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Determinants of the biodiversity patterns of ammonia-oxidizing archaea community in two contrasting forest stands

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Abstract
Purpose The variation in soil microbial community patterns is primarily influenced by ecological processes associated with spatial distance and environmental heterogeneities. However, the relative importance of these processes in determining the patterns of soil microbial biodiversity in different successional forests remains unclear.
Materials and methods Based on the species data from denaturing gradient gel electrophoresis (DGGE) analysis, we described the composition and beta diversity of ammonia-oxidizing archaea (AOA) community, an important functional microbial group in regulating nitrogen cycle, in a middle-succeed stand (60 years of secondary succession) and an undisturbed native stand in a subtropical forest in southern China. The composition pattern was examined using a multi-response permutation procedure (MRPP), and the beta diversity was described using the Sørensen index. The relative influence of edaphic, vegetational, spatial, and topographical factors on AOA composition and beta diversity was assessed by variation partitioning and multiple regression on distance matrices (MRM), respectively.
Results and discussion We did not find any stand-specific patterns in AOA community composition in the two stands; however, the influential variables were different between the two stands; 7.3 and 4.5 % of the total variation in AOA community composition could be explained by edaphic (i.e., available potassium and total phosphorus) and spatial variables, respectively, in the middle-succeed stand, while 3.7 and 2.8 % of the variation were explained by spatial variable and available phosphorus, respectively, in the native stand. Soil total phosphorus influenced the beta diversity of AOA community most in the middle-succeed stand, while genetic distance of tree species was found to be the most important factor in driving the beta diversity pattern in the native stand.
Conclusions Soil nutrients influenced the beta diversity of AOA community in the middle-succeed stand more than that in the native stand, while vegetation is more important in the native stand. The substantial unexplained variations were possibly due to the effects of other unmeasured variables. Nevertheless, dispersal process is more important in controlling AOA community composition in the native stand, while processes associated with environmental heterogeneities are more important in the middle-succeed stand in this subtropical forest.

Keywords Ammonia-oxidizing archaea · Beta diversity · Forest succession · Genetic distance · MRM · Subtropical forests

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1 Introduction

The microbial process of the transformation from ammonia (NH_3) to nitrite (NO_2^-) is the first and rate-limiting step of nitrogen (N) cycle (Hatzenpichler 2012), and it can be catalyzed by the monooxygenase coded by the functional gene of ammonia monooxygenase (amoA). With the first discovery of amoA gene in archaea (Konneke et al. 2005), the involvement of archaea in ammonia oxidation has been paid great attention and widely investigated in various environments (Treusch et al. 2005; Di et al. 2010; Zhang et al. 2012), and the ammonia-oxidizing archaea (AOA) community is increasingly considered as one of the most important functional microbial groups involved in N cycle. Although the importance of AOA and its counterpart, ammonia-oxidizing bacteria (AOB), in ammonia oxidation is still controversial, AOA community has been proved to be more important in influencing soil N turnover in acid environments (Nicol et al. 2008; Isobe et al. 2012; Zhalnina et al. 2012). For example, soils of the subtropical forests in southern China that have received chronic high N deposition are typically acidic, and the AOA community has been found to be the dominating functional microbial group in regulating nitrification rate in these forest soils (Isobe et al. 2012). In addition to the AOA abundance, Isobe et al. (2012) also reported different composition of AOA community in three subtropical forests in different successional stages from southern China. However, the exact patterns of AOA community composition and diversity and their influential factors in such successional forests are still unknown. According to Wessén et al. (2011), investigations of the microbial community composition and diversity by using spatial methods are important in helping us with the understanding of the ecosystem processes they performed and the underlying mechanisms in driving microbial distributions. Additionally, beta diversity, the variation in species composition among the sampling sites in the study areas, has been considered as a key factor for assessing the ecosystems functioning and exploring the managements in the biodiversity conservation (Martiny et al. 2011; Bahram et al. 2013). Thus, the characterization and explanation of the composition and beta diversity patterns of AOA community in the successional subtropical forests in southern China will be important for any programs aiming to managements and restorations of these disturbed forests.

The variation in microbial community patterns can usually be explained by the dispersal process related to spatial distance and complex processes, such as niche differentiation and environmental filtering, which are related to environmental heterogeneities (McArthur et al. 1988; Martiny et al. 2006). Abiotic environmental variables such as soil pH, soil N content, soil phosphorus (P) content, soil carbon (C) content, and soil moisture have proved to be the important predictors for the composition and diversity of soil microbial communities (Fu et al. 2012; Wang et al. 2015). Vegetational properties such as species composition, tree crown size, and roots are demonstrated as vital biotic variables in structuring microbial communities (Saetre 1999; Brant et al. 2006; Hu et al. 2010; Li et al. 2013). Recent researches even reported significant effects of plant genotypes on soil microbial communities (Madritch and Hunter 2002; Schweitzer et al. 2008), which may shed light on the underlying mechanisms in regulating the interactions between soil microbes and vegetation. Assessing the relative contributions of these predictors to the variation in the composition and biodiversity of soil microbial communities can help us with better understanding of ecological processes in driving soil microbial community biodiversity. However, such information is still limited, despite previous studies having focused on some aspects of this topic (Ramette and Tiedje 2007; Dumbrell et al. 2010; Bru et al. 2011).

