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Research Article

RESEARCH ARTICLE

Changes of soil prokaryotic communities after clear-cutting in a karst forest: evidences for cutting-based disturbance promoting deterministic processes

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One sentence summary: The bacterial community structure as well as the community assembly was significantly altered by clear-cutting, and became similar over time to a reference forest that was not subjected to logging.

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ABSTRACT

To understand the temporal responses of soil prokaryotic communities to clear-cutting disturbance, we examined the changes in soil bacterial and archaeal community composition, structure and diversity along a chronosequence of forest successional restoration using high-throughput 16S rRNA gene sequencing. Our results demonstrated that clear-cutting significantly altered soil bacterial community structure, while no significant shifts of soil archaeal communities were observed. The hypothesis that soil bacterial communities would become similar to those of surrounding intact primary forest with natural regeneration was supported by the shifts in the bacterial community composition and structure. Bacterial community diversity patterns induced by clear-cutting were consistent with the intermediate disturbance hypothesis. Dynamics of bacterial communities was mostly driven by soil properties, which collectively explained more than 70% of the variation in bacterial community composition. Community assembly data revealed that clear-cutting promoted the importance of the deterministic processes in shaping bacterial communities, coinciding with the resultant low resource environments. But assembly processes in the secondary forest returned a similar level compared to the intact

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primary forest. These findings suggest that bacterial community dynamics may be predictable during the natural recovery process.

Keywords: clear-cutting; bacterial community structure; archaeal community structure; diversity; community assembly; deterministic processes

INTRODUCTION

Clear-cutting, one of the most critical disturbances to forest ecosystems, has once been a widespread practice of commercial logging in many forests across the world (Young 1996; Harper *et al.* 2007). Its impact is highly complex and persistent on both the aboveground vegetation and belowground microbial communities (Keenan and Kimmins 1993; Kembel 2009; Hartmann *et al.* 2012). At the system level, the microbial communities mediate soil productivity and modulate the resilience of forest ecosystem to stresses (Felske *et al.* 2000; Kuramae *et al.* 2010; Bardgett and van der Putten 2014). It is likely that the microbial community structure, metabolic activities and gene expression patterns can serve as indicators of forest ecosystem status, which is vital to monitor forest ecosystems, to evaluate effects of anthropogenic disturbances and to detect changes in energy and nutrient flow patterns before they have irreversible effects (Harris 2003; van Dijk *et al.* 2009; Lewis *et al.* 2010).

Clear-cutting can exert direct effects on the soil microbial physiology and indirect effects by changing plants and soil properties (Carney and Matson 2005; Stegen *et al.* 2012). Site organic matter (OM) and soil porosity (SP) are two vital properties directly affected by clear-cutting (Grigal 2000; Marshall 2000). Clear-cutting usually causes severe soil compaction, especially in soils with low initial bulk density and high water saturation (Marshall 2000; Powers *et al.* 2005). Compacted soil directly affects the prokaryotic communities by limited oxygen availability, altered water regimes and reduced pore sizes (Schnurr-Pütz *et al.* 2006; Frey *et al.* 2011). OM removal and soil compaction tended to change carbon and nitrogen content, nutrient availability, diversity of meso- and macrofauna, leading to a low resource environment (Keenan and Kimmins 1993; Jurgensen *et al.* 1997). All these changes can subsequently reduce soil microbial biomass and indirectly exert an influence on soil microbial community composition and structure (Pennanen *et al.* 2004; Carney and Matson 2005).

Soil bacteria are highly responsive to soil nutritional changes (Pennanen *et al.* 2004; Carney and Matson 2005; Yao *et al.* 2014), while archaea are more resilient to energy stress (Valentine 2007). Many studies have demonstrated that soil nutrient conditions and vegetation communities become similar to surrounding intact primary forest ecosystems with natural regeneration (Schlesinger 1986; Johnson and Curtis 2001). As soil bacteria communities are mainly shaped by soil properties, thus dynamics of bacterial community structure and composition may present an obvious recovery trend toward the original state of surrounding primary forests after clear-cutting (Nemergut *et al.* 2007; Banning *et al.* 2011; Xiang *et al.* 2014). The low resource availability, induced by clear-cutting can restrict some sensitive taxa, and change community structure through niche-selection (Dvorský *et al.* 2011; Doležal, Yakubov and Hara 2013). When the environment conditions become less harsh with recovery, new ecological niches may emerge that drive the recovery of restricted species (Banning *et al.* 2011; Ferrenberg *et al.* 2013; Xiang *et al.* 2014).

It is well recognized that both deterministic and stochastic processes influence soil microbial community structure and composition (Tilman 2004; Zhou *et al.* 2014). Clear-cutting, as a disturbance event, kills or severely impacts numerous members of soil microbial community (Pietikäinen and Fritze 1995), which can be viewed as a 'reset' of the community assembly or as an 'environmental filter'. Many studies have demonstrated that nutrient availability has remarkable effects on bacterial community assembly processes, and stronger deterministic processes occur in more extreme environments (Horner-Devine and Bohannan 2006; Chase 2007; Kembel *et al.* 2011; Wiedenbeck and Cohan 2011; Van der Plas, Anderson and Olff 2012). Clear-cutting creates a relatively low nutrition environment, so it is likely that the importance of deterministic assembly processes of prokaryotic community may be increased by clear-cutting events.

Despite the above mentioned general relationships among soil factors and microbial community structure during the natural recovery processes (Kranabetter and Chapman 1999; Mariani, Chang and Kabzems 2006; Tan, Chang and Kabzems 2008; Hartmann *et al.* 2012; Willers, Jansen van Rensburg and Claassens 2015), it is not clear about the quantitative extent to which clear-cutting affects bacterial and archaeal community diversity and composition. It is also unclear to what extent the observed shifts in community structure and function are dependent on the altered soil properties. So far most of the previous work has been based on low resolution methods, such as microbial biomass, phospholipid fatty acid or single functional process, such as enzyme activity, litter decomposition rates and nitrogen mineralization potential (Tan, Chang and Kabzems 2008; Becker *et al.* 2015). Current high-throughput sequencing approaches make it possible to investigate forest soil microbial communities with far greater sample numbers, sequence coverage and phylogenetic resolution (Nacke *et al.* 2011; Baldrian *et al.* 2012). This allows us to discern whether particular taxonomic groups are more or less sensitive to environmental factors (Rui *et al.* 2015).

