Soil bacterial diversity and its determinants in the riparian zone of the Lijiang River, China

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This study was designed to analyse the soil bacterial community composition and diversity, as well as their relationships with various environmental factors in a riparian ecosystem. The 16S rRNA sequencing technology was applied to profile the bacterial composition of 120 samples from four different transects (long, moderate, less and rare inundation) in ten different study sites along the Lijiang riparian zone; the corresponding soil properties were also measured. The results indicated that, diversity was lowest in the rare inundation transect and there were high yet not significantly different bacterial community diversities in the long, moderate, and less inundation transects. The dominant bacterial groups of the four transects were similar, but there were great differences in the abundances of specific groups. Proteobacteria (29.28%), with the dominant classes of Beta- (15.65%), Delta- (5.75%), Gamma- (4.46%) and Alpha-proteobacteria (3.32%), was the most abundant phylum in the studied riparian soils. The genus *Candidatus Nitrososphaera* including ammonia-oxidizing archaea (AOA) and the genus *Nitrospira* including nitrite-oxidizing bacteria were both sensitive to inundation gradient changes. Redundancy analysis revealed that soil properties such as soil pH, inundation frequency, sand content, soil water content, total N and available N were significantly correlated with the bacterial community diversity and structure. The study suggests that the flood disturbance gradient and the spatial heterogeneity of soil properties affect the composition and diversity of bacterial communities in the Lijiang riparian zone.

Keywords: Bacterial diversity, inundation, river zone, 16S rRNA sequencing, soil physicochemical properties.

MICROBIAL communities play a key role in ecosystems and influence a large number of important ecosystem processes, including biogeochemical cycles, organic matter decomposition, nutrient acquisition, pollutant purification and soil structure maintenance[1,2]. However, the composition and diversity of the microbial community may be affected by environmental changes[3,4]. Spatially, on a fine scale of centimeters, microbial community composition can change and are mainly affected by soil pore structure[5], microbial interaction, rhizosphere effects[6]. On the scale from meters to kilometers, they are mainly affected by soil heterogeneity (e.g. soil pH, oxygen concentration and nutrition content)[7–9], vegetation[10] and topography[11] and other factors. On a larger scale, from hundreds to even thousands of kilometers, they are mainly affected by soil, climatic and geographical isolation conditions[12]. Soil microbial communities temporally change over shorter (e.g. rainfall process) or longer (e.g. seasonality, ecological succession) time scales. They are also affected by perturbations, flood disturbance, for instance, often affects microbial processes. Rinklebe and Langer[13] compared three types of floodplain soils with different flood regions and proposed that phospholipid fatty acids, soil microbial carbon, basal respiration and metabolic quotient were strongly influenced by gradients in elevation, type of soil, flooding duration, time since the various soils were last flooded, plant communities and their residues and by pH value, all of which may influence the microbial diversity. Moche *et al.*[14] concluded that the more stable properties of the bulk soil such as the magnitude of soil organic carbon, soil texture and associated flood duration had a stronger impact on soil microbial communities than monthly fluctuations of more dynamic properties, such as soil moisture and soil temperature. Wilson *et al.*[15] proposed that flooding caused significant changes in the microbial community structure, and the duration of the flooding was also important for carbon dynamics and microbial community structure.

Riparian zones are critical ecotones between the aquatic ecosystem and the terrestrial ecosystem[16]. Their characteristics of an edge effect and rich biodiversity make riparian zones some of the most dynamic, diverse and complex ecosystems on earth[17,18]. The interactions between hydrogeomorphic (flood dynamics) and ecological processes (biological succession) create a dynamic mosaic of habitat patches – from a bare gravel bar to a thick riparian forest. These patches, characterized by the contrasting and changing functional performance, such as differences in their inundation regime, stage of succession, soil properties, community composition, system metabolism and nutrient cycling, are defined as functional

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process zones[19,20]. High diversities of aerobic and anaerobic microbes have coexisted in the riparian soils, but this diversity is heterogeneously distributed among the functional process zones of the riparian zones[21]. Thus, riparian soils offer an ideal setting in which to study the spatial variations in the composition and diversity of soil microbes.

Lijiang River, as the soul of 'Guilin Best' and the world famous 'golden waterway', has a typical karst landscape, mountains surrounded by water, twists and turns of the natural scenery and abundant animal and plant resources, which make the Lijiang ecosystem have tremendous resources, environmental, economic and social value. However, a series of environmental problems, such as uneven distribution of water resources, cause seasonal flood and drought, vegetation degeneration, wetland shrinkage and water pollution along the Lijiang River[22]. Therefore, the protection and restoration of the Lijiang River ecosystem is an urgent necessity.

We hypothesized that the environmental conditions to which riparian soils have been exposed (mainly inundation regime) may affect soil properties and soil bacterial diversity and structure. Therefore, we have studied the following issues: (i) the spatial variations of soil bacterial diversity and community composition in the different transects (long inundation to rare inundation) of the Lijiang River riparian zone; (ii) the relationship between soil properties and bacterial structure and diversity within the Lijiang River riparian zone and the driving factors behind bacterial diversity and structure. In conclusion, we aim to study the composition and diversity of bacterial communities and their response and feedback to environmental changes.

Materials and methods

Study sites

The Lijiang River is located in the northeastern part of Guangxi Province, China (E110°18′–110°18′, N25°59′–23°23′) (Figure 1). It belongs to the Pearl River system and covers a distance of 214 km and a catchment area of 12 285 km^2 (ref. 23). The region of the Lijiang River is characterized by a subtropical humid monsoon climate. The average annual temperature is 17.8–19.1°C. The average annual precipitation is 1814–1941 mm. There is abundant annual runoff (mean annual runoff is 120–130 m^3/s) but the distribution is uneven throughout the whole year. The flood season lasts from March to August, during which the runoff accounts for 80% of total annual volume[24]. The Lijiang River basin has a karst landform, with widely exposed carbonate rocks. The river from Guilin to Yangshuo is the most typical karst development area. The river channel is composed of sand and pebbles. The soil type is red loam with high sand content.The trees are mainly *Pinus massoniana*, *Pterocarya tonkinensis*, *Cinnamomum burmannii*, *Phyllostachys heterocycla* cv. *Gracilis* and some other similar species; the shrubs are *Geumaleppicum* and *Vitex negundo* and the herbs are mainly *Cynodon dactylon*, *Polygonum hydropiper* and *Humulus scandens*[25].

