New Phytologist

Research

Microbiome-mediated response to pulse fire disturbance outweighs the effects of fire legacy on plant performance

Daniel Revillini[1], Aaron S. David[2], Eric S. Menges[2], Kevin N. Main[2], Michelle E. Afkhami[1]* and Christopher A. Searcy[1]*

[1]Department of Biology, University of Miami, 1301 Memorial Drive, Coral Gables, FL 33146, USA; [2]Archbold Biological Station, 123 Main Drive, Venus, FL 33960, USA

Author for correspondence:
Daniel Revillini
Email: dan.revillini@gmail.com

Received: *27 April 2021*
Accepted: *31 July 2021*

New Phytologist (2021)
doi: 10.1111/nph.17689

Key words: disturbance, fire, microbial mediation, plant performance, soil microbiome, succession.

Summary

- Fire plays a major role in structuring plant communities across the globe. Interactions with soil microbes impact plant fitness, scaling up to influence plant populations and distributions. Here we present the first factorial manipulation of both fire and soil microbiome presence to investigate their interactive effects on plant performance across a suite of plant species with varying life history traits.
- We conducted fully factorial experiments on 11 species from the Florida scrub ecosystem to test plant performance responses to soils with varying fire histories (36 soil sources), the presence/absence of a microbiome, and exposure to an experimental burn.
- Results revealed interactive 'pulse' effects between fire and the soil microbiome on plant performance. On average, post-fire soil microbiomes strongly reduced plant productivity compared to unburned or sterilized soils. Interestingly, longer-term fire 'legacy' effects had minor impacts on plant performance and were unrelated to soil microbiomes.
- While pulse fire effects on plant–microbiome interactions are short-term, they could have long-term consequences for plant communities by establishing differential microbiome-mediated priority effects during post-disturbance succession. The prominence of pulse fire effects on plant–microbe interactions has even greater import due to expected increases in fire disturbances resulting from anthropogenic climate change.

Introduction

Disturbance regimes have far-reaching effects on the ways in which organisms adapt and survive. Fire disturbance, in particular, is a key driver of plant evolution, biodiversity, and vegetation structure worldwide (Bond & Keeley, 2005; He *et al.*, 2019). Fires burn *c.* 3% of the Earth's vegetated surface every year, a value that is only expected to increase with anthropogenic climate change. For example, some of the most fire-prone regions of the USA are projected to experience a 200–400% increase in burned area with the next 1°C increase in temperature (Huang *et al.*, 2014; Jolly *et al.*, 2015). In fact, anthropogenic climate change has already doubled the burned area in these regions, mainly by increasing fuel aridity and lengthening fire seasons (Abatzoglou *et al.*, 2018). As fire regimes continue to shift and become more impactful in the Anthropocene, it becomes increasingly important that we develop a greater understanding of how this disturbance directly and indirectly impacts plant communities through ecological interactions that shape composition and persistence in the face of a changing environment.

Disturbance frequency, severity, size, and distribution directly affect plant successional processes (Sousa, 1980). Fire, particularly high intensity fire, has the potential to drastically reshape plant communities by opening up habitat for stress-adapted pioneer species to colonize (Franklin *et al.*, 2005). These taxa are eventually replaced by slower growing species that are superior competitors, adapted for a less abiotically stressful, but more biotically stressful, environment (De Deyn *et al.*, 2004). These successional trajectories have classically been understood in the context of plant–plant relationships, specifically plant–plant facilitation (Brooker *et al.*, 2008) or competition (Koffel *et al.*, 2018). However, plant performance after fire may depend on interactions with other organisms that are independently impacted by fire disturbance, namely the diverse assemblage of fungi, archaea, and bacteria within the soil microbiome (Wang *et al.*, 2012; Cordovez *et al.*, 2019). Despite the vital importance of plant–microbial interactions to plant health, community assembly and diversity, and ecosystem multifunctionality (Wagg *et al.*, 2014; Van Der Heijden *et al.*, 2016; Trivedi *et al.*, 2020), the role of microbiomes in shaping plant community assembly post-fire is largely unknown. Studies have begun to incorporate microbial communities and plant–microbial interactions with successional dynamics after fire (Menges & Hawkes, 1998; Bever *et al.*, 2010), but they largely lack manipulative treatments. Experiments that explicitly test the links between fire, plants, and belowground archaeal, bacterial, and fungal communities are

*These authors contributed equally to this work.

crucial to determining how the shifting influences of fire will affect primary producer communities.

Our understanding of the importance of microbes in plant biology has exploded in recent years, and it has become increasingly apparent that many advantageous traits and responses to stressors previously attributed to plants are in fact mediated or even generated by the microbes with which they associate (Friesen *et al.*, 2011; Johnson *et al.*, 2016; Trivedi *et al.*, 2020). Plants are often reliant on their microbiome to alleviate stressful conditions (David *et al.*, 2020). Beneficial microbial functions range in their response to abiotic and biotic stressors, and include interactions that increase plant drought tolerance (Kim *et al.*, 2012), alleviate plant nutrient requirements when resource availability is low (Allen *et al.*, 2020), and provide protection by increasing resistance to or warding off parasites and pathogens (Bakker *et al.*, 2018; Sharifi & Ryu, 2018). These relationships between plants and their microbial partners can function as a collective system including negative, neutral, and positive interactions (Vandenkoornhuyse *et al.*, 2015) and can regulate plant establishment, productivity, and survival over time (Keymer & Lankau, 2017).

The time horizon over which disturbance effects are experienced can range from immediate 'pulse' effects to long-term 'legacy' effects represented by immediate-to-prolonged changes in either abiotic or biotic conditions. High-intensity pulse disturbances can reset the successional process, creating unique habitat conditions (e.g. open space with increased solar exposure, decreased soil moisture, and altered physicochemistry) that favor certain species (Lucas-Borja *et al.*, 2019; Adkins & Miesel, 2021). Importantly, priority effects established in response to these pulse alterations of the environment may alter successional trajectories for years to come, even after any direct effects of the disturbance have dissipated (Fukami, 2015; Jacquet & Altermatt, 2020). Continuing legacy effects of disturbance, which alter the environmental conditions in which successional trajectories continue to play out, have similarly long-lasting effects on community composition (Collins *et al.*, 2017; Philippot *et al.*, 2021). Both pulse and legacy effects of disturbance on plant communities are well documented (Hillebrand & Kunze, 2020; Miller & Safford, 2020), but the relative importance of soil microbial mediation of pulse and legacy effects remains unclear.

The pyrogenic habitat of the Florida rosemary scrub provides an ideal system in which to untangle these relationships due to the presence of both active fire management through the use of controlled burns and a > 50 yr record of when fire disturbance has affected different habitat patches (Menges *et al.*, 2017b). We therefore set out to characterize pulse vs fire legacy effects of fire disturbance on the soil microbiome, experimentally test whether plant responses to fire are mediated through interactions with fire-selected soil microbiomes, and determine how short-term fire-driven shifts in plant–microbe interactions compare to long-term fire legacy effects on these associations. Based on the reported effects of fire legacy for plants and soil microbiomes separately (Pérez-Valera *et al.*, 2020; Qin *et al.*, 2020), we predict that fire legacy metrics are likely to have important consequences for microbial mediation of plant performance. However, the immediate and direct effect of pulse fire disturbance on the soil microbiome, though previously untested, may also have a strong impact on subsequent plant–microbial interactions. To investigate these factors, we factorially manipulated both presence/absence of the soil microbiome and exposure to recent high-intensity fire using a prescribed burn in the field (Supporting Information Fig. S1) across a suite of 11 plant species with varying life history traits. This allowed us to experimentally disentangle the influence of abiotic shifts caused by fire legacy from pulse fire-induced shifts in the microbiome, contributing to a more robust understanding of post-fire plant–microbial dynamics.

Materials and Methods

Study system

The Florida Scrub ecosystem has the highest rate of endemism in the southeastern USA and hosts a number of threatened species (Menges *et al.*, 2008). This ecosystem exhibits a range of habitat types, from open sand gaps and shrublands to mixed conifer flatwoods, in a relatively small area (Abrahamson *et al.*, 1984). Many of the rare and endemic plants in this ecosystem are found in the rosemary scrub habitat, where they occur in open sand gaps between the dominant, allelopathic shrub Florida rosemary (*Ceratiola ericoides* Michx.), and experience boom–bust cycles driven by the local fire regime (Quintana-Ascencio & Menges, 2000; Menges *et al.*, 2017a). The relationships between habitat, plant strategy, and fire are well-understood in the rosemary scrub habitat (Menges *et al.*, 2017a). Time since last fire (TSF) is positively related to species richness, and plants that specialize in open sand gaps are most abundant in the first decade after a fire (Dee & Menges, 2014).

