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Limited Evidence for a Microbial Signal in Ground-Level Smoke Plumes

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Key Points:

- Using aerosol samples collected from the western US, we found that microbial DNA was not elevated in air masses impacted by wildfire smoke
- Wildfire smoke events did not consistently impact the amounts or types of bacteria and fungi in near-surface aerosol samples
- Contrary to expectations, we did not detect a microbial signal associated with wildfire smoke in the near-surface atmosphere

Supporting Information:

Supporting Information may be found in the online version of this article.

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Abstract Recent studies have suggested that microbial aerosolization in wildfire smoke is an understudied source of microbes to the atmosphere. Wildfire smoke can travel thousands of kilometers from its source with the potential to facilitate the transport of microbes, including microbes that can have far-reaching impacts on human or ecosystem health. However, the relevance of longer-range detection of microbes in smoke plumes remains undetermined, as previous studies have mainly focused on analyses of bioaerosols collected adjacent to or directly above wildfires. Therefore, we investigated whether wildfire smoke estimated to originate >30 km from different wildfire sources would contain detectable levels of bacterial and fungal DNA at ground level, hypothesizing that smoke-impacted air would harbor greater amounts and a distinct composition of microbes as compared to ambient air. We used cultivation-independent approaches to analyze 150 filters collected over time from three sampling locations in the western United States, of which 34 filters were determined to capture wildfire smoke events. Contrary to our hypothesis, smoke-impacted samples harbored lower amounts of microbial DNA. Likewise, there was a limited signal in the composition of the microbial assemblages detected in smoke-affected samples as compared to ambient air, but we did find that changes in humidity were associated with temporal variation in the composition of the bacterial and fungal bioaerosols. With our study design, we were unable to detect a robust and distinct microbial signal in ground-level smoke originating from distant wildfires.

Plain Language Summary There have been recent reports that microbes can be released into the atmosphere during wildfires with the expectation that these microbes, including both bacteria and fungi, could possibly be detected in distant smoke plumes. However, we do not yet know if there is a detectable microbial "signal" in the near-surface atmosphere in smoke beyond the wildfire zone and whether there are broader implications of this mode of microbial dispersal, including possible aerosolization of allergens and pathogens in smoke plumes. We leveraged pre-existing air sampling efforts to investigate multiple smoke events observed at three sites in the rural western United States. We compared samples collected from ground-level smoke-impacted air to "ambient" air (no elevated smoke) using techniques targeting the DNA of bacteria and fungi recovered from the air filters. We expected to find more microbes and distinct types of microbes in air samples that were impacted by wildfire smoke. Instead, our results revealed limited evidence for a smoke-associated microbial signal in the near-surface atmosphere. While we cannot definitively claim that microbes are not associated with smoke plumes originating from distant fires, the microbial signal in the sampled ground-level smoke plumes was below our limits of detection.

1. Introduction

Understanding the amounts and types of microbes that can be found in the near-surface atmosphere is important given that airborne microbes can have important impacts on human and ecosystem health (Fröhlich-Nowoisky et al., 2016), including serving as allergens (Kim et al., 2018; Walser et al., 2015) or animal and plant pathogens (Rodríguez-Fernández et al., 2023). More generally, there is a growing interest in studying microbial dispersal through the atmosphere as a means by which bacteria and fungi are transported across geographic regions (Maki et al., 2019; Rodríguez-Fernández et al., 2023; Walters et al., 2022). We know that the amounts and types of bacteria and fungi found in the atmosphere can be highly variable across space and time (Barberán et al., 2015; Bowers et al., 2011; Šantl-Temkiv et al., 2022), with this variation often attributable to differences in atmospheric

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conditions or differences in the relative contributions of microbial source environments to the atmosphere. Important source environments include leaf surfaces, soils, water bodies, and animals, which contribute distinct microbial taxa to the near-surface atmosphere (Xie et al., 2020). Importantly, not all taxa are equally capable of being aerosolized and transported in the atmosphere (Burrows et al., 2009; Tong & Lighthart, 1998).

Wildfires represent a potentially important means by which microbes can be aerosolized and transported through the atmosphere. Incomplete combustion of soils and vegetation, combined with the intense convection often associated with large wildfires (Wagner et al., 2018) lead to microbial aerosolization in smoke plumes. This phenomenon has led to the introduction of the term "pyroaerobiology" (Kobziar et al., 2018), which emphasizes that wildfires are an under-recognized source of microbes to the atmosphere. The potential for aerosolization of microbes during wildfires and downwind detection in the resulting smoke plumes is of broad relevance because microbial allergens and pathogens could potentially be transported through the atmosphere in smoke plumes with corresponding impacts on populations and ecosystems distant from the wildfire (Kobziar & Thompson, 2020). As the intensity and severity of wildfires are expected to increase in many regions across the globe (Ellis et al., 2022) and smoke can travel thousands of kilometers from the wildfire source (Baars et al., 2021) it is important to understand if such smoke plumes do indeed harbor microbes distinct from those found in non-smoke impacted air.

Our objective with this study was to test whether we could detect a microbial "signal" in smoke-impacted air samples distant from wildfire sources. The idea that microbes can be aerosolized and are detectable in wildfire smoke plumes is derived from multiple lines of evidence. First, smoke captured from the burning of vegetation in laboratory settings and in tobacco smoke have previously been shown to contain viable bacteria and fungi (Larsson et al., 2008; Malayil et al., 2022; Mirskaya & Agranovski, 2020; Pauly & Paszkiewicz, 2011). Likewise, the air inside homes where biomass fuels are used for cooking and heating have been found to contain higher concentrations of bacterial endotoxins (Akila et al., 2020). Second, air samples collected near prescribed burns or wildfires can harbor higher concentrations of bacteria and fungi than adjacent non-smoke impacted air samples (Wei et al., 2019). For example, microbial cell numbers in smoke plumes above or adjacent to wildfires were higher than in air samples collected from outside the smoke plumes (Kobziar et al., 2022; Moore et al., 2021). Third, the composition of bacterial and fungal assemblages found in smoke plumes sampled near fire events appear to be distinct from those found in adjacent non-smoke impacted air samples (Kobziar et al., 2022). Finally, there is evidence that smoke originating from biomass burning can contain higher concentrations of fungi and fungal tracers (Mims & Mims, 2004; Yang et al., 2012). Together, these lines of evidence suggest that microbes are indeed aerosolized and emitted into the atmosphere from fire events, but what remains undetermined is whether these microbes are still evident as smoke plumes reach distant locations, that is, whether smoke-associated microbes can still be detected at the surface at sites located far from the wildfire event.

