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Contrasting elevational diversity patterns between eukaryotic soil microbes and plants

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Abstract. The diversity of eukaryotic macroorganisms such as animals and plants usually declines with increasing elevation and latitude. By contrast, the community structure of prokaryotes such as soil bacteria does not generally correlate with elevation or latitude, suggesting that differences in fundamental cell biology and/or body size strongly influence diversity patterns. To distinguish the influences of these two factors, soil eukaryotic microorganism community structure was investigated in six representative vegetation sites along an elevational gradient from forest to alpine tundra on Changbai Mountain in Northeast China, and compared with our previous determination of soil bacterial community structure along the same gradient. Using bar-coded pyrosequencing, we found strong site differences in eukaryotic microbial community composition. However, diversity of the total eukaryotic microorganism community (or just the fungi or protists alone) did not correlate with elevation. Instead, the patterns of diversity and composition in the total eukaryotic microbial community (and in the protist community alone) were closely correlated with soil pH, suggesting that just as for bacteria, acidity is a particularly important determinant of eukaryotic microbial distributions. By contrast, as expected, plant diversity at the same sites declined along our elevational gradient. These results together suggest that elevational diversity patterns exhibited by eukaryotic microorganisms are fundamentally different from those of plants.

Key words: Changbai Mountain, China; elevational diversity gradient; eukaryotic soil microbes; fungi; metazoans; protists; pyrosequencing; soil pH.

Introduction

The structure of a biological community (i.e., its species composition, evenness, richness, and species interactions) is closely linked with its ecological functioning (Loreau et al. 2001). Eukaryotic microorganisms, including fungi, protists, and metazoans play an essential role in trophic food webs and nutrient cycles in both terrestrial and aquatic habitats (Coleman et al. 2004, Dighton et al. 2010). However, we know much less about the structure and ecology of eukaryotic microbial communities than we do about macroorganism or bacterial communities. Soil is a complex environment and is likely to harbor abundant and diverse eukaryotic microorganisms, but until recently, studies have been methodologically constrained to focusing on specific groups within fungal or protist communities (Anderson and Cairney 2004, Bonkowski 2004). In the past few years, however, researchers have characterized the diversity and composition of total eukaryotic soil microbial communities using molecular methods including PCR-denaturing gradient gel electrophoresis (Moon-van der Staay et al. 2006), clone library (Fell et al. 2006), metatranscriptome (Bailly et al. 2007), and 18S rRNA gene pyrosequencing (Meadow and Zabinski 2012, Baldwin et al. 2013) techniques. These studies have yielded very useful insights, but as yet there has been no large-scale spatially explicit research specifically aimed at understanding the ecological patterns and controls on eukaryotic microorganism distributions.

The influence of elevation on biological diversity patterns is not only indispensable to a comprehensive understanding of basic ecology, but is also critical to predicting the potential influences of climate change on terrestrial ecosystems (Lomolino 2001, Rahbek 2005, Grytnes and McCain 2007, Malhi et al. 2010). Previous research focused exclusively on plant and animal taxa, showing that macroorganisms such as trees and

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mammals generally exhibit either monotonically decreasing or hump-shaped diversity patterns with increased elevation (Lomolino 2001, Rahbek 2005, Forister et al. 2010, Salas-Morales and Meave 2012). These elevational diversity patterns have been attributed to climatic factors (Hawkins et al. 2003, Currie et al. 2004, McCain 2007), spatial factors (e.g., decreasing area at higher elevations), reduced potential for biotic interactions (e.g., mutualism and competition), and historical impacts (evolutionary constraints) (Gaston 2000, Lomolino 2001). By contrast, microorganisms (soil bacterial communities at least) do not seem to vary with elevation in a corresponding manner (Zhang et al. 2009, Fierer et al. 2011, Shen et al. 2013, Yuan et al. 2014; although see Bryant et al. 2008 and Singh et al. 2012). Likewise, soil bacterial communities do not follow the typical diversity pattern of plants and animals across latitude (Chu et al. 2010). For bacteria, there is now a widespread consensus that soil pH is the primary determinant of large-scale patterns in diversity (Fierer and Jackson 2006, Lauber et al. 2009, Chu et al. 2010, Shen et al. 2013, Yuan et al. 2014). Do eukaryotic microbial community distribution patterns resemble those of bacteria or of macroorganisms? Certain studies report that the diversity and composition of nematode, testate amoeba, and fungal communities are influenced to some extent by soil features (Lauber et al. 2008, Wu et al. 2011, Tsyganov et al. 2013). Thus, we might expect to find some significant relationships between eukaryotic microbial communities and soil characteristics. In addition, plant communities may play a role in structuring eukaryotic microbial community composition, particularly for fungi that are often closely functionally linked to plants (Peay et al. 2013). However, body size differences between eukaryotic microorganisms and macroorganisms may have critical impacts on their community structures. Specifically, the shorter generation times, larger population sizes, and long-distance dispersal of microbial eukaryotes (Fenchel and Finlay 2004) may result in very different distribution patterns compared to macroorganisms. These considerations beg the question of whether communities of eukaryotic microorganisms are structured analogously to bacteria or to their closer taxonomic relatives. In other words, what is the relative influence of body size as compared to fundamental cell biology (i.e., bacteria vs. eukaryotes) in determining organism distribution patterns? To tease apart the relative importance of these two potential drivers, our study focused on eukaryotic microbes and compared their community structure along an elevational gradient with that of plants and soil bacteria.

The Changbai Mountain is the highest mountain in Northeast China and is one of very few well-protected and conserved natural ecosystems along a montane gradient on Earth (He et al. 2005). The vertical distribution of vegetation along the mountainside mirrors the latitudinal vegetation gradient from temperate to frigid zones on the Eurasian continent (Xu et al. 2004, Zhang et al. 2011). It thus provides an excellent opportunity for studying natural microbial distribution patterns on a regional scale. In this study, we investigated the composition and diversity of the total eukaryotic soil microbial communities, as well as particular functionally important component groups (fungi and protists) along an elevational gradient on Changbai Mountain. Plant species richness and diversity were measured simultaneously at the same sampling sites along the elevational gradient to enable a direct comparison between eukaryotic microorganism and macroorganism community patterns. We hypothesized that plant and eukaryotic microbial communities are similarly structured in that their richness and phylogenetic diversity decrease up an elevational gradient.

