Concurrent and rapid recovery of bacteria and protist communities in Canadian boreal forest ecosystems following wildfire

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ABSTRACT

Wildfire plays an important role in restructuring bacterial and protist taxonomic and functional communities in forest ecosystems. Yet, the recovery of bacterial and protist communities following wildfire has not been rigorously investigated across long-term wildfire chronosequences. We compared changes in bacterial and protist alpha diversity, community composition, co-occurrence networks and determinant edaphic factors between burnt and unburnt (reference) soils at 11 different sites representing a 76-year wildfire chronosequence in Canadian boreal forests. The fire increased bacterial and protist alpha diversity and altered community composition (beta diversity) as compared to the site-specific controls during the early recovery stage (<5 years since fire), but had no effect on bacterial and protist communities in the middle (8–20 years) and late (>30 years) recovery stages. Bacterial and protist communities showed similar patterns of change across the wildfire chronosequence. The post-fire recovery of bacterial communities was associated with changes in soil chemical properties (*e.g.,* nutrient status and pH) and vegetation succession. The protist community assembly was co-influenced by changes in bacterial communities and vegetation succession/soil properties. The concurrent recovery of bacterial and protist communities, and their influencing factors of vegetation and soil properties, imply the above- and belowground living communities are strongly linked in wildfire-perturbed forest ecosystems.

1. Introduction

Fire is an important ecosystem disturbance factor in boreal forests (5–20 million ha/yr is burnt) with a historical return frequency of approximately 20–200 years (Larsen and MacDonald, 1998; Stocks et al., 2002). The boreal forest biome occupies ~10% of the Earth's land surface and contains >30% of terrestrial carbon stocks (Apps et al., 1993). Forests across the boreal region (*e.g.,* Canada, Alaska, Russia, Scandinavia) are experiencing record high numbers of wildfires in recent years, with a pronounced increase in high severity fires (Kelly et al., 2013; Calef et al., 2015). Similar wildfire trends are occurring in

the boreal forest region in Canada, where half of the land area is occupied by forests (>400 million ha) that experience wildfire disturbances of ~2.5 million ha/yr. The regions where boreal forests grow are under escalating threat from wildfire disturbance as they have warmed at twice the rate of temperate forest ecosystems over the past 50 years (Markon et al., 2012).

Wildfire plays an important role in the perturbation of microbial communities in forest ecosystems, which affect several ecosystem functions such as carbon sequestration, net ecosystem productivity, nutrient cycling and climate regulation (Bond-Lamberty et al., 2007; Butler et al., 2018; Pellegrini et al., 2018). Microbial communities are

widely reported to be sensitive to wildfires, including decreased microbial biomass, decreased alpha diversity, altered community composition and propagation of responsive taxa such as *Massilia* sp. and *Penicillium* sp. in boreal forest soils (Allison et al., 2010; Xiang et al., 2015; Whitman et al., 2019). Changes in microbial communities were attributed to direct burning effects and/or fire-altered soil biogeochemical properties in both forest (Xiang et al., 2015; Sun et al., 2016) and grassland (Yang et al., 2020) ecosystems. Additionally, following high severity fires (stand-replacing fires), vegetation succession is expected to have a pronounced effect on the microbial community as vegetation is the primary source of organic matter inputs to support the heterotrophic microbial community (Hart et al., 2005; Kuzyakov et al., 2019). Vegetation alters the soil microclimate, physico-chemical soil properties (*e.g.*, rhizosphere), and host-specific relationships between specific plant species and microbes (Lareen et al., 2016; Chen et al., 2019).

Soil bacterial communities are sensitive to wildfire effects that can persist for many years after burning (Yang et al., 2020); however, the short-term recovery of bacterial communities relative to pre-fire conditions has received the most investigation (Griffiths and Philippot, 2013; Xiang et al., 2015). In general, high temperatures at the soil surface cause bacterial biomass reduction and decreased diversity, inducing high mortality and dormancy in the very short term (Wang et al., 2012). However, the short growth cycle, resistant cell structures (*e.g.,* spores, cysts) and wide niche breadth of bacteria generate a strong propensity to quickly re-colonize and adapt to altered soil environmental conditions (Dworkin, 2006; Allison and Martiny, 2008; Pérez-Valera et al., 2018). Additionally, wildfire causes considerable changes to soil conditions (*e. g.,* direct burn damage and vegetation loss), including overall soil C and N losses, increased ammonium availability, elevated soil pH and higher moisture and temperature (Choromanska and DeLuca, 2001; Hamman et al., 2008; Zhang et al., 2021). Given the close association among bacterial communities, soil chemical properties and vegetation succession, the recovery progress of bacterial communities is dependent on changes in both vegetation and edaphic factors during the post-fire recovery period (Krashevska et al., 2014; Dassen et al., 2017; Pressler et al., 2019).

Protists, a major component of the soil microbiome, consist of phagotroph, symbiotic, saprotroph and phototroph taxa, and play an important role in several forest soil functions. The unicellular phagotrophs feed on bacteria and fungi, thereby influencing soil biodiversity and microbially-regulated nutrient cycling (Geisen et al., 2018). The saprotrophs contribute to soil organic C degradation and the phototrophs function in soil C fixation (Seppey et al., 2017). Furthermore, symbiotic interactions of protists with plant roots stimulate plant performance (Rosenberg et al., 2009). Previous studies show that protists often exhibit a narrower habitat niche breadth than bacteria (Wu et al., 2018), making the protist community more sensitive to environmental disturbances than bacteria (Xiong et al., 2018; Zhao et al., 2019). While soil pH plays an important function in shaping bacterial communities (Xiong et al., 2018), the protist community composition is affected by both soil properties (*e.g.,* soil moisture, clay content and nutrient status) and associated bacterial communities (Zhao et al., 2019; Oliverio et al., 2020). To date, only a few studies have investigated the recovery of soil protist abundance and richness following wildfires, and the measurements applied, such as counting and fatty acid methyl esters, provided limited information on their response and recovery (Pressler et al., 2019). With the rapid development of high-throughput sequencing technologies, a deeper assessment of the protist community at the OTU level helps to 1) explain the short and long-term response during the post-fire recovery period, and 2) identify biomarkers for fire history in forest ecosystems. This approach also enables a rigorous comparison of concomitant bacteria and protist recovery patterns, and an investigation of interactions between bacterial and protist communities.

Herein, we collected soil samples from burnt and adjacent unburnt (*i. e.,* reference condition) soils along a wildfire chronosequence (0 → 76

years) (*i.e.,* recovery time from the last burn to sampling date) in a boreal forest of northwestern Canada. The recovery response of bacteria and protist to wildfire disturbance was interpreted in the context of three soil recovery/vegetation succession stages: early (<5 years), middle (8–20 years) and late (>30 years). This study aimed to: 1) compare alpha diversity, community composition and interactions among bacteria and protists in burnt and unburnt soils; 2) identify fire responsive taxonomic groups; and 3) explore the patterns and underlying mechanisms of recovery for bacterial and protist communities across the wildfire chronosequence. We hypothesized that wildfire-induced changes in bacterial diversity at the early recovery stage would disappear at middle and late stages due to their rapid recovery ability. Further, the recovery pattern (*i.e.,* diversity) of protist communities is posited to be linked with changes in bacterial communities.