We conducted our study with soils and seeds collected from Archbold Biological Station (Venus, FL, USA; 27°10′55.703″N, 81°21′6.903″W). Recent studies show that there are distinct soil microbiomes in rosemary scrub compared to the surrounding flatwoods habitat (Hernandez *et al.*, 2021), and that many of the rare, endemic plants that occur in the rosemary scrub are strongly influenced by interactions with the soil microbiome (David *et al.*, 2018, 2020). For this study, we collected seeds of 11 perennial, herbaceous plant species from across Archbold (Table S1) that vary across a spectrum of life history traits, and assessed the seeds for viability prior to planting.

Microbiome fire legacy and prescribed fire treatment

We identified 36 Florida rosemary scrub patches (i.e. open habitat patches dominated by *C. ericoides* that occur at relatively high elevations above the water table) with unique combinations of two aspects of fire legacy – TSF and total number of fires experienced within the last 52 yr (the period for which Archbold has conducted systematic fire inventories (Menges *et al.*, 2017b)) for soil microbiome collections. Time since last fire ranged from 1 to 92 yr (one collection site last burned before detailed fire records began in 1967, and TSF for this site has been estimated from historic aerials), and number of fires since 1967 ranged from 0 to 7

(Table S2). At each of the 36 rosemary patches, *c.* 2.35 l of soil was collected and placed in four foil trays (24 × 13 × 6.25 cm) using a sterile technique and covered with sterile aluminum foil. Soils were collected at least 1 m from the nearest Florida rosemary shrub and transferred to the trays, maintaining physical soil structure and ensuring that distinct biocrust layers, if present, remained on top. Three trays from each patch were transferred to a single site at Archbold composed of rosemary scrub habitat (27°8′2.553″N, 81°21′1.678″W) to undergo a prescribed burn, and were separated into three replicate 'burn blocks' to ensure uniform fire exposure across the 36 soil sources. The remaining replicate tray from each source patch was placed outside of the burn area for the same time period to serve as the unburned control treatment soils. All soils were collected from 6 to 7 May 2019 and the prescribed burn was carried out the following morning. To determine fire coverage and intensity, we placed nine Hobo temperature loggers (Onset Computer Corp., Bourne, MA, USA) randomly across each of the burn blocks at 2 cm below surface level. Ultimately, fire-treated soils from burn block 'Y' were selected for use in the subsequent grow room experiment, as this block received the most consistent and highest temperatures (Fig. S1). The fire moved quickly across the experimental burn blocks, with a total duration of *c.* 10 min, reaching temperatures of *c.* 500°C (Fig. S1), with peak temperatures lasting < 2 min. Following the prescribed burn, all soils were promptly collected from each tray and either sub-sampled and flash frozen in the field using liquid nitrogen for DNA extraction (later transferred to − 20°C), or placed on dry ice and stored at − 4°C (for *c.* 12 d) until their subsequent use as soil inoculum in the grow room experiment. All background soil for the grow room experiment was collected from a single rosemary scrub site at Archbold which last burned *c.* 20 yr prior (27°7′38.210″N, 81°20′57.562″W) and was sterilized prior to use (three times at 121°C).

Soil physicochemical analyses

Soils were analyzed for total soil organic matter content (%C) by loss on ignition (LOI), total Kjeldahl nitrogen (N), and Mehlich-3 extractable phosphorus (P) before and after prescribed fire treatment and across the 36 unique fire histories. Analyses of all 72 samples were performed at the University of Florida/Institute of Food and Agricultural Sciences (IFAS) Analytical Research Laboratory. Total organic carbon and N were measured from the same soil samples using an elemental carbon, hydrogen and nitrogen (CHN) analyzer, and plant-available P was measured with Mehlich-3 extractant and colorimetric determination of extracted P. One sample below the minimum detection limit for N and P was excluded from subsequent analyses.

Soil microbiome extraction, amplification, sequencing, and bioinformatic processing

DNA was extracted from homogenized soil samples ($n = 72$) using the DNeasy PowerSoil Pro QIAcube HT Kit (Qiagen; adapted protocol without QIAcube), and libraries were prepared for sequencing using a two-step dual indexing protocol (Gohl *et al.*, 2016). Briefly, we used *c.* 550 mg of soil for extractions, performed all wash and elution steps using S-Blocks (Cat. ID 19585) in an Avanti JXN-26 Centrifuge (Beckman Coulter Inc., Brea, CA, USA) at 4500 RCF, and performed final elution with 50 µl 10 mM Tris-HCl. DNA was quantified with a Qubit 4 fluorometer (Qiagen), and concentrations were normalized to 5 ng μl^{-1}. Polymerase chain reaction was targeted for archaeal/bacterial and broad fungal ribosomal DNA (rDNA) using primer pairs 515F-806R and ITS7o-ITS4, respectively. Polymerase chain reaction was carried out in 22 µl reactions containing 1 × Phusion Green Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA), an additional 1.5 mM $MgCl_2$ (3.0 mM total), 200 nM of each primer, ultra-pure water, and 2 µl of each normalized DNA sample. Polymerase chain reaction products were checked on 1% agarose gels, magnetic bead-purified, and diluted tenfold (1 : 10). Index and Illumina flowcell sequences were added in second-step PCR. Reaction conditions were the same for each amplicon, except we were using 100 nM for each primer, and 5 µl of PCR product. Products were checked on 1% agarose gel, magnetic bead-purified, and quantified via Qubit 4, and all targeted amplicon products were then pooled in equimolar quantities. The resulting pool was quantified using a Qubit 4 fluorometer and sent to the Duke University Microbiome Core Facility (Durham, NC, USA). Libraries were sequenced on a MiSeq Desktop Sequencer (v.3, 300 bp paired end; Illumina Inc., San Diego, CA, USA). Custom sequencing primers were used that matched the universal tail sequences from the first round of amplification (read 1: 5′-CCTATGTGGAGAGCCAG-TAAGCGATGCTATGGT-3′; read 2: 5′-GTCAACGC-TCACTACTGCGATTACCCAAG TCAG-3′; index 1: 5′-CTGACTTGGGTAATCGCAGT AGTGAGCGTTGAC-3′), similar to those in Alvarado *et al.* (2018).

Paired-end molecular sequence data were processed using QIIME2 v.2021.4 (Bolyen *et al.*, 2019). Briefly, denoising was performed with the DADA2 algorithm (Callahan *et al.*, 2016), which removes chimeric sequences and truncates 16S and ITS amplicon forward and reverse sequences to an equal length. Naive Bayes classifiers were constructed using the GREENGENES database v.13.8 (99%) and the UNITE database v.7.2 (99%) for archaeal/bacterial and fungal amplicons, respectively, and then amplicon sequence variants (ASVs) were classified using the SKLEARN algorithm (Pedregosa *et al.*, 2011). Multiple sequence alignments were performed using MAFFT v.7 (Katoh & Standley, 2013), an unrooted tree was created using FASTTREE2 (Price *et al.*, 2009), and the mid-point root method was then used to create a rooted tree for phylogeny-based analyses (e.g. weighted UniFrac). Amplicon sequence variants that were not present in greater than two samples were filtered out, and diversity metrics and dissimilarity matrices were calculated using the QIIME2 commands *diversity core-metrics-phylogenetic* (sampling depth = 6000) and *diversity core-metrics* (sampling depth = 10 000) for archaea/bacteria and fungi, respectively. All microbiome data from QIIME2 were read into R using the QIIME2R package (v.0.99.6).

Fire-selected microbiome experiment

Our 11 plant species from the rosemary scrub (Table S1) were grown in pots (66 ml Ray Leach cone-tainers; Stuewe & Sons, Tangent, OR, USA) inoculated with soil microbiomes from all factorial combinations of soil source (36 unique fire histories), fire treatment (unburned vs burned soil; described in the 'prescribed fire treatment' methods section above), and microbiome presence ('live' vs 'sterilized' soils) to determine the magnitude of microbial effects on plant performance and how these effects depend on exposure to fire. All sterilized soil and pots were autoclaved at 121°C three times over a period of 3 d. Each pot contained 50 ml of sterilized background soil plus 10 ml of the appropriate inoculum. This ensured that the majority of soil in each pot had identical abiotic and biotic properties, with only biotic factors (i.e. microbes) being able to disperse from the smaller amount of treatment soil (inoculum) and colonize the rest of the pot. After seeding directly into the inoculum soil, a 2 ml cap of sterilized background soil was added to avoid microbial desiccation. The number of seeds sown per pot (3–30 seeds per pot; Table S2) reflected previously determined differences in germination rates of these plant species (David *et al.*, 2020). Overall, our experiment included 36 microbiome sources in each of the four fire × microbial treatment combinations for each of the 11 plant species, totaling 1584 pots. All pots were watered daily with *c.* 2 ml sterile water for one month, and every other day afterwards. All pots were also quickly thinned to one plant if multiple seeds germinated. Plants were grown under full spectrum lights (*c.* 162 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR); calculated from Tran & Braun (2017)), with a 14 h : 10 h, light : dark photoperiod until harvest 4–7 months after the start of the experiment, depending on the natural histories of the plants (Table S1; as in David *et al.*, 2020). Germination percentages were determined based on species-specific seeding rates per pot, thus each pot was a replicate (Table S1). Shoot and root biomass (one plant per pot) were determined after oven-drying at 50°C until a constant mass was reached. Root : shoot biomass ratios were calculated to determine plant biomass allocation responses.

