

Pyroaerobiology: the aerosolization and transport of viable microbial life by wildland fire

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Abstract. The field of aerobiology is expanding due to a recognition of the diversity of roles microbes play in both terrestrial and atmospheric ecology. Smoke from global biomass burning has had significant and widespread ecological and human health consequences, but the living component of smoke has received little attention. Microbes aerosolized and transported by wildland fire may have profound effects on atmospheric and environmental factors, acting as nuclei for ice condensation, transporting pathogens or symbionts, and otherwise influencing ecosystems and human populations downwind. The potential for smoke to aerosolize and transport viable microbes is a virtually blank piece of the microbial biogeography puzzle with far-reaching implications. This study characterized the aerosolization of viable microbes via wildland fire smoke from burns in contrasting coniferous forests. Seventy aerosolized microbial morphotypes were recovered, and of these, a subset was identified using DNA analysis which revealed both pathogenic and non-pathogenic fungal species. Overall microbial colony-forming units decreased with increasing distance from smoke source, driven by bacterial abundance. Organisms were more abundant in smoke derived from mechanically treated fuels than intact forest floors and were most abundant in smoke from a dry, biennially burned *Pinus palustris* sandhill forest in Florida. Our findings of smoke-transported viable microbes have implications for ecosystem restoration/conservation, global biodiversity, meteorology, and human health.

Key words: aerobiology; atmospheric biology; bioaerosols; emissions; fire ecology; forest pathogen; fungal dispersal; microbial ecology; microbiology; prescribed fire; smoke; wildfire.

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INTRODUCTION

Long-distance transport of microbes has been documented across continents and oceans (Brown and Hovmoller 2002, Hara and Zhang 2012, Smith et al. 2012, 2013), as well as before and after storm winds and dust storms (Murata and Zhang 2014). These large-scale changes in microbial distribution demonstrate the likelihood for similar transport

mechanisms of viable microbial communities in a different type of atmospheric vector—wildland fire smoke. Global biomass burning is responsible for aerosolizing approximately 42.2 Tg of particulate matter (PM) annually (Andreae and Merlet 2001), yet the contribution of combustion-aerosolized and viable microbial organisms has received little scientific attention. Aerosolized microbes can be pathogenic or beneficial to plant and human

health (Roux and O'Brien 2001, Griffin 2007), or may act to change microbial communities and their roles in both atmospheric and terrestrial environments (Morris et al. 2013, Golan and Pringle 2017). These diverse, abundant, and adaptable organisms may be integral drivers of ecosystem resilience and recovery, especially as natural and human-induced changes to climate and disturbance regimes continue.

Although wildland fire smoke plume temperatures can reach maximums well over the thresholds for most life-forms (e.g., $>290^{\circ}\text{C}$ at 4.5 m above a typical grassland fire; Clements 2010), the mixing of burned and unburned fuels, fluctuations in oxygen availability, meteorological factors, and entrainment of ambient air result in a mosaic of fire intensities and temperatures across spatial and temporal scales (Hiers et al. 2009). High-energy convection columns carry a wide range of particle sizes due to intense vertical air mixing (Clark et al. 1998, Lynch et al. 2004) and can result in the aerosolization and transport of organic matter and even mineral soils (Pisaric 2002, Bormann et al. 2008). Bioaerosols, or airborne particles with biological origins, have the potential for long-range transport and are likely to be associated with particulates, as previous studies in continental dust transport have shown (Hara and Zhang 2012). Wildland fire produces uniquely suitable substrates for organisms that may not otherwise survive in smoke. For example, pyrogenic C particles have been shown to provide habitat for soil microbes (Pietikäinen et al. 2000), a role that may extend to PM within a smoke column. A recent review of the long-distance transport of fungi mentions the potential, yet unknown, role for fire as a biogeographical dispersal vector (Golan and Pringle 2017), because even prescribed fire can evoke smoke plume rise to >1 km above ground level (Liu 2014). Yet wildland fire behavior has received little attention for its singular potential to aerosolize living microbes and transport them via smoke plumes.

The viability of microorganisms in smoke plumes is likely to be controlled by a combination of atmospheric and fire conditions, including relative humidity; temperature; convective forcing; degree of mixing; ultraviolet (UV) radiation; and oxygen content. Of parallel importance are the traits of the source microbial community and the types of material aerosolized (e.g., fuel source, pigmentation, high G + C nucleic acid

content, high DNA repair ability, and UV protection; Mohr 2007). However, these complex associations have not been characterized in relation to wildfire or prescribed fire smoke presence. The composition of viable microbes transported by smoke may have significant implications for forest ecosystems and management. Understanding the fate of specific pathogenic and beneficial microbes can help direct broader restoration efforts for the conservation of affected ecosystems (Klopfenstein et al. 2010).

Of the numerous research publications pertaining to wildland fire smoke or aerobiology, we have only uncovered a single study that connects the two disciplines, and no exploration of this phenomenon in terms of microbiology, smoke science, fire behavior, and fire ecology from an interdisciplinary viewpoint (Fig. 1). Mims and Mims (2004) found a strong correlation ($r^2 = 0.78$) between fungal spores and aerosolized PM (assessed microscopically through visual counts of particles) deposited in Texas, USA, by smoke originating from wildfires on the Yucatán Peninsula, México. No assessment was conducted to verify that smoke particles were physically or biologically associated with the fungal spores. This case study also incorporated biological samples from a backyard experimental fire and measured higher numbers of colony-forming units (CFUs) on nutrient films exposed to smoke compared to those in ambient air. However, statistical tests were not conducted, and further study was not pursued by the authors.