2. Materials and methods

2.1. Site information

Soil samples were collected from 11 sites along a 76-year wildfire chronosequence in the Canadian boreal forest of Alberta (Fig. 1). The last fire event at each of the 11 sampling sites (1940, 1950, 1968, 1982, 1998, 2002, 2008, 2011, 2013, 2015 and 2016) created a wildfire chronosequence consisting of soil microbial community recovery times of 76, 66, 48, 34, 18, 14, 8, 5, 3, 1 years and 3 months after the wildfire event. Past fire maps compiled/verified by Alberta Agriculture and Forestry were used to delineate the burnt and unburnt sites (http://wildfire.alberta.ca/default.aspx), including the dates and fire perimeters. An Alberta government official who specialized in fire investigations assisted us with site access and sample collection, and to confirm no overlap among wildfires across sites. To avoid fire heterogeneity, we deliberately selected fire sites that were documented in the historical records compiled by Alberta Agriculture and Forestry as high severity, stand-replacing fires. The high severity burned conditions were evident from the even-aged stands (*i.e.,* not a mixture of young and old trees) corresponding to a given succession stage in boreal forest recovery.
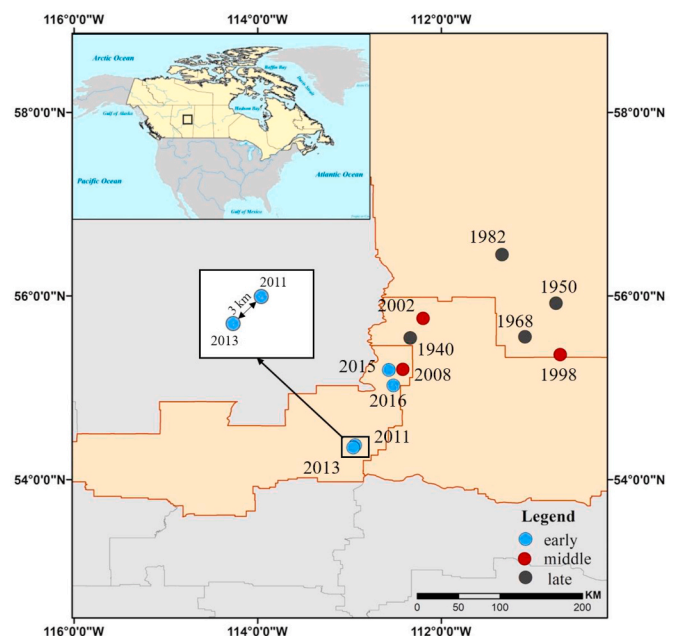

Fig. 1. Wildfire chronosequence (76-years) sampling sites in the boreal forest of Alberta, Canada. Values indicate the year of the last fire at each site; only one fire has occurred at each site within the past 76 years. Soil samples from burnt and unburnt (reference) areas at each site were located 100–500 m apart. The site 2011 was approximately 3 km away from the site 2013.

The sites had similar geographic properties and climatic conditions, and the same soil type. The mean annual temperatures across sites range from −0.2 to 2 °C and the mean annual precipitations range from 465 to 503 mm. Soils across the study area were classified as Alfisols based on USDA Soil Taxonomy and the parent materials were glacial till from mixed sedimentary deposits. All the sites were natural forests with no pre- or post-fire management in either the burned area or the adjacent unburned area. The climax forest across the wildfire chronosequence was dominated by white spruce (*Pinus glauca*), jack pine (*Pinus banksiana*) and trembling aspen (*Populus tremuloides*), with an extensive shrub understory dominated by ericaceous species. These boreal forest species are well adapted to fire and begin to colonize the burned area within the first few years following wildfire (Greene and Johnson, 1999).

2.2. Sample collection and characterization

In detail, both burnt (3–5 replicates) and adjacent unburnt (3–5 replicates) soil samples were collected at each site. If the wildfire area was large, we collected more than 3 replicates to better characterize the burned area. The unburnt plots were selected based on both historical records (wildfire history database for Alberta) and our field observations (*e.g.*, checking for the non-existence of fresh charcoal at the soil surface) to assure there was no recent fire disturbance. For both burnt and unburnt soils, plots were located near the center point of the fire area (not the fire border area) to assure that all samples were well within the fire perimeter. At each plot (a minimum of 100 m apart), 5–6 soil cores from the 20 cm mineral layer with litter layer removed were taken with an auger (inner diameter of 5 cm) and mixed into one composite sample (*i.e.*, one replicate). This sampling strategy aimed to use a large plot size to assure a representative set of samples, in spite of potential variability resulting from localized fire affects or natural pedogenic processes. Although the direct impact of fire (*i.e.*, soil heating) is usually apparent only in the first few centimeters of soil, the loss of vegetation and death of roots resulting from severe wildfires affects the entire rooting zone for several years following wildfire. Thus, we collected soil samples from the 0–20 cm layer (the primary rooting zone). This soil layer incorporates both the direct fire effects (first few centimeters), as well as the effects arising from post-fire vegetation recovery (*e.g.*, root death followed by recovery during vegetation succession).

We grouped the 11 sites into three recovery stages based on the post-fire vegetation succession pattern: early (3 months, 1, 3 and 5 years; hereafter called <5 years), middle (8, 14 and 18 years; hereafter called 8–20 years) and late (34, 48, 66 and 76 years; hereafter called >30 years). Recovery stage groupings were based on: 1) vegetation composition and cover and 2) the normalized difference vegetation index (NDVI) (Jiang et al., 2016) at the time of sampling. Madoui et al. (2015) characterize post-fire vegetation succession in the boreal forest as

typically consisting of rapid early-stage development with low height shrubs during the first ~10 years after disturbance; dominance by mixed shrub, coniferous and deciduous components in the 10–30 year recovery period; and coniferous species dominance beginning around 30 years post-disturbance (Madoui et al., 2015). This vegetation succession was fully consistent with the general vegetation characteristics recorded at the time of soil sampling. NDVI values determined from the Moderate Resolution Imaging Spectroradiometer (MODIS) for our recovery stage groupings showed a 0.09 decrease for early-stage sites, a 0.01 increase for middle-stage sites, and a 0.03 increase for late-stage sites relative to their respective non-burned plots (Table 1). Overall, these three recovery stages represent the preliminary recovery, near recovery and complete recovery of vegetation at fire-event sites, respectively.

Soil samples were transported on ice to the laboratory for subsequent chemical characterization and microbial community analyses. In the lab, samples were sieved to isolate the <2-mm fraction with visual removal of any remaining roots and woody detritus. Soil dissolved organic C (DOC) and dissolved organic nitrogen (DON) were extracted by deionized water (1:10 soil:water) and analyzed with a TOC/TN Analyzer (multi N/C 3100, Analytik Jena AG, Jena, Germany). Soil pH and electrical conductivity (EC) were measured in a water suspension of 1:2.5 soil:water (distilled-deionized water) ratio following a 30 min equilibration period using pH and electrical conductivity electrodes. Soil NH_4^+ and NO_3^- were extracted by 1.0 M KCl using 1:10 soil:solution, and concentrations were quantified by a continuous flow analyzer (Skalar SAN++ System, Netherlands).