Data analysis

Linear mixed effects models were employed to determine the effects of prescribed fire and fire legacy metrics on soil properties (%C, total N, and plant-available P), with soil collection site as a random effect. To determine the effects of prescribed fire and fire legacy on bacterial and fungal community composition, PERMANOVA stratified by collection site was performed using the *adonis2* function in R package VEGAN v.2.5-7 (Oksanen *et al.*, 2020) on weighted uniFrac and Bray–Curtis dissimilarity matrices, respectively. The terms used in the model were as follows: prescribed fire treatment, TSF, and number of fires (since 1967). Standardized effect sizes (SES) of significant predictor variables were calculated using the *permutest* function in VEGAN. To identify microbial taxa that responded particularly strongly to fire, differential analysis of microbial relative abundances from unburned and burned samples was performed using the *DESeq* function in R package DESEQ2 v.1.32 (Love *et al.*, 2014).

To understand how fire pulse and legacy effects on microbiomes impact plants, general linear models were used to determine microbiome, prescribed fire, and fire legacy effects on percent germination (arcsine transformed), root : shoot ratio (log-transformed), and total dry mass. Specifically, we identified plant performance responses to the following: presence or absence of soil microbiomes, whether or not soils were exposed to prescribed fire, and fire legacy represented by two metrics – TSF and number of documented fires. In order to meet the assumption of homogeneity of variances across species, we re-expressed all three response variables as standard normal deviates relative to their species means. Using these data, we first ran models for all plant species combined. Terms in these models included all of the above main effects and all two-way interactions with the microbial treatment, as well as plant species identity and interactions between plant species and all of the other terms. After finding significant interactions with plant species identity in the overall model, we used general linear models for each plant species individually. These models included the same four main effects and all two-way interactions with the microbial treatment. Models were performed in JMP v.15 (SAS Institute Inc., Cary, NC, USA, 2019), which uses Type III sums of squares, which are independent of the input order of predictor variables. In order to further examine variation in microbial mediation of fire across our 11 species, we used model selection to determine whether any of our known life history traits (seed mass, fecundity, degree of habitat specialization on the Florida rosemary scrub (David *et al.*, 2020), or whether the species forms a seed bank) predicted the species-specific parameter estimates for any of the microbe treatment × fire effects that exhibited significant variation among species. In each case, we identified the best model based on the corrected Akaike Information Criterion (AICc). Analyses were performed in JMP and R v.4.1.0 (R Core Team, 2020).

Results

Analyses of the soil microbiome and plant performance show that the effects of pulse fire disturbance were significant and strong, in contrast to those of fire legacy. There were no significant effects of prescribed fire treatment or fire legacy (TSF and historic number of fires) on any soil abiotic properties (%C, total N, or plant-available P; Fig. S2). Fire legacy exhibited no effects on either fungal or bacterial community composition. While pulse fire (prescribed burn) did not alter fungal community composition (SES = 1.04, pseudo-F = 0.89, $P = 0.118$), it did significantly alter bacterial community composition (SES = 1.94, pseudo-F = 1.46, $P = 0.035$). Differential abundance analysis revealed 13 highly responsive bacteria/archaea that significantly increased or decreased in relative abundance after prescribed fire, with $\log_2$ fold change (LFC) ranging from −23 to + 23 (Fig. 1a). Taxa in the families Conexibacteraceae and Pseudonocardiaceae (both in the phylum Actinobacteria) and one in the phylum Firmicutes increased most dramatically after fire (with *c.* +22 LFCs; Fig. 1a).

Surprisingly, the relative abundance of a different taxa within the Pseudonocardiaceae declined by −20 LFC, and taxa from the Chloroflexi and Verrucomicrobia both decreased substantially after fire, by −23 LFC (Fig. 1a). While the effect of the prescribed burn treatment on the overall fungal community was nonsignificant, some individual fungal taxa were strongly responsive. The relative abundance of 11 fungal taxa changed significantly after fire, with LFC ranging from −23 to +21 (Fig. 1b), comparable in strength to LFC of the bacterial fire-responsive taxa. Specifically, we found that *Gibberella fujikuroi* (order Hypocreales) had the greatest increase in relative abundance (+21 LFC), while an unidentified member of the order Sordariales and *Talaromyces diversus* (order Eurotiales) experienced the greatest decreases in relative abundance after prescribed fire (−23 and −20 LFC, respectively).

After observing shifts in the soil microbiome induced by the pulse fire treatment, we assessed the resulting impacts of the microbial shifts on plant performance in our manipulative plant growth experiment. In the analysis across all plant species (Fig. 2), germination was most significantly impacted by the interactive effect of the prescribed fire and microbial treatments ($P = 0.0003$), but also by the fire legacy effects of TSF ($P = 0.0130$) and number of documented fires ($P = 0.0265$). With respect to these latter factors, plants germinated more readily in more recently, but less frequently, burned soils. However, the lack of significant interactions between these fire legacy terms and the microbial treatment indicated that these effects were likely due to unmeasured abiotic changes to the soil. Effects of pulse fire disturbance, on the other hand, were microbially mediated. In particular, the largest contrast among the treatment combinations was between live and sterilized soils exposed to fire ($P = 0.001$; Fig. 3), with sterilized soils exposed to fire yielding the most positive effect on germination, while the fire-exposed microbiome yielded the most negative effect. The unburned treatment exhibited the opposite relationship ($P = 0.0585$), where plants grown with unburned microbiomes outperformed those grown with sterilized soils.

Plant total dry biomass exhibited the same response to fire legacy effects as germination percentage (i.e. greater biomass in more recently burned soils ($P = 0.0011$) and reduced biomass in soils that have historically experienced a greater number of fires ($P = 0.0023$; Fig. 2a)). Neither of these effects interacted with the

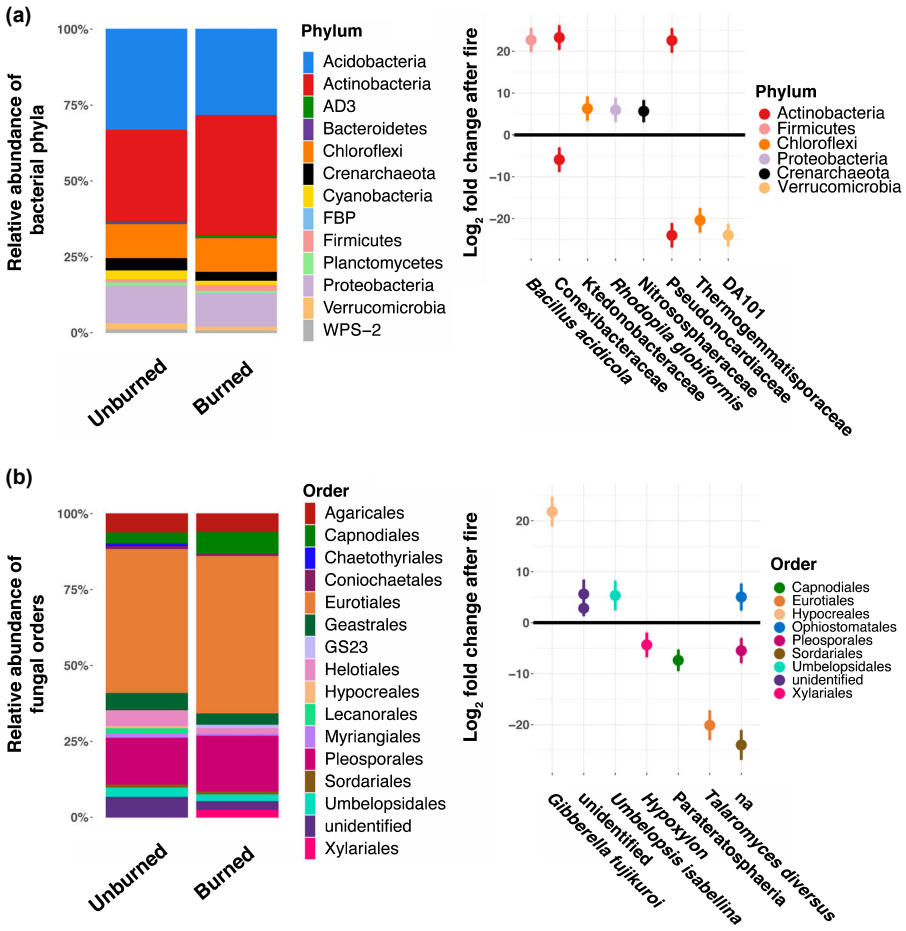
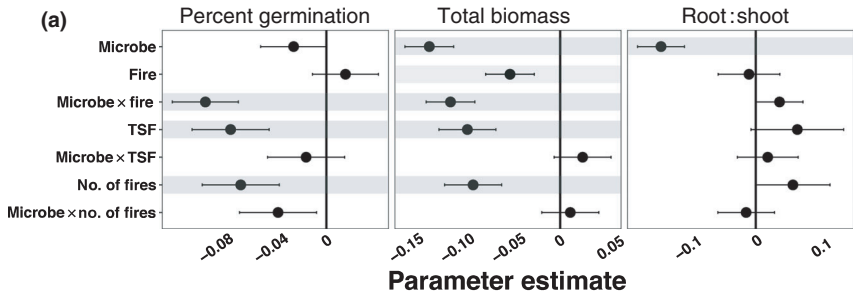