Methods

Site selection and soil sampling

Changbai Mountain (126°55′–129°00′ E, 41°23′–42°36′ N) is located in Jilin Province, Northeast China, close to the border with North Korea (see Plate 1). It is the highest mountain in Northeast China and lies at the head of three large rivers (the Songhua, Yalu, and Tumen). Topographic features differ on the mountain, with the northern slope being relatively moderate (average slope $<3\%$) compared to the others (average slope 10%). It has a typical continental temperate monsoon climate. Along the elevational gradient from 530 m to 2200 m, mean annual temperature decreases from 2.9°C to −4.8°C, and mean annual precipitation increases from 632 mm to 1154 mm (Tong et al. 2003).

These topographic and climatic features result in a vertical zonation of major forest types that is especially distinct along the northern slope. Below 1100 m lies a typical temperate forest, composed of Korean pine and hardwood species. Common hardwood species include aspen (*Populus davidiana* Dode), birch (*Betula platyphylla* Suk), basswood (*Tilia amurensis* Rupr), oak (*Quercus mongolica* Fisch), maple (*Acer mono* Maxim), and elm (*Ulmus pumila* L.). From 1100 to 1700 m is an evergreen coniferous forest, dominated by spruce (*Abies nephrolepis* (Trautv.) Maxim), and fir (*Picea jezoensis* (Siebold and Zucc.) Carrière), with the typical characteristics of boreal forests. From 1700 to 2000 m is a subalpine forest, dominated by mountain birch (*Betula ermanii* Cham.) and larch (*Larix olgensis* Henry). Above 2000 m is a unique alpine tundra that marks the southernmost occurrence of this ecosystem type on the eastern Eurasian continent. Many of the plant species there are relicts from the Quaternary glacial period, and the community is dominated by *Dryas octopetala* (L.), *Vaccinium uliginosum* (L.), and *Rhododendron chrysanthum* (Pall.) (Xu et al. 2004, He et al. 2005). The main climatic and ecological characteristics along the elevational gradient are summarized in Appendix A.

Soil samples were collected from the northern slope of Changbai Mountain on 18 June 2009. We chose six

elevations that represented the six typical vegetation types along the mountainside gradient. At each elevation, soil samples were collected from four independent replicate plots (10 × 10 m; about 100 m apart) that were representative of the typical local vegetation. In each plot, samples of the soil organic layer (~10 × 10 cm in area, and 0–5 cm depth directly below the litter layer) were collected at six random points using a sterile blade and composited together as a single sample. Visible roots and residues were removed prior to homogenizing the soil fraction of each sample. The fresh soil samples were sieved through a 2-mm sieve and divided into two subsamples. One was stored at 4°C to determine the physical and chemical properties, and the other was stored at −20°C prior to DNA extraction.

Soil nutrients and microbial biomass analyses

Soil pH was measured after shaking a soil water (1:5 mass/volume) suspension for 30 min. Soil moisture was measured gravimetrically. Total organic carbon (TOC) and total nitrogen (TN) were determined by dichromate oxidation and titration with ferrous ammonium sulphate (Walkley and Black 1934). Microbial biomass C (MBC) and biomass N (MBN) were analyzed by the chloroform fumigation and extraction method (Brookes et al. 1985), and calculated using the correction factors 0.35 (k_C) and 0.4 (k_N) (Jonasson et al. 1996). Soil characteristics are summarized in Appendix B.

Soil DNA extraction

Soil DNA was extracted from the 0.5 g wet soil using a FastDNA SPIN Kit for soil (MP Biomedicals, Santa Ana, California, USA) according to the manufacturer's instructions. This protocol has been successfully used for DNA extraction of eukaryotic soil microbes such as soil metazoans (Wu et al. 2011) and protists (Bates et al. 2013). The extracted soil DNA was dissolved in 50 μL TE buffer, quantified by spectrophotometer and stored at −20°C until use.

PCR and preparation of the amplicon libraries for 454 pyrosequencing

Primer set Euk1F (CTGGTTGATCCTGCCAG) and Euk516R (ACCAGACTTGCCCTCC) was used to amplify an 18S rRNA gene fragment for the 454 GS-FLX pyrosequencing platform (Diez et al. 2001). To perform 454 pyrosequencing with the GS-FLX System, the sequences of these oligonucleotides included the 454 Life Science A or B sequencing adapters (19 bp) fused to the 7-bp barcoded primer: Primer B (GCCTTGCCAGCCCGCTCAG) + barcode + forward primer; and Primer A (GCCTCCCTCGCGCCATCAG) + reverse primer. PCR was carried out in 50-μL reaction mixtures containing each deoxynucleoside triphosphate at a concentration of 1.25 mM, 2 μL (15 μM [each]) of forward and reverse primers, 2 μL of Taq DNA polymerase (TaKaRa, Otsu, Japan), and 50 ng of DNA. Each reaction mix contained 1 μL of genomic community DNA as a template, and the following cycling parameters were used: 35 cycles (95°C for 45 s, 56°C for 45 s, and 72°C for 1 min) were performed with a final extension at 72°C for 7 min. Triplicate reaction mixtures per sample were pooled, purified using the QIAquick PCR Purification kit (QIAGEN, Shenzhen, China), and quantified using NanoDrop ND-1000 (Thermo Scientific, Wilmington, North Carolina, USA). The bar-coded PCR products from all samples were normalized in equimolar amounts before pyrosequencing an aliquot (50 ng) of purified DNA by means of a Genome Sequencer FLX System platform (Life Sciences, Branford, Connecticut, USA).