We hypothesized that there would be higher total concentrations of microbial DNA and different types of bacteria and fungi, in air samples impacted by wildfire smoke than in non-smoke impacted air, with this microbial "signal" evident in ground-level aerosol samples collected at substantial distances from the wildfire source. We analyzed time-series collections of air filters from three independent sampling campaigns spanning multiple years that all captured smoke events, with the sampled smoke plumes estimated to have originated from wildfires that occurred 30–1,400 km away from the sampling site (Table S1 in Supporting Information S1). We then used cultivation-independent DNA-based analyses (quantitative PCR and marker gene sequencing) to quantify the amounts and types of bacteria and fungi found in smoke-affected versus non-smoke impacted air samples to determine whether we could detect smoke-associated microbes in the near-surface atmosphere. We found limited evidence to support our hypotheses, suggesting that, although a microbial signal might be evident in smoke sampled in close proximity to wildfire sources, such a signal is obscured, or at least below our threshold for detection, as smoke plumes are diluted across longer distances.

2. Materials and Methods

2.1. Sample Collection

Three independent atmospheric aerosol collection efforts were included in this study as we sought to assess the potential for microbial detection in smoke across multiple spatial and temporal scales (150 samples in total, with each of the three sample sets analyzed independently). We leveraged existing samples from sampling campaigns and a national air monitoring program to capture a diverse range of different wildfire-driven smoke

events with a high degree of variation in smoke impacts. The Boulder Lake, Wyoming (BOLA1) samples were collected by the Interagency Monitoring of Protected Visual Environments (IMPROVE) Network (IMPROVE, 2020; BOLA1 Site) from July 2 to 29 November 2020. BOLA1 filters were polytetrafluoroethylene (PTFE) filters fitted to a sampling apparatus with a PM_{10} inlet. Filters were used for gravimetric mass measurements of particulate matter (PM_{10}). The monitoring network sampled the air at ∼17 L min^{-1} for a 24-hr period (midnight to midnight). Samples are taken every third day with the samplers positioned ∼4 m above the ground. Notably, the IMPROVE network utilizes four separate filtering apparatuses with $PM_{2.5}$ particles collected on PTFE, nylon, and quartz for chemical analysis. For microbial analysis we analyzed DNA recovered from the PM_{10} air filters that are collected concurrently alongside all $PM_{2.5}$ filters used for chemical analyses. The PM_{10} air filters undergo strict handling protocols for chemical analysis to minimize contamination at each step of the process. This includes avoiding physical contact with the surface of filters, never leaving the filters exposed, and storing the filters in airtight containers. The BOLA1 samples were archived at room temperature for ∼10–14 months, and then shipped and stored at −20°C for 6 months until DNA was extracted for the microbial analyses. The aerosol chemical data associated with each sampling event were provided by IMPROVE and the relevant chemical metrics included in our study were $PM_{2.5}$ mass, organic carbon (OC), elemental carbon (EC) and PM_{10}, mass concentrations (Malm et al., 1994, 2011; Solomon et al., 2014). The IMPROVE chemical data used in this study can be accessed and downloaded from: http://views.cira.colostate.edu/fed/default.aspx. Summarized site and sample details are found in Table S2 of Supporting Information S1 and more comprehensive sample metadata, including chemical smoke proxies, can be found in Table S7.

Grand Teton National Park samples were collected as part of a previous study (Benedict et al., 2013) from August 1 to 21 September 2011, at two locations, the NOAA Climate Center (NC) near Moose Junction, Wyoming (GT-East) and the Grand Targhee Resort (GT-West). The filter samples from these two sites were collected daily for 24 hr using a high-volume sampler (Themo Anderson) that stood ∼1.3 m above the ground. The samplers draw ambient air at ∼850 L min^{-1} through a two-filter assembly to isolate the ambient aerosol into particles with aerodynamic diameters greater than and less than 2.5 μm. An impactor in combination with a slotted filter collects particles greater than $PM_{2.5}$, followed by a Whatman quartz filters (8"x10") to collect the $PM_{2.5}$ fraction. Only the $PM_{2.5}$ filters were analyzed for this study. The filters were wrapped in aluminum foil and pre-baked for 12 hr at 550°C before air sampling commenced. Blank samples were collected by loading a filter into the sampler for 2 min without air flow. Punches were taken from each filter to perform the chemical analyses, with measurements of OC, EC, and levoglucosan conducted as described previously (Sullivan et al., 2014, 2019; Sullivan, Frank, Kenski, & Collett, 2011; Sullivan, Frank, Onstad, et al., 2011). The filters were stored at −20°C for ∼10–11 years before subsampling for DNA extractions and microbial analyses. For all three sites included in this study (BOLA1, GT-West, and GT-East), we downloaded daily meteorological data from Visual Crossing Weather (Visual Crossing, 2022) for the sample collection periods at each site. Meteorological data used for analyses included minimum and maximum daily temperatures, average daily temperature, relative humidity, precipitation, wind gust, wind speed and wind direction. Site and sample summary details can be found in Table S2 of Supporting Information S1 and Table S7.

2.2. DNA Extractions

We extracted DNA from all air filters, and the corresponding field blanks, using the DNeasy Powersoil Pro Kit (Qiagen). We prepared the filters by cutting ½ of the 25 mm PTFE filter from BOLA1 and one 25 mm quartz filter punch from GT-West and GT-East filters with flame-sterilized scissors to ensure the filter would fit into microcentrifuge tubes for extraction. All filter sections were individually loaded into 2 mL screw cap microcentrifuge tubes, pretreated with 0.5–1 mL filter-sterilized phosphate buffer saline and Tween-80 (1%), and vortexed for 5–10 min. The resulting supernatant was then transferred into the 2 mL Powerbead Pro Tubes included with the DNeasy Powersoil Pro extraction kit to which we added 800 μL of solution CD1 and heated for 30 min at 65°C. We followed the manufacturer's extraction protocol with two modifications—the full volume of supernatant was transferred at every step to maximize DNA yields and the DNA was eluted into 50 μL rather than 100 μL DNase/RNase-free water at the final step to concentrate the DNA. Our modified DNA extraction protocol added in a wash buffer and a heating step, rather than bead beating alone, as recommended to improve DNA yields from low biomass aerosol samples (Luhung et al., 2021). Extraction blanks ($n = 16$) were included with every batch of DNA extractions to check for potential contaminants introduced during the extraction process. DNA yields were

visualized with PCR and gel electrophoresis and quantified using quantitative PCR (described below). DNA was stored at −20°C for downstream processing.