In this study, we investigated the AOA community in two stands in an evergreen broad-leaved forest (EBLF) forest in southern China. One stand was severely disturbed and has been conserved for nearly 60 years since 1956. The other stand was undisturbed and naturally developed. The composition and beta diversity patterns of AOA community were investigated and described in each stand, and the relative contributions of different environmental variables (i.e., edaphic, vegetational, topographical, and spatial variables) to the variation of these patterns were quantified and assessed to further explore the underling mechanisms in driving the distributions of AOA community in successional forests.

2 Materials and methods

2.1 Sample site and soil collection

The research was conducted in a forest located in the DHS National Nature Reserve (112° 30′ 39″–112° 33′ 41″ E, 23° 09′ 21″–23° 11′ 30″ N), which is also known as a famous tourist attraction. This forest is characterized by a south subtropical monsoon climate, with mean annual temperature of 20.9 °C and precipitation of 1929 mm. The soil is lateritic red earth (oxisol) formed from sandstone that is natively acidic. After long-time N deposition in this area, the soil is further acidified with pH ranging from 3.84 to 4.02 (Liu et al. 2010; Fang et al. 2011).

The climax vegetation of this forest is evergreen broad-leaved forest (EBLF). However, one part of the native vegetation has been degraded due to human disturbance and conserved in secondary succession for 60 years (thereafter the middle-succeed stand). The other part has never been disturbed as protected by the nearby Qinyun temple (therefor, the native stand). We focused our study areas on these two stands in this EBLF forest, and the general information about the soil, vegetation, and topography of the two stands and the EBLF forest was given (Table 1).

Table 1 Summaries of the edaphic, topographical and vegetational variables in this study

Variables	Middle-succeed stand (n=31) mean±SE	Native stand (n=31) mean±SE	EBLF forest (n=62) mean±SE
AOA abundance (Archaeal *amoA* copy numbers×10^9 g^{-1} dry soil)	1.12±1.89	1.77±1.90	1.44±1.91
Soil water content (SWC, %)	18.74±1.70	16.48±2.23	17.61±2.27
Soil bulk density (BD, g cm^{-3})	1.09±0.07	0.99±0.04	1.04±0.08
pH	3.79±0.08	3.67±0.07	3.73±0.10
Soil organic matter (SOM, mg kg^{-1})	50.81±5.64	73.76±11.42	62.28±14.61
Available potassium (AK, mg kg^{-1})	42.34±5.78	72.32±18.66	57.33±20.40
Available phosphorus (AP, mg kg^{-1})	2.63±1.08	0.80±0.23	1.71±1.21
Available nitrogen (AN, mg kg^{-1})	174.14±20.79	243.94±20.63	209.04±40.74
Total potassium (TK, g kg^{-1})	16.17±2.81	20.70±3.62	18.43±3.94
Total phosphorus (TP, g kg^{-1})	0.24±0.03	0.34±0.04	0.29±0.06
Total nitrogen (TN, g kg^{-1})	0.76±0.23	1.82±0.45	1.29±0.64
Altitude (m)	361±55	342±55	352±56
Slope (°)	29±6	39±7	34±8
Shannon-Wiener diversity (div.)	2.6±0.4	2.7±0.2	2.7±0.3
Species richness (Ric., #/quadrat)	25±6	30±7	28±7
Tree density (Den., #/quadrat)	116±42	142±53	129±49
Tree height (Hei., m)	4.9±0.6	4.6±0.4	4.7±0.5
DBH (cm)	7.3±1.9	5.8±1.1	6.6±1.7

In each stand, 31 quadrats (20×20 m^2) were selected (unaligned and randomly distributed) and recorded with the coordinate (Fig. 1 and Table S1, Electronic Supplementary Material). Three individual soil cores (approximately 500 g

