Rice Husk and Its Biochar Have Contrasting Effects on Water-Soluble Organic Matter and the Microbial Community in a Bamboo Forest Soil

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1. Materials and methods

1.1. Chemical and ^{13}C-CPMAS NMR analyses

The soil pH was measured at a soil/CaCl$_2$ (0.01 M) ratio of 1:5 (w:v) suspension using a pH meter. The electrical conductivity (EC) was analysed in an extracted solution of 1:5 (w:v) soil to deionized water suspension. The soil samples were extracted by 1 M NH$_4$OAc at pH 7.0 at a soil to solution ratio of 1:10 (w:v) and analysed using an inductively coupled plasma atomic emission spectrometer (ICP-OES) to determine the exchangeable Ca^{2+}, K$^+$, Mg^{2+}, and Na$^+$ concentrations. The cation exchange capacity (CEC) was calculated according to Brown [2].

The soil samples were pretreated prior to the ^{13}C-CPMAS NMR analysis. Briefly: 8 g of each soil sample was transferred into a 100-mL plastic tube, having 50 mL of HF solution (10% v/v). The tubes were closed and shaken for 1 h. Thereafter, the samples were centrifuged for 10 min at 1174 g. The supernatant was removed, discarded, and the residue was again treated with HF 8 times with shaking times of 1 h for the first four HF treatments, 12 h for the next three treatments, and 24 h for the last treatment. Finally, the remaining soil residues were washed four times using distilled water by centrifugation, oven-dried at 40 ºC, and ground to pass a 0.3-mm sieve. The NMR spectra were recorded using a Bruker Avance III-400 spectrometer (Bruker Bio-Spin Corporation, Switzerland), equipped with a magic angle spinning (MAS) probe. The samples were spun at 14 kHz, in the scanning region of -50 to 300 ppm, and ^{1}H ramp sequence was used before transference to ^{13}C at a 1.5 ms-contact time. One thousand scans were obtained with 6300 data points collected over the acquisition time of 10 ms with a 1 s-recycle delay/each spectrum.

1.2. Measurements of dissolved organic C (DOC), UV–visible spectra, and fluorescence EEMs,

   and PARAFAC modelling

For the extraction of WSOM of soil samples, 5 g of an air-dried soil sample was added to 50 mL of deionized water, shaken for 1 h at 170 rpm and 25 °C, and centrifuged at 3500 rpm (2040 g) for 20 min. Then the supernatant was filtered through 0.45 μm filters (Whatman Inc., Maidstone, UK). The freshly filtered extract was used for the ultraviolet (UV) absorbance, DOC, and fluorescence spectra measurements. The UV absorbance measurements were performed at 254 nm using an UV/VIS spectrophotometer (CADAS 200, Dr. Lange, Germany), and the specific UV absorbance (SUVA) was estimated according to Weishaar [11]. The DOC concentration was analyzed using a total organic C analyzer (Multi N/C 3100, Jena, Germany). The fluorescence excitation (Ex)–emission (Em) matrix was measured for all the WSOMs as well as blank solution of Milli-Q water at 250 to 500 nm at 5 nm intervals (Ex), and 250 - 550 nm at 1 nm increments (Em) using a fluorescence spectrometer (Hitachi F-7000 FL; Japan). The obtained fluorescence for the blank solution was deducted from the samples' fluorescence EEM data. The detailed procedure is described in previous studies [6,8,9]. The DOM indices were calculated as follows: Fluorescence index (FI) = the fluorescence intensities ratio at the Em of 450–500 nm to that at an Ex of 370 nm [12]; Y fluorescence index (a modified index from FI) = the ratio of the Em of 350-450 nm to that of Ex at 280 nm [13]; humification index = the ratio of the Em of 435–480 nm to 300–345 nm at an Ex of 254 nm [14]; and biological index = the ratio of Em at 380–430 nm to that of Ex at 310 nm [15]. The PARAFAC modelling was performed on the EEM data using MATLAB 7.6 (MathWorks, Natick, MA, USA), to identify and quantify different fluorescent components in different treatments.

1.3. High throughput 16S rRNA gene sequencing

The DNA was diluted to l μg μL^{-1} depending on the concentration. Using particular primers, such as 16S V4: 515F-806R, 18S V4: 528F-706R, 18S V9: 1380F-1510R, etc., the 16S rRNA genes of different regions (16S V4/16S V3/16S V3-V4/16S V4-V5) were amplified. All PCR reactions were carried out with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs); 0.2 μM of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s, and finally 72 °C for 5 min.