2.3. Marker Gene Amplicon Sequencing

To characterize the bacterial and fungal communities associated with the air samples, extracted DNA was PCR-amplified using barcoded primer pairs to permit multiplexing using primers targeting the hypervariable V4 region of the 16S rRNA bacterial gene (Caporaso et al., 2012) and barcoded primer pairs targeting the fungal internal transcribed spacer region (Emerson et al., 2015). PCRs were prepared in duplicate in 25 μL reaction volumes consisting of 12.5 μL Platinum™ II Hot-Start PCR Master Mix (Invitrogen, Carlsbad, CA, USA), 7.5 μL PCR H_2O, 1 μL of each 10 μM primer, and 4 μL of template DNA. PCR cycling conditions were at 94°C for 2 min, followed by 35 cycles of 94°C for 15 s, 60°C for 15 s and 68°C for 1 min with the final extension step at 72°C for 10 min. All samples were cleaned and normalized using the SequalPrep normalization kit (Thermo Fisher Scientific, Carlsbad, CA, USA) and pooled in equimolar concentrations to yield a ∼2 nM library for sequencing. Pooled libraries were sequenced on separate Illumina MiSeq runs (Illumina, California, USA) using a 2 × 150bp cycle kit for 16S rRNA sequencing of bacteria and the 2 × 250bp cycle kit for ITS sequencing of fungi at the Center for Microbial Exploration at the University of Colorado Boulder. In total, we extracted and sequenced 150 air samples, four field blanks (only available from GT-West), six PCR blanks, and 14 DNA extraction blanks. We included all of our associated blanks to identify potential contaminants in our pipeline.

Raw sequencing reads were processed through the DADA2 pipeline (v. 4.1., Callahan et al., 2016). Merged reads were quality filtered and clustered into amplicon sequence variants (ASVs) at 100% sequence identity. Bacterial taxonomy was assigned using a Bayesian Classifier (Wang et al., 2007) against the SILVA reference database (v. 138.1., Quast et al., 2012; Yilmaz et al., 2014) and fungal taxonomy was assigned against the UNITE reference database (v. 10.05.2021, Nilsson et al., 2019). Any samples or blanks with <1,000 reads were removed from downstream analyses along with those ASVs represented by <10 reads in total across all samples. For the bacterial analyses, this initial filtering threshold resulted in a final sample size of 150 air samples, two field blanks, three PCR blanks, and 12 extraction blanks (6,475,621 total reads). For the fungal analyses, the filtering threshold resulted in a final sample size of 150 air samples, two field blanks, zero PCR blanks and eight extraction blanks (3,470,739 total reads).

2.4. Processing of Sequence Data

For the downstream analyses of the bacterial 16S rRNA gene sequence data, we furthered filtered out bacterial reads associated with chloroplasts (751,334 reads; 265 ASVs), mitochondria (857,091 reads; 1,185 ASVs), and unidentified phyla (41,556 reads; 101 ASVs), retaining a total of 4,825,640 reads. The associated "blanks" contained 61,501 out of 4,825,640 total bacterial reads, with 181 ASVs shared between the blanks and all air samples out of a total of 17,989 ASVs. The number of reads associated with blanks made up 1.27% of reads associated with the air samples. We identified six ASVs, summed >2,000 reads across multiple blanks, which removed 8.36% of total reads across the entire bacterial data set (Table S6 in Supporting Information S1). The final bacterial 16S rRNA gene read count totaled 4,422,034 (4,391,782 only air) with median reads per blank of 1,142, and median reads per air sample of 29,268. We followed a similar filtering threshold for the fungal ITS sequence data, retaining only those samples with >1,500 reads and ASVs with >10 reads across all samples. After the initial filtering step, we had 3,470,739 ITS reads. We then filtered out unidentified phyla (32,383 reads; 299 ASVs). The associated "blanks" contained 26,807 reads out of 3,438,356 total fungal reads, with 69 ASVs shared between the blanks and all air samples out of a total of 8,386 ASVs. The number of fungal reads associated with blanks made up 0.78% of reads. Two fungal ASVs found across multiple blank samples with >1,500 reads were identified as lab-associated contaminants, which removed 2.6% of total reads across the fungal data set (Table S6 in Supporting Information S1). The final read count associated with our fungal data totaled 3,348,555 reads with median reads per blank of 1,687 and median reads per aerosol sample of 21,914.

2.5. Quantitative PCR

For estimating microbial biomass, we quantified bacterial and fungal gene copies using quantitative polymerase chain reaction (qPCR) on a Bio-Rad CFX Connect real-time system (Bio-Rad Laboratories, Hercules, CA, USA).

We used universal bacterial primers to target the 16S rRNA gene with 515f/806r (10 μM) and fungal primers FF390f/FR1r (10 μM) to target the fungal ITS region with primers and methods for qPCR previously described (Emerson et al., 2015). Both bacterial and fungal reaction mixtures were performed separately in 25 μL total reactions containing 12.5 μL 2X master mix (Thermo Scientific SYBR Green), 1.25 μL of forward and 1.25 μL of reverse primers, 6 μL of PCR H_2O and 4 μL of gDNA. We included 2 no-template controls on each 96-well plate and generated standard curves using genomic DNA from *Escherichia coli* to calculate bacterial genome equivalents ($R^2 = 0.98$) and *Aspergillus fumigatus* for fungal genome equivalents ($R^2 = 0.98$). Bacterial qPCR thermocycling conditions were as follows: 95°C for 15 min, with 40 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 1 min, and extension at 72°C for 1:30 min followed by a final extension step at 72°C for 10 min. Fungal qPCR thermocycling conditions were the same as bacterial, except we increased the annealing temperature from 50°C to 55°C for 1 min. Cycle quantity values (Cq) for samples above 29 for bacteria and 31 for fungal were excluded from microbial abundance analyses as they were below detection limits (this threshold also excluded all negative controls). The final sample size used for qPCR microbial abundance analyses was the following: (BOLA1 = 37, GT-West = 48 and GT-East = 44) and for fungi was BOLA1 = 43, GT-West = 50 and GT-East = 49. All qPCR results are reported as genome equivalents (either *E. coli* or *A. fumigatus*), but interpreted as estimates of total bacterial and fungal DNA per m^{-3} of air (Emerson et al., 2015).

