

Microbial responses to simulated water erosion in relation to organic carbon dynamics on a hilly cropland in subtropical China



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ABSTRACT

Water erosion significantly affects soil properties, and microbial communities are likely to respond to this disturbance, thereby considerably influencing soil organic carbon (SOC) dynamics. This study investigated the impact of water erosion on the soil microbial communities in a hilly, sloped cropland in subtropical China. To this end, 1 h rainfall was simulated, and the soil samples collected from three plots (I, II, and III) during and for 132 h after rainfall simulation were analyzed. The two-stage variations in soil microbial abundance and community structure were identified by quantitative polymerase chain reaction and denaturing gradient gel electrophoresis, respectively. During rain, severe water erosion significantly reduced the bacterial abundance (BA) and fungal abundance (FA) in plot II. Most of the soil properties of the entire land significantly changed because of erosion. The overall redundancy analysis results illustrate that during the subsequent 132 h, soil pH strongly controlled the Shannon index of bacterial diversity and soil moisture had a significant negative correlation with FA and the Shannon index of fungal diversity. By contrast, a positive correlation was found between BA, FA and SOC. These results suggest that the dynamics of microbial communities are closely related to erosion-induced changes in soil properties. Bacteria and fungi differentially respond to these changes. Thus, merely analyzing the variations in SOC pool content is therefore insufficient. Elucidating soil microbial dynamics and gaining insight into the dynamics of erosion-sensitive functional groups or species are necessary for evaluating eroded carbon dynamics.

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1. Introduction

Soil erosion, one of the main driving forces of soil carbon dynamics, has elicited tremendous concern because it directly and indirectly contributes to the global carbon cycle (Lal and Pimentel, 2008; Lü et al., 2012). The contribution of soil erosion in the pedosphere to atmospheric CO₂ has been investigated to explore either the effects of soil organic carbon (SOC) mineralization during transport as a CO₂ source (Lal et al., 2004) or effects of the deep burial of SOC in landscape as a CO₂ sink (Van Oost et al., 2007). Despite such efforts, however, considerable uncertainties as to how SOC pools

and dynamics are affected by water erosion remain (Lal, 2006; Van Oost et al., 2007). A key uncertainty is the implication of lateral movement and the mineralization of eroded SOC through the terrestrial system for the global carbon cycle (Berhe et al., 2007; Schwanghart and Jarmer, 2011).

A combined eco-geomorphologic and geomorphologic approach has been recognized as delivering a comprehensive explanation of the role of soil erosion in the global carbon cycle (Kuhn et al., 2009). This approach concerns interactions among the biosphere, landforms, and geomorphic processes at or near land surfaces (Otero et al., 2011; Viles, 1988). As a main component of the terrestrial biosphere, soil microorganisms play an important role in the decomposition of soil organic matter, which regulates soil CO₂ efflux (Abbasi and Khizar, 2012; Lopez-Sangil et al., 2011). Soil microorganisms undergo heterotrophic respiration, thereby releasing a large amount of SOC to the atmosphere (Schwanghart and Jarmer, 2011). Abiotic and biotic stresses, in turn, significantly affect the community structure of soil microorganisms. Microbial habitats experience a series of disturbances that are caused by

Abbreviations: BA, bacterial abundance; FA, fungal abundance; BH, Shannon index of bacterial diversity; FH, Shannon index of fungal diversity.

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water erosion through changes in soil properties (e.g., nutrient content, moisture, pH) (Liu et al., 2003) and substrate availability (i.e., SOC pools of different qualities and quantities) (Kuhn et al., 2009). The erosion-induced redistribution of soil microorganisms may considerably affect the dynamics of soil carbon pools because of its concurrent occurrence with the selectivity and enrichment effect of eroded SOC (Wang et al., 2010). Several experimental studies have shown that eroded SOC is potentially unstable because of aggregate disruption (Bernoux et al., 2006). Microbial contributions to SOC cycling is governed by the interactions among the amount of microbial biomass, the structure of microbial communities, microbial byproducts, and soil properties (Six et al., 2006), all of which may be significantly changed by water erosion.

Most studies on soil carbon erosion have focused on the SOC budget by directly analyzing the variations in SOC pools. The patterns of water erosion-induced redistribution of soil microorganisms and the extent to which this change is related to SOC dynamics have been disregarded. Meanwhile, as advanced molecular biological techniques, quantitative polymerase chain reaction (qPCR) and denaturing gradient gel electrophoresis (DGGE) have recently been applied to the study of soil ecosystems. These methods enable researchers to discern the dynamic development of microbial communities and regulate the effects of different treatment regimes on successive applications at the molecular level (Zhang et al., 2010). Through a simulation of rainfall and by using qPCR and DGGE methods, this study aims to (i) discuss the water erosion-induced redistribution of soil microorganisms in a hilly, sloped cropland in subtropical China during rain; (ii) investigate the relationship between the microbial communities dynamics and soil property distribution patterns shaped by water erosion; and (iii) evaluate the interaction between the microbial communities and SOC dynamics in the studied eroded cropland.

2. Materials and methods

2.1. Study site

The experiments were performed at the Soil and Water Conservation Monitoring Station (111°22' E, 27°03' N) of Shuangqing District, Shaoyang City, Hunan Province, China (Fig. 1). This region is characterized by a subtropical monsoon climate, featuring abundant precipitation in summer, with an annual precipitation and mean air temperature of 1327.5 mm and 17.1 °C, respectively. The soil in this area is classified as Quaternary red soil that is heavily weathered and is inherently of low SOC. Similar to most areas of red soil in south China (Dou et al., 2013), the study site, a typical sloping cropland established in about 1980 and left unused after October 2009, suffers from periodic drying/rewetting and severe erosion, especially in summer when high temperatures and frequent thunderstorms occur.

