New Phytologist

Research

Allelopathy-selected microbiomes mitigate chemical inhibition of plant performance

Daniel Revillini[1], Aaron S. David[2], Alma L. Reyes[1], Leslie D. Knecht[3], Carolina Vigo[1], Preston Allen[1], Christopher A. Searcy[1]* and Michelle E. Afkhami[1]*

[1]Department of Biology, University of Miami, 1301 Memorial Drive, Coral Gables, Florida 33146, USA; [2]Archbold Biological Station, 123 Main Drive, Venus, Florida 33960, USA; [3]Department of Chemistry, University of Miami, 1301 Memorial Drive, Coral Gables, Florida 33146, USA

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

Michelle E. Afkhami
Email: michelle.afkhami@miami.edu

Received: *11 July 2023*
Accepted: *1 August 2023*

New Phytologist (2023) **240:** 2007–2019
doi: 10.1111/nph.19249

Key words: allelochemical, disturbance frequency, microbial mediation, plant–microbial interaction, rhizobacteria.

Summary

- Allelopathy is a common and important stressor that shapes plant communities and can alter soil microbiomes, yet little is known about the direct effects of allelochemical addition on bacterial and fungal communities or the potential for allelochemical-selected microbiomes to mediate plant performance responses, especially in habitats naturally structured by allelopathy.
- Here, we present the first community-wide investigation of microbial mediation of allelochemical effects on plant performance by testing how allelopathy affects soil microbiome structure and how these microbial changes impact germination and productivity across 13 plant species.
- The soil microbiome exhibited significant changes to 'core' bacterial and fungal taxa, bacterial composition, abundance of functionally important bacterial and fungal taxa, and predicted bacterial functional genes after the addition of the dominant allelochemical native to this habitat. Furthermore, plant performance was mediated by the allelochemical-selected microbiome, with allelopathic inhibition of plant productivity moderately mitigated by the microbiome.
- Through our findings, we present a potential framework to understand the strength of plant–microbial interactions in the presence of environmental stressors, in which frequency of the ecological stress may be a key predictor of microbiome-mediation strength.

Introduction

Competition via allelopathy is a notable mechanism that structures plant communities (Inderjit *et al.*, 2011; Hierro & Callaway, 2021). Allelopathy has a broad taxonomic distribution, as a recent meta-analysis shows that 72% of all plant families are capable of producing bioactive secondary metabolites (allelochemicals; Kalisz *et al.*, 2021). Allelopathy is also common across ecosystems including grasslands (Ning *et al.*, 2016; da Silva *et al.*, 2017), shrublands (Mahall & Callaway, 1991; Hewitt & Menges, 2008), and both temperate and tropical forests (Ooka & Owens, 2018), and is an important factor in both agricultural and invasion ecology (Bais *et al.*, 2003). Meta-analysis has also shown that allelopathy reduces mean plant performance by 25% (Zhang *et al.*, 2021). These declines in plant fitness result from both direct effects of allelochemical inhibition and indirect effects such as decreasing soil nutrient availability, or through important yet largely unexplored alterations in the soil microbial communities that interact with surrounding plant roots (Cipollini *et al.*, 2012; Zhang *et al.*, 2019).

Soil microbes play outsized roles in plant health and survival (Berendsen *et al.*, 2012), and range from negative to positive effects on plant performance depending on environmental conditions (Hodge & Fitter, 2013; Trivedi *et al.*, 2020). Recent studies indicate that soil microbiomes can increase plant performance under stressful environmental conditions through amelioration of abiotic and biotic stressors (David *et al.*, 2020; Liu *et al.*, 2020). Plant response to abiotic and biotic sources of stress can act as a cue, sometimes described as a 'cry for help', that encourages recruitment of microbial communities and functions that ultimately enhance the plant's capacity to combat stress and maintain fitness (Bakker *et al.*, 2018). Abiotic stressors, such as abnormally high temperature or prolonged drought, can directly alter soil microbial community composition and shift allocation of plant carbon to mutualistic microbes in soil (Palta & Gregory, 1997). Despite the many potential beneficial microbial responses to this 'cry for help,' poststress plant microbial interactions can also lead to decreased microbiome multifunctionality and increased pathogen loads in the rhizosphere (Santos-Medellín *et al.*, 2017; Hinojosa *et al.*, 2019). Allelopathy can similarly impose stress-induced shifts in microbiome composition, with some studies indicating changes in functional capabilities

*These authors contributed equally to this work.

(Lorenzo *et al.*, 2013), and that allelochemicals may more strongly impact soil bacteria than fungi (Kong *et al.*, 2008). For example, some soil microbes have been shown to degrade phenolic allelochemicals (Zhang *et al.*, 2010), but little is known about the recruitment or increased reliance on these potentially beneficial microbes by plants. It is important to note that given this capacity for certain microbes to degrade allelochemicals, there are two potential pathways through which the soil microbiome can mediate plant performance responses to allelopathy: through allelopathy-selected compositional and functional shifts in the microbiome, or through the direct degradation or metabolization of an allelochemical (Fu & Oriel, 1999). Given the importance of microbiome functionality to plant health and stress resilience, understanding allelopathic effects on soil microbiomes is a crucial part of understanding plant community responses.

Our knowledge of microbial mediation of plant allelopathic interactions is notably limited in systems structured by native allelochemical inhibition. Much of what we know about the effects of allelopathy on plant–microbial interactions comes from studies of plant invasions. Allelopathy is very common among invasive species, with 51–67% of invasive plants reported to have allelopathic capacity (Zhang *et al.*, 2019; Kalisz *et al.*, 2021). Allelochemicals from invasive species have been shown to negatively impact bacterial abundance and community composition (Cipollini *et al.*, 2012; Zhang *et al.*, 2019), change microbial functionality in the rhizosphere (Qu *et al.*, 2021), and ultimately alter plant–soil feedbacks in agriculture (Hu *et al.*, 2018). It has been proposed and largely supported that naive native plant species are more vulnerable to negative impacts of non-native allelopathic plants because they have not adapted to the novel chemicals introduced in their system (novel weapons hypothesis; Callaway & Aschehoug, 2000). The likely corollary to this hypothesis, discussed in Callaway & Hierro (2006) and Mishra *et al.* (2013), is that plants in ecosystems *natively* structured by dominant allelopathic plants will have adaptations that minimize inhibition by allelopathy. We predict that plant–microbial interactions play a key role in this adaptation to allelopathy. For instance, allelopathy may select for soil microbiomes (through differential shifts in community members and associated functions) that mitigate or neutralize the inhibition of plant performance by allelochemicals (e.g. via increased beneficial interactions in stressful environments as in David *et al.*, 2020). Importantly, the strength of microbial mediation of allelochemical stress can fall along a continuum and may be dependent on plant species–microbe specificity (Revillini *et al.*, 2016).

Given the known importance of soil microbiomes for plant health and the global impacts of allelopathy (Wardle *et al.*, 1998; David *et al.*, 2018), we conducted a study to determine the direct effects of allelochemical addition on the soil microbiome, as well as the subsequent effects on performance of native plants from a habitat naturally structured by allelopathy. We address three questions: (1) Can allelochemicals alter bacterial and fungal community structure and function in soil? (2) How does a history of persistent allelochemical-selection on the soil microbiome impact native plant performance responses? And (3) which allelochemical-altered soil microbes and microbiome functions underpin changes in plant performance? We are interested in the potential for adaptation among native plant and soil microbial communities to allelopathy, a persistent and long-term stressor in this habitat, as there will have been consistent selection pressure for plant–microbial associations that are able to weather that stress. We predict that allelochemical addition to soils will more strongly alter bacterial than fungal communities due to previously noted bacterial susceptibility to allelochemicals (Lorenzo *et al.*, 2013; Niro *et al.*, 2016), and concomitant potential for greater fungal tolerance to allelochemicals (Barto *et al.*, 2011). Moreover, we expect that changes in microbial abundance as a response to allelopathy and relationships with increased plant performance will allow for the identification of microbial consortia that are adapted to mitigate inhibitory effects of native allelopathy in this system. We intend for this work to function as a template for future research in allelopathic systems by identifying core sets of allelochemical-selected soil microbiota and attendant microbiome-mediation of allelopathy.

Materials and Methods

Study system

The Florida Scrub ecosystem has the highest rate of endemism in the southeastern United States and hosts many threatened species (Dobson *et al.*, 1997; Menges *et al.*, 2008). This ecosystem exhibits a range of habitat types from open sand gaps and shrublands to mixed conifer flatwoods within 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* Michaux). Florida rosemary produces a suite of allelochemicals that can affect performance of other scrub species. Notably, Florida rosemary produces ceratiolin, a flavonoid that quickly decomposes into hydrocinnamic acid (HCA) and negatively affects plant germination and root length for many herbaceous Florida scrub plant species (Fischer *et al.*, 1994; David *et al.*, 2018). Ceratiolin and derivative HCA are documented as the dominant allelochemicals found in litter and soil of the Florida scrub habitat (Jordan, 1990) and have been credited with contributing to the patchy structure of the ecosystem (Hunter & Menges, 2002; Hewitt & Menges, 2008).

