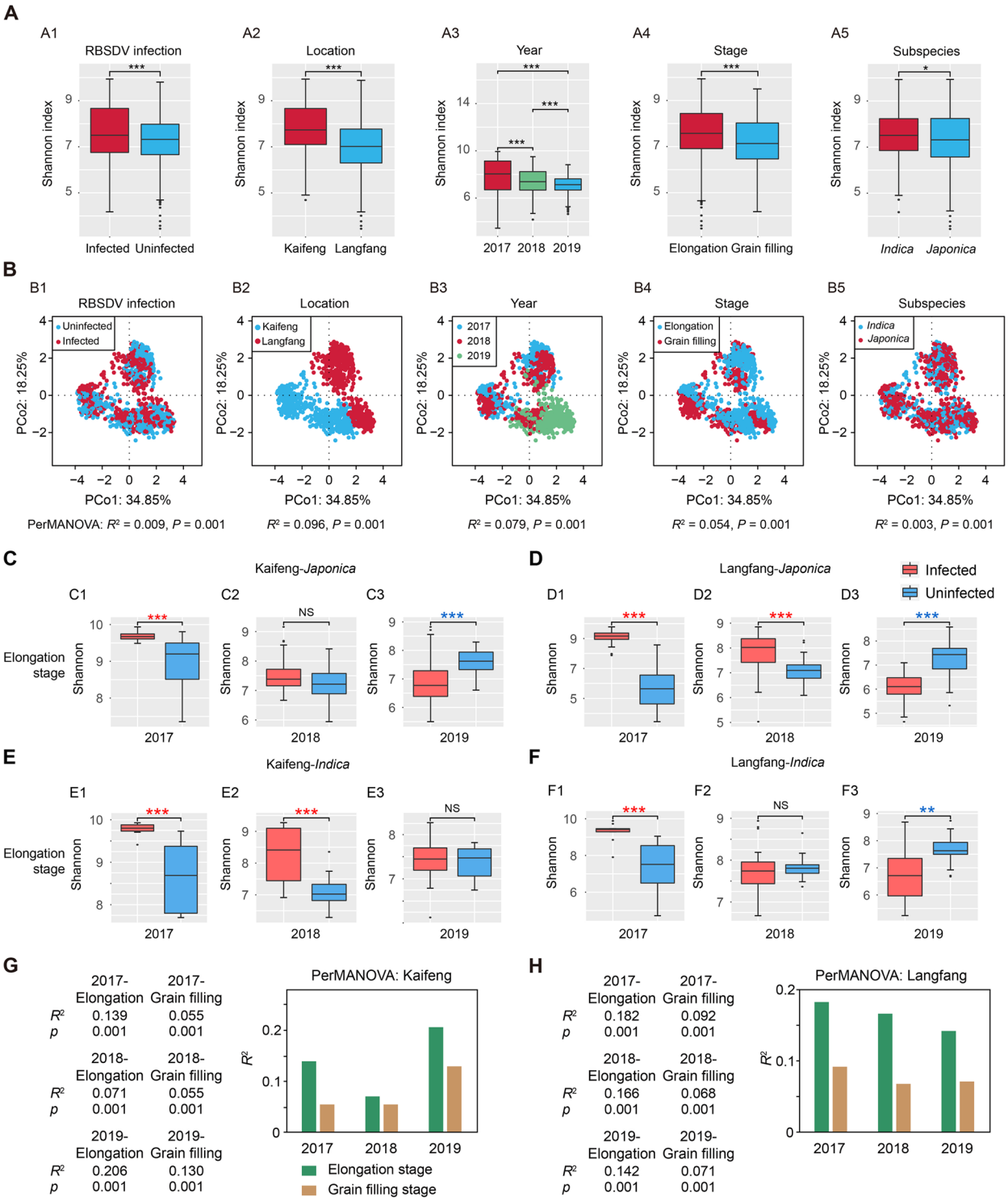

Fig. 2 (See legend on previous page.)

infected plants than from uninfected plants at Langfang in the same year (Fig. 2D2). All these results demonstrate that RBSDV infection on rice rhizosphere communities is susceptible to both rice-intrinsic and external conditions. Consequently, a cross-sectional study falls short in comprehensively unraveling the RBSDV-mediated plant-microbiome interactions.

Alterations in the diversity of rice rhizosphere communities in response to RBSDV infection showed "opposite characteristics" in 2017 and 2019

Interestingly, at the elongation stage in 2017, a significant increase in Shannon indices was observed for infected *indica* and *japonica* rhizosphere samples, in contrast to all uninfected samples, regardless of the location at Kaifeng and Langfang (Fig. 2C1, D1, E1, F1). However, the pattern reversed in 2019, with significantly higher Shannon indices observed in almost all uninfected samples at the elongation stage (Fig. 2C3, D3, F3). We refer to this change as "opposite characteristics." This phenomenon trend was particularly evident at the elongation stage and was independent of location or subspecies; i.e., it occurred in both locations and in the *japonica* and *indica* subspecies (Fig. 2C, D, E, F; Table S8). The opposite characteristics also extended to the bacterial composition. Specifically, the relative abundance of abundant genera in uninfected plants was higher compared to samples from infected ones at the elongation stage in 2017. By contrast, in 2019, the relative abundance of these abundant genera in uninfected samples was lower than in infected samples (Fig. 3A, B). The rare genera also showed opposite characteristics from 2017 to 2019 at the elongation stage, and their relative abundance was higher in infected samples than the uninfected in 2017 but lower in infected samples than in the uninfected in 2019 (Fig. 3A, B). *Pseudomonas* was the most abundant of all the rhizosphere genera, with an average relative abundance of 11.74%, increasing 7.67% in 2017 to 13.80% in 2018 and slightly decreasing to 13.09% in 2019. *Aeromonas* was the second most abundant genus (average 10.95%), but its relative abundance decreased from 2017 (17.97%) to 2019 (7.09%), followed by *Hydrogenophaga* with an average relative abundance of 8.14%, which increased from 3.94% (2017), 8.92% (2018), to 11.11% (2019) (Fig. 3C, D).