2.2. Rainfall simulation experiment and soil sampling design

The experiments (i.e., rainfall simulation and sampling) were carried out within 133 h in the summer of 2011. The design of the plots and sampling sites for the experiments is shown in Fig. 1. With the use of metal frames, a block of earth with dimensions of 4 m × 5 m (width × length) was bounded off on the sloping cropland, with a mean slope gradient of 1:10. The block was divided into five equivalently arranged plots, namely, A, B, C, D, and E (4 m width × 1 m length) along the sloping land. Prior to rainfall simulation, plant residues and sundries were removed from the soil surface. The uneroded plots were equally sprayed (without occurrence of erosion) with sterile water 24 h before the rainfall



Fig. 1. Location of the study site.

simulation to rewet the dry soil and avoid a considerable decrease in soil microorganisms because of sudden rewetting. The block was then carefully divided into two sub-blocks by using metal wayboards, of which one was used as rainfall sub-block and the other was used as control. The 1 h rainfall simulation was conducted at 7:00–8:00 am on July 16 of 2011. To generate rainstorm, four rainfall simulators were used and placed at the borders of the block (Fig. 2). The rainfall simulator, equipped with a SPRACO cone jet nozzle, was mounted on top of a fixed 4.57 m high stand pipe. The median rain drop size was 2.4 mm, with a uniformity of 89.7%, and the rainfall intensities adopted was 1.5–1.7 mm min⁻¹.

Soil sampling was conducted before and after the rainfall simulation on July 16, and at about 8:00 pm every day until July 21. Soil layers (0–10 cm depth) at plots A, C, and E (labeled plots I, II, and III in this paper) were randomly sampled, with their corresponding controls shown in Fig. 2; sampling was conducted using a sterile push probe with a diameter of 70 mm. Soil properties for each plot in 2011 are summarized in Table 1. The sub-samples collected from three separately arranged sites in each plot were selected as three replicates. All the soil samples were sealed in labeled sterile airtight Ziplock bags and immediately stored at –20 °C before use. The samples were used in the measurements of soil properties and microbial communities (i.e., microbial abundance and community structure).

2.3. Measurement of soil properties

A soil-to-water ratio of 1:10 (w/v) was adopted in measuring soil pH with a digital pH meter (Woonsocket, RI, USA). Soil moisture was determined by the vacuum freeze drying of soil samples at –60 °C and 6 Pa for 36 h. The freeze-dried soil samples were then used for SOC and total nitrogen (TN) analysis. SOC was determined using

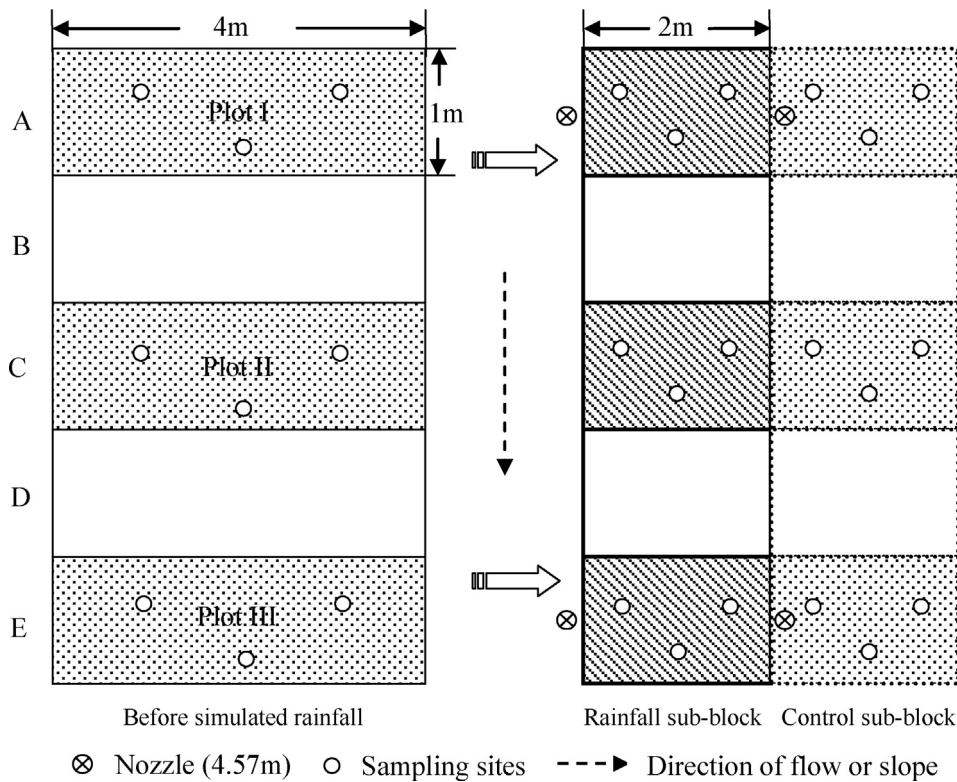


Fig. 2. Design of plots and sampling sites for the rainfall simulation.

the dichromate oxidation of Walkley and Black (Walkley and Black, 1934), and TN was measured using the Kjeldahl method (Kjeldahl, 1883). C/N ratio was calculated from the values of SOC and TN. Three replicates were used in SOC, TN, and pH analysis. Moisture analysis was performed in two replicates.