2.6. Microbial Analysis and Smoke Associated Taxa

We conducted all downstream data analyses in R (v. 4.2.2, R Core Team, 2022) and treated each sample set as independent data sets because samples from each site were collected across different years and locations. The R packages used for data analyses included *mctoolsr* (v. 0.1.1.9, Leff, 2016), *tidyverse* (v. 1.3.2, Wickham et al., 2019), *dplyr* (v. 1.1.0, Wickham et al., 2023), *vegan* (v. 2.6–5, Oksanen et al., 2023) and all visualizations were done with *ggplot2* (v. 3.4.0, Wickham, 2016) and *ggpubr* (v. 0.6.0, Kassambara, 2023). We rarefied samples for bacterial and fungal alpha diversity analysis with bacterial data sets rarefied to 5,000 sequences per sample and fungal data sets rarefied to 3,000 sequences (BOLA1), and 10,000 sequences (GT-East and GT-West). All other microbial analyses were performed on non-rarefied data to retain as many reads as possible (McMurdie & Holmes, 2014).

To test for statistically significant differences at all of the sites, we performed Wilcoxon Rank Sum statistical tests on microbial abundance and richness between smoke and non-smoke samples. We ran Pearson correlations to determine whether there was a linear relationship between elevated concentrations of OC and richness and abundances of microbes. We used permutational multivariate analysis of variance (PERMANOVA) with the "adonis2" function in *vegan* (Oksanen et al., 2023) for comparisons of microbial community composition between smoke and non-smoke samples and tested for the influence of abiotic and environmental factors on microbial community composition using multiple regression on distance matrices (MRM, Lichstein, 2007). Environmental data were scaled prior to applying MRM using *ecodist* (v. 2.0.9, Goslee & Urban, 2007). We sought to identify smoke associated taxa by setting a threshold that one ASV had to appear in a minimum of three smoke events within each site. We conducted Kruskal Wallis (Kruskal & Wallis, 1952) tests and corrected the p-values for multiple comparisons using the false discovery rate (FDR) post hoc test (Benjamini & Hochberg, 1995) to identify ASVs found to be significantly more abundant in smoke than in non-smoke samples.

3. Results

3.1. Sample Set Description

We analyzed 150 air filters collected from three different locations across two different years in Wyoming (USA) (Figure 1). Samples were collected over 24-hr intervals at each site for 1–5 months with total volumes of filtered air ranging between 2.34×10^4 to 2.74×10^6 L of air per sample (Table S2 in Supporting Information S1). We identified "smoke impacted" samples using a combination of chemical proxies and overhead satellite imagery for the validation of smoke events. For BOLA1, GT-West and GT-East, we considered "smoke impacted" samples to be those where OC concentrations exceeded 3.5 μg C m^{-3} (Figures 2a–2c), noting that OC is a significant fraction of the total $PM_{2.5}$ mass concentration and elevated $PM_{2.5}$ mass concentrations were correlated with the presence of wildfire smoke in a recent study of smoke health impacts (Childs et al., 2022). We cross-validated the OC-based inferences of smoke events with satellite imagery published by the National Oceanic and Atmospheric

Figure 1. Map of the western United States showing the three sampling sites. Each site is labeled by location: Boulder Lake, Wyoming (BOLA1), Grand Teton National Park West (GT-West), and Grand Teton National Park East (GT-East). Map dimensions span 40°–44°N and 107°–114°W.

Administration Hazard Mapping System (NOAA, 2022) to confirm that overhead smoke plumes were evident at the sampling locations on the respective sampling dates. For the BOLA1 samples, measured $PM_{2.5}$ concentrations were strongly correlated with OC concentrations ($R^2 = 0.99$, Table S3 in Supporting Information S1). We set a conservative minimum OC threshold at 3.5 μg C m^{-3} and applied the same strategy to both GT-West and GT-East where we only had OC measurements available to serve as a smoke proxy (Table S7). Our OC thresholds were secondarily validated using overhead satellite imagery of smoke, recognizing that NOAA's Hazard Mapping System cannot confirm smoke at ground level and cloud cover could obscure smoke events (Brey et al., 2018). We also note that the measured chemical proxies for smoke (OC) were strongly correlated across each sample set with other chemical indices including $PM_{2.5}$ (as mentioned above), PM_{10}, and levoglucosan concentrations (Table

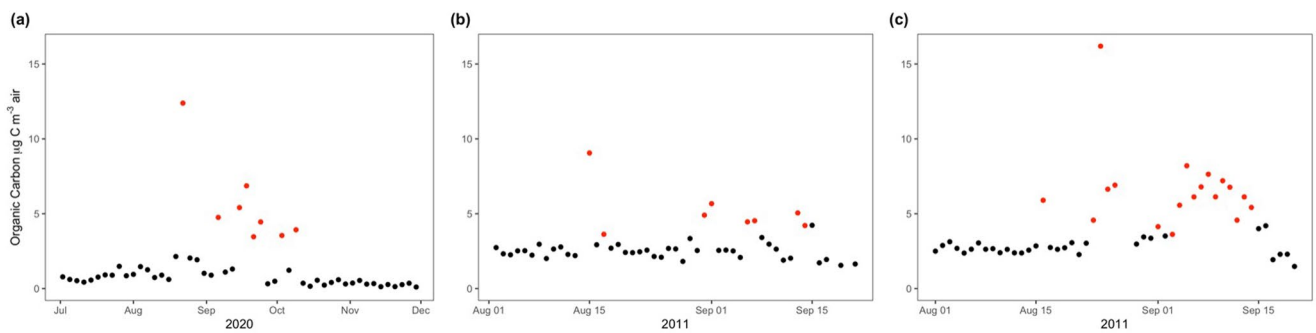

Figure 2. Temporal variation in chemical proxies for smoke presence at the sampling sites. (a) BOLA1, (b) GT-West, and (c) GT-East. Organic carbon (>3.54 μg C m^{-3}) thresholds were used to identify smoke events over time. The samples that were identified as impacted by smoke plumes ("smoke" samples) are indicated in red and the non-smoke impacted ("ambient") air samples are indicated in black.

