Applied Microbiology and Biotechnology (2019) 103:3215–3224
https://doi.org/10.1007/s00253-018-09582-1

ENVIRONMENTAL BIOTECHNOLOGY

Forest gaps influence fungal community assembly in a weeping cypress forest

Dehui Li[1,2] · Xianwei Li[1] · Yu Su[1] · Xiangzhen Li[3] · Haifeng Yin[1] · Xiangjun Li[1] · Maojin Guo[1] · Yunxiao He[2]

Received: 12 August 2018 / Revised: 11 December 2018 / Accepted: 12 December 2018 / Published online: 29 January 2019

Abstract

The forest gap crucially influences forest environments, but its effects on local fungal community assembly are not fully understood. In this study, the fungal community in a weeping cypress forest was investigated as a function of forest gap locations based on forest clearing, using amplicon sequencing of the ITS2 region. The results showed that the fungal community significantly varied with the variations in soil properties related to gap location. Deterministic processes played pivotal roles in fungal community assembly, which was mainly driven by the temperature, moisture, available nitrogen, and microbial carbon in soil. Beta diversity of the fungal community increased from the gap center to the closed canopy. The relative abundances of dominant orders such as *Microascales*, *Sordariales*, and *Chaetothyriales* regularly varied as a function of gap location, and they were potential indicators for different gap locations. Based on network analysis, gap locations caused distinct co-occurrence patterns of fungal communities. This study shed light on the roles of forest gaps in the assembly of local fungal communities and provided additional strategies to manage forest ecosystems.

Keywords Forest gap locations · Weeping cypress · Fungal community · Soil properties · Deterministic process

Introduction

The formation of forest gaps was usually caused by natural disasters and forest succession (Allen et al. 2010; Muscolo et al. 2014), and the artificial formation of forest gaps as effective forest management had recently increased (Mallik et al. 2014; Yang et al. 2017). Forest gaps played a crucial role in forest ecology by preventing the creation of low-efficiency forests caused by very dense distributions, and thus forest gaps provided spaces for the colonization of other species and preserving biodiversity (Gray et al. 2012; Muscolo et al. 2014). Forest soil underlying the forest ecology was also heavily influenced by forest gaps. Soil temperature and moisture at different gap locations were different because of distinct net radiation, rainfall, and plant transpiration (Ritter et al. 2005; Sariyildiz 2008; Zhu et al. 2003). Usually, the soil temperature at the gap center (GC) was higher than that at the gap border (GB) (Ritter et al. 2005), but the regulation of soil moisture by forest gaps was much more complicated, because lots of factors such as vegetation, rainfall, net radiation, and even microsite variation were involved (Gray et al. 2002; Ritter et al. 2005; Sariyildiz 2008; Zhu et al. 2003). Soil nutrient cycling also showed distinct scenarios at different forest gap locations. The availability of carbon, nitrogen, and phosphorus from the decomposition of plant litter decreased from GC to the closed canopy (CC) (He et al. 2016b), which was possibly related to the high decomposition rate of litter at the CC (Zhang and Zak 1995). Less litter input and root exudates were found in the GC than CC (Schliemann and Bockheim 2014; Xu et al. 2016), probably resulting in low nutrient levels in the soil. In

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00253-018-09582-1) contains supplementary material, which is available to authorized users.

✉ Xianwei Li
lxw@sicau.edu.cn

[1] Key Laboratory of State Forestry Administration on Forest Resources Conservation and Ecological Safety in the Upper Reaches of the Yangtze River; Forestry Ecological Engineering in the Upper Reaches of Yangtze River Key Laboratory of Sichuan Province, Sichuan Agricultural University, Chengdu 611130, China

[2] Sichuan Province Key Laboratory of Ecological Security and Protection, Mianyang Normal University, Mianyang 621000, China

[3] Key Laboratory of Environmental and Applied Microbiology, CAS; Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China

forest ecosystems, soil microbial communities played pivotal roles in litter decomposition and vegetation growth (Baldrian 2017; Peltoniemi et al. 2015; Singh et al. 1989), and were affected by forest gaps. From the GC to the CC, microbial activity increased (Zhang and Zak 1995), and the microbial compositions evaluated by phospholipid fatty acid analysis varied (Yang et al. 2017). Soil enzyme activity and substrate decomposition, which were partly regulated by microorganisms, had also been comprehensively investigated at forest gap locations (He et al. 2016a; Muscolo et al. 2007; Yang et al. 2017). Soil microorganisms were influenced by soil temperature, moisture, and nutrient levels (Baldrian 2017; Peltoniemi et al. 2015; Singh et al. 1989), and they played crucial roles in organic matter transformation and the development of vegetation in soil (Harantová et al. 2017). Both the deterministic processes (environmental selections) and stochastic processes (ecological drift and dispersal) underlying microbial community assembly were tightly related to the stability and succession of microbial community (Zhou et al. 2014) and thus were crucial for understanding the roles of forest gaps in microbial community assembly. Microorganisms with different phylogenetic affiliations probably employed distinct trophic strategies (Nguyen et al. 2016). Although there were some investigations into the effects of forest gaps on microbial communities (Yang et al. 2017; Zhang and Zak 1995), the assembly mechanism, taxonomic composition, and trophic strategies of microbial communities with forest gap locations were not fully understood. Fungal community usually forms mycorrhizal associated with vegetation and was the primary decomposers of vegetation litter (Harantová et al. 2017). Taken together, forest gaps crucially influenced fungal community in forest soil, and the investigation of fungal communities was essential to reveal the effects of forest gaps on forest ecosystem structure and function, yet elusive in the literature.

Weeping cypress (*Cupressus funebris*) has been comprehensively used for afforestation in China. The artificial formation of gaps in weeping cypress forests prevented not only the formation of low-efficiency forests but also disturbance to the local fungal community. However, rare attentions had been paid on the influences of gap locations of weeping cypress on local fungal community. In this study, the variation in fungal community along the gap locations in a weeping cypress forest was investigated based on amplicon sequencing of an internal transcribed spacer (ITS). The main goals were to reveal the (i) fungal diversity and composition in response to the gap locations, (ii) mechanisms (deterministic and stochastic processes) underlying fungal community assembly at the gap locations, and (iii) main environmental drivers and fungal indicators for specific gap locations.