2.3. Soil DNA extraction and sequencing

Soil DNA was extracted from field moist samples using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA) following manufacturer protocols. DNA quality was assessed by agarose gel electrophoresis and Qubit® 2.0 Fluorometer. Prepared DNA samples were sent to Novogene (Tianjin, China) for library preparation and amplicon 16S and 18S sequencing. The V4–V5 region of the 16s rRNA gene was targeted for bacterial community analyses using the 515F 5′-GTGCCAGCMGCCGCGGTAA -3′ and 907R 5′-CCGTCAATTCCTTT-GAGTTT-3′amplicon primers (Ren et al., 2015). The V4 region of 18s rRNA gene was targeted for the protist community analyses using the 528F: 5′-GCGGTAATTCCAGCTCCAA -3' and 706R: 5'-AATCCRA-GAATTTCACCTCT -3' amplicon primers (Cheung et al., 2010). Purified amplicons were pooled for paired-end sequencing on an Illumina HiSeq 2500.

Quantitative Insights into Microbial Ecology (QIIME) standard operation procedure was performed for raw sequence processing (Caporaso et al., 2010). After removal of adaptors and barcodes from sequences, raw sequencing reads were merged to paired-end reads using join_paired_ends.py. The alignments were then filtered, trimmed and

Table 1
Comparison of chemical soil properties and normalized difference vegetation index (NDVI) between burnt and unburnt sites at the three recovery stages. Standard deviations are shown in the brackets. The "*" represents a significant (p < 0.05) difference in soil properties between burnt and unburnt soils at each recovery stage as assessed by two-way analysis of variance (ANOVA).

Recovery stage	Fire treatment	NDVI	pH	EC (uS cm^{-1})	NH_4^+	NO_3^-	DON	DOC (g kg^{-1})
(mg kg^{-1})								
Early stage	Burnt	0.40*	5.38*	44.0	105	2.51	61.4	4.48*
		(0.05)	(0.84)	(26.5)	(91.1)	(2.94)	(19.6)	(1.45)
	Unburnt	0.49*	5.03*	42.4	97.8	3.33	66.1	6.03*
		(0.02)	(0.87)	(30.8)	(85.6)	(7.72)	(26.6)	(2.21)
Middle stage	Burnt	0.48	4.90*	36.5*	92.5	0.80	86.8	7.44
		(0.01)	(0.49)	(14.3)	(90.8)	(0.65)	(60.3)	(8.35)
	Unburnt	0.47	5.54*	70.6*	127	0.99	102	6.71
		(0.02)	(1.00)	(49.9)	(84.7)	(0.61)	(73.1)	(4.20)
Late stage	Burnt	0.46	5.16	39.5	104	0.93	79.8	9.27
		(0.05)	(0.65)	(23.4)	(63.5)	(1.12)	(38.6)	(6.16)
	Unburnt	0.43	5.08	48.5	123	0.88	80.2	8.04
		(0.02)	(0.73)	(28.3)	(82.5)	(0.93)	(25.5)	(3.16)

optimized as follows: 1) minimal overlapping length of 10 bp; and 2) mismatching ratio of an overlapped region of <0.2. UPARSE was used to remove the chimera from sequences (Edgar et al., 2013). Operational taxonomic units (OTUs) were clustered at >97% similarity. Representative sequences for each OTU were selected for taxonomic assignment (Edgar et al., 2010). Bacterial OTUs were assigned using the Ribosomal Database Project (RDP) database (Cole et al., 2014), and protist OTUs were taxonomically assigned by blasting against the Protist Ribosomal Reference (PR2) database (version_4.12) (Guillou et al., 2013) at 90% minimum similarity. All resulting sequences were rarefied at a minimum number of sequences per sample (48100 and 61880 for 16S and 18S, respectively) for downstream analysis. Eukaryote taxa that were not protist (*e.g.*, *Fungi*, *Rhodophyta*, *Streptophyta*, etc.) were removed from the protist OTU table. Raw data of 16S and 18S sequencing were deposited in the GenBank Sequence Read Archive with the identification numbers: PRJNA667812 and PRJNA667813.

2.4. Statistical analyses

Analysis of covariance (ANCOVA) using the unburnt plots as the covariate was conducted to determine differences in bacterial and protist alpha diversity between early, middle and late recovery stages. Two-way ANOVA tests evaluated the overall effects of fire, recovery stage and fire*recovery stage interactions on bacterial and protist alpha diversity prior to conducting analyses for each recovery stage. If the fire*recovery stage interactions on both bacterial and protist alpha diversity were found to be significant, we conducted an additional two-way analysis of variance (ANOVA) to evaluate the fire effects on bacterial and protist alpha diversity at each recovery stage. In addition, two-way ANOVA was conducted to evaluate the fire effects on soil physico-chemical properties. Analysis of similarities (ANOSIM) was conducted to explore differences in bacteria and protist community composition (*i.e.*, beta diversity) at each recovery stage. Visual results were presented in nonmetric multidimensional scaling plots (NMDS) based on the Bray–Curtis dissimilarity matrix, using "vegan" R package (Clarke, 1993). Pearson correlation analysis investigated relationships between bacterial community dissimilarity (*i.e.*, beta diversity) and protist community dissimilarity. Pearson correlations were also performed to investigate relationships between bacterial/protist community dissimilarity and soil chemical properties in burnt and unburnt soils.

Bacteria and protists with significant increases or decreases in relative abundances after wildfire were defined as positively or negatively responsive OTUs. The responsive OTUs were identified by linear discriminant analysis effect size (LEfSe) (http://huttenhower.sph. harvard.edu/lefse/), based on a relative abundance matrix from the OTU table, with an LDA threshold score of 2.0 and significance α of 0.05. Bacterial and protist co-occurrence networks for burnt and unburnt soils across the wildfire chronosequence were structured based on a Spearman's correlation matrix using OTU tables. Indirect correlation dependencies were distinguished using the network enhancement method (Wang et al., 2018). The threshold value was identified by random matrix theory, and the correlation significance was adjusted using the false discovery rate method (Barberán et al., 2012). We visualized bacterial-protist co-occurrence networks using Gephi and calculated topological parameters (*e.g.*, node number, edge number, average degree and average path length) using the igraph package (Csardi and Nepusz, 2006). All "differences" referred to in presentation of the results denote a statistically significant difference at P < 0.05, unless otherwise stated.