Recent studies show that there are distinct soil microbiomes in rosemary scrub compared with surrounding flatwoods habitat (Hernandez *et al.*, 2021) and that many of the rare, endemic plants occurring in the rosemary scrub are strongly influenced by interactions with the soil microbiome (David *et al.*, 2018, 2020). For this study, we used soils from 10 sites at Archbold Biological Station (Venus, FL, USA; 27.18°N, 81.35°W) and collected seeds of 13 perennial, herbaceous plant species from across Archbold that vary across a spectrum of life history traits.

Allelochemical treatment of the microbiomes

To capture the abiotic variation in our study system, we chose soils to test allelopathic effects across a realistic sampling range of

two important metrics in the system (Menges *et al.*, 2017). We collected soils from 10 Florida rosemary scrub patches (i.e. open habitat patches dominated by *C. ericoides* that occur at relatively high elevations above the water table; Supporting Information Table S1) with a range of fire histories – time since fire and total number of fires experienced within the last 52 yr. We collected *c.* 5 kg of soil from open sand gaps (at least 3 m from *C. ericoides* to minimize the effects of ambient environmental HCA) at each of the 10 sites, and then stored soils for 2 d before applying the allelochemical treatment. Hydrocinnamic acid concentrations have been shown to decrease rapidly with increasing distance (> 2 m) from the host plant in this ecosystem (Quintana-Ascencio & Menges, 2000). The allelochemical addition treatment was performed using 250 ppm hydrocinnamic acid (3-phenylpropionic acid; HCA) diluted in ultrapure H_2O. Hydrocinnamic acid concentration was selected based on previous studies from the field that identified natural concentrations of HCA ranging from 15 to 418 ppm (Jordan, 1990), and a manipulative study that found 250 ppm HCA effectively impacted plant performance (David *et al.*, 2018). 1250 ml of soil from each site was equally split among sterilized aluminum trays (34 cm × 24 cm × 7 cm, $n = 20$) to receive the control (ultrapure H_2O) or allelochemical addition (HCA+) treatment. Each tray was soaked with 50 ml (4% volume) using a sterile 2-l pump sprayer of either treatment 3 d per week in a temperature-controlled environment (25°C) for 5 wk, leading to a total HCA concentration of 150 ppm.

Soil microbiome extraction, amplification, sequencing and bioinformatic processing

DNA was extracted from homogenized soil samples after the allelochemical addition treatment concluded ($n = 20$; 10 soil sources and 2 allelochemical treatments) using the DNeasy PowerSoil Pro QIAcube HT Kit (Qiagen) with an adapted protocol without QIAcube (see Methods S1 section for full description; Revillini *et al.*, 2021). DNA was quantified with a Qubit 4 fluorometer (Qiagen) and normalized to 5 ng μl^{-1}. Libraries were prepared for sequencing using a two-step dual indexing protocol (Gohl *et al.*, 2016). PCR was targeted for archaeal/bacterial (16S) and broad fungal (ITS2) ribosomal DNA (rDNA) using primer pairs 515F-806R and ITS7o-ITS4, respectively. Index and Illumina flowcell sequences were added in second-step PCR. All targeted amplicon products were pooled in equimolar quantities, 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).

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 then the midpoint root method was used to create a rooted tree for phylogeny-based analyses (e.g. weighted UniFrac). Amplicon sequence variants that were not present in at least two samples were filtered out, and diversity metrics and dissimilarity matrices were calculated using the QIIME2 commands *diversity core-metrics-phylogenetic* (sampling depth = 6500) and *diversity core-metrics* (sampling depth = 9000) for archaea/bacteria and fungi, respectively. All microbiome data from QIIME2 was read into R v.4.1 (R Core Team, 2020) using the qiime2R package v.0.99.6 (https://github.com/jbisanz/qiime2R).

Allelochemical-selected microbiome–plant performance experiment

To determine the magnitude of microbial effects on plant performance in the rosemary scrub and how these effects depend on the microbiome's exposure to the dominant allelochemical (HCA) found in rosemary scrub soils (Fischer *et al.*, 1994), we conducted a 2 × 2 factorial growth room experiment manipulating microbiome presence (presence vs absence) and allelochemical selection on the microbiome (control vs HCA+) replicated using soil microbiomes collected from 10 rosemary scrub patches (see Methods S1 section for full description). We first sterilized half of the soil from each allelochemical treatment by autoclaving three times (121°C, 2 h). The 13 rosemary scrub plant species were each grown in sterilized pots (66 ml) inoculated with soil microbiomes from all 40 factorial combinations of soil source, allelochemical treatment, and microbiome presence. Each pot was filled with 50 ml of sterilized background rosemary scrub soil and topped with 10 ml of inoculum from one of the 40 treatment combinations. To ensure that the majority of soil in each pot had similar abiotic properties, and thus the only manipulation was the different soil microbiomes present in the inocula, background soil in this experiment was collected from a single large open sand gap at Archbold > 5 m from Florida rosemary and autoclaved 4× at 121°C. After seeding directly into inoculum soil, a 2-ml 'cap' of sterile, background soil was added to prevent seed desiccation. The number of seeds sown per pot reflected previously determined differences in germination rates among these plant species (David *et al.*, 2020; Revillini *et al.*, 2021), and all pots were thinned to one plant shortly after germination. Overall, our experiment included 10 microbiome sources in each of the four allelochemical × microbial treatment combinations, each with three replicates for each of the 13 plant species (except for *Liatris ohlingerae*, which had five microbiome sources due to lack of seed), totaling 1500 pots. All pots were watered with *c.* 2 ml of sterile water daily for 1 month and subsequently every other day. Plants were grown under full spectrum lights (*c.* 162 $(\mu mol\ m^2)^{-1}\ s^{-1}$ PAR; Tran & Braun, 2017), with a 14 h : 10 h, light : dark schedule until harvest *c.* 5 months after the start of the experiment (Table S2). Germination percentages were determined based on species-specific seeding rates per pot. Shoot and root biomass were determined after oven drying at 50°C until

reaching constant mass. Root : shoot biomass ratios were calculated to determine plant allocation responses.

Evaluating microbiome effects on hydrocinnamic acid concentration in soils

To determine the potential for microbial degradation of HCA in soils, we conducted a follow-up study with soils from the same 10 soil sources used in the larger experiment. We maintained either live microbiomes or sterilized the soils by autoclaving three times (121°C, 2 h), and then applied the same 250 ppm HCA solution over the same 5-wk time period using the same methods described in the Allelochemical treatment of the microbiomes section above. Soil samples were then stored at −80°C prior to High-Performance Liquid Chromatography (HPLC) analysis. We randomly selected five of 10 soil source pairs for HPLC analysis. To create a standard curve, we spiked autoclaved soil samples with HCA standards according to previously described methods (Jordan, 1990). Briefly, we added 2.5 ml of each HCA standard to 25 g of soil to a final concentration of 16, 32, 64, 100, or 200 $\mu g\, g^{-1}$ soil. The samples were equilibrated, in the dark, for 2 h without shaking. To extract the HCA from the soil, we added 50 ml of deionized water to each sample and incubated for 2 h at room temperature while shaking at 100 rpm. We then vacuum-filtered the mixtures with 0.2 µm Whatman filter paper followed by syringe filtration with a 0.45 µm filter. We analyzed the filtrate using a Dionex 3000 HPLC from Thermo Scientific equipped with an Agilent Zorbax Eclipse XDB-C 18 column (250 × 4.6 mm, 5 µm particle size). For the gradient elution, we used mobile phases (A) acetonitrile and (B) deionized water and acetic acid (99.5 : 0.5). The stepwise gradient was set from 50% A to 100% A over 7 min with a 1-min equilibration step between samples. The analysis was performed at 257 nm.

Data analysis

To identify a baseline for soil microbiome organization after allelochemical addition, we calculated the core microbiome for both bacteria and fungi using the *core* function from the 'MICROBIOME' package in R (http://microbiome.github.com/microbiome). The core microbiome here represents taxa with a > 0.1% relative abundance detection threshold that also occur in > 60% of all samples that experienced allelochemical addition (Busby *et al.*, 2017). The allelochemical effects on bacterial and fungal alpha diversity metrics (ASV richness, Shannon's *H*, Pielou's evenness, and Faith's phylogenetic diversity) were assessed using paired *t*-tests, with allelochemical addition as the factor of interest and microbiomes paired by soil source. To determine allelochemical effects on bacterial (weighted uniFrac) and fungal (Bray–Curtis dissimilarity) community composition, a PERMANOVA stratified by soil source was performed using the *adonis2* function in R package VEGAN v.2.5-7 (Oksanen *et al.*, 2020). To identify which microbial taxa responded strongly to allelochemical addition, analysis of differential microbial relative abundances from allelochemical control ('reference' factor level) to samples that underwent allelochemical addition was performed using the *DESeq* function in R package DESEQ2 v.1.32 (Love *et al.*, 2014).