Processing of pyrosequencing data

18S raw sequence data were processed using the QIIME 1.4.0 pipeline (Caporaso et al. 2010; *available online*).[8] Sequences were quality filtered on the basis of quality score, sequence length, and primer mismatch thresholds, and then assigned to soil samples based on unique 7-bp barcodes. Chimera checking and operational taxonomic unit (OTU) grouping were carried in QIIME using USEARCH (Edgar et al. 2011). Taxonomy was assigned to eukaryotic OTUs (clustered at 97% similarity) against the SILVA 104 database (more information *available online*)[9] classified with BLAST at a sequence similarity threshold of 0.90. All Viridiplantae (i.e., plant-derived nonmicrobial eukaryotic sequences) were removed from the 18S rRNA gene sequence data set prior to the subsequent analyses. After taxonomies had been assigned, OTUs that were not assigned to microbial eukaryotes were removed from the data set prior to subsequent analysis. Finally, data sets comprising of protistan taxa only (excluding fungi and metazoans) and fungi only (excluding protists and metazoans) were culled from all quality sequences for the individual analyses of the protist and fungal communities.

Statistical analyses of the soil eukaryotic community data

To determine if the different elevation samples formed unique phylogenetically related clusters, principal coordinates analysis (PCoA) of the Unifrac distance matrices were performed. The Unifrac algorithm computes the overall phylogenetic distances (across all taxonomically resolved levels) between all pairs of sample communities in the data set from neighbor-joining trees using either unweighted (i.e., presence/absence) and weighted (i.e., accounting for taxon relative abundance) data (Lozupone and Knight 2005). In addition, we tested for significant differences in community composition among elevations using analysis of similarities (ANOSIM) with R statistical software (R Development Core Team 2010).

[8] http://qiime.org/tutorials/processing_18S_data.html
[9] http://www.arb-silva.de/

PLATE 1. A view of the elevation transect in Changbai Mountain, Northeast China, from which all samples were collected. The photo was taken from an elevation of 2200 meters, and the view is straight down the ridge. The foreground is alpine tundra, which is followed by different forest types down the elevation. Photo credit: W. Cao.

To identify the environmental and biogeochemical factors that significantly correlated with community composition, we used Mantel tests of Bray-Curtis similarity distance values that were calculated on the presence/absence of the OTUs within each sample using the vegan package of R v.2.15.1 project (R Development Core Team 2010). To determine which significant environmental variables explained the observed OTU similarities between communities, the environmental data were further used in a distance-based linear model multivariate analysis (DistLM; McArdle and Anderson 2001). The contribution of each environmental variable was assessed using "marginal tests" to assess the statistical significance and percentage contribution of each variable taken alone, and then "sequential tests" to evaluate the cumulative effect of the environmental variables explaining microbial eukaryotic community similarity. Tests were performed using the computer program DISTLM_forward3 (Anderson 2003).

We compared community-level composition, number of phylotypes (the number of OTUs), nonparametric Chao1 index values (an estimate of the true OTU richness based on the frequencies of singletons and doubletons; Chao et al. 2009) and Faith's phylogenetic diversity index values (an indicator of the overall phylogenetic diversity across all taxonomic levels; Faith 1992) after rarifying all the data sets to the same level of sampling effort. The total eukaryotic community data set was rarefied using random selections of 1000 sequences per sample for downstream analyses, while the fungal and protistan-only data sets were rarefied to 500 sequences and 260 sequences per sample, respectively. These numbers of sequences for rarefaction in the different categories were determined according to the sample that yielded the lowest number of sequences in that category (Appendix C).

Sampling for plant community and diversity analyses

To characterize the elevational gradient in plant community structure, we sampled a total of 12 plots (four at the lowest elevation [530 m], and two at each of the other elevations where the soils were sampled) during the summer growing season of 2009. The plots (126°55′–129°00′ E, 41°23′–42°36′ N) were selected to include ecologically homogeneous and physiognomically representative zonal vegetation, without indications of significant recent disturbance. The plot size for each vegetation type was 600 m^2 (20 × 30 m), consisting of six 10 × 10 m subplots. In each plot, one of the six subplots was randomly selected for the study of the shrub layer, and a total of five 1 × 1 m quadrats (located at the four corners and the center of each plot) were used for herb

layer investigation. Latitude, longitude, altitude, aspect (degree to real north), inclination, and position on slope were recorded for each plot. All species of a plot were recorded using nomenclature following Fu (1995). In forested plots, we recorded tree species and individual diameters at breast height (dbh, breast height = 1.3 m) of all stems with dbh $\geq$3 cm. In the shrub layer and herb quadrats, abundance, cover, and mean height were recorded for each species (Fang et al. 2009, Wang et al. 2009). There were no tree and shrub layer quadrats in tundra. Species richness was based on number of species per plot, species diversity was quantified using the Shannon index using relative breast height basal area for trees or relative density for shrub and herb layers as the abundance measure.

Results

Total eukaryotic, fungal, and protistan community compositions across elevations

Across all soil samples, we obtained a total of 81 134 quality sequences with 1005–6787 sequences per sample (mean 3380), from which a total of 6595 OTUs were identified. Fungal (54.2%) and protistan (40.7%) sequences dominated the total eukaryotic community, while metazoans accounted for a small proportion (5.1%) (Appendix C). Within the fungi, Basidiomycota were extremely abundant, while the protists had high proportions of Cercozoa, Alveolata (mainly Apicomplexa, Ciliophora and Dinophyceae), and Stramenopiles (mainly Synurophyceae). Zygnemophyceae, Amoebozoa (mainly Mycetozoa), Chlorophyta, and so on were identified, but at relatively low abundances, and we also detected Apusozoa, Euglenozoa, Glaucocystophyceae, Heterolobosea, and Choanoflagellida, all of which have rarely been reported from soils (Appendix D). The Metazoa consisted mainly of Arthropoda and Nematoda (Appendix E).

