



Habitat heterogeneity induced by pyrogenic organic matter in wildfire-perturbed soils mediates bacterial community assembly processes

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Abstract

Although pyrogenic organic matter (PyOM) generated during wildfires plays a critical role in post-fire ecosystem recovery, the specific mechanisms by which PyOM controls soil microbial community assembly after wildfire perturbation remain largely uncharacterized. Herein we characterized the effect of PyOM on soil bacterial communities at two independent wildfire-perturbed forest sites. We observed that α -diversity of bacterial communities was the highest in wildfire-perturbed soils and that bacterial communities gradually changed along a sequence of unburnt soil → burnt soil → PyOM. The microbial communities reconstructed from unburnt soil and PyOM resembled the real bacterial communities in wildfire-perturbed soils in their α -diversity and community structure. Bacterial specialists in PyOM and soils clustered in phylogenetic coherent lineages with intra-lineage pH-niche conservatism and inter-lineage pH-niche divergence. Our results suggest that PyOM mediates bacterial community assembly in wildfire-perturbed soils by a combination of environmental selection and dispersal of phylogenetic coherent specialists with habitat preference in the heterogeneous microhabitats of burnt soils with distinct PyOM patches.

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Introduction

Wildfires are ubiquitous across the globe and annually burn an average of 348 Mha of vegetated land worldwide [1]. Given projections for future climate change, wildfires are predicted to become larger and more frequent and intense, causing severe perturbations to terrestrial ecosystems [2]. In particular, wildfires alter all aspects of the soil microbiome, including community structure and diversity [3]. In the days to weeks after fire disturbance, microbial diversity dramatically decreases with a recovery of community structure over time, which is attributed to initial soil sterilization followed by microbial regeneration and/or dispersal in the topsoil [4]. Several years after recovering from fire disturbance and following ecosystem stabilization, microbial diversity may exceed that of unburnt soil, whereas microbial community structure may achieve an alternative stable state [5]. These transformations are usually ascribed to wildfire-induced changes in vegetation and edaphic conditions (e.g., pH, organic carbon, nutrient availability) [6, 7]. Wildfire alterations to soil microbiomes play a vital role in terrestrial ecosystem resilience and recovery [8], highlighting the need to better understand responses of the soil microbiome to wildfire perturbations.

Research interest in pyrogenic organic matter (PyOM) has increased over recent decades in an effort to elucidate the impacts of wildfire perturbation on ecological processes [9]. PyOM is generated during fires, and consists of a broad continuum of carbon-enriched substances ranging from semi-charred biomass to charcoal and soot [10]. It is variously termed charcoal, black carbon or biochar in different contexts as they are all derived from biomass pyrolysis and have similar chemical structure and properties: enriched in carbon and aromatic compounds, and relatively resistant to microbial degradation [11]. Recent studies showed that, much like the long-term effects of wildfires, an application of anthropogenic PyOM (biochar) alters the community structure and diversity of microorganisms [12]. Similar to the soil changes wrought by wildfires, edaphic changes from biochar amendments include increases in nutrient availability and pH [13]. Overall, these studies suggest that long-term wildfire effects may be connected to PyOM through its role in regulating edaphic properties and the resulting soil microbiome, thereby having a strong feedback on terrestrial ecosystems.

It is unknown whether PyOM alters the soil microbiome indirectly through alterations of edaphic conditions or directly by serving as a microhabitat that regulates the colonization of microorganisms. The PyOM appears to be an important microhabitat for microorganisms in fire-perturbed soils as the porous structure of anthropogenic PyOM provides advantageous dwellings for microorganisms [14]. In boreal forests, microbes colonized in PyOM can also facilitate degradation of phenolic compounds retained by PyOM [15]. PyOM-associated microbial communities have a community structure distinctly different from the adjacent soil [16]. Furthermore, PyOM-induced microbial responses, as well as edaphic changes, demonstrate a steep distance-decay relationship at the interface between PyOM and bulk soil [17]. However, current studies on soil microbiome-PyOM interactions usually report the microbial response to overall edaphic changes caused by anthropogenic PyOM addition without specifically addressing habitat effects [17]. Moreover, previous studies either did not report community structure or microbial diversity [16], or lacked a rigorous conclusion due to limitations from inadequate sample size and/or neglected interactions between communities in PyOM and the adjacent soil [18]. Thus, our understanding of the linkages between PyOM microhabitats and fire-perturbed soil remains limited and in need of further study.

This study investigated bacterial communities in PyOM, burnt soils and unburnt soils at two wildfire-perturbed forest sites to examine the relationships between PyOM and soil bacteria, paying particular attention to community assembly processes and phylogenetic coherence. “Coherence,” in this sense, delineates the structured occurrence of habitat specialists under a specific phylogenetic lineage and potentially

reflects a lineage’s conserved habitat niche contrasting against diversification. The following hypotheses were tested: (1) PyOM forms a distinct microhabitat for some soil bacteria; (2) the heterogeneous PyOM-soil matrix mediates the bacterial community of burnt soils; and (3) habitat specialists in PyOM and soils have phylogenetic coherence. The relationships between PyOM and the bacterial community were explored through 16 S rRNA gene sequencing of soil samples collected from fire perturbed forests and additionally, through simulations wherein we reconstructed bacterial communities in burnt soils based on the bacterial communities identified in PyOM and unburnt soils to corroborate the bacterial community assembly found after wildfire perturbation. We combined these results to elucidate the role of PyOM in bacterial community assembly in wildfire-perturbed soils and to link observations of bacterial communities in burnt soils with microbial community assembly theories.

Materials and methods

Sample collection

Soil samples were collected post-fire in two forests in Zhejiang (30° 2′ 8″ N, 119° 39′ 24″ E) and Jiangxi (28° 14′ 35″ N, 117° 4′ 27″ E) provinces, China. The fire at the Zhejiang site occurred in August 2013 with soil sampling in March 2016. Soils were classified as Humults and had a sandy loam texture. The site had a slope of 55°, a mean annual precipitation of 1477 mm and a mean annual temperature of 16.7 °C. The fire at the Jiangxi site occurred in December 2017, and the soil was sampled in October 2018. Soils were classified as Humults and had a loam texture. The site had a slope of 30°, a mean annual precipitation of 1750 mm and a mean annual temperature of 18 °C. No historical wildfires were previously recorded at either site before the current fire events. Prior to the wildfires, both sites were vegetated by subtropical evergreen broad-leafed forests. Both fires burned at a moderate intensity with an estimated average temperature of 250–400 °C, consuming most of the litter layer and leaving an abundance of semi-charred and charred materials (Fig. S1); the wildfire was somewhat more severe at the Jiangxi forest compared to the Zhejiang forest site. Post-fire vegetation at the Zhejiang site was shrubs and low trees (Fig. S1c), while at Jiangxi the post-fire vegetation was primarily grasses or barren soil (Fig. S1d).