We used the PICRUSt2 algorithm (Douglas *et al.*, 2020) to calculate the predicted bacterial metagenome based on our 16S reads in order to assess the effect of allelochemical addition on important functional genes in nutrient release or transfer. We targeted analyses on genes associated with important carbon (C), nitrogen (N), and phosphorus (P)-cycling functions. Paired *t*-tests were performed on individual genes (e.g. nifQ or amoA) or sums of gene sets that comprise functional pathways for nitrite reduction (nirBDK), phosphonate (organic P) cleavage and transport (phnCDEJ), as well as phosphate transport (ugpACQ), to identify increases or decreases in predicted bacterial function after allelochemical addition.

To understand how allelochemical effects on the soil microbiome contributed to plant performance responses, we constructed linear mixed models. Our models considered how plant performance responded to the presence or absence of soil microbiomes and whether or not soils experienced allelochemical addition. All 13 plant species were included in analyses of germination rates, but two species with the lowest germination rates, *Hypericum cumulicola* (Small) P. Adams and *Paronychia chartacea* Fernald, were excluded from analyses of productivity or biomass allocation due to insufficient degrees of freedom. To meet the assumption of homogeneity of variances across species, *z*-scores were calculated for all plant response metrics within each plant species prior to analysis. Germination percentages were arcsine-square-root-transformed prior to *z*-score calculations to improve normality. Using these data, we first ran global models for all plant species combined. Terms in these models included microbiome presence (presence vs absence), allelochemical selection on the microbiome (control vs HCA+), and their interaction as well as plant species identity and interactions between plant species and all of the other terms. We also included a random effect of the soil collection site. After finding significant interactions with plant species identity in the global models, we constructed follow-up general linear models for each of 11 plant species individually. These models included the same microbiome and allelochemical main effects and their two-way interaction term. We conducted linear mixed models using the *lmer* function in R and model output was determined using Type III sums of squares, which are independent of the input order of predictor variables.

To identify the relationships between plant performance responses and the microbiome responses to allelochemical addition, each of the five measured plant performance metrics were regressed on the 23 bacterial and fungal ASVs (aggregated at the lowest taxonomic level and with 'unidentified' ASVs removed), and also on five predicted bacterial functional genes that were observed to change after allelochemical addition (nifQ, amoA, nir, ugp, and phn). Collection site (patch) and plant species identity were set as random effects. The Benjamini–Hochberg procedure was used to account for multiple comparisons. Finally, to determine whether there were differences in allelochemical concentration in the soil with or without a live soil microbiome from our degradation experiment, we performed a paired sample *t*-test.

Results

Allelochemical-selected microbiome

We identified 44 core bacterial ASVs and 42 core fungal ASVs in the allelochemical-selected microbiome. For bacteria, the allelopathic core microbiome was dominated by two taxa in the Burkholderiaceae – *Burkholderia tuberum* (34% of identified ASVs) and *Burkholderia byrophila* (29%) – with the remainder of core microbiome taxa coming from the Solibacteraceae, Mycobacteriaceae, and Nitrosphaeraceae (Table S3). Notably, four bacterial families in the control bacterial core microbiome fell below the core thresholds for soils experiencing allelochemical addition, and taxa in the Burkholderiaceae emerged only with allelochemical addition, becoming the second most prevalent allelopathic core member (Fig. 1). Of the 42 ASVs in the fungal core microbiome, 19% were from the genus *Talaromyces*, and the remaining ASVs were fairly equally distributed across 11 identified genera (Table S3). We observed the appearance of two new genera in the core fungal microbiome with allelochemical addition, *Gelasinospora* and *Chaetomium*, as well as increases in the prevalence of taxa in the *Gibberella* and *Veronaeopsis* (Fig. 1).

Allelochemical addition also significantly shifted overall bacterial community composition (pseudo-$F = 2.35$, $P = 0.002$; Fig. 2a), but did not significantly affect overall fungal community composition (pseudo-$F = 1.31$, $P = 0.08$; Fig. S1). However, differential abundance analysis of both bacteria and fungi revealed highly responsive taxa that significantly increased or decreased in relative abundance after allelochemical addition. Bacterial ASVs in the families Solibactereaceae (Acidobacteria) and Acetobacteraceae (Alphaproteobacteria) increased with allelochemical addition, with a 22 $\log_2$-fold change (LFC) for both, while abundance of three ASVs in the Thermogemmatisporaceae decreased by *c.* 20 LFC (Fig. 2b). Of the 65 total bacterial ASVs

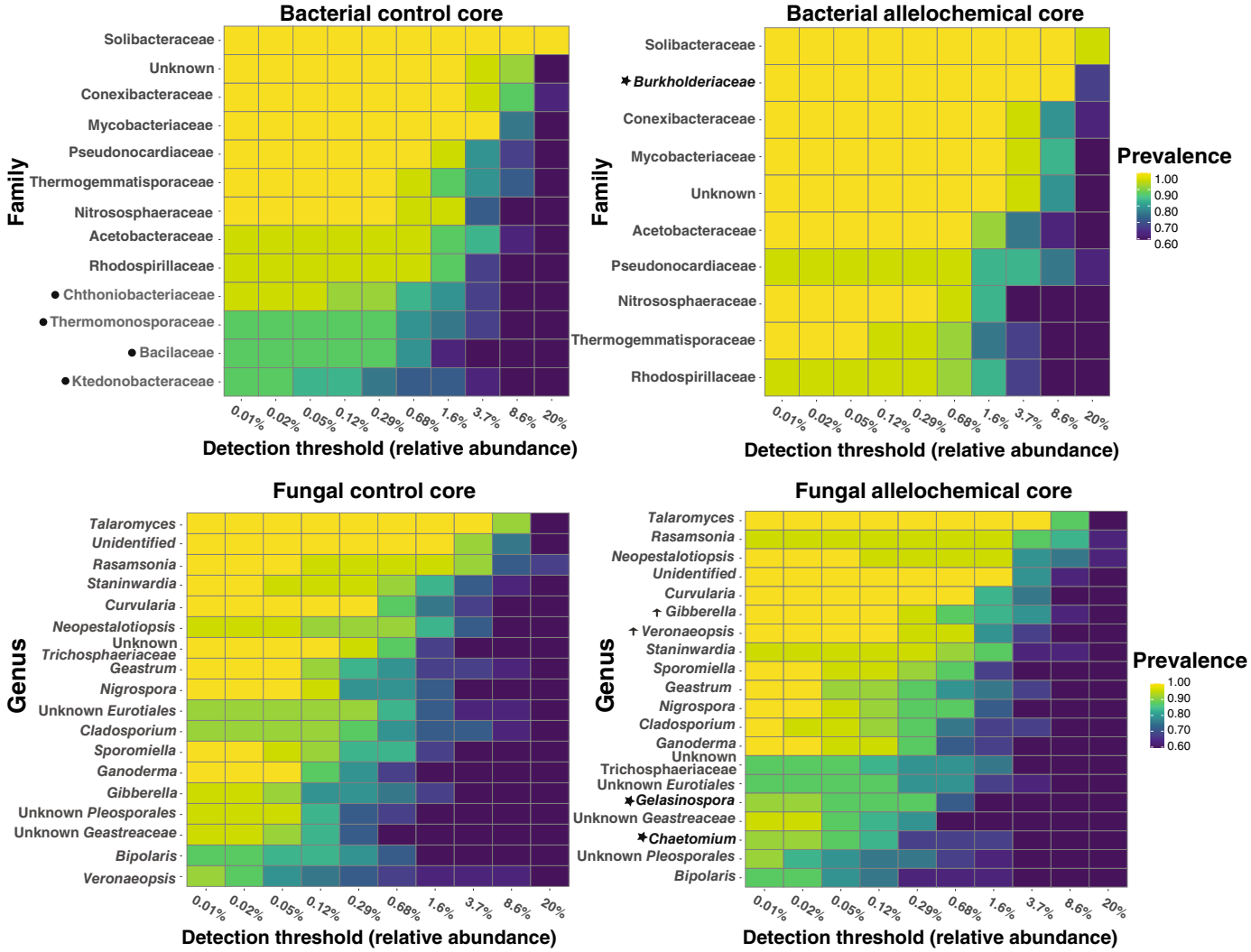

Fig. 1 Core microbial taxa without allelochemical addition (left) and with the addition of hydrocinnamic acid (right). Colored by prevalence and organized by relative abundance detection thresholds for core bacterial families (upper panels) and core fungal genera (lower panels). Stars, addition to core microbiome after allelochemical treatment; circles, removal from core microbiome after allelochemical treatment; arrows, increase in prevalence after allelochemical treatment.