3. Results

3.1. Bacterial and protist diversity and community composition

The analysis of covariance (ANCOVA) using the unburnt plot as a covariate showed that the recovery stage had significant effects on

bacterial and protist Shannon index (Fig. S1). The bacterial and protist Shannon index at the early stage was significantly larger than those at middle and late stages (Fig. S1). These findings indicate that any changes in bacterial and protist diversity were attributed to fire effects rather than spatial variation among sites. The two-way ANOVA evaluated the overall effects of fire, recovery stage (*i.e.*, early, middle and late), and fire*recovery stage interactions on bacterial and protist diversity across the wildfire chronosequence (Table S1). The fire*recovery stage effects for both bacterial and protist diversity were significant across the three recovery stages, indicating that the fire effects changed at different recovery stages (Table S1). Then, for each recovery stage, a two-way ANOVA focusing on fire effects showed that fire significantly increased the bacterial Shannon index by 0.25 during the early stage, while having no significant effects at the middle and late stages (Fig. 2a). Similarly, fire significantly increased the protist Shannon index by 0.31 in the early stage, while having no significant effects at the middle and late stages (Fig. 2b). In particular, during the early stage, fire
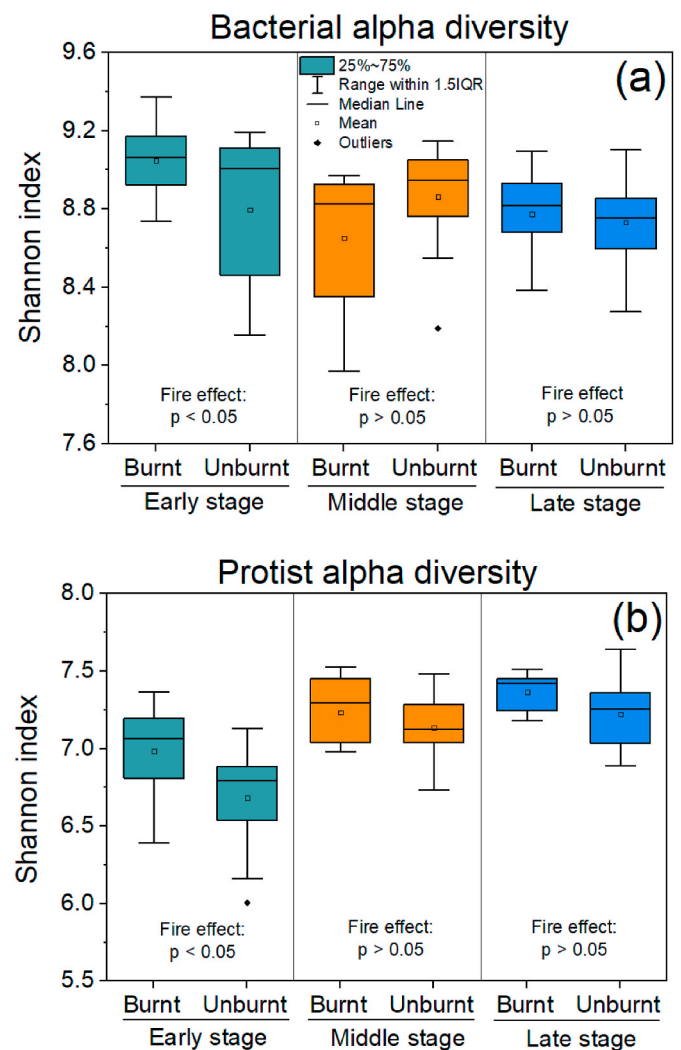
Fig. 2. Comparison of alpha diversity for soil bacteria and protist between burnt and unburnt forest soils at the early (3 months, 1, 3 and 5 years), middle (8, 14 and 18 years) and late (34, 48, 66 and 76 years) recovery stages. Two way ANOVA assessed the effects of fire on bacterial and protist alpha diversity for the three recovery stages. The p < 0.05 and p > 0.05 represent significant and non-significant differences in Shannon index between burnt and unburnt soils at each recovery stage. The comparison of bacterial and protist alpha diversity between early, middle and late recovery stages is shown in Fig. S1, which indicates the increases in bacterial and protist alpha diversity post fire at early stage was significantly larger than those at middle and late stages.

significantly increased the bacterial Shannon index at 3 months, 1 year and 5 years, while having no effects on the Shannon index at 3 years. The increase in Shannon index at 3 months was larger than those at other sites (Fig. S2). A similar pattern was observed for the protist Shannon index (Fig. S2).

The bacterial community composition (*i.e.,* beta diversity) in burnt soils was significantly ($p < 0.05$) different from that in unburnt soils during the early recovery stage (Fig. 3a), whereas fire did not alter ($p > 0.05$) the bacterial beta diversity during the middle and late stages (Fig. 3b and c). Similarly, the protist community composition (*i.e.,* beta diversity) in burnt soils was significantly ($p < 0.05$) different from that in unburnt soils at the early recovery stage (Fig. 3d), while fire did not alter ($p > 0.05$) the beta diversity at the middle and late stages (Fig. 3e and f). The bacterial community dissimilarity (*i.e.,* degree of community difference between burnt and unburnt treatments) had a significant positive correlation with the protist community dissimilarity (Fig. 4).

3.2. Bacterial and protist co-occurrence network

At the early recovery stage, fire considerably decreased the network complexity of the bacterial-protist co-occurrence network (Fig. 5). In response to fire, the node number, edge number, average degree and average path length of the network decreased by 122, 97, 0.40 and 0.33, respectively (Table 2). Notably, the edge linked between bacteria-protist decreased by 14, and the edge linked between bacteria-bacteria decreased by 84; however, the edge linked between protist-protist did not change (Fig. 5). For the middle recovery stage, fire did not change the overall network complexity of the bacterial-protist co-occurrence network (Fig. 5). Fire did not change the node number or edge number, but slightly increased the average degree and average path length by 0.20 and 0.17, respectively. By contrast, the edge linked between bacteria-protist and between bacteria-bacteria was not changed by fire. At the late recovery stage, fire slightly increased the overall network complexity of the bacterial-protist co-occurrence network (Fig. 5), as shown by an increased node number, edge number, average degree and
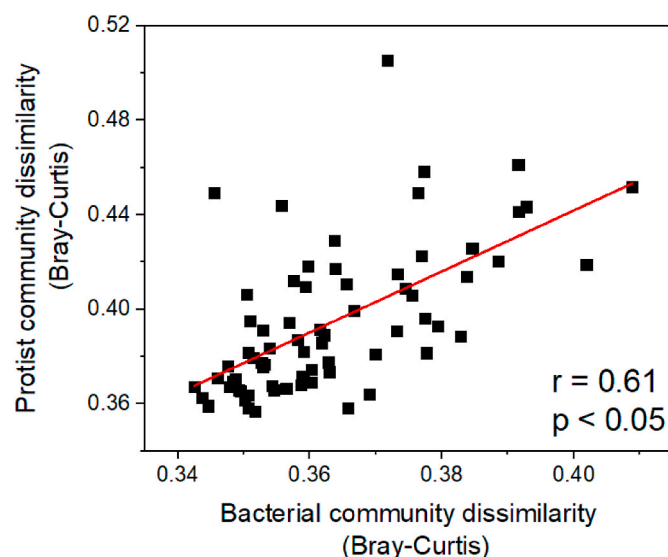

Fig. 4. Pearson correlations showing positive relationships between bacterial and protist Bray–Curtis community dissimilarities over recovery time. The community dissimilarity represents community differences between burnt and unburnt soils. Higher community dissimilarity indicates larger differences in bacterial or protist communities between burnt and unburnt soils.

clustering coefficient by 18, 27, 0.25 and 0.96, respectively. The edge linked between bacteria-bacteria showed a considerable increase of 36, whereas the edge linked between protist-bacteria decreased by 7 after fire (Table 2).