The relative abundances of each eukaryotic taxonomic group varied considerably among different elevations (Fig. 1; Appendices D and I). Principal coordinates analysis (PCA) of the pairwise UniFrac distances for the total eukaryotic communities in each sample indicated that overall phylogenetic structure tended to be relatively similar among samples within the same elevation and distinctly different among the different elevations (Fig. 2; Appendix H). Furthermore, community composition differed significantly among elevations according to the ANOSIM test (Table 1). Of all of the environmental variables examined, mean annual precipitation (MAP), mean annual temperature (MAT), elevation, and soil pH were most closely correlated with the total eukaryotic community composition as determined by Mantel tests (Table 2). Very similar patterns were observed in our individual analyses of the fungal and protist communities, except that the correlation between fungi and soil pH was relatively weak (Table 2; Appendix J). The distance-based multivariate linear model analysis (DistLM) indicated that only C/N ratio and total nitrogen (TN) were not significantly related to the variation in community composition when considered individually (Table 3). The sequential model indicated five significant variables (pH, MAP, elevation, MAT, TOC, $P < 0.001$ in all cases) that explained 43.2% of the total variation of the microbial eukaryotic community composition, with pH providing the greatest explanatory power (13.9% of the total variation; Table 3).

Diversity of total soil eukaryotes, fungi, and protists across elevations

Our measures of the total eukaryotic community diversity (i.e., OTU richness, and Chao1 diversity and Faith's phylogenetic diversity indices) were not significantly correlated with elevation (linear and second-order polynomial were used here; Fig. 3; Appendix G). Likewise, neither fungal nor protistan communities exhibited significant elevational gradients in diversity (Appendix G). Of all the soil and site environmental characteristics examined, only pH was significantly correlated with the Chao1 diversity and phylogenetic diversity of the total eukaryotic microbial community (Fig. 4; Appendix G). As in the Mantel test results described in the previous section, the relatively strong relationship with pH in the total eukaryotic community was driven mainly by the impact of acidity on the protist component (Fig. 4). The strong influence of pH was also observed with respect to the major phyla or classes of protists since the relative abundances of Cercozoa, Apicomplexa, Ciliophora, Zygnemophyceae, and Synurophyceae across all sites changed significantly along the soil pH gradient (Fig. 5; Appendix F). By contrast, fungal community OTU richness was most closely correlated (positively) with soil TOC and moisture content, and phylogenetic diversity was positively correlated with soil TOC, TN, and moisture content (Appendix G).

Plant species richness and diversity across elevations

Both total species richness and individual species richness for each of the different forest layer growth forms (trees, shrubs and herbs) significantly decreased with increasing elevation ($P < 0.001$; Fig. 3). Likewise, total species diversity (Shannon index), as well as tree and shrub layer diversity, significantly decreased with elevation ($P < 0.001$), but the herb layer diversity was not significantly related to elevation ($P = 0.182$; Appendix K).

Discussion

Elevational diversity patterns for total soil eukaryotes, fungi, and protists

Eukaryotic microorganism communities (either total, or fungi or protists alone) did not exhibit obvious elevational gradients in diversity along Changbai Mountain. By contrast, and as expected, plant diversity declined along the same gradient. Together, these results

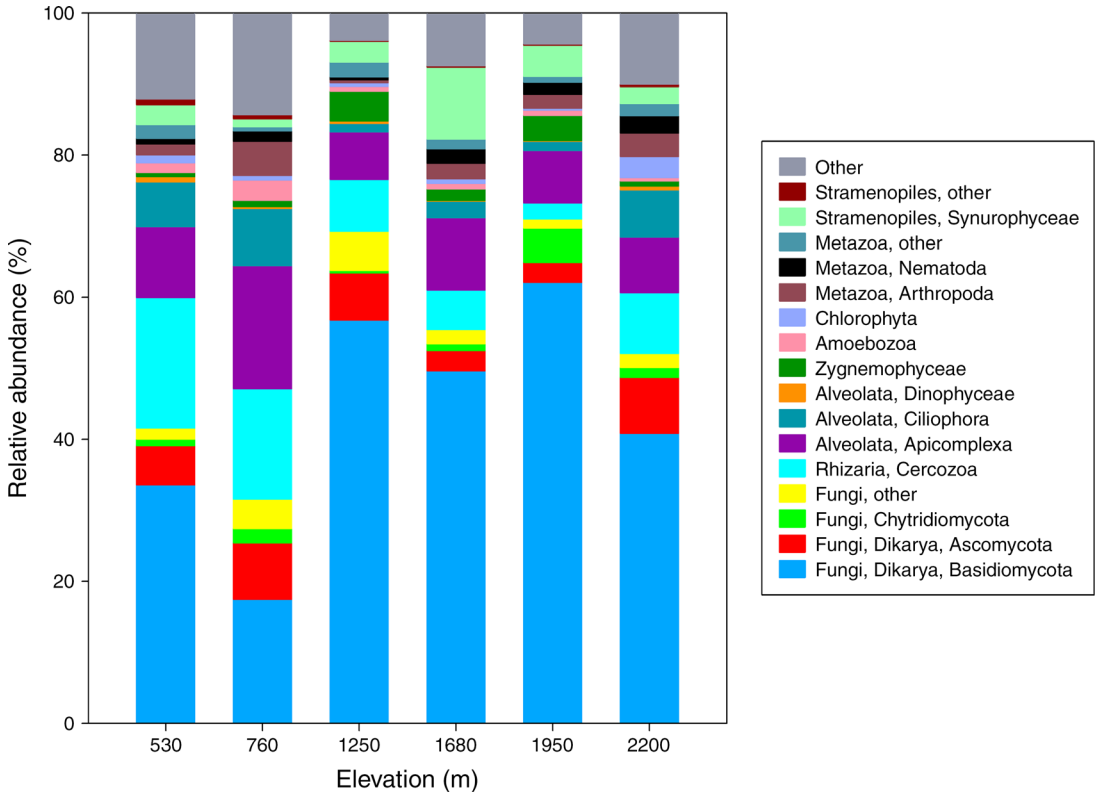

FIG. 1. Relative abundances of the dominant eukaryotic soil microbial groups at each of the six individual sampling locations along the elevational gradient on Changbai Mountain in Northeast China. Relative abundance is based on the proportional frequencies of those DNA sequences from all samples that could be classified at the phylum level. This figure is based on information provided in Appendices C and D. Sampling took place in summer 2009.