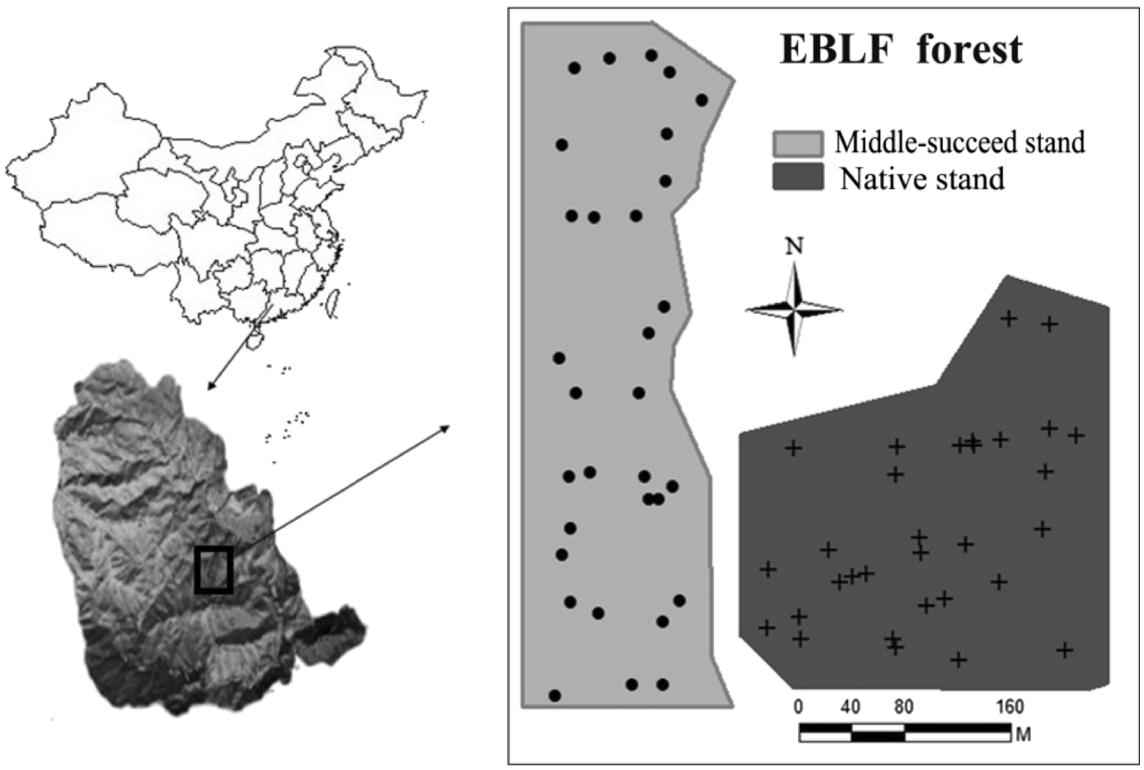

Fig. 1 Map of the sampling area in a subtropical evergreen broad-leaved forest (EBLF) divided into a middle-succeed and a native stand. Area filled with *light gray* represents the middle-succeed stand, and the native stand is marked by the *dark gray shade*. *Solid dots* and *plus signs* represent the sampling quadrats in the middle-succeed and native stand, respectively

per sample) were collected from the topsoil (0–10 cm) around the center of each quadrat by using a soil auger and then mixed. After the removal of litter, animals, and stones, these soil samples were sieved through a 2-mm mesh and divided into two parts. One part was saved at −20 °C for DNA extraction, and the other part was used for edaphic property analysis.

2.2 DNA extraction

Soil DNA was extracted as described by Krsek and Wellington (1999) with some modifications. In detail, 0.5 g of soil sample was firstly washed with PBS buffer (135 mM NaCL, 2.7 mM KCL, 1.5 mM NaH_2PO_4, 8 mM Na_2HPO_4, 20 mM EDTA, pH=7.4) three times after vibration for 3 min in a 2-ml sterile tube to improve the quality of the extracted DNA, followed by centrifugation for 5 min at 10,000 rpm (Zhao et al. 2005). Then, 0.9-ml extraction buffer and 50 μl proteinase K (100 mg/ml) were added, and the sample was then shaken for 30 min at 200 rpm and 37 °C. A total of 100 μl of 20 % sodium dodecyl sulfate (SDS) was added and the sample was incubated in a 65 °C water bath for 30 min with gentle end-over-end inversion every 10 min. The supernatant was collected after centrifugation at 10,000 rpm for 3 min at room temperature and transferred to another tube. Then, 0.2 volume of potassium acetate (KAc) (8 M) was added, followed by incubation in an ice bath for 30 min. The supernatant was collected after centrifugation at 13,000 rpm and 4 °C for 20 min, and 0.5 volume of polyethylene glycol (PEG) (50 %) with 0.1 volume of NaCl (5 M) was added. After incubation at 4 °C for 2 h, the mixture was centrifuged at 13,000 rpm and 4 °C for 20 min. The pellets were then washed with 70 % ethanol and dissolved in TE (10 mM Tris, 1 mM EDTA, pH 8.0). After that, the solution was mixed with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1; *v/v/v*). The aqueous phase was recovered by centrifugation and mixed with an equal volume of chloroform/isoamyl alcohol (24:1, *v/v*). The aqueous phase was again obtained by centrifugation and precipitated with 0.1 volume of sodium acetate (NaAc) (3 M) and 0.6 volume of isopropanol at 4 °C overnight. Finally, the pellet of crude DNA was formed by centrifugation at 10,000 rpm for 5 min, washed with 70 % ethanol, and suspended with 50 μl TE. DNA purity was verified using electrophoresis on a 1 % agarose gel and concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific).

