Resuscitation of the microbial seed bank alters plant-soil interactions

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ABSTRACT

While microorganisms are recognized for driving belowground processes that influence the productivity and fitness of plant populations, the vast majority of bacteria and fungi in soil belong to a “seed bank” made up of dormant individuals. Still, plant performance may be affected by microbial dormancy through its effects on the activity, abundance, and diversity of soil microorganisms. To test how microbial seed banks influence plant-soil interactions, we purified recombinant resuscitation promoting factor (Rpf), a bacterial protein that terminates dormancy. Then, in a factorially designed experiment, we applied the Rpf to soil containing field mustard (*Brassica rapa*), an agronomically important plant species. We found that plant biomass was ~33 % lower in the Rpf treatment compared to plants growing in the presence of an unmanipulated microbial seed bank. In addition, Rpf reduced soil respiration, decreased bacterial abundance, and increased fungal abundance. These effects of Rpf on plant performance were accompanied by shifts in bacterial community composition, which may have diluted mutualists or resuscitated pathogens. Our findings suggest that changes in microbial seed banks may influence the magnitude and direction of plant-soil feedbacks in ways that affect above- and below-ground biodiversity and function.

INTRODUCTION

Belowground soil microbial communities play a critical role in determining the productivity and fitness of aboveground plants. Plant roots are intimately associated with thousands of bacterial and fungal taxa that are involved in soil processes such as decomposition, nutrient cycling, and pathogen suppression (Bardgett & van der Putten, 2014; Berendsen, Pieterse, & Bakker, 2012; Wagg, Bender, Widmer, & van der Heijden, 2014). In addition to containing mutualistic symbionts, soils also harbor pathogenic microorganisms that reduce plant performance (Mansfield et al., 2012). Together, belowground microbial communities are responsible for generating plant-soil feedbacks that can influence the diversity and composition of plant communities (Bever, 2003). However, the direction and magnitude of soil microbial effects on plants is highly variable (Hoeksema et al., 2010; Kulmatiski, Beard, Stevens, & Cobbold, 2008) and is likely influenced by the complexity of belowground communities (van der Heijden, de Bruin, Luckerhoff, van Logtestijn, & Schlaeppi, 2016; Wagg et al., 2014).

One belowground feature that is commonly overlooked when considering plant-microbe interactions is the metabolic heterogeneity of soil microbial communities. Many microorganisms are capable of entering a reversible state of reduced metabolic activity (i.e., dormancy) when challenged by suboptimal environmental conditions (Lennon & Jones, 2011). A large proportion (≥90 %) of microorganisms are dormant in soils (Alvarez, Alvarez, Grigera, & Lavado, 1998; Blagodatsky, Heinemeyer, & Richter, 2000; Lennon & Jones, 2011). Collectively, these inactive individuals create a “seed bank”, which not only maintains biodiversity, but also affects ecosystem functioning. The incorporation of microbial dormancy into ecosystem experiments and models has been shown to improve predictions for soil microbial activity and nutrient

cycling (Salazar, Lennon, & Dukes, 2019; Wang et al., 2015). Few studies, however, have explored how soil microbial seed banks influence plant-soil interactions.

Microbial seed banks may prevent local extinctions when exposed to fluctuating and stressful conditions that are typical in soil habitats (Shoemaker & Lennon, 2018). If microbial mutualists can persist by entering a seed bank, then this may promote beneficial soil functions such as disease-suppression, induction of plant immune response, stimulation of root growth, and biofertilization, which together, can increase plant performance (Lugtenberg & Kamilova, 2009; Mendes et al., 2011; Peralta, Sun, McDaniel, & Lennon, 2018; Wagg et al., 2014). In contrast, seed banks may contain pathogenic microorganisms that can reduce plant performance. For example, it is well known that destructive pathogens can reside in soil for months to years after the demise of plants, rendering potentially arable soil unusable (Koike, Subbarao, Davis, & Turini, 2003; Peeters, Guidot, Vailleau, & Valls, 2013). Although microbial seed banks likely contain both mutualistic and pathogenic taxa, the net effect of dormancy transitions on plant performance remains to be determined.

Resuscitation is an essential process that regulates microbial seed-bank dynamics. Dormancy is often terminated via the interpretation of environmental cues or communication signals that are produced by other microorganisms (Dworkin & Shah, 2010). One such signal is a bacterial protein called resuscitation promoting factor (Rpf), a muralytic enzyme that degrades the β-(1,4) glycosidic bond in peptidoglycan, which is a major cell-wall component of virtually all bacteria (Mukamolova, Kaprelyants, Young, Young, & Kell, 1998). Rpf genes are broadly distributed among the G+C-rich Gram-positive Actinobacteria (Ravagnani, Finan, & Young, 2005; Schroeckh & Martin, 2006). In some soil samples, Rpf homologs can be found in up to 25 % of all genomes (Lennon & Jones, 2011). Rpf has cross-species effects that stimulate the

growth of closely related dormant bacteria (Puspita, Kitagawa, Kamagata, Tanaka, & Nakatsu, 2015; Schroeckh & Martin, 2006) either through its direct effects on cell wall integrity or through the release of small peptide fragments that cross-link peptidoglycan, which may serve as signaling molecules (Jonathan Dworkin, 2014). Under laboratory conditions, Rpf can stimulate the activity of some microbial taxa, but it has also been shown to inhibit the growth of other microorganisms (Mukamolova et al., 1998; Mukamolova et al., 2002), which is not surprising given that Rpf is homologous to lysozyme (Cohen-Gonsaud et al., 2005), an antimicrobial enzyme produced by many eukaryotic organisms. Overall, Rpf may have complex and interactive effects that influence plant-soil interactions.