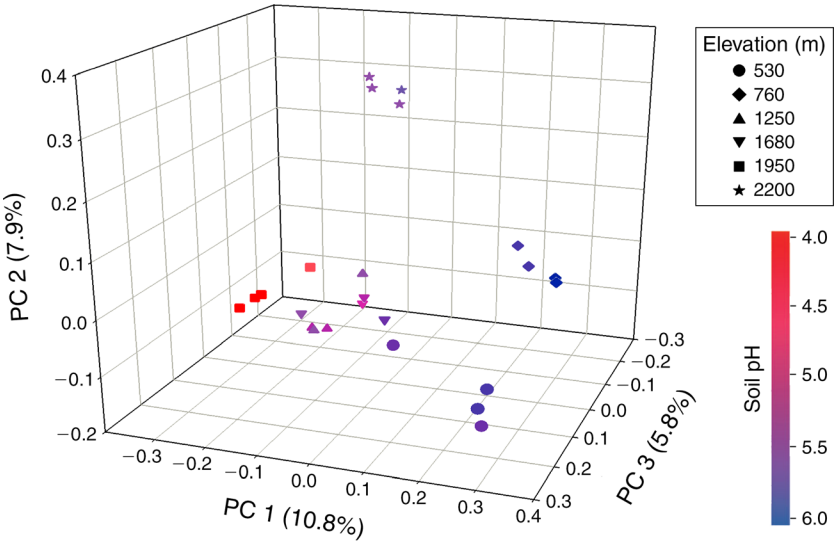

FIG. 2. A three-dimensional plot of principal coordinates analysis (PCA) of unweighted UniFrac distances of total eukaryotic soil microbial community comparing all 24 samples from the six different elevations on Changbai Mountain. Sites have been color-coded according to soil pH gradient. Sampling took place in summer 2009.

TABLE 1. Dissimilarities in total eukaryotic operational taxonomic unit (OTU) community composition between elevations on Changbai Mountain, Northeast China, as determined by analysis of similarities (ANOSIM) *R* values.

Elevation	760 m	1250 m	1680 m	1950 m	2200 m
530 m	**0.99**	**1**	**0.70**	**0.99**	**1**
760 m	n/a	**0.99**	**0.68**	**1**	**1**
1250 m		n/a	**0.49**	**0.77**	**1**
1680 m			n/a	0.43	**0.77**
1950 m				n/a	**0.99**

Notes: An *R* value near +1 means that there is dissimilarity between the groups, while an *R* value near 0 indicates no significant dissimilarity between the groups. Values in boldface type indicate significant dissimilarity ($P < 0.05$). Sampling took place in summer 2009.

refute our study hypothesis, and suggest that the primary controls on eukaryotic microbial communities are fundamentally different than those on macroorganisms. In a previous study, we found no trends in bacterial diversity in the same soil samples (that provided the microbial eukaryote data) from along the same elevational gradient (Shen et al. 2013). Furthermore, Yuan et al. (2014) recently found that soil bacterial diversity exhibited no elevational patterns along the south-facing slope of Nyainqentanglha Mountain on the Tibetan Plateau. To the best of our knowledge, there are only two studies of elevational gradients that simultaneously investigated the diversity of microorganisms and macroorganisms. Bryant et al. (2008) found that plant richness exhibited a unimodal pattern with a peak in species richness at mid-elevations, whereas bacterial (Acidobacteria only) richness decreased monotonically at higher elevations in the Colorado Rocky Mountains. This latter pattern was likely caused by the decreases in soil pH along elevation, given that Acidobacteria are known to be sensitive to pH change (Jones et al. 2009). In a much more comprehensive high taxonomic resolution study (using pyrosequencing as in our study here), Fierer et al. (2011) observed no consistent trend in soil bacterial diversity with elevation in the eastern Andes of Peru, whereas plants and animals showed marked decreases in diversity across the same montane gradient. Our eukaryotic microbial results here, along with our previous data on soil bacterial communities along the same elevational gradient on Changbai mountain, and the Fierer et al. (2011) study cited above, together suggest that the elevational diversity pattern observed for small-sized organisms (bacteria *and* eukaryotic microbes) is fundamentally different from those observed for macroorganisms. Ours is the first comprehensive study using high taxonomic resolution techniques to determine overall eukaryotic microbial diversity in soils along an elevation gradient. Clearly, further research using such high taxonomic resolution techniques and multiple replicate gradients is required to determine how widespread our pattern of results is.

We offer a series of related hypotheses to explain why elevational patterns of richness and diversity differ between eukaryotic microorganisms and macroorganisms. First, body size is probably a primary factor since small size strongly promotes high dispersal ability (Finlay et al. 1996, Hillebrand and Azovsky 2001). Furthermore, large population sizes and short generation times (Finlay and Clarke 1999*b*, Fenchel and Finlay 2004) result in relatively high abundances of individuals within microbial soil eukaryote populations that most

TABLE 2. Mantel test results for the correlation between community composition and environmental variables for total eukaryotes, fungi, and protists along the elevational gradient on Changbai Mountain.

Effect of controlling for	Total eukaryotes		Fungi		Protists	
	r	*P*	*r*	*P*	*r*	*P*
Elevation	0.53	**0.001**	0.50	**0.001**	0.50	**0.001**
MAT	0.54	**0.001**	0.50	**0.001**	0.50	**0.001**
MAP	0.54	**0.001**	0.53	**0.001**	0.49	**0.001**
pH	0.42	**0.001**	0.17	**0.048**	0.63	**0.001**
TOC	0.19	**0.018**	0.23	**0.014**	0.17	**0.017**
C/N	0.13	0.072	0.04	0.354	0.19	**0.011**
TN	0.14	0.119	0.02	0.345	0.24	**0.007**
Moisture	0.12	0.051	0.13	0.056	0.16	**0.027**

Notes: Abbreviations are: MAT, mean annual temperature; MAP, mean annual precipitation; TOC, total organic carbon; C/N, carbon/nitrogen ratio; and TN, total nitrogen. Values in boldface type indicate significant correlation ($P < 0.05$). Sampling took place in summer 2009.

