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EXPERIMENTAL ARTICLES

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## Microbiomes of Virgin Soils of Southern Vietnam Tropical Forests

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**Abstract**—Numbers of bacterial, archaeal, and fungal ribosomal gene copies and the taxonomic structure of prokaryotic communities in virgin tropical soils under weakly impacted monsoon forests at the CKat Tien National Park (Southern Vietnam) were determined, and their relation with the major physicochemical parameters of the studied soils were investigated. Samples were collected from genetic horizons of brown tropical (Cambisol) and dark-colored (Umbrisol) soils on volcanic deposits, red-yellow tropical soil (Regosol) on metamorphic slates, and alluvial sandy-loam soil (Fluvisol). The numbers of ribosomal gene copies in virgin soils of southern Vietnam tropical forests were up to  $10^{11}$ – $10^{12}$  gene copies per 1 g, which was comparable to the richest soils of the temperate zone. The highest numbers of microbial genes were found in the upper horizons (4–10 cm). Higher abundance of microbial ribosomal genes was found in volcanic soils, compared to red-yellow tropical and alluvial ones. The dominant prokaryotic phyla were *Proteobacteria* and *Acidobacteria* (subgroup 1, *Acidobacteriales*; subgroup 2; and *Solibacterales*). The share of *Acidobacteria* in soils correlated with pH and was highest in the most acidic red-yellow tropical soil. The share of *Verrucomicrobia* was highest in the surface soil layers and decreased with depth. The share of *Chloroflexi* increased with depth. Members of the recently described bacterial phylum *Rokubacteria* were revealed. The differences in soil-forming rocks (volcanic deposits, metamorphic slates, and alluvium) determined the differences in the chemical properties of soils and the taxonomic structure of their prokaryotic communities. Organic carbon content is probably the main factor determining both the abundance of microbial ribosomal genes and the taxonomic structure of prokaryotic communities from virgin forest tropical soils.

**Keywords:** tropical soils, volcanic soils, qPCR, sequencing, 16S rRNA, prokaryotes, *Verrucomicrobia*, *Acidobacteria*, *Rokubacteria*

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Tropical ecosystems are characterized by high diversity of flora and fauna, high biological productivity, and intense matter turnover. However, biodiversity in soil is lower than in overground zones; it was shown that biodiversity of both soil fauna (Bardgett and Putten, 2014) and prokaryotic communities (Miyashita et al., 2013) in the tropics did not exceed that in soils of temperate climate. Removal of natural forests often results in degradation of tropical soils and loss of their fertility. If the impacted regions are not turned into agrocenosis, secondary forests of different composition, herbaceous communities, and waste grounds develop in these areas. Deforestation in tropical areas caused radical changes in the taxonomic structure and diversity of soil microbial communities (Navarrete et al., 2015; Schneider et al., 2015; Kroeger et al., 2018). Taking into account an intense decrease in the tropical forest areas and agricultural application of tropical soils, little time remains for the study of microbial diversity in virgin tropical soils under native vegetation.

The present study was carried out at the Cat Tien National Park, the Dong Nai province (Southern Vietnam), which contains weakly impacted monsoon forests (Blanc et al., 2000). The Cat Tien preserve is characterized by abundance and high diversity of soil mesofauna (Anichkin, 2011). Aleksandrova and coworkers revealed high diversity of cultured soil fungi with absolute predominance of anamorphic ascomycetes and considerable differences in the fungal species composition in alluvial and ferralitic soils, as well as in soils under plain and mountain forests (Aleksandrova et al., 2011; Kalashnikova and Aleksandrova, 2015; Kalashnikova et al., 2016). The studies carried out in five different regions of Vietnam revealed that little differences in diversity of cultured actinomycetes from soils and plant litters (Hop et al., 2011). Direct cell enumeration using fluorescence microscopy revealed that the Cat Tien virgin soils were characterized by rather high abundance of bacteria (up to  $8 \times 10^9$  cells/g); however, the mycelium length of soil fungi was low (200–600 m/g) as compared with that in

**Table 1.** Physicochemical characteristics of the studied soils (according to Khokhlova et al., 2017): carbon and nitrogen contents, pH of water and salt extracts, and amounts of physical clay and silt