Twelve composite soil samples were collected from each site that spanned a gradient from the fire center to the perimeter. Composite soil samples consisting of triplicate cores were taken from the upper 8 cm of the A horizon after removal of any O horizon. We isolated PyOM particles (i.e., charred materials; 0.5–15 mm diameter) by handpicking from each soil sample to characterize their microbiomes and

distinct habitat conditions (Fig. S2). To investigate soil microbiomes free of any recent PyOM materials, 12 soil samples were collected from an adjacent unburnt location at each site that had similar soil, topography and pre-burn vegetation. Overall, 24 samples were collected at each site, comprising 12 samples from the unburnt locations ('unburnt' samples) and 12 samples from the burnt locations that were further subdivided into 12 "burnt" soil samples and 12 "PyOM" samples. In addition, 9 PyOM samples were taken from the Zhejiang site and washed with Milli-Q water (EMD Millipore, Germany) to investigate whether adhered soil impacted the PyOM microbiome characterization. Each soil sample was sieved through a 2-mm screen and split into three zip-lock bags stored at three different conditions: air-dried, frozen at -20°C and frozen at -80°C , for determination of pH and organic carbon (SOC), analysis of dissolvable organic carbon (DOC) and DNA extraction, respectively. Each isolated PyOM sample was split into two zip-lock bags and stored at two conditions: vacuum-dried and -80°C , for pH analysis and DNA extraction, respectively. Full details are given in Section 1.1 of the Supplemental Information (SI).

Bacterial 16 S rRNA gene sequencing and sequence data processing

Total DNA was extracted from the soil and PyOM samples using a FastDNA™ SPIN Kit for Soil following the manufacturer instructions (MP Biomedicals, USA). The V4–V5 regions (515F–907 R) of the 16 S rRNA gene were amplified with forward (515 F: 5'-GTG CCA GCM GCC GCG G -3') and reverse primers (907 R: 5'- CCG TCA ATT CMT TTR AGT TT -3') and sequenced with a MiSeq platform (Illumina Inc., USA). After raw data were quality screened, operational taxonomic units (OTUs) were identified using a 97% similarity criterion based on the UPARSE algorithm [19]. Phylogenies of OTUs were constructed using MUSCLE [20] and FastTree [21] and taxonomies were inferred using EzBioCloud [22]. Full details are provided in SI Section 1.2. OTU sequences from this study were deposited at DDBJ/EMBL/GenBank under the accession numbers KEKP000000000 for Zhejiang and KEKQ000000000 for the Jiangxi site.

Bacterial community analysis

Biodiversity measurements included α -diversity, β -diversity and phylogenetic clustering indices. The α -diversity indices included Shannon index, Faith's phylogeny diversity [23], Pielou's J [24] and observed OTU number. β -diversity was characterized using a non-constrained ordination, non-metric multidimensional scaling (NMDS) [25], based on a phylogenetic distance generalized UniFrac (GUniFrac) [26].

Procrustes analysis [27] was performed based on the NMDS results to assess correlations between the community structure of PyOM and adjacent soils. The washing procedure did not discernibly affect the microbial structure of PyOM samples (pseudo- $F = 0.65$, $P = 0.89$, PERMANOVA) (Fig. S3); thus, the data of washed and unwashed PyOM samples were pooled. Phylogenetic clustering indices included the α -nearest-taxon-index (α NTI, in each sample) and β -nearest-taxon-index (β NTI, between samples) [28]. Full analytical details are provided in SI Section 1.3. The source code of this and the following analyses are available at <https://github.com/Lujun995/Soil-PyOM-microbiome-studies.git>.

Phylogenetic coherence of habitat specialists

Habitat specialists were defined as OTUs preferring PyOM or soil habitats; specialist analysis was conducted after community-level analyses and was aimed to provide detailed information about OTU responses to habitat types. To select specialists, regression analysis was performed between OTU abundance (response variable) and a scale representing the PyOM's effect (explanatory variable). Scale values were 1, 2, and 3 for unburnt soils, burnt soils and PyOM, representing negligible, medium and high effect of PyOM, respectively. For regression analyses, OTUs with permutation-based false discovery rates below 0.01 [29] were deemed habitat specialists and all others were considered habitat generalists. Specialists that were positively associated with the PyOM scale were considered PyOM specialists, whereas those negatively associated were considered soil specialists. Sensitivity analyses [30] showed that specialists were responsible for 69% and 82% of the differences in ordinations for the Zhejiang and Jiangxi sites, respectively; generalists were responsible for the remaining 31% and 18% (Fig. S4). Furthermore, co-occurrence networks [31] showed potential co-occurrence patterns between specialists (Fig. S5), indicating they were good representatives. Full details are given in SI Section 1.4.

Phylogenetic coherence was used to distinguish heredity-induced niche conservatism from diversification-induced niche plasticity (Fig. 1a). To evaluate phylogenetic coherence of specialists, we visualized a phylogenetic tree and quantified phylogenetic conservatism using Fritz's D [32]. For convenience, the $1 - D$ metric was reported [33]; a larger $1 - D$ value indicates stronger phylogenetic conservatism. We also customized a hierarchical and agglomerative procedure to cluster the coherent lineages of specialists (Fig. 1b). The procedure was based on testing a statistical null hypothesis H_0 : the occurrence of specialists was random. Full details are presented in SI Section 1.5.

To contrast the niches of coherent lineages preferring PyOM versus soil habitats, we reviewed physiological traits of prokaryotic species in these lineages in order to access

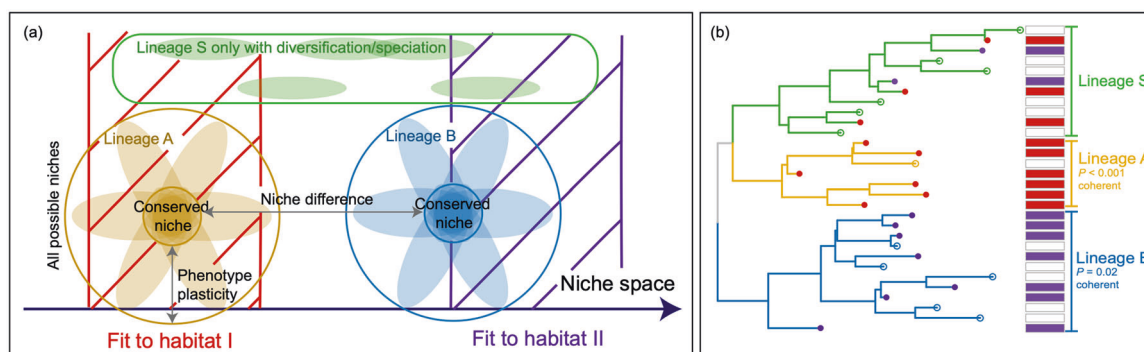


Fig. 1 Schematic diagram of conserved niche and its phylogenetic response. **a** Conserved niche is a convergent value of one functional trait of genetic variants in some lineages, representing a niche shared

by all the genetic variants in the lineages. **b** Conserved niche is recognized as coherence of habitat-preference in phylogenies. Statistical significance was tested using binominal tests.

data on their phenotypic traits (niches) with a focus on convergent traits of growth pH, halotolerance and endospore formation. A coherent lineage was excluded from the review if it was a predicted taxon without a reliable taxonomy or a class whose phenotypic traits were considered too diverse. Full details are given in SI Section 1.6.