3.3. Soil factors and bacterial/protist communities

At the early recovery stage, fire increased soil pH by 0.35 units and decreased soil DOC by 1.5 g kg^{-1}, while having no effects on other soil
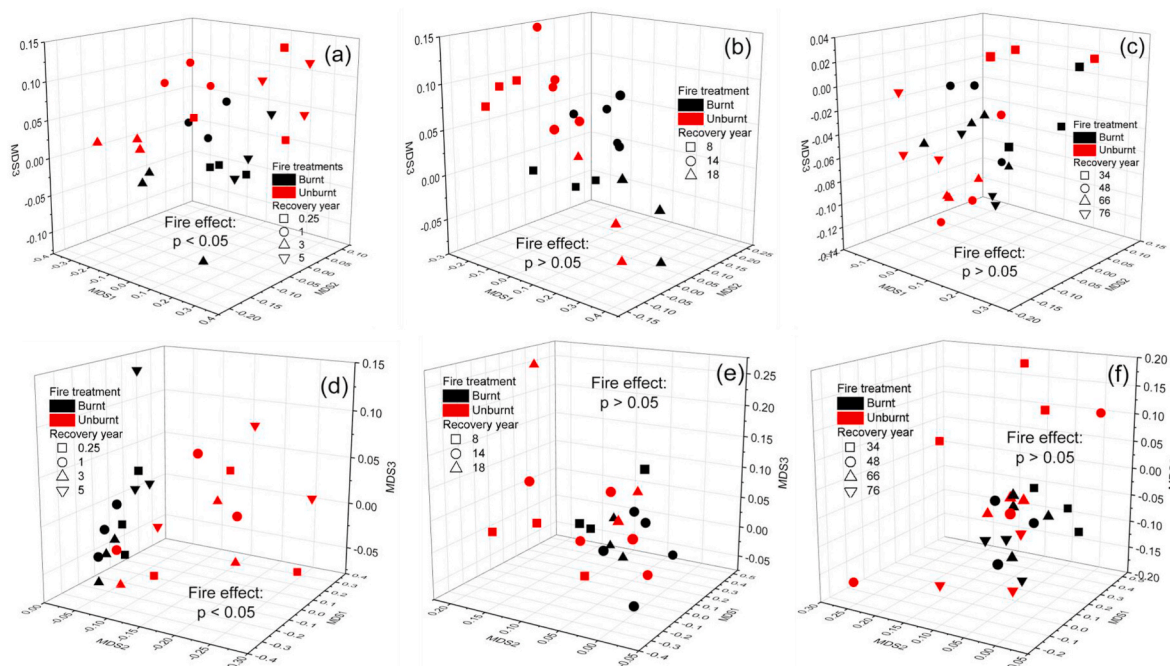

Fig. 3. Nonmetric multidimensional scaling plots (NMDS) based on Bray-Curtis distances of bacterial communities in burnt and unburnt soils at the early (a), middle (b) and late (c) recovery stages and protist communities at the early (d), middle (e) and late (f) recovery stages. Analysis of similarities (ANOSIM) assessed fire effects on bacterial and protist community composition for the three recovery stages. The $p < 0.05$ and $p > 0.05$ represent significant and non-significant differences in community composition between burnt and unburnt soils at each recovery stage. *Samples consisted of 3 replicates, except sites of 14 years (5 replicates) and 66 years (4 replicates). One replicate from the 66-year site was an outlier and deleted from the analysis.
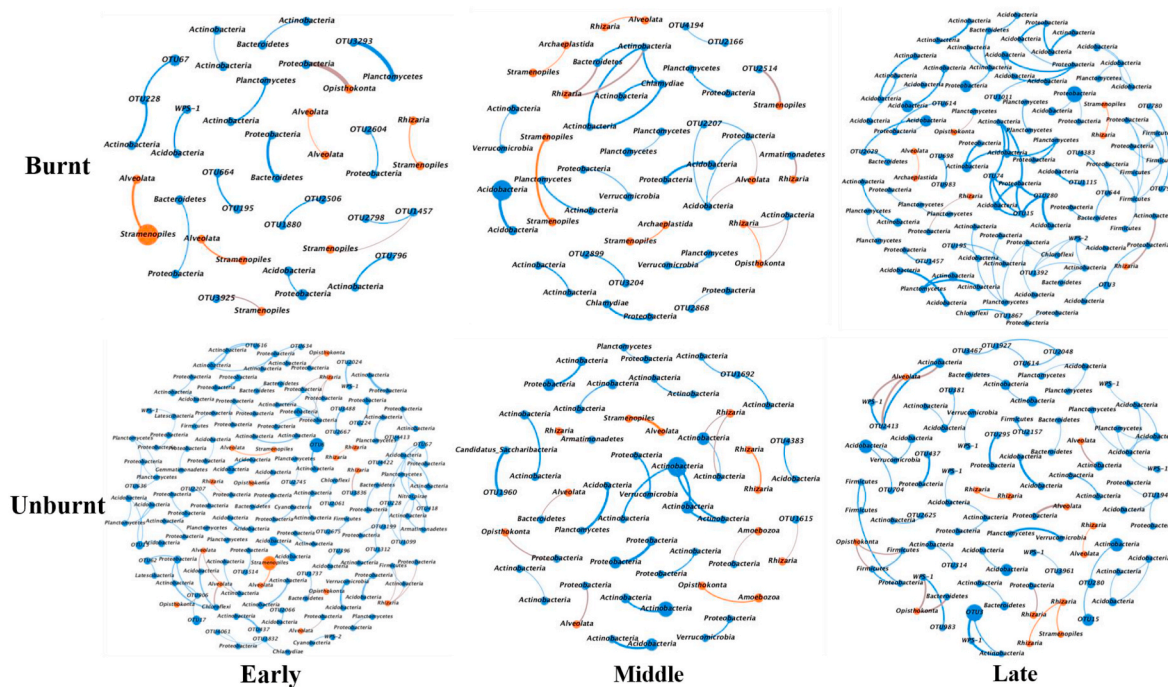
Fig. 5. Bacterial-protist co-occurrence networks in burnt and unburnt soils at the three recovery stages. Node size represents the relative abundance of each OTU. Blue nodes represent bacterial OTUs and orange nodes represent protist OTUs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2

Node number, edge number, average degree, average path length, edges linked with protist-protist, bacteria-protist and bacteria-bacteria in bacterial-protist co-occurrence networks in burnt and unburnt forest soils at the three recovery stages.

Network parameter	Early stage		Middle stage		Late stage	
	Burnt	Unburnt	Burnt	Unburnt	Burnt	Unburnt
Node number	42	164	51	58	112	94
Edge number	22	119	34	33	96	69
Average degree	1.05	1.45	1.33	1.14	1.71	1.47
Average path length	1.08	2.21	1.26	1.08	2.45	1.50
Linked edge number (protist-protist)	4	3	5	3	2	4
Linked edge number (protist-bacteria)	3	17	8	8	3	10
Linked edge number (bacteria-bacteria)	15	99	21	22	91	55

properties (Table 1). No differences were observed in soil properties at the middle and late recovery stages, except for pH and EC at the middle stage (Table 1). At the early stage, the bacterial community in the unburnt soils was linked to pH, EC, NH_4^+, NO_3^- and DON, as demonstrated by significant correlations between these parameters and bacterial community dissimilarity. In contrast, the bacterial community in the burnt soils was primarily explained by soil pH (Fig. 6a). Similarly, the protist community in the unburnt soils was linked to pH, EC, NH_4^+, NO_3^- and DON, while only soil pH contributed to the protist community in burnt soils (Fig. 6b). At the middle and late recovery stages, the contribution of soil parameters to bacterial communities in burnt soils was similar to that in the unburnt soils (Fig. 6a). No contributions of soil chemical properties to the protist community were observed at the middle and late recovery stages (Fig. 6b).

