

COMPARATIVE ANALYSIS OF FUNGAL DIVERSITY AND COMMUNITY STRUCTURE OF DIFFERENT VOLCANIC SOILS IN WUDALIANCHI, CHINA

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Abstract

The relationship between the fungal community characteristics and soil environmental factors of volcanic ecosystem in Wudalianchi, China were investigated. The soil fungal community structure and diversity of new, old, and non-erupting volcanos were explored through high-throughput sequencing technology. The result showed that the physical and chemical properties of three plots were significantly different. Through sequencing 578 species, 366 genera, 202 families, 89 orders, 32 classes, and 11 phyla were detected. Among them Ascomycota and Basidiomycota were the dominant fungi phyla. The relative abundance of various flora determined by phylum classification showed significant differences. The Shannon, Simpson, Ace, and Chao1 indices for the soil fungi in the three plots were also significantly different. Redundancy and correlation analyses showed that the α diversity of fungi was significantly correlated with pH, organic matter and total nitrogen in the soil. These results indicate that soil environmental factors influence the fungal diversity in the different volcanic ecosystems in Wudalianchi, China.

Introduction

Microorganisms, as the earliest life form on earth, determine the direction and progress of earth evolution, which promotes the occurrence and development of soil (Zhu *et al.* 2017). Soil fungi, as an important part of soil microorganisms, participate in the majority of soil ecological processes, such as geochemical cycle, decomposition, and transformation of organic matter (Leff *et al.* 2015). The formation and change of soil microbial community structure are also greatly affected by soil environmental factors, such as soil physical and chemical properties. Several soil microbial studies concentrated on areas with different soil types, such as forests, grasslands, wetlands, and water bodies (Han *et al.* 2017, Zhu *et al.* 2017, Li and Li 2019). However, few studies focused on volcanic areas, such as Mount Fuji Volcano, Etna (Sicily) Volcano, Chile Volcanoes, and other volcanic areas (Insam *et al.* 2017, Memoli *et al.* 2018, Valle *et al.* 2018).

Volcanic eruption, as the strongest representation of the earth's internal thermal energy on the surface, can directly destroy the green vegetation on the surface. (Zhou *et al.* 2011). The Wudalianchi National Geological Park (WNG), nominated as the world heritage, is an ideal place to study plants, soils, and microorganisms of volcanic ecosystems, because it maintains the intact inland single-genetic volcanic landform and complete vegetation ecological succession process. In this study, the fungi of new, old volcanic and non-volcanic eruption soils are measured through high-throughput sequencing. The intrinsic relationship between soil fungal diversity and soil

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environmental factors is discussed through analyzing the species, relative abundance, and composition relationship of soil fungi to reveal the interaction mechanism between soil environment and microbial community in a volcanic ecosystem.

Materials and Methods

The Wudalianchi National Geological Park (Fig. 1), located in the northern part of Heilongjiang Province, China, possessing a basaltic lava platform with an area of 65 km², in which 14 new and old volcanic geological landform erupted in different periods are formed in the past 21 million years (Yan *et al.* 2013). The Shankou lake Geological Park (Fig. 1) is located in the Sunwu fracture zone, 65km far from the Wudalianchi Geological Park. It is a granite-based low hill formed by fractures (Zhang 2012). The Wudalianchi area has a temperate continental monsoon climate with an annual average temperature of -0.5 °C, an annual average precipitation of 473 mm, an annual frost-free period of 121 days, and a zonal soil type of dark brown soil (Xie *et al.* 2019). The vegetation of Wudalianchi belongs to the temperate broad-leaved mixed forest. However, it is also affected by the European–Siberian coniferous forest area and the Eurasian steppe area. The main dominant plants are *Larix gmelini*, *Quercus mongolica*, *Betula platyphylla*, *Populus davidiana*, *P. koreana* and so on.