The transport of viable aerosolized microorganisms via wildland fire smoke, hereafter referred to as "pyroaerobiology" (PAB; Fig. 1), is an integration of micro- and aerobiology, smoke and atmospheric sciences, fire behavior, and fire ecology in a coherent effort to understand the ecological and societal impacts of smoke-vectored microbes. The objective of this study was to gain a foundational understanding of the capacity of wildland fire to aerosolize viable fungi and bacteria in smoke, and how different combustion processes and sources may affect the aerosolized communities. Although various microbiological methods could be used to assess microbial composition and abundance in air masses (Haig et al. 2016), a commonly employed method for assessing the likelihood that organisms would survive after being aerosolized (i.e., capacity of microbes to

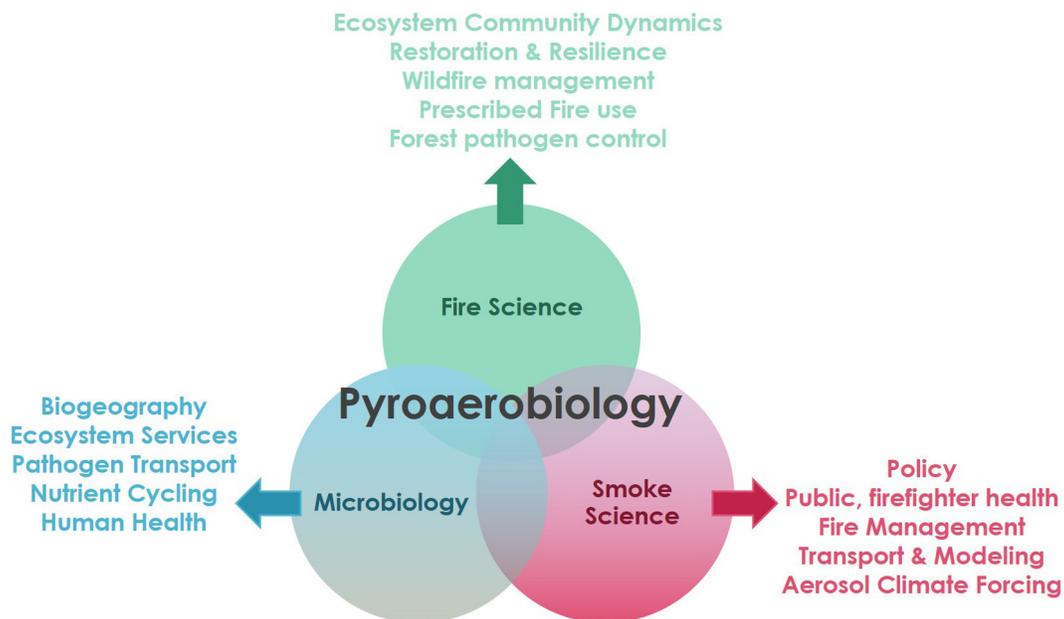


Fig. 1. Pyroaerobiology integrates theory and methods from microbiology, smoke and atmospheric sciences, and fire ecology, with a range of broader impacts and value added to each discipline.

influence the environment where they land) is the culturing of organisms (e.g., Yao and Mainelis 2007). This method is preferable because not only does it assess microbial presence, but it also allows for determination of post-fire viability. Because prescribed fires consume more biomass and typically burn more acreage than wildfires across the United States on an annual basis (NIFC 2018), and because prescribed fire scenarios allow for safe, direct access to the flaming front to control for differences in combustion type and fireline intensity, we performed this initial experiment using prescribed fires. To assess temperatures and determine whether mass loss corresponded to culturable microbial abundance, we conducted an additional study using burns in a controlled combustion laboratory using different fuel types. We tested three hypotheses about smoke-transported microbes during prescribed burns and laboratory combustion experiments by culturing impacted microbes, microscopic identification of morphotypes, and genetic analyses: (1) Viable microbe abundance as measured by CFUs will vary with increasing distance from the smoke source and will differ from ambient air; (2) viable species abundance will differ with the type of combustion (smoldering vs. flaming); and (3) viable species

abundance will differ by site, where historical fire frequency, management, or fuel types differ.

MATERIALS AND METHODS

Study sites

Two distinct studies with different methodology were conducted, based on limited available resources. In the Florida-based study, we utilized three 5- to 10-ha prescribed burns, while in Idaho, we transported forest floor samples and combusted them in a laboratory. The three burns were conducted in humid sub-tropical forests at the University of Florida's Austin Cary Forest approximately 18 km northeast of Gainesville, Florida, USA. The first burn was conducted on 3 April 2015 in a mature (70–90 yr old) *Pinus palustris* sandhill ecosystem (Myers and Ewel 1990) maintained by a two-year prescribed fire return interval since 2003 (Sandhill Biennial), while the second burn was on 6 August 2015 in a mature longleaf pine flatwoods (distinguished from sandhills by a higher water table and sub-surface spodic horizon) ecosystem that was burned annually since ca. 1990 (Flatwoods Annual). The third and final prescribed burn took place on 25 September 2015 in a hydric *P. palustris* and

Pinus elliotii flatwoods ecosystem that was previously (and incompletely) burned only once since 1940 (in 2012), characterized by a heavy buildup of surface and ground fuels (i.e., understory vegetation and organic soil horizons).

North Idaho forest floor sampling sites were located in semi-arid steppe forests at the University of Idaho Experimental Forest (UIEF) on the Palouse Range, about 20 km northeast of Moscow, Idaho, USA. These mixed-conifer forest stands consist of a diverse coniferous overstory dominated by *Pseudotsuga menziesii*, *Abies grandis*, *Thuja plicata*, and *Pinus ponderosa* var. *ponderosa*, and understory species dominated by shrubs, with fuel reduction treatments as described by Sparks et al. (2017).