The Nonggang National Nature Reserve, established in 1980, is located in southwest China, where the limestone seasonal rain forests are widely distributed as the typical virgin vegetation. Historically, the natural forests experienced massive clear-cutting before the establishment of the reserve. Some deforested areas have recovered naturally as secondary forests, while other areas have developed into shrub-grasslands due to repeated cutting disturbances on the edges of the reserve. These different recovery stages provide an opportunity to examine the changes of soil prokaryotic communities after a clear-cutting with a chronosequence (with space for time substitution). In this study, we aim to understand the changes in composition, structure and diversity of soil bacterial and archaeal communities in response to clear-cutting, as well as the main factors driving the changes. Specifically, we focus on the following three hypotheses.

(i) Soil bacterial and archaeal community structure would be strongly altered by clear-cutting driven by the major shifts

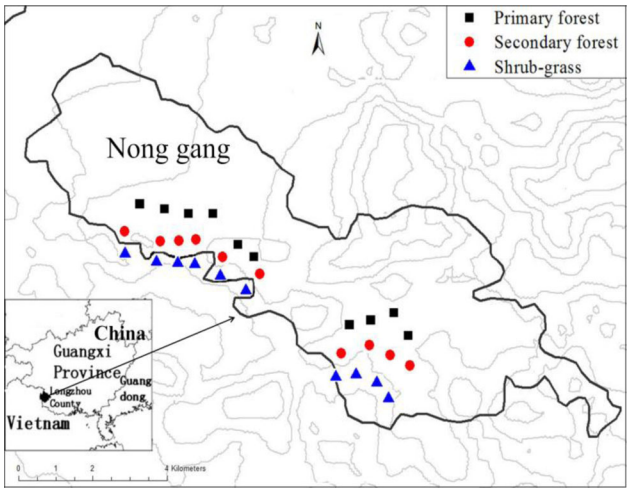

Figure 1. Geographical location of sampling sites in Nonggang Nature Reserve, Guangxi, PR. China.

in soil properties, and tend to be similar to the intact primary forest soil with rehabilitation recovery.

(ii) Soil bacterial and archaeal communities may have different responsive patterns following clear-cuttings.

(iii) Assembly processes in soil bacterial and archaeal communities may vary with recovery ages, and clear-cutting may increase the relative influence of deterministic factors.

This study will test the above three hypotheses and get further insights into the mechanism underlying the prokaryotic community succession and its important driving factors.

MATERIALS AND METHODS

Study site

The study site is located in the Nonggang Nature Reserve (5426 ha) Guangxi, China (106°42′-107°4′E, 22°13′-22°33′N). This area has distinct dry and rainy (May to September) seasons. The annual rainfall is from 1150 to 1750 mm with an average of 1430 mm, and the annual mean temperature is from 8°C to 30°C (averaged 22°C) (Zhou *et al.* 2006). The vegetation type is a limestone seasonal rain forest (Shu, Zhao and Huang 1988). The soil is an oxisol, a dark limy soil developed from limestone (State Soil Survey Service of China 1998).

Experimental design and soil sampling

The study site is a long strip of land, approximately 1800 ha, stretching from southeast to northwest. Half of the area was clear cut by a clear-cut in 1978 (before the establishment of the Reserve), and then regenerated naturally as a secondary forest. The remaining area on the edges has become a shrub-grassland due to periodic cutting disturbances. Inside the reserve is a typical primary forest that has had no logging disturbance for more than 400 years (Fig. 1). The representative species of the primary forest are *Ardisia quinquegona*, *Deutzianthus tonkinensis* and *Dracontomelon duperreanum*. The dominant species of secondary forest and shrub-grasslands are *Alchornea trewioides*, *Cipadessa cinerascens*, *Ficus hispida* and *Sterculia nobilis*. The secondary forest had been undisturbed for 35 years when the study was conducted in 2013 and the shrub-grassland had been undisturbed for 3 years. Thus, we defined the chronosequence as '3-year-old rehabilitation', '35-year-old rehabilitation' and 'intact primary forest' as a control.

A total of 30 quadrats (20 × 20 m), 10 each for the two recovery stages and the intact primary forest control were established (Fig. 1) and used for soil sampling in June of 2014. All the plots were on similar southwest aspects, with the elevation ranging from 215 to 355 m above the sea level. Intact primary forest, shrub-grassland and secondary regenerated forest sites were separated from each other by at least 400 m. In each plot, soil samples of 0–10 cm depth were collected at eight random points using a sterile blade, pooled together as a single sample, and kept on ice. Debris such as large roots and rocks was removed from samples before being passed through a 2 mm sieve. Each sample was divided into two parts: one was stored at 4°C for soil property measurements and another part at –40°C for DNA extraction.

Analyses of soil properties and microbial biomass

The analyses of soil properties were determined as described previously (Wang *et al.* 2013). Briefly, total organic carbon (TOC) was quantified with the dichromate digestion method and total N of soil was measured with the Kjeldahl method. To measure NH_4^+-N and NO_3^--N, 10 g dry weight of soil samples were removed and extracted in 50 ml of 2 M KCL solution while shaken for 1 h and then filtered. Soil moisture (SM) was measured by the gravimetric method after the soil samples were oven-dried at 60°C for 48 h. Soil total phosphorus (TP) and available phosphorus (AP) were determined with ultraviolet spectrophotometry method. Atomic absorption spectrophotometry was employed to measures oil total potassium (TK) and available potassium (AK) (Yao *et al.* 2014). Soil pH was measured in the soil-water slurry (1:5) using a pH meter. Cylinders (5-cm-diameter, 10-cm-high) used to determine soil porosity (Logsdon and Cambardella 2000). Soil microbial biomass C (MBC) was estimated by chloroform fumigation-extraction as described by (Vance, Brookes and Jenkinson 1987).

DNA extraction and MiSeq sequencing of 16S rRNA gene amplicons

DNA was extracted with the MO BIO Power Soil DNA Extraction kit (MO BIO Laboratories, Carlsbad, CA, USA), and quantified using a NanoDrop Spectrophotometer. PCR amplification was conducted with primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) with 12 nt unique barcode at 5′-end and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) designed to be universal for bacteria and archaea (Caporaso *et al.* 2011; Caporaso *et al.* 2012). Each 25 μl reaction volume contained 1 μl of each 10 μM primer, 10 ng of template DNA and 0.5 units of Accuprime high-fidelity Taq (Invitrogen, USA). Each sample was amplified in triplicate with 25 μl reaction under following 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 45 s, and extension at 72°C for 45 s; with a final extension at 67°C for 10 min. The PCR products from same sample were mixed in one tube and purified with the gel extraction kit (QIAGEN, USA). An equal molar amount of PCR product from each sample was pooled for library construction using Illumina Truseq DNA kit, and mixed with PhiX equal to 30% of the total DNA. The samples were sequenced using Illumina Miseq system with Reagent Kit v2 2 × 250 bp at Environmental Genome Platform of Chengdu Institute of Biology, CAS.