Materials and methods

Site description

The study was conducted in forests (31° 04′ N, 104° 25′ E, and 510 to 530 m above sea level) located in Hexin Town, Sichuan, China. The annual mean temperature ranged from 15 to 17 °C, with maximum and minimum temperatures of 25 °C and 5 °C, respectively. The annual precipitation is approximately 906 mm. The forests were mainly dominated by weeping cypress (*C. funebris*), which had been comprehensively used for afforestation in China. Due to high density and lack of proper management, the canopy had become low-efficiency forests. Other trees were German oak (*Quercus acutissima*), alder (*Alnus cremastogyne*), paper mulberry (*Moraceae broussonetia*), tungoil tree (*Vernicia fordii*), and privet (*Ligustrum lucidum*). The shrub layers were linden arrowwood (*Viburnum dilatatum*), Chinese sumac (*Rhus chinensis*), and pyracantha (*Pyracantha fortuneana*). The herb layers were brake (*Pteris multifida*), sedge (*Carex brunnea*), and hispid arthraxon (*Arthraxon hispidus*). Detailed information about local environments was given by Yulin (Yulin et al. 2014).

Experimental design and soil sampling

In April 2012, eight similar oval gaps (ca. 20 m × 14 m in size) were created by cutting trees in a low-efficiency forest of weeping cypress with an average tree height/diameter at breast height (DBH) of 6.5 m/8.0 cm and a canopy density greater than 0.8. The distances between the centers of adjacent gaps were at least 40 m. In forest gaps, other vegetations such as grasses and herbs were also removed. In October 2017 when sampling was conducted, in each forest gap, there was a similar vegetation cover such as some new establishments of grasses, herbs, and shrub. Soil samples were collected from three locations, the gap centers (GC), gap boarders (GB), and closed canopy (CC), within each gap. In each location, 3 plots (4 m apart from each other) were selected in areas without mushroom or other macrofungi. In each plot (1 m × 1 m), five soil cores (5 cm in depth, 5 cm in diameter) were collected. Then, all the 15 soil cores which were evenly distributed in one gap location were pooled together to form a composite sample. In total, 24 composite soil samples were collected from 3 locations in 8 gaps. The soil samples were sieved through a 2.0-mm mesh and then used for measurements of soil properties and genomic DNA extraction.

Soil property analysis

Soil temperature (ST) and soil moisture (SM) were recorded by a Thermochron iButton Device (DS1921-G, Maxim Integrated, San Jose, CA, USA). Soil organic carbon (SOC)

was measured by wet oxidation with potassium, soil total nitrogen (STN) by the Kjeldahl method, soil available nitrogen (SAN) by a modified Conway method (Stanford et al. 1973), soil total phosphorus (STP) using $HCLO_4$ digestion and colorimetric assay (Jackson 1958), and soil microbial carbon (SMC) and nitrogen (SMN) by the chloroform fumigation incubation method (Hart et al. 1994).

DNA extraction and MiSeq sequencing of ITS amplicons

Soil genomic DNA was extracted with the PowerSoil DNA kit (MoBio, Carlsbad, CA, USA). Two-round PCR amplification was conducted with universal primers ITS4 (5′-TCCT CCGCTTATTGATATGC-3′) and gITS7F (5′-GTGA RTCATCGARTCTTTG-3′), which target the fungal ITS2 region of the ribosome encoding genes (Žifčáková et al. 2016). The details of the PCR procedure were described previously (Žifčáková et al. 2016). The PCR products were pooled and purified with the QIAquick Gel Extraction Kit (Omega, Norcross, GA, USA). In total, 24 samples were prepared for sequencing with the MiSeq platform (Illumina, San Diego, CA, USA).

Data analysis

Sequence data analysis

The analysis of amplicon sequence was conducted with QIIME Pipeline version 1.9.0 (Caporaso et al. 2010). All sequence reads were sorted based on their unique barcodes, trimmed for sequence quality, and clustered at 97% similarity for identification of operational taxonomic units (OTUs). The removal of chimera sequences was conducted with the UCHIME algorithm (Edgar et al. 2011). The phylogenetic affiliation of each sequence was conducted based on the Ribosomal Database Project classifier. Each sample was resampled to equal numbers of sequences (12,276 reads per sample) for downstream analysis. The original sequencing data were available at the European Nucleotide Archive by accession at PRJE25917 (http://www.ebi.ac.uk/ena/data/view/PRJEB25917).

Statistical analysis

Principal coordinate analysis and permutational multivariate analysis of variance (PerMANOVA) based on the Bray-Curtis distance were conducted in R (http://www.r-project.org/) to evaluate the general changes in the fungal community at different gap locations. Mantel and partial Mantel tests, Spearman's correlation, the Wilcoxon rank sum test, random forest algorithms with 9999 trees, and the indicator analysis based on the function *multipatt* were conducted in R. p values were adjusted by the methods of FDR (Benjamini and Hochberg 1995). The deterministic process underlying fungal community assembly (Zhou et al. 2014) was evaluated by a "null model test" at the pipeline (http://ieg.ou.edu/microarray/) by keeping alpha and gamma diversity constant, using the Bray-Curtis distance of the fungal community, without a phylogenetic tree which is highly unreliable based on ITS sequences. Prediction of the trophic mode of the fungal community was performed with FUNGuild (Nguyen et al. 2016) by retaining only the taxa with confidences of probable and highly probable. Co-occurrence network analysis was performed based on OTUs with an occurrence > 2 and average relative abundance > 0.1% in each gap location. Spearman's correlations between two taxa with a correlation coefficient > |0.6| and an adjusted p value (Benjamini and Hochberg 1995) < 0.05 were considered statistically robust. Meanwhile, 9999 Erdös–Rényi random networks (Erdos and Rényi 1960) with the same number of nodes and edges as each correspondingly observed network were generated using the function *erdos.renyi.game* in R. The visualizations of networks were realized by Gephi software (https://gephi.github.io/)

Results

Soil properties

Principal coordinate analysis and PERMANOVA tests showed that forest gaps significantly affected local soil properties ($R^2 = 0.964$, $p < 0.001$). Compared with CC, forest gaps (GC and GB) resulted in significantly lower SM, STN, SAN, SMC, and SMN but higher ST in their soil (Table 1).