No.	Soil	Horizon	Depth, cm	C, %	N, %	pH (H <sub>2</sub> O)	pH (KCl)	Physical clay, %	Silt, %
I	Dark-colored	A1	0–6 (10)	3.18	0.45	4.9	4.2	53.7	27.0
		AB	6 (10)–20 (25)	1.54	0.25	4.7	3.8	78.5	55.0
		BC (C)	55 (60)–115	1.01	0.21	4.9	3.9	78.0	58.0
II	Brown tropical	A1	0–6 (10)	3.11	0.48	5.4	4.1	52.8	19.4
		AB	6 (10)–25 (35)	1.07	0.13	5.5	4.4	53.1	21.5
		BmC	4 (55)–85 (105)	0.61	0.22	5.6	4.7	72.9	57.9
III	Red-yellow tropical	A1	0–4 (6)	1.90	0.32	3.8	3.2	68.9	25.3
		A1C	4 (6)–50 (60)	0.60	0.19	3.7	3.1	80.5	29.1
		C1	50 (60)–80	0.43	0.21	4.6	3.7	82.3	25.6
IV	Alluvial	A1	0–6 (8)	0.59	0.16	5.0	4.3	14.0	5.0
		AB	6 (8)–15	0.27	0.09	5.2	4.6	10.0	4.9
		C2	45 (50)–75	0.22	0.05	5.4	4.3	14.6	7.1

the forest soils of temperate climate (Lysak et al., 2017). No evaluation of taxonomic structure and diversity of soil prokaryotic communities (including the use of molecular biological methods) in this region have been carried out previously.

Location of the main soil types at the Cat Tien preserve depends mainly on soil-forming rocks and relief: red-yellow tropical soils (Regosol) were located on metamorphic slates; brown tropical (Cambisol) and dark-colored humic (Umbrisol) soils were found on volcanic deposits; alluvial loam or sandy soils (Fluvisol) occurred on the river alluvium (Khokhlova et al., 2017). These four soil types were taken as the subjects of study in this work.

The goal of the present work was to evaluate abundance of archaea, bacteria, and fungi by determining the numbers of their ribosomal gene copies and to study the taxonomic structure and diversity of prokaryotic communities in the main types of virgin soils of monsoon forests at the Cat Tien preserve.

## MATERIALS AND METHODS

**The subjects of the study** were soil samples taken from the reference soil profiles at the Cat Tien preserve, which have been earlier described (Khokhlova et al., 2017):

(1) dark-colored clay soil on volcanic deposits, Skeletic Greyzemic Umbrisol (Clayic), further designated as “dark-colored” (11°25′40″ N, 107°25′32″ E);

(2) brown tropical clay shallow soil on volcanic deposits, Distric Skeletic Rhodic Cambisol (Clayic), further designated as “brown tropical” (11°25′43″ N, 107°25′39″ E);

(3) red-yellow tropical shallow immature clay soil on metamorphic slates, Dystric Regosol (Clayic), further designated as “red-yellow tropical” (11°26′56″ N, 107°26′29″ E);

(4) alluvial sandy-loam soil, Dystric Fluvisol (Arenic, Drainic), further designated as “alluvial” (11°26′48″ N, 107°26′26″ E).

All soil samples were withdrawn under cover of weakly impacted monsoon forests. The dark-colored and brown tropical soils were located at the watershed area formed by volcanic deposits (basalt and tuff); red-yellow tropical soil was located at the peak of a ridge formed by metamorphic clay slates; alluvial soil was located at the near-riverbed swell of the Dong Nai River. Physical and chemical characteristics of the soils are given in Table 1.

**Sampling and DNA isolation.** Soil samples for microbiological examination were taken in November 2015. Samples were withdrawn in triplicate from the walls of soil sections in the surface (A), subsurface (AB or AC), and mineral (BC or C) horizons of soil profiles (Table 1). Before analysis, the samples were stored at –70°C.

Total DNA was isolated from a soil sample (0.5 g) by using the FastDNA SPIN Kit for soil (MP Biomedicals, United States) according to the manufacturer’s protocol with modified homogenization performed on a Precellus 24 homogenizer at 6500 rpm for 30 s. The DNA isolation was verified using electrophoresis in agarose gel (1% solution in Tris-acetate-EDTA (TAE) buffer) with subsequent visualization under UV illumination.

**Determining the number of microbial ribosomal genes.** The abundance of bacteria, archaea, and fungi

in soil was evaluated by quantitative polymerase chain reaction (PCR) of their ribosomal genes on an iCycler amplifier (Bio-Rad, United States) using the Eub338 and Eub518 primers to the 16S rRNA genes for bacteria (Fierer et al., 2005), the 915f and 1059r primers for archaea (Yu et al., 2005), and the ITS1f and 5.8s primers to the ITS region for fungi (Fierer et al., 2005). The PCR was carried out on a Real-Time CFX96 Touch amplifier (Bio-Rad, United States), and the fluorescence intensity in the mixture was measured in each reaction cycle. The reaction mixture was prepared based on the BioMaster HS-qPCR SYBR Blue preparation (Biolabmix, Russia). Solutions of cloned ribosome operon fragments of *Escherichia coli* for bacteria, *Halobacterium salinarum* FG-07 for archaea, and *Saccharomyces cerevisiae* Meyen 1B-D1606 for fungi were used as concentration standards. The initial number of gene copies was determined with the aid of the CFX Manager software package. The number of gene copies in DNA preparations was calculated per 1 g soil of field humidity taking into account the dilution used and the amount of soil taken.