In this study, we explored how seed-bank dynamics affect plant-microbe interactions by manipulating the process of resuscitation. Throughout the lifespan of a host plant (*Brassica rapa*), we applied purified recombinant Rpf to soils containing complex microbial communities. The experiment was guided by a conceptual model that considers how active and inactive microorganisms interact and potentially create feedbacks with plants (Fig. 1). We hypothesized that resuscitating the microbial seed bank with Rpf would alter plant performance through changes in the abundance, activity, and composition of soil microbial communities. On the one hand, Rpf treatment could enhance plant performance by waking up mutualistic taxa in the soil microbial community. On the other hand, resuscitating dormant microorganisms could negatively influence plant performance by diluting the benefits of mutualistic taxa or through the recruitment of pathogenic microorganisms. Here, we test these hypotheses while also considering other effects of Rpf on plants, microbes, and their interactions.

METHODS

Experimental design — We conducted a growth chamber experiment to test the effect of Rpf on plant-soil interactions using *Brassica rapa* L. (Brassicaceae), an economically important plant that consists of a variety of cultivated subspecies. We sowed 16 *B. rapa* seeds into individual pots in June 2016. Seeds used in this experiment were obtained from Wisconsin Fast Plants™ (Standard stock seeds, Wisconsin Fast Plants Program, University of Wisconsin, Madison, WI, USA). While *B. rapa* is non-mycorrhizal, it still associates with a diverse microbial community that influences plant growth (Lau & Lennon, 2012). For our experiment, we used plastic pots (17 cm diameter, 13 cm height) filled with autoclaved (121 °C, 15 PSI, 16 h) soil medium (1 part greenhouse metro-mix: 1 part vermiculite), which was inoculated with live field soil collected from Indiana University Research and Teaching Preserve (DMS 39 °11'56.5"N 86 °30'40.5"W). For the +Rpf treatment, we added 1 mL of recombinant Rpf protein (10 µM) to the soil surface each week in three equidistant locations 5 cm away from main plant stem. The same was done for the -Rpf treatment (negative control), except we used protein buffer (20 mM Tris-HCl, 100 mM NaCl) instead of recombinant Rpf. Our experiment was conducted in a Percival model PGC-15 growth chamber with high efficiency light lamps (Philips series 700 32 watts 4100K F32T8/TL741 AltoII). The growth chambers were set to constant and full light intensity (1100 µmol m^{-2} s^{-1}) with controlled humidity (60 %) and temperature (28 °C). Pots were arranged in a fully randomized design where each plant was assigned to a resuscitation treatment (i.e., -Rpf vs. +Rpf). All pots were watered with equal amounts of filtered and de-ionized water every other day.

Recombinant Rpf — To manipulate microbial seed banks, we overexpressed Rpf from *Micrococcus luteus* sp. KBS0714, a bacterial strain isolated from agricultural soil at the W.K. Kellogg Biological Station, Hickory Corners, MI (MSU) (Kuo, Shoemaker, Muscarella, & Lennon, 2017; Lennon, Aanderud, Lehmkuhl, & Schoolmaster Jr, 2012). *Micrococcus luteus* sp. KBS0714 is a close relative of *M. luteus* NCTC 2662 (99 % sequence 16S rRNA sequence similarity, NCBI CP001628.1), a model organism used for studying Rpf (Mukamolova et al., 1998). To create a Rpf expression host, we amplified and cloned the *rpf* gene from KBS0714 into the expression vector pET15b. First, we extracted KBS0714 genomic DNA from pure culture using a Microbial DNA isolation kit (MoBio, Carlsbad, CA) for PCR amplification of the open reading frame of the *rpf* gene using two primers, Upper-F 5' GCC CAT ATG GCC ACC GTG GAC ACC TG 3' and Lower-R 5' GGG GAT CCG GTC AGG CGT CTC AGG 3', with incorporated restriction sites EcoRI, NdeI (forward primer) and BamHI (reverse primer) (Koltunov et al., 2010; Mukamolova et al., 1998). We amplified the *rpf* gene sequence with the following PCR conditions: initial: 95 °C for 5 m, 30 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 m, and one final extension at 72 °C for 7 m. The *rpf* gene amplicon was ligated into pET15b (Invitrogen) as an EcoRI/BamHI fragment and transformed into *Escherichia coli* TOP10 (a non-expression host). We then identified positive clones with correct *rpf* sequence and orientation by Sanger sequencing at the Indiana University Center of Genomics and Bioinformatics (IU-CGB). Next, we extracted purified pET15b plasmids with the *rpf* gene from transformed TOP10 *E. coli* using the QIAprep Spin Miniprep Kit (Qiagen) following the manufacturer's protocol. Finally, we transformed the purified pET15b expression vector with the *rpf* gene into the *E. coli* Origami BL21 (DE3) expression host incorporated with a polyhistidine-tag on the N-terminus of the recombinant protein.