Table 1 Soil properties among different gap locations

	ST	SM	SOC	STN	SAN	STP	SMC	SMN
GC	22.288 ± 0.164^a	74.198 ± 0.558^a	52.754 ± 0.718^a	2.528 ± 0.074^a	151.351 ± 3.422^a	0.223 ± 0.027^a	491.25 ± 9.483^a	79.25 ± 4.559^a
GB	21.025 ± 0.167^b	79.544 ± 0.175^b	60.168 ± 3.995^b	1.863 ± 0.036^b	161.8 ± 4.368^b	0.251 ± 0.02^b	625.125 ± 54.02^b	50.875 ± 4.704^b
CC	19.788 ± 0.113^c	85.923 ± 0.353^c	59.363 ± 1.34^b	3.196 ± 0.063^c	184.838 ± 4.777^c	0.251 ± 0.025^b	959.25 ± 25.9^c	85.625 ± 3.503^c

Data are expressed as the average ± SD. Different letters in the same column denote statistically significant at $p < 0.05$

Specifically, SM, SAN, and SMC significantly decreased, but ST increased from GC to CC (Table 1). In consideration of that only ST, SM, SAN, and SMC regularly responded to gap locations, it was deduced that the environmental variations caused by gap locations were mainly represented by the four soil properties.

Overall variations in the fungal community

The complete coverages of fungal communities were confirmed by rarefaction curves (Supplementary Fig. S2). Principal coordinate analysis and PERMANOVA tests showed significant variations ($R^2 = 0.21674$, $p = 0.001$) in fungal communities at different gap locations (Fig. 1 and Supplementary Table S1). All the soil properties except STN and SMN significantly contributed to the variations in fungal communities. The Mantel and partial Mantel tests were used to further evaluate the relation between the fungal community and soil properties (Table 2). Based on the Mantel test, all the soil properties except SOC, STP, and SMN showed significant positive correlations with the fungal community. However, the results of a partial Mantel test showed that when other soil properties were separately controlled, in most of cases, only ST, SM, SAN, and SMC still significantly correlated with the fungal community. Consequently, ST, SM, SAN, and SMC significantly contributed to the differentiation of fungal communities among different forest gaps.

A null model method was used to evaluate the effects of deterministic vs. stochastic processes on fungal community assembly. All the observed fungal community assemblies were significantly distinguished from the random null expectation ($p < 0.01$), indicating that the fungal communities were mainly shaped by deterministic processes (Table 3). Thus, gap locations influenced the fungal community through deterministic processes, and these processes were likely to be mainly driven by ST, SM, SAN, and SMC. Standardized effect size (SES) is commonly used as a quantitative index for deterministic processes (Zhou et al. 2014), and it was greatest at GC ($p < 0.01$), followed by GB and CC (Table 3), which indicated that the effects of deterministic processes on fungal community assemblies decreased from GC to CC. Interestingly, a shift in the SES corresponded well with changes in ST, SM, SAN, and SMC as a function of gap location.

The beta diversity of the fungal community was lowest at GC ($p < 0.05$), followed by GB and CC (Supplementary Fig. S3), indicating that the highest similarity (lowest variation) among fungal communities was at GC, followed by those at GB and CC. The beta diversity significantly correlated with ST, SAN, and SMC (Supplementary Table S2). This shift in beta diversity was usually determined by environmental heterogeneity and/or the trade-off between stochastic and deterministic processes.

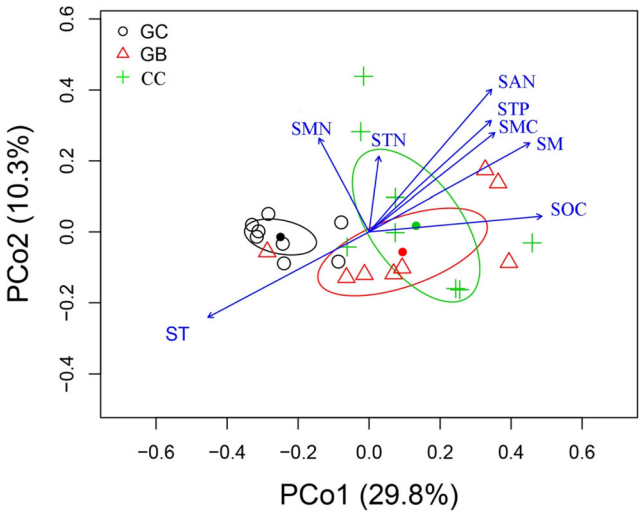

Fig. 1 The principal coordinate analysis of fungal community based on Bray-Curtis distance among different groups. The solid point represents the centroid in each group. The circle represents 95% confidence interval

Variations in fungal composition

The alpha diversities of fungal communities showed nonsignificant differences among different gap locations and nonsignificant correlations with nearly all the soil properties (Supplementary Table S3), indicating that gap locations barely influenced the alpha diversities of fungal communities.

Based on FUNGuild, the trophic modes of fungal communities were predicted (Supplementary Table S4). Only the trophic modes of pathotrophs and saprotrophs showed significant differences among different gap locations. The relative abundance of pathotrophs increased from GC to CC, and it showed significantly positive correlations with SM and SOC. However, the relative abundance of saprotrophs decreased from GC to CC, and showed significant correlations with ST, SM, SOC, SAN, and SMC. The variations in fungal trophic modes were undoubtedly related to variations in fungal composition. At the phylum level, all the fungal communities were dominated by *Ascomycota* (> 86%) and *Basidiomycota* (> 9%), and the relative abundances of nearly all the phyla showed nonsignificant differences among different gap locations and nonsignificant correlations with soil properties (Supplementary Table S5). At the order level, the relative abundances of *Microascales* and *Sordariales* decreased from GC to CC, but that of *Chaetothyriales* showed the opposite trend (Fig. 2). The relative abundances of the above three orders showed significant correlations with all the soil properties except STN and SMN (Supplementary Table S6). Most of the OTUs in the three orders were saprotrophic (Supplementary Table S6). The relative abundance of *Verrucariales* was significantly higher ($p < 0.01$) at the CC (11.3%) than at forest gaps (GC = 2.7%; GB = 1.9%), but it did not regularly

Table 2 Mantel and partial Mantel tests for the correlations between soil properties and fungal community based on Bray-Curtis distance

Mantel test		ST 0.2201**	SM 0.2592**	SOC 0.1556	STN 0.1763*	SAN 0.3962**	STP 0.1366	SMC 0.3528**	SMN 0.1153
Controlling variables in the partial Mantel test	ST	NA	0.1404	0.04475	0.07413	0.3576**	0.1144	0.2979**	0.07497
	SM	− 0.000265	NA	0.04891	0.01004	0.3511**	0.1287	0.2754**	0.05417
	SOC	0.1636*	0.2152*	NA	0.1559*	0.3693**	0.1008	0.3205**	0.09264
	STN	0.1526*	0.1932*	0.1318	NA	0.3698**	0.1409*	0.3178**	− 0.03009
	SAN	− 0.125	− 0.1729	0.01961	− 0.08866	NA	0.08281	0.0006107	0.02527
	STP	0.2074**	0.2553**	0.1256	0.1796*	0.3832**	NA	0.3431**	0.1085
	SMC	− 0.09811	− 0.1241	0.007458	− 0.07197	0.1927**	0.1056	NA	0.0268
	SMN	0.2025**	0.2395**	0.1398	0.1375	0.3823**	0.1309	0.3366**	NA