### 3.4. Fire-responsive bacteria and protist OTUs

Wildfire elicited 29 positive- and 1 negative-responsive bacterial OTUs during the early recovery stage. The number of positive-responsive bacterial OTUs decreased to 6 and 10 in the middle and late stages, respectively. In contrast, negative-responsive bacterial OTUs increased to 23 and 11 in the middle and late stages, respectively (Fig. S3). The positive-responsive bacterial OTUs with the largest changes (top three) in abundance under fire disturbance were primarily from *Firmicutes* (phylum), *Bacilli* (class) and *Bacillales* (order) at the early stage; *Acidobacteria* (phylum), *Rhodospirillales* (order) and *Gp1* (order) at the middle stage; and *Acidobacteria_Gp3* (class), *Gemmatimonadetes* (phylum) and *Acidobacteria_Gp7* (class) at the late stage (Table 3).

Wildfire elicited 20 positive- and 26 negative-responsive protist OTUs at the early recovery stage. Meanwhile, the number of positive-responsive protist OTUs decreased to 7 and 5 in the middle and late stages, respectively, whereas the number of negative-responsive protist OTUs decreased to 0 and 5 in the middle and late stages, respectively (Fig. S3). The positive-responsive protist OTUs with the largest changes (top three) in abundance following fire disturbance were primarily from *Rhizaria* (domain), *Cercozoa* (phylum) and *Hypotrichia* (order) at the early stage; *Archaeplastida* (domain), *Chlorophyta* (phylum) and *Chlorophyceae* (class) at the middle stage; and *Filosa_Imbricatea* (class), *Thaumatomonadidae* (family) and *Euglyphidae* (family) and at the late stage (Table 3).

4. Discussion

4.1. Recovery of bacteria communities

Previous studies showed high resistance and resilience in microbial biomass C, microbial respiration and bacterial growth following wildfire disturbance in both boreal forest (Allison et al., 2010) and Mediterranean pasture (Velasco et al., 2009). Bacterial diversity in Mediterranean ecosystems increased during the early stages of recovery following fire disturbance and gradually decreased to pre-fire conditions across a
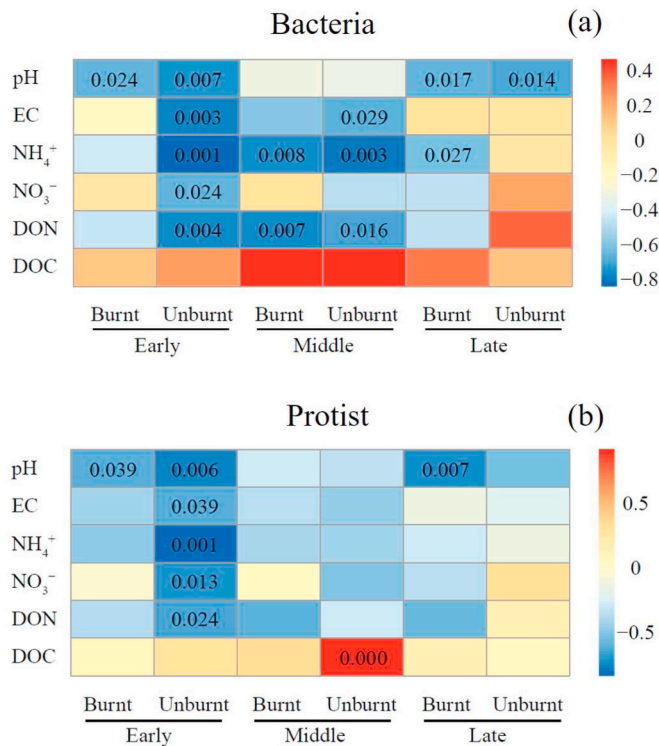
Fig. 6. Heat map of Pearson correlations examining relationships between bacterial community and soil chemical properties in burnt and unburnt soils at the early, middle and late recovery stages (a), and relationships between protist community and soil chemical properties at the early, middle and late recovery stages (b). Red and blue colors represent positive and negative correlations, respectively. The p values (<0.05) shown in color rectangles present the significant correlations between bacterial community and soil chemical parameters. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

prominently to the recovery of ecosystem functions, such as nutrient cycling.

Although wildfire (*e.g.,* soil heating) has a direct destructive effect on bacteria cells, bacteria can rapidly re-colonize soil environments due to: 1) a short bacterial growth cycle with rapid reproduction rates (Madigan et al., 2008) that induce a fast recovery of bacterial biomass and diversity (Velasco et al., 2009); 2) resistant cell structures, such as spores, cysts and akinete, that are resistant to fire/heating and promote rapid bacterial reproduction (Dworkin, 2006), and 3) wider niche breadth that makes bacteria less prone to environmental filtering than other organisms, and provides higher plasticity in metabolic abilities and environmental tolerance (Wu et al., 2018). Wildfire also affects the soil microbial community by 1) altering post-fire soil chemical properties (*e.g.,* increased pH and nutrient availability) (Wang et al., 2015; Dai et al., 2017; Yang et al., 2020); and 2) altering the post-fire biological environment such as vegetation-microbial interactions in the rhizosphere (Pérez-Valera et al., 2017). Hence, alterations in soil chemical properties during soil and vegetation recovery play an important role in bacterial community recovery to pre-fire conditions (Fig. 7). A number of studies show that soil pH and nutrient status are key parameters affecting soil bacterial diversity and community composition across diverse ecosystems (Rousk et al., 2010; Liu et al., 2014, 2015; Dai et al., 2018). Our results showed that both soil nutrient status and pH were significantly correlated to bacterial communities in unburnt soils in early recovery stage soils, whereas only soil pH was linked to protist communities after wildfire (Fig. 6). This suggests that fire reduces the effects of soil nutrient status on bacterial communities. During long-term recovery periods, the soil nutrient status (*e.g.,* NH_4^+ and DON) and soil pH contributed to bacterial communities in both burnt and unburnt soils, indicating that the quick recovery of bacterial communities was associated with the recovery of soil nutrient status and pH.