2.4. qPCR and PCR-DGGE analysis

Total DNA was extracted from the freeze-dried soil samples (approximately 8 g) according to the method described previously (Yang et al., 2007). After purified with a Purification Kit (Biotek, Beijing, China), the extracted DNA was dissolved in 100 μ L of TE buffer and stored at -20°C before being used. Primers 338f and 518r as well as NS1 and Fung (Zhang et al., 2010) were used for the quantification of bacterial and fungal community gene abundance, respectively. The qPCR was conducted on an iCycler IQ5 Thermocycler (Bio-Rad, USA). Amplification was performed in triplicate using a total volume of 20 μ L containing 10 μ L of $2 \times$ SYBR real-time PCR

premixure (Invitrogen, USA), 0.5 μ L of each primer (10 μM), 0.5 μ L of BSA (10 mg/mL), 1 μ L of purified-DNA template, and 6.5 μ L of sterile water. Cycling conditions were 95°C for 2 min, followed by 40 cycles of 20 s at 95°C , 30 s at 55°C for bacteria or 40 s at 55°C for fungi, and 40 s at 72°C . Data was retrieved at 72°C . Ten-fold serial dilutions of linearized plasmids containing cloned bacterial or fungal genes were used to produce the standard curves for qPCR. The order of each magnitude was 1.0×10^4 to 1.0×10^9 copies of template. All reactions were completed with a melting curve to verify amplicon specificity.

For performing PCR-DGGE, the bacterial 16S rDNA gene and fungal 18S rDNA gene were amplified using primers 338f and 518r as well as Fung and NS1, with the forward primers 338f and Fung attached to a GC clamp to prevent complete separation of the DNA strands during DGGE (Muyzer and Smalla, 1998). Mixture preparation and PCR amplification procedure design were conducted as described by Zhang et al. (2010). The PCR amplification was run on a MyCycler thermal cycle (Bio-Rad, USA).

Table 1

Soil properties (0–10 cm) of the plots I, II and III. Values are mean \pm standard error, $n = 3$.

Properties	Plot I	Plot II	Plot III
SOC (g C kg^{-1} dry soil)	8.81 ± 0.21	5.88 ± 0.54	4.54 ± 0.21
TN (g N kg^{-1} dry soil)	0.78 ± 0.01	0.53 ± 0.01	0.49 ± 0.08
C/N	11.27 ± 0.33	11.00 ± 0.92	9.35 ± 0.32
pH	4.88 ± 0.01	4.95 ± 0.02	4.90 ± 0.01
Moisture (g water g^{-1} dry soil)	0.19 ± 0.01	0.21 ± 0.01	0.19 ± 0.01
BA ^a ($\text{lg}(\text{bacteria gene copies g}^{-1}$ dry soil))	7.23 ± 0.56	6.53 ± 0.08	6.43 ± 0.53
FA ^b ($\text{lg}(\text{fungi gene copies g}^{-1}$ dry soil))	5.71 ± 0.05	5.77 ± 0.04	5.77 ± 0.19
BH ^c	1.51 ± 0.00	1.63 ± 0.00	1.05 ± 0.00
FH ^d	2.65 ± 0.00	2.56 ± 0.00	2.55 ± 0.00

^a Bacterial abundance.

^b Fungal abundance.

^c Shannon index of bacterial diversity.

^d Shannon index of fungal diversity.

and DCode™ Universal Detection System according to the manufacturer's instructions (Bio-Rad, USA), respectively. PCR sample (20 μ L) containing approximately equal amounts of PCR amplicons was loaded onto the 1-mm-thick 8% (w/v) polyacrylamide gels with a denaturing gradient of 30–70% for bacterial and 20–50% for fungal PCR samples. Electrophoresis was performed in 1 \times TAE buffer at 60 °C, 80 V for 12 h. After stained with Du-red nucleic acid gel stain, the gels were scanned and analyzed with the Quantity One software V 2.0 (Bio-Rad, USA).

2.5. Statistical analysis

Prior to analysis, microbial abundance (i.e. gene copies per gram of dry soil) was normalized using \log_{10} transformations. Band numbers and relative intensity (within the lane detected by DGGE) were quantified by the Quantity One software (version 2.0, Bio-Rad, Hercules, CA, USA), as described by Zhang et al. (2010). The bands with relative intensity below 1% were excluded from the analysis. The Shannon diversity index (H) was chosen to detect the dynamics of microbial community composition (Zeng et al., 2011). This index is calculated as

$$H = - \sum \left(\frac{N_i}{N} \right) \ln \left(\frac{N_i}{N} \right)$$

where N_i is the relative intensity of each band i , i is the number of bands in each DGGE profile, and N is the sum of all the relative intensities in a given DGGE profile.

One-way ANOVA using SPSS (version 18, SPSS, Chicago, IL) was conducted to evaluate the effects of water erosion on soil microbial communities and soil properties during the rainfall simulation. The variations in soil properties and microbial communities, as well as the differences in these parameters among treatments and plots, during the 132 h after the rainfall simulation were assessed with repeated measures ANOVA. The eroded and control samples were then compared. Redundancy analysis (RDA) was performed using Canoco (version 4.5) to distinguish the effects of erosion and erosion-induced changes in soil properties on the microbial dynamics. Forward selections were performed to verify which soil properties significantly influence the microbial communities in the study site. Variation partitioning was carried out using a partial RDA model to estimate the proportion of variations explained by each factor (Zhang et al., 2010). Reduced-model Monte Carlo tests, with 499 unrestricted permutations, were performed to evaluate the significance of the first canonical axis and all canonical axes. A $P < 0.05$ level was considered statistically significant.