2.3 Real-time PCR amplification, DGGE, and phylogenetic analysis of amoA gene sequences

The real-time PCR was performed using the PCR protocol described as Zhang et al. (2012) on an ABI 7500 thermocycler system with Primer pairs CrenamoA616r/CrenamoA23f (Tourna et al. 2008). All the 62 samples were analyzed using denaturing gradient gel electrophoresis (DGGE) method with three replications for each sample and as much as 16 DGGE gels were used in this study. Prior to DGGE analysis, a PCR amplification of archaea amoA gene was conducted on a Lab Cycler system (SENSO) with primer pairs of CrenamoA616r/CrenamoA23f in 50-μl reaction mixtures, consisting of 2 μL of each primer (10 mmol/L), 4 μL dNTPs (2.5 mmol/L for each), 5 μl 10× buffer, 0.3 μL Taq polymerase, 0.05 mg bovine serum albumin, 3 μL of tenfold purified DNA (1–10 ng) as a template, and 35.7 μL double-distilled water (DDW). The thermal profile of PCR was as follows: initial 5 min at 95 °C, followed by 35 cycles of 45 s at 95 °C for denaturing, 45 s at 53 °C for annealing, and 1 min at 72 °C for extension. The DGGE analysis was performed on a JY-TD331 denaturing gradient gel electrophoresis system (JUNYI, Beijing, China). PCR products were loaded onto an 8 % polyacrylamide gel with a linear gradient of 30–60 % to run the electrophoresis at 60 °C for 12 h with a constant voltage of 80 V, and then silver stained (McCaig et al. 2001). After that, a total of 20 different predominant DGGE bands were excised and placed into sterile micro centrifuge tubes with 30 μl TE, which were saved at 4 °C for 12 h and then 5 μl liquid was used as DNA template for AOA amoA gene re-amplification with the same primers described earlier. A touchdown PCR procedure was used for the re-amplification to improve the production efficiency and all the products were saved in −20 °C for sequencing. The 20 sequences obtained directly by sequencing the product of band re-amplification were involved in following analyses, because these sequences probably dominate in the DGGE bands (Muyzer et al. 1993; Zhang et al. 2012). The number of the DGGE bands for each sample lane was recorded as species richness of AOA community.

After BLAST on the NCBI, sequences which were most similar to those obtained from DGGE analysis were selected and downloaded as the references. Phylogenetic tree was then constructed using neighbor-joining method in MEGA 5.1 (Tamura et al. 2011) with 1000 bootstrap tests for every node based on the 20 AOA amoA gene sequences from this study and the reference sequences. Nucleotide sequences of AOA amoA genes obtained in this study were deposited in the EMBL database under the following accession numbers: HG514287-HG514306.

2.4 Edaphic, topographic, and vegetational variables

Soil water content (SWC) was examined by drying fresh samples to a constant weight at 105 °C. Soil bulk density (BD) was measured using stainless-steel containers. Testing of soil pH values were performed in a soil to water ratio of 1:2.5 using a pH meter. For determining soil total organic matter (SOM), available potassium (AK), available phosphorus (AP), available nitrogen (AN), total nitrogen (TN), total phosphorus (TP), and total potassium (TK), the specific methods were used as described by Liu et al. (1996). Topographical

variables including altitude, slope, and convexity were obtained using the methods described as Ma et al. (2014).

For the vegetational variables, we recorded the diameter at breast height (DBH) and height of all free-standing plants, and calculated the tree density, species diversity, and species richness for these 62 quadrats in which our soil samples were collected. The Shannon-Wiener diversity index was determined using the method as Liu et al. (2011), and the number of tree species in each quadrat was counted and recorded as species richness. Trees with height ≥10 m in each stand were selected and defined as the canopy layer. Principal component analysis (PCA) was used to obtain the species composition of the canopy layer. The first two axis of PCA (therefore, the veg-PC1 and veg-PC2) were selected and regarded as the variables of canopy tree composition. The tree species that was nearest to the three sampling sites in each quadrat was recorded, and the sequences of the DNA barcoding regions (*ITS*) of these species were downloaded from NCBI based on the accession numbers from Liu et al. (2015). The pairwise genetic distance (GD) of these tree species were calculated based on the alignment of these sequences in MEGA 5.1 (Tamura et al. 2011), and the genetic distance matrix of each stand was considered in the analysis described below (i.e., multiple regression on distance matrices (MRM)). Since the EBLF forest was mainly divided into the middle-succeed and native stand (Fig. 1), the average values of the total 62 samples from these two stands of the soil, vegetational and topographic features were calculated and presented as the general information of the EBLF forest (Table 1).

2.5 Spatial variables

The spatial variables were yield from the interquadrat distances using the method of Moran's eigenvector maps (MEMs) in R 3.1.0 with the "spacemakeR" packages, which formerly called principal coordinates of neighbor matrices (PCNM) (Dray et al. 2006). The eigenvectors that model positive spatial correlation (Moran's I larger than E (I)) were retained and used as the explanatory spatial variables in the following analyses (i.e., variation partitioning and MRM).