Reconstructing the bacterial community of burnt soils

Our second hypothesis implies that the bacterial community in burnt soils originated from the interactions of communities found in PyOM and in unburnt soils. In order to examine this relationship, we reconstructed the bacterial community in burnt soils via simulations that used data from the unburnt soils and PyOM. We projected the coexistence of soil and PyOM specialists by averaging their abundance in PyOM and unburnt soils and generated the generalists by extracting their abundance from either PyOM or unburnt soils. These two components were then combined and rarefied to the sequencing depth of burnt soils. Subsequently, the simulated communities were contrasted to the real communities measured in burnt soils using Shannon, α NTI and GUniFrac distance indices. Full details are presented in SI Section 1.7.

Results

Edaphic properties and α -diversity

The pH among samples followed an order of PyOM \geq burnt soils $>$ unburnt soils for both sites (Dunn-test, $P < 0.05$) (Fig. 2a and 2g). However, even though it followed the same pattern, no significant difference was observed between PyOM and burnt soils for the Jiangxi site (Dunn-test, $P = 0.41$). Burnt soils had decreased DOC concentrations at both sites (Welch's t test, $P = 0.005$) (Fig. S6); fire

history appeared to increase SOC at Jiangxi (Welch's t test, $P < 0.001$), but not at the Zhejiang site (Welch's t test, $P = 0.42$) (Fig. S6). Bacterial communities in burnt soils generally had higher α -diversity than unburnt soils and PyOM, although the differences were not always statistically significant (Fig. 2b–2e, 2h–2k). Burnt soils' Shannon and observed OTU counts were significantly higher than both unburnt soils and PyOM in Zhejiang samples (Dunn-test, $P < 0.05$). All other α -diversity measures aside Faith's diversity at the Jiangxi site showed diminished differences at one or both directions (Dunn-test, $P \geq 0.05$). Phylogenetic clustering indices indicate that the bacterial communities in PyOM were the most phylogenetically scattered with lower α -NTI values than both burnt and unburnt soils at both sites (pairwise t test, $P < 0.01$) (Fig. 2f and 2l).

The relationship between Shannon indices and pH values followed an inverted-U shape at Zhejiang (quadratic regression, $P < 0.001$ for the quadratic coefficient) (Fig. 3b), but was relatively flat at Jiangxi (quadratic regression, $P = 0.09$ for the quadratic coefficient) (Fig. 3d). Neutral environmental pH and low clustering of phylogenetic structure did not necessarily lead to increases in bacterial diversity; PyOM had pH values close to neutral and the least clustered phylogenetic structures (Fig. 2a, 2g, 2f and 2l), but did not have a higher biodiversity than the burnt soil (Fig. 3b and 3d). All biodiversity indices followed an inverted-U relationship with NMDS1 scores (see below; quadratic regression, $P < 0.01$ for quadratic coefficients) (Fig. 3e–3l).

β -diversity of bacterial communities

The bacterial community structure in burnt soils was significantly different from those in unburnt soils and PyOM (PERMANOVA, $P < 0.001$ for both sites) along the first NMDS axis (Fig. 4a and 4b). The bacterial community structure at Zhejiang was differentiated by the first and third NMDS axes (Fig. 4a), whereas at Jiangxi it was differentiated by the first and second NMDS axes