The significances are tested based on 999 permutations. $^{**}p < 0.01$; $^{*}p < 0.05$. The italic entries represent the controlled variable. NA means inapplicable

respond to gap locations (Fig. 2). Nearly all OTUs in *Verrucariales* were symbiotrophic. The relative abundance of *Eurotiales* was lower at GC than at GB and CC (Fig. 2) and significantly positively correlated with only SOC (Supplementary Table S6). The other orders rarely showed significant correlations with soil properties (Supplementary Table S6). Thus, it seemed that gap locations mainly influenced the distributions of the orders *Microascales*, *Sordariales*, and *Chaetothyriales*. At the OTU level, the OTUs discriminating among different gap locations were identified based on a random forest analysis (Fig. 3). Among the OTUs, the relative abundances of 16 OTUs affiliated with *Scedosporium* (OTU74, OTU91, OTU206, and OTU4979), *Ascomycota* (OTU487, OTU557, and OTU3832), *Agaricomycetes* (OTU34 and OTU449), *Eurotiomycetes* (OTU44 and OTU3470), *Microascaceae* (OTU39 and OTU670), *Phialemonium* (OTU89 and OTU670), and *Aspergillus* (OTU231) decreased from GC to CC and significantly positively correlated with ST but negatively correlated with SM, SOC, SAN, and SMC (Fig. 3). However, the relative abundance of OTU362 (*Phialophora*) increased from GC to CC and significantly positively correlated with SM, SOC, STN, SAN, STP, and SMC but negatively correlated with ST (Fig. 3). Furthermore, there were more indicator species at GC than GB and CC (Supplementary Table S7). The indicator species at GC were mainly affiliated with *Dothideomycetes*, those at GB were mainly affiliated with *Penicillium*, and those at CC were mainly affiliated with *Phialophora*. Although, the indicator species in each gap location showed high diversity at OTU level, most of them were affiliated with the orders *Microascales* and *Chaetothyriales*. Consequently, the distributions of these OTUs were crucially influenced by gap locations.

Co-occurrence of fungal microorganisms

All the three networks at different gap locations clearly showed greater network modularity than the corresponding Erdös–Rényi random networks (Fig. 4), suggesting modular structures in the three networks (Newman 2006). Fungal co-occurrence patterns were clearly distinct at different gap locations. For example, the co-occurring taxa affiliated with the six orders showed different distributions of degree at gap locations (Fig. 4). The network of GB had the most nodes and edges, followed by those of CC and GC (Fig. 4 and Supplementary Table S9), which indicated that the fungal co-occurrence was stronger at GB than at other gap locations. There were fewer connections at GC than GB and CC, which indicated the effects of forest on fungal co-occurrence. There were not OTUs affiliated with the order *Chaetothyriales* in the network at GC, which was probably related with its low relative abundance at GC. However, there were not OTUs affiliated with the order *Microascales* in the any of networks, even though it was abundant despite gap locations.

Table 3 The significance test of centroid differences between the observed communities and the null model simulations based on Bray-Curtis distance

	Actual centroid	Null centroid	*F*	*p*	Standardized effect size (SES)[a]
GC	0.31	0.578	112.573	< 0.001	46.137 ± 10.156
GB	0.388	0.582	25.665	< 0.001	20.283 ± 8.135
CC	0.426	0.576	15.957	0.001	9.935 ± 4.468

[a] Mean significant differences ($p < 0.01$) of SES among groups

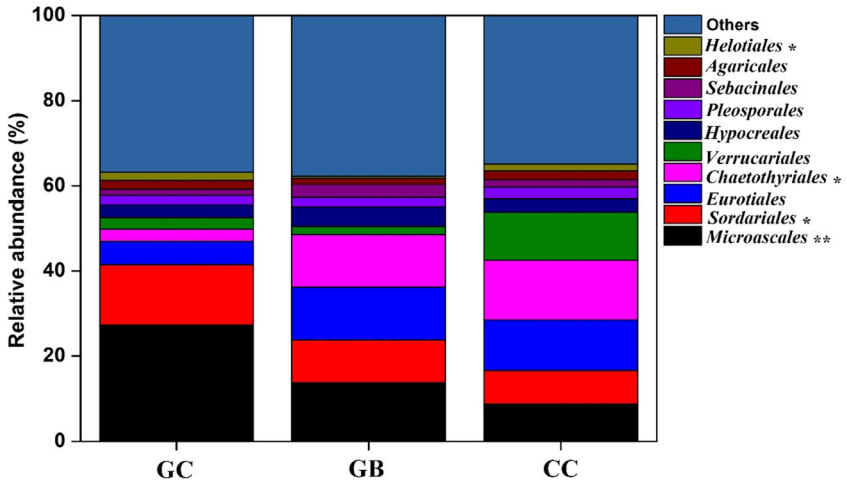

Fig. 2 The relative abundance of fungal taxa at order level. Only the taxa with average relative abundance of > 0.1% are shown. Significant differences were shown. Single asterisk indicates $p < 0.05$, and double asterisks indicate $p < 0.01$

Discussion

Differential responses of fungal taxa to specific gap locations

Fungi at different taxonomic levels differentially responded to distinct gap locations. At the phylum level, there were not significant differences among distinct gap locations, which was probably due to the overdominance of *Basidiomycota* and especially *Ascomycota* at all gap locations. Overdominances of *Ascomycota* and *Basidiomycota* in soil fungal communities had been observed in many studies (Buee et al. 2009; Mandarano et al. 2018), and they were two large phyla containing thousands of species with distinct environmental adaptions (Schoch et al. 2009; Watkinson et al. 2015). Thus, although fungal species differentially responded to diverse environments (Fig. 1), such distinct responses are rarely observed at phylum level.