Soil sample collection—mixed-conifer forest in Idaho

Soil samples of the entire organic horizon (O horizon) were collected from three forest stands planted in 1982. Each stand had two treatments including understory fuel reduction (all surface fuels and small trees shredded and left on site—masticated; see Sparks et al. 2017), and one left untreated—control. Three soil samples were collected to the entire depth of the organic soil horizon (including O_i, O_e, and O_a layers) using a 17 cm diameter ring at three randomly located plots within both treatments in each stand ($n = 18$). Samples were kept cold for seven days (2°C), air-dried for 48 h in the laboratory under a sterilized closed laminar flow hood, and then composited by treatment by stand prior to combustion. Compositing was used to account for high spatial variability within sub-sections, and to achieve sufficient masses for continuous flaming and residual smoldering combustion subsequently in the combustion laboratory ($n = 6$).

Bioaerosol sampling—combustion laboratory in Idaho

Because we sought to culture the microbes, sampling durations were limited to 2 min in order to reduce the potential for desiccation and damage to the organisms (Mainelis and Tabayoyong 2010). To assess background levels of aerosolized microbes, ambient air samples were taken in the field at each sampling location by exposing one Petri dish with sterilized potato dextrose agar medium at one meter above the ground surface,

quickly sealing it with Parafilm, and storing it in a cooler for immediate transport to the laboratory ($n = 18$). A combustion laboratory (IFIRE Lab, University of Idaho, Idaho, USA) was used for the burn experiments. The laboratory is comprised of a preparation and control room where data are monitored, and a separate combustion chamber within a climatically controlled room containing an over-sized dedicated fume hood. O horizon samples were transported to the combustion laboratory, and using sterile techniques, ~100–200 g of soil was transferred into sterilized metal pans prior to ignition. Pan contents were burned on a table scale to measure real-time mass loss rates (per second, and as percent of initial mass). Three type-K, 20-AWG fiberglass-sheathed thermocouple wires (Omega Engineering, Stamford, Connecticut, USA) were positioned within the fuel bed at 0, 15, and 60 cm above the fuel bed to monitor temperatures during burning using a datalogger in the preparation room. During flaming and then smoldering combustion, three Petri dishes were suspended approximately 50 cm to 1 m above the fuel source for a total of nine replicate smoke samples for each stand × fuel treatment combination (total number of Petri dishes subjected to smoke in laboratory = 18, with nine smoldering and nine flaming combustion samples). Two additional dishes were exposed for the same time period prior to any ignitions to serve as laboratory blanks. After exposing the dishes to smoke and ambient laboratory air, dishes were sealed, stored in a cooler, and transported to a microbiology laboratory for incubation using sterile handling techniques.

Bioaerosol sampling—prescribed burns in Florida

The three prescribed burns were conducted within the late-spring-to-late-summer growing season. All prescribed burns were ignited using a combination of flanking and strip head fire ignition patterns. Flame lengths (indicative of fire intensity and ranging from 0.5 to 3 m) were estimated by ocular comparison to a 1-m pole (Kreye and Kobziar 2015) and ranged from an average value of 0.9 m in the Flatwoods Annual site, 2.3 m in the Sandhill Biennial site, to 3.8 m in the Long-Unburned site.

All samples were collected via passive impaction onto malt extract agar (MEA) medium in Petri dishes manually oriented into the wind.

Sampling locations at each burn were established when consistent fire rate of spread was observed, and outside of direct ignition zones. Samples were collected at the origin (approximately 0.5 m) and increasing distances from the flaming front relative to flame length. Sterilized Petri dishes with MEA were suspended approximately 1–2 m above ground level on platforms attached to extension poles facing upwind into smoke plumes for two minutes, closed, sealed with Parafilm, and stored in a cooler for 1–2 h before transportation to the laboratory. During the burns, three to four ambient air samples per burn were taken at a minimum of 9 m upwind and away from any perceivable influence of smoke, but within the same hour and site conditions. Samples were also collected during smoldering combustion at 0.5–1 m from the source of combustion. No smoldering samples were collected at the Flatwoods Annual site due to the insufficient source of smoldering combustion. The number of samples taken was dictated by opportunities for safe entry into the burn zone, and the total duration of each burn.

Microbial culture processing—both locations

Samples were transported to the laboratory and kept at room temperature (~23°C) in the dark until colonies had developed, 72 h and 7–14 d for Florida and Idaho samples, respectively. Plates were visually examined under a 40× microscope, classified into morphotypes, and CFUs for fungi and yeasts were counted. After approximately one week, a colony from each morphotype from the Florida sites was subcultured on acidified potato dextrose agar (APDA) to enable targeted analysis of morphological characteristics (e.g., spore production, colony features). Of seventeen different fungal morphotypes, eight randomly selected morphotypes were subcultured on sterilized cellophane on APDA for seven days, to facilitate growth and harvesting of material for DNA extractions. Standard sterile technique and analysis within a laminar air flow hood were used throughout with all cultures maintained at ~23°C in the dark.

DNA extraction, amplification, and sequencing—Florida samples

The randomly selected unique morphotypes from the Florida smoke samples had DNA

extracted by harvesting a portion of the colony from the cellophane, using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. The nuclear ribosomal internal transcribed spacer (ITS) region from fungal morphotypes was amplified by polymerase chain reaction (PCR) with primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; White et al. 1990) using PCR conditions from Sena et al. (2018), Sanger-sequenced at the University of Florida Genetics Institute in Gainesville, Florida, USA, and compared to sequences in GenBank. The Idaho samples were not sequenced due to resource limitations.

Statistical analysis

Data were explored and analyzed in the R environment (R Core Team 2016). Total CFUs from the Florida study were analyzed pooled and separate for each site in order to evaluate the relationships between colonized microbes and fire or site factors, respectively. The total CFUs were then analyzed by growth forms. To meet statistical assumptions, total CFU data were transformed by $\log(x + 1)$ and then compared with an ANOVA against combustion type and distance from flaming front within each ecosystem. When split into organism types, we used Kruskal–Wallis tests as the data did not fit all assumptions of ANOVA. Where applicable, transformed CFU data were tested in a regression model against distance from the flaming front. Total CFUs from the Idaho study were transformed by $\log(x + 1)$ to meet ANOVA assumptions and tested against fuel type, organism types, and regressed against temperatures and mass loss (%) during combustion.