Experimental design and sampling

Ten study sites were set on the left riparian zone along the Li River of Guilin to Yangshuo (Figure 1). From the upper stream to downstream, three sites (C1, C2 and C3) were established in the city, four sites (S1, S2, S3 and S4) in the suburbs and the last three sites in the mountain tourist zone (M1, M2 and M3). To assess the effects of the inundation regime on the soil bacteria, each site was divided into four types of transects called long inundation (Lon-inu), moderate inundation (Mod-inu), less inundation (Les-inu) and rare inundation (Rar-inu) according to the different flooding frequencies, distance to the river and vegetation types (Figure 2). Close to the river, Lon-inu corresponds to inundation approximately nine months per year with high hydraulic energy, Mod-inu corresponds to inundation seven months per year, Les-inu

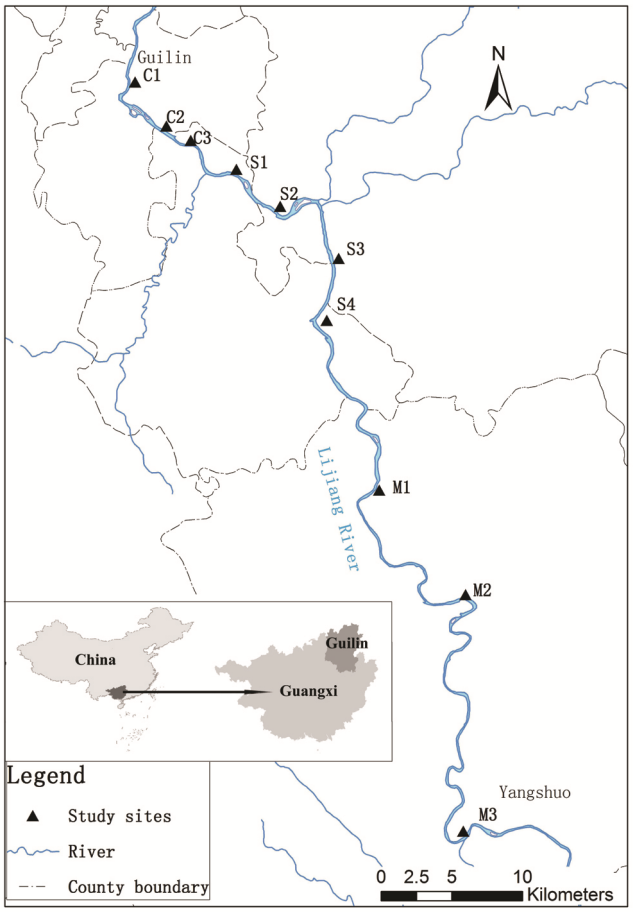

Figure 1. Locations and sampling sites in the riparian zone of the Lijiang River. C1-3, S1-4, M1-3 respectively, represent city, suburban and mountain tourist zones.

corresponds to inundation four months per year and Rar-inu corresponds to inundated approximately 1.5 months per year. The corresponding vegetation types were bare gravel beach (scattered herbs of *Cynodon dactylon* and *Polygonum hydropiper* L.), grassland (*Cynodon dactylon*, *Polygonum hydropiper* L. and *Humulus scandens*), shrubland (*Geum aleppicum*, *Nerium indicum* Mill and *Vitex negundo* L.) and woodland (*Pterocarya tonkinensis*, *Cinnamomum burmannii* and *Phyllostachys heterocycla* cv. *Gracilis*). The widths of Lon-inu, Mod-inu and Les-inu transects are 20–45 m, while that of Rar-inu is from approximately 50 to 100 m.

Three replicate points were chosen randomly for soil sampling within each inundation transect in September 2014 (avoiding the rainy season). Along an S-shape sampling route, mineral soils in the upper 15 cm were collected from 5 to 8 locations within each sample points and they were then mixed to compose a soil sample. A total of 120 soil samples were obtained. Soil was placed in a portable refrigerator and transported to the laboratory. Each soil sample was sieved (2 mm), homogenized and separated into two subsamples. One subsample was used for analysis of soil physicochemical properties after being air-dried and the other was kept frozen at –80°C for DNA extraction and molecular analysis.

Soil physicochemical properties

The physicochemical properties of the soil were analysed according to the standard methods[26]. Soil pH was determined by the potential method with 1 : 2.5 ratio of soil to water. Soil water content (SWC) was measured after over-drying the soil at 105°C for 24 h. Soil organic matter (SOM, g kg^{-1} dry soil) was determined by the dichromate oxidation method. Total N was measured by Kjeldahl digestion procedures (TN, g kg^{-1} dry soil). Total P was determined colorimetrically by the ascorbic acid method at a wavelength of 700 nm (TP, g kg^{-1} dry soil). Total K was measured by means of a flare photometer (TK, g kg^{-1} dry soil). Available N was determined by alkaline hydrolysis diffusion (AN, mg kg^{-1} dry soil). Available P was determined by the hydrochloric acid–sulphuric acid extraction method (AP, mg kg^{-1} dry soil). Available K was measured by ammonium acetate extraction and flame photometry (AK, mg kg^{-1} dry soil). All soil physicochemical properties are determined according to the standard methods[26]. Each sample was measured three times.

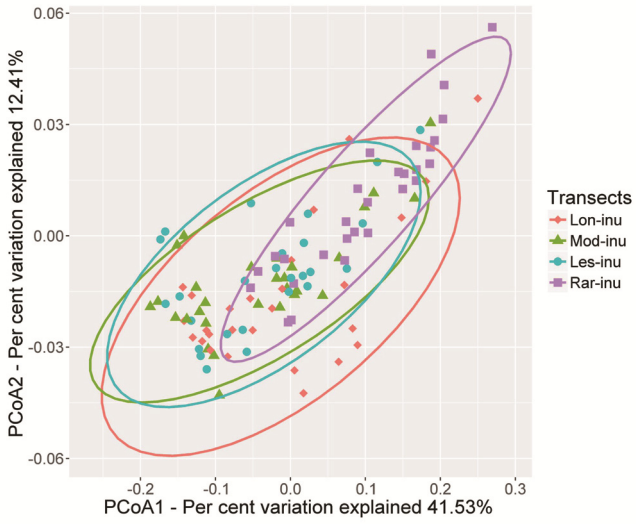

Figure 2. Sectional sketch map of the riparian zone of the Lijiang River.