**Preparation of gene libraries and sequencing the 16S rRNA gene amplicons.** For preparing the 16S rRNA gene libraries, in each DNA sample amplification with universal primers for variable V3–V4 region of the 16S rRNA gene: F515 (GTGCCAGCMGCCGCGG-TAA) and R806 (GGACTACVSGGGTATCTAAT) (Bates et al., 2011) was carried out with addition of linker- and barcode-containing sequences according to the Illumina method. The PCR was performed in the reaction mixture (15  $\mu$ L) containing Phusion Hot Start II High-Fidelity polymerase (0.5–1.0 U), 1 $\times$  Phusion buffer (Thermo Fisher Scientific, United States), forward and reverse primers (5 pM each), DNA template (10 ng), and dNTPs (2 nM each) (Life Technologies, United States).

The mixture denaturation at 94°C for 1 min was followed by 35 cycles: 94°C (30 s), 50°C (30 s), and 72°C (30 s). Final elongation was carried out at 72°C for 3 min. PCR products were purified by the Illumina method using the AM Pure XP reagents (Beckman Coulter, United States). Further formation of libraries was carried out using MiSeq Reagent Kit Preparation Guide (Illumina, United States) according to the manufacturer's instructions. The libraries were sequenced on an Illumina MiSeq device using MiSeq Reagent Kit v3 (600 cycle) with paired-end reading.

**Statistical data processing and bioinformatic analysis.** The sequencing data were processed using the QIIME software package (Caporaso et al., 2010). The trimming (the sequence filtration according to the read quality) was performed using the Trimmomatic program (Bolger et al., 2014); forward and reverse reads were united using the Fastq-join algorithm (Aronesty, 2013). Searching and filtration of chimeric sequences was carried out using the Vsearch algorithm (Rognes et al., 2016). The sequences with 97% nucle-

otide similarity were combined into operational taxonomic units (OTUs) using the SILVA database, v. 132, 2018 (<https://www.arb-silva.de/download/archive/qiime>). Singletons (OTUs containing only one nucleotide sequence), as well as OTUs of chloroplasts and mitochondria were eliminated. After data processing, from 8606 to 23265 (on the average, 15186) 16S rRNA gene sequences were obtained for each soil sample. Taxonomic structure of microbiomes was characterized by using the mean data of triplicate measurements for each soil sample.

To evaluate the general diversity of microbial communities (alpha diversity), the following parameters were calculated: the number of OTUs detected, the Chao1 index (estimation of the real number of OTUs in a microbiome) (Collwell and Coddington, 1994), and the Shannon's index. The difference between microbiomes (beta diversity) was assessed using the weighted UniFrac metric (Lozupone, 2011). The results of the beta diversity analysis were visualized using nonmetric multidimensional scaling (NMDS) in a two-dimensional space. For evaluation of alpha and beta diversity, the data were normalized over 8606 sequences for each sample.

Relationship between various microbiological characteristics (the abundance of microbial ribosome genes, alpha diversity, and the share of individual prokaryotic phyla) and physicochemical characteristics of soil was assessed using the Spearman's rank correlation coefficient at the significance level  $p = 0.05$  using the STATISTICA program. To assess correlations with the shares of prokaryotic phyla, median proportion was calculated from thrice-repeated measurements for each sample.

Evaluation of the correlation between phylogenetic structure of communities (based on the weighted UniFrac method) and soil characteristics (depth, pH of the water and salt extracts, the contents of carbon, nitrogen, physical clay, and silt) was performed by the Mantel test using the QIIME software package with the permutation number of 999.

## RESULTS

**Abundance of microbial ribosomal genes.** In all soil profiles studied, the numbers of bacterial, archaeal, and fungal ribosomal gene copies decreased with depth along the profile (Table 2).

The highest number of bacterial genes ( $1.1 \times 10^{12}$  16S rRNA gene copies per 1 g of soil in the upper horizon) was found in the brown tropical soil on basalt deposits. Lower gene numbers were revealed in the dark-colored soil on basalt and tuff and alluvial soil ( $3.3 \times 10^{11}$ /g and  $2.6 \times 10^{11}$ /g, respectively, in horizons A). The red-yellow tropical soil on metamorphic slates was characterized by the lowest number of bacterial 16S rRNA gene copies ( $2 \times 10^{11}$ /g in horizon A).