TABLE 3. Results of distance-based multivariate linear model (DistLM) for microbial eukaryotic community composition showing the percentage of variation explained by environmental variables: each variable analyzed individually (ignoring other variables); and forward-selection of variables, where amount explained by each variable added to model is conditional on variables already in the model.

Variable	%Var	Pseudo-*F*	*P*	Cum(%)
Variables individually				
pH	13.93	3.56	**0.001**	
Elevation	12.96	3.28	**0.001**	
MAT	12.69	3.2	**0.001**	
MAP	12.6	3.17	**0.001**	
TOC	7.12	1.69	**0.013**	
Moisture	7	1.65	**0.027**	
C/N	5.51	1.28	0.115	
TN	5.17	1.2	0.156	
Variables fitted sequentially				
pH	13.93	3.56	**0.001**	13.93
MAP	9.94	2.74	**0.001**	23.87
Elevation	7.64	2.23	**0.001**	31.51
MAT	6.27	1.91	**0.001**	37.78
TOC	5.41	1.71	**0.001**	43.19
TN	3.59	1.15	0.258	46.78
C/N	3.27	1.04	0.417	50.05
Moisture	2.99	0.96	0.574	53.04

Notes: The percentage of variance in species data explained by that variable is abbreviated as "%Var," and the cumulative percentage of variance explained is abbreviated as "Cum(%)." Variable abbreviations as in Table 2. Values in boldface type indicate significant correlation ($P < 0.05$). Sampling took place in summer 2009.

likely increase their chance of long-distance dispersal (Finlay 2002). Our finding here parallels a study of protozoa and diatoms that showed no correlations between species richness and latitude (Hillebrand and Azovsky 2001). Importantly, these authors concluded that the strength of the relationship between richness and latitude was positively correlated to the size of the organisms. Data compiled across a wide range of eukaryotic taxonomic groups (e.g., amoebae, diatoms, and mollusks, among others) from freshwater pond and shallow marine bay showed that the local : global species ratio decreased consistently with mean body size, indicating that small organisms (<1 mm in length) tend to have a cosmopolitan distribution (Finlay and Fenchel 2004). Thus, we hypothesize that body size does not constrain a eukaryotic microorganism's dispersal rate, population density, and range size, whereas it does somewhat constrain those of larger organisms.

Second, environmental factors influencing community diversity with elevation might fundamentally differ between eukaryotic microorganisms and macroorganisms. On the one hand, climate (especially temperature and precipitation) are strong predictors of plant and animal diversity along elevational gradients (Currie et al. 2004, McCain 2007). Although there is some evidence that temperature effects on metabolic rate may strongly influence bacterial speciation rates in marine latitudinal gradients (Pommier et al. 2007, Fuhrman et al. 2008), it seems unlikely that these processes would be as important in soils because they have more complex chemical and physical features than seawater (Fuhrman 2009). On the other hand, a strong and positive relationship between soil pH and bacterial diversity has been consistently reported in many studies of latitudinal and elevational distributions (Fierer and Jackson 2006, Chu et al. 2010, Shen et al. 2013, Yuan et al. 2014). Recently, close correlations between eukaryotic microbial community and soil pH, as well as other characters like soil C:N ratio and moisture, were found in studies across local (Tsyganov et al. 2013), regional (Mulder et al. 2005), and global scales (Wu et al. 2011, Bates et al. 2013). Here, we also found significant correlation between the diversity of the eukaryotic microbial community and soil pH. These results suggest that local soil environmental conditions are much more important than broad-scale factors in determining species richness of eukaryotic microorganisms.

Third, the lack of a discernable elevational trend for eukaryotic microorganisms may be due to insufficient taxonomic resolution (Green and Bohannan 2006). Our study relied on pyrosequencing, which is one of the best techniques available, but we cannot preclude the possibility that future even higher resolution techniques as well as a more comprehensive BLAST eukaryotic microbial sequence identification database may yield alternative patterns.

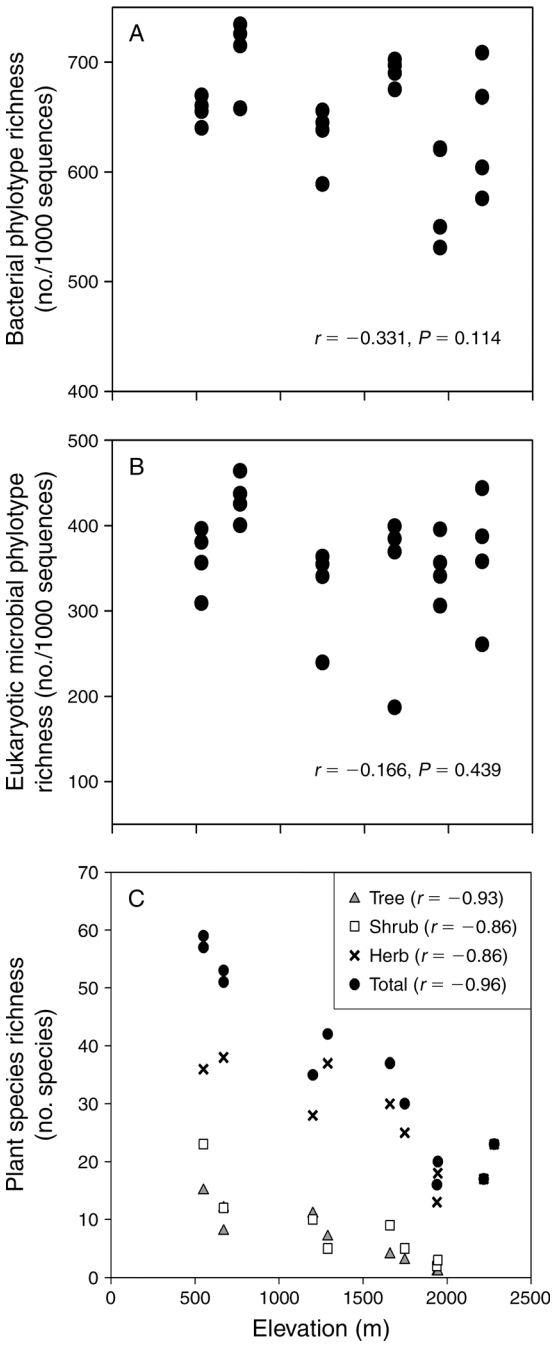

FIG. 3. (A) Bacterial phylotype richness (data from a previous study Shen et al. 2013), (B) eukaryotic microbial phylotype richness, and (C) plant species richness across the elevational gradient on Changbai Mountain. Sampling took place in summer 2009.