DNA extraction, 16S rRNA gene amplification and barcoded sequencing

DNA extraction. The DNA was extracted with a Power Soil DNA Extraction Kit (MO BIO Laboratories USA) according to the manufacturer's instructions. To quantify and check the DNA integrity, a 5 µl aliquot was subjected to electrophoresis on a 1% agarose gel and quantified using 2 µl of Low DNA Mass Ladder (Invitrogen Technology China).

16S rRNA gene amplification and barcoded sequencing. The universal primer set 515F/806R was used to amplify the bacterial and archaeal specific V4 hypervariable region of the 16S rRNA genes[27] with a 12-bp barcode specific to the soil subsample on R806. Samples were amplified in triplicate following the thermal cycling described previously[28]. Replicate PCR reactions for each sample were pooled and purified using a QIAquick Gel Extraction Kit (Qiagen, Chatsworth, CA, USA). The purified composite DNA sample was sequenced using an Illumina MiSeq platform with paired end reads of 250 bp in length.

Processing of sequencing dataset. Raw data were processed with pipeline coupling Mothur[29] and QIIME[30] software. The commands shhh.flows[31], pre.cluster[32] and chimera.uchime[33] were used to denoise the sequence data, reduce sequencing error and identify and remove chimeras, respectively. Operational taxonomic units (OTUs) were identified with uclust at the 97% sequence similarity level[34]. Subsequently, a representative sequence was selected from each OTU and the taxonomic assignment was achieved using the Ribosomal Database Project (RDP) Classifier[35] with a minimum confidence interval of 80%. The alpha diversity (OTUs, Chao1, Faith's phylogenetic diversity (PD), Shannon and Invsimpson) and beta diversity (unweighted and weighted UniFrac distances) were estimated on the basis of a subset of 3,700 randomly selected sequences per community with 100 iterations. UniFrac-based principal-coordinate analysis (PCoA) was used to find clusters and the most important axes of variation among samples.

Statistical analysis

Different α-diversity indices (OTUs, Chao1, phylogenetic diversity, Invsimpson and Shannon) were calculated using Mothur[29]. To evaluate differences in the soil physicochemical properties and bacterial α diversity, ANOVA and Tukey's test ($P < 0.05$) were performed. To explore dissimilarities in the bacterial community based on the Bray-Curtis distances, principal coordinate analysis (PCoA) was applied using the vegan package of R-language[36].

To determine the impact of the soil physicochemical properties on bacterial community α-diversity and the relative abundances of the dominant phyla, redundancy analysis (RDA) and Monte Carlo permutation tests ($P < 0.05$) were performed using the vegan package[36]. Bacterial α-diversity matrices and the dominant phyla relative abundance matrices were alternatively used as response matrices, the soil physicochemical dataset was used as an explanatory matrix. The forward selection procedure was used during the analysis to identify the attributes with the highest discriminatory power[5,36].

Results

Soil physicochemical properties

Soil physicochemical properties varied across the study area (Table 1). The soils of the Lijiang River riparian zone were sand-based, with contents ranging from approximately 74.99% to 88.44%, far higher than both silt and clay. With the decrease of inundation frequency, the sand content decreased while the clay and silt content increased gradually. Soil pH value, SWC, AN, AP and AK increased initially and subsequently decreased with the decline of the inundation frequency. Additionally, the pH was highest in Mod-inu (7.71) and lowest in Rar-inu (6.99), while the other five variables were largest in Les-inu and lowest in Lon-inu. SOM and TN increased gradually with the decrease of inundation frequency.

Alpha diversity of bacterial communities

We used barcoded sequencing to analyse the bacterial and archaeal 16S rRNA genes across all of the soil samples, generating 2,213,657 quality sequences, with the sequences for individual samples ranging from 3,734 to 44,344. In total, 89,925 OTUs were identified in the complete data set, with an average of 1242 to 1880 OTUs per sample. A comparison of OTUs, Chao1, Faith's phylogenetic diversity (PD), Shannon and Invsimpson revealed differences in the bacterial communities' diversity of the studied soil groups (Table 2). The number of OTUs was in Rar-inu soils and the Rar-inu also showed lowest community diversity, while the remaining three transects (Lon-inu, Mod-inu and Les-inu) exhibited higher community diversity but no significant differences between them. The other diversity parameters (PD, Shannon and Invsimpson) are consistent with these results.

Beta diversity of bacterial communities

The principal coordinate analysis (PCoA) showed that there were strong differences in the soil bacterial community among the different transects (Figure 3), which is evident by 53.94% of the total variance in the weighted UniFrac distances. The bacterial communities displayed clear clustering on the ordination plot according to the studied soil groups; the samples of Rar-inu clearly separated from the other three transects (Lon-inu, Mod-inu and Les-inu). This indicates that different sampling sites in the riparian zone may have certain effects on the soil bacterial community composition.

Composition of bacterial communities

A total of 76 phyla were detected in the complete data set, and most of them could be assigned to the domain Bacteria. The top 11 phyla were defined as dominant (average relative abundance ≧ 1%); among them, *Proteobacteria* (29.28%) was the predominant phylum in all soils, followed by *Acidobacteria*, *Crenarchaeota*, *Verrucomicrobia*, *Planctomycetes*, *Bacteroidetes*, *Nitrospirae*, *Chloroflexi*, *Actinobacteria*, *Gemmatimonadetes* and *Firmicutes*, accounting for 19.15%, 15.94%, 7.48%, 6.04%, 5.56%, 4.51%, 3.51%, 2.20%, 2.12% and 1.02% of total quality sequences respectively (Figure 4). The class level distributions of *Proteobacteria* showed that *Betaproteobacteria* (15.65%), *Deltaproteobacteria* (5.75%), *Gammaproteobacteria* (4.46%) and *Alphaproteobacteria* (3.32%) were the four major classes within this phylum.

The structure of the bacterial communities differed among the different types of transects (Figure 4). On the phylum level, with the decreasing of inundation frequency, the relative abundance of the phyla *Proteobacteria* and *Bacteroidetes* increased initially and then decreased, while the relative abundances of *Acidobacteria*, *Crenarchaeota* and *Planctomycetes* decreased initially and subsequently increased. The relative abundance of *Verrucomicrobia* tended to gradually increase, while those of *Nitrospirae* and *Chloroflexi* decreased. This indicated that these bacterial phyla are responsive to changes in the inundation gradient.

