16S and ITS rRNA analysis of topsoil and rhizosphere microbial communities

Total DNA was extracted from each soil sample using the E.Z.N.A. Soil DNA Kit (Omega Bio-tek Inc., Norcross, USA) according to the manufacturer's protocols. The final DNA concentration and purification were determined using a Nano Drop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. PCR amplification was carried out using a Thermocycler PCR system (Gene Amp 9700, ABI, USA). The V3-V4 hypervariable regions of the 16S rRNA gene were amplified with bacterial primers 338F 5'-ACTCCTACGGGAGGCAGCAG-3' and 806R 5'-GGACTACHVGGGTWTCTAAT-3' (Chen et al., 2018). Fungal rRNA gene amplification was performed in the fungal ITS sequence region using the primers ITS1F 5′-CTTGGTCATTTAGAGGAAGTAA-3′ and ITS2R 5′-GCTGCGTTCTTCATCGATGC-3′ (Chen et al., 2018). The PCR products of bacteria were extracted from a 2% agarose gel, further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) and quantified using QuantiFluor-ST (Promega, USA) according to the manufacturer's protocol. The bacteria and fungi PCR products were purified and pooled in equimolar amounts and paired-end sequenced (2×300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw fastq files of bacterial and fungal reads obtained from MiSeq sequencing were quality-filtered by Trimmomatic and merged by FLASH (Bolger et al., 2014; Magoč and Salzberg, 2011). The processed sequences were subsequently clustered into operational taxonomic units (OTUs) with a minimum of 97% similarity using UPARSE (version 11) (Gdanetz et al., 2017). The taxonomy of the bacterial sequence was analyzed by the RDP Classifier algorithm against the SILVA database (silva 138/16S-bacteria database) with a confidence threshold of 70%. The taxonomy of the fungal sequence was analyzed by the RDP Classifier algorithm (version 2.11) against the United States database (unite 8.0/ its-fungi database) using a

confidence threshold of 70% (Wang et al., 2007). For alpha diversity analysis, sobs index and shannon index were calculated to estimate community richness and diversity.