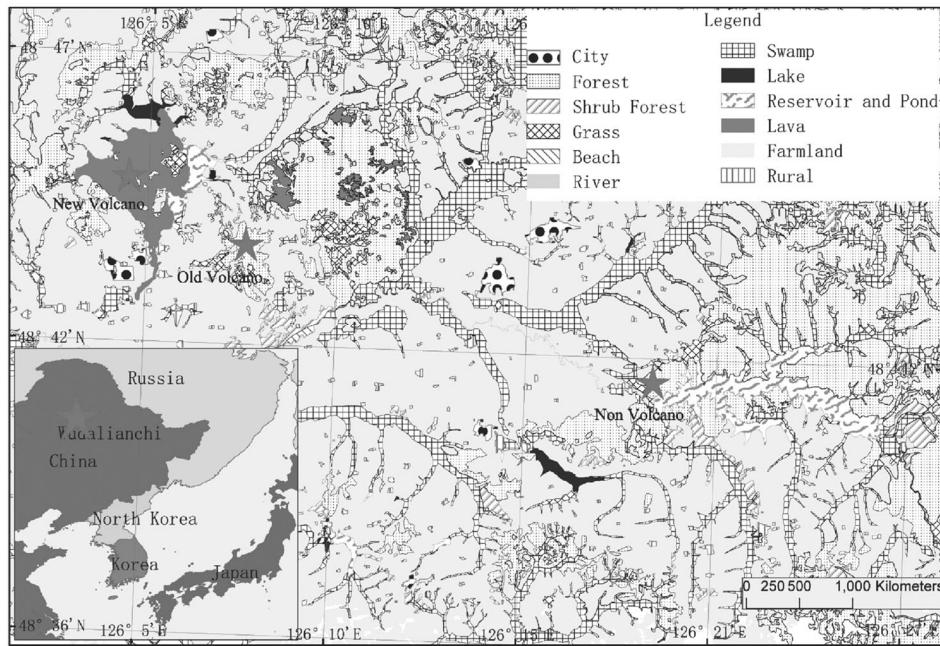


Fig. 1. Location of three sampling sites in the Wudalianchi and Shankou lake Geological Park.

In July 2019, typical representative plots were selected for soil sample collection in the new volcano (NV), the old volcano (OV), and the Shankou lake Geological Park (Non-volcanic eruption, Non-V) of the WNG Park (Table 1). Three 20 m × 20 m standard plots were set, five sampling points from the four corners and the center in each plot were selected, the litter and humus layer were removed. The soil with a depth of 0–10 cm form a soil drill with a diameter of 10 cm was collected. The litter, fine roots, and small stones were removed. They were passed

through a 2 mm nylon sieve; and divided into two parts. Some samples were placed in a freezer at 4°C and then stored at -80 °C for soil microbial determination after being delivered to the laboratory. Other soil samples were returned to the laboratory for the determination of soil physicochemical properties. The laboratory test was completed in the laboratory Heilongjiang academy of sciences in August 2019.

Soil moisture content was determined by the drying method. The pH value was determined by the electrometric method, and the soil total nitrogen and organic carbon were determined by the CN element analyzer (Analytik Jena, Germany). Soil total phosphorus was determined by molybdenum antimony colorimetry, soil available phosphorus was determined by NaHCO₃ extraction colorimetry, and soil ammonium nitrogen and nitrate nitrogen were determined by potassium chloride-flow analyzer (Skalar, Netherlands) (Chao *et al.* 2019).

Table 1. General characteristic of the study areas.

Samples	Plots	Location	Eruption time	Soil type	Vegetation	Altitude
NV	Laohei volcano	48°42'32"N 126°07'06"E	300 years ago	Volcanic ash	<i>Populus</i> and <i>Betula</i> forest	328 m
OV	East jiaodebu volcano	48°39'13"N 126°16'30"E	0.17-0.19 Ma	Dark brown soil	<i>Populus</i> and <i>Betula</i> forest	375 m
Non-V	Shankou lake	48°28'20"N 126°30'30"E	Non-Eruption	Dark brown soil	<i>Populus</i> and <i>Betula</i> forest	306 m

The fungi DNA isolated from the soil was extracted using the American Strong Soil Kit (Qiagen, USA) following the protocols in the manual. Using 50 ng DNA as a template, universal primers ITS1F (5'-CTTGGTCATTAGAGGAAGTAA-3') and ITS2R(5'-GCTGCGTTCTT CATCGATGC-3) were used to amplify in the V4-V5 highly variable region. The PCR product was recovered by cutting the gel using AxyPrep DNA gel recovery kit. The Miseq High-throughput sequence were performed at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

Data were processed using QIIME (version 1.17 <http://qiime.org/>) software. Operational taxonomic units (OTUs) were divided into standards based on 97% similarity using Usearch (version 7.1, <http://drive5.com/aparse/>). Using the RDP classifier Bayesian algorithm for taxonomic analysis of OTU representative sequence, the confidence threshold is 0.7. Sequence coverage was used to obtain a sequence depth index. Community abundance was indicated using Ace and Chao indices. The richness and diversity of the Fungi community species were represented by the Simpson and Shannon indices, respectively. All indices were analyzed by use of mothur software. Redundancy analysis using R language vegan package (RDA) and ggplot package mapping for redundancy analysis. (R Development Core Team. 2018).