New Phytologist

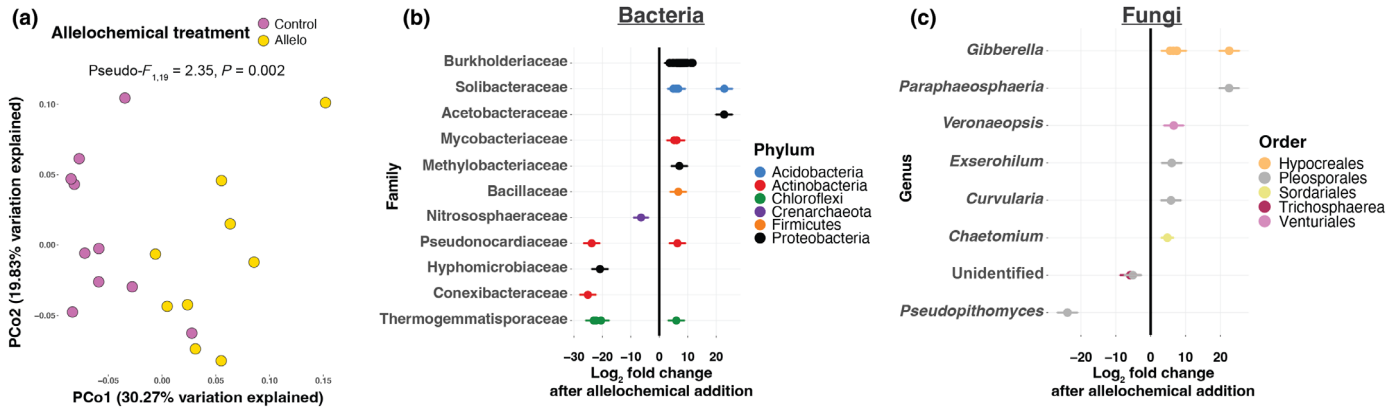

Fig. 2 Microbial responses to allelochemical addition in this experiment. (a) Principal coordinate analysis of bacterial community composition (weighted UniFrac) colored by allelochemical treatment. Bacterial composition is significantly different after allelochemical addition (Allelo). Significant $\log_2$ fold change (LFC) of bacterial abundance at the family level, colored by bacterial phylum (b), and of fungal abundance at the genus level, colored by fungal order (c). Points represent mean LFC and lines represent SE from DESEQ2.

that shifted after allelochemical addition, the majority (24 and 15 ASVs) were identified as two species: *B. tuberum* and *Burkholderia bryophila*, respectively.

Differential abundance analysis showed that 19 fungal ASVs also significantly responded to allelochemical addition (18 fungal taxa across eight genera plus one 'unidentified'; Fig. 2c). Changes in abundance of fungal taxa after allelochemical addition were comparable in strength to LFCs observed in the more allelochemical-responsive bacterial community (−23 to +21 LFC). Two taxa in the genera *Gibberella* and *Paraphaeosphaeria* and an unidentified taxon in the order Eurotiales all increased in abundance by approximately +20 LFC, and the putative plant pathogen, *Pseudopithomyces*, had the largest decrease after allelochemical addition (Fig. 2c).

Of the 11 bacterial functional genes/gene sets associated with C, N, and P cycling we examined, five were significantly affected by allelochemical addition; nifQ, amoA, nir, phn, and ugp (Fig. 3). Allelochemical addition increased predicted gene abundances for nitrogen fixation (nifQ; $P = 0.003$) and decreased predicted gene abundances for ammonia oxidation (amoA; $P = 0.039$), nitrite reduction (nirB, nirD, and nirK; $P = 0.004$), phosphate transport (ugpA, upgC, and upgQ; $P = 0.004$), and phosphonate uptake and breakdown (phnC, phnD, phnE, and phnJ; $P = 0.022$).

Plant performance responses

Across all plant species, effects of allelochemical addition on productivity were microbially mediated, where allelochemical-selected microbiomes mitigated negative impacts to total plant biomass (Table 1). Both shoot and total biomass were significantly reduced by allelochemical addition ($P = 0.019$ and 0.026, respectively), but for total biomass, this response was significantly mitigated by the soil microbiome ($P = 0.034$; Fig. 4a), whereas microbial mitigation was marginal for shoot biomass ($P = 0.073$; Table S4). Plant species varied significantly in their response to the microbiome treatment across all plant performance metrics that we examined ($P \leq 0.009$; Figs S2, S3), but only for root : shoot ratio was there significant interspecific variation in the degree to which allelochemical treatment modulated this response ($P = 0.016$; Table 2), ranging from a 106% decrease (for *Balduina angustifolia* (Pursh) B. L. Robinson) to a 137% increase (for *Liatris tenuifolia* Nuttal) in root biomass investment when the microbiome was present to mediate allelopathic effects (Fig. S3). Surprisingly, individual plant species models revealed only one species with a significant allelochemical × microbiome treatment interaction (Fig. 4b; Table S5). In *B. angustifolia*, the allelochemical × microbiome interaction was significant for three plant performance responses: Both aboveground shoot biomass and total plant biomass were significantly higher when a microbiome was present to alleviate the effects of allelochemical addition ($P = 0.0001$ and 0.027, respectively), while investment in roots was significantly lower when the microbiome was present to mediate allelopathic effects ($P = 0.0001$; Table S5).

Relationships between microbiomes and plant performance responses

Of the 23 microbial taxa and four bacterial functions significantly affected by allelochemical addition, we identified six microbial taxa (three bacterial and three fungal) and two bacterial functions that had significant relationships with at least one of the measured plant responses: germination, total biomass, or root : shoot biomass ratio (Fig. 5). The fungal species *Exserohilum rostratum*, which increased after allelochemical addition, had the strongest positive relationship with both shoot biomass and total biomass ($t = 2.27$, $P = 0.03$; $t = 2.12$, $P = 0.034$, respectively), while the bacterial genera *Rhodoplanes* and *Bacillus*, which decreased and increased after allelochemical addition, respectively, had the strongest negative effects on shoot biomass and root biomass ($t = -3.15$, $P = 0.009$; $t = -2.14$, $P = 0.032$, respectively). The two predicted bacterial functions nir and ugp, both of which decreased after allelochemical addition, also explained variation in plant performance that was significant, but moderate (Fig. 5). Nir genes, which code for nitrite reduction, had slight negative effects on germination ($P = 0.012$), while ugp genes, which code