To overexpress Rpf, we grew the *E. coli* expression host in Lysogeny Broth (LB) with appropriate antibiotics (ampicillin 100 µg/mL, kanamycin 15 µg/mL, and tetracycline 12.5 µg/mL). During logarithmic growth, recombinant protein production was induced with Isopropyl β-D-1-thiogalactopyranoside (IPTG) (100 µM final concentration). We confirmed overexpression of Rpf with Western blots (Fig. S1). Cells were then collected by centrifugation, lysed by sonication, and filtered through a Ni-NTA Purification System (Invitrogen) using a 10 mL gravity fed column with a 2 mL resin bed to purify recombinant Rpf with the N-terminus polyhistidine-tag. Recombinant Rpf protein was washed with 5 mM imidazole buffer (300 mM NaCl, 50 mM Tris-HCl, 5 mM imidazole) and then eluted with 125 mM imidazole buffer. Rpf protein was purified by buffer exchange using a 10 mL Zeba Spin Desalting Columns (Thermo Fisher) with protein buffer (20 mM Tris-HCl, 100 mM NaCl) following manufacturer's instructions and then passed through a 0.2 µm syringe filter before adding to soil as described above.

Plant responses — We censused the plants every day and recorded germination and flowering date. After most plants had finished flowering, we documented flower number, plant height, and specific leaf area (SLA = leaf area/dry mass) as previously described (Lau & Lennon, 2012). All open flowers in each experimental unit were hand-pollinated by other open flowers in the same treatment group with a soft paint brush, which was cleaned with 30 % isopropyl alcohol between treatment groups to prevent gene flow. Plants and seeds were harvested at the end of the six-week growing period when most individuals ceased flowering and had begun to senesce. As an estimate of female fitness, we removed plant seed pods and counted the number of seeds produced. To estimate male fitness, we counted the number of flowers per plant. In addition, we

measured total, aboveground, and belowground biomass of each plant after drying plants at 65 °C for 2 d. We performed two-tailed Student's *t*-test to evaluate the effect of Rpf on total biomass, aboveground (i.e., shoot) biomass, root biomass, root : shoot ratio, SLA, and plant fitness (i.e., flower and seed numbers).

Soil microbial activity —We measured soil respiration on a weekly basis to determine the effect of Rpf treatment on belowground microbial activity. For each pot, we transferred 1 g of soil into glass vials with a silicon membrane septum cap. After incubating the soil samples at 25 °C in the dark for 24 h, we measured the CO_2 concentration (ppm) from 1 mL of headspace gas using an infrared gas analyzer (IRGA) (LI-COR Environmental). We calculated of CO_2 concentration in our samples based on values generated from a standard curve of known CO_2 concentrations. We performed repeated-measures ANOVA (RM-ANOVA) using an AR(1) covariance structure to test for the main effects and interaction of Rpf and time on rates of soil respiration.

Microbial abundance — We estimated bacterial and fungal abundance using quantitative PCR (qPCR) on DNA extracted from soil samples. For each pot, we collected 1 g of soil pooled from three subsamples and immediately stored the soil samples at -80 °C. We extracted the DNA from 1 g of soil using a PowerSoil® Total RNA Isolation Kit with DNA Elution Accessory Kit (MoBio) following manufacturer's protocol, and the DNA was subsequently quantified using a Take5 *Synergy* microplate reader (BioTek). We then conducted qPCR assays (Lau & Lennon, 2011) that consisted of 20 μL reactions containing 1 μL of DNA template (2.5 ng μL^{-1}), 1 μL of each primer (10 μmol L^{-1}), 7 μL of molecular-grade water, and 10 μL iQTM SYBR Green

SuperMix (Bio-Rad Laboratories Hercules, CA, USA). For bacteria we used Eub338 (forward) and Eub518 (reverse) primers to amplify the 16S rRNA gene, and for fungi we used ITS1f (forward) and 5.8S (reverse) primers to amplify the ITS gene region (Fierer, Jackson, Vilgalys, & Jackson, 2005). qPCR assays were performed using Eppendorf Mastercycler Realplex system using previously reported thermal cycle conditions (Fierer et al., 2005). The coefficients of determination (r^2) of our assay ranged from 0.95 and 1, while amplification efficiencies fell between 0.93 and 0.99. Based on melting curve analyses, we found no evidence for primer dimers. We estimated fungal and bacterial abundance based on the estimated gene copy number from their respective standard curves generated from bacterial and fungal isolates as described elsewhere (Lau & Lennon, 2012). We performed a two-tailed Student's *t*-test to determine the effect of the Rpf treatment on soil bacterial (16S rRNA) and fungal (ITS) gene copy abundances, as well as the fungal to bacterial ratio (F : B).