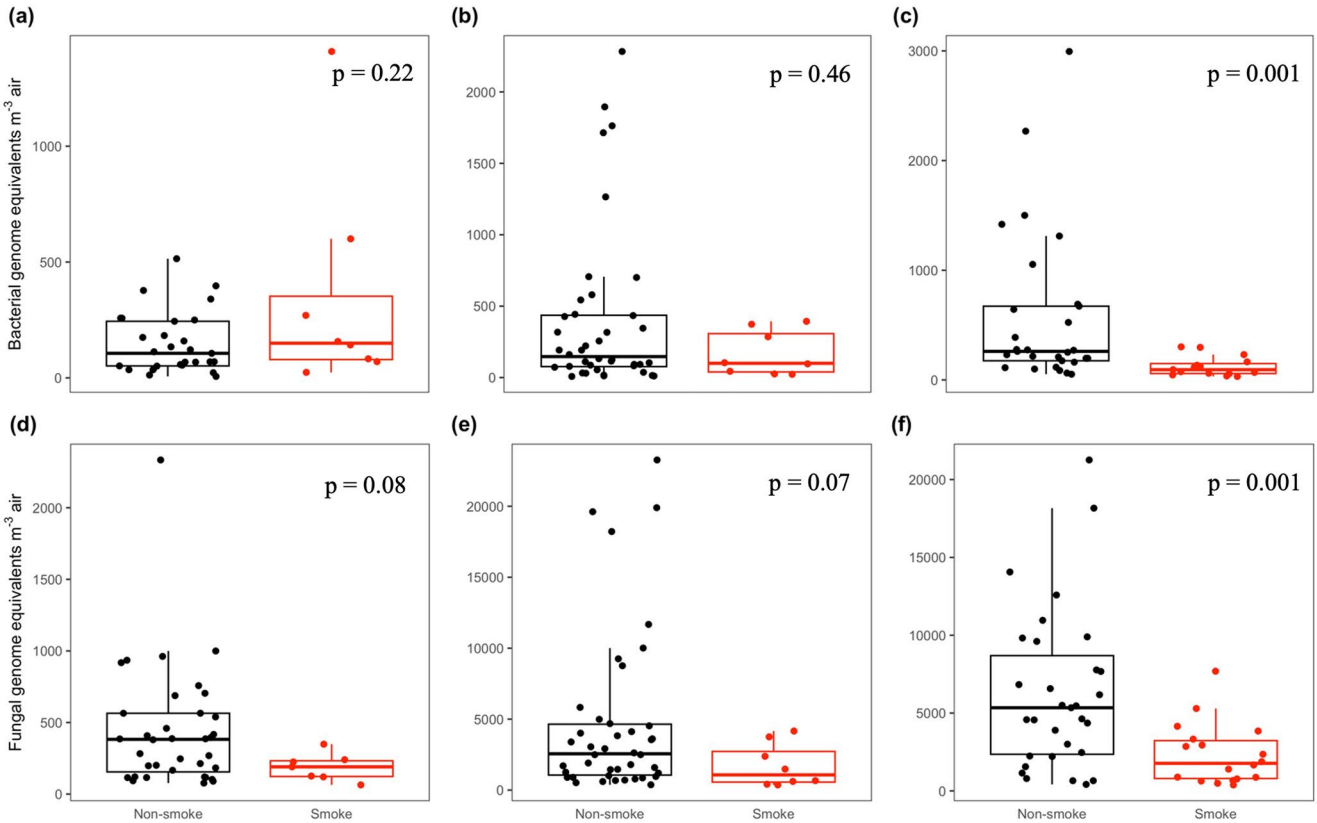

Figure 3. Quantitative PCR-based measurements of bacterial and fungal DNA concentrations between non-smoke and smoke impacted samples across all three sites. Panels (a–c) include bacterial quantitative polymerase chain reaction (qPCR) results and panels (d–f) include fungal qPCR results. Panels (a and d) = Boulder Lake, Wyoming (BOLA1), (b and e) = Grand Teton National Park West (GT-West), and (c and f) = Grand Teton National Park East (GT- East). P values to assess the statistical significance of observed differences between non-smoke and smoke impacted samples were determined using Wilcoxon rank sum exact tests.

S3 in Supporting Information S1). Thus, using the chemical proxies of smoke concentration and the satellite imagery associated with each sampling date where smoke plumes were captured at the sampling sites, we were able to establish that 34 of the 150 samples had captured smoke events (Figure 2) and that those 34 smoke events likely originated from wildfires occurring at least 30 km away from the respective sampling locations (Table S1 in Supporting Information S1). However, the number of smoke events captured per site varied depending on the site in question (BOLA1 = 8, GT-West = 8, GT-East = 18). Importantly, we recognize the limitations of using pre-determined threshold concentrations of smoke proxies to categorize samples into either "smoke-impacted" or "ambient" aerosol samples. For this reason, we complemented the categorical analyses with correlation-based analyses where we compared microbial metrics directly against measured concentrations of the smoke proxies, as detailed below.