RESULTS

Florida prescribed burn sites

Across the three Florida prescribed burn sites, distance was negatively related to the average number of CFUs for all organism types during flaming combustion ($n = 36$, $r^2 = 0.77$, $P < 0.001$; Fig. 2). Although there were higher CFU counts for filamentous fungi compared to bacteria and yeast ($n = 123$, 47, and 25, respectively), the relationships between CFUs and distance (Fig. 2) were driven by bacteria. Overall, filamentous

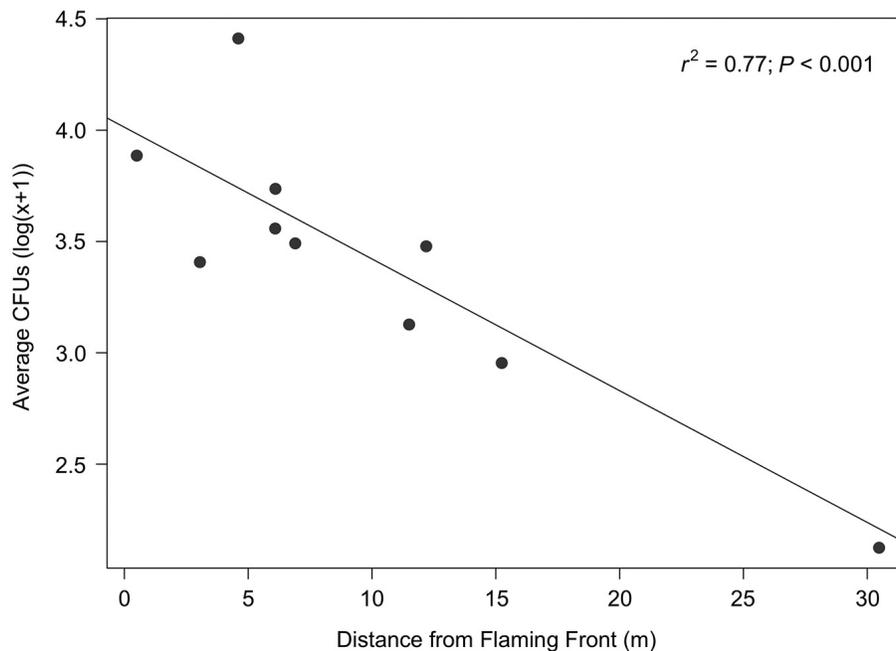


Fig. 2. Number of colony-forming units averaged for each distance from the flaming front of experimental prescribed fires in all north Florida sites ($n = 7, 8, 7, 2, 4, 2, 2, 2, 2, 2$, from 0.5 to 30 m, respectively). Total number of samples taken = 38.

fungus and yeast CFUs were not significantly related to distance or distance divided by flame length when analyzed independently ($P > 0.05$). Smoke samples from the Sandhill Biennial site showed a significant ($P < 0.001$) and negative correlation ($r^2 = 0.40$) between bacterial CFUs and distance from the flaming front. The Flatwoods Long-Unburned site showed significant differences ($P < 0.01$) in CFUs among distances from the flaming front, which were lowest at the origin and outside of the smoke (at 30 m) but highest at 3- and 6-m (2–3 times the flame lengths) collection points, suggesting convective wind vortices may have aerosolized organisms from the abundant and tall (>1 m) understory vegetation unique to this site.

Samples taken during flaming combustion yielded higher CFUs compared to ambient samples ($P < 0.05$) but were not significantly different from smoldering samples (ambient $n = 7$, flaming $n = 28$, smoldering $n = 22$; Fig. 3). Total CFUs, regardless of combustion type, were highest in the driest (based on soil type) burned site (Sandhill Biennial: 171, $n = 30$) and the mesic Flatwoods Annual (104, $n = 14$) sites, compared

to the Flatwoods Long-Unburned site (69, $n = 27$). Colony-forming units were significantly higher in the Sandhill Biennial site compared to Flatwoods Long-Unburned site ($P < 0.001$). In the Sandhill Biennial site, CFUs were highest and most variable when aerosolized by flaming combustion; they were significantly lower in ambient samples when compared to both types of combustion ($P < 0.05$; Fig. 3). Eight unique fungal morphotypes isolated from smoke samples and identified using ITS sequences show a diverse group of fungi, representing several orders and ranging from pathogens to non-pathogens with diverse ecological roles (Table 1).

Smoke samples collected during laboratory experiments on soils from Idaho bore unique morphotypes in all treatments: These were highest in masticated fuel sites (ambient samples contained 15 total morphotypes with five unique; burned samples had 11 morphotypes with five unique). The unique morphotype assemblages in burned, unburned, and masticated fuel beds suggest that combustion aerosolized microbes that would not be found in ambient air in the conditions and season in which we sampled.