To address which bacterial genera in the rhizosphere were significantly affected by RBSDV infection, we identified biomarkers of RBSDV infection using the tool LEfSe [60]. The results showed that the number of significantly enriched bacterial genera in rhizosphere samples from infected plants at elongation gradually decreased from 2017 to 2019 in both locations (Kaifeng: 227, 219, and 79 genera; Langfang: 418, 211, and 46 genera in the respective years) but increased in sample from uninfected rice (Kaifeng: 52, 60, and 140 genera; Langfang: 35, 81, and 211 genera in the respective years) (Fig. 3E, F). This is because some genera significantly enriched in infected samples in 2017 were instead significantly enriched in uninfected samples in 2019. For example, of the genera significantly enriched in the infected samples of Langfang in 2017, only 12 remained enriched in infected samples in 2019, whereas 151 were instead enriched in the uninfected samples (Fig. 3F). We refer to bacteria with altered enrichment as "reversible dominant microbes," regardless of the direction of the change. At Kaifeng, 42 reversible dominant bacterial genera were found, such as Rikenellaceae Blvii28, unclassified *Kryptoniales* MSB-3C8, unclassified Bacteroidales, *Desulfovibrio*, and *Ruminiclostridium* (Figure S4A). At Langfang, 158 reversible dominant bacterial genera were found, such as *Propionivibrio*, unclassified Thermodesulfovibrionia, *Acholeplasma*, Dehalococcoidia Sh765B-TzT-20, and *Altererythrobacter* (Figure S4B).

RBSDV infection enhanced the correlation between bacterial communities in rice rhizosphere and root endosphere

It is well established that bacterial and fungal pathogen infestations alter the rhizosphere microbial community through the plant root system [22–24], whereas whether virus diseases have a similar effect is still rarely reported. We hypothesized that changes in rice rhizosphere communities due to RBSDV infection are related to root endosphere communities and root exudates or metabolites in rhizosphere soil. We analyzed α-diversity and the composition of root endosphere communities as done for the rhizosphere of infected and uninfected plants collected at elongation in 2017 in both two locations. The results showed that the Shannon indices of the root endosphere communities were significantly higher (t-test, $p < 0.05$) in infected samples than in uninfected samples in all combinations except the Kaifeng-*indica* samples (Fig. 4A, B), illustrating that RBSDV infection increased the α-diversity of rice root endosphere communities in that year. PCoA and PerMANOVA showed that the composition of root endosphere communities changed significantly in infected samples at Kaifeng and Langfang (Kaifeng: $R^2 = 0.26$, $p = 0.001$; Langfang: $R^2 = 0.10$, $p = 0.001$) (Fig. 4C). Furthermore, significant correlations were found between rhizosphere and root endosphere communities in all rice samples (Mantel, $p < 0.05$) (Fig. 4D). Interestingly, the correlation coefficients for infected samples were higher than that for uninfected samples, indicating RBSDV infection led to a closer correlation between rhizosphere and endosphere communities. These findings provide evidence that the rice-intrinsic conditions are regulating the RBSDV-mediated plant-microbiome interactions. We speculate that this phenomenon occurred because the roots of infected rice have acquired more microbes from the soil (the α-diversity of both the rhizosphere and endosphere communities was higher for infected rice than uninfected rice at the elongation stage in 2017), thus contributing to greater similarity between the two communities.