3. Results

3.1. Variations in soil properties during and after erosion

During the rainfall simulation, soil properties of the sloping land significantly changed. In plots I and II, erosion increased SOC by 34.8% ($P < 0.05$) and 13.8% ($P < 0.001$), respectively, whereas in plot III, SOC decreased by 24.0% ($P < 0.05$). However, when the data from the three plots were combined to represent the SOC level in the entire sloping land, no significant variation was observed ($P > 0.05$) (Fig. 3a). Plot I exhibited an especially pronounced erosion-induced effect on TN (increased by 23.5%, $P < 0.05$), whereas the other two plots ($P > 0.05$) and the entire land scale ($P > 0.05$) (Fig. 3b) showed no significant erosion-induced changes. Water erosion reduced the C/N ratio in plot II and plot III by 25.5 and 17.3% ($P < 0.05$), respectively. By contrast, no significant change in this ratio was observed in plot I ($P > 0.05$) and at the entire land scale ($P > 0.05$) (Fig. 3c). Water erosion significantly affected the soil pH in plot I ($P < 0.001$) and at the entire land scale ($P < 0.05$), but imposed no remarkable

effects on the soil pH in plots II and III ($P > 0.05$). Soil moisture visibly changed at all scales in the sloping land, with increases of 107.8, 65.0, 53.8, and 76.2% in plots I ($P < 0.01$), II ($P < 0.05$), III ($P < 0.05$), and the entire sloping land ($P < 0.05$) (Fig. 3e), respectively.

During the 132 h after the rainfall simulation, SOC and C/N ratio significantly increased (by 26.4%, $P < 0.001$ and 23.0%, $P < 0.001$, respectively) at the entire land scale. By contrast, TN, pH, and moisture did not significantly differ from those of the corresponding controls at the same scale (Table 2). SOC dynamics was characterized by marked discrimination among the three plots ($P < 0.001$), with SOC increasing to a greater extent in plot I (by 31.6%, $P < 0.001$) than in plot II (by 20.3%, $P < 0.05$) and plot III (by 25.2%, $P < 0.05$). The variations in TN were especially pronounced in plots I ($P < 0.05$) and II ($P < 0.05$), decreasing by 14.1 and 8.5%, respectively. By contrast, no distinct difference in TN between plot III ($P > 0.05$) and its control was detected. The C/N ratio in all the three plots significantly increased during the succeeding 132 h, with the highest value occurring in plot II (by 29.5%, $P < 0.05$) and the lowest in plot I (by 16.3%, $P < 0.05$). Moisture showed minimal changes at the plot and entire land scales ($P > 0.05$) (Table 1).

3.2. Dynamics of soil microbial communities during and after erosion

During the 1 h rainfall simulation, bacterial abundance (BA) and fungal abundance (FA) significantly decreased (by 17.2%, $P < 0.05$ and 28.4%, $P < 0.05$, respectively) in plot II (Fig. 3f and g). The variations in these parameters were less pronounced in the other two plots and at the entire land scale (Fig. 3f and g). During the 132 h after the rainfall simulation, BA increased ($P < 0.05$) by 4.8% in the entire sloping land, but FA exhibited no significant changes ($P > 0.05$) (Table 2) at the same scale. At the plot scale, the variation pattern of FA in plot I ($P > 0.05$) differs from those in the other two plots; i.e., significant variations in this measure were found in plots II ($P < 0.001$) and III ($P < 0.05$) (Table 2). However, the BA in all the three plots was nonsignificantly different from the control at the $P < 0.05$ level (Table 2).

The structures of bacterial and fungal communities were analyzed by DGGE and the Shannon diversity index. Fig. 3h and g show that the Shannon indices of bacterial and fungal diversity (denoted as BH and FH, respectively) exhibited minimal changes during the 1 h rainfall simulation in the entire sloping land ($P = 0.05$). During the succeeding 132 h, however, FH showed remarkable dynamics (increased by 21.1%, $P < 0.05$) in the entire land, whereas BH did not ($P > 0.05$) (Table 2). At the plot scale, no significant change in the structures of the microbial communities was found (Table 2).

3.3. Relationship between microbial communities and soil property dynamics

The RDA results were listed in Table 3. The variation percentage (i.e., eigenvalue $\times 100\%$) explained by each measured property represents the individual contribution of that property without consideration for interaction among properties. Significant discrepancy was associated with the effects of different soil properties on soil microbial communities. The forward selection illustrates that BA was significantly related to erosion and SOC, which jointly contributed to 59.5% ($P < 0.01$) of the variances in BA. The individual contribution of these two variables accounted for 8.6% ($P < 0.05$) and 16.0% ($P < 0.05$) of the variations in BA, respectively. SOC and moisture were significant properties, jointly contributing to 38.8% of the variances in FA. However, only moisture had a statistically significant individual contribution to these variations (i.e., 13.8%, $P < 0.05$). The property critically contributed to the variations in BH was pH ($P < 0.05$), contributing 19.4% of the variations; it