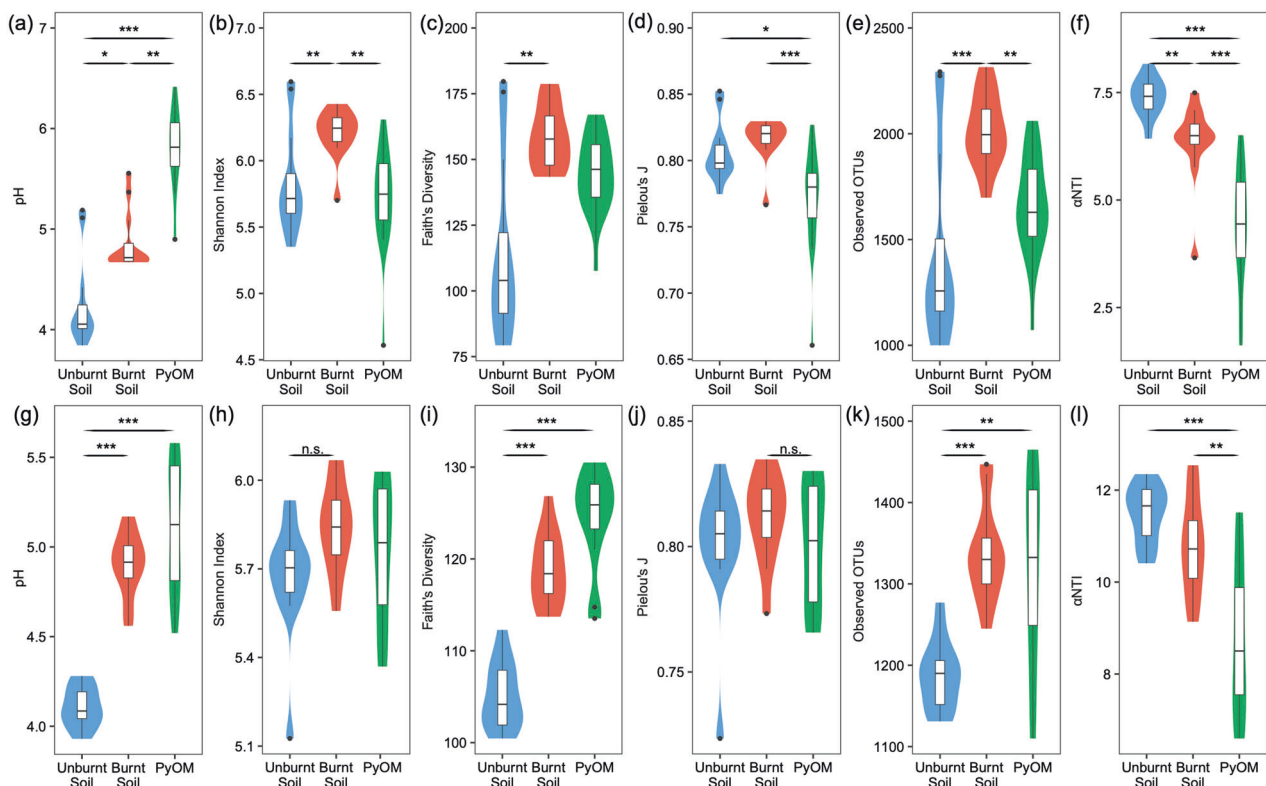


Fig. 2 pH and α -diversity of samples at the Zhejiang and Jiangxi sites. (a–f) The Zhejiang site. (g–l) The Jiangxi site. An α NTI value >2 is considered phylogenetic clustering. The curvilinear polygons indicate estimates of frequency densities. The upper/lower hinges of boxes represent the 75th/25th quantiles, and the lines inside boxes indicate

the median value. The whiskers above/below boxes extend to the maximum/minimum values within ≤ 1.5 inter-quantile-range, whereas data points outside the range are plotted separately. *, ** and *** indicate significance at $P \leq 0.05$, ≤ 0.01 and ≤ 0.001 , respectively.

(Fig. 4b). The first NMDS axis was strongly correlated with pH (Pearson correlation, $P < 0.001$ at both sites) (Fig. 3a and 3c). Bacterial community structure in the burnt soils was strongly related to that in PyOM (Procrustes analysis, $P = 0.003$ for Zhejiang and $P < 0.001$ for Jiangxi) (Fig. 4c and 4d).

Relative abundances of phyla differed between sample types at both sites (Fig. 5). Overall, differentiation of bacterial community structure was strongly influenced by the relative abundances of Acidobacteria, Bacteroidetes and Streptophyta at both sites (Kruskal–Wallis test with Bonferroni correction, $P < 0.01$). The relative abundances of Bacteroidetes and Streptophyta were higher in burnt soils and PyOM, but Acidobacteria abundance followed the order of unburnt $>$ burnt $>$ PyOM (Fig. 5).

Simulation-based reconstruction of burnt soil communities

To link the bacterial community found in burnt soils to that in PyOM and unburnt soils, we reconstructed burnt soil communities via simulations based on the identified abundance data from the PyOM and unburnt soils. The simulated

communities resembled the measured communities in the burnt soils at both sites when NMDS1 and NMDS3 scores were considered (Kolmogorov–Smirnov test, $P \geq 0.05$) (Fig. 6b and 6d). Shannon indices, observed OTU numbers, α NTI of the mock communities were considered equal to (Kolmogorov–Smirnov test, $P \geq 0.05$) or moderately different from (Kolmogorov–Smirnov test, $0.01 \leq P \leq 0.05$) those of measured communities in burnt soils at both sites (Fig. 6). However, disparities were found in NMDS2 scores (Kolmogorov–Smirnov test, $P < 0.001$) at both sites (Fig. 6b and 6d).

Phylogenetic coherence of specialists in PyOM and soils

Specialists in soil and PyOM had strong phylogenetic signals but the signal strength varied across phyla (Fig. 7). The $1 - D$ metric (Fritz's D) for the Zhejiang site was 0.26 for PyOM specialists and 0.51 for soil specialists. That for the Jiangxi site was 0.41 for PyOM specialists and 0.39 for soil specialists ($P < 0.01$, based on label permutation, Table S1). Notably, 45% of soil specialists and 35% of PyOM specialists were phylogenetically coherent at Zhejiang, while