Sequence analyses

The sequence data were split based on the unique sample barcodes, trimmed for sequence quality, and denoised using QIIME Pipeline (http://qiime.org/tutorials/tutorial.html). Operational taxonomic units (OTUs) were classified clustered at 97% identity for OTUs using USEARCH v7.0 (http://www.drive5.com/usearch/download.html). In the sequence analyses, the following criteria were used: sequences of length <200 bp were removed; a minimum average quality score of 30 was allowed in read; a maximum number allowed of primer mismatches was 2; no errors in barcode were allowed; no ambiguous bases were allowed. Chimera sequences and singletons were removed from clustered sequences with USEARCH. Resampling to the same sequence depth (8000 sequences per sample) was performed using daisychopper.pl (http://www.festinalente.me/bioinf/downloads/daisychopper.pl) for calculating the relative abundance of bacteria/archaea in total prokaryotes (sum of bacteria and archaea). As the sequence number of the bacteria/archaea varied among samples, we randomly resampled to the same sequences depth for each sample (5647 sequences for bacteria and 829 sequences for archaea) for further analysis. The phylogenetic affiliation of each sequence was analyzed by an RDP Classifier at a confidence level of 80% (Wang *et al*. 2007). A phylogenetic maximum likelihood–approximation tree was generated using the generalized time-reversible model in FastTree 2.1.1 (Price, Dehal and Arkin 2010). Phylogenetic diversities (PD) were estimated by Faith's index (Faith 1992), which provides an integrated index of the phylogenetic breadth across taxonomic levels. Phylotype richness, phylogenetic diversities, diversity indices and a weighted UniFrac distance matrix were calculated using QIIME pipeline v1.7.0 (Lozupone and Knight 2005; Lozupone, Hamady and Knight 2006).

Statistical analyses

Principal coordinates analysis (PCoA) in Fast UniFrac was used to evaluate the overall structural change of soil prokaryotic communities. The statistical significance of community structure among different recovery stages was assessed by PerMANOVA in PAST. Differences in relative abundances of taxonomic units, soil properties and alpha diversity between samples were tested by one-way analysis of variance (ANOVA). Compositional similarity of communities among different rehabilitation stages was calculated by the Bray-Curtis dissimilarity metric (Bray and Curtis 1957) using the varpart procedure in the vegan package in R (R Project 3.1, v.2.3-1) (R Core Team 2015).

To test the effects of soil parameters on microbial community composition, forward selection was used for redundancy analysis (RDA) to select a combination of soil variables that explained most of the variation observed in the prokaryotic composition. A series of constrained RDA permutations were performed in Canoco (version 4.5 for Windows, PRI Wageningen, Netherlands) to determine which variables best explained the community composition's variation, using manual forward selection (permutations = 999).

Analyses of prokaryotic community assembly

Using phylogenetic information to evaluate ecological processes requires that the phylogenetic distance between taxa is similar to their ecological niche difference (for example, the difference in habitat requirements). Previous work suggests that closely related prokaryotic microbes have similar habitat associations (Fierer, Bradford and Jackson 2007; Andersson, Riemann and Bertilsson 2010; Philippot *et al*. 2010). Thus, we used patterns of phylogenetic communities composition to calculate community assembly processes (Fierer, Bradford and Jackson 2007; Kraft *et al*. 2007). First, if community composition and structure are primarily stochastic, community phylogenetic composition and structure should not be significantly different from the expectations based on stochastic community assembly. Second, if environmental filtering is the most influential process, coexisting taxa should be closely related and not randomly distributed (Stegen *et al*. 2012).

We employed the net relatedness index (NRI) and nearest taxon index (NTI) (Webb *et al*. 2002; Fine and Kembel 2011; Stegen *et al*. 2012) to infer prokaryotic community assembly using the 'taxa label shuffling' approach implemented in the picante package ('ses.mpd' and 'ses.mntd') (Fine and Kembel 2011; Stegen *et al*. 2012) in R (R Project 3.1, v.2.3-1). The NTI can show the clustering degree of taxa at a relatively finer phylogenetic scale, while NRI defines the broad clustering degree of taxa across the whole phylogenetic tree. The NRI is a standardized measure of the mean pairwise phylogenetic distance between all pairs of taxa in a sample, relative to the regional species pool and is calculated using the following equation:

$$\mathrm{NRI} = -(\mathrm{MPD}_{\mathrm{obs}} - \mathrm{MPD}_{\mathrm{rand}})/\mathrm{sd\_MPD}_{\mathrm{rand}}, \quad (1)$$

where $\mathrm{MPD}_{\mathrm{obs}}$ is the average of all pairwise phenotypic distances between all the taxa in a local community weighed by their abundances, $\mathrm{MPD}_{\mathrm{rand}}$ is the average of MPD calculated in the null model (in our case, the sum of all taxa identified in all plots, 999 randomizations), and $\mathrm{sd\_MPD}_{\mathrm{rand}}$ is the standard deviation of $\mathrm{MPD}_{\mathrm{rand}}$. The NTI is a standardized measure of the phylogenetic distance to the nearest taxon for each taxon in the sample, relative to a phylogeny of the null model and is calculated using the following equation:

$$\mathrm{NTI} = -(\mathrm{MNTD}_{\mathrm{obs}} - \mathrm{MNTD}_{\mathrm{rand}})/\mathrm{sd\_MNTD}_{\mathrm{rand}}, \quad (2)$$

where MNTD is the average of phylogenetic distance to the nearest taxon in the phylogeny of the pool; $\mathrm{MNTD}_{\mathrm{obs}}$, $\mathrm{MNTD}_{\mathrm{rand}}$ and $\mathrm{sd\_MNTD}_{\mathrm{rand}}$ are calculated as for MPD. NRI or NTI value close to zero ($-2 <$ NRI/NTI < 2) suggests that community assembly is highly stochastic and neutral processes are more important in structuring the community. Larger positive or negative null deviations (NRI/NTI < -2 or > 2) suggest that deterministic/niche-based processes are more important, or environmental filters, could have strong influences on community assembly (Webb *et al*. 2002; Kembel 2009; Placella, Brodie and Firestone 2012; Stegen *et al*. 2012).

RESULTS

Soil properties among vegetation types

All tested soil chemical properties except soil pH were significantly altered by clear-cutting (Table 1). Clear-cutting significantly reduced soil TN, C/N ratio, NH_4^+-N, TP, TK, AN, AP and SP (Table S1, Supporting Information), so that after 35 years of recovery, these soil properties were still significantly lower than that in primary forest (Table S1, Supporting Information). TOC, NO_3^+-N and AK, as well as SM, was lower in shrub-grassland

Table 1. Soil properties under different vegetation types.