At the order level, the relative abundances of only *Microascales*, *Sordariales*, and *Chaetothyriales* regularly varied as a function of gap location, indicating their sensitivity to environmental variations. *Microascales* and *Sordariales* were abundant at GC and mainly performed as saprotrophs. Thus, these two orders, especially *Microascales*, contributed greatly to the high relative abundances of saprotrophs at GC. These two orders were reported to favor high temperatures (Langarica-Fuentes et al. 2014) at which saprotrophy is probably enhanced. Thus, temperature was probably a crucial driver in determining the distribution of the two orders as a function of gap location. The taxa in *Chaetothyriales* mainly performed saprotrophic and a combination of pathotrophic and saprotrophic modes. In consideration of the low relative abundance of *Chaetothyriales* at GC, *Chaetothyriales* contributed less to both the trophic modes than the other two varying orders. Consequently, *Microascales*, *Sordariales*, and *Chaetothyriales* were indicator taxa differentiating different gap locations, and the two formers mainly caused the shift in saprotrophs in the fungal community.

Among the discriminatory OTUs, only 17 OTUs regularly varied as a function of gap location. Most of these OTUs were affiliated in the orders *Microascales*, *Sordariales*, and *Chaetothyriales*, which partly supported the finding that the three orders were indicators for gap locations. The relative abundances of *Scedosporium apiospermum* (OTU91 and OTU206) and *Scedosporium prolificans* (OTU74 and OTU4979) decreased from GC to CC. Although the two species were pathogenic (Cooley et al. 2007), they probably contributed less to pathotrophs whose relative abundances differed nonsignificantly as a function of gap location. *S. apiospermum* was recently regarded as an indicator for ecosystems exposed to anthropogenic influence (Al-Yasiri et al. 2017). Here, the artificial forest gap possibly resulted in the high abundance of *S. apiospermum*. Additionally, the 17 OTUs significantly correlated with ST, SM, SOC, SAN, and SMC, further indicating that the environmental gradient caused by forest gaps crucially affected the distributions of these OTUs. Interestingly, at both the order and OTU levels, these indicators significantly correlated with multiple soil properties such as ST, SM, SAN, SMC, and SOC, whereas in partial correlations controlling the other soil properties, almost all indicators did not significantly correlate with any of soil properties (Supplementary Table S8). Thus, it was deduced that the integrative effects caused by these soil properties constitute an environmental gradient as a function of gap location to drive fungal distribution. The results based on Mantel and partial Mantel tests (Table 2) further ensured the crucial roles of ST, SM, SAN, and SMC in shaping fungal communities. The co-occurrence patterns of fungal communities were also crucially influenced by gap location. Microbial co-occurrence usually infers tight relationships between microorganisms (Banerjee 2016). Thus, forest gaps crucially influenced not only fungal composition but also relationships between individuals.

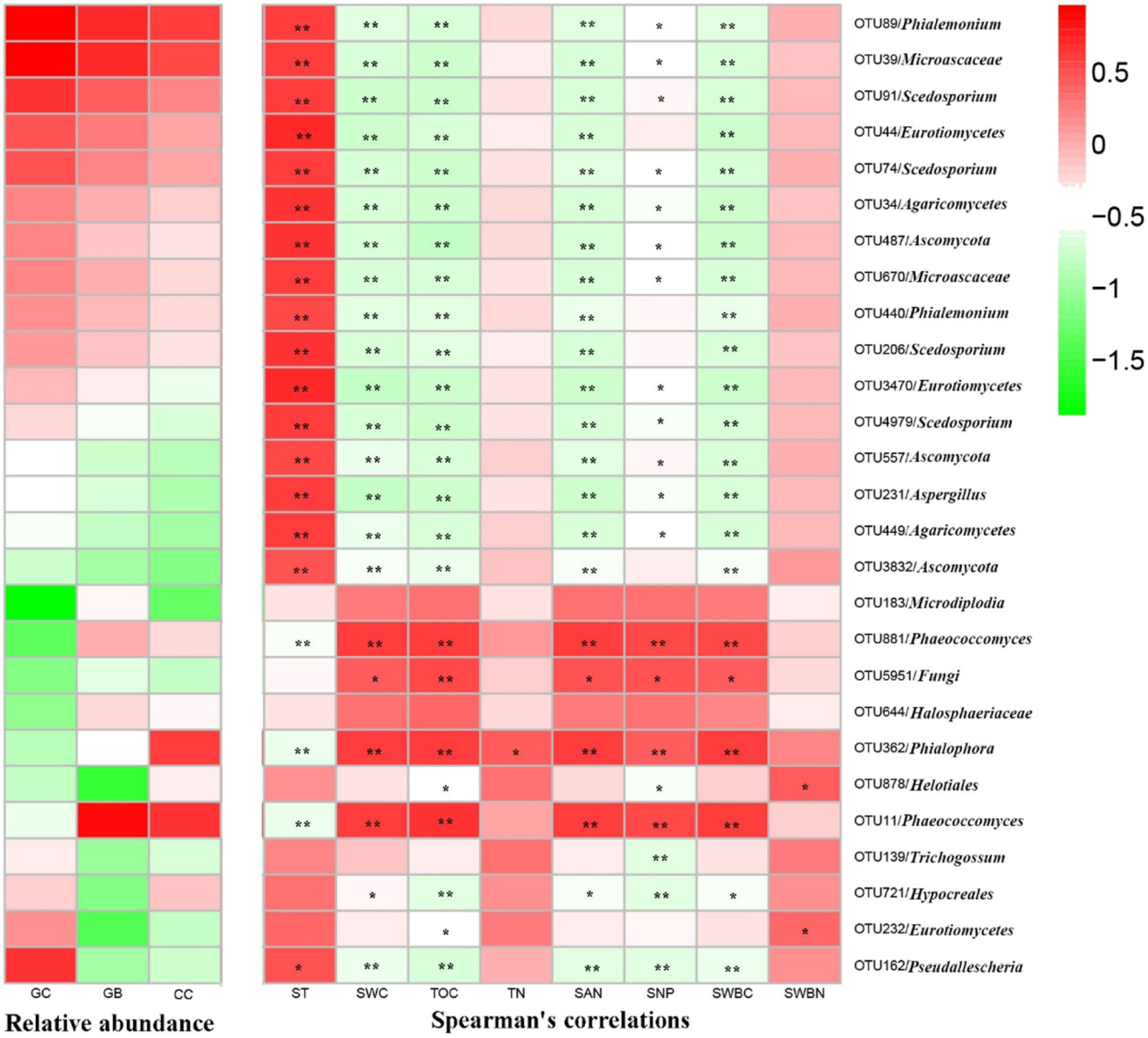

Fig. 3 The heat map depicts the relative abundances of OTUs selected by random forest and Spearman's correlations between OUT abundances and soil properties. The top 50 OTUs based on the values of mean decrease in the Gini index are selected and only the ones with average relative abundances of > 0.1% are shown. The cells are colored based on $\log_{10}$ (relative abundances) and correlation coefficients, respectively. Double asterisks indicate $p < 0.01$; single asterisk indicates $p < 0.05$. The lowest annotation level of OTU is shown aside the OTU number