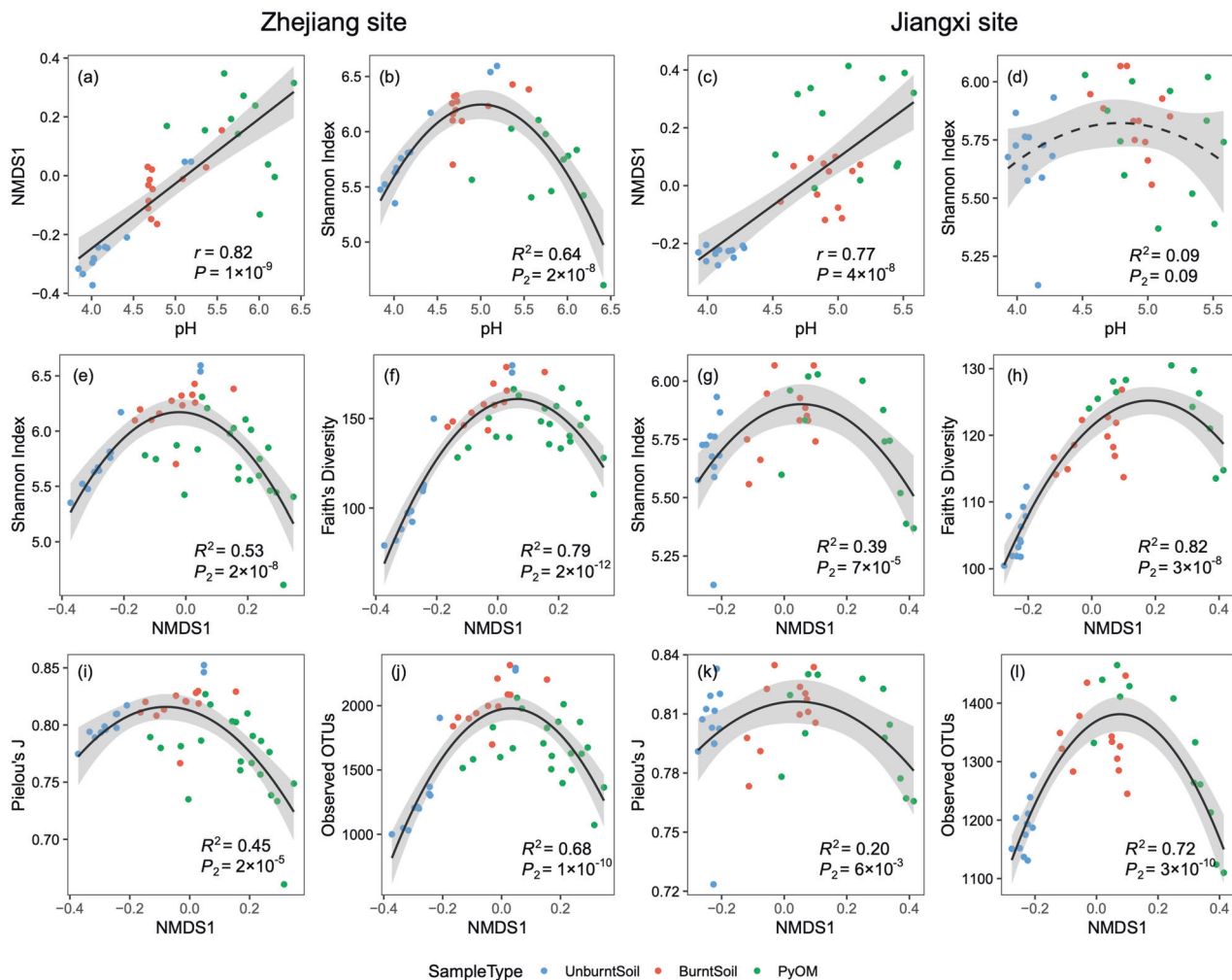


Fig. 3 Correlations among environmental pH, α -diversity and NMDS1 scores. (a, b, e, f, i, j) The Zhejiang site. (c, d, g, h, k, l) The Jiangxi site. Relationships were tested using linear (a and c) or quadratic (b, d, e–j) regressions. A solid curve or line indicates statistical significance at the 0.05 level with the dashed line indicating

insignificance. The gray shading represents the 95% confident interval for regressions. Notations and abbreviations: r , Pearson's r statistics; P_2 , P values for the quadratic coefficients, which reflect the inverted-U shape.

38% of soil specialists and 22% of PyOM specialists were phylogenetically coherent at Jiangxi (Fig. 7 and Table S2). Coherent lineages found at both sites included some lineages (e.g., EU445199_o and Acidobacteriaceae) in Acidobacteria, EU335336_g (a predicated genus) and *Acidibacter* in Proteobacteria for soil, and Frankiales, Geodermatophilaceae and Micrococcales in Actinobacteria for PyOM (Table 1). In nearly every coherent lineage, a consistent optimum pH of its bacterial species was found, regardless of year and location of isolation (Tables S2–S5). There was an observable pH-niche difference between coherent lineages preferring soil and PyOM. Coherent lineages of PyOM were chiefly neutrophilic (optimum growing pH 6–8); conversely, lineages of soil were primarily acidophilic (optimum growing pH ≤ 5.5) (Tables 1 and S2). The relationship between pH niches and habitat

preferences was non-random (Fisher-exact-test for count data, $P < 0.001$). However, sporulation and halotolerance were not associated with habitat preferences of coherent lineages investigated in this study (Fisher's-exact-test for count data, $P \geq 0.10$). Virtually, all the lineages were categorized as non-endospore-forming and non-halotolerant to moderately halotolerant (Tables S2–S5).

Discussion

PyOM-mediated bacterial community assembly after wildfire perturbation

Bacterial community structure in burnt soils differed from those in unburnt soils and PyOM, but could not be fully

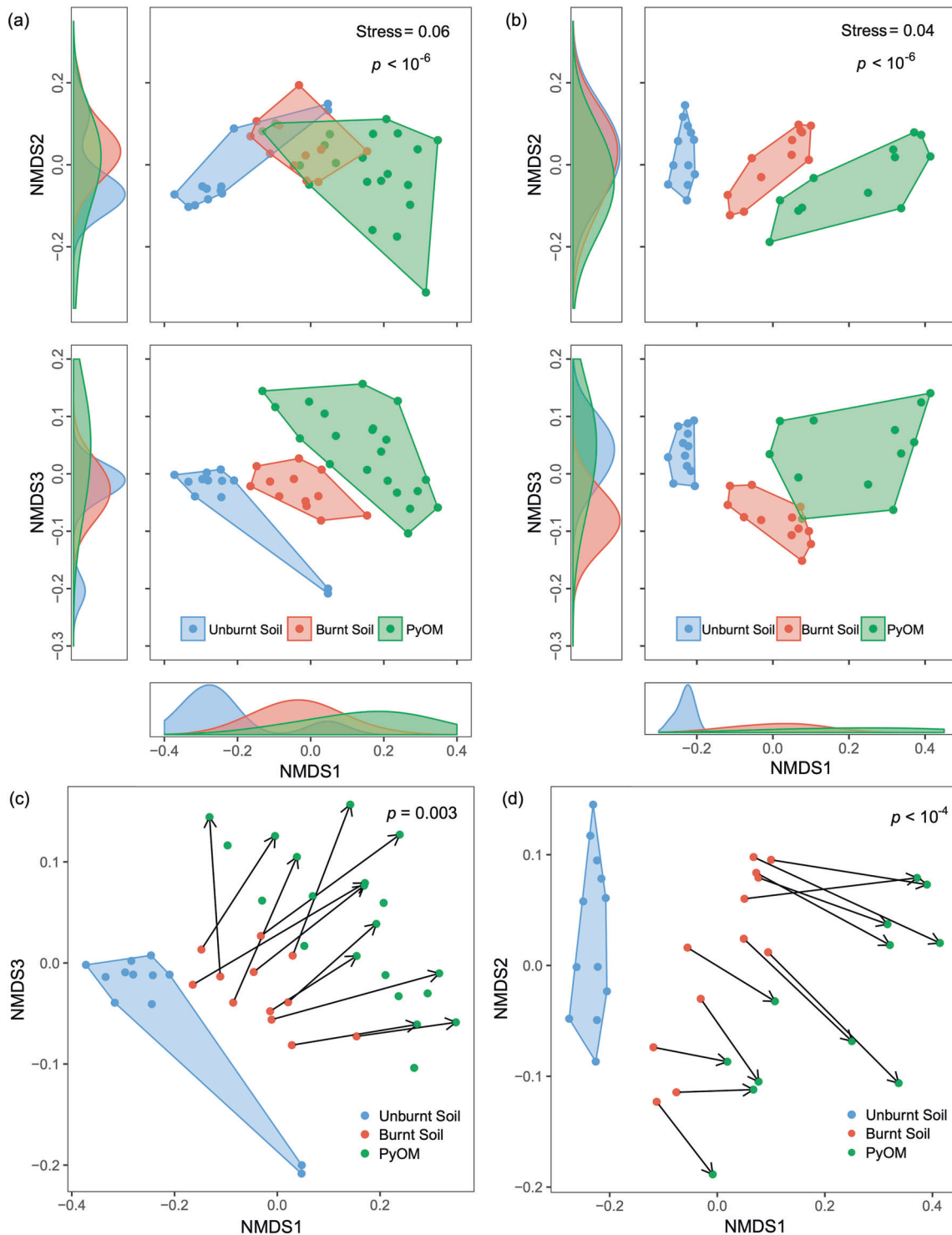


Fig. 4 Microbial community changes after wildfire perturbation. Unconstrained ordinations (NMDS) among all samples based on generalized UniFrac distances for Zhejiang (a) and Jiangxi (b) sites. Statistical significance was tested using PERMANOVA by assigning 3 (high), 2 (medium) and 1 (no) influence of PyOM, to PyOM, burnt

soils and unburnt soils. Curves next to and below the ordinations are estimations of frequency densities for the corresponding axes. Procrustes analysis between PyOM and its adjacent burnt soils at Zhejiang (c) and Jiangxi (d) sites. The adjacency relationship is indicated by arrows.

explained by changes in edaphic conditions. Given that meta-communities inhabit burnt soils comprising both soil and PyOM matrices, we propose an explanation

incorporating spatial co-existence [34, 35] and community assembly theories [36] to reveal the effects of PyOM on bacterial community assembly in burnt soils.