On the genus level, the structure of the bacterial communities also differed among the transects (Figure 5). *Candidatus Nitrososphaera* was the most dominant genus in each transect soil, and the relative abundance changed from 10.01% (Les-inu) to 22.18% (Rar-inu), indicating that it may be sensitive to the change of the riparian flood inundation. Next was *Nitrospira*, whose relative abundance

Table 1. Average values and standard errors of chemical parameters in different groups of riparian soils

Transects	Lon-inu	Mod-inu	Les-inu	Rar-inu	*P*
Sand (%)	88.44 ± 0.45d	81.59 ± 1.08c	77.70 ± 1.16b	74.99 ± 1.33a	***
Silt (%)	6.17 ± 0.29a	10.00 ± 0.57b	12.16 ± 0.74c	14.07 ± 0.98d	***
Clay (%)	5.40 ± 0.36a	8.40 ± 0.56b	10.14 ± 0.52c	10.94 ± 0.42d	***
SWC (%)	13.52 ± 2.39a	21.43 ± 3.20b	24.01 ± 2.91b	16.86 ± 1.62a	***
pH	7.32 ± 0.11b	7.71 ± 0.15c	7.45 ± 0.07b	6.99 ± 0.12a	***
SOM (g kg^{-1})	23.72 ± 3.17a	26.38 ± 3.55a	26.76 ± 2.83a	32.68 ± 1.98b	***
TN (g kg^{-1})	0.93 ± 0.25a	1.40 ± 0.17b	1.22 ± 0.14ab	1.35 ± 0.27b	***
TP (g kg^{-1})	0.54 ± 0.18a	0.60 ± 0.05a	0.54 ± 0.03a	0.57 ± 0.04a	*
TK (g kg^{-1})	89.68 ± 7.66bc	76.92 ± 2.39a	94.71 ± 5.77c	83.39 ± 3.90ab	***
AN (mg kg^{-1})	41.72 ± 12.61a	81.92 ± 16.75b	109.56 ± 12.88c	100.00 ± 13.48c	***
AP (mg kg^{-1})	6.01 ± 1.35a	10.71 ± 1.30bc	11.84 ± 1.49c	8.86 ± 1.76b	***
AK (mg kg^{-1})	30.78 ± 5.67a	44.53 ± 7.77b	71.77 ± 12.61d	57.25 ± 7.68c	***
Inundation frequency in 2014 (months)	9	7	4	1.5	

Average values followed by the same letter are not significantly different between soil groups according to two-way ANOVA and Tukey's test.
SWC, Soil water content; SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; AK, available potassium. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant.

Table 2. Average values and standard errors of the OTUs, Chao1, PD, Shannon and Invsimpson indexes of the bacterial communities in different groups of riparian soils

	Alpha diversity				
Transects	OTUs	Chao 1	PD	Shannon	Invsimpson
Lon-inu	1722 ± 37b	4669 ± 155.95b	181 ± 3.23b	6.582 ± 0.11b	182.014 ± 41.53b
Mod-inu	1728 ± 32b	4477 ± 170.40a	178 ± 3.07b	6.579 ± 0.11b	181.090 ± 39.16b
Les-inu	1743 ± 34b	4670 ± 145.13b	178 ± 3.34b	6.579 ± 0.11b	181.090 ± 41.28b
Rar-inu	1572 ± 37a	4406 ± 148.76a	162 ± 3.39a	6.558 ± 0.10a	172.122 ± 37.98a
P	***	**	***	***	***

Average values followed by the same letter are not significantly different between soil groups according to two-way ANOVA and Tukey's tests.
OTUs, Operational taxonomic units; PD, Faith's phylogenetic diversity. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant.

decreased with the decline of the riparian inundation frequency. The relative abundances of *Dechloromonas* and *Geobacter* first increased and then decreased as the inundation frequency declined.

Association between soil properties and bacterial communities

The redundancy analysis showed that pH, Inu_fre, SWC, TK, AK, Dist_city, TN, TP and sand showed the highest correlation with the indexes of the bacterial diversity (Figure 6 *a*). Of the total variation, 39.88% was explained by axis 1 and axis 2 accounted for only 4.22%. Environmental variables explained 44.88% of the total variation. Axis 1 of RDA (most variance explained by pH, Inu-fre, Sand, SWC) was significantly correlated with the OTUs, PD, Invsimpson and Shannon indices of the bacterial communities diversity measures, whereas axis 2 of RDA (variance mostly explained by TN) was significantly correlated with the Chao1 index of the bacterial communities of soils. Thus, we conclude that soil pH, Inu_fre, Sand and TN had a high influence on diversity indices of the bacterial communities. Moreover, Inu_fre was positively correlated with bacterial diversity indicating that the riparian bacterial diversity of the Lijiang River trended from high to low diversity in the direction away from the water body.

Additionally, Inu_fre, SWC, pH, Dist_city, TN, Sand, AN, AK and TK had the highest correlation with the relative abundance of the dominant phyla using the forward selection methods (Figure 6 *b*). Axis 1 and axis 2 explain 18.27% and 8.63% respectively, of the total variation, while the environmental variables account for 36.81%. Axis 1 of RDA (most variance explained by pH) was significantly correlated with the relative abundances of *Proteobacteria*, *Bacteroidetes*, *Crenarchaeota*, *Planctomycetes*, *Acidobacteria*, *Chloroflexi* and *Nitrospirae*. Axis 2 of RDA (variance mainly explained by AN, SWC, Sand) was significantly correlated with the relative abundances of *Verrucomicrobia* and *Firmicutes*. Thus, we conclude that soil pH was significantly affected by the relative abundances of the *Proteobacteria*, *Bacteroidetes*, *Crenarchaeota*, *Planctomycetes*, *Acidobacteria*, *Chloroflexi* and *Nitrospirae*. Similarly, AN, SWC and sand was significantly affected by the relative abundances of *Verrucomicrobia* and *Firmicutes*, therefore revealing

that the structure of the bacterial communities was influenced.