3.2. Microbial DNA Concentrations

We hypothesized that air samples which captured smoke events would have higher concentrations of microbial DNA than the non-smoke "ambient" samples. This hypothesis was not supported by our results. We found significant differences in the qPCR-based estimates of bacterial and fungal DNA concentrations between smoke and non-smoke impacted samples only from GT-East (Figures 3c and 3f). However, in GT-East the pattern was the opposite of what we expected as bacterial and fungal DNA concentrations were lower in smoke samples than in non-smoke samples (Wilcoxon rank sum test, $p < 0.001$, Figures 3c and 3f). The results from our comparisons between "smoke" and "non-smoke" samples were consistent with correlation-based analyses where we compared bacterial and fungal DNA concentrations to inferred smoke proxy concentrations (using measured OC concentrations as a proxy for smoke concentrations). No significant correlations were found between bacterial or fungal DNA concentrations and OC concentrations at any of the three sampling locations. With the exception of

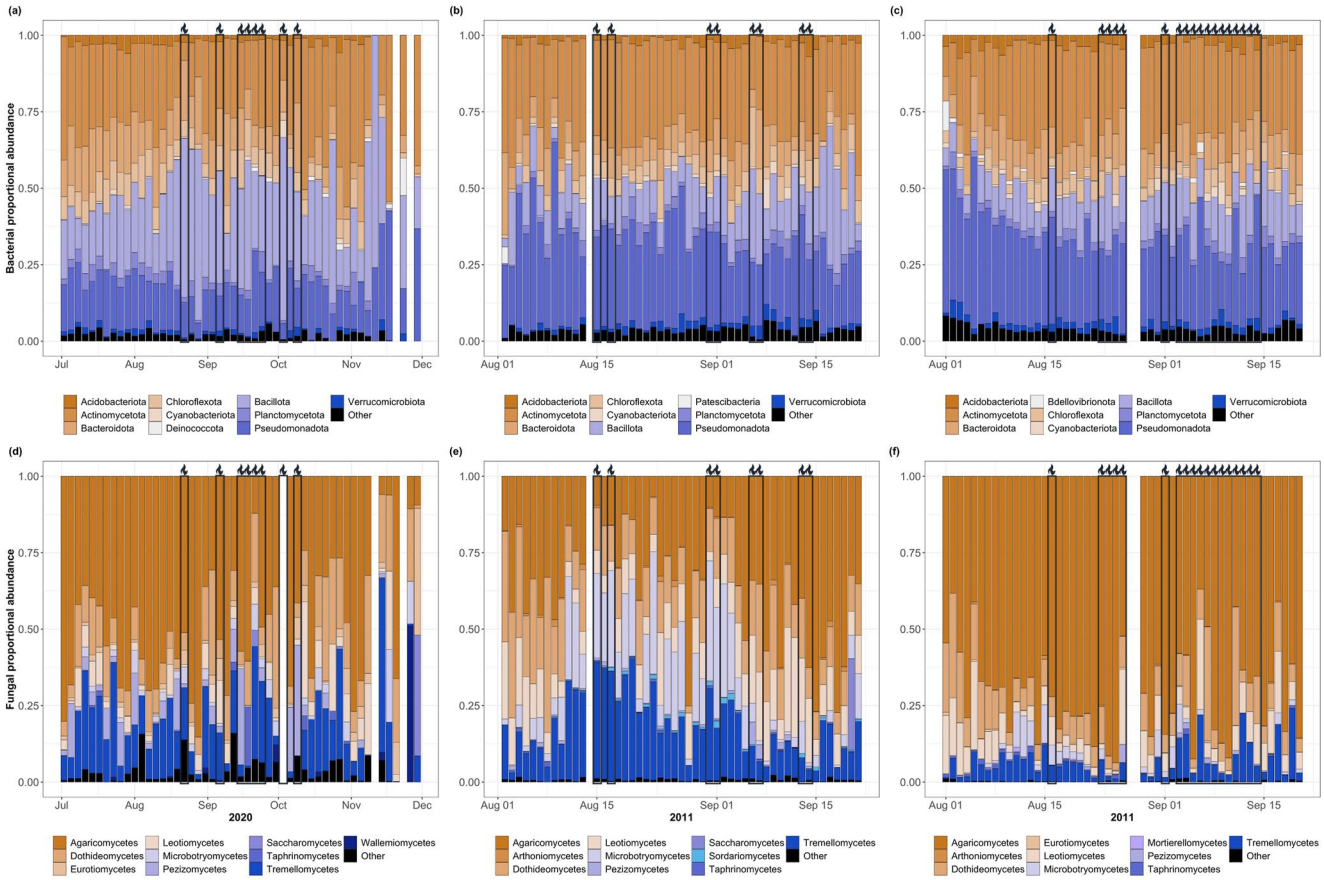

Figure 4. Changes in the proportional abundances of bacterial phyla and fungal classes across all four sites over time. Panels (a–c) include bacterial results and panels (d–f) include fungal results. Panels (a and d) = Boulder Lake, Wyoming (BOLA1), (b and e) = Grand Teton National Park West (GT-West), and (c and f) = Grand Teton National Park East (GT- East). Smoke events are outlined by dark gray borders capped with gray fire symbols.

bacterial abundances at BOLA1, all of the other sites were negatively correlated with OC concentrations (Table S4 in Supporting Information S1).

3.3. Bacterial and Fungal Richness

We hypothesized that air samples that captured smoke events would have a greater diversity of taxa detected in smoke as compared to corresponding non-smoke impacted samples collected from the same locations. We did not detect a higher diversity of bacterial or fungal taxa in smoke-impacted samples compared to the corresponding non-smoke impacted air samples (Figure S1 in Supporting Information S1). At GT-East, bacterial richness was significantly lower in smoke than in non-smoke samples (Wilcoxon, $p = 0.029$, Figure S1c in Supporting Information S1), while fungal richness was significantly lower in the smoke impacted samples than in the non-smoke impacted air samples at BOLA1 and GT-East (Wilcoxon, $p < 0.05$, Figures S1d and S1f in Supporting Information S1). Likewise, if we consider smoke concentrations as a continuous variable (inferred from the measured OC concentrations), we found no significant correlations between smoke concentrations and either bacterial or fungal richness levels. Our results suggest that these ground-level smoke events are not contributing additional microbial diversity to the sampled air masses.