Fungal community assembly driven by forest gaps

In this study, forest gaps resulted in significant environmental heterogeneity in soil, mainly by regulating ST, SM, SAN, and SMC. The high ST and low SM at GC were mainly attributed to solar irradiation, and low SAN and SMC were probably due to less litter input and root exudates at these sites than at other sites (Schliemann and Bockheim 2014; Xu et al. 2016). Interestingly, the four soil properties also mainly contributed to significant differences in the fungal community assembly at different gap locations. ST and SM were physicochemical properties that were crucial for influencing the microbial community (Baldrian 2017; Peltoniemi et al. 2015), and SAN and SMC indicate nutrient level (Singh et al. 1989). Thus, the four soil properties could be agents that influence fungal community assembly in soil at the forest gaps. However, how forest gaps influence fungal community assembly by regulating the four soil properties should be further determined.

The deterministic process affecting community assembly was mainly environmental filtering (Chase and Myers 2011). Many studies had described environmental filtering crucially affecting fungal community assembly (Clemmensen et al.

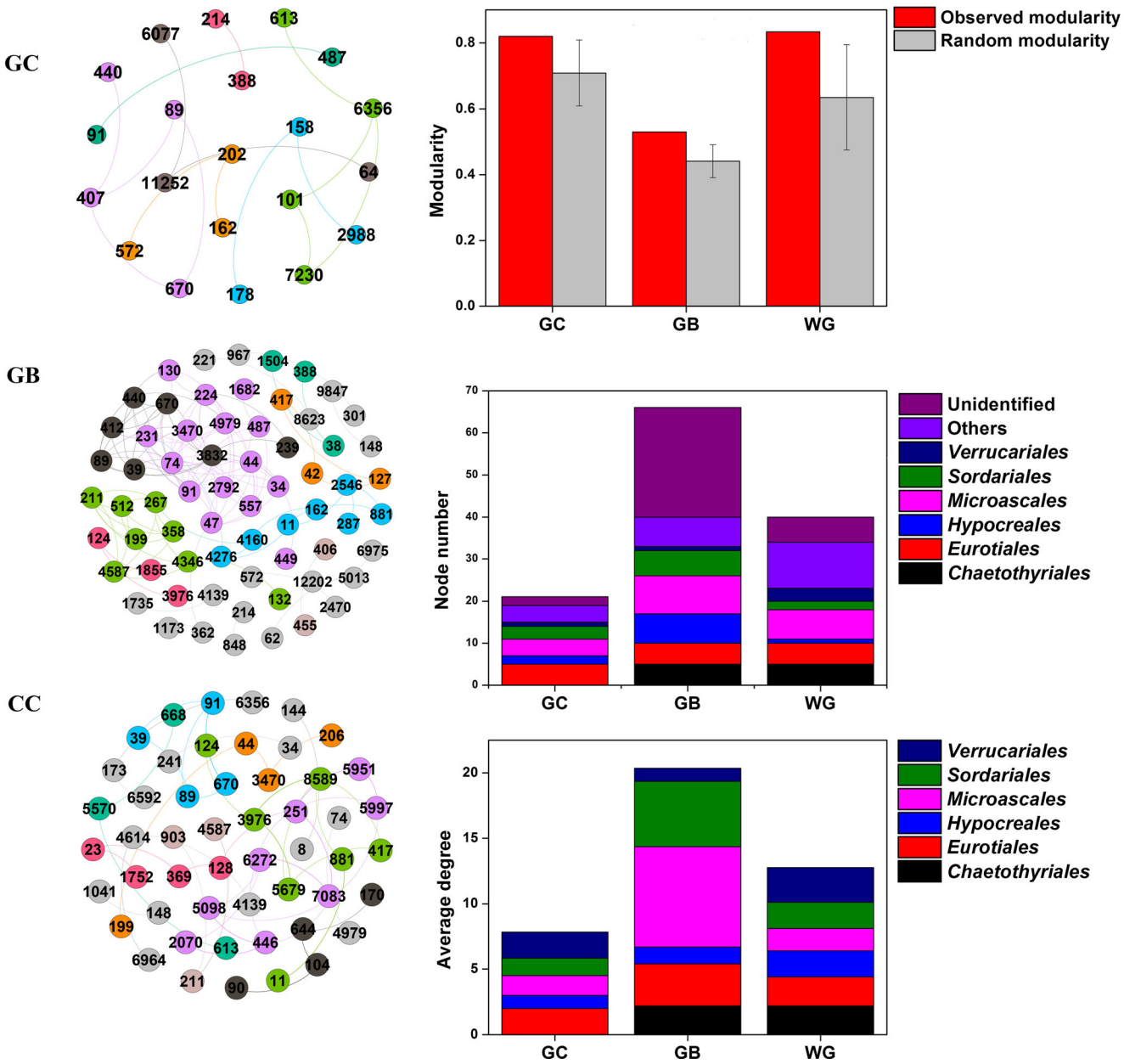

Fig. 4 Network analyses of fungal communities at different gap locations. The nodes in the networks are colored by module class

2015; Harantová et al. 2017; Kivlin et al. 2014; Urbanová et al. 2015). The shift in the SES was in accordance with the SM, SAN, and SMC values but opposite to the ST trend, further supporting the crucial roles of environmental filtering in a deterministic process in fungal community assembly. Fungi were strictly heterotrophic (Boer et al. 2005), and they heavily depended on the nutrient availability in soil. The low nutrient availability at GC created relatively harsh environments where only adaptable fungi could survive, which undoubtedly enhanced environmental filtering. The high ST and low SM at GC related to other sites might increase pressure on fungi (Baldrian 2017; Peltoniemi et al. 2015) and strengthen deterministic processes. The deterministic processes drove the assembly of communities with similar adaption to environmental pressures and promoted high similarity among fungal communities with similar assembly processes. This reason likely explained the decrease in the beta diversity of fungal communities from CC to GC. Additionally, at CC, the uneven distributions of trees, litter input, and root exudates probably resulted in enhanced heterogeneity in local environments, which increased the beta diversity of the fungal community. SAN and SMC positively correlated with the beta diversity, which further implied that high nutrient levels improved the dispersion of fungal communities. Surprisingly, although forest gaps crucially influenced the

beta diversity of the fungal community, alpha diversity did not show significant differences among different gap locations, even without significant correlations between alpha diversities and nearly all the soil properties. Some studies also showed that the alpha diversity of fungal communities was to some extent resistant to environmental heterogeneity (She et al. 2018; Zhang et al. 2018; Zhang et al. 2017). In this manner, alpha diversity prevented from great variations caused by forest gaps and even the loss of alpha diversity in fungal communities.