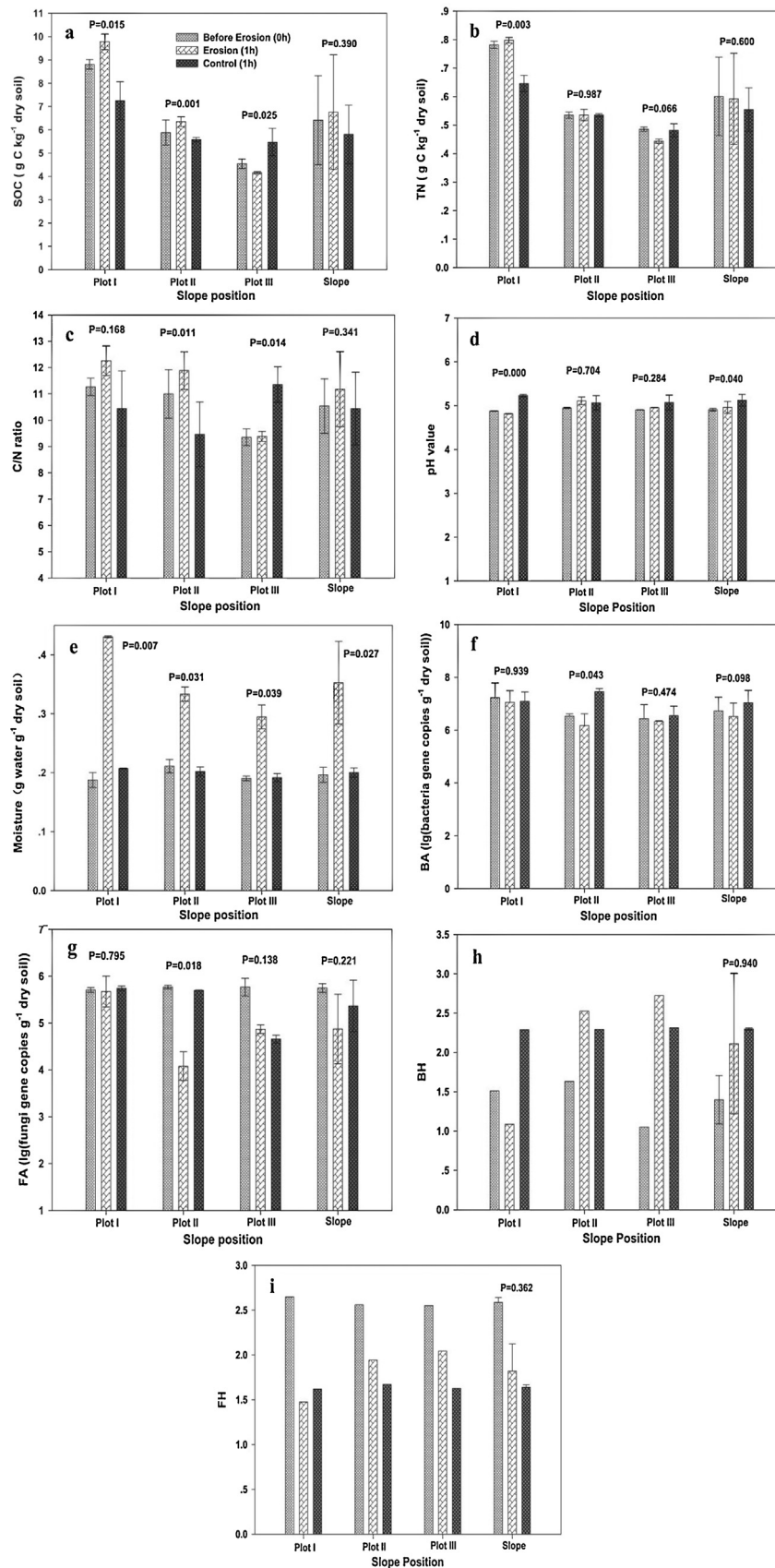


Fig. 3. Dynamics of soil properties and microbial communities in the sloping land during the rainfall simulation. The *P* value represents significant difference between the eroded and the control samples after rainfall. The error bars represent the standard errors of the means. BA, bacterial abundance; FA, fungal abundance; BH, Shannon index of bacterial diversity; FH, Shannon index of fungal diversity.

Table 2

Summary statistics of repeated measures ANOVA examining the effects of erosion on soil properties and microbial communities during the 132 h after the rain at different scales. Values are mean \pm SE. Values with the same letters are not significantly different at the $P < 0.05$ level as determined using a LSD test in repeated measures ANOVA model by SPSS 18 version. All of the comparisons were conducted between eroded and control samples.