Fig. 1 Microbiome composition in unburned and burned treatments (left), and microbial taxa that exhibited significant changes in relative abundance after fire (right) for (a) archaea/bacteria and (b) fungi. Taxa on the *x*-axis (right) are presented with the highest taxonomic resolution for both (a) and (b). Points represent mean $\log_2$ fold change, bars are standard errors. na, no available classification beyond fungal order. Three additional bacterial and one additional fungal taxa are not presented as they could not be identified to phylum or order, respectively.



(b)

Variation among plant species

Performance metric	Species†	Species × microbe	Species × fire	Species × microbe × fire	Species × TSF	Species × no. of fires	Species × microbe × TSF	Species × microbe × no. of fires
Germination	***0.0001***	***0.0001***	***0.0001***	***0.0458***	0.0694	0.4368	0.5820	0.0517
Biomass	***0.0001***	***0.0001***	***0.0001***	***0.0035***	***0.0365***	0.3200	0.3985	0.0941
Root:shoot	***0.0001***	0.2563	***0.0001***	***0.0001***	0.8902	0.7612	***0.0411***	0.2630

Fig. 2 Results of global general linear models examining: (a) mean effects of microbial, prescribed burn, and fire legacy treatments, and (b) variation among plant species in percent germination, total dry biomass, and root : shoot biomass ratio across all 11 studied plant species. Points in (a) represent standardized β-coefficients, bars are SE, and shaded boxes indicate significant effects ($P < 0.05$). Pulse fire effects were microbially mediated for two out of three plant performance metrics, while neither fire legacy × microbe interaction was significant in any of the models examined. In (b), *P*-values are presented, with significant *P*-values bolded and italicized. †, *P*-values for variation among species means are based on raw values prior to within-species standardization. Fire, 'pulse' fire effects from prescribed burn; TSF, time since last fire.

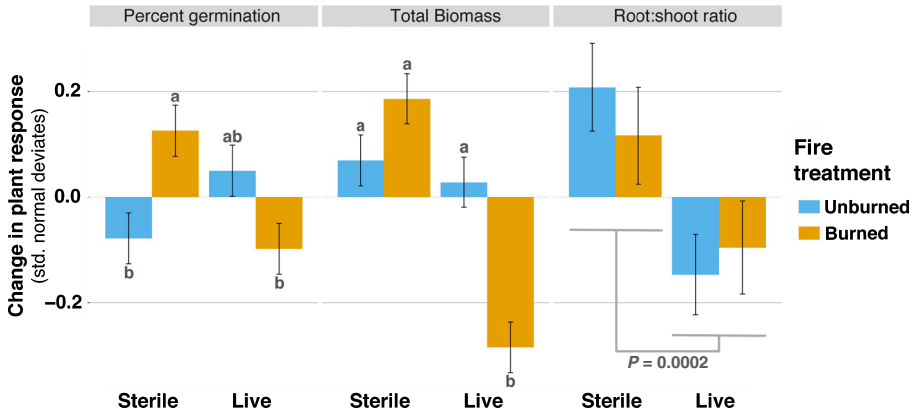


Fig. 3 Effects of prescribed fire and microbiome sterilization on plant percent germination, plant productivity, and root : shoot biomass ratio across all plant taxa. For total biomass, the burned soil microbiome yielded lower plant performance than all other treatment combinations (the lowercase letters represent significance groupings from Tukey's method). When a significant interaction was not present, we show *P*-values indicating significant microbiome main effects. Error bars are SE. All response variables are expressed in standard normal deviates, illustrating treatment effects relative to the grand mean and in units of mean SD across species.

microbial treatment, however, again suggesting that they were due to abiotic shifts in the soil. Similar to the germination results, the effects of immediate fire disturbance were microbially mediated, with 39% lower productivity for plants grown in the treatment combination with both recently fire-treated soils and a live microbiome (compared to mean productivity from all other treatment combinations; $P \leq 0.0001$; Fig. 3). Across species, the allocation of biomass to roots was found to be significantly lower when the soil microbiome was present (Fig. 3), but had no response to the pulse fire treatment or fire × microbial treatment interaction (Fig. 2a).

In addition to these common effects across plant species, there was also significant variation among species' responses to immediate fire disturbance, microbiomes, and their interaction (Fig. 2b). Of particular import was the significant inter-species variation in the response of plant productivity to the interaction of the prescribed fire and microbiome treatments ($P = 0.0035$; Fig. 4), as detecting such interactive effects between fire disturbance and the soil microbiome was the main goal of the experiment. Unfortunately, model selection failed to identify any of the plant life history traits as significant predictors of this interspecific variation. This was similarly true for all other microbe treatment × fire effects that exhibited significant interspecific variation. However, the species-specific models consistently showed the importance of pulse fire effects relative to fire legacy effects on plant performance. In the individual plant species models, the prescribed fire × microbiome treatment interaction was significant for 7 of the 11 species, while the microbiome treatment

interaction with both TSF and the number of documented fires previously experienced by the soils was each significant for only 2 of the 11 species (Table 1).

Discussion

Our experiment demonstrated a major role for microbial mediation of plant species' response to pulse fire disturbance. Despite the diverse fire legacy of our soil sources, there were no legacy effects on the microbiome, and instead we show that pulse fire disturbance selected most strongly on the microbiome and its interactions with the plant community. In the plant–microbiome experiment, we found a significant interaction between pulse fire disturbance and presence of a soil microbiome for 7 of the 11 plant species examined, more than for any other factor except the main effect of microbiome presence, which was also observed in seven species. To our knowledge, this research is the first to specifically test the effects of fire-altered microbiomes on plant performance in a fully manipulative experiment. Plant performance was, on average, negatively affected by soil microbiomes that experienced pulse fire disturbance (Fig. 2), but the strength, and in some cases direction, of effects was dependent on plant species identity. Those species most able to tolerate the negative effects of the post-fire microbiome may experience a relative advantage with respect to their competitors, which could establish priority effects important for successional dynamics. In the following paragraphs, we discuss the responses of the soil microbiome to pulse fire disturbance, impacts of the post-fire microbiome on plant performance, and implications for plant community development.

Soil microbes in our study exhibited significant changes after an experimental prescribed burn, including shifts in abundance of taxa that could prove critical in plant–microbial interactions that ultimately determine plant fitness after disturbance. Previous studies have shown that fire can alter soil microbial communities (Knelman *et al.*, 2015; Reazin *et al.*, 2016; Certini *et al.*, 2021), influence bacterial assembly patterns (van der Voort *et al.*, 2016), and also shift microbial carbon, nitrogen, and phosphorus cycling capacities (Wang *et al.*, 2012; Pérez-Valera *et al.*, 2020), but we knew very little about the immediacy with which fire could alter the soil microbiome, or the subsequent impact of these fire-induced changes on plant performance. Here, we show that pulse fire disturbance significantly altered bacterial community composition within minutes after fire, including substantially shifting the relative abundances of > 20 bacterial and fungal taxa. Some of the most striking shifts occurred among the fungal taxa *T. diversus* and *G. fujikuroi*. *Talaromyces diversus*, a known P-solubilizing fungal species and pathogen antagonist (Raaijmakers *et al.*, 2009; Della Mónica *et al.*, 2018) experienced the greatest decrease in relative abundance after the prescribed fire treatment (−23 LFC), while a virulent plant pathogen, *G. fujikuroi* (O'Donnell *et al.*, 2000), exhibited the greatest increase in relative abundance (+21 LFC). Previous studies have suggested that in early stages of succession the ratio of pathogens to mutualists is high (Hannula *et al.*, 2017), which aligns with this result. Among bacteria, there was a surprising decrease after fire in abundance

