Supplementary Materials for the article:

Bacterial community structure in tobacco rhizosphere soil at different altitudes in Hubei Province, China

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Supplementary Note 1: Quantity and Quality Analysis of Extracted Genomic DNA

The quantity and quality of genomic DNA extracted from tobacco rhizosphere soil samples (low altitude <800 m, medium altitude 800–1300 m, high altitude >1300 m) were determined using a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively, with three biological replicates and three technical replicates for each sample.

1.1 DNA quantity detection (NanoDrop NC2000)

The absorbance values of the extracted DNA samples were measured at 260 nm, 280 nm and 230 nm to determine DNA concentration ($ng \cdot \mu L^{-1}$) and purity (A260/A280, A260/A230). The qualified standard for soil microbial DNA was set as: A260/A280 ratio of 1.8–2.0 (indicating low protein contamination) and A260/A230 ratio >1.7 (indicating low organic acid and salt contamination). All DNA samples in this study met the above purity criteria, with the concentration of extracted genomic DNA ranging from 58.2 to 126.5 $ng \cdot \mu L^{-1}$, which was sufficient for subsequent PCR amplification and high-throughput sequencing.

1.2 DNA quality detection (agarose gel electrophoresis)

1% agarose gel (containing GoldView nucleic acid stain) was prepared for electrophoresis analysis. 5 μL of each DNA sample was loaded with 1 μL of 6× loading buffer, and electrophoresis was performed at a constant voltage of 120 V for 20 min in

1× TAE buffer. The gel was observed and photographed under a gel imaging system (Bio-Rad, Hercules, CA, USA).The results showed that all extracted DNA samples presented a single, clear and non-diffuse high-molecular-weight band at about 23 kb, without obvious smearing or degradation. No RNA or protein impurity bands were detected, indicating that the genomic DNA was intact and free of degradation, and the quality met the requirements of subsequent molecular experiments.

1.3 Experimental repeatability

The coefficient of variation (CV) of DNA concentration and purity among three technical replicates of each sample was <5%, and the CV among three biological replicates of each altitude group was <8%, indicating good repeatability of the DNA extraction method and high reliability of the extracted DNA samples.