**Table 2.** The number of microbial ribosomal gene copies (billion per 1 g soil) and characteristics of alpha diversity of prokaryotic communities: the Shannon's index, the number of OTUs revealed, and the Chao1 index (a range of values from three replicates)

Soil	Depth, cm	16S rRNA genes of bacteria, 10 <sup>9</sup> /g	16S rRNA genes of archaea, 10 <sup>9</sup> /g	ITS genes of fungi, 10 <sup>9</sup> /g	Diversity of prokaryotes		
					the Shannon's index	the number of OTU	the Chao1 index
I Dark-colored	4	297–354	219–373	13–17	8.0–9.0	1272–1716	2053–2711
	16	112–302	68–126	9–12	7.2–8.6	773–1430	1205–2184
	90	31–169	31–67	6–10	7.7–7.9	997–1170	1530–1927
II Brown tropical	4	854–1347	231–375	11–17	7.8–8.2	1343–1430	2175–2407
	19	259–869	98–134	14–17	8.0–8.2	1131–1253	1935–2167
	68	7–12	9–14	3–4	7.7–7.8	930–1006	1347–1413
III Red-yellow tropical	3	197–218	96–195	18–21	8.3–8.5	1040–1144	1572–1687
	30	22–80	30–66	6	7.4–8.0	676–1062	956–1627
	68	11–14	19–34	4–5	6.9–7.2	536–610	711–847
IV Alluvial	4	136–418	104–131	18–21	8.7–9.0	1357–1515	2035–2353
	11	94–252	58–76	11–13	8.1–8.7	1086–1356	1593–2026
	60	7–9	11–28	3–4	7.5–7.7	690–784	892–1130

In the upper horizons of all soils, the number of archaeal ribosomal genes reached 10<sup>11</sup>/g. In volcanic soils, the number of archaeal genes was two to three times higher than that in the red-yellow and alluvial soils.

The number of fungal ribosomal genes in the upper horizons reached 10<sup>10</sup>/g and varied slightly in the studied soils (Table 2).

According to the Spearman's coefficient, the numbers of microbial genes showed a positive correlation with carbon content and a negative correlation with depth (Table 3).

**Taxonomic structure and the microbiome diversity.** The obtained 16S rRNA gene sequences were deposited with the BioProject of the NCBI database under the accession number PRJNA518023 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA518023>). After data editing and removal of singletons, 6070 operational taxonomic units (OTUs) were revealed in all the samples. The estimated species abundance of microbiomes in the studied soil samples was 536–1716 OTUs revealed among accessible 8850 nucleotide sequences; the real OTU number according to the Chao1 index ranged from 762 to 2711 (Table 2). Prokaryotic diversity was the lowest in horizons of red-yellow tropical soil and the highest in dark-colored and brown tropical soils on volcanic deposits. The

upper horizons of all soils had the highest prokaryotic diversity. All diversity characteristics (the OTU number, the Shannon's index, and the Chao1 index) showed negative correlation with depth (Table 3).

By using the SILVA taxonomic database, 38 different prokaryotic phyla were found in the studied microbiomes. However, most of the phyla were found only in some samples and were characterized by low relative abundance (the percentage of the total number of the 16S rRNA gene sequences). Bacterial phyla *Acidobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Chloroflexi*, *Actinobacteria*, *Firmicutes*, *Planctomycetes*, *Rokubacteria*, *Nitrospirae*, *Gemmatimonadetes*, and *Bacteroidetes*, as well as the archaeal phylum *Thaumarchaeota* had the shares exceeding 1% (Fig. 1). In all the studied microbiomes, 33 to 65% nucleotide sequences belonged to two dominant phyla, *Acidobacteria* and *Proteobacteria*. The share of *Acidobacteria* was higher in lower horizons than in the surface ones and differed markedly in various soil types: it was minimal (8–16%) in brown tropical and maximal (34–52%) in red-yellow soil. The studied soils differed considerably in the taxonomic composition of *Acidobacteria*: in red-yellow and alluvial soils, the most abundant were the members of subgroup 2 with no cultured members (up to 36%), *Acidobacteriales* (up to 13%), and *Solibacteriales* (up to 6%) (Fig. 2). In brown tropical and dark-colored soils, *Acidobacteriales* prevailed; the share of

**Table 3.** The Spearman's rank correlation coefficients for characteristics of soil (depth, pH of water and salt extracts, contents of carbon, nitrogen, physical clay, and silt) and the microbiome (the numbers of bacterial, archaeal, and fungal ribosomal gene copies, the shares of individual prokaryotic phyla, and values of alpha diversity). Statistically significant correlation ( $p < 0.05$ ) is shown in bold