Microbial structure — To account for the variation in metabolic activity, we characterized soil bacterial communities using RNA and DNA. DNA is a relatively stable molecule contained in intact cells irrespective of their metabolic status. Accordingly, we interpret 16S rRNA sequences recovered from the DNA pool as the "total" community. In contrast, RNA is a more ephemeral molecule that is required for protein synthesis by actively growing cells. Therefore, we interpret 16S rRNA sequences recovered from the RNA pool after complementary DNA (cDNA) synthesis as the "active" community (Jones & Lennon, 2010). Following protocols outlined elsewhere (Lennon, Placella, & Muscarella, 2017), we characterized the effects of Rpf on soil bacterial communities using high-throughput sequencing. We amplified the V4 region of the 16S rRNA gene from bacterial DNA and cDNA to characterize total and active communities,

respectively, using barcoded primers (515F and 806R) designed to work with the Illumina MiSeq platform (Caporaso et al., 2012). We removed residual DNA from RNA samples using DNase 1 (Invitrogen) following manufacturer's protocol and synthesized cDNA by reverse transcribing RNA using iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad). We cleaned the sequence libraries using the Agencourt AMPure XP purification kit (Beckman Coulter, Brea, CA, USA), quantified the resulting product using the QuanIt PicoGreen kit (Invitrogen), and pooled libraries at equal molar ratios (final concentration: 10 ng per library). We then sequenced the pooled libraries with the Illumina MiSeq platform using paired end reads (Illumina Reagent Kit v2, 500 reaction kit) at the IU-CGB. Paired-end raw 16S rRNA sequence reads were assembled into contigs using the Needleman algorithm. After quality trimming with a moving average quality score (window 50 bp, minimum quality score 35), we aligned the sequences to the Silva Database (v 128) using the Needleman algorithm. Chimeric sequences were removed using the UCHIME algorithm (Edgar, Haas, Clemente, Quince, & Knight, 2011). We created operational taxonomic units (OTUs) by first splitting the sequences based on taxonomic class (using the RDP taxonomy) and then binning sequences into OTUs based on 97 % sequence similarity. All sequence processing was completed using the software package *mothur* (version 1.39.5) (Schloss et al., 2009).

Using the 16S rRNA sequences, we determined the effect of Rpf treatment on bacterial richness (i.e., the number of OTUs in a sample) using a resampling approach that subsampled 1,000 sequence observations per sample, resampled 999 additional times, and then calculated the average number of OTUs to estimate per sample richness (± SEM) (Muscarella, Jones, & Lennon, 2016). Similarly, we estimated the effect of Rpf treatment on taxa evenness (i.e., the equitability in relative abundance of OTUs in a sample) using the same resampling approach

with Simpson's index. We then determined the effect of Rpf treatment on the total and active bacterial pools using multivariate statistical procedures. Specifically, we conducted a Principal Coordinates Analysis (PCoA) with Bray-Curtis dissimilarity metric using $\log_{10}$-transformed relative abundances to visualize the effects of Rpf on bacterial communities. To test the hypothesis that Rpf affected the structure of the total (DNA) and active (RNA) bacterial pools, we used permutational multivariate analysis of variance (PERMANOVA) with Bray-Curtis dissimilarity metric implemented with the 'adonis' function in the *vegan* package (Oksanen et al., 2011) in the R statistics environment (v 3.2.3). In the PERMANOVA, we coded pot number as a factor to account for paired-sample design given that DNA and RNA were co-extracted from the same soil sample within each experimental unit. To identify influential taxa driving structural changes in response to the Rpf treatments, we calculated the correlation coefficient of each OTU along PCoA axes using the 'add.spec.scores' function in the *BiodiversityR* package (Kindt, 2016) and implemented a coefficients cut-off (> |0.7|) (Fowler, Cohen, & Jarvis, 2013). We then performed a permutation test (n = 999) to evaluate the correlations of each OTU along PCoA axes using the 'envfit' function on the *vegan* package (Oksanen et al., 2011).

RESULTS

Plant responses — Plants in +Rpf treatment had less total (t_7 = 2.84, P = 0.013), aboveground (t_7 = 2.70, p = 0.017), and belowground biomass (t_7 = 2.30, P = 0.049) than plants in the -Rpf treatment (Fig. 2). While recombinant protein addition led to a ~33 % reduction in root biomass, Rpf no effect on other plant traits including SLA (t_7 = 0.80, P = 0.442), root : shoot ratio (t_7 = -1.20, P = 0.250), and shoot height (t_7 = 1.11, P = 0.286). Similarly, there was no effect of Rpf on the number of seeds produced per plant (t_7 = 0.83, P = 0.421), but we detected a marginal

decrease in the number of flowers produced per plant in the +Rpf treatment (t_7 = 1.79, P = 0.097).

Microbial activity — Rpf altered the activity of the belowground microbial community based on measurements of soil respiration. We detected a significant main effect of Rpf ($F_{1, 84}$ = 9.68, P = 0.002) and time ($F_{5, 84}$ = 21.60, P < 0.001), but no interaction ($F_{5, 84}$ = 1.22, P = 0.308), on soil respiration (Fig. 3). While soil respiration increased throughout the experiment in both treatments, based on marginal means, soil respiration was 24 % lower in the +Rpf treatment than in the -Rpf treatment.