3.4. Taxonomic Structure of the Bacterial and Fungal Assemblages

We found appreciable temporal variation in the composition and relative abundances of the bacterial and fungal taxa detected in the collected samples (Figure 4). Overall, the air samples were dominated by the bacterial phyla Actinomycetota, Pseudomonadota, Bacillota, Bacteroidota, and Chloroflexota (Figures 4a–4c), while the fungal

assemblages were dominated by Basidiomycota followed by Ascomycota, specifically, the fungal classes of Agaricomycetes, Tremellomycetes, Dothideomycetes and Leotiomycetes (Figures 4d–4f). We found significant differences in the microbial assemblages between smoke and non-smoke impacted samples at GT-East for bacteria (PERMANOVA, $R^2 = 0.025$, Pseudo-F = 1.21, $p = 0.03$) and BOLA1 and GT-East for fungi (PERMANOVA, $R^2 = 0.03$, Pseudo-F = 1.53, $p = 0.003$ and $R^2 = 0.04$, Pseudo-F = 2.07, $P = 0.001$, respectively). However, the variation in the bacterial and fungal assemblages within sites was weakly associated with the presence of smoke ($R^2 < 0.03$), instead the composition of the bacterial and fungal assemblages was more strongly associated with variation in local atmospheric conditions (as explained below). These minimal effects of smoke plumes on bacterial and fungal assemblages are also qualitatively evident in Figure 4, which shows that the proportional abundances of major taxa varied temporally within each sample set, but the samples that captured smoke events do not stand out as consistently unique. Proportional abundances of all bacterial phyla and fungal classes per site can be found in Tables S8a–S8f.

To complement the analyses described above, we also examined the correlation between the amount of smoke on a given sampling date (inferred from OC concentrations) and the dissimilarity in assemblage composition. We found that heavier smoke days and days with little to no smoke had assemblages that were no more dissimilar than would be expected by chance (MRM, $R^2 < 0.01$ and $p > 0.1$ in all cases, except $p = 0.08$ for fungi at BOLA1, Table S5 in Supporting Information S1). Despite observing variation by site regarding the effect of smoke on the overall composition of bacterial and fungal assemblages, the compositional shifts observed over time at BOLA1 were most strongly associated with differences in humidity and air temperature (MRM - $R^2 > 0.15$, $p < 0.001$, Table S5 in Supporting Information S1), which we attribute to the seasonal variation captured with the longer time series of the BOLA1 samples. Humidity best explained the variation in fungal assemblages at GT-West and GT-East (MRM—$R^2 > 0.21$, $p < 0.001$, Table S5 in Supporting Information S1). These results highlight that the composition of bacterial and fungal assemblages was not random, nor was smoke the main driver, but that other meteorological factors best explained the observed variation (Table S5 in Supporting Information S1).

3.5. Smoke-Associated Taxa

To further investigate the potential effects of smoke events on the types of microbes found in the near-surface atmosphere, we determined if there were specific taxa that might be consistently more abundant in smoke versus non-smoke samples. These analyses were conducted on each sample set independently. Of the ASVs that met our criteria for inclusion in these analyses (see Methods), only a very small fraction of ASVs were significantly associated with smoke events (Table 1) and these taxa were more likely to be rare, accounting for <0.5% of the overall abundance at each site with minimal taxonomic overlap between sites. At BOLA1, out of 6,630 bacterial ASVs and 2,318 fungal ASVs detected across all samples, only seven bacterial ASVs and one fungal ASV met our statistical threshold for being considered significantly more abundant in smoke versus non-smoke samples (Kruskal Wallis, FDR corrected $p < 0.05$). These included ASVs assigned to the bacterial genera *Thermoactinomyces*, *Segetibacter*, *Blastococcus*, *Solibacillus*, *Ammoniphilus*, and *Lysinibacillus* and one fungal genus *Phanerochaete* (Table 1). At GT-West, we identified six bacterial ASVs and one fungal ASV (out of 7,432 bacterial and 3,928 fungal ASVs in total) that were found to be associated with smoke events, including bacterial ASVs assigned to the following groups: *Solibacillus*, Thermoanaerobaculaceae, *Pseudomonas*, *Amnibacterium*, *Intrasporangium*, and *Dydobacter*, and one fungal ASV assigned to the genus *Taphrina* (Table 1). We did not identify any bacterial or fungal ASVs significantly associated with smoke (out of 7,040 bacterial and 3,730 fungal ASVs in total) at GT-East (Kruskal Wallis, FDR corrected $p > 0.05$), despite there being 18 smoke events to measure at that site (Figure 2).

4. Discussion and Conclusion

There is growing interest in determining whether wildfires represent an important (and previously unrecognized) source of microbes to the atmosphere (Kobziar & Thompson, 2020; Kobziar et al., 2018), with wildfire smoke events representing a potentially important mode of microbial dispersal. However, we found limited evidence for a smoke-associated microbial signature in diluted smoke sampled near the ground surface. We expected to find elevated bacterial and fungal abundances in smoke-impacted samples as compared to non-smoke impacted samples, but this was not the case and, in fact, we observed lower concentrations of bacteria and fungi in the smoke-affected samples at some of the sites (Figure 3). This may indicate that smoke or the environmental

Table 1
Taxa Identified as Being "Smoke Associated" and the Differences in Their Relative Abundances Between Smoke-Impacted and Non-Smoke Air Samples

	Site	Phylum	Genus	Non-smoke, %	Smoke, %
Bacteria	BOLA1	Bacillota	*Thermoactinomyces*	0.05	0.23
		Bacillota	*Solibacillus*	0.01	0.02
		Bacillota	*Ammoniphilus*	0.01	0.08
		Bacillota	*Lysinibacillus*	0.08	0.37
		Actinomycetota	*Blastococcus*	0.00	0.10
		Bacteroidota	*Segetibacter*	0.01	0.50
Fungi		Basidiomycota	*Phanerochaete*	0.08	0.49
Bacteria	GT-West	Bacillota	*Solibacillus*	0.00	0.10
		Acidobacteriota	Thermoanaerobaculaceae *Subgroup 10*	0.00	0.04
		Actinomycetota	*Amnibacterium*	0.00	0.15
		Actinomycetota	*Intrasporangium*	0.00	0.10
		Bacteroidota	*Dyadobacter*	0.00	0.06
		Pseudomonadota	*Pseudomonas*	0.01	0.05
Fungi		Ascomycota	*Taphrina*	0.00	0.11