Parameter	Depth	pH (H <sub>2</sub> O)	pH (KCl)	C	N	Physical clay	Silt
The number of bacterial 16S rRNA gene copies	<b>-0.73</b>	0.08	0.01	<b>0.71</b>	0.42	-0.35	-0.28
The number of archaeal 16S rRNA gene copies	<b>-0.85</b>	-0.13	-0.14	<b>0.73</b>	0.52	0.34	-0.27
The number of fungal ITS gene copies	<b>-0.84</b>	-0.12	-0.02	0.53	0.29	-0.41	-0.36
<i>Thaumarchaeota</i>	0.00	<b>0.59</b>	0.45	-0.04	-0.13	-0.41	-0.36
<i>Acidobacteria</i>	0.31	<b>-0.61</b>	-0.51	<b>-0.63</b>	-0.46	0.37	-0.05
<i>Actinobacteria</i>	-0.22	<b>0.64</b>	0.50	<b>0.63</b>	0.47	-0.23	0.17
<i>Bacteroidetes</i>	<b>-0.83</b>	-0.01	0.02	<b>0.58</b>	0.47	-0.50	-0.32
<i>Chloroflexi</i>	<b>0.83</b>	0.22	0.14	-0.08	-0.12	0.49	<b>0.70</b>
<i>Firmicutes</i>	-0.20	<b>0.71</b>	<b>0.71</b>	0.13	-0.16	<b>-0.68</b>	-0.36
<i>Gemmatimonadetes</i>	<b>0.63</b>	<b>0.59</b>	0.53	-0.23	-0.42	-0.08	0.18
<i>Nitrospirae</i>	<b>0.94</b>	0.16	0.09	-0.50	-0.39	0.39	0.45
<i>Planctomycetes</i>	<b>-0.83</b>	-0.39	-0.20	0.13	0.11	-0.35	-0.57
<i>Proteobacteria</i>	<b>-0.88</b>	-0.02	0.05	<b>0.59</b>	0.38	-0.44	-0.32
<i>Rokubacteria</i>	<b>0.85</b>	0.29	0.13	-0.43	-0.27	0.42	0.34
<i>Verrucomicrobia</i>	<b>-0.79</b>	0.23	0.21	<b>0.67</b>	0.43	-0.54	-0.33
The Shannon's index	<b>-0.79</b>	0.10	0.29	0.40	0.15	<b>-0.62</b>	-0.41
Total number of OTUs	<b>-0.73</b>	0.22	0.31	0.52	0.28	<b>-0.62</b>	-0.36
The Chao1 index	<b>-0.64</b>	0.28	0.31	<b>0.59</b>	0.32	-0.56	-0.28

subgroup 2 was lower, whereas general diversity of *Acidobacteria* was higher. The share of the phylum *Verrucomicrobia* represented mainly by the orders *Chthoniobacterales* and *Pedosphaerales* was rather high (11–22%) in the upper horizons of the studied soils and decreased with depth to 2–7% in the lower horizons. The share of *Actinobacteria* varied from 3–6% (red-yellow soil) to 12–15% (brown tropical soil). The share of the recently described phylum *Rokubacteria* ranged from 1 to 6% of the prokaryotic community and increased in the lower horizons of all soils (Fig. 1).

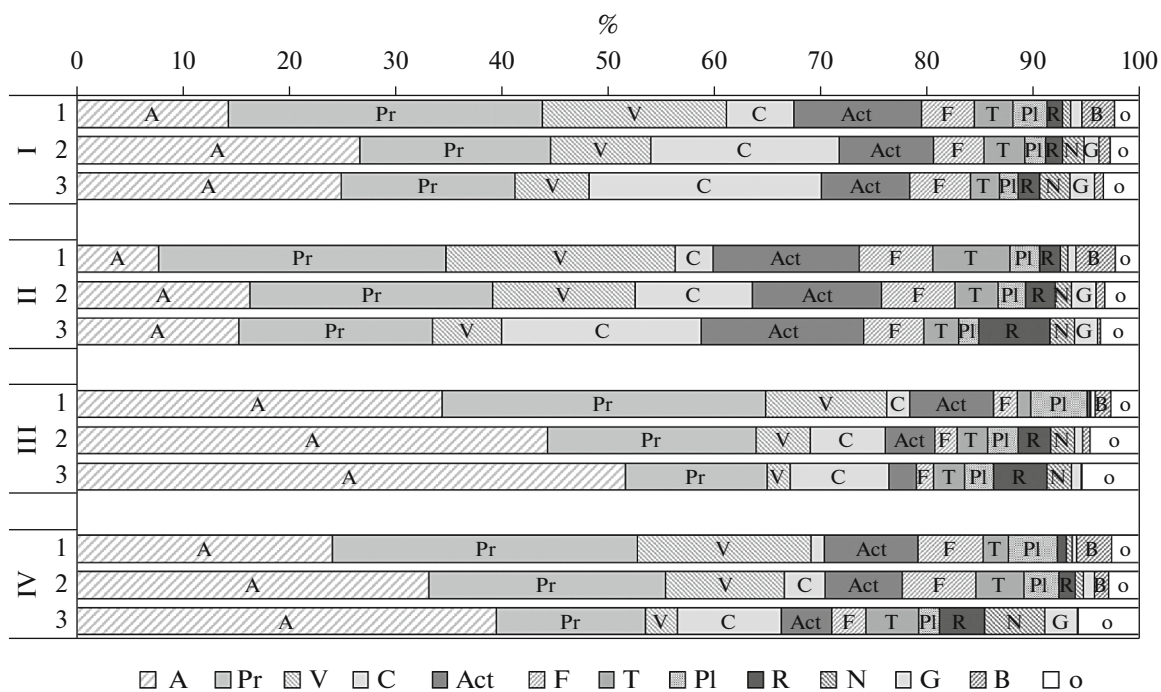
Soil depth was the main factor determining the share of prokaryotic phyla in microbiomes (Table 3). With increasing depth, the shares of the phyla *Bacteroidetes*, *Planctomycetes*, *Proteobacteria*, and *Verrucomicrobia* decreased, whereas the shares of *Chloroflexi*, *Gemmatimonadetes*, *Nitrospirae*, and *Rokubacteria* increased. A significant correlation was also found between the shares of some phyla and pH, carbon content, and amounts of physical clay and silt (Table 3).