In summary, forest gaps significantly influenced the fungal community, mainly by regulating ST, SM, SAN, and SMC. The environmental gradient created by forest gaps significantly influenced the fungal community in terms of its beta diversity, deterministic processes, abundances of species, and co-occurrence patterns. However, it should be noted that the forest gaps were generated for only 5 years, so this study could shed light on the initial effects of forest management on fungal community assembly.

Authors' contributions DL and XwL designed the study. YS, HY, XL, MG, and YH performed the experiments. DL, YS, HY, XjL, and MG analyzed the data. XzL improved the manuscript, and DL wrote the paper.

Funding This study was funded by German Government Loans for Sichuan Forestry Sustainable Management (G1403083), a Pillar Project of the "12th" Five-Year Plan for China (2011BAC09B05), and a Project of the Sichuan Provincial Education Department (No. 18ZB305)

Compliance with ethical standards

Competing interests The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH, Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim JH, Allard G, Running SW, Semerci A, Cobb N (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manag 259(4):660–684. https://doi.org/10.1016/j.foreco.2009.09.001

Al-Yasiri MH, Normand A-C, Mauffrey J-F, Ranque S (2017) Anthropogenic impact on environmental filamentous fungi communities along the Mediterranean littoral. Mycoses 60(7):477–484. https://doi.org/10.1111/myc.12612

Baldrian P (2017) Forest microbiome: diversity, complexity and dynamics. FEMS Microbiol Rev 41(2):109–130. https://doi.org/10.1093/femsre/fuw040

Banerjee S, Kirkby CA, Schmutter D, Bissett A, Kirkegaada JA, Richardson AE (2016) Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. Soil Biol Biochem 97:188–198. https://doi.org/10.1016/j.soilbio.2016.03.017

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Stat Soc B 57(1):289–300

Boer WD, Folman LB, Summerbell RC, Boddy L (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol 29(4):795–811. https://doi.org/10.1016/j.femsre.2004.11.005

Buee M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F (2009) 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytol 184(2):449–456. https://doi.org/10.1111/j.1469-8137.2009.03003.x

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Met 7(5):335–336. https://doi.org/10.1038/nmeth.f.303

Chase JM, Myers JA (2011) Disentangling the importance of ecological niches from stochastic processes across scales. Philos T R Soc B 366(1576):2351–2363. https://doi.org/10.1098/rstb.2011.0063

Clemmensen KE, Finlay RD, Dahlberg A, Stenlid J, Wardle DA, Lindahl BD (2015) Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. New Phytol 205(4):1525–1536. https://doi.org/10.1111/nph.13208

Cooley L, Spelman D, Thursky K, Slavin M (2007) Infection with *Scedosporium apiospermum* and *S.prolificans* Australia. Emerg Infect Dis 13(8):1170–1177. https://doi.org/10.3201/eid1308.060576

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16):2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Erdos P, Rényi A (1960) On the evolution of random graphs. Publ Math Inst Hung Acad Sci 5(1):17–60

Gray AN, Spies TA, Easter MJ (2002) Microclimatic and soil moisture responses to gap formation in coastal Douglas-fir forests. Can J For Res 32(2):332–343. https://doi.org/10.1139/X01-200

Gray AN, Spies TA, Pabst RJ (2012) Canopy gaps affect long-term patterns of tree growth and mortality in mature and old-growth forests in the Pacific Northwest. For Ecol Manag 281:111–120. https://doi.org/10.1016/j.foreco.2012.06.035

Harantová L, Mudrák O, Kohout P, Elhottová D, Frouz J, Baldrian P (2017) Development of microbial community during primary succession in areas degraded by mining activities. Land Degrad Dev 28(8):2574–2584. https://doi.org/10.1002/ldr.2817

Hart S, Stark J, Davidson E, Firestone M, Weaver R, Angle S, Bottomley P, Bezdiecek D, Smith S, Tabatabai A (1994) Microbiological and biochemical properties. In: Hart S (ed) Methods of soil analysis, part 2. Soil Science Society of America, Madison, pp 985–1017

He W, Wu FZ, Yang WQ, Tan B, Zhao YY, Wu QQ, He M (2016a) Lignin degradation in foliar litter of two shrub species from the gap center to the closed canopy in an alpine fir forest. Ecosystems 19(1):115–128. https://doi.org/10.1007/s10021-015-9921-6

He W, Wu FZ, Yang WQ, Zhang DJ, Xu ZF, Tan B, Zhao YY, Justine MF (2016b) Gap locations influence the release of carbon, nitrogen and phosphorus in two shrub foliar litter in an alpine fir forest. Sci Rep UK 6:11. https://doi.org/10.1038/srep22014

Jackson ML (1958) Soil chemical analysis. Prentice-Hall, Upper Saddle River Inc,Englewood Cliffs

Kivlin SN, Winston GC, Goulden ML, Treseder KK (2014) Environmental filtering affects soil fungal community composition more than dispersal limitation at regional scales. Fungal Ecol 12:14–25. https://doi.org/10.1016/j.funeco.2014.04.004

Langarica-Fuentes A, Zafar U, Heyworth A, Brown T, Fox G, Robson GD (2014) Fungal succession in an in-vessel composting system characterized using 454 pyrosequencing. FEMS Microbiol Ecol 88(2):296–308. https://doi.org/10.1111/1574-6941.12293

Mallik AU, Kreutzweiser DP, Spalvieri CM (2014) Forest regeneration in gaps seven years after partial harvesting in riparian buffers of boreal mixedwood streams. For Ecol Manag 312:117–128. https://doi.org/10.1016/j.foreco.2013.10.015

Mandarano AH, Giloteaux L, Keller BA, Levine SM, Hanson MR (2018) Eukaryotes in the gut microbiota in myalgic encephalomyelitis/chronic fatigue syndrome. PeerJ 6:18. https://doi.org/10.7717/peerj.4282

Muscolo A, Sidari M, Mercurio R (2007) Influence of gap size on organic matter decomposition, microbial biomass and nutrient cycle in Calabrian pine (*Pinus laricio*, Poiret) stands. For Ecol Manag 242(2-3):412–418. https://doi.org/10.1016/j.foreco.2007.01.058