Results and Discussion

Table 2 shows that the soil pH values of the three plots are weakly acidic with significant differences. The pH in the OV is the largest. The soil organic matter, total nitrogen, total phosphorus, ammonium nitrogen, and nitrate nitrogen were significantly different among the three plots, but the trend was the same. The NV and OV soils mainly developed from volcanic rocks-basalts. With soil development and soil weathering, the concentrations of SOC, TN, AN, NN, TP, and AP in the OV plot were significantly higher than those in the NV, which may be associated

with the rock parent material (Fu *et al.* 2015). The soil water content and available phosphorus (AP) were the minimum in the NV, and no significant difference was found between the OV and Non-V, this result might be due to the shallow soil layer in the NV, the permeable properties of basalt lava and volcanic ash reduce the surface run off and cause insufficient soil moisture (Kuritani *et al.* 2013).

The total number of valid sequences of soil samples was 1,125,884 after the optimization to filter the low-mass sequence. The sampled soil samples were clustered by OTU at 97% similarity after split and de-redundation. A total of 1746 OTUs were obtained. The figure shows that the sample dilution curves of soil with different types are flat but not yet saturated. This result shows that the tested samples and data are reasonable, and the actual environment fungal community structure in the soil is of high confidence, which can truly reflect the fungal community of the soil samples.

Table 2. Physical and chemical characteristics of soil in volcano.

Samples	pH	WC (%)	TOC (g/kg)	TN (g/kg)	TP (g/kg)	AP (mg/kg)	AN (mg/kg)	NN (mg/kg)
NV	5.08 ± 0.02 ^b	8.50 ± 0.21 ^b	74.05 ± 2.67 ^c	1.38 ± 0.05 ^c	0.36 ± 0.01 ^c	10.20 ± 0.96 ^b	4.17 ± 0.09 ^c	6.45 ± 0.22 ^c
OV	5.45 ± 0.02 ^a	50.38 ± 1.01 ^a	363.12 ± 2.03 ^a	13.29 ± 0.10 ^a	0.95 ± 0.01 ^a	19.30 ± 3.10 ^a	70.30 ± 2.46 ^a	104.45 ± 0.98 ^a
Non-V	4.68 ± 0.01 ^c	51.96 ± 1.65 ^a	183.59 ± 1.45 ^b	6.74 ± 0.16 ^b	0.81 ± 0.01 ^b	20.67 ± 0.81 ^a	35.29 ± 1.70 ^b	15.37 ± 0.44 ^b

There is a significant difference in delivery of different lowercase letters in the same column ($p < 0.05$), the same below. NV: New volcano; OV: old volcano; Non-V: Non-volcanic eruption; WC: Water content; TOC: Total organic carbon TN: Total nitrogen; AN: Ammonium nitrogen; NN: Nitrate nitrogen; TP: Total phosphorus; AP: Available phosphorus.

The Venn of the soil fungi in the three plots (Fig. 2) shows that the total number of OTUs in each plot was OV > Non-V > NV, in which the soil fungi OTU in OV was the highest, which is 1313, accounting for about 75.20% of all OTUs. The total number of OTUs in the soil fungi in the three plots was 105, accounting for 6.01% of the total OTUs. The number of OTUs in the NV and Non-V was the lowest at 128, whereas the total number of OTUs in the OV and Non-V was up to 304. This result indicates that the OTU distribution of soil fungi in the three plots is different, and the OV and Non-V are similar.

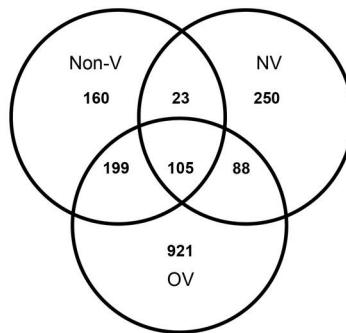


Fig. 2. OTUs comparison of soil fungi in each sample site.

Under the similarity level of 97%, DNA sequencing revealed 578 species, 366 genera, 202 families, 89 orders, 32 classes, and 11 phyla. As shown in Fig. 3, The fungal communities of the three plots are similarly composed of five dominant fungi communities ($\geq 10\%$), including Basidiomycota, Ascomycota, Rozellomycota, Mortierellomycota, and unknown fungi communities. The relative abundance of seven other phylum fungi communities is below 1%. This phenomenon is related to the vegetation types of *Populus davidiana* and *Betula platyphyllea*, which are the same in all three plots, indicating that the fungal structure and plant type are closely related (Kasel *et al.* 2008). Ascomycetes and Basidiomycetes are the major decomposers in the soil (Yelle *et al.* 2010). In this study, Basidiomycota and Ascomycota are the dominant fungi communities of soil fungi in Wudalianchi, and their abundance in the NV is 98.01%–98.98%. The new volcanic lava platform is featured by less water and good permeability, which are consistent with the characteristics of Basidiomycetes and Ascomycetes that prefer soil permeability (Liu *et al.* 2019).