The PCR products were detected by electrophoresis on 2% agarose gel, mixed in equal ratios, and then purified with a Qiagen Gel Extraction Kit (Qiagen, Germany). Library preparation and sequencing were generated using a TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, California, USA), assessed on the Qubit@2.0 Fluorometer (Thermo Scientific, Waltham, U.S.) and Agilent Bioanalyzer 2100 system, and sequenced on an Illumina NovaSeq platform.

A dilution curve for Operational Taxonomic Units (OTUs) was created based on a random sampling of a certain amount of sequencing data from the sample, counting the number of species they represent (that is, the number of OTUs), and constructing the curve based on the amount of sequencing data extracted and the corresponding number of species. The dilution curve directly reflects the rationality of the amount of sequencing data and indirectly reflects the richness of species in the sample. When the curve becomes flat, the amount of sequencing data is reasonable, and more data will only produce a small number of new species of OTUs.

Table S1. Soil microbial diversity in the soils treated with rice husk (RH) and its biochar (RHB) at two rates (10 and 30 t ha^{-1})

Treatment	Shannon	Simpson	Chao1	ACE	Coverage
Control	8.4±0.2a	1.0±0.0a	1927.8±99.1a	1944.6±102.5a	1.0±0.0a
RH10	8.5±0.2a	1.0±0.0a	1999.1±173.4a	2021.1±179.9a	1.0±0.0a
RH30	8.4±0.2a	1.0±0.0a	1791.7±362.7a	1815.6±373.9a	1.0±0.0a
RHB10	8.5±0.2a	1.0±0.0a	1931.1±162.2a	1955.5±169.6a	1.0±0.0a
RHB30	8.6±0.3a	1.0±0.0a	2065.5±181.5a	2085.6±178.1a	1.0±0.0a

Data are means ± standard deviation. Different letters after the standard deviations represent significant differences among the treatments at $p < 0.05$.
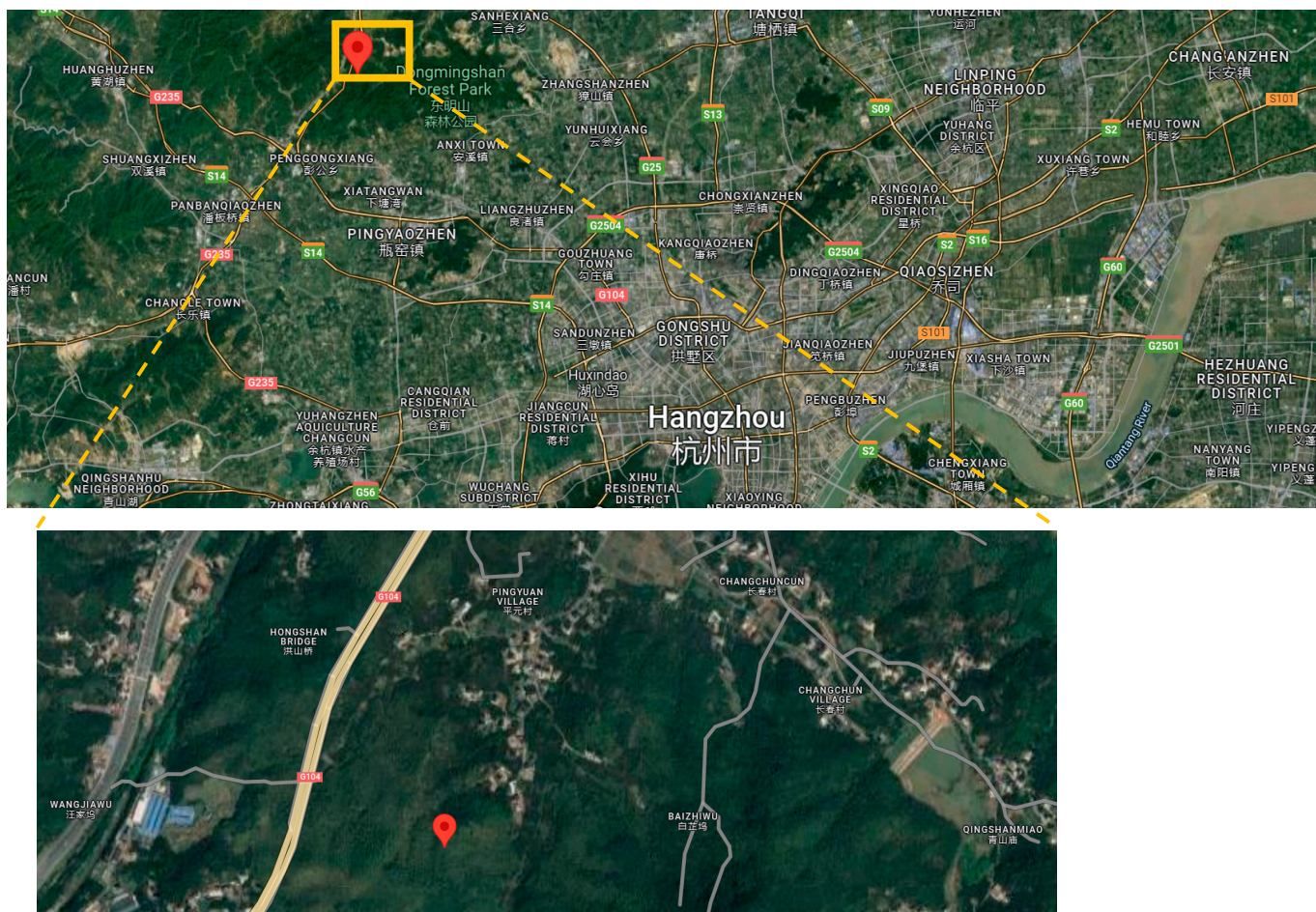
Figure S1. Site of soil sample collected from forest soil in Hangzhou (30° 27′ N, 119° 57′ E), Zhejiang, China and used in the experiment.