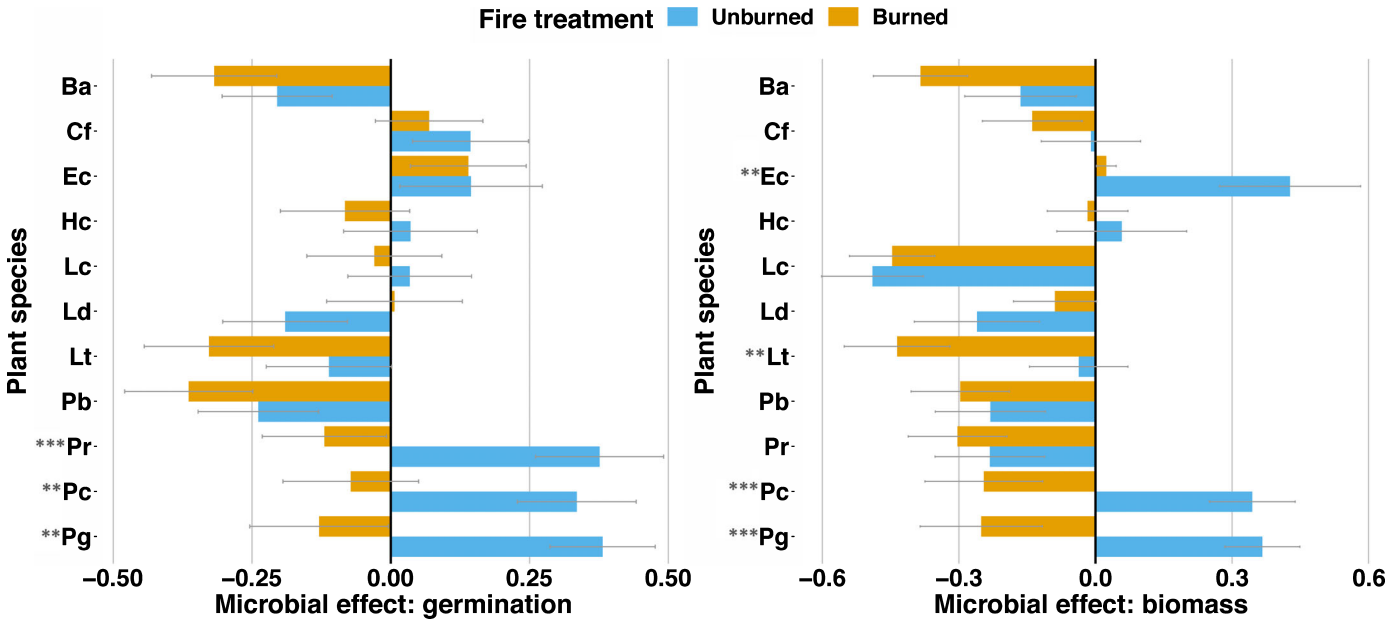


Fig. 4 Microbial effects in burned and unburned treatments for all plant taxa. The zero line represents no effect of the soil microbiome on plant germination (left) or plant biomass (right). Microbiome-mediated plant responses to fire were generally negative, but there was significant variation among species. Microbial effect was calculated as the mean of the live treatment – the grand mean for the relevant species × fire treatment combination. Error bars represent the SE of the mean parameter estimate. Asterisks indicate a significant difference between microbial effects for burned vs unburned treatments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Ba, *Balduina angustifolia*; Cf, *Chamaecrista fasciculata*; Ec, *Eryngium cuneifolium*; Hc, *Hypericum cumulicola*; Lc, *Lechea cernua*; Ld, *Lechea deckertii*; Lt, *Liatris tenuifolia*; Pb, *Polygonella basiramia*; Pr, *Polygonella robusta*; Pc, *Paronychia chartacea*; Pg, *Pityopsis graminifolia*.

Table 1 Results from general linear models (GLMs) examining microbial, prescribed burn, and fire legacy effects on plant percent germination, total dry biomass, and root : shoot biomass ratio in individual plant species.

GLM response	Plant species	Microbe	Fire	Microbe* Fire	TSF	TSF* Microbe	No. of fires	No. of fires* Microbe
Germination	*Balduina angustifolia*	0.0004 (−)	0.0001 (+)	–	–	–	0.0404 (−)	0.0052 (−)
	Chamaecrista fasciculata	–	0.0001 (−)	–	–	–	–	–
	Eryngium cuneifolium	–	–	–	–	0.0383 (+)	–	–
	Lechea cernua	–	0.0171 (−)	–	–	–	–	–
	Lechea deckertii	–	–	–	0.0102 (−)	–	–	–
	Liatris tenuifolia	0.0079 (−)	–	–	–	–	–	–
	Paronychia chartacea	–	–	0.0138 (−)	–	–	–	–
	Pityopsis graminifolia	–	0.004 (+)	0.0012 (−)	–	–	–	0.0184 (−)
	Polygonella basiramia	0.0003 (−)	–	–	–	–	–	–
	Polygonella robusta	–	–	0.0023 (−)	–	–	–	–
Biomass	*B. angustifolia*	0.0006 (−)	–	–	0.0185 (−)	–	0.0016 (−)	–
	C. fasciculata	–	0.0001 (−)	–	–	–	–	–
		0.003 (+)	0.0054 (−)	0.0077 (−)	0.0363 (+)	–	–	–
	Hypericum cumulicola	–	–	–	0.0258 (−)	–	–	–
	L. cernua	0.0001 (−)	0.0223 (−)	–	–	–	–	–
	L. deckertii	0.0336 (−)	–	–	0.0103 (−)	–	–	–
	L. tenuifolia	0.0038 (−)	–	0.0142 (−)	–	–	–	–
	P. chartacea	–	–	0.0004 (−)	–	–	–	–
	P. graminifolia	–	0.0392 (+)	0.0001 (−)	–	–	–	0.0115 (−)
	P. basiramia	0.0014 (−)	–	–	–	–	–	–
	P. robusta	0.0013 (−)	–	–	–	–	–	–
Root : shoot	*B. angustifolia*	0.0115 (−)	0.0236 (−)	0.0004 (−)	–	–	–	–
	C. fasciculata	–	0.0298 (−)	–	–	–	–	–
	L. cernua	–	0.0001 (+)	0.0289 (+)	–	–	–	–
	L. deckertii	–	–	–	–	0.0282 (+)	–	–
	P. graminifolia	–	–	–	–	–	–	0.0193 (−)
	P. robusta	0.0013 (−)	0.0343 (+)	0.012 (+)	–	–	–	–

Microbial effects and their interactions with the prescribed burn treatment were common, while fire legacy effects (and their interactions) were comparatively weak. Plant performance metrics that were not significantly related to any of the model terms were omitted from the table. Fire, 'pulse' fire effects from the prescribed burn treatment; TSF, time since last fire. Reported values are *P*-values followed by direction of the effect in parentheses. *(column headings) indicates model interaction term.

(−20 LFC; Fig. 1) of a putative thermophilic taxon in the family Thermogemmatisporaceae (Yabe *et al.*, 2017). More predictably, two bacterial taxa in the families Conexibacteraceae and Pseudonocardiaceae, found commonly in disturbed habitats (Shange *et al.*, 2012) and known to respond positively to pyrogenic organic matter content in soil (Nielsen *et al.*, 2014; Khodadad *et al.*, 2011), increased in relative abundance after prescribed fire (+23 and +22 LFC, respectively). Overall, changes to the microbiome inhibited plant performance, which has the potential to limit plant survival in the recently disturbed, stressful environment. In future work, it would be valuable to identify individual microbial taxa responsible for these effects on plant performance.

In this study, fire shifted the effects of the soil microbiome towards less beneficial, or even harmful, interactions for most of the plant taxa examined. Collectively, post-fire microbiomes caused performance responses to switch from positive to negative for 5 of 11 plant taxa in our experiment (Fig. 4), suggesting 'pulse' disturbance disruptions to plant–microbial interactions. This is a particularly interesting outcome given that our previous work in this system that did not examine pulse fire effects has shown that associating with a microbiome can improve plant performance across a wide range of plant taxa (David *et al.*, 2018, 2020) and in some cases can even be crucial for plant population persistence (David *et al.*, 2019). A recent meta-analysis found that wildfires reduce mycorrhizal colonization of plant roots globally by 21% (Dove & Hart, 2017). In another study, high-severity fire increased the proportion of pathogenic fungi and decreased plant performance, thus altering plant establishment patterns in the Arctic tundra (Hewitt *et al.*, 2016). By contrast, Prendergast-Miller *et al.* (2017) found that fire disturbance increased the abundance of putative mutualists in the soil microbiome. While all of these studies provide important glimpses into the composition of post-fire microbiomes, none of them factorially manipulated both fire and microbiome presence/absence, and thus microbiome mediation of plant fitness after fire remained unknown. We found that such interactive effects are indeed present, and most importantly we have demonstrated that plant responses to the soil microbiome are strongest after pulse fire disturbance and are unaffected by soil fire legacy, adding a new dimension to our understanding of how fire and microbes interact to influence plant performance.