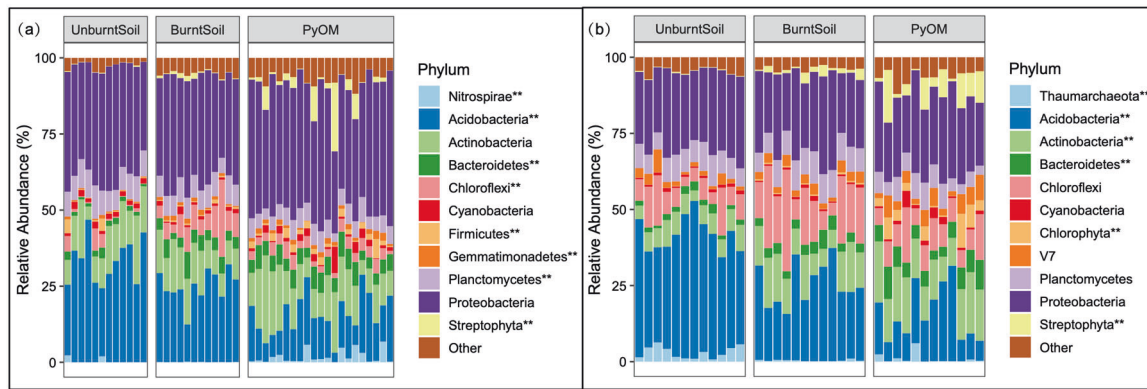


Fig. 5 Relative abundances of dominant phyla. (a) The Zhejiang site. (b) The Jiangxi site. Phyla are selected as dominant phyla if their relative abundance is >1% in at least one sample type (unburnt soils,

burnt soils or PyOM) at each site. Phyla are labeled with ** if their relative abundance is associated with a specific sample type (Kruskal–Wallis test with Bonferroni correction, $P < 0.01$).

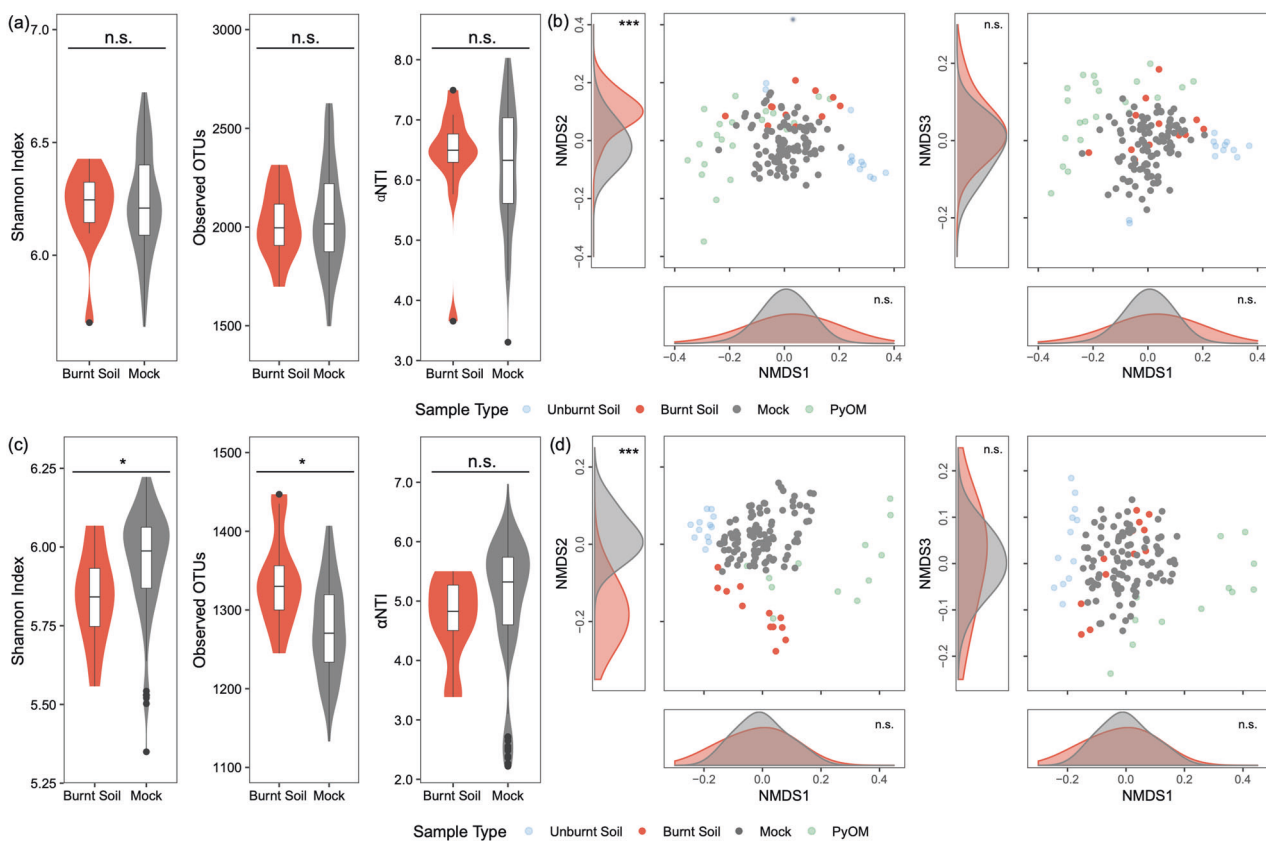


Fig. 6 Comparison of reconstructed and measured communities of the burnt soils. Statistical significance was tested using Kolmogorov–Smirnov tests. Symbols and abbreviations: n.s., * and *** stand for insignificant at $P = 0.05$, $P < 0.05$ and $P < 0.001$, respectively. **a**, **c**

Biodiversity metrics. The shapes have the same meaning as in Fig. 1. **b**, **d** Unconstrained ordinations using NMDS based on generalized UniFrac. The curves next to and below the ordinations are estimations of frequency densities for the corresponding axes.