Figure S2. Collection of composite samples from the surface layer (0-20 cm) of each pot using a stainless-steel auger at the end of the experiment.
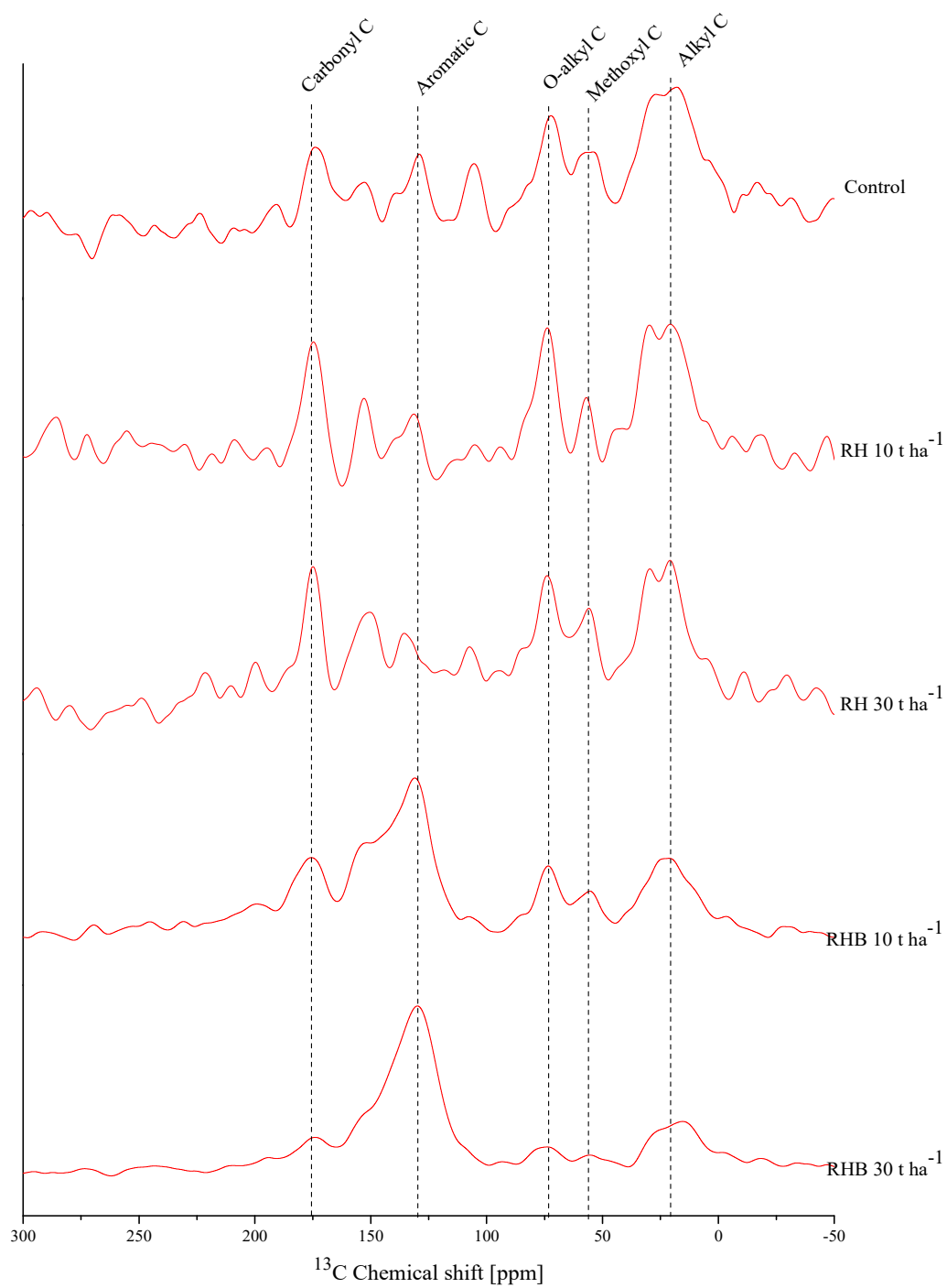
Figure S3. Effects of the addition of rice husk (RH) and its biochar (RHB) at two