Despite strong fire legacy effects on plant distributions in the Florida scrub (Menges & Kohfeldt, 1995; Menges *et al.*, 2017a), we found soil microbiome mediated fire legacy effects on plant performance were weaker and less predictable than those

mediated through the prescribed fire treatment (Table 1; Fig. 2). We suspect that multiple factors may explain the generally muted fire legacy effect on the microbiome and plant–soil microbiome interactions in our study, including limited penetration of fire along a soil depth gradient, and the small spatial scale of fire management units in this system. Wildfires and prescribed burns have been shown to impose the strongest effects on surface soils and microbiomes (Bruns *et al.*, 2020). Due to the limited volumes of organic matter present in the Florida scrub habitat, prescribed burns do not penetrate deeply into the soil (Carrington, 2010), leaving potential for microbial recolonization from short vertical distances below the surface, thus minimizing the duration of fire legacy effects on the soil microbiome. Prescribed burns in the Florida scrub also have relatively small spatial scales, which may allow for quick microbial dispersal and recolonization of burned areas across horizontal space as well as the vertical space (Chaudhary *et al.*, 2020; Evans *et al.*, 2020). Given these scaling factors, it might not be surprising that fire legacy effects were less pronounced in our study compared to some other studies. More broadly, these results emphasize the importance of considering differences in ecological characteristics and management practices between systems when predicting the importance of microbiomes in fire legacy effects on plants.

Even if short-lived, the immediate 'pulse' impacts of fire on the microbiome, and consequent effects on plant recruitment and performance, could prove critical to the successional trajectories of plant communities through changes in priority effects (Duhamel *et al.*, 2019). After disturbance, differential early establishment of plants can influence the abiotic and biotic conditions that drive community development through time (Fukami, 2015; Weidlich *et al.*, 2021). Early establishing plants can alter availability of limiting resources to other plants (e.g. availability of light, water, and nutrients in soils), which then influences interspecific plant–plant and plant–soil interactions (Van de Voorde *et al.*, 2011; Suding *et al.*, 2013) that lead to nonneutral plant community assembly (Fargione *et al.*, 2004). Early colonizers can also affect the belowground microbiome through the input of plant-specific exudates and interactions with selected microbial partners (Sasse *et al.*, 2018; Jones *et al.*, 2019). Filtering of the local abiotic and biotic conditions by early colonizers immediately after fire could perpetuate an environment that either inhibits or facilitates competitor plant establishment and productivity, thus altering plant community assembly and successional dynamics (Wubs *et al.*, 2019), even if fire effects on microbiome composition or plant–microbiome interactions are relatively short-term.

While the evidence for post-fire soil microbiome impacts on plant performance is strong in this experiment, there are some potential alternative hypotheses that should be addressed in future studies. While none of the soil abiotic factors measured in this experiment (%C, N, P) were affected by the prescribed fire treatment or fire legacy, soil pH and other micronutrients can respond to fire (Reinhart *et al.*, 2016; Alcañiz *et al.*, 2018), and play important roles in shaping microbial community structure and function (Egidi *et al.*, 2016; Pérez-Valera *et al.*, 2020). These additional soil characteristics as well as associated microbial functions could be analyzed in tandem to continue to advance our understanding of both abiotic and biotic contributions to disturbance effects on plant–microbial interactions in the future. It is also important to note that experimental soil sterilization via autoclave may not completely remove all members of the microbiome (Berns *et al.*, 2008), though this method is notably effective at eliminating microbial activity and is recommended for microbial manipulation studies (Otte *et al.*, 2018). Extremophile bacteria and fungi have the potential to survive this sterilization treatment, which could lead to a reduced but persistent microbiome with potential to influence microbial reassembly (van der Voort *et al.*, 2016). Despite this caveat, the experimental design employed here with a majority of sterilized background soil present in all pots and thus microbial treatment effects being driven almost exclusively by the 'live' treatment's unsterilized soil inoculum allows for robust conclusions.

This study's demonstration of the importance of pulse fire effects on plant–microbe interactions is particularly pressing given the expected increase in pulse fire disturbances as a result of anthropogenic climate change (Bowman *et al.*, 2020; Kelly *et al.*, 2020). Even more concerning is that these effects were generally negative, indicating that fire is not only releasing carbon previously stored in live biomass, but it may also be selecting for a microbiome that inhibits resequestration of some of that carbon back into the plant tissue (Lasslop *et al.*, 2019). Targeted studies in the future should begin to quantify the impact of post-disturbance microbiome mediation on plant carbon cycling (Chen *et al.*, 2017). One positive note is that these negative effects appear to be short-lived, with no indication of prolonged fire legacy effects over a time-since-fire gradient of 1–92 yr. Future studies could also work to more finely partition the recent time-since-fire side of this gradient, examining soils that have burned over a scale of several weeks to months rather than several decades. This would further clarify how microbial communities are structured by deterministic vs stochastic processes over time (Ferrenberg *et al.*, 2013), and how quickly the soil microbiome (and its attendant interactions with the plant community) recovers from this type of pulse disturbance. In conclusion, we advocate for more plant–soil ecological studies using a range of temporally distinct disturbance regimes and manipulative disturbance treatments to better understand the role of post-disturbance microbiome mediation of plant succession.

Acknowledgements

This work was funded by an award to MEA and CAS from the U.S. National Science Foundation (NSF) DEB-1922521, and a research grant to DR from the Florida Native Plant Society. Many thanks to Stephanie Koontz and KT Charton for invaluable support at Archbold; Vivienne Sclater, who supplied important metadata for Archbold fire legacy analyses; members of the Afkhami and Searcy labs for their insights during the preparation of this manuscript; and three anonymous reviewers and editor Dr. Amy Austin for constructive comments, which greatly improved the manuscript. Special thanks to Kasey Kiesewetter and Hunter Howell for assistance with field work. Extra special thanks to undergraduate research assistants Brooke Boyd,

Carolina Vigo, and Preston Allen for all their work on the plant–microbiome experiment through setup and harvest.

Author contributions

DR, ASD, MEA, and CAS planned and designed the research. DR and CAS performed the experiment and analyzed data, with contributions from MEA. KNM performed the experimental burn treatment. DR, MEA, and CAS wrote the manuscript. ASD and ESM edited the manuscript. MEA and CAS contributed equally to this work.

ORCID

Michelle E. Afkhami https://orcid.org/0000-0001-7207-6256
Aaron S. David https://orcid.org/0000-0002-0802-7799
Daniel Revillini https://orcid.org/0000-0002-0380-4106

Data availability

Microbiome sequencing data have been submitted to the NCBI SRA database (PRJNA750476). Plant performance data will be available in the Dryad database after 16 February 2022 at https://doi.org/10.5061/dryad.rn8pk0pbb.

References

Abatzoglou JT, Williams AP, Boschetti L, Zubkova M, Kolden CA. 2018. Global patterns of interannual climate–fire relationships. *Global Change Biology* **24**: 5164–5175.

Abrahamson WG, Johnson AF, Layne JN , Peroni PA. 1984. Vegetation of the Archbold Biological Station, Florida: an example of the southern Lake Wales ridge. *Florida Scientist* **47**: 209–250.

Adkins J, Miesel JR. 2021. Post-fire effects of soil heating intensity and pyrogenic organic matter on microbial anabolism. *Biogeochemistry* **154**: 555–571.

Alcañiz M, Outeiro L, Francos M, Úbeda X. 2018. Effects of prescribed fires on soil properties: a review. *Science of the Total Environment* **613–614**: 944–957.

Allen K, Fisher JB, Phillips RP, Powers JS, Brzostek ER. 2020. Modeling the carbon cost of plant nitrogen and phosphorus uptake across temperate and tropical forests. *Frontiers in Forests and Global Change* **3**: 1–12.

Alvarado P, Teixeira MDM, Andrews L, Fernandez A, Santander G, Doyle A, Perez M, Yegres F, Barker BM. 2018. Detection of *Coccidioides posadasii* from xerophytic environments in Venezuela reveals risk of naturally acquired coccidioidomycosis infections article. *Emerging Microbes and Infections* **7**: 1–13.

Bakker PAHM, Pieterse CMJ, de Jonge R, Berendsen RL. 2018. The soil-borne legacy. *Cell* **172**: 1178–1180.

Berns AE, Philipp H, Narres HD, Burauel P, Vereecken H, Tappe W. 2008. Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. *European Journal of Soil Science* **59**: 540–550.