Microbial biomass, diversity and communities often display strong associations with vegetation dynamics, such as vegetation biomass and diversity (Van Der Heijden et al., 2008; Chen et al., 2019; Ma et al., 2021). Vegetation interactions include 1) alteration of soil physico-chemical properties, such as pH, porosity and moisture (Liang et al., 2019; Bennett et al., 2020); 2) inputs of organic matter to support the heterotrophic microbial community, and 3) formation of mutualistic symbiosis between microorganisms and plant roots. Thus, the recovery of vegetation and microbial communities are likely to proceed concurrently post-wildfire. Our study observed that the high severity fires characteristic of boreal forests dramatically damage vegetation at the early stage, as indicated by the 0.09 decrease in NDVI measured for the early stage recovery period. However, the vegetation quickly recovered to pre-fire status after ~8 years, as shown by the increases in NDVI for the middle (+0.01) and late (+0.03) stage recovery periods (Table 1). These rapidly changing vegetation dynamics may affect the bacterial community recovery at the early stage, as vegetative dynamics after fire are the drivers of microbial community structure in forest soils (Hart et al., 2005; Wang et al., 2016). However, no changes in bacterial communities occurred at the middle and late recovery stages following

20-year wildfire chronosequence (Pérez-Valera et al., 2018). These findings are consistent with our study showing increased bacterial diversity and altered community composition in the early recovery stage (<5 years) and no changes in these parameters between burnt and unburnt soils after 8 years (Fig. 2 and 3). In contrast, some studies showed that fire decreased soil bacterial diversity in short-term, post-fire periods (Pérez-Valera et al., 2017; Adkins et al., 2020; Dove et al., 2021). These differences are probably attributable to different ecosystem types, climatic conditions and fire severity. Given that soil N cycling and inorganic N availability in post-fire soils are regulated by microorganisms (Smithwick et al., 2005; Turner et al., 2007), we infer that the rapid recovery of bacterial communities in boreal forest soils contribute

Table 3

Bacterial and protist responsive OTUs identified by linear discriminant analysis effect size (LEfSe) with the largest abundance increase in burnt soils (compared to unburnt soils) at the three different recovery stages.

| Recovery stage	Bacteria			Protist		
	Phylum	Finest level identified	LDA score (log10)	Domain:phylum	Finest level identified	LDA score (log10)
Early	*Firmicutes*	*Firmicutes (p)*	3.07	*Rhizaria:*	*Rhizaria*	3.82
	Firmicutes	*Bacilli (c)*	3.07	*Rhizaria:Cercozoa*	*Cercozoa*	3.81
	Firmicutes	*Bacillales (o)*	3.06	*Alveolata:Ciliophora*	*Hypotrichia*	3.60
Middle	*Acidobacteria*	*Acidobacteria (p)*	3.82	*Archaeplastida:*	*Archaeplastida*	2.99
	Proteobacteria	*Rhodospirillales (o)*	3.22	*Archaeplastida:Chlorophyta*	*Chlorophyta*	2.99
	Acidobacteria	*Gp1 (o)*	3.21	*Archaeplastida:Chlorophyta*	*Chlorophyceae*	2.95
Late	*Acidobacteria*	*Acidobacteria_Gp3 (c)*	3.04	*Rhizaria:Cercozoa*	*Filosa_Imbricatea*	2.91
	Gemmatimonadetes	*Gemmatimonadetes (p)*	2.89	*Rhizaria:Cercozoa*	*Thaumatomonadidae*	2.72
	Acidobacteria	*Acidobacteria_Gp7 (c)*	2.87	*Rhizaria:Cercozoa*	*Euglyphidae*	2.33
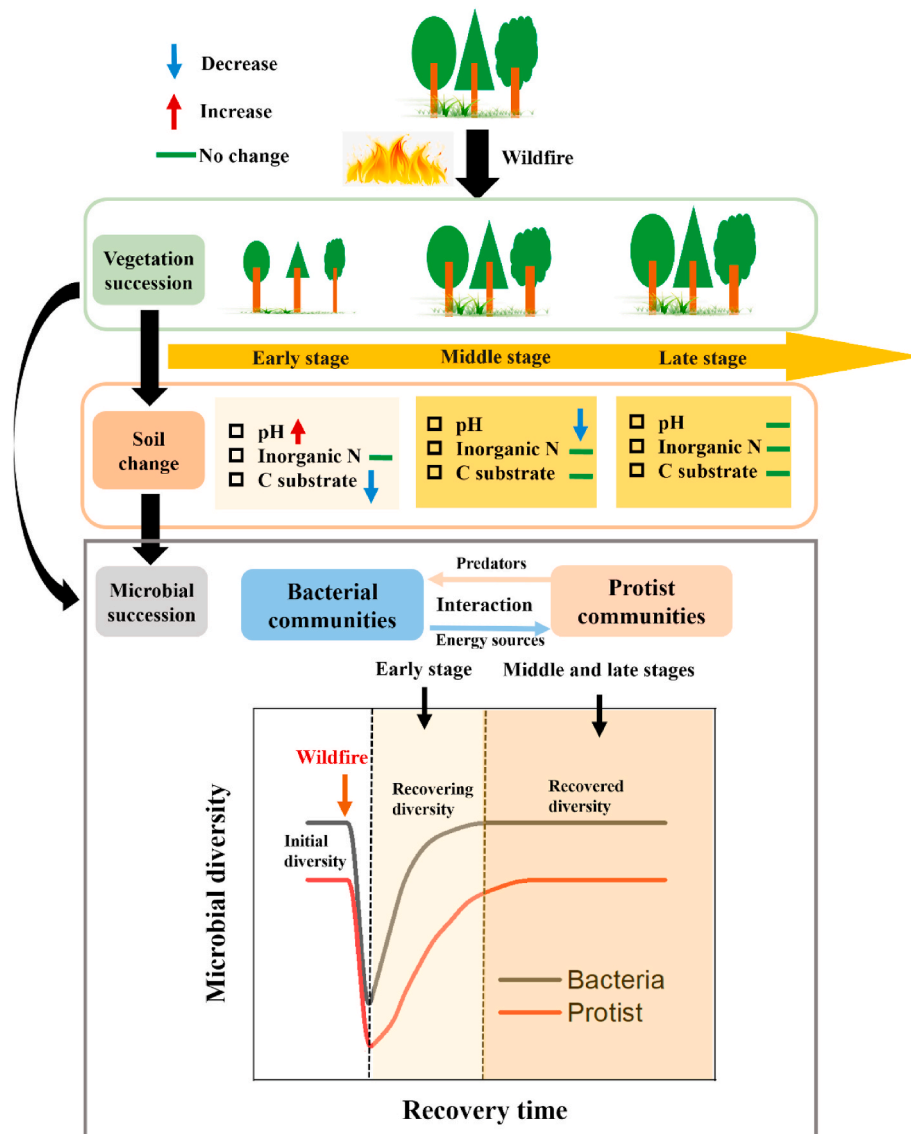
Fig. 7. Summary diagram illustrating vegetation succession and changes in soil chemical properties affecting post-fire recovery of bacterial and protist communities, and the concurrent recovery of bacterial and protist communities following wildfire across the 76-year wildfire chronosequence.

vegetation recovery (Fig. 7). Thus, our study shows that bacterial recovery was likely associated with vegetation recovery after fire perturbation, implying a potential linkage between the aboveground and belowground living communities in wildfire-perturbed forest ecosystems.