	PF	SG	SF
pH	6.75 (0.20)a	6.68 (0.26)a	6.53 (0.28)a
SM (%)	39.73 (3.34)a	30.83 (4.83)b	37 (4.39)a
SP (%)	74.99 (3.35)a	47.36 (3.84)c	60.64 (4.43)b
TOC (g kg^{-1})	61.79 (6.60)a	49.97 (5.86)b	58.71 (5.88)a
TN (g kg^{-1})	4.85 (0.47)a	2.72 (0.51)c	4.13 (0.55)b
C/N (%)	12.75 (0.81)c	16.86 (1.18)a	14.40 (1.20)b
NH_4^+-N (mg kg^{-1})	65.03 (2.75)a	36.82 (5.29)c	53.12 (3.03)b
AN (mg kg^{-1})	377.66 (26.49)a	231.67 (21.66)c	295.98 (37.90)b
NO_3^--N (mg kg^{-1})	29.97 (3.44)a	11.66 (2.21)b	23.97 (1.92)a
AP (mg kg^{-1})	1.60 (0.21)a	0.47 (0.26)c	0.83 (0.26)b
TP (mg kg^{-1})	1.61 (0.25)a	0.55 (0.23)c	1.01 (0.14)b
TK (mg kg^{-1})	10.58 (1.41)a	3.68 (0.46)c	8.61 (0.72)b
AK (mg kg^{-1})	115.19 (24.92)a	70.26 (15.78)b	106.11 (15.56)a
MBC (mg kg^{-1})	613.80 (54.59)a	411.47 (38.94)c	512.10 (430.09)b

PF: primary forest; SG: shrub-grassland; SF: secondary forest; SML soil moisture; SP: soil porosity; TOC: total organic carbon; TN: total nitrogen; AN: available nitrogen; NH_4^+-N: ammonium nitrogen; NO_3^--N: nitrate nitrogen; TP: soil total phosphorus; AP: available phosphorus; TK: soil total potassium; AK: available potassium; MBC: microbial biomass carbon. Data (means ± SE, $n = 10$) followed by different letters indicate significant different at $P = 0.05$.

soils than control sites, but they became similar between the 35-year-old rehabilitation stage and the intact old-growth control (Table S1, Supporting Information). Clear-cutting significantly reduced microbial biomass carbon, with 33% reduction in shrub-grassland and 17% reduction in secondary forest soils, compared to the intact primary forest (Table 1, Supporting Information).

Prokaryotic community structure and Bray-Curtis similarity

PCoA based on weighted UniFrac showed that clear-cutting had different effects on bacterial and archaeal communities (Fig. 2). The bacterial communities clustered according to the different rehabilitation stages (Fig. 2A), demonstrating that site differences within the same rehabilitation stage were less relative to differences between different rehabilitation stages. The PERMANOVA analyses of weighted UniFrac distances also revealed significant differences among the bacterial communities at different rehabilitation stages ($P = 0.018$). Clear-cutting appeared to have a significant effect on the overall bacterial community structure, contributing 56.7% of total variation (Fig. 2A). By contrast, there were no significant differences in archaeal communities at the different recovery stages ($P = 0.423$; Fig. 2C).

To further elucidate the trend of recovery of the bacterial and archaeal communities after clear-cutting, the similarity of community composition at the OTU level between different rehabilitation stages and primary forest soils was calculated. All bacterial communities in the primary forest had an average Bray-Curtis similarity of 54.3%. The average Bray-Curtis similarity of bacterial community between the different rehabilitation stages and the primary forest was 39.6% for shrub-grassland and 45.7% for secondary forest, respectively (Fig. 2B). This suggests a trend of increasing similarity of soil bacterial communities from the shrub-grassland to the secondary forest compared to the intact primary forest. However, the average Bray-Curtis similarity of soil archaeal community between different rehabilitation stages and primary forest was consistent in response to clear-cutting (Fig. 2D).

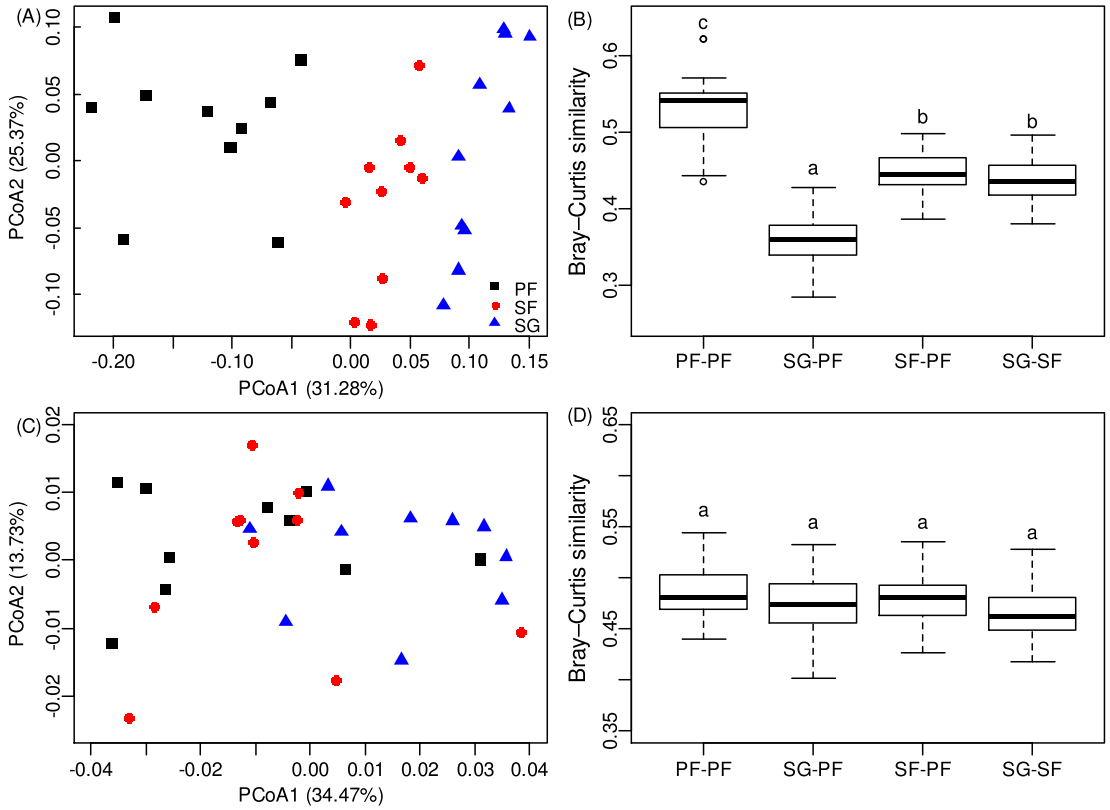

Figure 2. Principal coordinate analyses of weighted UniFrac distances of bacterial (**A**) and archaeal communities (**C**). Bray-Curtis similarity of bacterial communities (**B**) and archaeal communities (**D**) at the OTU level between rehabilitation age and primary forest communities. PF: primary forest; SG: shrub-grassland; SF: secondary forest.