Properties	Scale of entire sloping land		Scale of plot					
	Erosion	Control	Plot I-Erosion	Plot I-Control	Plot II-Erosion	Plot II-Control	Plot III-Erosion	Plot III-Control
SOC (g C kg ⁻¹ dry soil)	7.878 \pm 0.070 ^b	6.232 \pm 0.086 ^a	10.418 \pm 0.122 ^a	7.914 \pm 0.149 ^b	6.817 \pm 0.122 ^a	5.667 \pm 0.149 ^b	6.399 \pm 0.122 ^a	5.113 \pm 0.149 ^b
TN (g N kg ⁻¹ dry soil)	0.601 \pm 0.003 ^a	0.594 \pm 0.003 ^a	0.808 \pm 0.005 ^a	0.694 \pm 0.006 ^b	0.507 \pm 0.005 ^a	0.592 \pm 0.006 ^b	0.489 \pm 0.005 ^a	0.497 \pm 0.006 ^a
C/N	12.980 \pm 0.139 ^b	10.555 \pm 0.170 ^a	13.041 \pm 0.241 ^a	11.213 \pm 0.295 ^b	13.058 \pm 0.241 ^a	10.079 \pm 0.295 ^b	12.868 \pm 0.241 ^a	10.374 \pm 0.295 ^b
pH	4.963 \pm 0.017 ^a	5.021 \pm 0.021 ^a	4.879 \pm 0.029 ^a	4.951 \pm 0.036 ^a	5.075 \pm 0.029 ^a	4.966 \pm 0.036 ^a	4.936 \pm 0.029 ^a	5.145 \pm 0.036 ^b
Moisture (g water g ⁻¹ dry soil)	0.224 \pm 0.076 ^a	0.185 \pm 0.019 ^a	0.221 \pm 0.119 ^a	0.183 \pm 0.019 ^a	0.226 \pm 0.065 ^a	0.195 \pm 0.013 ^a	0.221 \pm 0.043 ^a	0.176 \pm 0.022 ^a
BA ^a (lg(bacteria gene copies g ⁻¹ dry soil))	6.864 \pm 0.069 ^b	7.199 \pm 0.069 ^a	7.410 \pm 0.119 ^a	7.385 \pm 0.149 ^a	6.639 \pm 0.122 ^a	7.215 \pm 0.149 ^a	6.544 \pm 0.122 ^a	6.997 \pm 0.149 ^a
FA ^b (lg(fungi gene copies g ⁻¹ dry soil))	5.567 \pm 0.022 ^a	5.603 \pm 0.022 ^a	5.782 \pm 0.037 ^a	5.832 \pm 0.037 ^a	5.348 \pm 0.037 ^a	5.608 \pm 0.037 ^b	5.570 \pm 0.037 ^a	5.371 \pm 0.037 ^b
BH ^c	1.864 \pm 0.157 ^a	1.941 \pm 0.210 ^a	1.408 \pm 0.084 ^a	1.690 \pm 0.256 ^a	1.882 \pm 0.276 ^a	1.529 \pm 0.205 ^a	2.301 \pm 0.274 ^a	2.603 \pm 0.430 ^a
FH ^d	2.335 \pm 0.084 ^a	1.927 \pm 0.129 ^b	2.261 \pm 0.210 ^a	1.896 \pm 0.209 ^a	2.370 \pm 0.114 ^a	1.799 \pm 0.311 ^a	2.375 \pm 0.122 ^a	2.087 \pm 0.152 ^a

^a Bacterial abundance.

^b Fungal abundance.

^c Shannon index of bacterial diversity.

^d Shannon index of fungal diversity.

provided a contribution of 37.7% ($P < 0.01$) in combination with some other properties. Water erosion ($P < 0.05$) and moisture ($P < 0.05$) individually contributed 23.6 and 18.6% of the variations in FH, respectively. The combined contribution of these two variables accounted for 46.1% ($P < 0.05$) of the variances in FH.

Although no definite individual explanation for the effects of these variables was generated, they cannot be disregarded. Their interaction with other variables may exert significant effects, as evidenced by the fact that the combined explanation for all the variables reflects 65.6% ($P < 0.05$), 43.4% ($P > 0.05$), 55.0% ($P < 0.05$), and 46.3% ($P < 0.05$) as the proportions accounting for variations in BA, FA, BH, and FH, respectively. According to the RDA results (Fig. 4), BA and FA were positively correlated with SOC, TN, and C/N ratio but negatively correlated with moisture. The Shannon index of microbial diversity (i.e., BH and FH) was positively correlated to pH. A negative correlation was found between BH and C/N ratio and SOC and TN. FH was positively correlated with C/N ratio and SOC but negatively correlated with soil moisture (Fig. 4).

4. Discussion

The main purpose of this study is to investigate how microbial communities of topsoil (0–10 cm) vary under stress caused by water erosion in sloping lands during and a short period after rain. The main finding is that the microbial communities at different positions of the studied sloping land markedly differed in terms of susceptibility to stress from water erosion. Both microbial abundance and community structures were significantly dynamic during the rainfall simulation and for 132 h after. The perturbation induced by water erosion during the rainfall simulation changed the soil properties and distribution of soil microorganisms in the sloping land. SOC and C/N ratio significantly changed in the entire sloping land during the 132 h after the rainfall simulation. At the plot scale, most of the soil properties and microbial communities exhibited dynamic changes with sampling time. A close statistical relationship among these measures was also detected, indicating that water erosion-induced changes in soil properties may profoundly affect the dynamics of microbial communities in the studied sloping land.

4.1. Microbial responses to water erosion and SOC dynamics during rainfall simulation

After the rainfall simulation, the qPCR results reveal that BA and FA significantly decreased in plot II, which suffered severe erosion. These results support our hypothesis that water erosion may contribute to microbial community redistribution in the sloping land. A direct explanation is that the topsoil, in which more microorganisms survived than in the subsoil given superior conditions (Fengxue et al., 2000), was eroded and transported out of the plot. As a major factor that affects the vertical and horizontal movements of microorganisms in soil, rainfall caused serious microbial migration on sloping soil surfaces through runoff action (Abu-Ashour and Lee, 2000). The water potential associated with rewetting a dry soil can kill a large fraction of microorganisms (Schimel et al., 1999). To mitigate the influence of drying and sudden rewetting on microbial activity, the studied land (both erosion plots and their controls) was pretreated by evenly spraying sterile water on the soil surface. Another explanation for the decrease in microbial abundance is that microorganism death/lysis was caused by the splash (Turner et al., 2003), collision, and shear force induced by raindrops and runoff, especially for the microorganisms released from broken soil aggregates. These microorganisms therefore lacked protection. Nonetheless, the bacteria and fungi differed in their ability to resist

Table 3

Partial RDAs testing of the sole influence on microbial communities by each factor and all significant factors, respectively. For each partial model in Partial RDAs, the other factors were used as covariables. Plot and erosion are used as qualitative explanatory variables (i.e., nominal variables), the *P* values was estimated using Monte Carlo permutations with *n* = 499.