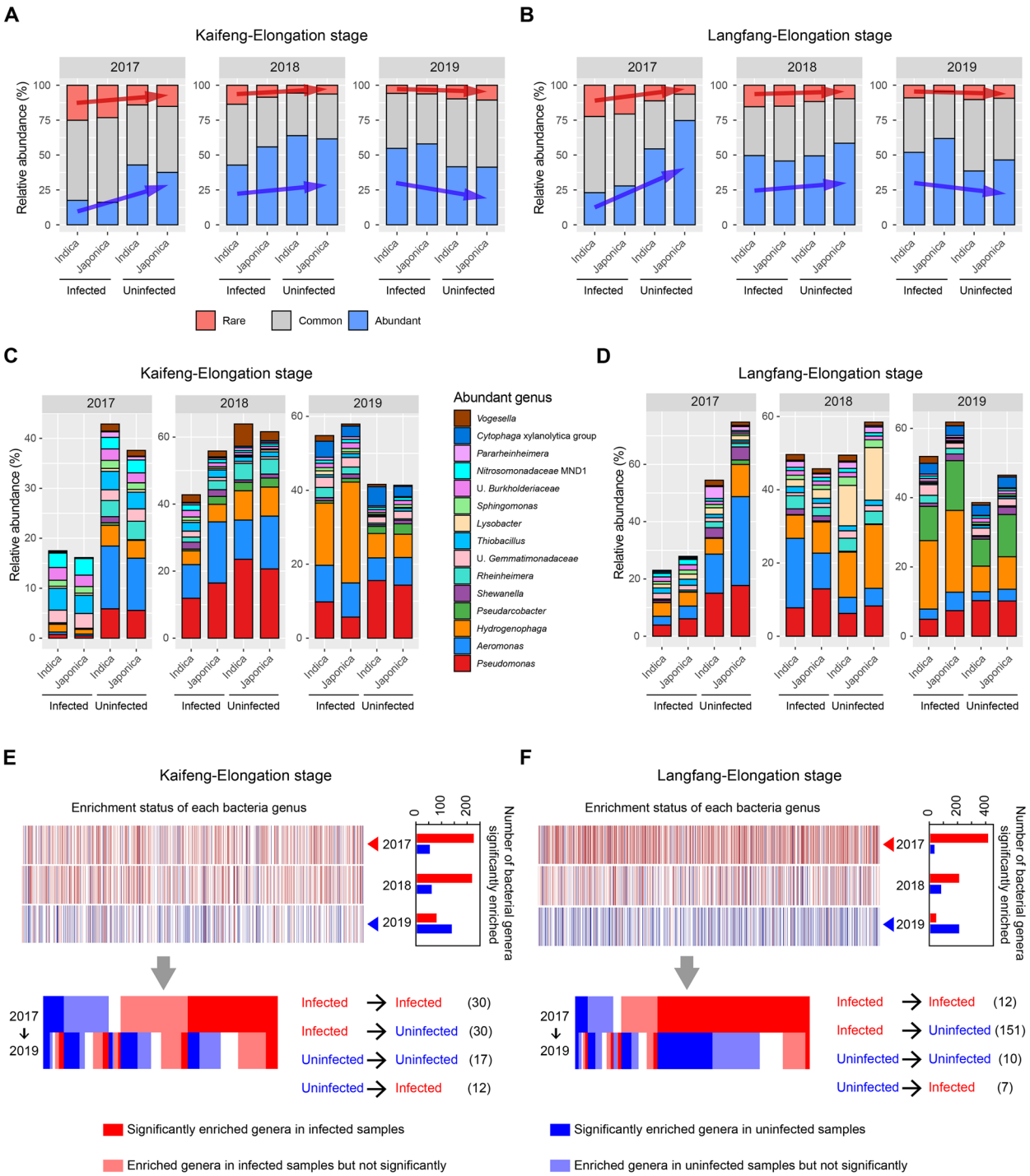

Fig. 3 The rhizosphere bacterial communities in elongation stage showed opposite response characteristics after being infected by RBSDV in 2017 and 2019. **A**, **B** Relative abundance of rare (color in red, relative abundance < 0.1%), common (color in gray), and abundant (color in blue; relative abundance > 1%) genera in Kaifeng (**A**) and Langfang (**B**). **C**, **D** Relative abundance of the abundant genera in Kaifeng (**C**) and Langfang (**D**). The opposite trend in the rhizosphere communities after RBSDV infection was shown by the total relative abundance of abundant and rare bacteria genera in 2017 compared with 2019. **E**, **F** Enrichment status of bacteria genera in Kaifeng (**E**) and Langfang (**F**). Some genera that were significantly enriched in infected samples in 2017 were instead significantly enriched in uninfected samples in 2019. Red or blue indicates that relative abundance of a genus in the infected plant is higher or lower, respectively, than in the uninfected. Taxa significantly enriched in rhizosphere from infected or uninfected plants are shown as dark red or dark blue, respectively; lighter colors indicate no significant difference between samples, and white indicates zero or no change in the relative abundance of the genus