Discussion

Bacterial communities diversity

The bacterial communities of the Lijiang River riparian soils showed significant spatial complexity. The first three transects (Lon-inu, Mod-inu and Les-inu) have the same and higher α-diversity of the bacterial communities present, and the bacterial community diversity in Rar-inu was lower. PCoA indicated that the bacterial communities can be separated clearly from the other three transects (Lon-inu, Mod-inu and Les-inu). As the critical ecotones between aquatic ecosystem and terrestrial ecosystem, riparian zones show an obvious edge effect, resulting in the great biological diversity and habitat complexity. The Lon-inu, Mod-inu and Les-inu are located in the centre of the riparian zone, where inundation frequency is quite high, and thus there are lots of anaerobic and aerobic bacteria coexisting in the soil, leading to higher bacterial diversity; while the Rar-inu are near the land, where inundation frequency is much lower, and thus there is much less anaerobic bacteria in soil, leading to a lower bacterial diversity. The flood pulse theory also suggests that the flood pulse enhances the biodiversity in riparian ecosystem[37]. In addition, some studies showed that the microbial diversity is significantly positive correlated with soil pH within certain range[38], and the lower diversity occurs under acidic conditions while peak diversity is associated with near-neutral pHs[39]. Soil pH in Rar-inu is lower than the other three transects, and that may also be the reason of the lower bacterial diversity.

Bacterial community composition

We detected 80 different bacterial phyla in the studied samples, but more than 83.44% of the sequences in each sample belonged to only six phyla (*Proteobacteria*, *Acidobacteria*, *Crenarchaeota*, *Verrucomicrobia*, *Planctomycetes*, *Bacteroidetes*), indicating that less than one-fifth of sequences represented rare taxa. A similar trend has been observed in previous studies in freshwater[40], both created and natural wetland soils[41,42] and marine sediments[43]. This suggested that even though there were differences among sampling sites and the research objectives, there are similar bacterial groups under similar habitat conditions. *Proteobacteria*, dominated by *Beta-*, *Delta-* and *Gamma*-classes, was the most common phylum in the riparian zones of the Li River also. Earlier studies have found *Gamma-*, *Delta-* and *Betaproteobacteria* to be dominant in wetlands soils and sediments[40–42], while another study showed that *Alpha-*, followed by *Delta-* and

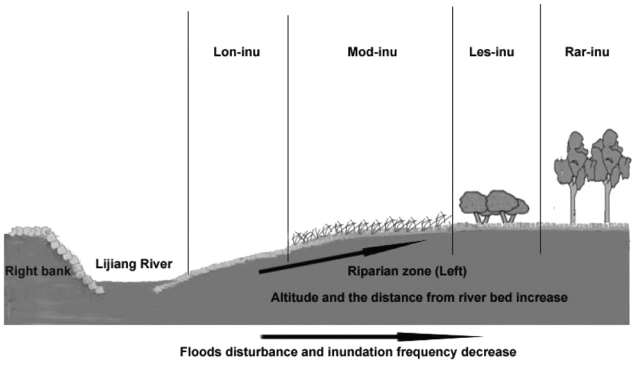

Figure 3. PCoA results coloured according to the different transects with a weighed UniFrac distance matrix comparing the 120 soil samples. The percentages of variation in the samples described by the plotted principal coordinates are indicated on the axes.

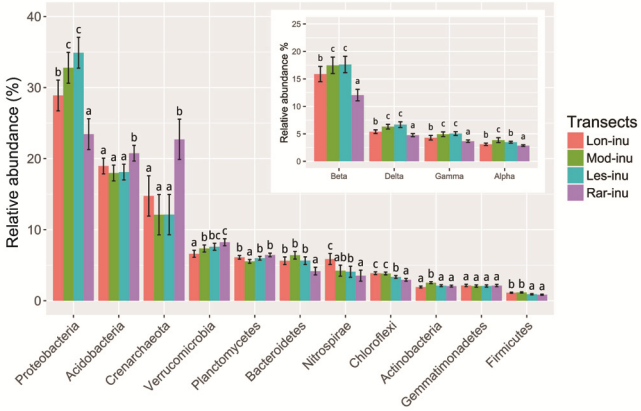

Figure 4. Relative abundances of dominant phyla in different groups of the riparian soils. The relative abundances of the Alpha-, Beta-, Delta- and Gamma-proteobacteria classes are also shown. The bars indicate the standard errors of relative abundances of the each of the phyla.

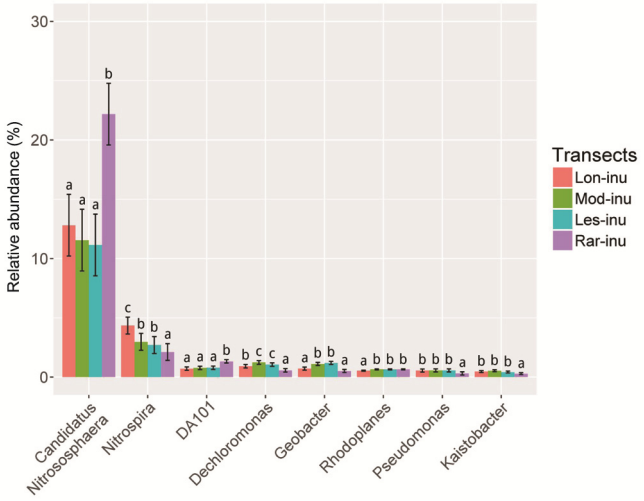

Figure 5. Relative abundances of the dominant genera in the different groups of the riparian soils. The genera with an average abundance of ≧0.5% in at least one group are defined as dominant ones. The bars indicate the standard errors of relative abundances of the each genera.