Muscolo A, Bagnato S, Sidari M, Mercurio R (2014) A review of the roles of forest canopy gaps. J For Res 25(4):725–736. https://doi.org/10.1007/s11676-014-0521-7

Newman MEJ (2006) Modularity and community structure in networks. P Natl Acad Sci USA 103(23):8577–8582. https://doi.org/10.1073/pnas.0601602103

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol 20:241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Peltoniemi K, Laiho R, Juottonen H, Kiikkila O, Makiranta P, Minkkinen K, Pennanen T, Penttila T, Sarjala T, Tuittila ES, Tuomivirta T, Fritze H (2015) Microbial ecology in a future climate: effects of temperature and moisture on microbial communities of two boreal fens. FEMS Microbiol Ecol 91(7). https://doi.org/10.1093/femsec/fiv062

Ritter E, Dalsgaard L, Einhorn KS (2005) Light, temperature and soil moisture regimes following gap formation in a semi-natural beech-dominated forest in Denmark. For Ecol Manag 206(1-3):15–33. https://doi.org/10.1016/j.foreco.2004.08.011

Sariyildiz T (2008) Effects of gap-size classes on long-term litter decomposition rates of beech, oak and chestnut species at high elevations in Northeast Turkey. Ecosystems 11(6):841–853. https://doi.org/10.1007/s10021-008-9164-x

Schliemann SA, Bockheim JG (2014) Influence of gap size on carbon and nitrogen biogeochemical cycling in Northern hardwood forests of the Upper Peninsula, Michigan. Plant Soil 377(1-2):323–335. https://doi.org/10.1007/s11104-013-2005-5

Schoch CL, Sung GH, Lopez-Giraldez F, Townsend JP, Miadlikowska J, Hofstetter V, Robbertse B, Matheny PB, Kauff F, Wang Z, Gueidan C, Andrie RM, Trippe K, Ciufetti LM, Wynns A, Fraker E, Hodkinson BP, Bonito G, Groenewald JZ, Arzanlou M, de Hoog GS, Crous PW, Hewitt D, Pfister DH, Peterson K, Gryzenhout M, Wingfield MJ, Aptroot A, Suh SO, Blackwell M, Hillis DM, Griffith GW, Castlebury LA, Rossman AY, Lumbsch HT, Lucking R, Budel B, Rauhut A, Diederich P, Ertz D, Geiser DM, Hosaka K, Inderbitzin P, Kohlmeyer J, Volkmann-Kohlmeyer B, Mostert L, O'Donnell K, Sipman H, Rogers JD, Shoemaker RA, Sugiyama J, Summerbell RC, Untereiner W, Johnston PR, Stenroos S, Zuccaro A, Dyer PS, Crittenden PD, Cole MS, Hansen K, Trappe JM, Yahr R, Lutzoni F, Spatafora JW (2009) The *Ascomycota* tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst Biol 58(2):224-239 https://doi.org/10.1093/sysbio/syp020

She W, Bai Y, Zhang Y, Qin S, Feng W, Sun Y, Zheng J, Wu B (2018) Resource availability drives responses of soil microbial communities to short-term precipitation and nitrogen addition in a desert shrubland. Front Microbiol 9:186. https://doi.org/10.3389/fmicb.2018.00186

Singh J, Raghubanshi A, Singh R, Srivastava S (1989) Microbial biomass acts as a source of plant nutrients in dry tropical forest and savanna. Nature 338(6215):499–500. https://doi.org/10.1038/338499a0

Stanford G, Carter JN, Simpson EC, Schwaninger DE (1973) Nitrate determination by a modified conway microdiffusion method. J Assoc Offic Ana Chem 56(6):1365–1368

Urbanová M, Šnajdr J, Baldrian P (2015) Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. Soil Biol Biochem 84:53–64. https://doi.org/10.1016/j.soilbio.2015.02.011

Watkinson SC, Boddy L, Money N (2015) The fungi. In: Academic Press,Cambriadge,Massachusetts,UK

Xu JX, Xue L, Su ZY (2016) Impacts of forest gaps on soil properties after a severe ice storm in a *Cunninghamia lanceolata* stand. Pedosphere 26(3):408–416. https://doi.org/10.1016/s1002-0160(15)60053-4

Yang YG, Geng YQ, Zhou HJ, Zhao GL, Wang L (2017) Effects of gaps in the forest canopy on soil microbial communities and enzyme activity in a Chinese pine forest. Pedobiologia 61:51–60. https://doi.org/10.1016/j.pedobi.2017.03.001

Yulin Y, Xianwei L, Yigui Z, Yunke L (2014) Effects of gap thinning on growth and diversity of a cypress plantation in the hilly region of central Sichuan. Chin J Appl Environ Biol 19(19):922–928 (in Chinese with English abstract). https://doi.org/10.3724/SP.J.1145.2014.05004

Zhang Q, Zak JC (1995) Effects of gap size on litter decomposition and microbial activity in a subtropical forest. Ecology 76(7):2196–2204

Zhang ZQ, Zhou X, Tian L, Ma LN, Luo SS, Zhang JF, Li XJ, Tian CJ (2017) Fungal communities in ancient peatlands developed from different periods in the Sanjiang Plain. China PLoS One 12(12):16. https://doi.org/10.1371/journal.pone.0187575

Zhang HF, Wang LL, Liu HM, Zhao JN, Li G, Wang H, Lai X, Li J, Xiu WM, Yang DL (2018) Nitrogen deposition combined with elevated precipitation is conducive to maintaining the stability of the soil fungal diversity on the *Stipa baicalensis* steppe. Soil Biol Biochem 117:135–138. https://doi.org/10.1016/j.soilbio.2017.11.004

Zhou JZ, Deng Y, Zhang P, Xue K, Liang YT, Van Nostrand JD, Yang YF, He ZL, Wu LY, Stahl DA, Hazen TC, Tiedje JM, Arkin AP (2014) Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. P Natl Acad Sci USA 111(9):E836–E845. https://doi.org/10.1073/pnas.1324044111

Zhu J-j, Matsuzaki T, Lee F-q, Gonda Y (2003) Effect of gap size created by thinning on seedling emergency, survival and establishment in a coastal pine forest. For Ecol Manag 182(1-3):339–354. https://doi.org/10.1016/S0378-1127(3)00094-X

Žifčáková L, Větrovský T, Howe A, Baldrian P (2016) Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Environ Microbiol 18(1):288–301. https://doi.org/10.1111/1462-2920.13026