2.6 Statistical analysis

To test the significance of the difference of AOA community composition between the middle-succeed stand and the native stand, a multi-response permutation procedure (MRPP) was used in PC-Ord version 5 (MjM Software Design, Gleneden Beach, OR) with the raw binary data matrix of AOA composition (Table S1, Electronic Supplementary Material; 1 means the presence of a specific species in the sampling sites, and 0 representing this species is absence). MRPP is a nonparametric approach for testing the hypothesis of no differences between two or more groups. The significance of differences is tested by comparing the average distance between all pairs of points within each of the pre-defined groups to a Pearson type III continuous distribution of all possible partitions of the data. The MRPP procedure will yield a chance-corrected within-group agreement (A) statistic which ranging from 1 to −1 and a p value assessing the significance of the homogeneous among groups, and the more positive A is, the more similar groups are and the greater confidence in the p value (O'Hanlon and Harrington 2012). Additionally, an indicator species analysis (ISA) in the program PC-Ord was used to tell which populations were diagnosed as the most abundant for each group. The statistical significance of the indicator values were tested using Monte Carlo tests with 1000 randomizations (Flinn et al. 2008).

The beta diversity (beta-sor; Sørensen index) of AOA community in each stand was calculated based on the presence-absence data (Table S1, Electronic Supplementary Material) (Southwood and Henderson 2000; Koleff et al. 2003). Then, the relative influence of environmental variables on the variation in beta diversity was determined by using multiple regression on distance matrices (MRM) in R 3.1.0 with the "ecodist" packages (Legendre et al. 1994; Goslee and Urban 2007; Lichstein 2007). Four MRM models were constructed to detect the partial effects: (i) the edaphic model, (ii) the edaphic + vegetation model, (iii) the spatial + vegetation + spatial model , and (iv) the edaphic + vegetation + spatial + topography model. As suggested by Florencio et al. (2014), we firstly used spearman correlations to assess the relationships between environmental distance matrixes and the dissimilar matrix (beta diversity) of AOA community; then, the significant explanatory variables are identified using a forward-selection procedure; finally, the significance of MRM models were tested by 1000 permutations on the objects of the response distance matrix. The r^2 from MRM analyses was used to assess the variation in the beta diversity of AOA community explained by these significant environmental variables and the significantly correlated variables were excluded within each group of variables (edaphic, vegetational, spatial, and topographic variables).

To detect the relative contributions of different environmental factors to the variation in AOA community composition in each of the two stands, a multivariate variation partitioning method was used in R 3.1.0 with the "varpart" packages (Peres-Neto et al. 2006; Gilbert and Bennett 2010). All the environmental variables involved in this analysis were transformed to meet the normal distribution. Prior to partitioning, forward selections of all the environmental variables including edaphic, vegetational, spatial and topographical variables were performed in R 3.1.0 using the "packfor" packages (Legendre et al. 1994), and only the variables that

significantly influenced AOA composition were presented in the final variation partitioning results.

3 Results

3.1 AOA community composition in the two contrasting forest stands

Phylogenetic trees of the AOA amoA gene sequences obtained from DGGE analysis are shown in Fig. 2. A total of 20 sequences dominated in both the middle-succeed and native stands. All the sequences were grouped into two clusters falling within soil and sediment lineages. The sequences in cluster 1 were similar to those found in the Pine forest (Isobe et al. 2012), the Peachester forest (Long et al. 2011, unpublished data in GenBank), as well as the sediment from the deep-sea of west pacific ocean (Dang et al. 2009). Sequences belonging to cluster two were correlated with those recovered from evergreen broadleaf forest, pine forest, mixed (pine and broadleaf) forest in the Dinghushan (Isobe et al. 2012), sediment, and acid soils (Lu and Jia 2013).

According to the MRPP test (A=0.011, p<0.05), the distribution of AOA species between the two stands was significantly less heterogeneous than expected by chance. Additionally, the results of indicator species analysis (Table S2, Electronic Supplementary Material) suggested only the species retrieved from band14 to band17 had significantly greater indicator values in the middle-succeed stand, which could be defined as the dominant species in this stand.

3.2 Variation partitioning of AOA community composition

In the middle-succeed stand, 8.2 % of the total variation in composition of AOA community could be explained by the