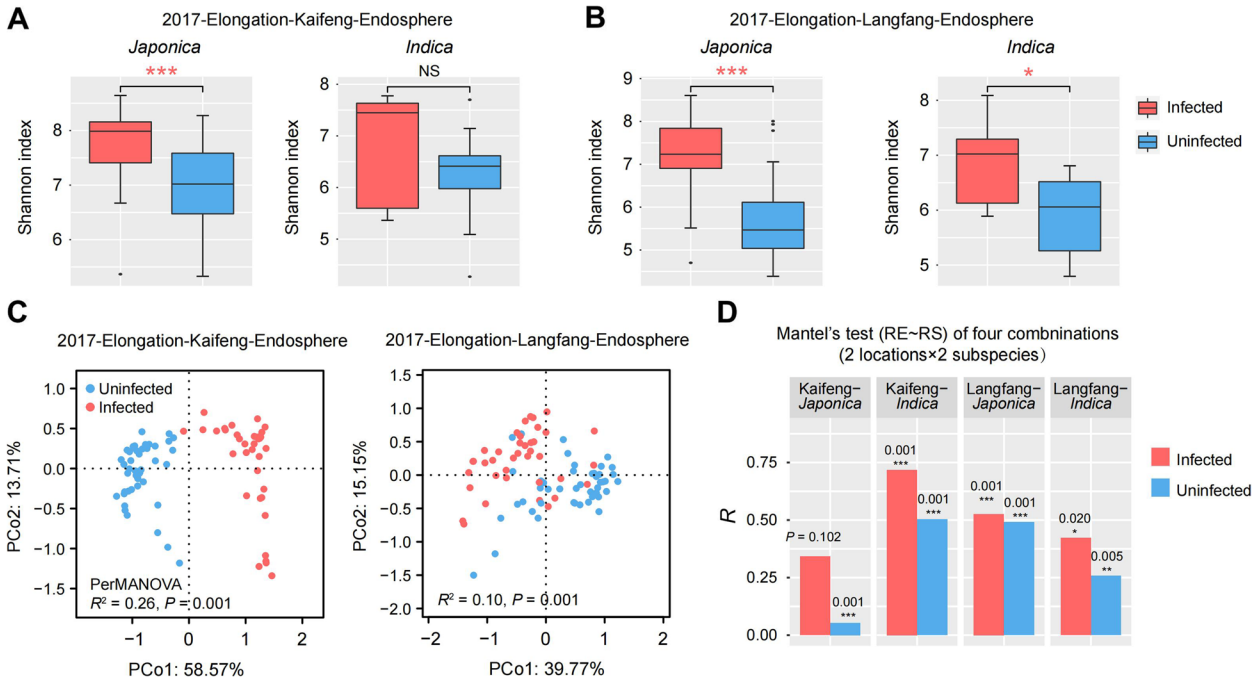

Fig. 4 Correlation comparison of bacterial communities in rhizosphere and root endosphere between RBSDV-infected and uninfected plants from Kaifeng and Langfang at elongation stage in 2017. **A**, **B** Shannon indices of rice root endosphere bacterial communities in infected (red) and uninfected (blue) *japonica* and *indica* plants from Kaifeng (**A**) and Langfang (**B**) (*T*-test, *$p<0.05$, **$p<0.01$, ***$p<0.001$, NS $p>0.05$). **C** PCoA and PERMANOVA of root endosphere bacterial community composition in uninfected and infected rice plants from Kaifeng and Langfang. **D** Mantel's test showing significant correlations between rice rhizosphere (RS) and root endosphere (RE) bacterial communities in infected (red) and uninfected (blue) *japonica* and *indica* plants from Kaifeng and Langfang (Mantel's test, *$p<0.05$, **$p<0.01$, ***$p<0.001$)

RBSDV infection-induced alterations of bacterial communities associated with the changes of metabolites in rhizosphere soil

To elucidate whether metabolites are involved in altering the composition and diversity of bacterial communities in the rhizosphere soil after rice infection with RBSDV, rhizosphere soil from uninfected or infected rice plants was analyzed by GC-MS to detect volatile, thermally unstable, low to medium polar substances, and highly polar substances containing hydroxyl, carboxyl, amino, and imino groups [52]. A total of 201 peaks, which were annotated among 16 major classes of compounds such as lipids and lipid-like molecules, organic acids and their derivatives, benzene ring-type compounds, phenylpropanoids, and polyketides, were detected across all samples. The substances exhibited significant differences between the infected and uninfected samples, as evidenced by the results of OPLS-DA (Fig. 5A). Among the compounds identified by GC-MS, 13 were found to be significantly higher in uninfected samples compared to infected samples (variable importance in projection ($VIP>1$ and $p<0.05$). These compounds include pinitol, isoleucine, leucine, isocitric acid, glutamine dehydrated, 5′-deoxy-5′-methylthioadenosine, salicylic acid, gallocatechin, resveratrol, melezitose, 2-deoxytetronic acid, myo-inositol, and aconitic acid (Figs. 5B, S5A).

Metabolites in the rhizosphere soil of uninfected and infected plants were also analyzed by LC-MS, which has a broader range of detection targets than GC-MS does and is applicable to the vast majority of compounds [53]. Of 18,182 peaks, 3035 were aligned with spectra in the metabolomics BinBase database to match to known compounds [54]. The OPLS-DA of the total metabolites showed significant differences between uninfected and infected plants (Fig. 5C). Among the 173 differential compounds identified, 101 were significantly higher in the uninfected plants, and 72 were significantly higher in the infected plants (Figs. 5D, S5B).

We then used a Mantel's test to determine whether the alterations of the metabolites in rhizosphere soil were statistically significant and involved in the changes in rhizosphere communities that resulted from RBSDV infection. Results showed a strong and significant correlation between the metabolites and rhizosphere communities ($R^2=0.81$, $p=0.05$) (Fig. 5E). Further, a stronger correlation was found between differential metabolites ($VIP>1$ and $p<0.05$) and rhizosphere communities ($R^2=0.91$, $p=0.02$) than for the total metabolites results