Microbial abundance — The Rpf treatment significantly altered the abundance of soil bacteria (t_8 = 2.71, P = 0.016) and fungi (t_8 = -3.27, P = 0.007) relative to the control (Fig. 4). Bacterial abundance estimated as 16S rRNA gene copy number decreased by ~30 % in the +Rpf treatment relative to the -Rpf treatment. In contrast, fungal abundance estimated as the ITS gene copy number increased 2.8-fold in the +Rpf treatment. Consequently, soil F : B ratio increased 4.8-fold under +Rpf treatment relative to the control treatment (t_8 = -2.84, P = 0.018).

Bacterial structure — Since Rpf production is thought to be restricted to the Actinobacteria, we hypothesized that Rpf treatment would disproportionally affect the diversity and composition of taxa belonging to this group. Therefore, we estimated alpha- and beta-diversity of 16S rRNA sequences belonging to the Actinobacteria phylum. Rpf had no effect on the OTU richness of Actinobacteria regardless of whether sequences came from the total ($F_{1, 8}$ = 0.80, p = 0.397) or active community ($F_{1, 8}$ = 0.14, p = 0.722) (Table S1). Similarly, Rpf had no effect on the

evenness of the total ($F_{1, 8}$ = 0.69, p = 0.429) or active ($F_{1, 8}$ = 0.41, p = 0.538) actinobacterial taxa (Table S1). We detected a small and marginally significant reduction in the relative abundance of actinobacterial sequences in the +Rpf treatment relative to the -Rpf treatment (t_9 = 1.36, P = 0.097) (Fig. A2). However, PERMANOVA revealed that actinobacterial composition was significantly affected by metabolic status (i.e., active vs total) ($F_{1, 19}$ = 16.08, r^2 = 0.33, P = 0.001) and Rpf treatment ($F_{1, 19}$ = 3.25, r^2 = 0.07, p = 0.010). The PERMANOVA also indicated that actinobacterial composition was marginally influenced by an interaction between metabolic status and Rpf treatment ($F_{1, 19}$ = 1.96, r^2 = 0.04, P = 0.055). This interaction reflected an Rpf effect on the active community (i.e., RNA) that was not evident in total (i.e., DNA) community (Fig. 5). Based on this trend, we identified 17 actinobacterial taxa, all of which belonged to a single order (*Solirubrobacterales*), in the active community that had positive (r > 0.7) and significant (p < 0.001) correlations with both PCoA axis 1 and PCoA axis 2 (Table A2).

DISCUSSION

Dormancy is an important life-history trait that influences the diversity, composition, and function of soil microbial communities. After generating recombinant protein with a gene from an environmental isolate, we applied resuscitation promoting factor (Rpf) to soils to test the hypothesis that microbial seed-bank dynamics alter plant growth and fitness traits by driving changes in the belowground microbial community (Fig. 1). The Rpf treatment decreased plant biomass (Fig. 2) most likely by altering the activity (Fig. 3), abundance (Fig. 4), and composition (Fig. 5) of soil microbial communities. Our findings are consistent with the view that soil microbial seed banks can influence plant performance by disrupting interactions with beneficial microorganisms or through the recruitment of pathogens. In the following sections, we discuss

these findings while exploring other potential ways in which Rpf may affect plant-microbe interactions in soil environments.

Rpf indirectly affects plant performance — Resuscitation of microbial seed banks led to a 33 % reduction in plant biomass (Fig. 2). For the following reasons, we infer that Rpf effects on plant performance is likely to be indirect. Rpf hydrolyzes glyosidic linkages in peptidoglycan (Cohen-Gonsaud et al., 2005; Mukamolova et al., 2006).While this polymer is found in the cell walls of virtually all bacteria, it is absent from plant tissues. Nevertheless, we conducted an experiment with *Arabidopsis thaliana*, a close relative of *B. rapa*, to evaluate whether Rpf can directly affect plant performance. We grew seedlings on microbe-free agar while being exposed to recombinant Rpf at concentrations similar to those in our plant-soil experiment with *B. rapa*. A lack of an effect (Fig. S5) led us to conclude that Rpf influenced plants primarily via its effect on the activity, abundance, or composition of the soil microbial community.

Rpf altered fungal-bacterial interactions — Rpf may have altered plant performance by modifying fungal-bacterial interactions. We observed a nearly three-fold increase in fungal abundance in response to Rpf additions (Fig. 4). As a result, soil F : B ratio increased under Rpf treatment (Fig. 4), which may also explain the reduction in soil microbial activity (Fig. 3) given that there are often differences in carbon-use efficiency between bacteria and fungi (Sakamoto & Oba, 1994; Whitaker et al., 2014). Shifts in F : B may also reflect the way that Rpf influences microorganisms with different cell-wall properties. Like plants, fungi do not contain peptidoglycan, the substrate which Rpf acts upon. Instead, the fungal cell wall is primarily composed of chitin. Therefore, an increase in fungal abundance could arise if Rpf decreased the

competitive ability of soil bacteria in our system. In principle, the muralytic activity of Rpf may have even lysed some bacterial cells. If this occurred, the resulting necromass could be scavenged by fungi to meet their metabolic demands (Bradley, Amend, & LaRowe, 2018). Our results, however, do not support this hypothesis. We found that bacterial abundance from three different soils increased with Rpf concentration up to 4 µM g^{-1} soil. At higher concentrations (8 µM g^{-1}), we no longer observed a growth-stimulating effect, but bacterial abundance never dropped below levels observed in control soils without Rpf (Fig. S6). Rpf was much lower (40 nM g^{-1} soil) in our *Brassica* experiment, yet above concentrations shown to resuscitate dormant bacteria (Cohen-Gonsaud et al., 2005; Mukamolova et al., 1998). Based on these findings, it seems unlikely that reductions in bacterial abundance (Fig. 4) were due to lysis or direct inhibition of the soil microbial community.