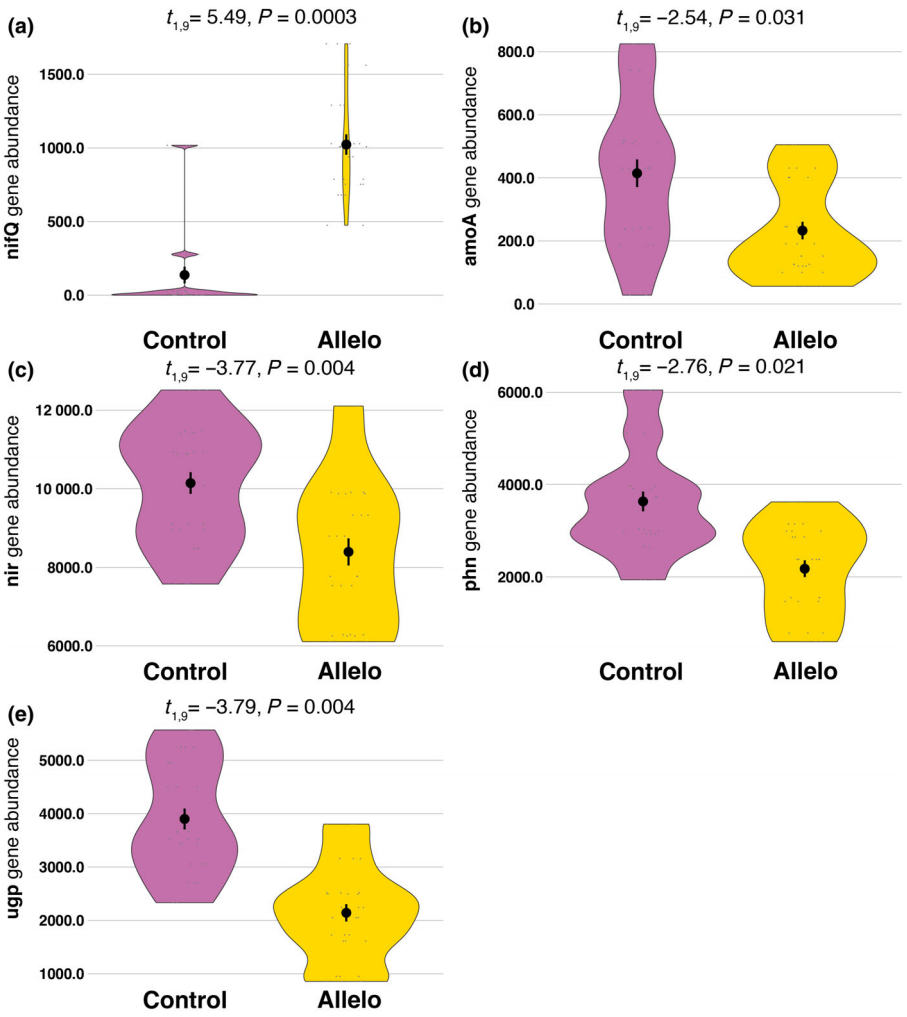

Fig. 3 Predicted bacterial functional genes that responded significantly to allelochemical addition (Allelo). A nitrogen fixation gene, nifQ, increased with allelochemical addition (a), and amoA, responsible for ammonia oxidation, decreased after allelochemical addition (b). Sums of genes responsible for nitrite reduction (nirB, nirD, and nirK) are presented for 'nir' (c), sums of genes responsible for the uptake and breakdown of phosphonates (phnC, phnD, phnE, and phnJ) are presented for 'phn' (d), and sums of genes responsible for phosphate transport (ugpA, ugpC, and ugpQ) are presented for 'ugp' (e). Black dots represent mean gene abundance and lines represent SE.

Table 1 Significant results from linear mixed models of *z*-score standardized plant performance responses.

Response	Plant species[1]	Microbiome	Allelochemical (Allelo)	Microbiome × Allelo	Plant × Microbiome	Plant × Allelo	Plant × Microbiome × Allelo
Germination	< 0.0001	–	–	–	0.008	–	–
Total biomass	< 0.0001	–	0.029	0.034	0.0001	–	–
Root : Shoot	< 0.0001	–	–	–	0.005	0.002	0.016

Model predictor terms are column headers, *P*-values are presented when $P \leq 0.05$.
[1]*P*-values presented are from performance responses prior to within plant species standardization.

for organic P solubilization, had slight positive effects on germination and negative effects on root : shoot biomass ratio ($P = 0.024$ and 0.025, respectively).

Microbial degradation of dominant soil allelochemical

A paired *t*-test revealed no difference in the allelochemical concentration in soils with a sterile or live soil microbiome after 5 wk of allelochemical addition. In fact, the trend was in the direction of more allelochemical being present in the live soil treatment, indicating that the soil microbiome in these soils does not significantly degrade the native allelochemical ($t_4 = -0.643$, $P = 0.55$; Fig. S4).

Discussion

Allelopathy strongly affected soil microbiome structure and predicted functions in this study. The allelopathic chemical derived from the dominant native shrub (*C. ericoides*) in this system altered the core microbiome, bacterial composition, and relative abundances of bacterial and fungal taxa. Allelochemical-treated microbiomes also showed evidence of functionally important

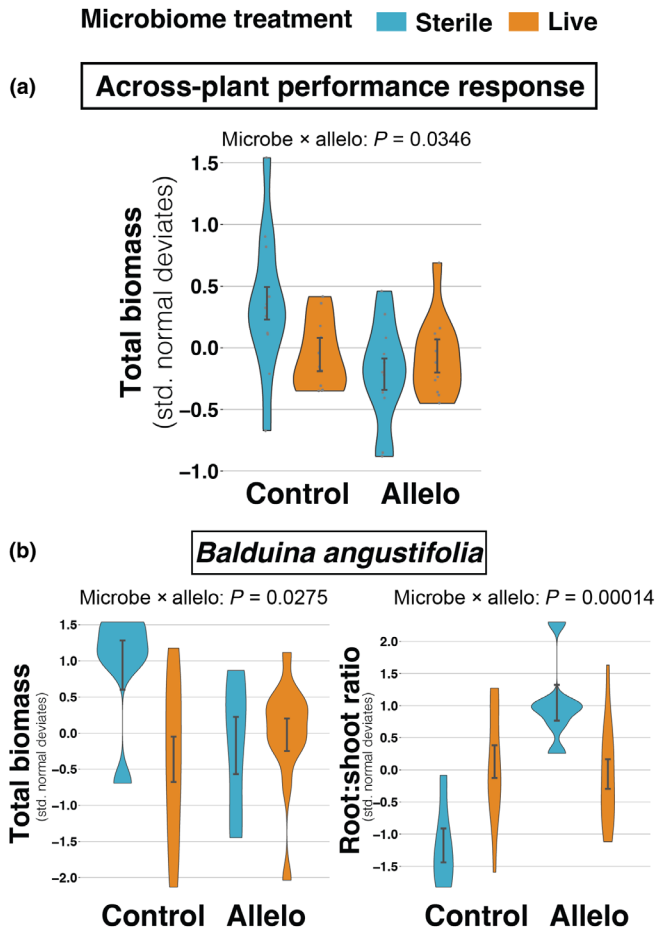

Fig. 4 Plant performance responses to allelochemical addition (Allelo) treatments, colored by microbiome treatment. (a) Plant total biomass responses for all plant species combined. The figure presents mean values for each plant species ($n = 11$) per treatment combination as dots. Overall, total biomass exhibited microbiome-mediated effects of allelochemical addition. (b) Microbial-mediation of allelochemical effects in *Balduina angustifolia*. Total biomass of *B. angustifolia* was less inhibited by allelochemical addition in the presence of a microbiome. The allocation of biomass to roots was significantly lower when the microbiome was present to mediate allelochemical effects. All data were converted to *z*-scores prior to analysis to standardize results within each plant species and are presented as standard normal deviates from the mean. Bars in (a, b) equal the mean and SE.

changes, such as notable increases in abundance of putative beneficial bacteria (i.e. Burkholderiales) and putative fungal pathogens (Fig. 2) as well as shifts in predicted bacterial functional genes including an almost sevenfold increase in the abundance of the nifQ gene, coding for N_2-fixation. Notably, we determined that the native soil microbiome did not degrade the allelochemical tested in our study and suggests an important role of microbial compositional or functional shifts in the mediation of plant performance responses under allelopathy. We found significant, but overall weak effects of allelochemical-altered microbiomes on plant performance responses (productivity) in the manipulative growth experiment (i.e. Fig. 4a). Similar to previous studies, the microbiome exhibited a net positive effect on plant productivity, one of the most important performance metrics for perennial germinants in this habitat (Menges & Kohfeldt, 1995), in the presence of allelopathy (Cipollini *et al.*, 2012; Mishra *et al.*, 2013). Our study explicitly reveals the link between allelochemical-altered soil microbes and plant performance. While previous studies have identified allelopathy-induced shifts in microbiomes and/or plant performance, the vast majority could only infer microbiome-mediation (but also see Hu *et al.*, 2018). However, the effect sizes we observed tended to be fairly modest, such that when combined with the smaller sample sizes within individual species, only one of the 11 species (*B. angustifolia*) registered a significant effect. We also suspect the *lack of novelty* of the allelochemical weapon in this ecosystem has led to previous adaptive responses of these plants that allow them to tolerate allelochemical-induced shifts in the microbiome (i.e. representing a stable community exhibiting weak–neutral responses; Shade *et al.*, 2012). The novelty of an ecological weapon has a direct relationship with the frequency of a stressor, where high frequency would present a more common weapon and low frequency would represent a more novel weapon. We propose that the strength of microbial mitigation or exacerbation of plant responses to disturbance is negatively related to the frequency of the ecological stressor in question (Fig. 6).

Microbial composition shifted distinctly with allelochemical addition, indicating strong direct effects of allelochemical addition on soil microbiomes. Soil bacteria were notably more responsive to allelochemical addition than fungi, as has been found in previous studies (Kong *et al.*, 2008), and also appear to have shifted towards a structure and functions that would promote greater plant growth. In particular, we found significant increases in Burkholderia (many putative N-fixers), as well as *Rhidopila globiformis* (Acetobactereaceae), a nitrogen-fixer that may contribute to alternative N_2-fixation via the vanadium-dependent nitrogenase pathway (Imhoff *et al.*, 2018). By contrast, fungi exhibited increases in multiple putative pathogens after allelochemical addition (Wang *et al.*, 2021). These included an increase in prevalence of *Gibberella*, a known fungal pathogen (Bai *et al.*, 2021). Interestingly, there was also an increase in the dark septate endophytic genus *Veronaeopsis*, which has been shown to mitigate infectivity of other fungal pathogens (Khastini *et al.*, 2012). Thus, the increased relative abundance of this taxon may indicate a fungal mechanism for reducing allelochemical-induced stress to plant roots. These shifts reveal increased dominance for putative beneficial bacteria and putative pathogenic fungi among their respective soil consortia, and this apparent positive–negative balance of representative microbial taxa might contribute to the weakly positive microbial mediation effect of the soil microbiome on plant performance responses in our manipulative growth experiment (Vandenkoornhuyse *et al.*, 2015).