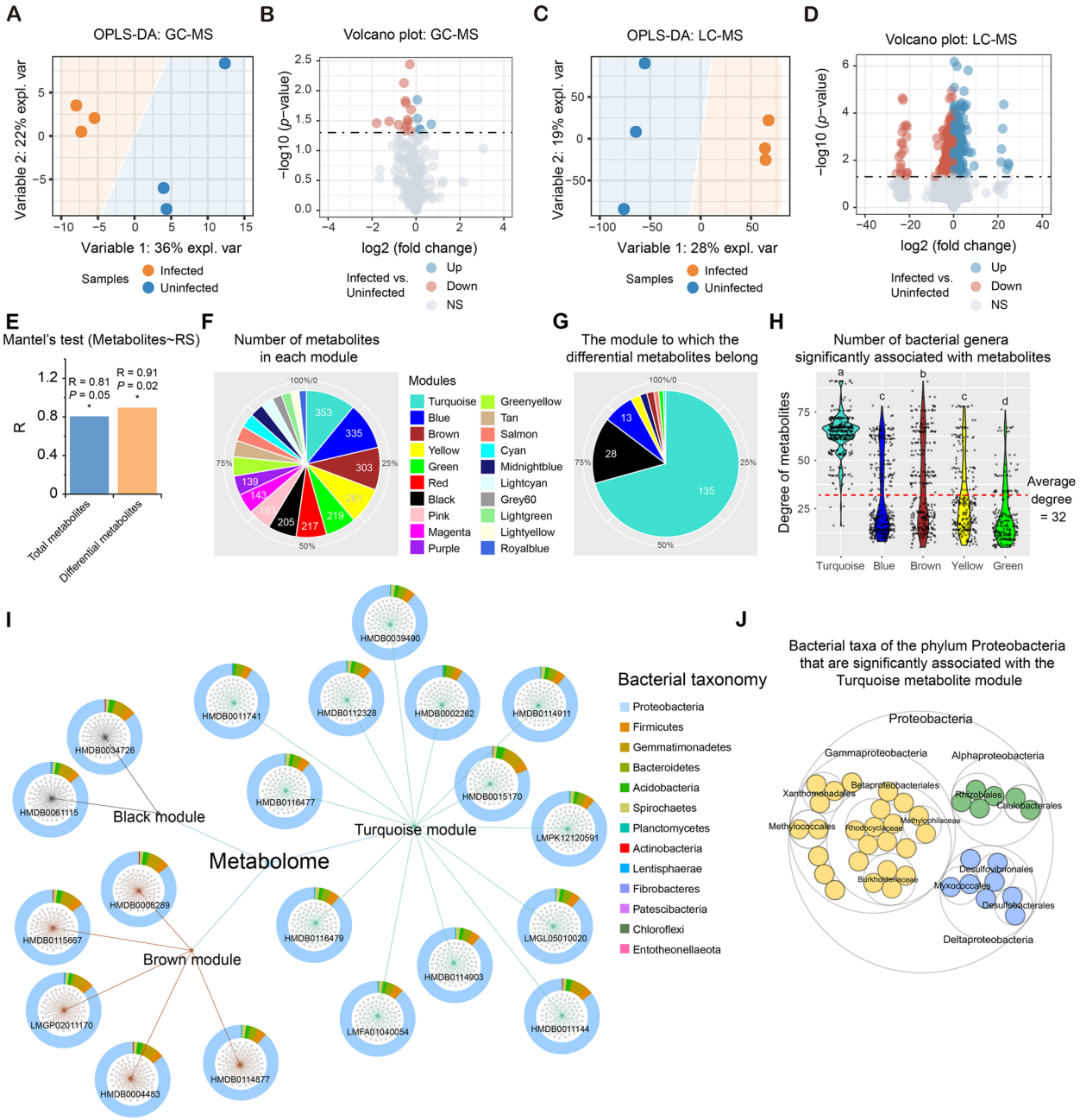

Fig. 5 RBSDV infection-induced changes in rice rhizosphere communities associated with the discrepant metabolites. **A**, **C** OPLS-DA for the metabolites obtained by GC-MS (**A**) and LC-MS (**C**) showing differences in the rhizosphere metabolite profiles for infected rice and uninfected rice plants. **B**, **D** Volcano plots of enrichment and depletion of root metabolites in the infected samples compared with uninfected samples by GC-MS (**B**) and LC-MS (**D**) ($p < 0.05$, variable importance in projection (VIP) > 1). **E** Mantel test's showing a higher correlation of the rhizosphere communities with differential metabolites rather than with total metabolites. **F** The 3236 metabolites with known information were divided into 20 modules using WGCNA. **G** The number of differential metabolites contained in each module was counted. **H** The number of bacteria genera significantly associated with a certain metabolite was calculated using the Spearman method, and the average number of metabolites in turquoise module was higher than that in other modules. **I** The top 20 metabolites most closely associated with the rhizosphere communities were presented, indicating the metabolite-related genera mainly belonged to phylum Proteobacteria. **J** The main categories of Proteobacteria to which the bacteria significantly associated with the turquoise metabolite module belong were counted

obtained by GC-MS and LC-MS (Fig. 5E), demonstrating that the alterations of the metabolites in rhizosphere are associated with the changes in the rhizosphere bacterial communities of RBSDV-infected plants.

Using WGCNA, we modularized all 3236 compounds with annotation information, resulting in a total of 20 modules (Fig. 5F). We then counted the number of differential compounds contained in each module and found that 70% (135/191) of the differential compounds were distributed in the turquoise module (Fig. 5G), indicating that the turquoise module was the most sensitive to RBSDV infection. To further clarify which compounds may be key rhizosphere metabolites in regulating microbial communities, we calculated how many bacterial genera each compound was significantly associated with. The results showed that each compound was significantly associated with an average of 32 bacterial genera, but the compounds of the turquoise module were significantly associated with 64 bacterial genera on average, which was much higher than the other modules (Fig. 5H). This suggests that the turquoise module is not only the most sensitive to RBSDV infection but also closely associated with changes in the relative abundance of rice rhizosphere communities.

We selected the top 20 compounds most closely associated with the rhizosphere bacterial community and counted the bacterial taxa that were significantly correlated with them (Fig. 5I). Thirteen of these 20 compounds belonged to the turquoise module, 5 to the Brown module, and 2 to the Black module. These 20 metabolites were classified into 9 glycerophospholipids, 2 steroids and steroid derivatives, 2 carboxylic acids and derivatives, 2 prenol lipids, and others (Table S9). Correlation analyses showed that microbes in the phylum Proteobacteria were the most closely related to metabolites, with relative abundance of metabolite-related genera of the phylum Proteobacteria exceeding 80% (Fig. 5I). Further, we revealed which microbial taxa in phylum Proteobacteria were significantly associated with the turquoise module. The results showed that the genera significantly associated with the turquoise module mainly belong to the microbial taxa Rhodocyclaceae, Burkholderiaceae, Methylophilaceae, Rhizobiales, and Xanthomonadales (Fig. 5J).