Rpf altered microbial structure – While seed banks are known to promote biodiversity by buffering ecological and evolutionary dynamics, microbial dormancy may have complex effects that influence plant-soil dynamics. The influence of Rpf on communities of interacting species is likely dependent on the enzyme specificity. Very few studies have investigated Rpf effects on a broad range of microorganisms, but some evidence suggests cross-species resuscitation using recombinant Rpf generated from actinobacteria belonging to *Mycobacterium* and *Brevibacterium* (Mukamolova et al., 1998; Puspita et al., 2015). In our study, we found that Rpf treatment had broad-scale effects on bacterial communities. The composition of Actinobacteria (Fig. 5), Gram-positive bacteria (Fig. A3), and the entire domain of sequenced Bacteria (Fig. A4) were affected by the six-week application of recombinant Rpf. Treatment effects were also apparent when we compared the composition of active Acintobacteria represented by sequences from the RNA

pool, with those recovered from the DNA pool, which includes individuals spanning the full spectrum of metabolic activity (i.e., active, dormant, dead) (Lennon et al., 2018; Fig. 5). This divergence in actinobacterial composition was due to changes in the relative abundance of 17 influential OTUs belonging to the *Solirubrobacterales* (Table A2). Members of this group are aerobic, non-spore-forming, and non-motile bacteria that are recovered in forest and agricultural soils (Kim et al., 2007; Reddy & Garcia-Pichel, 2009; Seki, Matsumoto, Ōmura, & Takahashi, 2015). Little is known about the role of *Solirubrobacterales* in the context of plant-microbe interactions, but some representatives assimilate simple organic compounds (e.g., glucose, maltose, sucrose, xylose, and arginine) that are commonly found in the rhizosphere (Reddy & Garcia-Pichel, 2009). Interestingly, the *Solirubrobacterales* are only distantly related to *Micrococcus* KBS0714, the soil isolate containing the Rpf gene that we used for producing the purified recombinant protein. This suggests that Rpf functionality is not tightly restricted to close kin. Additional work is needed to unveil the network of interactions between Rpf producers and responders, in addition to whether or not the evolutionary history of Rpf genes maps onto phylogenetic relationships of Actinobacteria.

Implications of seed-bank dynamics for plant-microbe interactions – Our study demonstrates that resuscitation promoting factor (Rpf) modified plant performance through its effects on soil microbial communities. Reductions in plant growth were accompanied by shifts in soil microbial community properties, which may have included the recruitment of pathogens. Although we did not observe root lesions or other clear signs of infection, we cannot rule out the possibility the seed banks harbor pathogenic or non-mutualistic taxa that could decrease plant performance. Such a view is consistent with reports of microbial pathogens persisting in soil for long periods

of time, likely in a state of reduced metabolic activity (Sharma & Reynnells, 2016). More explicit tests would include the targeting of known pathogens combined perhaps with measurements of plant immune responses to altered microbial seed banks. Alternatively, our findings could be explained by a dilution of mutualists in the soil or a general disruption of beneficial plant-microbe interactions, which would likely be reflected in the profiling of plant exudates and microbial metabolites.

While our study provides strong empirical support for the notion that microbial seed banks are important for plant performance, it also raises a number of questions for future exploration. We observed that a one protein from a single strain of bacteria can have cascading effects on plant-microbe interactions. However, some microorganisms may be responsive to certain families of Rpf, while others may not. This likely is due to the mechanisms by which Rpf operates in a community context, which is a topic that received little attention to date. More generally, metabolic transitions into and out of dormancy are influenced by mechanisms besides Rpf (Lennon & Jones, 2011) many of which are tied to the interpretation of environmental triggers (e.g., soil rewetting) that are known to resuscitate microorganisms (Aanderud, Jones, Fierer, & Lennon, 2015). Finally, there is a need to explore interactions with other plants. Generalities should be sought by examining the effects of Rpf outside of the Brassicaceae given that these plants tend to lack mycorrhizal symbionts, which are important mutualists found among most flowering plants. It is not difficult to imagine that more complex outcomes could emerge when altered microbial seed banks are considered in diverse plant communities (Fig. 1). Nevertheless, our study highlights a potentially important but overlooked component of plant-microbe interactions. It is estimated that soil microbial communities can be dominated by dormant taxa, yet changes in activity can be fast, suggesting that seed banks may be an important

factor contributing to plant-soil feedbacks, which is thought to be an important mechanism maintaining landscape patterns of biodiversity (Bever, 2003).