Property	Eigen value				<i>P</i> value			
	BA ^a	FA ^b	BH ^c	FH ^d	BA	FA	BH	FH
Plot	0.006	0.001	0.016	0.006	0.844	0.998	0.688	0.884
Erosion	0.086	0.002	0.041	0.236	0.036	0.792	0.148	0.018
SOC	0.160	0.004	0.028	0.083	0.012	0.654	0.244	0.114
TN	0.002	0.010	0.011	0.005	0.740	0.528	0.482	0.640
C/N	0.013	0.005	0.025	0.044	0.430	0.662	0.260	0.228
pH	0.008	0.021	0.194	0.004	0.466	0.404	0.008	0.728
Moisture	0.032	0.138	0.022	0.186	0.136	0.034	0.304	0.016
Above factors together	0.656	0.434	0.550	0.463	0.004	0.106	0.020	0.076
All significant factors together	0.595	0.388	0.377	0.461	0.002	0.014	0.002	0.008

^a Bacterial abundance.

^b Fungal abundance.

^c Shannon index of bacterial diversity.

^d Shannon index of fungal diversity.

erosion stress given that FA decreased to a greater extent than did BA during erosion. This finding can be attributed to by the fact that fungi are usually located at the outer part of soil aggregates (i.e., in large pores and on aggregate surfaces), whereas bacteria are located in small pores (Gordon et al., 2008), making the former more sensitive to soil disturbance.

A number of factors can indicate why the SOC levels in all the three plots significantly differed from those in the corresponding controls after the 1 h rainfall simulation. First, the serious erosion and transportation caused by raindrops and runoff are likely important factors. SOC can be eliminated by a selective process of water erosion, redistributed over the landscape, and deposited in depression sites. Second, nutrient leaching from topsoil may be related to SOC dynamics (Zhang et al., 2006). The increased amount of dissolved organic substrates derived from aggregate slaking and microbial death may stimulate SOC leaching, thereby changing its distribution pattern in the topsoil (Gordon et al., 2008). Third, as an unobservable process (except through modeling), the depletion of SOC by decomposition and mineralization during erosion is, to a certain extent, responsible for the SOC changes in the studied sloping land. Previous research has shown that abundant SOC is physically protected in the soil aggregates, which are sensitive to water erosion (Shi et al., 2010). Perturbation by erosion can disrupt soil aggregates and expose SOC to microbial attacks. The metabolism of decomposers may cause considerably dissociative SOC in runoff to be mineralized during transport. Although this phenomenon has attracted considerable attention, the mechanisms responsible for SOC depletion and the extent to which dissociative SOC can be degraded during erosion remain unclear. The results of this study suggested that significant attention should be paid to these dissociative microorganisms as eroded SOC dynamics is traced.

4.2. Microbial dynamics in relation to SOC variation

As evidenced in this study, most of the properties of the sloping soil were significantly influenced by water erosion during the rainfall simulation. The variations in soil microbial communities across the landscape were often strongly correlated with the heterogeneity and dynamics of the soil properties (Rousk et al., 2010). These properties may be directly or indirectly related to the variations in microbial communities through individual effects or through interaction effects among them. Erosion (i.e., the nominal variable in the RDA model) and SOC significantly explained the changes in BA. These two variables jointly contributed to 59.5% of the BA variations, of which 34.9% can be attributed to the interaction between

them. On the one hand, this finding is consistent with that of Six et al. (2006), who suggested that microbial dynamics is directly influenced by soil texture, soil structure, and the pore-size distribution in soil aggregates; such dynamics can be severely disturbed by water erosion and simultaneous sediment movement (Quinton et al., 2010; Shi et al., 2012). On the other hand, this finding indicates that erosion may affect bacterial dynamics mainly by causing SOC redistribution in sloping land. Therefore, the results support our previous hypothesis that SOC distribution in the land shaped by water erosion may interact with soil microbial dynamics. Moisture individually contributed to 13.8% of the variances in FA but did not significantly affect BA. This result is consistent with that of Griffiths et al. (2003), who found that soil fungal biomass is more susceptible to water stress than is bacterial biomass in the eroded soil after erosion. This susceptibility is attributed to the adaptation of bacterial cells to moisture variations through the regulation of cellular activity (Gordon et al., 2008). In the present study, SOC positively influenced FA, but this effect was caused by its interaction with the other variables. By contrast, SOC exerted no significant individual influence on FA variations. This finding is consistent with that of Bossuyt et al. (2001), who suggested that substrate quality can alter fungal/bacterial ratios, with high carbon content favoring fungi and low carbon content favoring bacteria. In the current work, a positive correlation was detected between SOC and BA, as well as between SOC and FA. Thus, the enrichment in SOC caused by selective migration induced by erosion may stimulate microbial dynamics because of substrate availability.

Chowdhury et al. (2011) suggested that microbial community structure is more resistant to soil disturbance than is microbial abundance. However, the authors conducted their study at the total microbial community scale. In the present study, the FH in the studied land significantly increased across the sampling period after erosion, but BH did not show any statistical change. This result indicates that fungal community structure is more sensitive to the stress from water erosion. This finding is consistent with that of Gordon et al. (2008), who found that fungal, rather than bacterial and total microbial, community structure is significantly dynamic under the stress induced by soil drying and rewetting. The RDA results in the current research illustrate that only pH was positively correlated with BH and significantly contributed to 19.4% of its variances; it imposed no significant influence on FH. Similarly, Rousk et al. (2010) found that bacterial community structure is closely defined by soil pH, whereas fungal community structure is only weakly related to this property. Significant influence of erosion and moisture on FH was observed in the present work, with the combined contribution of these two properties accounting