Bulk soil microbiota influenced RBSDV infection-induced changes in rice rhizosphere bacterial communities and disease incidence in the greenhouse

There is still a lack of evidence to support that soil microbiota can influence RBSDD, and that native soil microbiota alter the effects of RBSDV infection on rhizosphere communities. A two-batch greenhouse pot experiment was carried out to test our hypothesis (Fig. 6A). The results of Batch 1 in pot experiment showed that 78 rice plants survived in natural soil; 18 of these were confirmed as infected by RT-PCR (23.08%) compared with 85 plants in sterile soil and 32 infected (37.65%). That is, significantly more plants were infected in sterile soil than in natural soil (z-test, $p<0.05$), meaning that bulk soil microbiota may be an influential factor in plant resistance to RBSDV. Infection significantly altered rhizosphere communities (PerMANOVA, $p<0.05$) (Fig. 6B, D) and root endosphere communities ($p<0.05$) in both natural and sterile soil (Fig. 6C, E), but the difference was greater in natural soil (rhizosphere soil: $R^2=0.334$, $p=0.001$; root endosphere: $R^2=0.196$, $p=0.001$) than in sterile (rhizosphere soil: $R^2=0.186$, $p=0.001$; root endosphere: $R^2=0.164$, $p=0.001$) as indicated by the PCoA and PerMANOVA based on the ASVs profile. RBSDV infection also had a significant influence on the rhizosphere communities of the natural soil in Batch 2, but not as great as in Batch 1 based on R^2 of 0.334 for Batch 1 and 0.062 for Batch 2 (Fig. 6B, D, F), implying that the legacy of natural soil microbiota might interfere with the effects of RBSDV infection on rhizosphere communities. Further, in natural soil, the R^2 value of Batch 2 (0.062) dropped sharply compared to Batch 1 (0.334), while in sterilized soil, the R^2 value of Batch 2 (0.200) remained at similar levels to Batch 1 (0.196) (Fig. 6B). The same dynamic trend was found in root endosphere communities; the influence of RBSDV infection largely decreased from Batch 1 ($R^2=0.186$, $p=0.001$) to Batch 2 ($R^2=0.075$, $p=0.025$) in natural soil and was maintained in sterile soil ($R^2=0.164$ in Batch 1, $p=0.002$; $R^2=0.179$ in Batch 2, $p=0.049$) (Fig. 6C). All the above results demonstrated that extrinsic soil conditions, such as the bulk soil microbiota, regulated RB-mediated plant-rhizosphere microbiome interactions.

Discussion

The composition of host-associated microbes is altered by many environmental and biological factors [17–19]. The microbiota associated with rice roots varied significantly across 2 years at a California site [15] and during the vegetative stage in two locations in China [21]. However, the present study is the first to characterize the effects of rice infection by an insect-transmitted reovirus under multiple rice-intrinsic and external conditions on bacterial abundance and composition in the root-associated microbiomes. Our observations indicated that the influence of RBSDV infection on the rhizosphere bacterial community in rice was discernible and significant under certain conditions. Nevertheless, this influence was highly susceptible to the rice-intrinsic and external factors, including year, location, growth stage, and subspecies, which could even yield