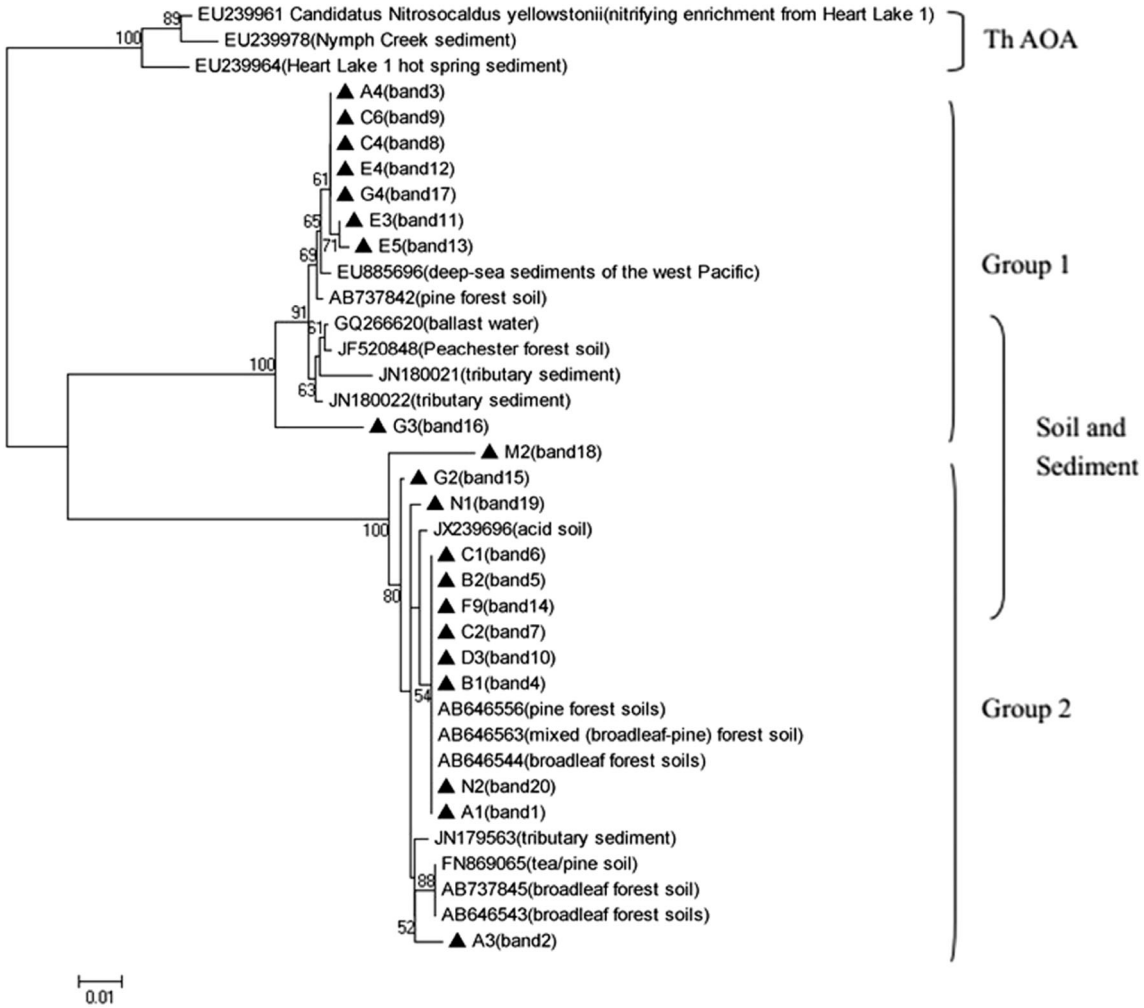

Fig. 2 Phylogenetic analysis of archaeal amoA gene retrieved from both the middle-succeed and native stand in this study. Neighbor-joining tree is constructed, and bootstrap values higher than 50 % are indicated at nodes. Sequences derived from this study are marked by *triangles in bold* and indicated by DGGE band number which named by experimental order

environmental variables, with 3.7 % attributed to the pure effects of AK and TP, 1 % related to the pure effect of spatial variable, and 3.5 % caused by the combination of edaphic and spatial factors. In the native stand, only 5.6 % of the total variation in the composition of AOA community composition could be explained, with 2.8 and 1.8 % of the variation related to the spatial variable and AP content, respectively, and only 0.9 % of the variation jointly explained by the spatial variables and edaphic properties (Fig. 3). When combining the two stands as an EBLF forest, 12.2 % of the total variation of AOA composition could be explained by these factors. Particularly, 6.9 % of the variation was spatially structured, 3.4 % of the variation could be attributed to the AOA community abundance, TN and AN, and 1.8 % of the variation was jointly explained by the spatial variables and edaphic properties (Fig. 3). Nevertheless, a vast majority of the variation of AOA community composition distribution still remained unexplained, which might be due to some unmeasured environmental variables.

3.3 Relative contributions of environmental and spatial factors to the variations in beta diversity of AOA community

In the middle-succeed stand, the beta diversity of AOA community was most influenced by TP, with high absolute coefficients in both the edaphic and edaphic + vegetation models. Veg-PC2 was found to be marginally significant in the edaphic + vegetation model which totally explained 3.4 % of the variation in AOA beta diversity. None of the spatial and topographic variables showed significant influence on the beta diversity pattern of AOA community. In the native stand, only TP influenced the beta diversity in the edaphic model, with marginally significance. GD showed the highest absolute coefficients in the other three models and therefore best explained the variation in AOA beta diversity. V4 and convexity were added in the edaphic + vegetation + spatial + topography model, and amount of 7.7 % of the variation in beta diversity of AOA community could be explained in this final model (r^2=0.077, Table 2).