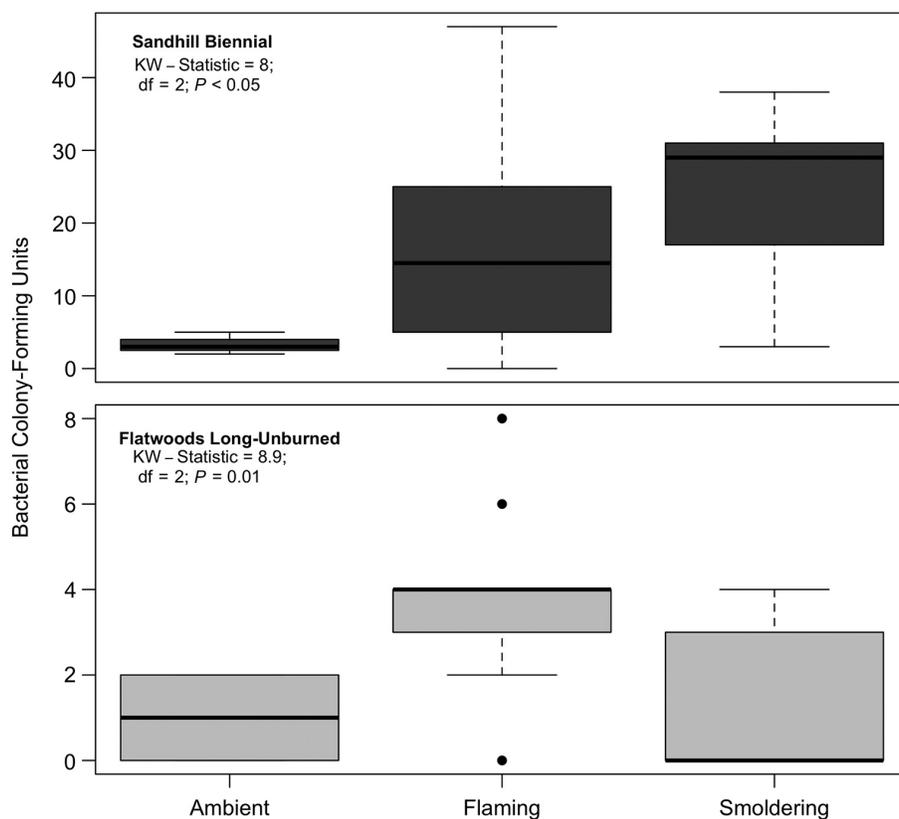


Fig. 3. Bacterial colony-forming units collected during ambient, flaming, and smoldering combustion in Florida prescribed burns in Sandhill Biennial sites ($n = 3, 18, 9$ for ambient, flaming, and smoldering combustion types, respectively) and Long-Unburned Flatwoods ($n = 4, 10, 13$ for ambient, flaming, and smoldering combustion types, respectively). The average flame length at the Sandhill Biennial site was 2.3 m with a relative humidity (RH) of 39%, and 3.8 m at the Long-Unburned Flatwoods sites with an RH of 57%. The Flatwoods Annual site was not included because it lacked sufficient smoldering combustion.

Table 1. Fungal identifications for eight unique morphotypes from prescribed fire smoke samples in a biennially burned Sandhill pine ecosystem and Long-Unburned Flatwoods pine ecosystems in north Florida.

Best BLAST	GenBank no.	Identities	Ecological function
<i>Trichoderma strigosum</i>	EU718074	601/604	Functions in nutrient and mineral uptake, genus important in agricultural remediation
<i>Dothideomycete</i> sp. 1	EU680480	546/546	Often found as pathogens, endophytes, or epiphytes of living plants. Saprobes degrade cellulose and other complex carbohydrates in dead or partially digested plant matter
<i>Dothideomycete</i> sp. 2	HQ631008	584/595	As above
<i>Pestalotiopsis</i> sp.	KX757719	546/546	Parasitic fungus that targets ants, also plant pathogens
<i>Epicoccum nigrum</i>	MF687186	541/541	Endophyte and plant pathogen, produces anti-fungal and anti-bacterial compounds
<i>Neopestalotiopsis australis</i>	KY398730	547/547	Endophytic fungus capable of breaking down and digesting polyurethane, can metabolize under anaerobic conditions
<i>Hypocreales</i> sp.	KP306921	437/451	Diverse functions by species
<i>Penicillium lagena</i>	NR_153223	549/549	Non-pathogenic fungi present around mycorrhizal roots

Notes: Identities are based on best BLAST matches to the NCBI database using the internal transcribed spacer region. Identities are nucleotide matches between the morphotype and GenBank accession. Ecological function is based on brief literature review of the best BLAST match.

Unlike trends in the Florida samples, Idaho forest microbial CFUs in smoke derived from flaming combustion did not differ significantly between combustion types nor when compared with the ambient samples. The composition of morphotypes, however, differed between the ambient and the combusted samples, with eight unique morphotypes occurring only in smoke samples. In addition, the masticated fuel type aerosolized more CFUs compared to untreated fuel, but neither had significantly different CFU numbers from the ambient samples ($P < 0.01$). While the quality of the fuel source (i.e., masticated or not) of burned organic matter had a significant influence on the number of CFUs in smoke, the quantity burned (% mass loss or total mass loss [g]) did not correlate with total CFUs ($n = 18$, $r^2 = 0.30$, $P = 0.26$). Idaho smoke samples showed higher mean CFUs per sample of fungi than bacteria (13 and 4, respectively). Temperatures were not measurably correlated with morphotype composition or number of CFUs; however, the maximum temperatures of flaming vs. smoldering combustion from the fuel bed thermocouples were higher (528°C vs. 395°C, respectively), and temperatures at 60 cm height, where samples were taken, at times exceeded 60°C during both flaming and smoldering combustion.

DISCUSSION

Pyroaerobiology, a term we introduce in this study, represents an interdisciplinary and little-researched line of inquiry, integrating terrestrial ecology, aerobiology, smoke science, microbiology, fire behavior, and fire ecology in a coherent effort to understand the impacts of aerosolized live pyrogenic material. Because this line of inquiry is a new application of aerobiology, our exploratory study was designed to provide evidence for the potential for smoke to aerosolize and transport viable microorganisms and to test some basic hypotheses. Our study was inherently limited by the specific source/fuels sampled, fire behavior characteristics, sampling duration and methods, and processing methods, so that specific results should not be extrapolated to other fires. We used culture-based methods to capture and grow viable microbes from smoke using a single medium in each study. It is well established that the media used will affect the

microbes recovered and that most microbes are unculturable. We used a general growth medium able to grow many fungi and bacteria but presumably only cultured a small portion of the potentially viable microbes in the smoke. In addition, different sampling durations would likely lead to different results (Mainelis and Tabayooyong 2010).