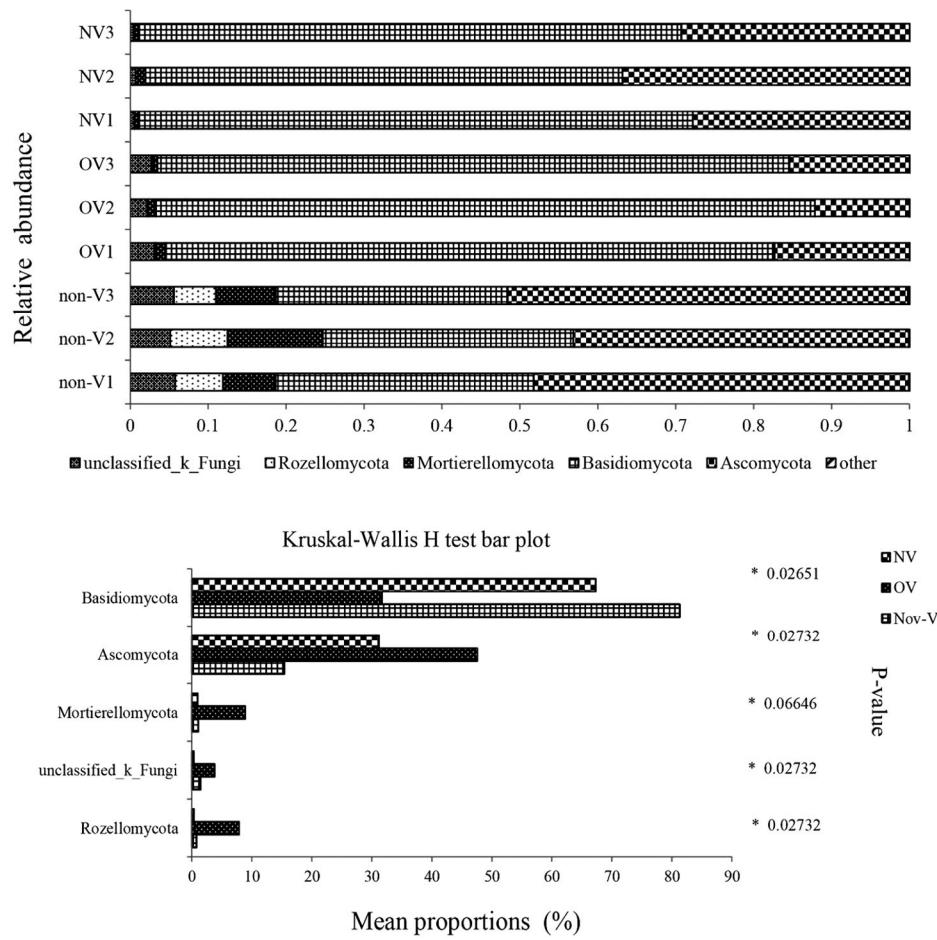


Fig. 3. Composition and test of fungi community at phylum level.

However, the abundances of Basidiomycetes, Ascomycetes, Rozellomycota, and unknown fungi community in the three plots are significantly different. This might be due to the differences in soil nutrients, moisture content, and pH (Truu *et al.* 2009). The abundance rates of Basidiomycota are most dominant fungi in the Non-V (81.26%), the abundance rates of

ascomycota is most dominant fungi in the Non-V (47.55%). In addition, the relative abundance of Mortierellomycota in the OV is higher, which can reach up to 5.40 - 7.37%, whereas the relative abundance in the NV and Non-V is less than 0.65%.

As shown in Table 3, the coverage index of soil sequencing in the three plots was between 0.994 and 0.998 and of no significant difference, indicating that the sequencing results of this experiment can accurately reflect the real situation of the soil samples tested. The Ace, Chao1, and Shannon indices of the forest soil fungi in the three plots were significantly different and are the highest in the OV soil, showing that the total number of species, community complexity, and information in the OV soil fungi are the highest. The Simpson index of OV soil fungi was the lowest, indicating that the soil fungal community diversity is higher with higher uniformity. In Table 4 it is apparent that the Shannon index was positively correlated with soil pH, organic matter, total nitrogen, ammonium nitrogen, and nitrate nitrogen. The Simpson index is significantly or significantly negatively correlated with soil pH and nitrate nitrogen. The Ace and Chao1 indices show a significant or highly significant positive correlation with the seven soil properties, except AP (Table 4).