Bever JD, Dickie IA, Facelli E, Facelli JM, Klironomos J, Moora M, Rillig MC, Stock WD, Tibbett M, Zobel M. 2010. Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution* **25**: 468–478.

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F *et al.* 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* **37**: 852–857.

Bond WJ, Keeley JE. 2005. Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends in Ecology and Evolution* **20**: 387–394.

Bowman DMJS, Kolden CA, Abatzoglou JT, Johnston FH, van der Werf GR, Flannigan M. 2020. Vegetation fires in the Anthropocene. *Nature Reviews Earth & Environment* **1**: 500–515.

Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres LA, Kunstler G, Liancourt P, Tielbörger K, Travis JMJ, Anthelme F. *et al.* 2008. Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology* **96**: 18–34.

Bruns TD, Chung JA, Carver AA, Glassman SI. 2020. A simple pyrocosm for studying soil microbial response to fire reveals a rapid, massive response by Pyronema species. *PLoS ONE* **15**: 1–20.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Carrington ME. 2010. Effects of soil temperature during fire on seed survival in Florida Sand Pine Scrub. *International Journal of Forestry Research* **2010**: 1–10.

Certini G, Moya D, Lucas-Borja ME, Mastrolonardo G. 2021. The impact of fire on soil-dwelling biota: a review. *Forest Ecology and Management* **488**: 118989.

Chaudhary VB, Nolimal S, Sosa-Hernández MA, Egan C, Kastens J. 2020. Trait-based aerial dispersal of arbuscular mycorrhizal fungi. *New Phytologist* **228**: 238–252.

Chen G, Hayes DJ, David MA. 2017. Contributions of wildland fire to terrestrial ecosystem carbon dynamics in North America from 1990 to 2012. *Global Biogeochemical Cycles* **31**: 878–900.

Collins SL, Ladwig LM, Petrie MD, Jones SK, Mulhouse JM, Thibault JR, Pockman WT. 2017. Press–pulse interactions: effects of warming, N deposition, altered winter precipitation, and fire on desert grassland community structure and dynamics. *Global Change Biology* **23**: 1095–1108.

Cordovez V, Dini-Andreote F, Carrión VJ, Raaijmakers JM. 2019. Ecology and evolution of plant microbiomes. *Annual Review of Microbiology* **73**: 69–88.

David AS, Quintana-Ascencio PF, Menges ES, Thapa-Magar KB, Afkhami ME, Searcy CA. 2019. Soil microbiomes underlie population persistence of an endangered plant species. *American Naturalist* **194**: 488–494.

David AS, Thapa-Magar KB, Afkhami ME. 2018. Microbial mitigation–exacerbation continuum: a novel framework for microbiome effects on hosts in the face of stress. *Ecology* **99**: 517–523.

David AS, Thapa-Magar KB, Menges ES, Searcy CA, Afkhami ME. 2020. Do plant–microbe interactions support the Stress Gradient Hypothesis? *Ecology* **101**: 1–10.

De Deyn GB, Raaijmakers CE, van der Putten WH. 2004. Plant community development is affected by nutrients and soil biota. *Journal of Ecology* **92**: 824–834.

Dee JR, Menges ES. 2014. Gap ecology in the Florida scrubby flatwoods: effects of time-since-fire, gap area, gap aggregation and microhabitat on gap species diversity. *Journal of Vegetation Science* **25**: 1235–1246.

Della Mónica IF, Godoy MS, Godeas AM, Scervino JM. 2018. Fungal extracellular phosphatases: their role in P cycling under different pH and P sources availability. *Journal of Applied Microbiology* **124**: 155–165.

Dove NC, Hart SC. 2017. Fire reduces fungal species richness and in situ mycorrhizal colonization: a meta-analysis. *Fire Ecology* **13**: 37–65.

Duhamel M, Wan J, Bogar LM, Segnitz RM, Duncritts NC, Peay KG. 2019. Plant selection initiates alternative successional trajectories in the soil microbial community after disturbance. *Ecological Monographs* **89**: e01367.

Egidi E, McMullan-Fisher S, Morgan JW, May T, Zeeman B, Franks AE. 2016. Fire regime, not time-since-fire, affects soil fungal community diversity and composition in temperate grasslands. *FEMS Microbiology Letters* **363**: 1–11.

Evans SE, Bell-Dereske LP, Dougherty KM, Kittredge HA. 2020. Dispersal alters soil microbial community response to drought. *Environmental Microbiology* **22**: 905–916.

Fargione JE, Brown CS, Tilman D. 2004. Community assembly and invasion: an experimental test of neutral versus niche processes. *Proceedings of the National Academy of Sciences, USA* **101**: 8916–8920.

Ferrenberg S, O'Neill SP, Knelman JE, Todd B, Duggan S, Bradley D, Robinson T, Schmidt SK, Townsend AR, Williams MW *et al.* 2013. Changes in assembly processes in soil bacterial communities following a wildfire disturbance. *ISME Journal* **7**: 1102–1111.

Franklin J, Syphard AD, He HS, Mladenoff DJ. 2005. Altered fire regimes affect landscape patterns of plant succession in the foothills and mountains of southern California. *Ecosystems* **8**: 885–898.

Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, Martinez-Romero E. 2011. Microbially mediated plant functional traits. *Annual Review of Ecology, Evolution, and Systematics* **42**: 23–46.

Fukami T. 2015. Historical contingency in community assembly: integrating niches, species pools, and priority effects. *Annual Review of Ecology, Evolution, and Systematics* **46**: 1–23.

Gohl DM, Vangay P, Garbe J, MacLean A, Hauge A, Becker A, Gould TJ, Clayton JB, Johnson TJ, Hunter R *et al.* 2016. Systematic improvement of amplicon marker gene methods for increased accuracy in microbiome studies. *Nature Biotechnology* **34**: 942–949.

Hannula SE, Morriën E, De Hollander M, Van Der Putten WH, Van Veen JA, De Boer W. 2017. Shifts in rhizosphere fungal community during secondary succession following abandonment from agriculture. *ISME Journal* **11**: 2294–2304.

He T, Lamont BB, Pausas JG. 2019. Fire as a key driver of Earth's biodiversity. *Biological Reviews* **94**: 1983–2010.

van der Heijden MGA, De BS, Luckerhoff L, Van Logtestijn RSP, Schlaeppi K. 2016. A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. *ISME Journal* **10**: 389–399.

Hernandez DJ, David AS, Menges ES, Searcy CA, Afkhami ME. 2021. Environmental stress destabilizes microbial networks. *ISME Journal* **15**: 1722–1734.

Hewitt RE, Hollingsworth TN, Stuart Chapin F, Lee TD. 2016. Fire-severity effects on plant-fungal interactions after a novel tundra wildfire disturbance: Implications for arctic shrub and tree migration. *BMC Ecology* **16**: 1–11.

Hillebrand H, Kunze C. 2020. Meta-analysis on pulse disturbances reveals differences in functional and compositional recovery across ecosystems. *Ecology Letters* **23**: 575–585.

Huang Y, Wu S, Kaplan JO. 2014. Sensitivity of global wildfire occurrences to various factors in the context of global change. *Atmospheric Environment* **121**: 86–92.

Jacquet C, Altermatt F. 2020. The ghost of disturbance past: long-term effects of pulse disturbances on community biomass and composition. *Proceedings of the Royal Society B: Biological Sciences* **287**: 20200678.

Johnson NC, Gehring CA, Jansa J. 2016. *Mycorrhizal mediation of soil: fertility, structure, and carbon storage*. Amsterdam, Netherlands: Elsevier.

Jolly WM, Cochrane MA, Freeborn PH, Holden ZA, Brown TJ, Williamson GJ, Bowman DMJS. 2015. Climate-induced variations in global wildfire danger from 1979 to 2013. *Nature Communications* **6**: 1–11.

Jones P, Garcia BJ, Furches A, Tuskan GA, Jacobson D. 2019. Plant host-associated mechanisms for microbial selection. *Frontiers in Plant Science* **10**: 1–14.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software v.7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.

Kelly LT, Giljohann KM, Duane A, Aquilué N, Archibald S, Batllori E, Bennett AF, Buckland ST, Canelles Q, Clarke MF *et al.* 2020. Fire and biodiversity in the Anthropocene. *Science* **370**: 6519.

Keymer DP, Lankau RA. 2017. Disruption of plant–soil–microbial relationships influences plant growth. *Journal of Ecology* **105**: 816–827.

Khodadad CL, Zimmerman AR, Green SJ, Uthandi S, Foster JS. 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. *Soil Biology and Biochemistry* **43**: 385–392.

Kim Y-C, Glick B, Bashan Y, Ryu C-M. 2012. Enhancement of plant drought tolerance by microbes. In: Aroca R, ed. *Plant responses to drought stress SE – 15*. Berlin, Germany: Springer, 383–413.