Habitat heterogeneity between PyOM and soil was a strong selection factor for microbiome community assembly. At the community level, the bacterial community progressively changed along the unburnt soil → burnt soil → PyOM gradient (Fig. 4a and 4b). These results are consistent with recent observations that PyOM alters bacterial communities, although changes in microbial abundance

demonstrated no clear trends [9]. In our present study, community differences were influenced by changes in the relative abundances of Acidobacteria, Bacteroidetes and Streptophyta at both sites (Fig. 5). These changes are in accordance with those induced by variations in environmental pH [37]. pH is a likely driver of the selection process induced by PyOM–soil habitat heterogeneity, given that

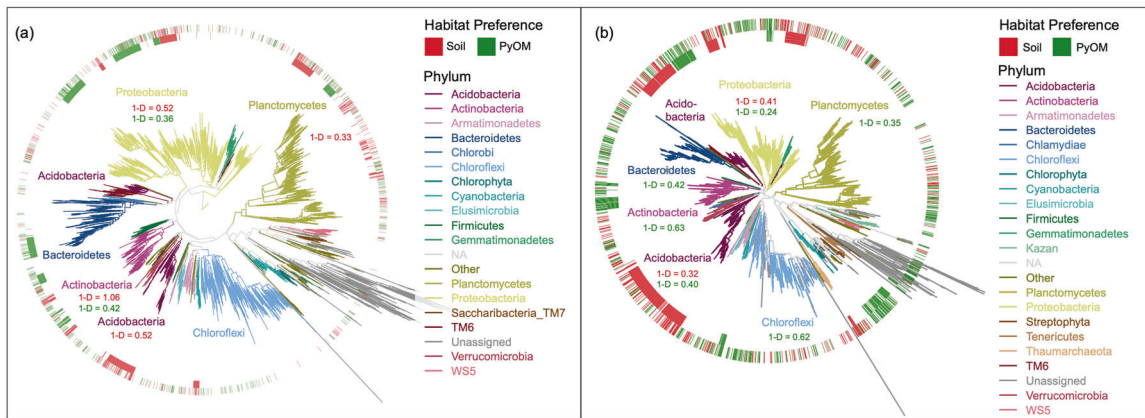


Fig. 7 Phylogenetic coherence and conservatism of specialists. (a) The Zhejiang site. (b) the Jiangxi site. Markers in the outer ring of the aura indicate habitat preference of OTUs with blank areas indicating a neutral response. The coherent markers in the inner ring indicate

coherent lineages (Table S2) and their habitat preference. The statistic inside the aura is Fritz’s $1 - D$, which is noted in the 6 largest phyla of phylogenetic conservatism ($P < 0.01$, based on label permutation). A higher $1 - D$ value indicates stronger phylogenetic conservatism.

Table 1 Summary of the phenotypic traits of coherent lineages.

Type	Coherent lineages	Taxonomy level	Phenotypic traits	Site
Soil	Acidobacteriaceae	Family	Acidophilic	Zhejiang, Jiangxi
	<i>Acidipila</i>	Genus	Acidophilic	Zhejiang
	<i>Aciditerrimonas</i>	Genus	Acidophilic	Zhejiang
	<i>Aquisphaera</i>	Genus	Neutrophilic	Zhejiang
	Acetobacteraceae	Family	No convergence	Zhejiang
	<i>Acidibacter</i>	Genus	Acidophilic	Zhejiang, Jiangxi
PyOM	Frankiales	Order	Neutrophilic	Zhejiang, Jiangxi
	Geodermatophilaceae	Family	Neutrophilic	Zhejiang, Jiangxi
	Micrococcales	Order	Neutrophilic to slightly alkalophilic	Zhejiang, Jiangxi
	<i>Sphingomonas</i>	Genus	Neutrophilic to slightly alkalophilic	Jiangxi
	Chitinophagaceae	Family	Neutrophilic	Zhejiang
	Rhizobiales	Order	No convergence	Zhejiang
	Beijerinckiaceae	Family	No convergence	Zhejiang
	Bradyrhizobiaceae	Family	Neutrophilic	Zhejiang
	Comamonadaceae	Family	Neutrophilic to slightly alkalophilic	Zhejiang
	Oxalobacteraceae	Family	Neutrophilic	Zhejiang

Coherent lineages whose optimum growing pH was available in Table S2 were summarized in this table. If optimum growing pH was absent but a pH range for growth was reported, the midpoint of the range was used as the optimum value. Description of phenotypic traits: acidophilic, optimum growing pH ≤ 5.5 ; neutrophilic and neutrophilic to slightly alkalophilic, optimum growing pH 6–8.5; No convergence, taxa comprising more than 1 lineages under the taxon with discernible gaps in their optimum growing pH. The relationship between coherent lineage type and phenotypical traits is non-random ($P < 0.001$, Fisher’s-exact-test for count data).

NMDS1 scores were strongly correlated with this environmental factor (Fig. 3a and 3c). Significant (NTI > 2) phylogenetic clustering indices α NTI and β NTI (Figs. 2f, 2l and S7) provide further support for pH being a strong selection factor [38]. In contrast to these common tendencies, site-specific responses to PyOM and wildfire perturbation (Figs. 2b-2f, 2h-2l, 3b, 3d and 5) may reflect the contribution of site biogeographical history (e.g. vegetation,

topography or indigenous microbiomes) to bacterial community assemblage [12].

The close association between bacterial communities in PyOM and the surrounding burnt soils suggests that dispersal accounts for the relatedness between PyOM and its surrounding fire-perturbed soil (Fig. 4c and 4d). Dispersal homogenizes the local communities in regions between heterogeneous habitat patches, resulting in a decrease of

β -diversity and an increase of community similarity [39]. Therefore, a necessary indicator of dispersal requires that wildfire-perturbed soil communities appeared to be a homogenization of PyOM and unburnt soil communities; this argument is in line with our homogenized reconstructed communities resembling the measured communities in NMDS1 and NMDS3 (Fig. 6b and 6d). Microorganisms appear to primarily distribute passively, such as via water flow, aerosols [40], dust [41] or fauna transport/mixing [42]. However, it is noteworthy that PyOM can influence surrounding edaphic conditions [16], thus modifying the soil niche via the dispersal of ions and organic compounds, in addition to dispersal of microorganisms.