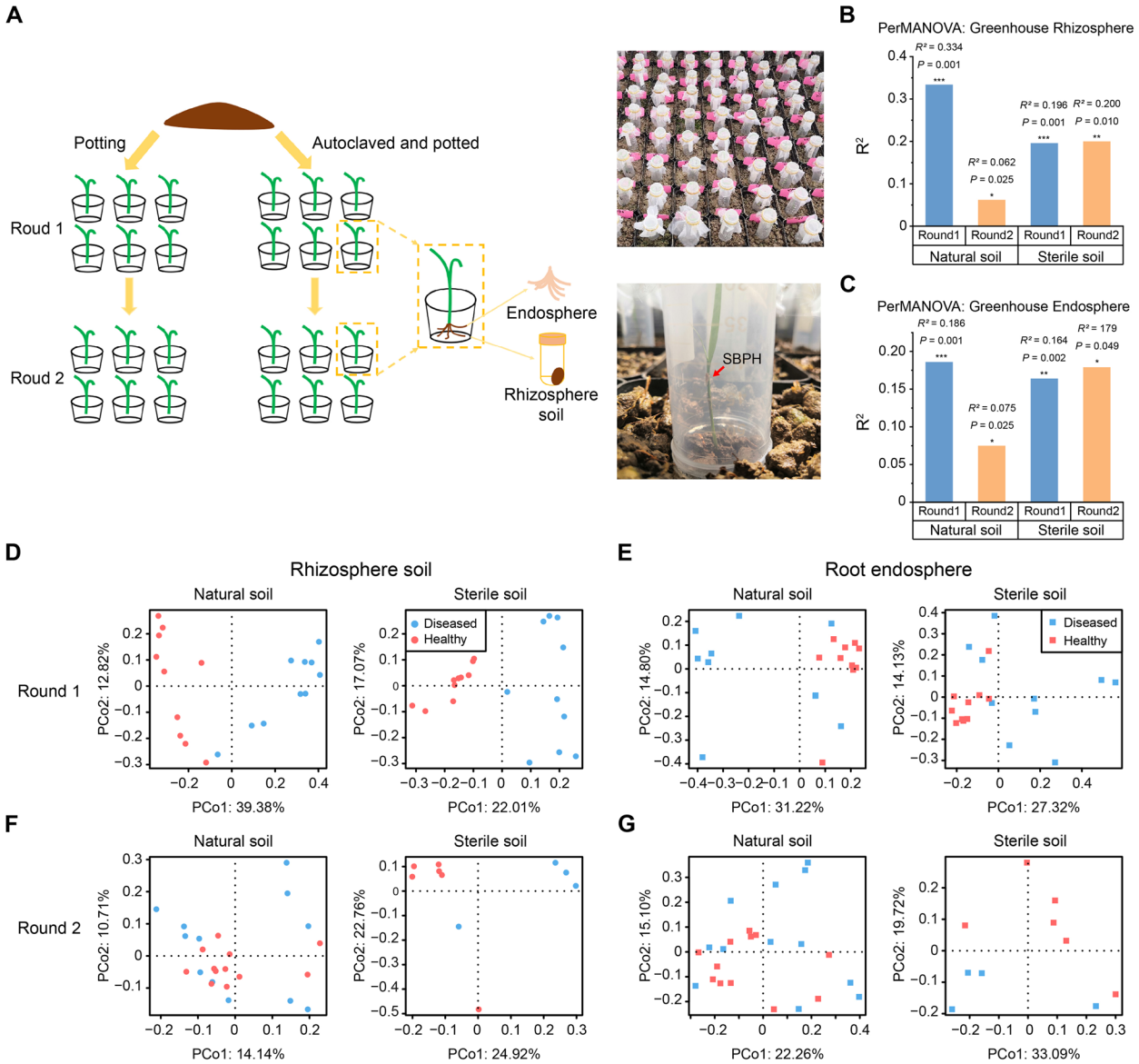

Fig. 6 Bulk soil microbiota influenced RBSDV infection-induced changes in rice rhizosphere and endosphere bacterial communities in the greenhouse. **A** A two-batch greenhouse pot experiment was carried out using soil collected from the same field in Langfang. The soil was sieved and thoroughly mixed, then half of it was autoclaved three times and used for the sterile soil treatment, and the other soil was used for the natural soil treatment. All seedlings were exposed to viruliferous SBPHs, and infection status was detected by RT-PCR. The soil in the pots was then sterilized or used directly in another batch (Batch 2) of transplanting and RBSDV inoculation. **B**, **C** PERMANOVA and **D**, **E**, **F**, **G** PCoA showing significant differences between infected and uninfected samples for both rhizosphere (**B**, **D**, **F**) and endosphere communities (**C**, **E**, **G**) in natural soil and sterile soil. R^2 values for PERMANOVA of rhizosphere bacterial community samples showing a sharp decrease from the Batch 1 to Batch 2 in natural soil but not in sterile soil. The same trend was found in root endosphere communities

contrasting characteristics of the RBSDV-mediated rhizosphere bacterial communities. To substantiate the authenticity, we further examined the root endosphere bacterial communities and rhizosphere metabolites at the elongation stage in 2018. The results revealed that both the two were significantly influenced by RBSDV infection and exhibited significant correlations with the rhizosphere bacterial communities. Notably, the correlations were more significant in infected plants. Subsequently, we designed greenhouse experiments and discovered that the bulk soil microbial communities might influence the incidence rate of RBSDD and regulated the RBSDV-mediated rhizosphere bacterial communities. This study holds profound implications

for future research exploring virus-mediated plant-microbiome interactions.

Because RBSDV infection will impact rice metabolic processes [42, 43] and root exudates are significantly affected by many factors including pathogen infection [67], we are not surprised that the metabolites in rhizosphere soil from infected plants are altered and associated with changes in the microbiome. In response to pathogens, plants produce various sugars, amino acids, organic acids, fatty acids, secondary metabolites, and hormones that are secreted by the roots [68–70]. Generally, these metabolites can attract or repel microbes to the rhizosphere from the bulk soil and are an important driving force for changes in microbial abundance and composition in the rhizosphere and bulk soil [68, 70–72]. Our analyses for GC-MS and LC-MS results showed significant differences of the metabolites in rhizosphere soil between the infected and uninfected plants (Fig. 5A, B, C, D). Furthermore, the differential metabolites in rhizosphere soil caused by virus infection contributed more than the total metabolites to explain the change in the composition of the rhizosphere bacterial community (Fig. 5E). Some organic acids (isocitric acid, dihydroxymalonic acid, aconitic acid), amino acids (isoleucine, leucine), flavonoids (gallocatechin), and hormones such as salicylic acid differed significantly in the metabolome between the uninfected and infected rice samples (Figure S5). These compounds are known to affect the interactions between plants and microorganisms in the rhizosphere and the development of some diseases [69, 73, 74]. Together, our data indicate that the chemical composition of the metabolites in rhizosphere soil is impacted by virus infection, and the discrepant metabolites are associated with changes in the bacterial diversity and composition in rice rhizosphere.

In response to invasion of some pathogenic bacteria and fungi, plants gain additional resistance by seeking the assistance of beneficial microorganisms residing in the soil [26, 31, 39]. These beneficial microorganisms accumulate in soils to form a soil-borne "legacy" that safeguards against subsequent infections by the same pathogens [16, 26, 27, 32, 75]. Our study is the first to show that the rhizosphere microbiome changes when a plant is infected by an insect-transmitted virus by statistically analyzing rice spanning 3 years, 2 locations, 18 varieties, and 2 developmental stages. However, the question of whether viral plant diseases can similarly establish a soil-borne "legacy" of resistance remains unanswered and necessitates further investigation. Interestingly, our findings demonstrate some possibilities that may inspire future research efforts. The incidence of RBSDD in each rice variety in the two fields decreased gradually during the 3-year experiment (Figure S1). Concurrently, relative abundance of *Hydrogenophaga*, the known plant growth-promoting bacteria [76], showed an increasing trend in rice rhizosphere during 2017–2019 (Fig. 3C, D). The higher abundance of bacteria in the phylum of Bacteroidales (including the family Rikenellaceae), which plays a central role in the utilization of carbon monoxide, and in response to soil amended with biochar [77, 78], and of the genus *Desulfovibrio*, which play a very specific role in biological nitrogen fixation and promotes plant growth [79], was found in the top 10 reversible dominant bacterial genera in this study (Figure S4). During two batches of greenhouse pot experiments, the rice plants grown in natural bulk field soil had significantly lower disease incidence than those in the sterilized soil. Moreover, the influence of RBSDV infection on the bacterial composition in both rhizosphere and endosphere of the plants in natural soil tended to lessen by the second batch, but did not in the sterile soil (Fig. 6), suggesting that the bulk soil microbiota could regulate the RBSDV-mediated plant-rhizosphere microbiome interactions. In future, beneficial candidates, especially involving persistent enrichment of specific taxa over time, could be selected for isolation and further characterization.