Functional changes in soil microbiomes after allelochemical addition indicate a range of responses to allelopathy that also likely contributed to the neutral-to-positive microbial mediation of plant performance observed here. Multiple bacterial functional genes shifted after allelochemical addition, with increases in potential N-fixation via the nifQ gene, which donates molybdenum to nifH for biosynthesis of the FeMo nitrogenase enzyme (Hernandez *et al.*, 2008). On the contrary, we observed decreases

Table 2 Individual plant species general linear model results.

Response	Plant species	Microbiome	Allelochemical	Microbiome × Allelo
Germination	*Chapmannia floridana*	$F = 8.32$, $P = 0.003$	$F = 3.95$, $P = 0.046$	–
	Eryngium cuneifolium	$F = 5.41$, $P = 0.019$	$F = 8.33$, $P = 0.003$	–
	Polygonella robusta	$F = 4.61$, $P = 0.031$	–	–
Total biomass	*Balduina angustifolia*	–	–	$F = 4.85$, $P = 0.027$
	Chamaecrista fasciculata	$F = 14.68$, $P = 0.0001$	–	–
	Eryngium cuneifolium	$F = 4.5$, $P = 0.033$	–	–
	Polygonella robusta	$F = 10.89$, $P = 0.0009$	–	–
Root : Shoot	*Balduina angustifolia*	–	$F = 13.216$, $P = 0.0002$	$F = 18.75$, $P < 0.0001$
	Chamaecrista fasciculata	$F = 27.9$, $P < 0.0001$	–	–
	Chapmannia floridana	$F = 12.09$, $P = 0.0005$	–	–
	Eryngium cuneifolium	–	–	$F = 3.82$, $P = 0.05$
	Lechea cernua	–	$F = 5.17$, $P = 0.022$	–
	Polygonella robusta	$F = 5.1$, $P = 0.023$	–	–

For each performance response, only plant species with a significant effect are presented ($P \leq 0.05$). Shading in gray indicates a negative main effect on plant performance, while main effects without shading indicate a positive main effect.

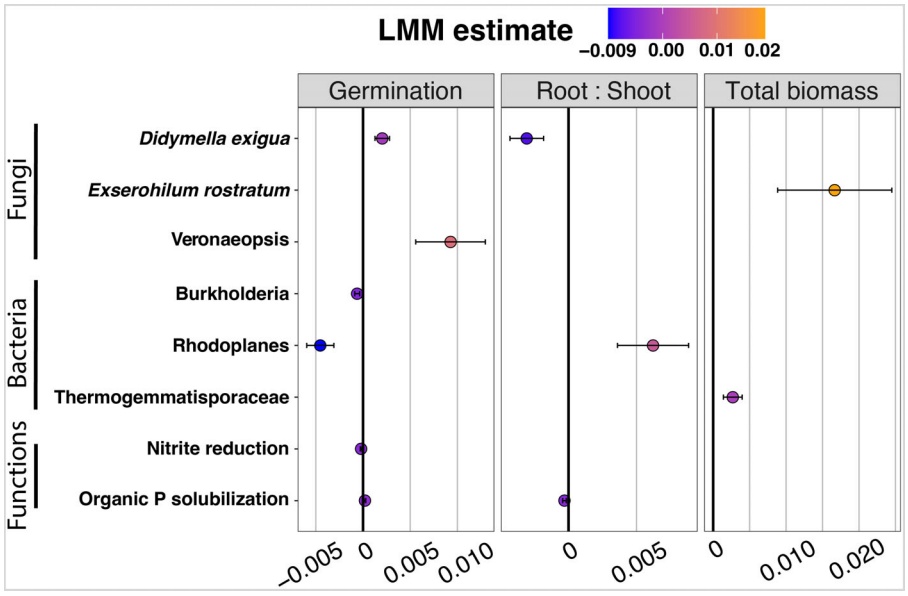

Fig. 5 Significant ($P < 0.05$) linear mixed-effects model (LMM) estimates between three plant performance responses and relative abundance of microbial taxa and predicted bacterial functions that responded significantly to allelochemical addition. Points represent mean LMM parameter estimates and lines represent SE.

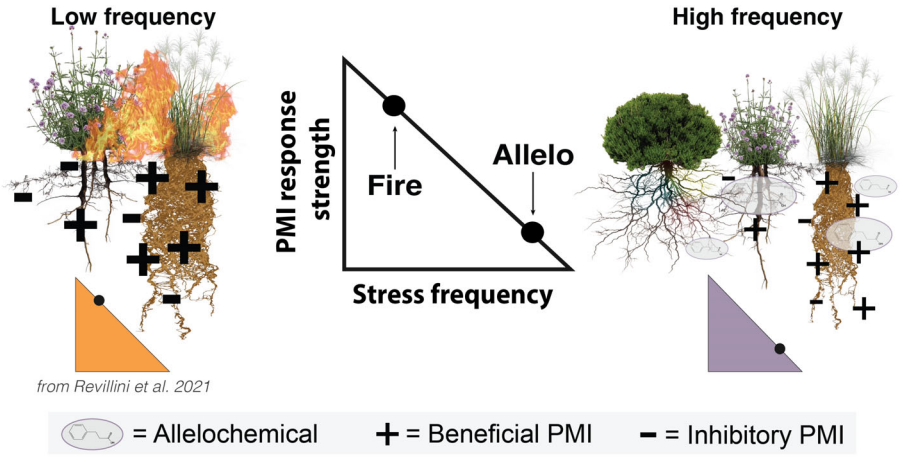

Fig. 6 Potential framework explaining the relationship between stress frequency and post-disturbance plant–microbial interaction (PMI) responses. We propose that infrequent stressors can strongly affect belowground communities (as in Revillini *et al*., 2021), which leads to equally strong effects on microbial mediation of plant performance (size of PMI), while a frequent stressor ultimately results in moderate-to-weak microbial mediation of plant performance (this study). Size of PMI interaction (±) is relative to microbial mediation effect under different stress conditions.

in ammonia oxidation, nitrite reduction, phosphonate reduction, and phosphate transport that suggest a suppressive effect of allelopathy on bacterial N and P cycling belowground (Fig. 3). While these results are predicted using the PICRUSt2 algorithm, which can underestimate certain gene frequencies (Toole *et al.*, 2021), they still indicate a functional mechanism – via increased N_2-fixation – that may have contributed to the mitigation of allelopathic stress on plant productivity found in our across plant species analysis (Fig. 4). To build on these findings, we advocate for future research exploring differential responses of bacterial and fungal functions to allelopathy using targeted methods such as metagenomics or quantitative stable isotope probing to assess impacts on microbiome functional responses and subsequent plant–microbial interactions (Hungate *et al.*, 2015).

Significant relationships between allelochemical-responsive microbial taxa (6 out of 23) and bacterial functions (2 out of 5) and plant performance may help identify individual microbial taxa that could be important for the resilience and persistence of the rare, endemic plants in this system (Fig. 5). Interestingly, our results relating plant performance with specific members and functions of the microbiome revealed that not all taxa or functions considered putatively beneficial or inhibitory influence host performance as expected. For instance, the fungal taxon with the strongest positive effects on shoot and total biomass, *E. rostratum*, is a putative plant pathogen that causes root rot across many plant families (Sharma *et al.*, 2014). Although the majority of research indicates that this species negatively impacts plant productivity, a recent study found an *E. rostratum* variant that was beneficial for plant growth in sunchokes (Khaekhum *et al.*, 2021) suggesting that this taxon can act as a mutualist under certain conditions. Negative microbial relationships with plant performance were also surprising, because many were found for taxa known to contribute to plant-growth promotion including those in the Burkholderiaceae and Rhodoplanes (Adesemoye *et al.*, 2009; Carrión *et al.*, 2018; Anzuay *et al.*, 2021). These relationships between members of the microbiome and plant performance metrics suggest that: (1) many allelochemical-responsive microbial taxa and functions may play outsized roles impacting plant performance; (2) putative functional categorizations of members of the soil microbiome are likely oversimplified; and (3) functional relationships between individual plants and members of the soil microbiome should be studied further to identify patterns of context-dependency across systems experiencing disturbance.