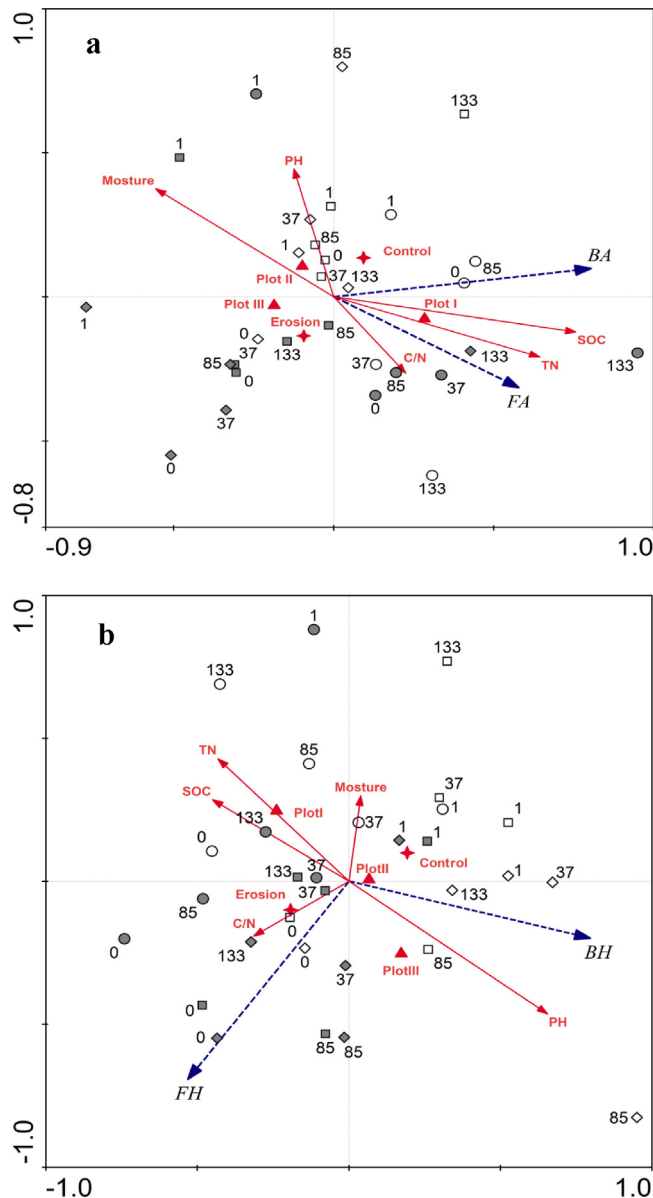


Fig. 4. Overall RDA of the correlation among water erosion (a nominal variable in the RDA model), plot (a nominal variable in the RDA model), soil properties, microbial abundance (a), and diversity index (b), as determined by Canoco. The circle, square, and diamond represent the samples from plots I, II, and III, respectively. The charcoal gray and white fill colors of the aforementioned shapes represent the eroded and control samples, respectively. Numbers refer to sampling hours. The intersection angle between vector lines represents the significance of the correlation between their corresponding variables, in which a sharp angle represents positive correlation, an obtuse angle represents negative correlation, and a right angle represents no significant correlation. BA, bacterial abundance; FA, fungal abundance; BH, Shannon index of bacterial diversity; FH, Shannon index of fungal diversity.

for 36.1%. In the same manner, Zhang et al. (2010) observed that fungal, rather than bacterial, community structure is significantly correlated with moisture. Fungal diversity is likely more vulnerable to moisture content change than bacterial diversity. As revealed by the experiments in the current study, water erosion exerted a more pronounced effect the fungal community structure than on its abundance; the opposite was true for bacteria. As two main agents that mediate soil carbon transformations (Reineke and Knackmuss, 1979), therefore, bacteria and fungi may considerably regulate the carbon cycling of eroded soil because these microorganisms differentially respond to water erosion in terms of abundance and

community structure. We suggest that future research on quantifying the impact of erosion on soil carbon link the observed changes in SOC to the quantification of microbial community function. Providing insight into the dynamics of a certain functional group or species that is sensitive to erosion is a valid approach to clarifying the complex biological mechanisms related to eroded carbon dynamics.

5. Conclusion

The effects of water erosion on the soil microbial communities and properties of a sloping land in southern China were examined. One hour rainfall was simulated, and soil samples (0–10 cm) were collected from three plots along the sloping land. Microbial abundance and most of the soil properties were substantially influenced by water erosion. A combination of physical mechanisms that occurred during the rainfall simulation can explain the variations in microbial abundance in the sloping land. Because of out-migration with runoff from the plots, a considerable amount of microorganisms, which may have been lost rather than redistributed in the sloping land during the rainfall simulation, could not be detected by plot experiment. Therefore, further prospective studies are required to investigate water erosion effects at a large scale. During the 132 h after water erosion, the erosion-induced changes in soil properties caused flowing microbial community dynamics. The RDA results reveal that water erosion significantly affected BA and FH. A positive correlation was observed between SOC and BA, as well as between SOC and FA. Although our study suggests that the selected variables (i.e., SOC, TN, C/N, pH and moisture) satisfactorily explains the variations in soil microbial communities after erosion, a small part of variations in microbial communities cannot be accounted for by such variables. This unexplained component is typically interpreted as the variations caused by unmeasured environmental variables, including soil temperature, soil aeration, and complex interactions in biological processes. Because bacteria and fungi differentially respond to water erosion in terms of abundance and community structure, our work suggests that providing insight into the dynamics of a certain functional group or species that is sensitive to erosion is necessary for clarifying the complex biological mechanisms related to the eroded carbon budget.

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