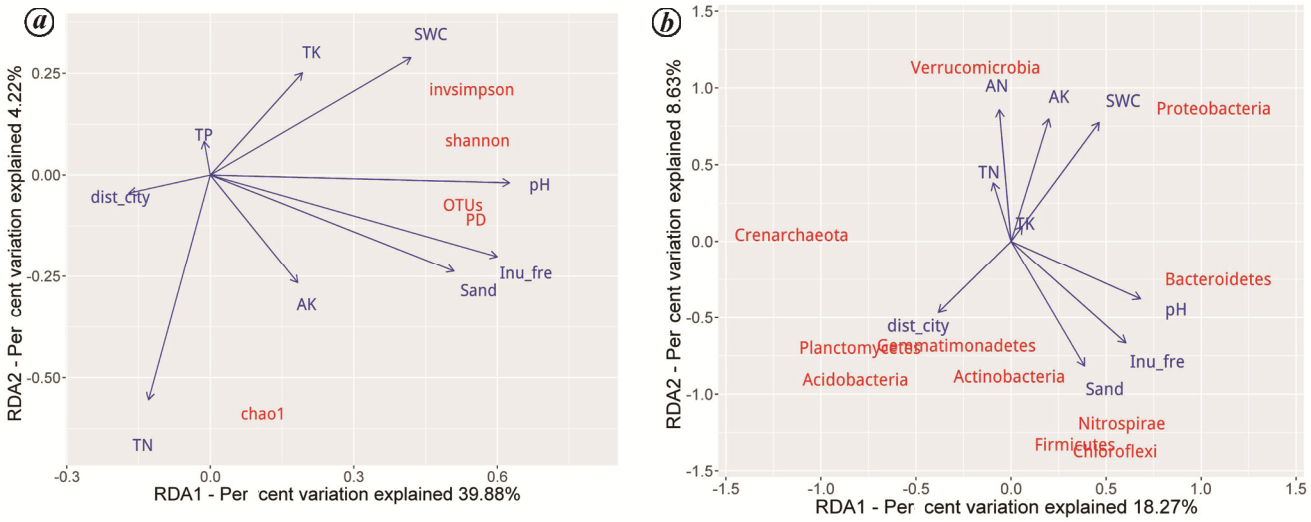

Figure 6. Redundancy analysis (RDA) illustrating the relationships between soil physicochemical properties and bacterial community diversity (***a***) and the relative abundances of dominant the phyla (***b***). The arrows represent physicochemical attributes as follows: Dis_city, Site C1 as the original point, the other nine sites' distances to the origin; Inu_fre, inundation frequency; Sand, sand content; SWC, Soil water content; pH; SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; AK, available potassium. The spots represent diversity indexes of bacterial community, as follows: OTUs, Chao1, PD, Shannon and Invsimpson.

Betaproteobacteria were dominated in created and natural wetland soils[42]. Among them, *Beta-*, *Gamma-* and *Deltaproteobacteria* were the dominant classes; these bacteria have a rich metabolic diversity and play an important role in the circulation of C, N, S, Fe and other elements in the Lijiang River riparian zone. A large number of medium-temperature *Crenarchaeota* was detected in the Lijiang River riparian soil. The phylum *Crenarchaeota* and the genus *Candidatus Nitrososphaera*, as an ammonia-oxidizing archaea, is widely distributed in a multitude of environments, including marines, lakes and soil[44,45]. Ammonia-oxidizing archaea (AOA) may play a major role in nitrification on earth; these microorganisms are capable of oxidizing ammonia (NH_3) to nitrite (NO_2^-)[46–48]. Nitrospirae and its most abundant genus Nitrospira are nitrite-oxidizing bacteria (NOB), which can oxidize nitrite (NO_2^-) to nitrate (NO_3^-). Both these genera are key players in the nitrification process ($NH_3 \rightarrow NO_2^- \rightarrow NO_3^-$), yet there is a less intensively studied group of NOB: *Crenarchaeota* and *Nitrospirae*, both may be involved in the process of soil nitrification in the riparian zone. Nitrification bacteria are important to the wetland environment; if there is a lack of nitrification bacteria, the ammonia nitrogen/nitrate/nitrite circulation system is interrupted and will cause destruction of the aquatic environment and fish death; *Nitrospira* in a sewage-treatment plant and laboratory reactor is a primary nitrite-oxidizing bacteria. *Acidobacteria* are physiologically diverse and abundant members of soil bacterial communities with a few characterized strains[49], but their role in soils has not been well documented[50]. The abundance of *Acidobacteria* is correlated with soil pH, and its abundance will significantly increase at a pH < 5.5 (refs 39, 49). However, pH is not the main reason behind the accumulation of *Acidobacteria* in the soil. Recent studies of freshwater sediment reported that the abundance of the *Acidobacteria* ranged from 4% to 6%, which is similar to our results, and none of these samples was acidic[40,43]. In addition to *Acidobacteria*, many saprophytic microbes preferring eutrophic conditions were present in the riparian soils, such as *Actinobacteria*, *Bacteroidetes* and *Firmicutes*. A number of photoautotrophic microbes, e.g. *Chloroflexi*, also exist in riparian environments. In conclusion, this special community structure provided insight into isolating microbes with biodegradation potential from riparian soils, even though many strains had been obtained without clear prior knowledge.

The relative abundance of *Proteobacteria* decreased to 23.44% (Rar-inu) from 33.63% (Les-inu), while that of *Crenarchaeota* increased from 11.19% (Les-inu) to 22.71% (Rar-inu). In terms of genera, *Candidatus Nitrososphaera* increased from 10.01% (Les-inu) to 22.18% (Rar-inu), and *Nitrospira* decreased from 4.35% (Lon-inu) to 2.12% (Rar-inu). These changes due to different flood disturbance frequencies led to the change of the soil environment and in turn the variation among the bacterial community structures among the transects. Ahn and Peralta[51] also found that hydrology may play a vital role in determining the structure of bacterial communities in wetlands.

Factors determining soil bacterial communities

Bacterial community diversity and structure can be affected by many factors, such as vegetation type, soil structure, chemical properties and disturbance. In our

studies, there have been different bacterial community diversities and structures in different Lijiang River riparian transects. The ultimate reason is that the riparian zone was subjected to different flood dynamics in horizontal, which leads to a series of vegetation changes, changes in soil properties and other ecological processes, which further influenced the varied diversity and structure of the bacterial populations among the four transects. Redundancy analysis (RDA) illustrated that soil pH, Inu_fre, sand, SWC, TN and AN had a high influence on the bacterial diversity and structure. Soil pH has been found to affect the composition of the bacterial communities in different soils[12,52,53], including that of wetland soils[42,54]. Near-neutral pH has peaked diversity in soils, and to some degree, the structure of soil bacterial communities is predictable[39]. Our study also showed that the diversity of riparian microbe was higher in the neutral or slightly alkaline environments (Lon-inu, Mod-inu and Les-inu). Soil structure (sand, silt and clay) depends on the association between mineral soil particles and organic matter, in which aggregates of different size and stability are formed[55]. The structural organization of soil particles provides a spatially heterogeneous habitat for microorganisms characterized by different substrate, nutrient and oxygen concentrations; water contents; and pH values, thus affecting bacterial diversity and structure[56]. According to Gestel *et al.*[57] higher nutrient availability in smaller particles may have caused higher bacterial diversities. However, our study was in the special environment of the riparian zone, and the flood invasion and edge effects have led to high bacterial diversity in the area with large particle sizes.