We predicted that the nature of the allelopathy stress in this study system – functioning as a persistent stressor – would lead to beneficial plant–microbial interaction responses, and our results supported this prediction. Allelopathy is persistent in the rosemary scrub, leading to increased opportunities for plant and microbial adaptation via increased interaction frequency (i.e. familiarity) compared with infrequent disturbances. We propose that stress frequency is critical in determining the strength of the plant–microbial interaction response (Fig. 6). More specifically, we expect that microbial mediation of plant response to stress becomes more muted with increased stress frequency, as the community experiences persistent selection for greater stability (weaker interactions and responses) in the face of such a common stressor. This is in contrast to effects observed from novel or infrequent disturbances (e.g. species introductions, fire, drought) on plant–microbial interactions. For instance, our research was conducted in a fire-dependent system (Menges & Kohfeldt, 1995), where fire is a naturally occurring disturbance with a return interval of *c.* 16 yr (Menges, 2007). In a previous study testing the ability of soil microbiomes to mediate plant performance responses to prescribed fire with many of the same plant species used here, we showed much stronger mediation effects of the postfire soil microbiome on plant performance (Revillini *et al.*, 2021). This difference in the strength of microbial-mediation of allelopathic vs fire stress within the same ecosystem could be a feature of their local adaptation to the dominant and *persistent* allelochemical stress as opposed to relatively infrequent fire disturbance (Fig. 6). To test this prediction, future research should strive to identify the continuum under which plant–microbial interactions respond to stressors along a frequency gradient. Finally, to more broadly confirm our results regarding plant–microbial interaction responses to allelochemical addition, we feel it would be valuable to investigate the total effects of the source allelopathic plants (incorporating roots and the full suite of phytochemicals) on microbial mediation of plant responses in future experimental manipulations.

Microbial resistance and resilience to stress, resulting legacies in soil, and microbiome-mediation of plant responses to stress are still emerging lines of research in soil ecology (Bakker *et al.*, 2018; Kiesewetter & Afkhami, 2021; Philippot *et al.*, 2021), but it is becoming apparent that the strength and frequency of ecological stressors should be considered a major contributing factor to the functional relationships between the soil microbiome and aboveground communities. We have shown here that plant–microbial interaction responses to persistent allelopathy stress are subtle and neutral-to-positive for plant performance. It is still possible in other systems that microbial mitigation of allelopathic effects may be stronger if microbial degradation of allelochemicals reduces the direct effect of allelopathy, but here we reveal that allelochemical selection on the soil microbiome can lead to mitigation of allelochemical stress to plants. While previous studies have begun to identify patterns of microbial resistance and resilience to disturbance in a broad global sense (Shade *et al.*, 2012; Rocca *et al.*, 2018), our research focuses this field by directly testing the link between the stress-selected microbiome and plant performance responses. Our work suggests that the soil microbiome has great potential to mitigate plant responses to abiotic stress, and emphasizes the importance of future work identifying functional roles of the soil microbiome that mediate environmental stress.

Acknowledgements

Many thanks to the Plant Ecology Program at Archbold Biological Station, and particularly Stephanie Koontz for invaluable help with seed collection. Thanks to the members of the Afkhami and Searcy laboratories for feedback on manuscript preparation and figures, as well as manuscript editor Dr. Stéphane Hacquard and three

anonymous reviewers. This research was funded by National Science Foundation grant DEB-1922521 awarded to MEA and CAS, and a research award to DR from the Florida Native Plant Society.

Competing interests

None declared.

Author contributions

DR, ASD, CAS, and MEA planned and designed the research. DR, PA, CV, and CAS performed the main experiment and analyzed data, with contributions from MEA. MEA and CAS performed the allelochemical degradation experiment. ALR and LDK performed the HPLC optimization and analysis. DR, CAS, and MEA wrote the manuscript. ASD edited the manuscript. DR, CAS, and MEA revised the manuscript after review. CAS and MEA 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
Leslie D. Knecht https://orcid.org/0000-0001-9504-1769
Daniel Revillini https://orcid.org/0000-0002-0380-4106

Data availability

All raw plant and soil data are available on the Dryad database (doi: 10.5061/dryad.f4qrfj71z), and all molecular data used for microbiome analyses are available at the NCBI SRA under project accession PRJNA1008570.

References

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.

Adesemoye AO, Torbert HA, Kloepper JW. 2009. Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. *Microbial Ecology* **58**: 921–929.

Anzuay MS, Viso NP, Ludueña LM, Morla FD, Angelini JG, Taurian T. 2021. Plant beneficial rhizobacteria community structure changes through developmental stages of peanut and maize. *Rhizosphere* **19**: 100407.

Bai H, Si H, Zang J, Pang X, Yu L, Cao H, Xing J, Zhang K, Dong J. 2021. Comparative proteomic analysis of the defense response to Gibberella stalk rot in maize and reveals that ZmWRKY83 is involved in plant disease resistance. *Frontiers in Plant Science* **12**: 694973.

Bais HP, Vepachedu R, Gilroy S, Callaway RM, Vivanco JM. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* **301**: 1377–1380.

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

Barto EK, Antunes PM, Stinson K, Koch AM, Klironomos JN, Cipollini D. 2011. Differences in arbuscular mycorrhizal fungal communities associated with sugar maple seedlings in and outside of invaded garlic mustard forest patches. *Biological Invasions* **13**: 2755–2762.

Berendsen RL, Pieterse CMJ, Bakker PAHM. 2012. The rhizosphere microbiome and plant health. *Trends in Plant Science* **17**: 478–486.

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.

Busby PE, Soman C, Wagner MR, Friesen ML, Kremer J, Bennett A, Morsy M, Eisen JA, Leach JE, Dangl JL. 2017. Research priorities for harnessing plant microbiomes in sustainable agriculture. *PLoS Biology* **15**: 1–14.

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.

Callaway RM, Aschehoug ET. 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* **290**: 521–523.

Callaway RM, Hierro JL. 2006. Resistance and susceptibility of plant communities to invasion: revisiting Rabotnov's ideas about community homestasis. In: Reigosa MJ, Pedrol N, Gonzalez L, eds. *Allelopathy: a physiological process with ecological implications*. Dordrecht, the Netherlands: Springer Netherlands, 395–414.

Carrión VJ, Cordovez V, Tyc O, Etalo DW, de Bruijn I, de Jager VCL, Medema MH, Eberl L, Raaijmakers JM. 2018. Involvement of Burkholderiaceae and sulfurous volatiles in disease-suppressive soils. *The ISME Journal* **12**: 2307–2321.

Cipollini D, Rigsby CM, Barto EK. 2012. Microbes as targets and mediators of allelopathy in plants. *Journal of Chemical Ecology* **38**: 714–727.

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**: e03081.

Dobson AP, Rodriguez JP, Roberts WM, Wilcove DS. 1997. Geographic distribution of endangered species in the United States. *Science* **275**: 550–553.

Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI. 2020. PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology* **38**: 685–688.

Fischer NH, Williamson GB, Weidenhamer JD, Richardson DR. 1994. In search of allelopathy in the Florida scrub: the role of terpenoids. *Journal of Chemical Ecology* **20**: 1355–1380.

Fu W, Oriel P. 1999. Degradation of 3-phenylpropionic acid by *Haloferax* sp. D1227. *Extremophiles* **3**: 45–53.

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.

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

Hernandez JA, Curatti L, Aznar CP, Perova Z, Britt RD, Rubio LM. 2008. Metal trafficking for nitrogen fixation: NifQ donates molybdenum to NifEN/NifH for the biosynthesis of the nitrogenase FeMo-cofactor. *Proceedings of the National Academy of Sciences, USA* **105**: 11679–11684.

Hewitt RE, Menges ES. 2008. Allelopathic effects of *Ceratiola ericoides* (Empetraceae) on germination and survival of six Florida scrub species. *Plant Ecology* **198**: 47–59.

Hierro JL, Callaway RM. 2021. The ecological importance of allelopathy. *Annual Review of Ecology, Evolution, and Systematics* **52**: 25–45.

Hinojosa MB, Laudicina VA, Parra A, Albert-Belda E, Moreno JM. 2019. Drought and its legacy modulate the post-fire recovery of soil functionality and microbial community structure in a Mediterranean shrubland. *Global Change Biology* **25**: 1409–1427.

Hodge A, Fitter AH. 2013. Microbial mediation of plant competition and community structure (D. Robinson, Ed.). *Functional Ecology* **27**: 865–875.

Hu L, Robert CAM, Cadot S, Zhang X, Ye M, Li B, Manzo D, Chervet N, Steinger T, van der Heijden MGA *et al.* 2018. Root exudate metabolites drive plant–soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications* **9**: 2738.

Hungate BA, Mau RL, Schwartz E, Caporaso JG, Dijkstra P, Van Gestel N. 2015. Quantitative microbial ecology through stable isotope probing. *Applied and Environmental Microbiology* **81**: 7570–7581.