4.2. Recovery of protist communities and their interactions with bacterial communities

In general, protists are consumers (*i.e.*, predators) in the soil food web, decomposers of soil organic matter and contributors to plants root interactions (Geisen et al., 2018). The quick recovery (after 8 years) of protist communities to pre-fire status indicates a limited influence of wildfire-altered protist communities on regulating forest soil functions from a long-term perspective. However, at the early recovery stage, fire perturbations on the protist community may affect soil nutrient cycling, as protists enhance the microbial loop (*i.e.*, releasing nutrients) and increase plant performance (*e.g.*, plant pathogen protection) (Rosenberg et al., 2009). Protist communities are generally influenced by climatic factors (*e.g.*, annual mean rainfall and temperature) (Oliverio et al., 2020), soil moisture, clay content and nutrient status (Zhao et al., 2019,

2020), but appear to be not overly sensitive to soil pH (Fiore-Donno et al., 2019). Our results showed that the effects of soil nutrient status, but not soil pH, on protist communities were reduced during the early stage after fire (Fig. 6). This was consistent with previous finding demonstrating that protist communities were more susceptive to the fire-caused changes in soil nutrient status rather than soil pH.

No correlations were found between soil chemical properties and the protist community at middle and late recovery stages indicating that recovery of the protist community was probably associated with soil biological properties, such as interactions with the bacterial community (Fig. 6). Similar responses between bacteria and protist alpha diversity and community composition to wildfire (Fig. 2) and the strong positive correlations between bacteria and protist communities (Fig. 3) reveal a concurrent recovery and a close association between these microbial communities (Figs. 2 and 3). Given that protists are predators of bacteria in bacterial-protist food webs, we infer that the protist community's rapid recovery to its pre-fire status was strongly linked to the recovery of the bacterial community. Protists were identified as the central hub in the bacterial-protist co-occurrence network with linkages to diverse bacterial populations (Xiong et al., 2018). During the early recovery stage, most of the dominant bacterial taxa (*e.g.*, OTU6, OTU17 and

OTU41) with the highest abundances (i.e., largest node size in Fig. 5) in unburnt networks were not found in burnt networks. This indicates that the dominant bacteria were diminished by the wildfire event, and their interactions with vegetation and other microorganisms were attenuated. In general, the linked edge number suggests the degree of interaction between a specified bacterial/protist node with others (Barberán et al., 2012). The diminished initial abundance of bacterial prey for protists resulted in a considerable decrease of interactions between bacteria-protists (*i.e.,* edge linked between bacteria-protists decreased by 14) and enhanced competition between protist communities for bacterial prey (*i.e.,* increased edge linked between protists-protists) (Fig. 5 and Table 2). As the bacterial community recovered coincident with vegetation/soil recovery, bacteria-bacteria interactions returned to pre-fire levels (indicated by no changes in network complexity between burnt and unburnt soils, Table 2). Correspondingly, bacterial-protist interactions did not change in the middle stage recovery, as shown by the same edge numbers for both burnt and unburnt treatments (Fig. 5 and Table 2). During the late recovery stage, although the bacterial communities returned to their initial status, the bacteria-protist interactions did not completely return to pre-fire status (Table 2). We posit that some dominant protists with a narrow niche width may require longer periods for full recovery (Geisen et al., 2018). Overall, bacteria appear to adapt more quickly to wildfire-altered vegetation and soil conditions, whereas the recovery of the protist community is slightly lagged due to its dependence on bacterial community recovery, in addition to the recovery of specific vegetation and soil conditions (Fig. 7). The concurrent recovery of bacterial and protist communities, including alpha/beta diversity and their interactions, implies a close association with food web dynamics after wildfire perturbation.

4.3. Fire-responsive bacteria and protist taxa

Although the overall bacterial community did not change in the mid-to long-term recovery stages, some bacterial OTUs remained positively or negatively responsive to wildfire perturbations. These sensitive responders are thereby especially important in indicating the fire history of certain forest ecosystems. In general, bacterial taxa are influenced by nutrient status and environmental condition (*e.g.,* soil pH). For instance, *β-Proteobacteria* and *Bacteroidetes* were found to be more dependent on soil nutrient availability than *Acidobacteria* (Fierer et al., 2007). Soil pH affects the abundance of taxa from *Acidobacteria* and *Actinobacteria* (Meyer et al., 2018). The positive wildfire responders with highest abundance during the early recovery stage were from *Firmicutes* (Table 3), which was possibly attributed to large changes in soil properties post-fire, as taxa such as *Bacillales* are adaptable to relatively extreme environments (Madigan et al., 2008). However, our study showed that positive wildfire responders during the middle and late recovery stages were very diverse, ranging among *Firmicutes, Bacteroidetes*, *Proteobacteria* and *Acidobacteria* (Table 3). This was supported by the lack of change in soil chemical properties between burnt and unburnt soils at middle and late recovery stages (Table 1). The stochastic selection of bacterial responsive OTUs during late vegetation recovery may explain the specific bacteria responses.

There is a paucity of information concerning protist response to wildfire. We identified *Rhizaria (domain): Cercozoa* (phylum) and *Alveolata (domain): Ciliophora* (phylum) as both early and late-stage responsive OTUs for fire-disturbance/recovery in forest soils (Table 3). These identified protist OTUs (eukaryotes) can be used to ascertain the fire history of forest ecosystems, in conjunction with prokaryote microorganisms. Regarding soil functions, *Cercozoa* and *Ciliophora* are widely distributed in soil ecosystems as phagotrophs (Foissner, 2009). They feed on bacteria, fungi and other protists and animals, thereby affecting microbial diversity and microbially-regulated nutrient cycling. These functions are consistent with the strong relationship found with soil biological properties (*e.g.,* microbial biomass and bacterial number) and soil chemical properties (*e.g.,* organic C/N) (Fiore-Donno et al.,

2019). The protist responder, *Chlorophyta*, was a phototroph, which can fix carbon in forest ecosystems following wildfire. *Oxytrichidae* and *Urostylidae* at the family level (Fig. S3) were identified as responsive OTUs in burnt soils and provide a higher resolution to trace historic fire events and the associated recovery stage of forest ecosystems. Given these insights, further investigation of the soil functions associated with these responsive protists and the deterministic environmental factors contributing to their wildfire responsiveness are strongly warranted. Overall, identifying the protist responders to wildfire not only helps to trace historic fire events in forest ecosystems, but also predict soil functions such as nutrient cycling during post-fire, recovery periods.

5. Conclusions

This study demonstrated that the fire-altered bacterial and protist communities returned to pre-fire levels prior to the mid-to long-term recovery periods (<8 years). The recovery of protist communities was associated with bacterial community recovery. Our study showed that vegetation and bacterial community both changed following fire, while the causal link between them should be confirmed by further more controlled observations. The influencing factors of vegetation succession and soil properties imply strong linkages between above- and below-ground living communities in wildfire-perturbed forest ecosystems. Notably, protist evolution and their interactions with bacteria after a wildfire enhance our understanding of the role of protists in the regulation of nutrient cycling and energy transfers following wildfire in forest ecosystems. Despite the lack of differences in overall bacterial and protist communities between burnt and unburnt soils during the middle and late recovery stages, the stochastic assembly of bacterial and protist communities occurred in burnt soils (Ferrenberg et al., 2013). The underlying mechanisms related to bacterial and protist assembly and the contributions of stochastic assembly and deterministic factors are multifaceted and provide a fruitful area for further investigation.

Data availability

Raw data of 16S and 18S sequencing have been deposited in the GenBank Sequence Read Archive with the identification numbers: PRJNA667812 and PRJNA667813. Other datasets from this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.soilbio.2021.108452.

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