Factors influencing eukaryotic soil community structure

Although the overriding importance of soil pH in controlling soil bacterial diversity and community composition has been well demonstrated (Fierer and Jackson 2006, Lauber et al. 2009, Chu et al. 2010,

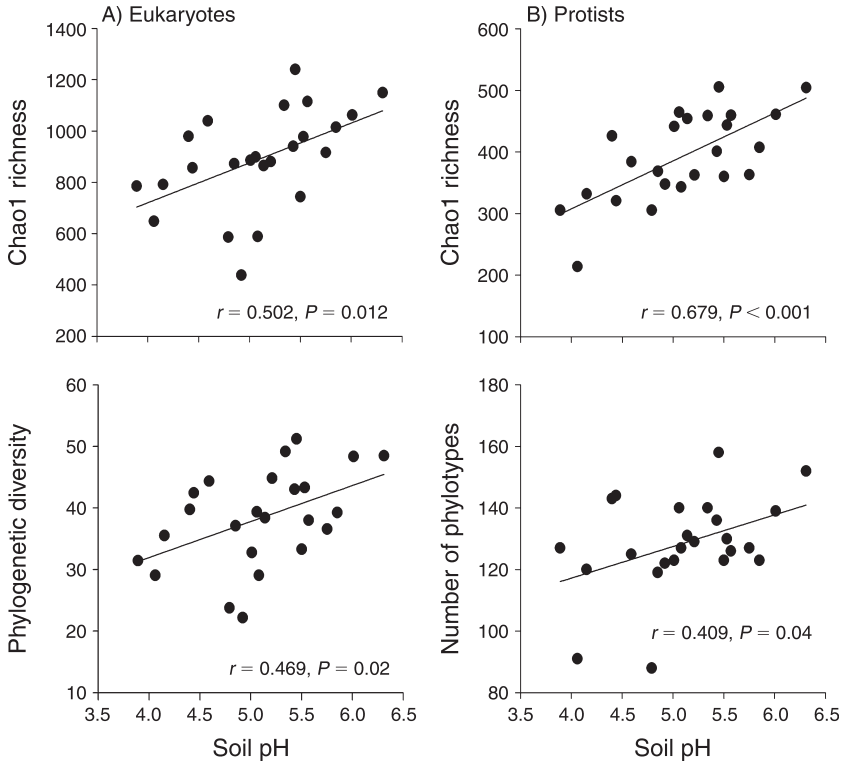

FIG. 4. The diversity of total (A) eukaryotes (1000 randomly selected sequences per sample, Chao1 richness, and phylogenetic diversity indices) and (B) protists (260 randomly selected sequences per sample, Chao1 richness, and number of phylotypes) in relation to soil pH on Changbai Mountain. Sampling took place in summer 2009.

Griffiths et al. 2011, Shen et al. 2013), we know very little about the influence of pH on eukaryotic soil microbes. Recent studies have documented the influence of pH on the diversity and community composition of metazoans and protists. For example, nematode and microarthropod abundances were positively and negatively (respectively) correlated with soil pH across 284 sandy soil samples along a pH gradient in The Netherlands (Mulder et al. 2005). Wu et al. (2011) found that the percentage of nematodes in 42 plots from around the globe was positively correlated with soil pH and negatively correlated with soil C:N ratio, whereas the percentage of arthropods was inversely correlated with the same two variables. In addition to pH, soil moisture also seems to be a strong determinant of protist community structure (Tsyganov et al. 2013). Bates et al. (2013) found that soil protist diversity was only marginally influenced by pH, and shifts in community composition for protists were most strongly correlated with annual soil moisture availability rather than pH. In our study, we found that pH was significantly and fairly strongly correlated with total eukaryotic and protist-only community composition, and only weakly correlated with fungal community composition. Likewise, the various diversity indices of total eukaryotic microbes and protists significantly correlated with pH, whereas the diversity of fungi did not. Soil fungi seem to have a relatively wide pH optimum compared to bacteria. For example, a previous study across a pH gradient in an arable soil reported that the fungal community composition was significantly related to soil pH, but the influence was far weaker than for the bacterial community (Rousk et al. 2010). Mulder et al. (2005) also found that fungi tended to be much more acid tolerant than bacteria. Actually, fungal community composition is often most closely associated with changes in soil nutrient status (Lauber et al. 2008). Together, these results suggest that pH is an important factor determining the protist component of eukaryotic soil microbial community distributions, but that the fungal component seems to be more strongly influenced by organic matter substrate-related characteristics such as carbon and nutrient quality.