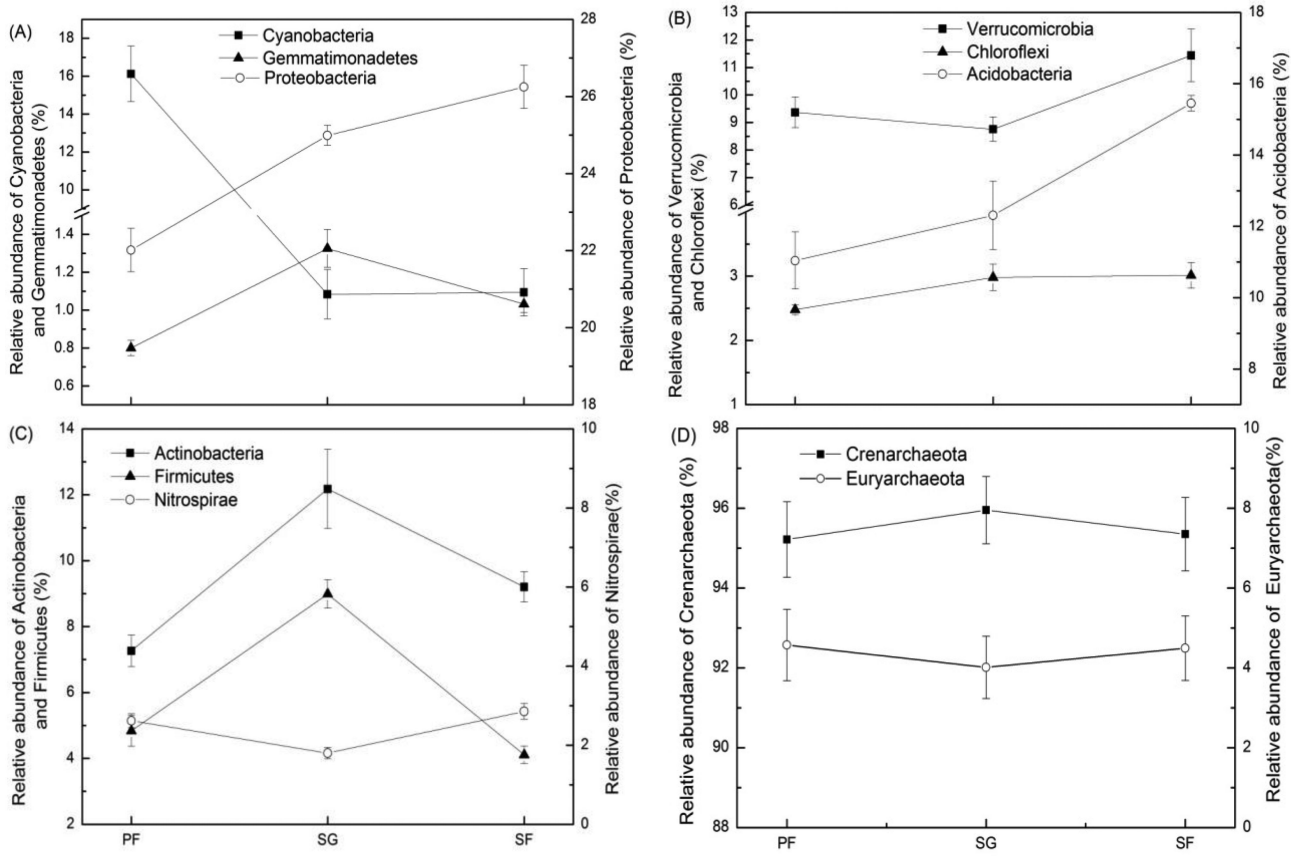

Figure 3. The relative abundance of dominant bacterial (**A**, **B** and **C**) and archaeal phyla (**D**) at different rehabilitation stages. Error bars denote standard deviation. PF: primary forest; SG: shrub-grassland; SF: secondary forest.

Phylogenetic diversity and composition of soil prokaryotic community

Sequencing data showed that the relative abundance of bacteria accounted for 86.6% −89.1% of total prokaryotes, while archaea accounted for 10.9%−13.4%. The alpha-diversity indices based on phylotype richness (i.e. number of OTUs) and phylogenetic diversity (Faith 1992) of soil bacterial and archaeal communities exhibited different changing trends (Fig. S1, Supporting Information). Clear-cutting decreased bacterial phylotype richness and phylogenetic diversity in the shrub-grassland, but after 35-year recovery, these parameters were significantly higher in the secondary forest than those in primary forest (Fig. S1A and B, Supporting Information). By contrast, no significant differences among different rehabilitation stages were observed for archaeal alpha-diversity indices (Fig. S1C and D, Supporting Information).

The dominant phyla of bacterial communities among all samples were Proteobacteria (24.4 ± 1.6%; means ± SD), followed by Acidobacteria (13.3 ± 1.3%), Verrucomicrobia (9.9 ± 1.4%), Bacteroidetes (9.6 ± 1.6%) and Actinobacteria (9.5 ± 1.4%), accounting for more than 88.9% of total bacterial sequences in all the samples (Table S2, Supporting Information). Clear-cutting significantly increased the relative abundance of Proteobacteria, Actinobacteria, as well as Firmicutes and Gemmatimonadetes, while decreasing the relative abundances of Nitrospirae and Cyanobacteria (Fig. 3A and C). After 35-year recovery, only half of these dominant phyla (Actinobacteria, Firmicutes and Nitrospirae) in the secondary forest returned to a similar level compared to the intact primary forest (Fig. 3C). Interestingly, the highest relative abundances of Acidobacteria, Verrucomicrobia and Chloroflexi were found in the secondary forest soils, although clear-cutting did not significantly shift their abundances (Fig. 3B; Table S2, Supporting Information). These findings indicated a partial recovery of bacterial communities at the phylum level through the 35-year rehabilitation after clear-cutting.