Evaluation of the phylogenetic structure similarity of prokaryotic communities based on the weighted UniFrac method showed that communities of two volcanic soils formed a separate cluster, which differed from the microbiomes of red-yellow and alluvial soils (Fig. 3). The structure of microbiomes of alluvial sandy-loam soil was the most different from that of volcanic soils. Table 4 shows results of the Mantel test,

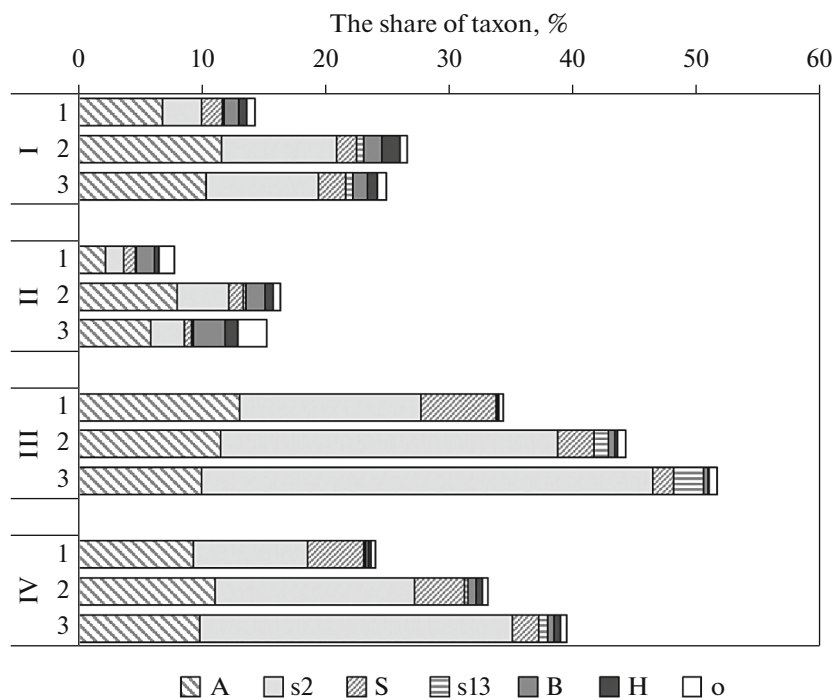
which indicated correlation between chemical and physical characteristics of soil and differences in the structure of prokaryotic communities assessed by the weighted UniFrac method. The structure of soil microbiome depended first of all on carbon content; the other significant factors ( $p < 0.05$ ) were nitrogen content and soil depth.