Hunter ME, Menges ES. 2002. Allelopathic effects and root distribution of *Ceratiola ericoides* (Empetraceae) on seven rosemary scrub species. *American Journal of Botany* **89**: 1113–1118.

Imhoff JF, Rahn T, Künzel S, Neulinger SC. 2018. New insights into the metabolic potential of the phototrophic purple bacterium *Rhodopila globiformis* DSM 161T from its draft genome sequence and evidence for a vanadium-dependent nitrogenase. *Archives of Microbiology* **200**: 847–857.

Inderjit WDA, Karban R, Callaway RM. 2011. The ecosystem and evolutionary contexts of allelopathy. *Trends in Ecology & Evolution* **26**: 655–662.

Jordan ED. 1990. *Determination of allelochemicals in the environment surrounding Ceratiola ericoides*. In: Fischer NH, ed. Dissertation, Baton Rouge, LA, USA: Louisiana State University and Agricultural and Mechanical College.

Kalisz S, Kivlin SN, Bialic-Murphy L. 2021. Allelopathy is pervasive in invasive plants. *Biological Invasions* **23**: 367–371.

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

Khaekhum S, Ekprasert J, Suebrasri T, Mongkolthanaruk W, Riddech N, Jogloy S, Boonlue S. 2021. The first member of *Exserohilum rostratum* beneficial for promoting growth and yield of sunchoke (*Helianthus tuberosus* L.). *Rhizosphere* **19**: 100379.

Khastini RO, Ohta H, Narisawa K. 2012. The role of a dark septate endophytic fungus, *Veronaeopsis simplex* Y34, in Fusarium disease suppression in Chinese cabbage. *Journal of Microbiology* **50**: 618–624.

Kiesewetter KN, Afkhami ME. 2021. Microbiome-mediated effects of habitat fragmentation on native plant performance. *New Phytologist* **232**: 1823–1838.

Kong CH, Wang P, Zhao H, Xu XH, Zhu YD. 2008. Impact of allelochemical exuded from allelopathic rice on soil microbial community. *Soil Biology & Biochemistry* **40**: 1862–1869.

Liu H, Brettell LE, Qiu Z, Singh BK. 2020. Microbiome-mediated stress resistance in plants. *Trends in Plant Science* **25**: 733–743.

Lorenzo P, Pereira CS, Rodríguez-Echeverría S. 2013. Differential impact on soil microbes of allelopathic compounds released by the invasive *Acacia dealbata* Link. *Soil Biology & Biochemistry* **57**: 156–163.

Love M, Anders S, Huber W. 2014. Differential analysis of count data – the DESeq2 package. *Genome Biology* **15**: 10–1186.

Mahall BE, Callaway RM. 1991. Root communication among desert shrubs. *Proceedings of the National Academy of Sciences, USA* **88**: 874–876.

Menges ES. 2007. Integrating demography and fire management: an example from Florida scrub. *Australian Journal of Botany* **55**: 261.

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. 2017. Dynamics of gaps, vegetation, and plant species with and without fire. *American Journal of Botany* **104**: 1825–1836.

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.

Mishra S, Upadhyay RS, Nautiyal CS. 2013. Unravelling the beneficial role of microbial contributors in reducing the allelopathic effects of weeds. *Applied Microbiology and Biotechnology* **97**: 5659–5668.

Ning L, Yu F-H, van Kleunen M. 2016. Allelopathy of a native grassland community as a potential mechanism of resistance against invasion by introduced plants. *Biological Invasions* **18**: 3481–3493.

Niro E, Marzaioli R, De Crescenzo S, D'Abrosca B, Castaldi S, Esposito A, Fiorentino A, Rutigliano FA. 2016. Effects of the allelochemical coumarin on plants and soil microbial community. *Soil Biology & Biochemistry* **95**: 30–39.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P *et al.* 2020. *VEGAN: community ecology package (2.5-7)* [Computer software]. [WWW document] URL https://r-forge.rproject.org/projects/vegan/ [accessed 17 February 2023].

Ooka JK, Owens DK. 2018. Allelopathy in tropical and subtropical species. *Phytochemistry Reviews: Proceedings of the Phytochemical Society of Europe* **17**: 1225–1237.

Palta JA, Gregory PJ. 1997. Drought affects the fluxes of carbon to roots and soil in ^{13}C pulse-labelled plants of wheat. *Soil Biology & Biochemistry* **29**: 1395–1403.

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.

Philippot L, Griffiths BS, Langenheder S. 2021. Microbial community resilience across ecosystems and multiple disturbances. *Microbiology and Molecular Biology Reviews* **85**: e0002620.

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.

Qu T, Du X, Peng Y, Guo W, Zhao C, Losapio G. 2021. Invasive species allelopathy decreases plant growth and soil microbial activity. *PLoS ONE* **16**: e0246685.

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

Revillini D, David AS, Menges ES, Main KN, Afkhami ME, Searcy CA. 2021. Microbiome-mediated response to pulse fire disturbance outweighs the effects of fire legacy on plant performance. *New Phytologist* **233**: 2071–2082.

Revillini D, Gehring CA, Johnson NC. 2016. The role of locally adapted mycorrhizas and rhizobacteria in plant–soil feedback systems. *Functional Ecology* **30**: 1086–1098.

Rocca JD, Simonin M, Blaszczak JR, Ernakovich JG, Gibbons SM, Midani FS, Washburne AD. 2018. The microbiome stress project: toward a global meta-analysis of environmental stressors and their effects on microbial communities. *Frontiers in Microbiology* **9**: 3272.

Santos-Medellín C, Edwards J, Liechty Z, Nguyen B, Sundaresan V. 2017. Drought stress results in a compartment-specific restructuring of the rice root-associated microbiomes. *mBio* **8**: e00764.

Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H, Huber DH, Langenheder S, Lennon JT, Martiny JBH *et al.* 2012. Fundamentals of microbial community resistance and resilience. *Frontiers in Microbiology* **3**: 1–19.

Sharma K, Goss EM, Dickstein ER, Smith ME, Johnson JA, Southwick FS, van Bruggen AHC. 2014. *Exserohilum rostratum*: characterization of a cross-kingdom pathogen of plants and humans. *PLoS ONE* **9**: e108691.

da Silva ER, Overbeck GE, Soares GLG. 2017. Something old, something new in allelopathy review: what grassland ecosystems tell us. *Chemoecology* **27**: 217–231.

Toole DR, Zhao J, Martens-Habbena W, Strauss SL. 2021. Bacterial functional prediction tools detect but underestimate metabolic diversity compared to shotgun metagenomics in southwest Florida soils. *Applied Soil Ecology* **168**: 104129.

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.

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.

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.

Wang Y, Yao J, Li Z, Huo J, Zhou S, Liu W, Wu H. 2021. Genome sequence resource for *Nigrospora oryzae*, an important pathogenic fungus threatening crop production. *Molecular Plant–Microbe Interactions* **34**: 835–838.

Wardle DA, Nilsson M-C, Gallet C, Zackrisson O. 1998. An ecosystem-level perspective of allelopathy. *Biological Reviews of the Cambridge Philosophical Society* **73**: 305–319.

Zhang P, Li B, Wu J, Hu S. 2019. Invasive plants differentially affect soil biota through litter and rhizosphere pathways: a meta-analysis. *Ecology Letters* **22**: 200–210.

Zhang Z, Liu Y, Yuan L, Weber E, van Kleunen M. 2021. Effect of allelopathy on plant performance: a meta-analysis. *Ecology Letters* **24**: 348–362.

Zhang Z-Y, Pan L-P, Li H-H. 2010. Isolation, identification and characterization of soil microbes which degrade phenolic allelochemicals. *Journal of Applied Microbiology* **108**: 1839–1849.

Supporting Information

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

Fig. S1 Principal coordinate analysis of Bray–Curtis dissimilarity for fungal communities that experienced allelopathy (Allelo) or control treatments.

Fig. S2 Total biomass (*z*-score) response of all plant species in the study under sterile and live soil conditions that experienced allelochemical or control treatments.

Fig. S3 Root : shoot ratio (*z*-score) response of all plant species in the study under sterile and live soil conditions that experienced allelochemical or control treatments.

Fig. S4 Concentration of hydrocinnamic acid (HCA), the allelochemical tested in this study, in soils with live or sterile microbiomes after 5 wk of HCA addition.

Methods S1 Detailed methods for soil microbiome extraction, amplification, sequencing and the allelopathy-selected microbiome–plant performance experiment.

Table S1 Rosemary scrub patch coordinates for soil collection.

Table S2 Native plant species seeding rates and mean number of days until harvest.

Table S3 Core bacterial and fungal taxa after allelochemical addition.

Table S4 Significant results from linear mixed models of *z*-score standardized plant performance responses.

Table S5 Individual plant species general linear model results.

Please note: Wiley is 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.