Knelman JE, Graham EB, Trahan NA, Schmidt SK, Nemergut DR. 2015. Fire severity shapes plant colonization effects on bacterial community structure, microbial biomass, and soil enzyme activity in secondary succession of a burned forest. *Soil Biology and Biochemistry* **90**: 161–168.

Koffel T, Boudsocq S, Loeuille N, Daufresne T. 2018. Facilitation- vs competition-driven succession: the key role of resource-ratio. *Ecology Letters* **21**: 1010–1021.

Lasslop G, Coppola AI, Voulgarakis A, Yue C, Veraverbeke S. 2019. Influence of fire on the carbon cycle and climate. *Current Climate Change Reports* **5**: 112–123.

Love MI, Anders S, Huber W. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**: 550.

Lucas-Borja ME, Miralles I, Ortega R, Plaza-Álvarez PA, Gonzalez-Romero J, Sagra J, Soriano-Rodríguez M, Certini G, Moya D, Heras J. 2019. Immediate fire-induced changes in soil microbial community composition in an outdoor experimental controlled system. *Science of the Total Environment* **696**: 134033.

Menges ES, Craddock A, Salo J, Zinthefer R, Weekley CW. 2008. Gap ecology in Florida scrub: species occurrence, diversity and gap properties. *Journal of Vegetation Science* **19**: 503–514.

Menges ES, Crate SJH, Quintana-Ascencio PF. 2017a. Dynamics of gaps, vegetation, and plant species with and without fire. *American Journal of Botany* **104**: 1825–1836.

Menges ES, Hawkes CV. 1998. Interactive effects of fire and microhabitat on plants of Florida scrub. *Ecological Applications* **8**: 935–946.

Menges ES, Kohfeldt N. 1995. Life history strategies of Florida scrub plants in relation to fire. *Bulletin of the Torrey Botanical Club* **122**: 282–297.

Menges ES, Main KN, Pickert RL, Ewing K. 2017b. Evaluating a fire management plan for fire regime goals in a Florida landscape. *Natural Areas Journal* **37**: 212–227.

Miller JED, Safford HD. 2020. Are plant community responses to wildfire contingent upon historical disturbance regimes? *Global Ecology and Biogeography* **29**: 1621–1633.

Nielsen S, Minchin T, Kimber S, van Zwieten L, Gilbert J, Munroe P, Joseph S, Thomas T. 2014. Comparative analysis of the microbial communities in agricultural soil amended with enhanced biochars or traditional fertilisers. *Agriculture, Ecosystems & Environment* **191**: 73–82.

O'Donnell K, Nirenberg HI, Aoki T, Cigelnik E. 2000. A multigene phylogeny of the *Gibberella fujikuroi* species complex. *Mycoscience* **41**: 61–78.

Oksanen AJ, Blanchet FG, Kindt R. 2020. *Vegan: community ecology package v.2.5-7*. [WWW document] URL https://rdrr.io/cran/vegan/

Otte JM, Blackwell N, Soos V, Rughöft S, Maisch M, Kappler A, Kleindienst S, Schmidt C. 2018. Sterilization impacts on marine sediment-Are we able to inactivate microorganisms in environmental samples? *FEMS Microbiology Ecology* **94**: 1–14.

Pedregosa F, Varaquaux G, Gramfort A, Michel V, Thirion B, Grisel O, Blondel M, Prettenhofer P, Weiss R, Dubourg V *et al.* 2011. Scikit-learn: machine learning in python. *Journal of Machine Learning Research* **12**: 2825–2830.

Pérez-Valera E, Verdú M, Navarro-Cano JA, Goberna M. 2020. Soil microbiome drives the recovery of ecosystem functions after fire. *Soil Biology and Biochemistry* **149**: 107948.

Philippot L, Griffiths BS, Langenheder S. 2021. Microbial community resilience across ecosystems and multiple disturbances. *Microbiology and Molecular Biology Reviews* **85**: e00026–e00120.

Prendergast-Miller MT, de Menezes AB, Macdonald LM, Toscas P, Bissett A, Baker G, Farrell M, Richardson AE, Wark T, Thrall PH. 2017. Wildfire impact: natural experiment reveals differential short-term changes in soil microbial communities. *Soil Biology and Biochemistry* **109**: 1–13.

Price MN, Dehal PS, Arkin AP. 2009. Fasttree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution* **26**: 1641–1650.

Qin C, Zhu K, Chiariello NR, Field CB, Peay KG. 2020. Fire history and plant community composition outweigh decadal multi-factor global change as drivers of microbial composition in an annual grassland. *Journal of Ecology* **108**: 611–625.

Quintana-Ascencio PF, Menges ES. 2000. Competitive abilities of three narrowly endemic plant species in experimental neighborhoods along a fire gradient. *American Journal of Botany* **87**: 690–699.

R Core Team. 2020. *R: a language and environment for statistical computing v.4.1.0*. Vienna, Austria: R Foundation for Statistical Computing. [WWW document] URL https://www.R-project.org/.

Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz Y. 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil* **321**: 341–361.

Reazin C, Morris S, Smith JE, Cowan AD, Jumpponen A. 2016. Fires of differing intensities rapidly select distinct soil fungal communities in a

Northwest US ponderosa pine forest ecosystem. *Forest Ecology and Management* **377**: 118–127.

Reinhart KO, Dangi SR, Vermeire LT. 2016. The effect of fire intensity, nutrients, soil microbes, and spatial distance on grassland productivity. *Plant and Soil* **409**: 203–216.

Sasse J, Martinoia E, Northen T. 2018. Feed your friends: do plant exudates shape the root microbiome? *Trends in Plant Science* **23**: 25–41.

Shange RS, Ankumah RO, Ibekwe AM, Zabawa R, Dowd SE. 2012. Distinct soil bacterial communities revealed under a diversely managed agroecosystem. *PLoS ONE* **7**: e40338.

Sharifi R, Ryu CM. 2018. Revisiting bacterial volatile-mediated plant growth promotion: lessons from the past and objectives for the future. *Annals of Botany* **122**: 349–358.

Sousa WP. 1980. The response of community to disturnace: the importance of successional age and species' life histories. *Oecologia* **81**: 72–81.

Suding KN, Stanley Harpole W, Fukami T, Kulmatiski A, Macdougall AS, Stein C, van der Putten WH. 2013. Consequences of plant-soil feedbacks in invasion. *Journal of Ecology* **101**: 298–308.

Tran TM, Braun DM. 2017. An inexpensive, easy-to-use, and highly customizable growth chamber optimized for growing large plants. *Current Protocols in Plant Biology* **2**: 299–317.

Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. 2020. Plant–microbiome interactions: from community assembly to plant health. *Nature Reviews Microbiology* **18**: 607–621.

Van de Voorde TFJ, van der Putten WH, Martijn BT. 2011. Intra- and interspecific plant-soil interactions, soil legacies and priority effects during old-field succession. *Journal of Ecology* **99**: 945–953.

van der Voort M, Kempenaar M, van Driel M, Raaijmakers JM, Mendes R. 2016. Impact of soil heat on reassembly of bacterial communities in the rhizosphere microbiome and plant disease suppression. *Ecology Letters* **19**: 375–382.

Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. 2015. The importance of the microbiome of the plant holobiont. *New Phytologist* **206**: 1196–1206.

Wagg C, Bender SF, Widmer F, van der Heijden MGA. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences, USA* **111**: 5266–5270.

Wang Q, Zhong M, Wang S. 2012. A meta-analysis on the response of microbial biomass, dissolved organic matter, respiration, and N mineralization in mineral soil to fire in forest ecosystems. *Forest Ecology and Management* **271**: 91–97.

Weidlich EWA, Nelson CR, Maron JL, Callaway RM, Delory BM, Temperton VM. 2021. Priority effects and ecological restoration. *Restoration Ecology* **29**: 1–11.

Wubs ERJ, van der Putten WH, Mortimer SR, Korthals GW, Duyts H, Wagenaar R, Bezemer TM. 2019. Single introductions of soil biota and plants generate long-term legacies in soil and plant community assembly. *Ecology Letters* **22**: 1145–1151.

Yabe S, Sakai Y, Abe K, Yokota A. 2017. Diversity of Ktedonobacteria with actinomycetes-like morphology in terrestrial environments. *Microbes and Environments* **32**: 61–70.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Temperatures experienced by the soil sources during the prescribed burn treatment across replicate burn sites measured with on-site HOBO temperature data loggers.

Fig. S2 Responses of measured soil physicochemical properties to prescribed fire, historic number of fires, and time since last recorded fire.

Table S1 Plant species, seeding rates per pot, and length of growing time in the grow room experiment.

Table S2 Locations of rosemary scrub patches at Archbold Biological Station that were selected as soil sources for the grow room experiment.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.