These initial studies using prescribed burns and laboratory experiments show that fire aerosolizes and smoke transports a variety of viable, culturable microbes, and these assemblages are dissimilar in composition and abundance from the communities aerosolized by background aerosolization drivers (e.g., wind, gravity, spore propulsion) in paired samples. While our study addressed forest stand-level transport of organisms, longer-distance transport and its implications would depend on fire behavior and sources of the microbes (e.g., the microbial community), season (which affects sporulation, activity, and probably survival), environmental conditions (recent rain events, winds affect background levels of aerosolized organisms), and the physiological hardiness and growth potential of the organisms or propagules aerosolized (e.g., fungal hyphae, spores, and their dispersal mechanisms; Golan and Pringle 2017).

Two of our hypotheses were supported by our data, including that abundance (CFUs) varied with distance from the smoke sources and that species abundance and composition would differ by site/site conditions (e.g., mastication). Our results suggest that the more frequently burned sites have higher numbers of viable aerosolized organisms in smoke overall, which may reflect fire history and associated microbial fire adaptations (Glassman et al. 2016), or differences in the types of fuels combusted. Grasses and pine litter drove fire behavior in the frequently burned sites in contrast to shrubs, grasses, and even small trees in the Long-Unburned site. Comparisons of the source microbial populations among sites would be needed to draw conclusions about whether the differences in aerosolized communities are a function of source, fire behavior, or even sampling protocols (including culture medium used). These results suggest that future PAB research should include an assessment of smoke source communities in order to derive predictions for the potential impact on atmospheric and downwind terrestrial

communities. Other improvements would include larger sampling sizes, methods parameterized for expected aerosol densities, and employing metagenomic analyses to reduce bias against non-culturable species.

The Idaho mixed-conifer forest samples demonstrated that masticated fuels produced more CFUs and unique microbial communities than non-treated stands, as indicated by distinct morphotypes, and that both differed from ambient communities. Mastication changes individual fuel (soil O horizon) surface areas, fuel packing ratios, moisture content, and depth (Kreye et al. 2013), hence changing the microhabitat. As such, fuels treatments may have an impact on microbial communities that then extends to aerosolized and dispersed microbes when and if fire occurs. Mixing of the soil organic horizons due to heavy equipment used for fuel reduction treatments may expose organisms whose habitats would otherwise be unavailable for aerosolization via combustion.

Our second hypothesis that species abundance differed with combustion phase was only supported in the Florida sites. Flaming combustion burned smaller diameter woody litter, soil organic horizons, and surface fuels including shrubs, grasses, and herbaceous vegetation. In contrast, smoldering combustion samples were necessarily obtained from residual fuel, often larger woody debris after the initial passage of the fire front. The source of the microbial materials was therefore different between the two phases of combustion; we cannot isolate the effect of the fuel source from that of the phase of combustion. That the different phases of combustion did not produce significant results in the laboratory burns using Idaho mixed-conifer O horizon samples, along with the lack of a temperature effect, implies that the energetic differences between the combustion phases were not significant for the microbes we were able to culture.

Heating from wildfire and prescribed fire events have poorly understood physiotemporal effects on soil microbial populations (Pingree and Kobziar 2019). In forests devoid of regular fire disturbances, prescribed burning employed as a restoration effort may negatively impact ecosystem processes. For example, soil heating may be substantially increased where organic soil horizons are deeper, as in the Long-Unburned Florida

site, leading to prolonged heating and increased temperatures (Varner et al. 2005), and increased potential for greater numbers of microbes to be aerosolized. Indirectly, the exclusion of frequent, low-severity fires may favor the proliferation of a soil microbial community with lower temperature thresholds and disturbance adaptability compared to a frequently burned forest soil community (Hart et al. 2005). These altered microbial communities may also be transported and relocated via aerosolization or particle-mediated transport in smoke with unknown consequences for adjacent ecosystems. Efforts to measure and characterize wildland fire effects on microbial species can help to improve management of sensitive and rare ecosystems where recurrent fire and adapted microbial species are closely coupled with ecosystem function (Glassman et al. 2016).

Societal impacts of smoke-transported living microbes could be both indirect (e.g., ecosystem services) and direct (human health). Microorganisms provide integral functions in forest ecosystems including decomposition and C cycling, nutrient cycling, production and consumption of greenhouse gases, development of soil structure and maintenance, and effects on other soil biota. Understanding the fate of specific pathogenic and beneficial microbes could help direct broader restoration efforts for the conservation of affected ecosystems (Klopfenstein et al. 2010). Theoretically, managers could retard spreading of detrimental pathogens and promote dissemination of beneficial mycorrhizae or nitrogen-fixing bacteria, or other microbes that would benefit society.

The viability and composition of microbes transported by smoke may have significant implications for forest health. For example, the fungal pathogen *Cronartium ribicola* (J.C. Fisch.), which causes white pine blister rust and threatens the endangered whitebark pine (*Pinus albicaulis* Engelm.), was spread to new hosts in the western United States via long-distance dispersal by atmospheric transport (Frank et al. 2008). If this pathogen is viable in smoke, disease spread may be vectored by smoke as well. It is currently unknown what role smoke and wildfire play in the transport of forest pathogens. These consequences may in fact be an undesired impact of management practices. For example, a recommended practice to dispose of biomass infected with plant pathogens (e.g., *Phytophthora ramorum*,

which causes Sudden Oak Death) is to burn the material (Agrios 2005). Such attempts at pathogen control may actually disperse pathogens depending on environmental conditions (e.g., as has been shown in a study of wheat field burning; Roux and O'Brien 2001). With additional knowledge, managers could plan burns when conditions are unlikely to transport pathogens present in a stand to uninfected areas.