Eukaryotic soil microbial communities

Our study is one of the first surveys of soil eukaryotic microorganisms across different vegetation types. Along our elevational gradient from forest and tundra, we found most soil eukaryotic taxa that had previously been identified at other sites by other molecular techniques (Fell et al. 2006, Moon-van der Staay et al. 2006). Our results indicating fungal dominance by species from the Basidiomycota phylum in the five lower elevation tree-dominated sites except for the

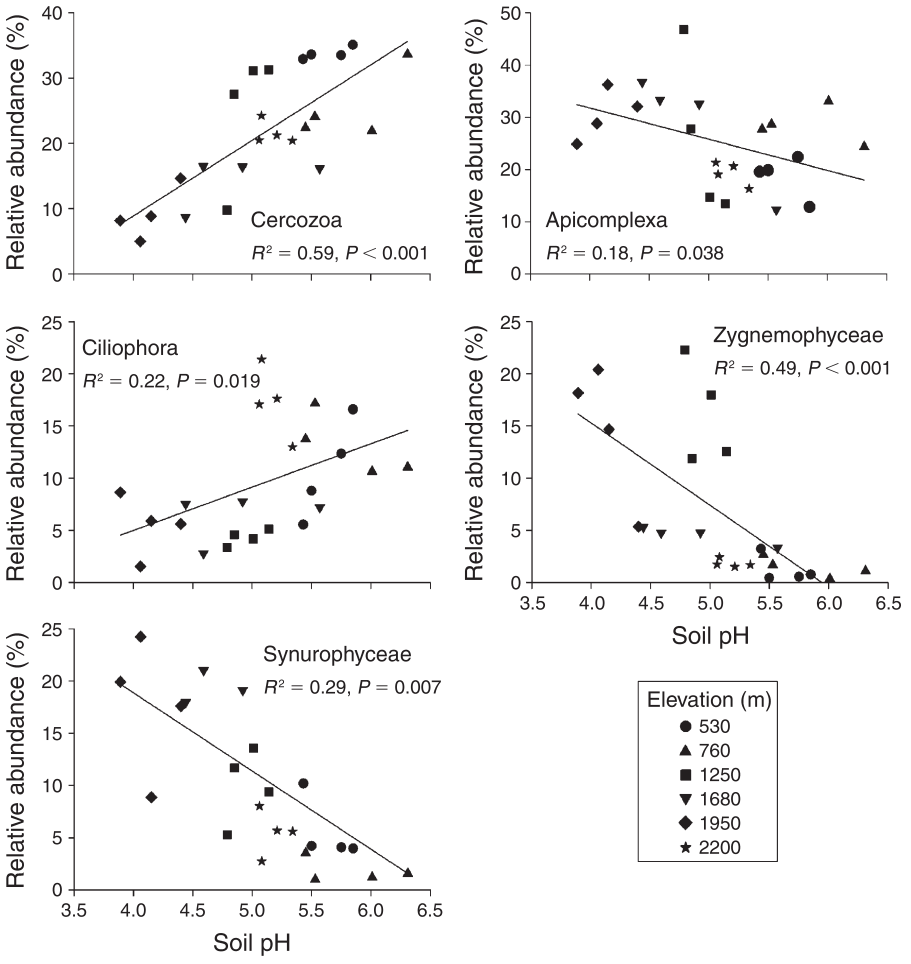

FIG. 5. The relative abundances of the dominant protistan phyla at each elevation on Changbai Mountain in relation to soil pH. The strength of each relationship given is based on the linear regression equation. Sampling took place in summer 2009.

mixed coniferous forest at 760 m is in agreement with previous studies in forests (O'Brien et al. 2005, Bailly et al. 2007).The Basidiomycota were also the most abundant phylum at our uppermost tundra vegetation site, whereas Schadt et al. (2003) reported a predominance of Ascomycota in a North American alpine tundra site. Climatic differences between these two regions may explain these data but in addition, our method with its higher resolution may also be an important factor, given that Schadt et al. (2003) found a large number of unknown groups using the clone library technique. Furthermore, differences in sampling timing may be an explanatory factor since our results are based on mid-summer data, and Zinger et al. (2009) found that the ratio of Basidiomycota to Ascomycota in alpine tundra differed between the snow-covered and summer seasons.

Arthropoda (mainly acarid) and Nematoda dominated the Metazoa at our sites, just as they do at many other locations (Wu et al. 2011, Meadow and Zabinski 2012). For protists, we identified Cercozoa and Apicomplexa, both of which are common members of soil communities as determined by direct observation (Adl and Gupta 2006) and pyrosequencing studies (Bates et al. 2013). Likewise, our observations of Eucoccidiorida, which accounted for 84% of Apicomplexa sequences, and Heterocapsaceae, which represented 99% of Dinophyceae sequences, were basically consistent with the reports from other soils (Bates et al. 2013). It is noteworthy that Synurophyceae occupies a major proportion of Stramenopiles in both forest and tundra soils. The Synurophyceae, also known as scaled chrysophytes (Siver et al. 2013), are important indicators of the past ecology in lacustrine environments (Wujek 2013) and have long been recognized as ubiquitous in aquatic environments (Finlay and Clarke 1999*a*). Our results here indicate that Synurophyceae are also abundant in soils. Furthermore, other rare eukaryotic microorganisms such as members of the Apusozoa, Euglenozoa, Glaucocystophyceae, Heterolobosea, and Choanoflagellida were also detected in our study. These rare groups, as well as Stramenopiles, were not uncovered in another study using metatranscriptome method (Bailly et al. 2007), suggesting the particular

potential of the pyrosequencing technique for the examination of eukaryotic soil microbial diversity.

Conclusion

Ours is the first study to document the overall pattern of eukaryotic soil microbial diversity along an elevation gradient, and our results indicate that microbes do not follow the elevational diversity pattern of macroorganisms. Meanwhile, significant correlations between the diversity of the microbial eukaryotic community and pH were found in this study, suggesting that pH strongly influences not only bacterial communities but also eukaryotic (primarily the protist) component of soil microbial communities. Characterizing these similarities and differences among microorganisms and macroorganism communities that live alongside each other in the same terrestrial environments is fundamental to advancing our understanding of ecology. We anticipate that our results here will lead to new simultaneous investigations of prokaryotic and eukaryotic soil communities along multiple replicate elevational (and latitudinal) gradients to more fully elucidate the patterns and underlying mechanisms determining soil biological diversity. Furthermore, our data provide the starting point for establishing diversity resource databases for eukaryotic soil microbes (and bacteria), as well as for plants along the Changbai Mountain, against which the impacts of future climate changes and ecosystem management practices can be assessed. Given the remoteness of this region and the fact that it has been relatively undisturbed by anthropogenic activities in the last 150 years, these databases will have particular ecological science and conservation value.

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Supplemental Material

Ecological Archives

Appendices A–K are available online: http://dx.doi.org/10.1890/14-0310.1.sm