Based on the evidence from this study, we speculate the occurrences of RBSDV since its first report in 1952 is potentially correlated with the dynamic changes in rice rhizosphere microbiomes that are shaped by virus infection. Once infected by viruses, rice plants undergo physiological and metabolic changes that alter the chemical composition of the root exudates. These molecules can act as signals to recruit beneficial microbes, which accumulate to generate a soil "memory" or soil-borne "legacy" that will contribute to the defense of the next generation of plants against the virus. However, this defense gradually diminishes over time because fewer and fewer plants are infected, and the root exudates change, causing the microbial communities to change. When the epidemic conditions become suitable again (i.e., high density of viruliferous vector insects at the rice seedling stage) [6], plants become infected, continuing the cycle of intermittent epidemics. This novel interpretation indicates the potential of manipulating microbial communities as a strategy to confer virus resistance/tolerance to field crops for the sustained, environmentally safe protection of rice and other crops.

Conclusions

In this 3-year field study of RBSDV-induced changes in root-associated microbiome at the elongation and grain filling stages of *japonica* and *indica* rice plants at two locations in China, all results consistently demonstrated that RBSDV infection significantly impacted the rice rhizosphere microbiome assembly but to different

extents in the order location > year > growth stage > virus infection > rice subspecies. The influence of virus infection on the rhizosphere community showed contrasting characteristics at the elongation stage in 2017 and 2019, as indicated by the variations in bacterial diversity, abundance, and numbers of enriched genera in the infected and the uninfected plants. The rhizosphere bacterial communities were significantly correlated with both the rhizosphere metabolites and root endosphere bacterial communities, particularly in RBSDV-infected plants. Glycerophosphates, amino acids, steroid esters, and triterpenoids showed the most closely association with the rhizosphere bacterial community. They primarily linked to the taxa of Proteobacteria, particularly those belonging to the following familes: Rhodocyclaceae, Burkholderiaceae, and Xanthomonadales. Our greenhouse pot experiments, using sterilized and untreated bulk field soil, further proved that the microbiota in bulk soil affected the composition of the rhizosphere and endosphere communities and regulated the RBSDV-mediated plant-rhizosphere bacterial community interactions.

Abbreviations

ASVs	Amplicon sequence variants
GC-MS	Gas chromatography-mass spectrometry
LC-MS	Liquid chromatography-mass spectrometry
LEfSe	Linear discriminant analysis effect size
LMM	The linear-mixed model analysis
OPLS-DA	Orthogonal partial least-squares discrimination analysis
PerMANOVA	Permutational multivariate analysis of variance
PCoA	Principal coordinate analysis
RBSDD	Rice black-streaked dwarf disease
RBSDV	Rice black-streaked dwarf virus
SBPH	Small brown planthopper
VIP	Variable importance in projection

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40168-024-01910-0.

Additional file 1: Figure S1. Incidence rates of rice black streak dwarf disease (RBSDD) in Kaifeng and Langfang from 2017 to 2019. Figure S2. Alpha-diversity of rhizosphere bacterial communities of infected and uninfected plants during grain filling stage of rice in Kaifeng and Langfang from 2017 to 2019. Figure S3. Beta-diversity of rice rhizosphere bacterial communities affected by RBSDV infection. Figure S4. Relative abundance (%) of the top 10 genera with reversed enrichment patterns in Kaifeng (A) and Langfang (b). Figure S5. Comparisons of the discrepant metabolites in rhizosphere soil between infected and uninfected rice plants at elongation stage (japonica variety: Zhendao 99).

Addtional file 2: Table S1. Soil physicochemical properties in two experimental fields. Table S2. Information of rice varieties used in the field experiments. Table S3. Statistical tests of the influence of five abiotic and biotic factors on α-diversity (Shannon index) of rice rhizosphere bacterial communities (n = 1,075). Table S4. Influence of five abiotic and biotic factors on α-diversity of rice rhizosphere bacterial communities were tested with a linear-mixed model (LMM). Table S5 PERMANOVA by Adonis of all rice rhizosphere bacterial communities (n = 1,075). Table S6. Influence of five abiotic and biotic factors on main characteristics of rice rhizosphere bacterial communities were tested with a linear-mixed model (LMM). Table S7. Influence of RBSDV infection status on α-diversity (Shannon index) of rice rhizosphere bacterial communities was tested with a linear mixed model (LMM) when other abiotic and biotic factors were considered. Table S8. Alpha-diversity changes of rice rhizosphere soil bacterial community by RBSDV infection under multiple factors. Table S9. The top 20 metabolites most closely associated with the rhizosphere bacterial community.

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Authors' contributions
N.W. and W.S. contributed equally to this work. N.W., W.S., Y.R., Z.G. and X.W. designed the research. N.W., W.S., L.Z., H.W., W.L., Y.R., X.L., Z.G. and W.X. performed the experiments. N.W., W.S. and X.W. analyzed the data. N.W., W.S., Z.G. and X.W. wrote and revised the manuscript. All authors reviewed the manuscript.

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Availability of data and materials
The raw sequencing data are publicly available in the NCBI Sequence Read Archive (SRA; BioProject ID: PRJNA890875).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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