The vital activities of microorganisms require not only energy but also nutrients. When the soil microbial growth is limited by nitrogen, fertilization can stimulate that growth because the growth of a microbe requires a high C : N source[58]. There was a positive correlation between the amounts of most soil microbes and the soil nitrogen content. Nitrogen can directly or indirectly affect soil microbe growth and activity, subsequently altering the quantity, diversity and structure of the microbial species present[59].

1. Fierer, N., Schimel, J. P. and Holden, P. A., Variations in microbial community composition through two soil depth profiles. *Soil Biol. Biochem*., 2003, **35**, 167–176.
2. Douterelo, I., Goulder, R. and Lillie, M., Soil microbial community response to land management and depth, related to the degradation of organic matter in English wetlands: implications for the *in situ* preservation of archaeological remains. *Appl. Soil Ecol.*, 2010, **44**, 219–227.
3. Hansel, C. M., Fendorf, S., Jardine, P. M. and Francis, C. A., Changes in bacteria and archaeal community structure and functional diversity along a geochemically variable soil profile. *Appl. Environ. Microb*., 2008, **74**, 1620–1633.
4. Peralta, A. L., Matthews, J. W. and Kent, A. D., Microbial community structure and denitrification in a wetland mitigation bank. *Appl. Environ. Microb*., 2010, **76**, 4207–4215.
5. Borcard, D., Gillet, F. and Legendre, P., *Numerical Ecology with R. Matisse*, 2011, **77**, 332–334.
6. Marschner, P., Yang, C. H., Lieberei, R. and Crowley, D. E., Soil and plant specific effects on bacterial community composition in the rhizosphere. *Soil Biol. Biochem*., 2001, **33**, 1437–1445.
7. Kuang, J. L., Huang, L. N., Chen, L. X., Hua, Z. S., Li, S. J. and Hu, M., Contemporary environmental variation determines microbial diversity patterns in acid mine drainage. *ISME J*., 2013, **7**, 1038–1050.
8. Ludemann, H., Arth, I. and Liesack, W., Spatial changes in the bacterial community structure along a vertical oxygen gradient in flooded paddy soil cores. *Appl. Environ. Microb*., 2000, **66**, 754–762.
9. Zhou, J. Z., Xia, B. C., Huang, H., Palumbo, A. V. and Tiedje, J. M., Microbial diversity and heterogeneity in sandy subsurface soils. *Appl. Environ. Microb*., 2004, **70**, 1723–1734.
10. Yu, Y., Wang, H., Liu, J., Wang, Q., Shen, T., Guo, W. and Wang, R., Shifts in microbial community function and structure along the successional gradient of coastal wetlands in Yellow River Estuary. *Eur. J. Soil Biol*., 2011, **49**, 12–21.
11. Wang, J. T., Zheng, Y. M., Hu, H. W., Zhang, L. M., Li, J. and He, J. Z., Soil pH determines the alpha diversity but not beta diversity of soil fungal community along altitude in a typical Tibetan forest ecosystem. *J. Soil Sediment*, 2015, **15**, 1224–1232.
12. Fierer, N. and Jackson, R. B., The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. USA*, 2006, **103**, 626–631.
13. Rinklebe, J. and Langer, U., Microbial diversity in three floodplain soils at the Elbe River (Germany). *Soil Biol. Biochem*., 2006, **38**, 2144–2151.
14. Moche, M., Gutknecht, J., Schulz, E., Langer, U. and Rinklebe, J., Monthly dynamics of microbial community structure and their controlling factors in three floodplain soils. *Soil Biol. Biochem*., 2015, **90**, 169–178.
15. Wilson, J. S., Baldwin, D. S., Rees, G. N. and Wilson, B. P., The effects of short-term inundation on carbon dynamics, microbial community structure and microbial activity in floodplain soil. *J. Microbiol. Meth*., 2011, **27**, 213–225.
16. Naiman, R. J. and Décamps, H., The ecology of interface: Riparian zones. *Annu. Rev. Ecol. Syst*., 1997, **28**, 621–658.
17. Gregory, S. V., Swanson, F. J., McKee, W. A. and Cummins, K. W., An ecosystem perspective of riparian zones focus on links between land and water. *BioScience*, 1991, **41**, 540–551.
18. Malanson, G. P., *Riparian Landscapes*, Cambridge University Press, New York, 1993.
19. Naiman, R. J., Bechtold, J. S., Drake, D. C., Latterell, J. J., O'Keefe, T. C. and Balian, E. V., Origins, patterns, and importance of heterogeneity in riparian systems. In *Ecosystem Function in Heterogeneous Landscape* (eds Lovett, G. M. *et al.*), Springer, New York, 2005, pp. 279–309.
20. Samaritani, E. *et al.*, Heterogeneity of soil carbon pools and fuxes in a channelized and a restored floodplain section (Thur River, Switzerland). *Hydrol. Earth Syst. Sci*., 2011, **15**, 1757–1769.
21. Thorp, J. H., Thoms, M. C. and Delong, M. D., The riverine ecosystem synthesis: Biocomplexity in river networks across space and time. *River Res. Appl.*, 2006, **22**, 123–147.
22. Huang, W. J., Liu, X. Z. and Cai, D. S., Researches on Lijiang River Ecosystem. *Ecol. Environ.*, 2007, **3**, 130–135.
23. Liu, J. R., Feng, H., Yu, X. L., Song, G. J. and Ye, Q., A preliminary discussion on the historic change of the name for the Lijiang river system. *Carsologica Sin*., 2003, **22**, 77–83.
24. Huang, Z. W., Chen, Y. D., Jiang, Y. P. and Cheng, Y. P., Analysis on water resources of Lijiang River Basin. *J. Guangxi Acad. Sci.*, 2005, **21**, 56–60.
25. Li, Y., Wang, D. M. and Xin, Z. B., Spatial distribution of vegetation and soil in aquatic-terrestrial ecotone, Li River. *Trans. CSAE*, 2013, **29**, 121–128.