**Table 4.** The correlation coefficient for the pairwise distance matrix ( $R$ ) and the correlation significance level ( $p$ ) for the differences between the community structure assessed by the weighted UniFrac method and soil characteristics (depth, pH of water and salt extracts, contents of carbon, nitrogen, physical clay, and silt) determined by the Mantel test

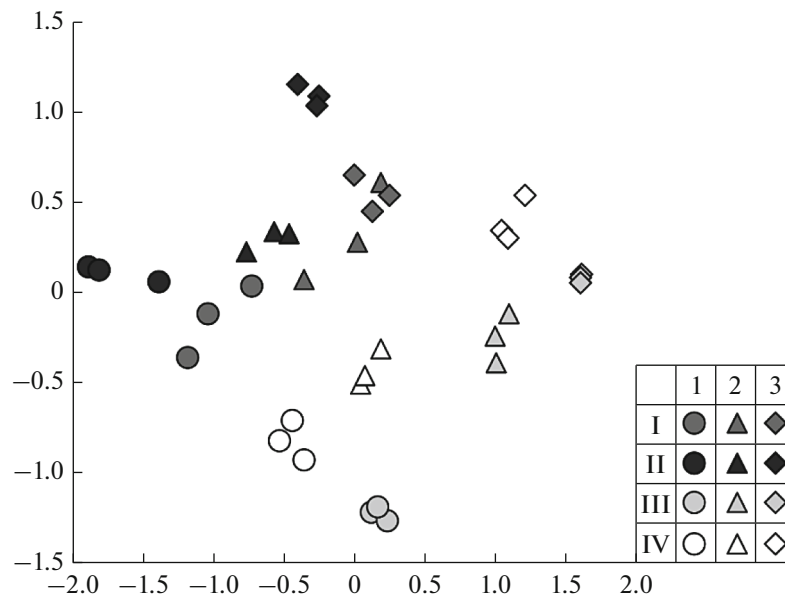
Soil characteristics	$R$	$p$
C	0.39	0.006
N	0.33	0.021
Depth	0.27	0.044
pH (H <sub>2</sub> O)	0.16	0.315
pH (KCl)	0.07	0.657
Silt	-0.08	0.694
Physical clay	-0.03	0.967



**Fig. 1.** Taxonomic structure of microbiomes (at the level of prokaryotic phyla) in the surface (1), subsurface (2), and mineral (3) horizons of the studied soils: dark-colored (I), brown tropical (II), red-yellow tropical (III), and alluvial (IV) soils. The phyla with the share of above 1%: *Acidobacteria* (A), *Proteobacteria* (Pr), *Verrucomicrobia* (V), *Chloroflexi* (C), *Actinobacteria* (Act), *Firmicutes* (F), *Thaumarchaeota* (T), *Planctomycetes* (Pl), *Rokubacteria* (R), *Nitrospirae* (N), *Gemmatimonadetes* (G), *Bacteroidetes* (B), and the others (o) are shown. The mean data for three replicated samples are given.



**Fig. 2.** Taxonomic structure of the *Acidobacteria* phylum in the surface (1), subsurface (2), and mineral (3) horizons of dark-colored (I), brown tropical (II), red-yellow tropical (III), and alluvial (IV) soils. The shares of *Acidobacteriales* (A), subgroup 2 (s2), *Solibacteriales* (S), subgroup 13 (s13), *Blastocatellia* (B), *Holophagae* (H), and other acidobacteria (o) are shown.



**Fig 3.** Two-dimensional diagrams of the microbiome similarity in the surface (1), subsurface (2), and mineral (3) horizons of dark-colored (I), brown tropical (II), red-yellow tropical (III), and alluvial (IV) soils. The diagrams are constructed by the NMDS method based on the weighted UniFrac metric similarity.

## DISCUSSION

According to the estimated number of ribosomal gene copies, tropical soils under weakly impacted monsoon forests at the Cat Tien preserve were characterized by high abundance of microorganisms, which was comparable with that in the richest soils of the temperate zone (e.g., chernozems) (Semenov et al., 2018). However, the highest numbers of microbial ribosomal genes were revealed in the surface humus horizons (4–10 cm). High numbers of archaeal 16S rRNA gene copies (up to  $10^{11}$ /g in the upper horizons of all soils) are noticeable. The number of fungal ITS gene copies was lower than that of bacterial and archaeal 16S rRNA genes and varied slightly in four soil types. It had been earlier shown that the length of fungal mycelium in the Cat Tien soils was two to three times less than that in forest soils of the temperate zone (Lysak et al., 2017), which was possibly due to the absence of forest litter on the surface of tropical soils. In tropical forests, unlike the forests of the temperate zone, the litter is rapidly degraded by fauna, e.g., termites, which assimilate up to 50% of the leaf litter (Matsumoto and Abe, 1979; Brauman, 2000); therefore, supply of plant biomass available for degradation by saprophytic soil fungi was lower. Unlike nutrient-depleted red-yellow tropical and alluvial soils, volcanic soils, which contained higher amounts of organic substances and macrolelements and had higher pH value, were characterized by higher numbers of bacterial and archaeal 16S rRNA genes. Composition of the tree stand differed slightly in the studied zones; differences in the chemical composition of soils were mainly determined by the type of soil-forming rocks:

volcanic deposits, alluvium, or clay slates (Khokhlova et al., 2017). Thus, abundance of microorganisms in these soils is apparently determined by the carbon content, which depends on the lithological basis and, possibly, on the water regime.