rates (10 and 30 t ha^{-1}) to the soil on ^{13}C-CPMAS NMR spectra of soils
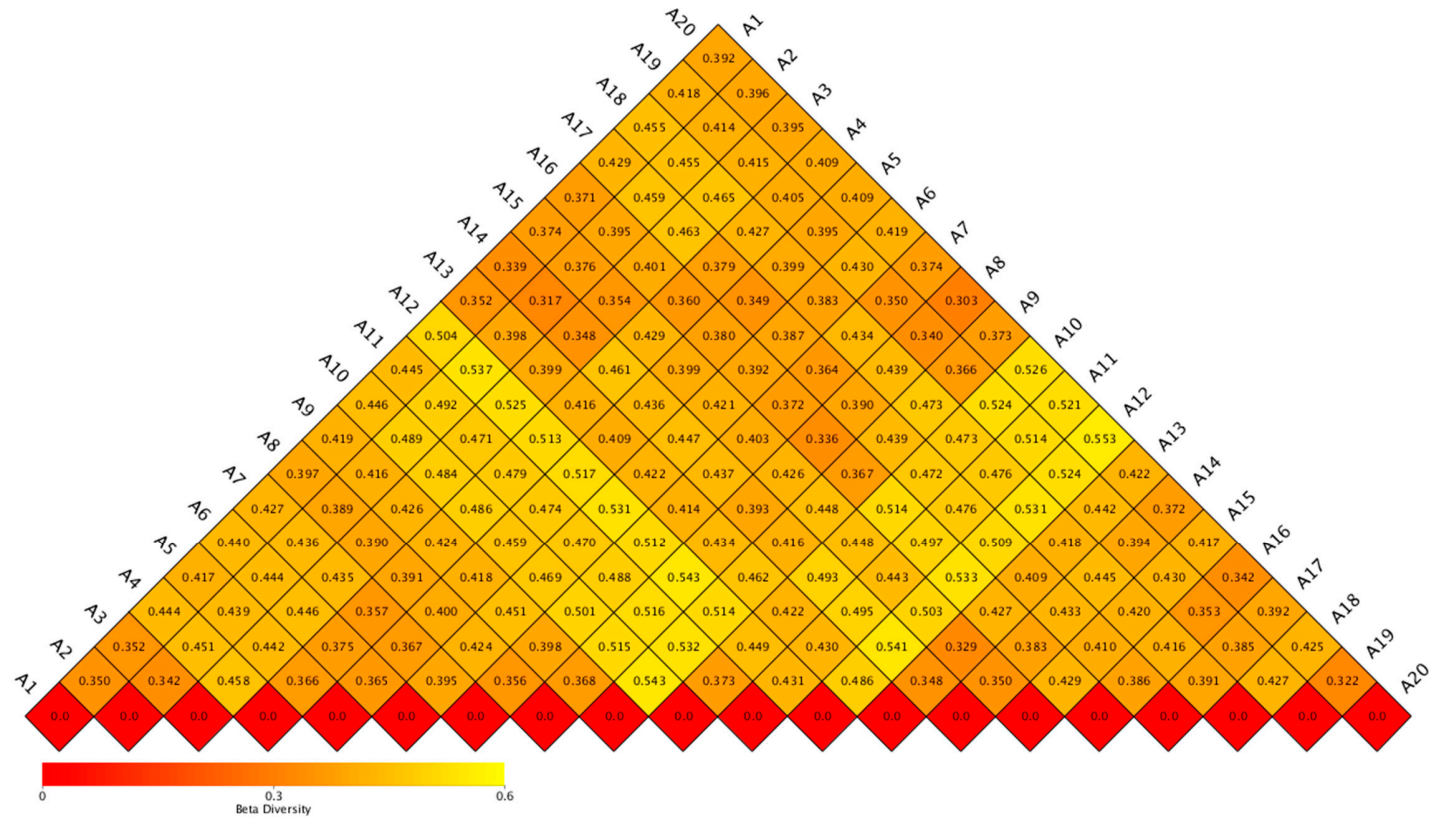
Figure S4. Effects of the addition of rice husk (RH) and its biochar (RHB) at two rates (10 and 30 t ha^{-1}) to the soil on beta diversity index heat map. Numbers