26. Lu, R. K., *Method of Analysis in Soil and Agrochemistry*, China Agricultural Science and Technology Press, Beijing, 2000.
27. Bates, S., Berg, L. D., Caporaso, J., Walters, W., Knight, R. and Fierer, N., Examining the global distribution of dominant archaeal populations in soil. *ISME J.*, 2010, **5**, 908–917.
28. Fierer, N., Hamady, M., Lauber, C. L. and Knight, R., The influence of sex, handedness, and washing on the diversity of hand surface bacteria. *Proc. Natl. Acad. Sci. USA*, 2008, **105**, 17994–17999.
29. Schloss, P. D. *et al.*, Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microb.*, 2009, **75**, 7537–7541.
30. Caporaso, J. G. *et al.*, QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods*, 2010, **7**, 335–336.
31. Quince, C. *et al.*, Noise and the accurate determination of microbial diversity from 454 pyrosequencing data. *Nat. Methods*, 2009, **6**, 639–641.
32. Huse, S. M., Welch, D. M., Morrison, H. G. and Sogin, M. L., Ironing out the wrinkles in the rare biosphere through improved OTU clustering. *Environ. Microbiol.*, 2010, **12**, 1889–1898.
33. Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C. and Knight, R., UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, 2011, **27**, 2194–2200.
34. Edgar, R. C., Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 2010, **26**, 2460–2461.
35. Wang, Q., Garrity, G. M., Tiedje, J. M. and Cole, J. R., Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microb.*, 2007, **73**, 5261–5267.
36. Oksanen, J. *et al.*, Vegan: Community Ecology Package. R Package Version 2.3-0.2. 2015; http://CRAN.R-project.org/package=vegan
37. Junk, W. J., Bayley, P. B. and Sparks, R. E., The flood pulse concept in river-floodplain systems. *Can. J. Fish. Aquat. Sci.*, 1989, **106**, 110–127.
38. Lal, R., Agricultural activities and the global carbon cycle. *Nutr. Cycling Agroecosyst.*, 2004, **70**, 103–106.
39. Zhalnina, K. *et al.*, Soil pH determines microbial diversity and composition in the park grass experiment. *Microbial Ecol.*, 2015, **69**, 395–406.
40. Röske, K., Sachse, R., Scheerer, C. and Röske, I., Microbial diversity and composition of the sediment in the drinking water reservoir Saidenbach (Saxonia, Germany). *Syst. Appl. Microbiol.*, 2012, **35**, 35–44.
41. Ligi, T., Oopkaup, K., Truu, M., Preem, J. K., Nölvak, H. and Mitsch, W. J., Characterization of bacterial communities in soil and sediment of a created riverine wetland complex using high-throughput 16S rRNA amplicon sequencing. *Ecol. Eng.*, 2014, **72**, 56–66.
42. Peralta, R. M., Ahn, C. and Gillevet, P. M., Characterization of soil bacterial community structure and physicochemical properties in created and natural wetlands. *Sci. Total Environ.*, 2013, **443**, 725–732.
43. Wang, Y., Sheng, H. F., He, Y., Wu, J. Y., Jiang, Y. X., Tam, N. F. Y. and Zhou, H. W., Comparison of the levels of bacterial diversity in freshwater, intertidal wetland, and marine sediments by using millions of Illumina tags. *Appl. Environ. Microb.*, 2012, **78**, 8264–8271.
44. Spang, A. *et al.*, Distinct gene set in two different lineages of ammonia-oxidizing archaea supports the phylum Thaumarchaeota. *Trends Microbiol.*, 2010, **18**, 331–340.
45. Brochier-Armanet, C., Boussau, B., Gribaldo, S. and Forteere, P., Mesophilic crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. *Nat. Rev. Microbiol.*, 2008, **6**, 245–252.
46. Hatzenpichler, R. *et al.*, A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. *Proc. Natl. Acad. Sci. USA*, 2008, **105**, 2134–2139.
47. Könneke, M., Bernhard, A. E., de la Torre, J. R., Walker, C. B., Waterbury, J. B. and Stahl, D. A., Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature*, 2005, **437**, 543–546.
48. Treusch, A. H., Leininger, S., Kletzin, A., Schuster, S. C., Klenk, H. P. and Schleper, C., Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. *Environ. Microbiology*, 2005, **7**, 1985–1995.
49. Jones, R. T., Robeson, M. S., Lauber, C. L., Hamady, M., Knight, R. and Fierer, N., A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *ISME J.*, 2009, **3**, 442–453.
50. Newton, R. J., Jones, S. E., Eiler, A., Mcmahon, K. and Bertilsson, S., A guide to the natural history of freshwater lake bacteria. *Microbiol. Mol. Biol. R.*, 2011, **75**, 14–49.
51. Ahn, C. and Peralta, R. M., Soil bacterial community structure and physicochemical properties in mitigation wetlands created in the Piedmont region of Virginia (USA). *Ecol. Eng.*, 2009, **35**, 1036–1042.
52. Nacke, H. *et al.*, Pyrosequencing-based assessment of bacterial community structure along different management types in German forest and grassland soils. *PLoS ONE*, 2011, **6**, 1–12.
53. Preem, J. K. *et al.*, Bacterial community structure and its relationship to soil physico-chemical characteristics in alder stands with different management histories. *Ecol. Eng.*, 2012, **49**, 10–17.
54. Hartman, W. H., Richardson, C. J., Vilgalys, R. and Bruland, G. L., Environmental and anthropogenic controls over bacterial communities in wetland soils. *Proc. Natl. Acad. Sci. USA*, 2008, **105**, 17842–17847.
55. Tisdall, J. M. and Oades, J. M., Organic matter and water stable aggregates in soils. *Soil Sci.*, 1982, **33**, 141–163.
56. Ladd, J. N., Foster, R. C., Nannipieri, P. and Oades, J. M., Soil structure and biological activity. *Soil Biochem.*, 1996, **9**, 23–78.
57. Gestel, M. V., Merckx, R. and Vlasse, K., Spatial distribution of microbial biomass in microaggregates of a silty-loam soil and the relation with the resistance of microorganisms to soil drying. *Soil Biol. Biochem.*, 1996, **28**, 503–510.
58. Aber, J. D., Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. *Trends Ecol. Evol.*, 1992, **7**, 220–223.
59. Vanotti, M. B., Leclerc, S. A. and Bundy, L. G., Short-term effects of nitrogen fertilization on soil organic nitrogen availability. *Soil Sci. Soc. Am. J.*, 1995, **59**, 1350–1359.

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