4 Discussion

AOA community showed similar composition in the two stands, and only the two species retrieved from band14 and band17 were demonstrated as the indicator species in the middle-succeed stand. These results may be caused by the reason that the composition of microbial community could be affected by the historical legacy which could maintain the initial functional differences between microbial communities over time (Yan et al. 2007; Keiser et al. 2011). For instance, the historical depositional environments could play an important role in shaping the microbial communities in sediment (Wang et al. 2008). In this study, the two nearby stands belonged to a same EBLF forest before disturbance and

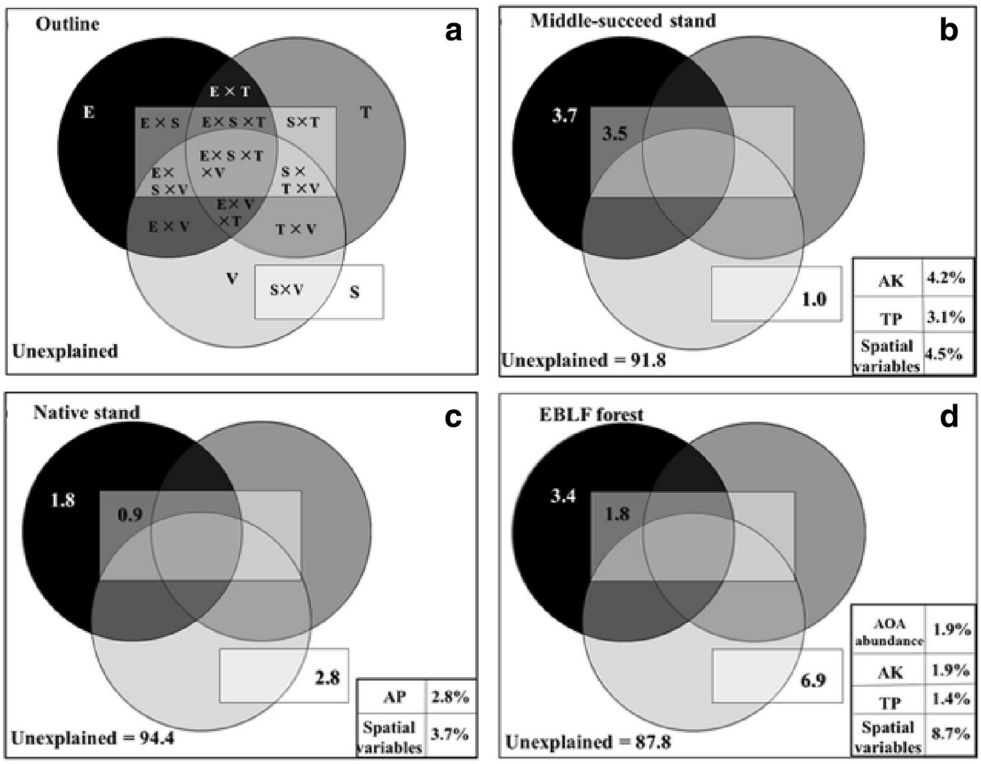

Fig. 3 Variation partitioning of the distribution of AOA community composition into the relative effects of four groups of environmental factor: edaphic factors (*E*), topographical factors (*T*), vegetational factors (*V*), and the spatial factor (*S*). **a** The outline instruction. **b** The results of variation partitioning in the middle-succeed stand. **c** The results of variation partitioning in the native stand. **d** The results of variation partitioning in the EBLF forest. The significant variables selected in each group are presented in the table of each subgraph with the contributed variation

Table 2 Different multiple regression models predicting the responses of AOA community β-diversity to the variables including edaphic variables; edaphic and vegatational variables; edaphic, vegetational and spatial variables; and edaphic, vegatational, spatial and topographic variables

Middle-succeed stand				Native stand			
Model	Variables	Coefficient[a]	r^2	Model	Variables	Coefficient[a]	r^2
Edaphic	TP	−0.136 m.s.	0.021[b]	Edaphic	TP	−0.167 m.s.	0.008 m.s.
	AOA abundance	−0.0003 m.s.		Edaphic + Vegetation	TP	−0.176 m.s.	0.033[b]
Edaphic + Vegetation	TP	−0.133 m.s.	0.034[b]		GD	0.203[b]	
	AOA abundance	−0.0004[b]		Edaphic + Vegetation + Spatial	TP	−0.182 m.s.	0.062[b]
	Veg-PC2	−0.023 m.s.			GD	0.203[b]	
					V4	0.0006[b]	
				Edaphic + Vegetation + Spatial + Topography	TP	−0.160 m.s.	0.077[b]
					GD	0.201[b]	
					V4	0.0005[b]	
					Convexity	−0.004 m.s.	