Wildfire induced higher α -diversity for bacterial communities

Bacterial α -diversities displayed an inverted U relationship with the PyOM gradient (Fig. 3e–3l) and tended to be higher in burnt soils than the unburnt soils or PyOM (Fig. 2). However, such a result should be interpreted with caution due to the narrow differences in α -diversity and the inconsistent result for Faith's diversity at Jiangxi. The increased biodiversity in burnt soils is frequently attributed to an increase in pH induced by PyOM [43]. This follows as soils with neutral pH generally have higher bacterial biodiversity than acidic soils [44]. Similarly, higher bacterial biodiversity is frequently reported in soils amended with anthropogenic PyOM (i.e., biochar), especially in acidic soils [43].

Bearing the connection between pH and increased biodiversity in mind, our observations in this study suggest how more microbes may co-exist. The burnt soils have a more heterogeneous environment providing both soil and PyOM microhabitats (Figs. S1e and S1f). Accordingly, microbial communities inhabiting more heterogeneous environments with diverse microhabitats will develop into a meta-community with higher overall biodiversity. Hence, bacterial diversity in burnt soils is expected to be characterized as the co-existence of generalists, PyOM specialists and soil specialists. Specialists preferring PyOM or soil microhabitats may spatially co-exist in burnt soils via their pH-niche differences, which is consistent with the environmental pH tendency (unburnt soils < burnt soils \leq PyOM) (Fig. 2, Table 1 and S2). Our simulations projected such a co-existence by averaging abundance data of specialists in PyOM and unburnt soils and remarkably reproduced the biodiversity patterns found in the microbial communities of burnt soils (Fig. 6a and 6c). These inferences are congruous with previous studies investigating ecotones, the transition zone between two or more ecosystems with distinct traits, such as rhizospheres among rhizocompartments of bulk soil, plant roots and root nodules [45] and seawater-

hydrothermal fluid mixing zones at hydrothermal vents [46]. A typical community in an ecotone would comprise specialists from both habitats and some specialists of the ecotone itself [46].

Phylogenetic coherence in shaping habitat preference of specialists in PyOM and soils

Coherence, synonymous with conservatism [47] and congruence [48], is typically portrayed as an association between phylogenetic relatedness and functional traits of species. Several previous studies emphasize using a universal overall indicator for coherence of the phylogenetic tree [47]. This study also found that phylogenetic coherence may reflect the conserved niche of specific lineages (Fig. 7a and 7b), in contrast to diversification and phenotype plasticity. Indicators for phylogenetic coherence of specialists in both PyOM and soils were statistically significant at both sites (Table S1). Notably, many of the specialists fall within coherent lineages (Table 1 & Fig. 7) in which pH niches are greatly conserved (Tables S2–S5). Therefore, in these lineages, even if diversification plays a role in forming bacterial pH niche and habitat preference, it is expected to play a minor role compared with coherence.

Alternatively, selection may be dominated by the diversification process, yielding incoherent phylogenetic patterns. Although overall coherence indicators were significant in this study (Table S1), the strength was not uniform (Fig. 7) when some lineages, such as *Edaphobacter* at Jiangxi and *Conexibacter* at Zhejiang (Fig. S8), were considered. Since *Edaphobacter* is consistently reported as acidophilic [49], members of this clade are expected to proliferate in acidic forest soils, becoming soil specialists. As expected, OTUs associated as *Edaphobacter* were coherent soil specialists at the Zhejiang site. In comparison, some OTUs of *Edaphobacter* preferred either a PyOM or a soil habitat at the Jiangxi site (Fig. S8a). A corresponding phenomenon was also found for *Conexibacter*; some were presumed to be PyOM specialists because *Conexibacter* is neutrophilic [50], but some were found as soil specialists at Zhejiang (Fig. S8b). Such phylogenetic incoherence is likely caused by diversification (or speciation) [36], which is a universal mechanism for bacteria to adapt to their environment [51, 52].

Overall, both coherence and diversification shape niches of bacteria. Therefore, the contribution of coherence is relative to that of diversification under various scenarios where the ancestors' niche might be more or less conserved in the descendants. In addition to phylogenetic lineage characteristics, for example, more complex traits, such as pH range expansion and salinity preference, appear to be more conserved than less complex traits, such as viral resistance [47], which often evolves independently via

diversification [53]. Altogether, extensive recent observations of strong phylogenetic coherence in microbial studies [54] call for further examination and application of phylogenetic methods in microbial ecology, since phylogenetic coherence indicates microbial niche conservatism [55].

There are some limitations and caveats associated with our approach. First, the dynamics of meta-communities in soil are still largely unknown because we understand little about the dispersal of bacteria. While some studies have explored this topic [40], there is a lack of dispersal rates and mechanisms for different microorganisms in complex soil systems. Thus, future investigations are critically needed to address bacterial dispersal, especially following major soil/ecosystem perturbations. Second, diversification is a dynamic process that may generate coherent lineages during the long course of evolution, forming new species [56]. However, when clustering coherent lineages, we regarded diversification as static and independent of the phylogenetic tree. Thirdly, there were differences in other attributes besides PyOM between the burnt and unburnt soils at each site, such as differences in vegetation, litter, microclimate (i.e., soil water and temperature) and soil nutrient availability. These additional factors potentially shaped the soil microbiome [57] and might contribute to the differences associated with NMDS2 and NMDS3 (Fig. 4b and 4d) at the community level. Although these differences were mathematically orthogonal to NMDS1 (Fig. S4), they might substantially influence soil specialists. Furthermore, even though we collected phenotypical traits to the best of our knowledge, our communal understanding of the microbial universe is limited and some coherent lineages in Tables S2–S5 are without reliable taxonomy and/or phenotypical traits. These missing values may cause a bias in Table 1 and our analyses. Finally, the microbiome in burnt soils likely displays a temporal pattern during ecosystem succession following a fire event [4]. However, a previous study indicated that such a microbial succession would not last for more than 1 year [58].

Overall, this study bridges the gaps between observations of soil physical-chemical alterations by wildfire, microbial community perturbations from wildfire and ecological community assembly theories. A determinant role of PyOM in soil bacterial community assembly after wildfire perturbation was clearly demonstrated. PyOM microhabitats were distinctly different from those of the adjacent wildfire-perturbed soil and soil-PyOM heterogeneity was a strong selection factor for microbial assembly. Ecological dispersal likely contributed to the relatedness between PyOM and adjacent soils. The role of PyOM in bacterial community assembly in burnt soils was supported by reconstructing bacterial communities in burnt soils based on the specialists identified in PyOM and unburnt soil. A strong contrast in pH niche was found in coherent lineages preferring soil or

PyOM. Habitat preferences of bacterial taxa were associated with both their conserved niche and diversification, resulting in a relative coherence in different lineages. Our findings highlight that PyOM contributes a strong control on bacterial community assembly processes in burnt soils after wildfire perturbation through the selection and dispersal of specialists with divergent pH-niche preference in heterogeneous microhabitats.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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