conditions surrounding smoke events diminishes the diversity and abundance of microbes found in the aerobiome, a result that contradicts our initial expectation. Likewise, we expected to find that smoke plumes harbored distinct amounts and types of bacteria and fungi as compared to non-smoke impacted air, and while we observed some significant differences at two sites for fungi, and one site for bacterial, based on our PERMANOVA results (Figure 4), there was limited evidence to support a distinct smoke-associated microbial assemblage. Instead, we observed that changes in microbial composition over time was most strongly associated with changes in relative humidity—(Table S5 in Supporting Information S1), a result that is consistent with results from other studies which have examined temporal dynamics of microbes in the near-surface atmosphere (de Groot et al., 2021; Gusareva et al., 2019). We were able to identify a small number of taxa that were indicative of smoke, but it is important to point out that these taxa were generally rare, and only evident in two of the three sites (Table 1). At one of the sites (GT-East) where we had samples from 18 smoke events, we did not identify a single bacterial or fungal taxon associated with smoke. However, we do want to highlight that one smoke-associated bacterial group (members of the genus *Solibacillus*) were found to be indicative of smoke at both the BOLA1 and GT-West locations. Although this taxon was detected in low abundances (representing 0.02%–0.10% of 16S rRNA gene reads in smoke-impacted samples from these two sites), further investigation of this group and its potential link to wildfire smoke are warranted as this organism has been noted in other aerobiome studies (Ruiz-Gil et al., 2020).

Our results challenged our expectations that a clear and distinct microbial signal would be detectable in smoke originating from distant wildfire sources, especially considering that previous research has demonstrated the capacity for long-range transport of fungal pathogens and dust-associated microbes (Maki et al., 2019; Nicolaisen et al., 2017). We note that previous studies which have detected a microbial signal associated with wildfire smoke (Kobziar et al., 2018, 2022; Moore et al., 2021) collected samples above or in close proximity to wildfire events where there is a high degree of convection and atmospheric turbulence associated with wildfire events (Heilman, 2023). In contrast, the samples we analyzed captured smoke events originating from distant wildfire sources where the smoke was diluted at ground level. While we did not investigate transport along a smoke plume, account for the variation of smoke distance traveled, or smoke source, we expected to find a detectable microbial signature associated with smoke events. It is possible that the microbial signal in smoke is attenuated as smoke plumes travel through the atmosphere, either due to the removal or degradation of microbes (and their DNA) during transport or simply because any microbes that may be present in the smoke are diluted to the point where the signal becomes undetectable above background temporal variation in non-smoke impacted air conditions. Either way, although bioaerosol samples collected in close proximity to wildfire events may exhibit a measurable

microbial signal (Kobziar et al., 2022) a microbial signature in ground level samples collected in smoke further from the wildfire events was of insufficient magnitude to be evident above background temporal variation.

There are some important caveats associated with our study. First, we did not differentiate between viable and non-viable microbial cells with our DNA-based methods. It is possible that smoke harbors more viable cells, or different types of viable taxa, than would be found in non-smoke impacted air samples. Second, we sampled smoke plumes in the near-surface atmosphere where the dilution of smoke may have obscured any potential microbial signal that might be evident in samples collected from less diluted smoke plumes. Third, we took advantage of sample sets that happened to capture smoke events and there was important underlying variation in the types of wildfire events, the distances from the wildfire source, smoke concentrations, and the background atmospheric conditions across each of the sampling campaigns. Fourth, the samples used in this study were not initially collected for microbial analyses. Instead, we leveraged existing sample sets to try to capture the variability associated with wildfire smoke originating from different sources.

We recognize the limitations associated with using archived aerosol samples, including a lack of field blanks distributed sufficiently across our sample sets as well as potential degradation of DNA over extended periods of time on preexisting air filters. To account for this underlying concern, we applied a series of quality control measures consistently across all samples during our microbial processing pipeline and were able to identify meteorological variables associated with the microbial patterns reported above. However, we do acknowledge that there are underlying sources of variability that we could not account for with our study design that could have contributed to challenges we had in identifying a clear microbial signal associated with ground-level smoke events.

Overall, the field of "pyroaerobiology" needs more evidence to support the emerging idea that long range microbial aerosolization in smoke plumes is of ecological significance. We cannot rule out the possibility that there are differences between the amounts and types of microbes observed in smoke-impacted versus non-smoke impacted air, but those differences were not detectable with our study design. More extensive and intentional microbial sampling of smoke plumes from different wildfire events spanning different vegetation types, fire intensities, and smoke concentrations would be valuable. Moving forward, it would be useful for future studies to collect samples from plumes at varying distances from wildfire events (or sampling individual plumes as they move through the upper atmosphere) to better assess the importance of microbial dispersal in smoke and the smoke concentrations at which smoke-associated microbes might be detectable.

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Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

The bacterial and fungal data presented in this study are available at the Sequence Read Archive through the National Center for Biotechnology Information (NCBI) under BioProject accession PRJNA1066330 (Gering, 2024).

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Erratum

The originally published version of this article contained a few typographical errors in the online version. The PDF was unaffected. In Section 3.1 the figure citation "(Figures 2a, 2b and 2d)" in the third sentence should be changed to "(Figures 2a–2c)." Also in Section 3.1, the sentence beginning "We set a conservative minimum OC threshold . . ." should be changed to "We set a conservative minimum OC threshold at 3.5 μg C m^{-3} and applied the same strategy to both GT-West and GT-East where we only had OC measurements available to serve as a smoke proxy (Table S7)." In Section 3.2 the figure citation "(Figures 3d and 3h)" in the third sentence should be changed to "(Figures 3c and 3f)." The caption for Figure 3 should be changed from "Quantitative PCR-based measurements . . ." to "Quantitative PCR-based measurements of bacterial and fungal DNA concentrations

between non-smoke and smoke impacted samples across all three sites. Panels (a–c) include bacterial quantitative polymerase chain reaction (qPCR) results and panels (d–f) include fungal qPCR results. Panels (a and d) = Boulder Lake, Wyoming (BOLA1), (b and e) = Grand Teton National Park West (GT-West), and (c and f) = Grand Teton National Park East (GT- East). P values to assess the statistical significance of observed differences between non-smoke and smoke impacted samples were determined using Wilcoxon rank sum exact tests." In Section 4 the word "assemblages" in the fifth sentence should be corrected to "assemblage." The errors have been corrected, and this may be considered the authoritative version of record.