Relationships between microbial transport and smoke composition may thereby help guide smoke management decisions with significant consequences (Bowman and Johnston 2005). Smoke plumes from wildland fires impact natural resource management decision-making, public opinion, and public safety and have catalyzed immense planning and coordination efforts by multiple stakeholders (Hardy et al. 2001). Future investigation into targeted species of special concern to human health impacts is also warranted, because aerosolized microbes are well known to aggravate patients with asthma and even cause illness in otherwise healthy individuals (Griffin 2007).

CONCLUSIONS

The addition of viable organisms to the atmosphere may alter bioaerosol species composition, activity, and growth, with effects on biogeochemical cycling, atmospheric cloud development, and weather (Morris et al. 2013, Krumins et al. 2014). Our study provides an initial foundation for a broad spectrum of future inquiries. Under what conditions does wildland fire smoke transport and deposit active plant pathogens to adjacent or distant locations, and what are the potential consequences? Can human pathogens and allergens be transported in smoke to such an extent that they affect sensitive populations, and more immediately, wildland fire personnel? With these and other questions in mind, the establishment of appropriate conceptual and methodological guidelines for this line of inquiry is needed. The approach should be grounded in the established principles and theory of aerobiology, which emphasize the importance of addressing the complete aerobiological pathway. Ultimately, pyroaerobiology should take into consideration the source, characteristics of aerosolization (i.e., launch), atmospheric transport, deposition, and

direct and indirect subsequent ecological impacts (Edmonds 1979).

We suggest the following considerations be applied to future studies:

1. PAB sampling systems should sample smoke plumes from multiple fire types at increasing heights and distances from the source using mobile platforms in smoke columns; these would enable smoke communities to be sampled specifically, the influence of ambient air entrainment to be characterized, and the degree of transport to be quantified;
2. PAB sampling should incorporate a wide range of microbiological diversity assessments including community sequencing, various media, and baiting. Phylogenetic analyses coupled with physiological/morphological examinations of the species identified may shed light on the evolution of the pyroaerobiome.
3. PAB sampling strategies should integrate environmental and aero-habitat conditions (e.g., PM levels, relative humidity, temperature, UV exposure) concurrently with sample extraction in order to characterize and compare aero-habitats;
4. A variety of sampling techniques (e.g., impaction, filtering, impingement) and durations need to be laboratory- and field-tested for maximum capture of all viable organisms to determine appropriate sampling duration and volume for the unique habitat of wildland fire smoke;
5. To link these effects with predictive models of smoke transport and effects, an understanding of fire behavior, source substrates, and how they interact to aerosolize microbes is needed. PAB must include all sub-disciplines to address the questions of relevance to ecological systems and processes, as well as potential human health impacts.

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LITERATURE CITED

- Agrios, G. 2005. Plant pathology. Fifth edition. Elsevier Academic Press, Amsterdam, The Netherlands.
- Andreae, M. O., and P. Merlet. 2001. Emission of trace gases and aerosols from biomass burning. *Global Biogeochemical Cycles* 15:955–966.
- Bormann, B. T., P. S. Homann, R. L. Darbyshire, and B. A. Morrisette. 2008. Intense forest wildfire sharply reduces mineral soil C and N: the first direct evidence. *Canadian Journal of Forest Research* 38:2771–2783.
- Bowman, D. M. J. S., and F. H. Johnston. 2005. Wildfire smoke, fire management, and human health. *Eco-Health* 2:76–80.
- Brown, G., and A. Mohr. 2016. Fate and transport of microorganisms in air. Pages 3.2.4-1–3.2.4-12 in M. Yates, C. Nakatsu, R. Miller, and S. Pillai, editors. *Manual of environmental microbiology*, fourth edition. American Society of Microbiology, Washington, D.C., USA.
- Brown, J. K. M., and M. S. Hovmoller. 2002. Epidemiology—Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* 297:537–541.
- Clark, J. S., J. Lynch, B. J. Stocks, and J. G. Goldammer. 1998. Relationships between charcoal particles in air and sediments in west-central Siberia. *Holocene* 8:19–29.
- Clements, C. B. 2010. Thermodynamic structure of a grass fire plume. *International Journal of Wildland Fire* 19:895–901.
- Edmonds, R. L., editor. 1979. *Aerobiology: the ecological systems approach*. US/IBP Synth. Ser. 10. Dowden. Hutchinson & Ross, Stroudsburg, Pennsylvania, USA.
- Frank, K. L., B. W. Geils, L. S. Kalkstein, and H. W. Thistle. 2008. Synoptic climatology of the long-distance dispersal of white pine blister rust II. Combination of surface and upper-level conditions. *International Journal of Biometeorology* 52:653–666.
- Glassman, S. I., C. R. Levine, A. M. DiRocco, J. J. Battles, and T. D. Bruns. 2016. Ectomycorrhizal fungal spore bank recovery after a severe forest fire: Some like it hot. *ISME Journal: Multidisciplinary Journal of Microbial Ecology* 10:1228–1239.
- Golan, J. J., and A. Pringle. 2017. Long-distance dispersal of fungi. *Microbiology Spectrum* 5. <https://doi.org/10.1128/microbiolspec.FUNK-0047-2016>
- Griffin, D. W. 2007. Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. *Clinical Microbiology Reviews* 20:459–477.
- Haig, C. W., W. G. Mackay, J. T. Walker, and C. Williams. 2016. Bioaerosol sampling: sampling mechanisms, bioefficiency and field studies. *Journal of Hospital Infection* 93:242–255.
- Hara, K., and D. Zhang. 2012. Bacterial abundance and viability in long-range transported dust. *Atmospheric Environment* 47:20–25.
- Hardy, C. C., R. D. Ottmar, J. L. Peterson, J. E. Core, and P. Seamon, editors. 2001. *Smoke management guide for prescribed and wildland fire: 2001 edition*. National Wildfire Coordination Group, Boise, Idaho, USA.
- Hart, S. C., T. H. DeLuca, G. S. Newman, M. D. MacKenzie, and S. I. Boyle. 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. *Forest Ecology and Management* 220:166–184.
- Hiers, J. K., J. J. O'Brien, R. J. Mitchell, J. M. Grego, and E. L. Loudermilk. 2009. The wildland fuel cell concept: an approach to characterize fine-scale variation in fuels and fire in frequently burned longleaf pine forests. *International Journal of Wildland Fire* 18:315–325.
- Klopfenstein, N. B., et al. 2010. Invasive forest pathogens: summary of issues, critical needs, and future goals for forest service research and development. In M. E. Dix and K. Britton, editors. *A dynamic invasive species research vision: opportunities and priorities 2009-29*. GTR-WO-79/83, U.S. Department of Agriculture, Research and Development, Washington, D.C., USA.
- Kreye, J. K., and L. N. Kobziar. 2015. The effect of mastication on surface fire behaviour, fuels consumption, and tree mortality in pine flatwoods of Florida, USA. *International Journal of Wildland Fire* 24:573–579.
- Kreye, J. K., L. N. Kobziar, and W. C. Zipperer. 2013. Effects of fuel load and moisture content on fire behaviour and heating in masticated litter-dominated fuels. *International Journal of Wildland Fire* 22:440–445.
- Krumins, V., G. Mainelis, L. J. Kerkhof, and D. E. Fennell. 2014. Substrate-dependent rRNA production in an airborne bacterium. *Environmental Science & Technology Letters* 1:376–381.
- Liu, Y. 2014. A regression model for smoke plume rise of prescribed fires using meteorological conditions.