inside the squares represent unweighted unifrac values. A1-4: control, A5-8: RH10, A9-12: RH30, A13-16: RHB10, and A17-A20: RHB30.
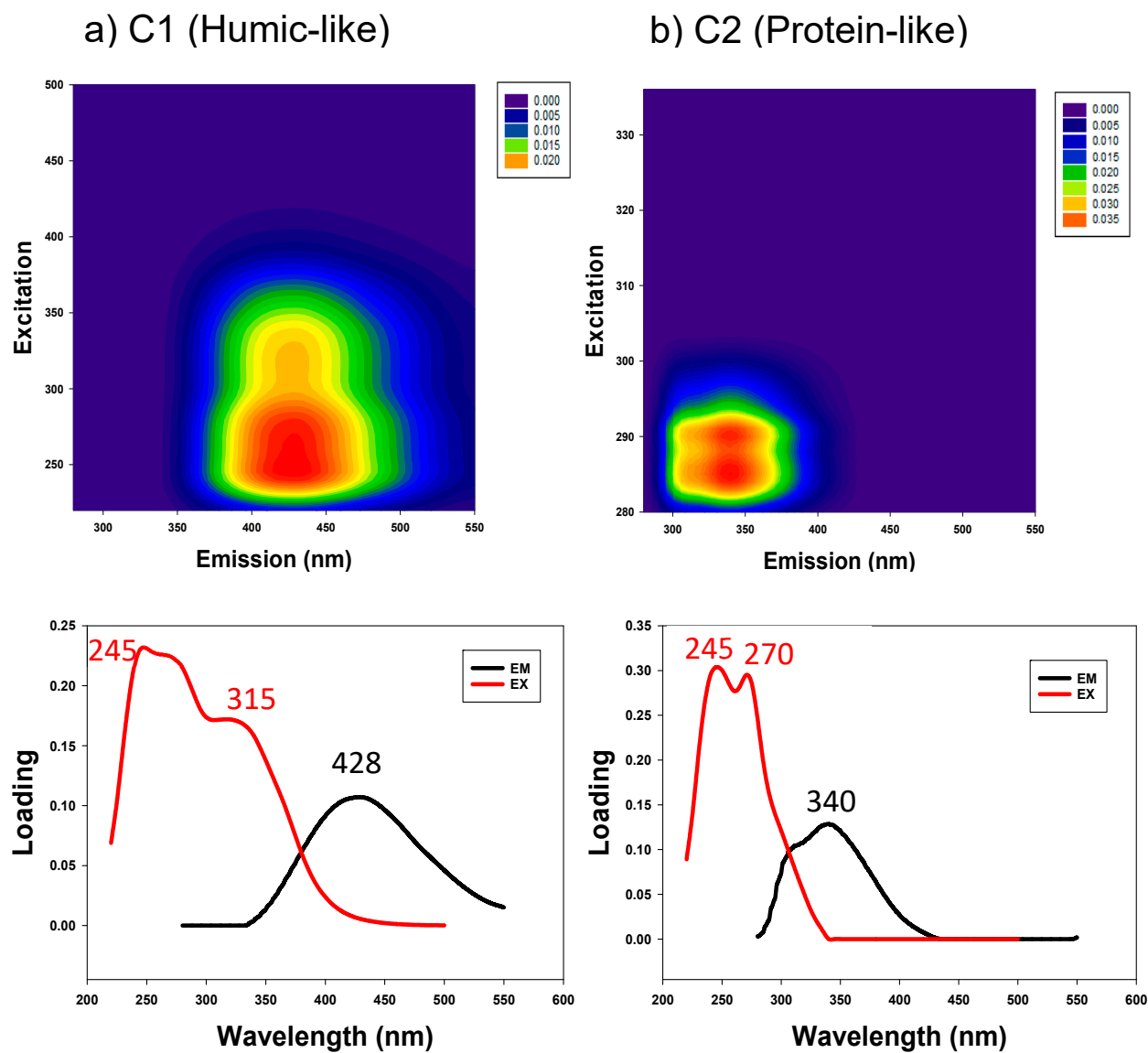
Figure S5. The EEM fluorescence spectra and PARAFAC components (C1: humic-like, C2: protein-like)