A further examination of the bacterial composition changes revealed that these significantly changed phyla occurred in some specific bacterial groups at the class or lower phylogenetic levels. The significant increase of the Proteobacteria was mainly driven by increased abundances of the class Alphaproteobacteria and Deltaproteobacteria, while Betaproteobacteria showed lower abundance in response to clear-cutting (Table S2, Supporting Information). The increase of the phylum Verrucomicrobia in the secondary forest was mainly driven by the genus *Xiphinematobacter* and *Opitutus* (Table S2, Supporting Information). The decrease of the Cyanobacteria in response to clear-cutting was mainly due to the dramatical decrease in the abundances of dominant OTUs, such as OTU907, OTU5794 (Table S2, Supporting Information) and OTU1304. The increase of Actinobacteria in the shrub-grassland and its recovery in the secondary forests soils were driven by all OTUs except OTU3560, 186 and 3299. The responsive pattern of the phylum Firmicutes were mainly driven by all OTUs except OTU3461, 10269 and 84621. Similarly, all the OTUs in Nitrospirae except OTU 18872, OTU 25027 and OTU 27195 also showed recovery from the previous reduction induced by the clear-cutting.

Archaea were mainly distributed in the phylum Crenarchaeota (95.8% of total archaeal reads) and Euryarchaeota (4.0%

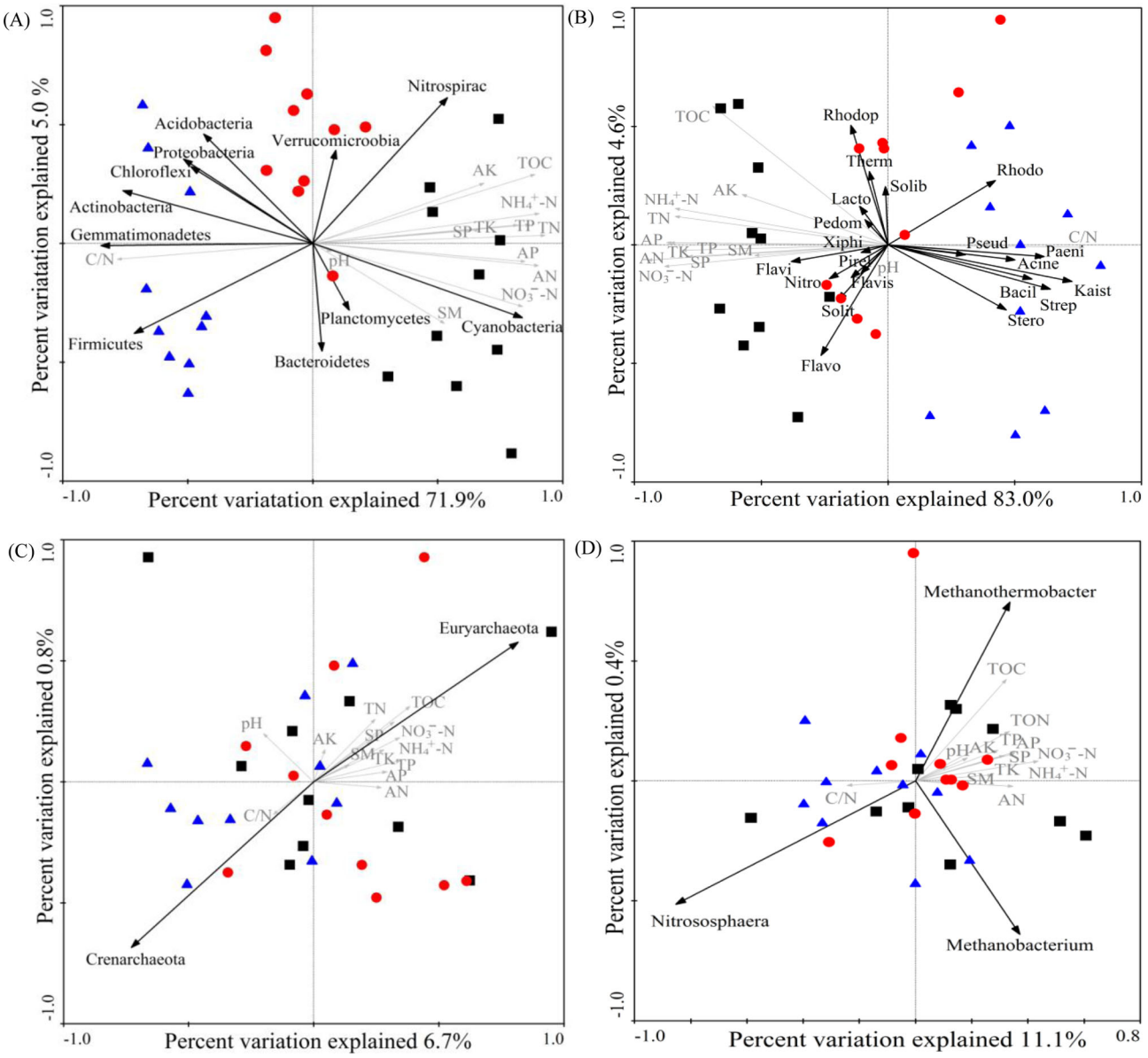

Figure 4. RDA showing the effect of soil properties on bacterial on the phylum level (**A**) and genes level (**B**) and archaeal on the phylum level (**C**) and genes level (**D**). Only taxa above 0.5% of total average relative abundance are shown. Black square: primary forest; blue triangle: shrub-grassland; red cycle: secondary forest; grey arrow: soil properties; dark arrow: soil prokaryotes.

of total archaeal reads). Their relative abundances were not significantly different among all the rehabilitation stages and intact primary forest (Fig. 3D). In addition, no significant changes occurred at the class or lower levels except for OTU8099 and OTU10699 (Table S3, Supporting Information).

Relationships between prokaryotic community composition and soil factors

Mantel test was used to determine the effects of the rehabilitation stages, elevation and geographic distance on soil prokaryotic community composition. Geographic distance ($P = 0.278$) and elevation ($P = 0.342$) had no effect on both bacterial and archaeal communities. Rehabilitation stage was found to be a significant factor affecting soil bacterial community composition ($P < 0.01$) (Table S4, Supporting Information) but not archaea ($P = 0.23$).

Forward selection on RDA was used to understand the potential effects of soil factors on bacterial composition at phylum level. Overall, all measured parameters formed a clear gradient along the chronosequence (Fig. 4A). Individual selection of variables revealed that soil pH had a weak effect on the bacterial community composition ($P < 0.05$) among all the measured soil chemical parameters, whereas other soil variables (e.g. TOC, TN, AN, NH_4^+-N, NO_3^--N and C/N) significantly explained the patterns of community composition. Collectively, all the soil properties adequately explained the patterns of community composition (76.9% of total variation) (Fig. 4A), with NH_4^+-N, NO_3^--N and C/N together accounting for 64.6% of the explained variation. Mantel test further confirmed the relationships between the community composition at the phylum level and soil factors (Table S4, Supporting Information). Notably, Spearman correlations between individual soil variables and the relative abundances of main phyla demonstrated that Actinobacteria,

Firmicutes, Nitrospirae, as well as Cyanobacteria and Gemmatimonadetes, were significantly correlated with soil nutrients (Table S5, Supporting Information).