Table 3. Diversity index of each soil sample at similarity of 97%.

Samples	Shannon index	Simpson index	Ace index	Chao1 index	Coverage index
NV	3.194 ± 0.144 ^b	0.093 ± 0.013 ^b	450.8 ± 53.7 ^b	431.410 ± 36.030 ^b	0.998 ± 0.000 ^a
OV	4.045 ± 0.098 ^a	0.049 ± 0.004 ^c	1122.9 ± 50.9 ^a	1101.798 ± 46.249 ^a	0.994 ± 0.000 ^a
Non-V	2.945 ± 0.044 ^b	0.153 ± 0.007 ^a	478.9 ± 47.4 ^b	457.393 ± 33.250 ^b	0.998 ± 0.000 ^a

Different letters in the same group indicate significant difference ($p < 0.05$).

Table 4. Correlation coefficients between soil fungi α -diversity indices and soil physicochemical properties.

Samples	pH	WC	TOC	TN	TP	AP	AN	NN
Shannon index	0.895**	0.260	0.787*	0.739*	0.485	0.143	0.734*	0.910**
Simpson index	-0.947**	0.113	-0.519	-0.449	-0.126	0.194	-0.437	-0.733*
Ace index	0.815**	0.487	0.917**	0.887**	0.688*	0.422	0.868**	0.975**
Chao1 index	0.825**	0.492	0.926**	0.895**	0.693*	0.414	0.877**	0.985**

* $p < 0.05$; ** $p < 0.01$.

The spatial differentiation of microorganisms can be attributed to differences in soil nutrient and environmental conditions (Allison *et al.* 2013). According to the RDA ranking results (Fig. 4), all samples are more clearly divided into three groups with smaller distance between each sample group. However, the samples in the three groups are distributed in different quadrants, indicating that the soil fungi among the three plots are of great composition differences. The contribution rates of the two axes were 88.03% and 10.80%, respectively, and the cumulative contribution rate was 98.83%. Ascomycota was positively correlated with soil pH, whereas Basidiomycetes were negatively correlated with soil pH, organic matter, total nitrogen, and nitrate nitrogen. These results indicate that the soil pH and nutrient content of the Wudalianchi volcano can affect the total number of species of microbial communities, as well as the richness and uniformity of soil fungi (He *et al.* 2014). In addition, the structure and composition of the fungal community are

selective for the soil environment, and further study needs to be carried out on factors affecting the selectivity of the fungi.

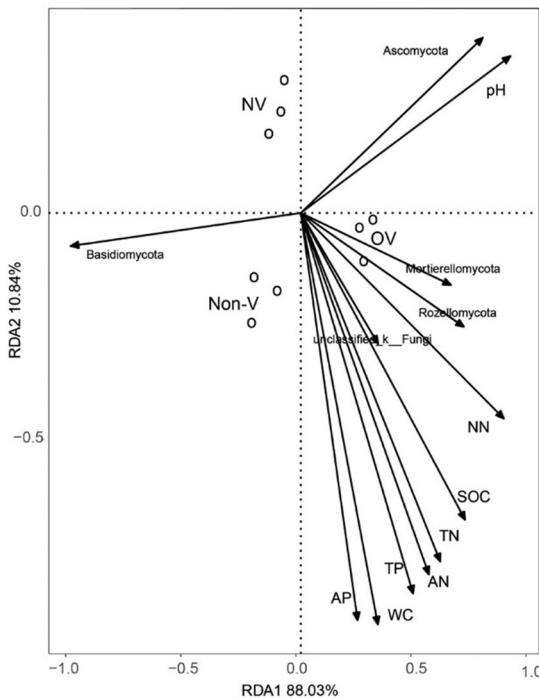


Fig. 4. RDA based on soil fungi community at Phylum level and environmental factors of the soil.

In this study, the soil fungi in Wudalianchi volcanoes were analyzed through high-throughput sequencing technology, the results showed that the fungal community characteristics and diversity differed significantly in the NV, OV and Non-V, and the main factors were pH, organic matter and nitrogen in soil. Volcanic eruption directly leads to the diversity of microbial metabolic types and functions by changing the volcanic material group of sedimentary layer, and then affects the bacterial community structure (Byloos *et al.* 2018). Further studies are needed to understand the mechanism of soil microbial activity in volcanic system.

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