In the studied soils, prokaryotic phyla *Acidobacteria*, *Proteobacteria*, *Verrucomicrobia*, and *Actinobacteria* prevailed in the upper horizons; these data are in agreement with the results of other researches: based on the 16S rRNA gene sequencing, domination of the phyla *Acidobacteria* and *Proteobacteria* was revealed in tropical soils from various regions; high abundance of *Verrucomicrobia*, *Actinobacteria*, *Planctomycetes*, and *Firmicutes* has been also demonstrated in a number of studies (Miyashita et al., 2013; Navarrete et al., 2015; Schneider et al., 2015; Kroeger et al., 2018). It is remarkable that the share of *Acidobacteria* is rather high (up to 52%), which is typical of acidic soils (Laubert et al., 2009; Jones et al., 2009). Acidic soils of humid climate differed from the neutral and alkaline arid soils by prevalence of *Acidobacteria*; the latter soils were characterized by significantly lower shares of *Acidobacteria* and higher shares of *Actinobacteria* (Laubert et al., 2009; Chernov et al., 2018; Tikhonovich et al., 2018). *Acidobacteria* were represented mainly by the groups *Acidobacteriales* (subgroup 1), subgroup 2, and *Solibacterales*. Subgroup 1 was represented by acidophiles, mainly obligate aerobes exhibiting hydrolytic activity towards a wide range of biopolymers (Dedysh and Sinnighe Damsté, 2018). *Solibacterales* are also acidophilic oligotrophic bacteria; according to metagenomic studies of soils carried out in Germany, *Solibacterales* and *Acidobacteriales* contained a wide range



of genes responsible for mobilization of mineral phosphorus (Bergkemper et al., 2016); this capability is a great advantage for bacterial growth in red-yellow and alluvial soils with low concentration of mobile phosphorus (Khokhlova et al., 2017). High relative abundance of acidobacteria belonging to subgroup 2 was revealed in the Brazil soils under tropic forests (Navarrete et al., 2015) and savanna (Catão et al., 2014); the share of these bacteria correlated with the concentration of  $Al^{3+}$ , which was high in many tropical soils (Kielak et al., 2016). In the studied soils, subgroup 2 predominated among acidobacteria in poor nonvolcanic soils (red-yellow and alluvial). It can be considered that acidobacteria of subgroup 2 are typical of the organic matter-depleted acidic tropical soils of different genesis.

The share of the phylum *Verrucomicrobia* represented by widespread soil bacteria with poorly studied ecology, was shown to decrease with depth. Low relative abundance of *Verrucomicrobia* in deep mineral soil layers have also been revealed in the other soil types (Semenov et al., 2018; Chernov et al., 2018). The maximum share of *Verrucomicrobia* was sometimes found in the subsurface soil horizons at the depth of 10–50 cm (Eilers et al., 2012; Zhang et al., 2017); based on these results, it was assumed that members of the phylum *Verrucomicrobia* were oligotrophs adapted to growth under deficiency of organic substances. In the studied tropical soils, we did not reveal the maximum development of *Verrucomicrobia* in subsurface layers, most probably because of low depth of the humus horizons (4–10 cm).

An appreciable share of prokaryotic communities in these soils was represented by *Rokubacteria*, a group of poorly investigated bacteria, which have been recently assigned to a separate phylum (Becraft et al., 2017). Information is available on the presence of *Rokubacteria* in the Amazonian soils under pluvial tropical forests (Kroeger et al., 2018). In this study, we used the SILVA (2018) taxonomic database, whereas in its previous version (2017), *Rokubacteria* were not designated as a separate bacterial phylum and had no referent sequences. Thus, continuing development of systematics, together with the instability of prokaryotic taxonomy result in considerable dependence of experimental results on the used taxonomic system and complicate comparison of the results on metagenomics and metabarcoding obtained with the use of different versions of taxonomic databases.

Evaluation of the taxonomic structure similarity in prokaryotic communities showed similarity of the microbiomes in volcanic soils (dark-colored and brown tropical). The taxonomic structures of microbiomes in red-yellow tropical and alluvial soils differed both from those in volcanic soils and from each other. The NMDS diagrams (Fig. 3) clearly show that the structures of prokaryotic communities were changed in two directions depending on both the soil type

(from brown tropical to alluvial sandy-loam soil) and depth (from surface humus to lower mineral horizons). A key factor that determined the different structures of prokaryotic communities and the microbial abundance in tropical soils at the Cat Tien preserve was most probably the organic carbon content, which differed considerably in various horizons of the soil profiles and in soils on various soil-forming rocks.

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#### COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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