At the genus level, soil properties significantly affected bacterial communities. Soil properties contributed 87.6% of the total variations in bacterial community structure (Fig. 4B) and the NH_4^+-N, AN, NO_3^--N and C/N were major drivers. This was consistent with the strong relationships between dominant bacterial genes (the top 20) and soil nutrient content, such as Rhodococcus, Acinetobacter, Steroidobacter, Flavihumibacter, Bacillus and Paenibacillus (Table S6, Supporting Information).

RDA showed that soil factors had no effects on archaeal community composition at both phylum and genus level (Fig. 4C and D).

Prokaryotic community assembly processes

Bacterial and archaeal community assembly processes were assessed by NRI and NTI. There were significant shifts in NRI and NTI values in response to clear-cutting for bacteria ($P = 0.001$; Fig. 5A and B) but not for archaea ($P = 0.213$; Fig. 5C and D). In the shrub-grass land, both NRI and NTI value for bacterial communities was dramatically higher than that in the primary forest ($P < 0.001$). In the secondary forests, however, both indices were not significantly different compared to the intact primary forest ($P = 0.187$; Fig. 5A and B).

DISCUSSION

Recovery patterns of bacterial community structure and diversity

Despite the essential role that microbes play in ecosystem recovery, there is limited experimental evidence to show that there are predictable patterns in microbial communities occur during secondary succession or ecosystem restoration (Fierer *et al.* 2010; Bardgett and van der Putten 2014). In this context, our results could provide a better understanding of the recovery patterns of bacterial dynamics by showing that clear-cutting caused dramatic changes in soil bacterial community composition and structure, and became more similar to its original state of the intact primary forest through the 35 years of rehabilitation (Figs 2A, B and 3). This finding supports the hypothesis that there are predictable successional patterns in bacterial community recovery after a disturbance, which is similar to the successional patterns of plant communities. The 'restoration ecology' theory (Hobbs and Norton 1996; Palmer, Ambrose and Poff 1997), which assumes that ecosystem degeneration is not irreversible and the recovery after disturbance will proceed toward a primary, unperturbed state, is true in this soil bacterial case. It indicates that the bacterial communities could rebound to its original state after clear-cutting disturbance under certain mechanisms. In fact, the time frame we present for the recovery of soil bacterial community may require at least 30 years.

Our results also indicate that different taxa might recover at different rates and directions, leading to changes in the relative abundances of certain taxa (Fig. 3). The intraspecific variability in taxon abundance may provide the potential for a bacterial community to respond rapidly and reversibly to disturbance events through flexible adjustment (species sorting) (Rui *et al.* 2015). The shifts on different taxa support the hypothesis that the niche-selection induced by clear-cutting, changes on bacterial community structure mainly through species sorting.

Bacterial phylogenetic diversity has been suggested as a powerful proxy for assessing the role of microbial diversity in shaping ecosystem functioning, because it takes into account (phylogenetically linked) ecological differences among species (Srivastava *et al.* 2012). Our results found that clear-cutting did not significantly decrease the diversity of soil bacterial communities (Fig. S1, Supporting Information), which is consistent with the previous study that discovered that residue management regimes have no significant effect on bacterial diversity (He, Xu and Hughes 2006). Strikingly, the bacterial communities had the highest phylogenetic diversity in the 35-year recover stage forest (Fig. S1, Supporting Information). Lower diversity observed at the shrub-grassland was associated with respective high disturbance while the primary forest was associated with respective low disturbances (Doležal, Yakubov and Hara 2013). Similar results have been reported in both bacteria and plant community studies (Hart and Chen 2008; Xiang *et al.* 2014; Huang *et al.* 2015), which supports the intermediate disturbance hypothesis. This hypothesis proposes that species diversity should be low after high disturbance, or under stress condition, or low resource availability, as only a few species can survive (Dvorský *et al.* 2011; Doležal, Yakubov and Hara 2013). With a decrease in disturbance intensity and habitat stress or an increase in resource availability, species diversity should increase to a maximum, but low species diversity will occur beyond this maximum point because only a few highly competitive species will become dominant and consequently suppress the others (Yodzis 1986; Doležal, Yakubov and Hara 2013). Our results indicate that this unimodal relationship between species diversity and disturbance or recovery may be true for soil bacterial communities.

Disturbance promotes the importance of deterministic processes

Clear-cutting provides an opportunity to examine the relative importance of stochastic versus deterministic assembly processes of bacterial communities following clear-cutting. In this study, both NRI and NTI values for bacterial communities were significantly higher in the shrub-grassland than in the primary forest. However, there was no significant difference between the secondary forest and the intact primary forest (Fig. 5A and B). This result indicates that clear-cutting significantly increased deterministic processes, but after 35 years of recovery, the degree of deterministic processes returned to a similar level as the intact primary forest. Previous studies also found that disturbances such as drought and dietary restrictions, would lead to the increase of niche-based processes (Chase 2007; Jiang and Patel 2008). Our results go beyond these findings by showing that the relative importance of deterministic processes would recover during natural regeneration, which supports the model proposed by Cadotte (2007) and Zhou *et al.* (2014).

While addressing the issue of why clear-cutting disturbance promotes the relative influence of deterministic processes, we come up with a few ideas. Cutting directly changes soil microclimate (Keenan and Kimmins 1993; Jurgensen *et al.* 1997), vegetation, litter quantity and quality, decreases soil organic carbon and soil nutrient availability (Grigal 2000). Therefore, the soils of the shrub-grassland may represent an extreme, low resource environment relative to the intact primary forest (Table 1). As expected, when the environment became less harsh through the certain time of recovery, the degree of deterministic processes returned to a state as the primary forest. Furthermore, we found that there was a strong relationship between the