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FIGURE CAPTIONS

Fig. 1. Conceptual framework depicting the role of the soil microbial seed bank in local plant-microbe interactions. Members of the soil microbial community can transition between the metabolically active pool (M_a) and the seed bank (M_d) through initiation into dormancy and resuscitation into an active state. Both M_a and M_d pools have associated levels of microbial abundance (N) and local diversity (α). While only members in M_a can reproduce, the baseline rate of mortality (m_d) in M_d is reduced relative to the rate for M_a (m_a). Active microorganisms (M_a) and plants (P) can directly interact with one another through a diverse suite of mechanisms, such as mutualism, parasitism, root colonization, and secretion of phytochemicals. We envision that the M_d pool has minimal effects on plants but is likely influenced by the release of compounds via plant rhizodeposition.

Fig. 2. Influence of resuscitation promoting factor (Rpf) on (A) *Brassica rapa* shoot biomass, (B) root biomass, and (C) flower number produced per plant. We compared plant traits from individuals that were exposed to weekly additions of recombinant Rpf (+Rpf) to those exposed to a protein buffer control (-Rpf). Black symbols represent the mean ± 1 $SE_{x\square}$. Grey symbols represent the individual observations.

Fig. 3. Effect of resuscitation promoting factor (Rpf) on soil microbial activity. Soil respiration was measured after applying Rpf (+Rpf) or protein buffer control (-Rpf) to soils on a weekly basis. Symbols represent the mean ± 1 $SE_{x\square}$.

Fig. 4. Effect of resuscitation promoting factor (Rpf) on (A) soil bacterial 16S rRNA copy number, (B) fungal ITS copy number, and (C) the fungi : bacteria gene copy ratio. Gene copy number was measured from soil after six weekly applications of recombinant Rpf (+Rpf) or protein buffer control (-Rpf) treatment. Black symbols represent the mean ± 1 $SE_{\bar{x}}$. Grey symbols represent the individual observations.

Fig. 5. Principal Coordinate Analysis (PCoA) plot depicting composition of active (i.e., RNA) and total (i.e., DNA) Actinobacteria from soil that were exposed to +Rpf and -Rpf treatments at the end of a six-week experiment. The ellipses were generated by 'ordiellipse' function using the standard deviation of PCoA point scores to visualize the spread of each treatment.

FIGURES

Fig. 1

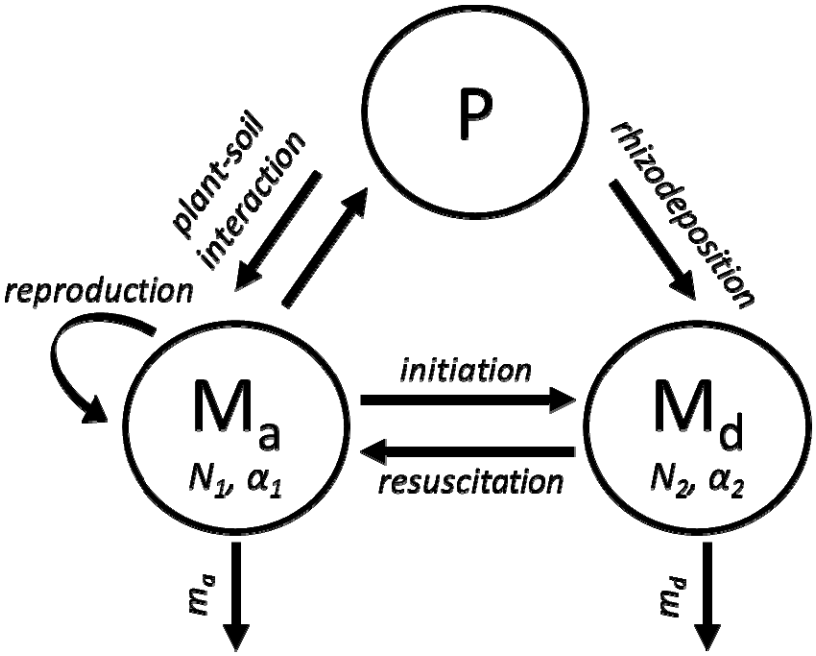


Fig. 2

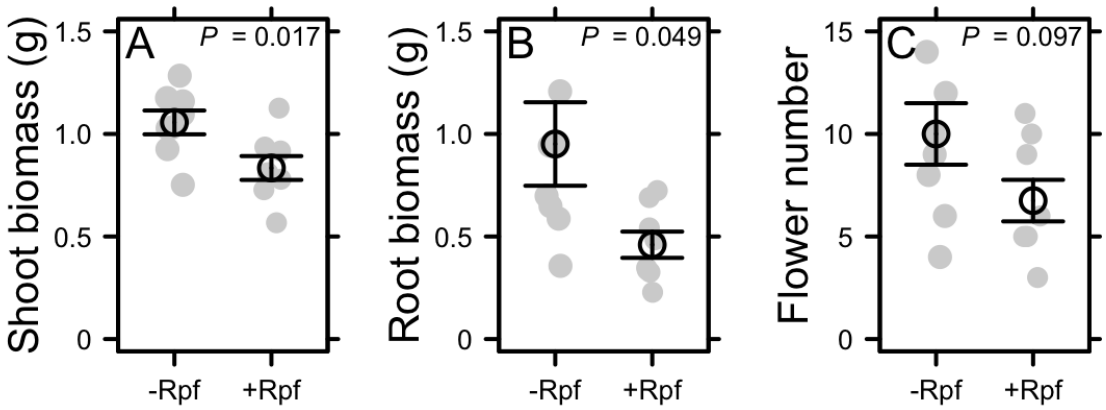

A
P = 0.017
Shoot biomass (g)
1.5
1.0
0.5
0
-Rpf
+Rpf
B
P = 0.049
Root biomass (g)
1.5
1.0
0.5
0
-Rpf
+Rpf
C
P = 0.097
Flower number
15
10
5
0
-Rpf
+Rpf