[a] Coefficients of Spearman correlations, r^2 (ranged 0–1), 10,000 permutations

[b] $p<0.05$

m.s marginally significant ($p<0.1$), *V4* Eigenvectors extracted from the interquadrat distances among the sampling quadrats based on the Moran's eigenvector maps, *TP* soil total phosphorus, *AOA* abundance, Archaeal *amoA* copy numbers per gram dry soil, *Veg-PC2* canopy tree composition, *GD* genetic distance between tree species

experienced the same soil microbial development process owing to the homogenous soil parent materials and vegetational conditions in the initial stage. Thus, similar AOA genetic lineages may exist in soil from the two stands; despite that, the changes in environmental variables after forest disturbance or during forest restoration may alter the relative abundance of different AOA species (Liang et al. 2014). In addition, since the two stands were adjacent to each other, the dispersal barriers caused by distance likely to be easily overcome, and the high similarity in microbial community composition would be possible as a result of inoculation from the adjacent environments.

We found the edaphic, spatial, and vegetational variables influenced the AOA community composition and beta diversity significantly, and the relative contributions of these variables were different between the two stands. Previous researches suggested that the variation in microbial community composition could be both determined by the process based on spatial factors (i.e., spatial dispersal) and processes associated with environmental heterogeneities, such as niche differentiation and environmental filtering (McArthur et al. 1988; Martiny et al. 2006), whereas, the relative importance of these factors was controversial. In this study, results of variation portioning showed more influence of edaphic properties (i.e., AK and TP) (7.2 %) could exert on AOA composition variation in the middle-succeed stand than other variables, while the pure spatial effect was more important in the native stand (2.8 %). Soil P and potassium (K) are usually less available in soil from disturbed forests than the native forests, as deforestation might exacerbate the deficiencies of soil nutrients such as K and P (Hedin et al. 2003). AOA community composition may change with the environmental gradients caused by soil P and K contents (de Gannes et al. 2014; Chen et al. 2015). Consequently, the distribution heterogeneities of these two nutrients in the middle-succeed stand may act as environmental filters filtering out the AOA individuals that were not favored by the site-specific environmental conditions, leading to the niche differentiations of different AOA species. Contrastingly, the pure spatial effects in the native stand may be generated from the spatial processes of AOA community such as biotic interactions and autocorrelation (Borcard and Legendre 1994).

Total P content significantly influenced the beta diversity pattern of AOA community in both stands, which was similar with the results from variation partitioning showing significant influence of TP and AP on AOA composition. These results confirmed that soil P content was an important edaphic variable in driving AOA community assemblage in the this EBLF forest, which may attributed to the strong immobility of soil P in this area caused by low soil pH after severe N deposition (Huang et al. 2009; Liu et al. 2013). Besides the edaphic and spatial variables, GD was identified as a significant variable in influencing the beta diversity of AOA community in the native stand. To our knowledge, this was probably the first case to link the turnover in AOA community species to the genetic distance of tree species in forests, although previous studies have suggested that the genotypes of individual plant could influence the associated belowground soil microbial community (Hooper et al. 2000; Wardle et al. 2004). This result suggested that the feedback between AOA community diversity and tree species might be more stable and more pronounced in the native stand than that of the middle-succeed

stand. In forests, changes in tree species may alter the quality and quantity of leaf litter composition, consequently, influence the soil microbial compositions (Merila et al. 2010). Moreover, different tree species may favor specific microbial groups through altering the biotic and abiotic environments underground with root exudates or production (Haichar et al. 2008; Prosser and Nicol 2012). These hypotheses can help us with the understanding of our finding showing significant correlation between GD and beta diversity of AOA community in the native stand, whereas further experiments are still needed to explore the specific underlying mechanisms in determining the relationships between the genetic distances of tree species and the variation in AOA community diversity. Although the explanatory power was weak, the amounts of variations in beta diversity and composition of AOA community that could be explained by the pure effects of edaphic, vegetational, and spatial variables belonged to the range of what has been reported in previous studies in spatial analyses of microbial communities (Ramette and Tiedje 2007; Wang et al. 2008; Bru et al. 2011; Martiny et al. 2011; Bahram et al. 2013). However, substantial proportions of the total variation in both of AOA composition and diversity in the two stands remain unexplainable, which is possibly caused by the pure effects or the spatial arrangement of other important environmental variables that we did not measure in this study (Gilbert and Bennett 2010), such as soil salinity and temperature (Zhalnina et al. 2012).

5 Conclusions

The current study demonstrated no significant difference of AOA community composition between the middle-succeed stand with 60 years succession and the undisturbed native stand within an EBLF forest, which might be attributed to historical events and frequently inoculation between the adjacent environments. TP showed significant effects on AOA community structure and beta diversity in both stands, and the genetic distance between tree species was found to be an important predictor for the beta diversity pattern of AOA community in the native stand. More of the variation in AOA community composition could be explained by spatial variable in the native stand, while the effects of edaphic properties were evidenced more importantly in the middle-succeed stand. From these results, we can further confirm that the genotypes of plants can exert significant effects on AOA community diversity and the relative role of the dispersal and niche-related processes in shaping AOA community during forest succession are different. The dispersal process based on spatial distance may dominate in the undisturbed native stand, while the processes based on environmental heterogeneity may dominate in the middle-succeed stand.

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