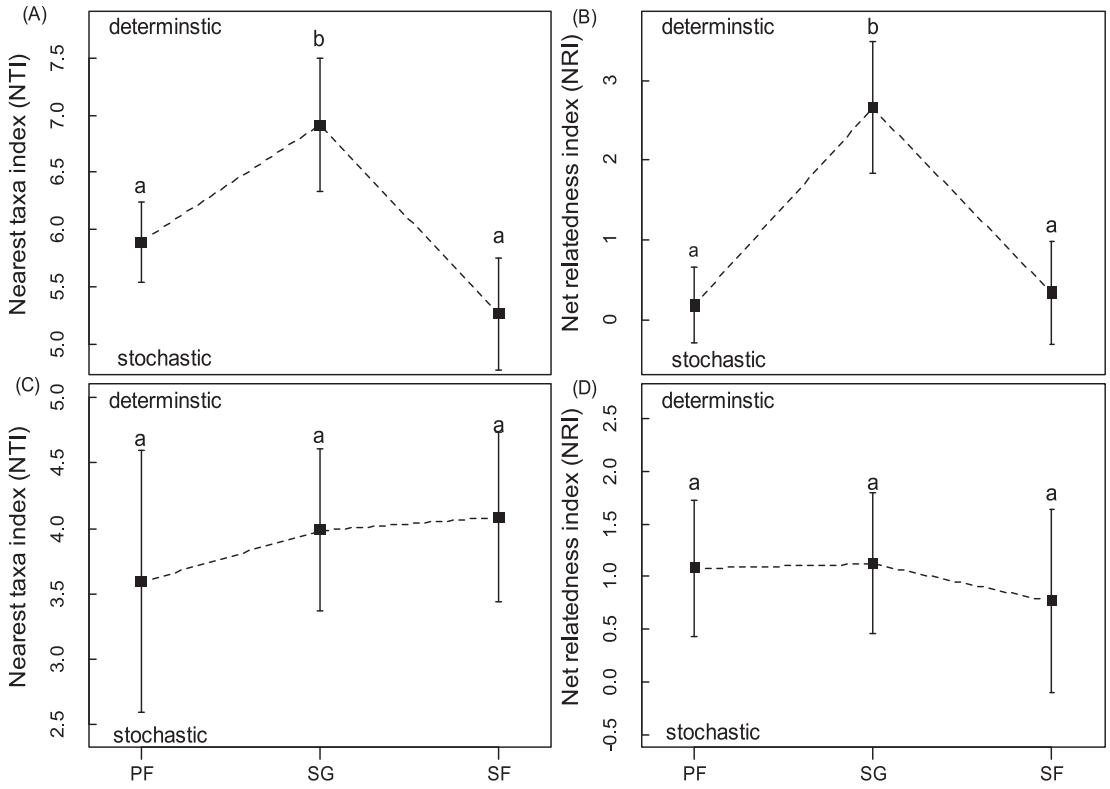

Figure 5. The NTI and NRI of bacterial (A&B) communities and archaeal communities (C&D) at different rehabilitation stages. Different letters indicate significant differences in NTI/NRI between vegetation types based on the permutation tests ($P < 0.05$). PF: primary forest; SG: shrub-grassland; SF: secondary forest.

composition of bacterial communities and soil factors. All these findings provide convincing evidence that clear-cutting increases the importance of deterministic processes working as a dominant role of environmental filtering (Kembel 2009). The key inference is that one or more soil factors limit community membership whereby there is closely related and ecologically similar taxa co-occurrence to a greater degree than expected from the null model. This inference is consistent with previous work suggesting that there are greater deterministic processes in low or extreme resource environments (Chase 2007; Gerisch *et al.* 2012; Van der Plas, Anderson and Olff 2012).

Dynamics of bacterial community was driven by biotic and abiotic variables

Linking bacterial community dynamics to environmental conditions may provide insights into how abiotic variables influence the composition and distribution of taxa along the rehabilitation stages following a disturbance (Chase 2003; Ferrenberg *et al.* 2013). In our study, significant relationships were found between all tested soil properties, except soil pH, and microbial community composition (Fig. 4, Tables S5 and 6, Supporting Information). Soil pH was previously identified as the most important factor affecting soil bacterial communities (Ferrenberg *et al.* 2013; Shen *et al.* 2013), but the effect of clear-cutting on soil pH is often low. Here, we did not observe any significant effect of soil pH on soil bacterial communities. In this study, our results indicate that soil nutrient availability, especially variations in NH_4^+-N, NO_3^--N and C/N, have an important role in shaping soil bacterial communities in response to clear-cutting.

In addition to be driven by abiotic factors, that is, the succession of bacterial communities might be partially autogenic (Archer *et al.* 1988; Walker and Del Moral 2003; Dini-Andreote *et al.* 2014). This indicates that the bacterial community can alter the characteristics of its surroundings and then influence secondary colonizing species through competition for nutrients, facilitation or antagonism. For instance, cyanobacteria introduces ammonia into the system (nitrogen fixation), and creates favorable growth conditions for other organisms (Schütte *et al.* 2009). Thus, this is possibly extendible to other ecological interpretations concerning functional interdependencies and niche occupancy within a community. In addition, our results show that communities contain closely related species during ecosystem disturbance, indicating interspecific competition and niche occupancy is much stronger in the shrub-grassland as explained by interspecific competition and niche theory (Case and Gilpin 1974). The principle that interspecific competition could lead to species dispersal (Armstrong and McGehee 1980; Webb *et al.* 2002) is verified by the bacterial communities having a larger genetic distance at later succession stage than at prior succession stage, which indicates the importance of biotic interactions in bacterial community succession after a disturbance.

Species sorting of archaeal communities is highly conservative to cutting-based disturbance

The widespread distribution of archaea in forest ecosystems implies their potential contribution to global biogeochemical cycles (Schleper, Jurgens and Jonuscheit 2005). In this study, Archaea accounted for 10.9%–13.4% of total prokaryotes in Karst soils at different recovery stages, which is similar to the previous reports of high relative abundances (5% –15.6%) of archaea in wet tropical forests soils, compared to relatively low abundances

(averaging 2%) across 146 sites in North and South America and Antarctica (Bates *et al.* 2011; Cao *et al.* 2012).

No significant shifts of archaeal community composition and structure occurred in response to clear-cutting (Figs 2C, D and 3D). This is in agreement with previous observations in different vegetation and soil types (Chu *et al.* 2011; Cao *et al.* 2012). The lack of archaeal response is in agreement with previous studies that archaea are superior to bacteria in tolerating soil nutritional changes and energy stress (Valentine 2007). Therefore, as the soil nutrient status declines due to clear-cutting, bacterial composition changes rapidly, while archaea survive and remain stable even in these less fertile deforested soils.

CONCLUSIONS

In summary, our results showed that soil bacterial and archaeal communities had entirely different response patterns to clear-cutting disturbance. No significant shifts were observed in archaeal communities. While changes of bacterial communities after a disturbance were predictable, returning to an intact primary state during the natural recovery processes. This recovery pattern of bacterial community structure and diversity follows the conceptual framework observed for plants. Succession of bacterial communities was mostly driven by shifts in soil properties. Clear-cutting increased the importance of deterministic processes in shaping bacterial community composition, coinciding with low resource environments. This means that changes in soil factors can influence the community composition by promoting deterministic processes and working as an 'environmental filter'. Further research is needed to include more rehabilitation stages, and integrate local and regional perspectives at the structure and functional levels to better understand soil microbial succession patterns and assembly processes under disturbance.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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