- Journal of Applied Meteorology and Climatology 53:1961–1975.
- Lynch, J. A., J. S. Clark, and B. J. Stocks. 2004. Charcoal production, dispersal, and deposition from the Fort Providence experimental fire: interpreting fire regimes from charcoal records in boreal forests. *Canadian Journal of Forest Research* 34:1642–1656.
- Mainelis, G., and M. Tabayoyong. 2010. The effect of sampling time on the overall performance of portable microbial impactors. *Aerosol Science and Technology* 44:75–82.
- Mims, S. A., and F. M. Mims. 2004. Fungal spores are transported long distances in smoke from biomass fires. *Atmospheric Environment* 38:651–655.
- Morris, C. E., D. C. Sands, C. Glaux, J. Samsatly, S. Asaad, A. R. Moukahel, F. L. T. Gonçalves, and E. K. Bigg. 2013. Urediospores of rust fungi are ice nucleation active at greater than -10°C and harbor ice nucleation active bacteria. *Atmospheric Chemistry and Physics* 13:4223–4233.
- Murata, K., and D. Zhang. 2014. Transport of bacterial cells toward the Pacific in Northern Hemisphere westerly winds. *Atmospheric Environment* 87:138–145.
- Myers, R. L., and J. J. Ewel. 1990. *Ecosystems of Florida*. University of Florida Press, Gainesville, Florida, USA.
- NIFC [National Interagency Fire Center]. 2018 August 20. “Statistics”. https://www.nifc.gov/fireInfo/fireInfo_statistics.html
- Pietikäinen, J., O. Kiikkilä, and H. Fritze. 2000. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos* 89:231–242.
- Pingree, M. R. A., and L. N. Kobziar. 2019. The myth of the biological threshold: a review of biological responses to soil heating associated with wildland fire. *Forest Ecology and Management* 432:1022–1029.
- Pisarcic, M. F. J. 2002. Long-distance transport of terrestrial plant material by convection resulting from forest fires. *Journal of Paleolimnology* 28:349–354.
- R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Roux, C., and O. O’Brien. 2001. Karnal bunt of wheat detected in South Africa. *Plant Protection News* 58:15–17.
- Sena, K. L., T. J. Dreaden, E. Crocker, and C. D. Barton. 2018. Detection of *Phytophthora cinnamomi* in forest soils by PCR on DNA extracted from leaf disc baits. *Plant Health Progress* 19:193–200.
- Smith, D. J., D. A. Jaffe, M. N. Birmele, D. W. Griffin, A. C. Schuerger, J. Hee, and M. S. Roberts. 2012. Free tropospheric transport of microorganisms from Asia to North America. *Microbial Ecology* 64:973–985.
- Smith, D. J., H. J. Timonen, D. A. Jaffe, D. W. Griffin, M. N. Birmele, K. D. Perry, P. D. Ward, and M. S. Roberts. 2013. Intercontinental dispersal of bacteria and Archaea by transpacific winds. *Applied and Environmental Microbiology* 79:1134–1139.
- Sparks, A. M., A. M. S. Smith, A. F. Talhelm, C. A. Kolden, K. M. Yedinak, and D. M. Johnson. 2017. Impacts of fire radiative flux on mature *Pinus ponderosa* growth and vulnerability to secondary mortality agents. *International Journal of Wildland Fire* 26:95–106.
- Varner, J. M., D. R. Gordon, F. E. Putz, and J. K. Hiers. 2005. Restoring fire to long-unburned *Pinus palustris* ecosystems: novel fire effects and consequences for long-unburned ecosystems. *Restoration Ecology* 13:536–544.
- White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. 38 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols*. Academic Press, San Diego, California, USA.
- Yao, M., and G. Mainelis. 2007. Analysis of portable impactor performance for enumeration of viable bioaerosols. *Journal of Occupational and Environmental Hygiene* 4:514–524.