Fig. 3

586

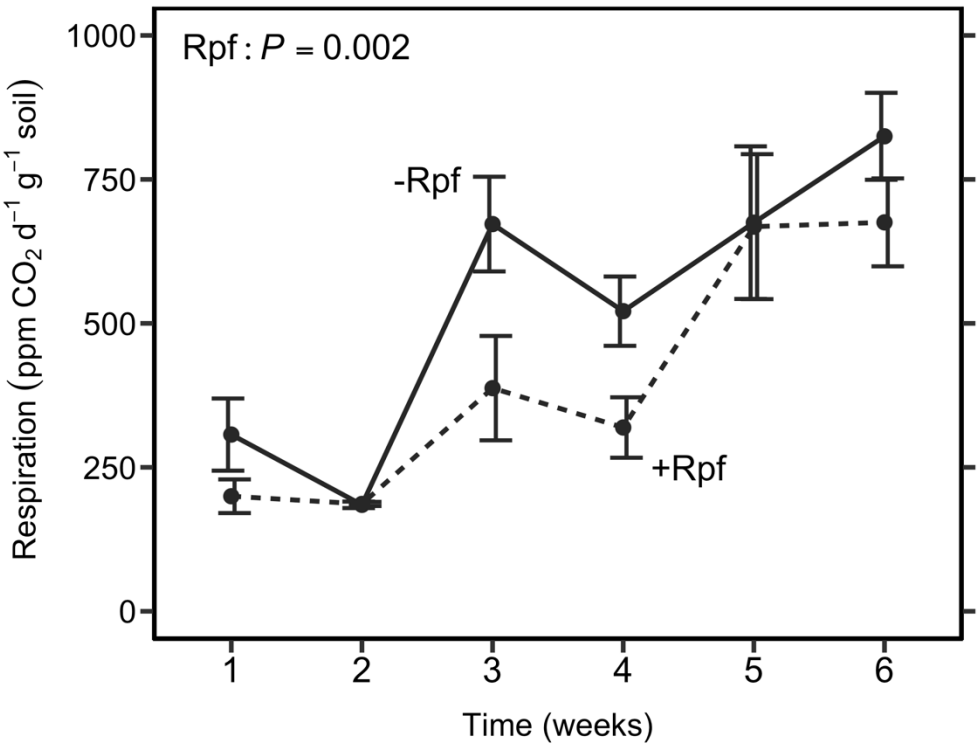
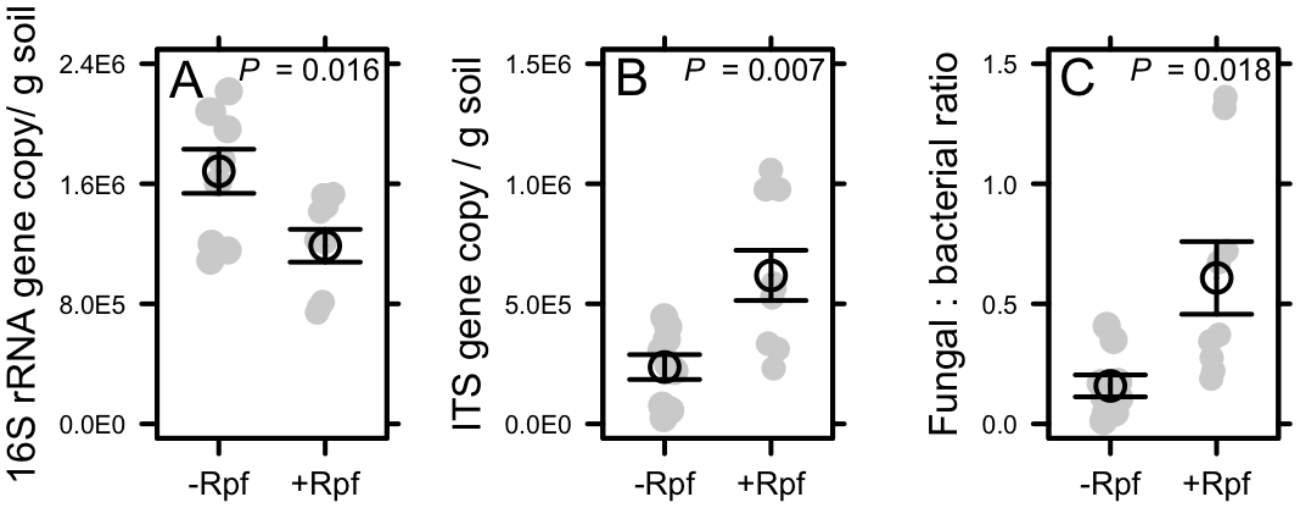
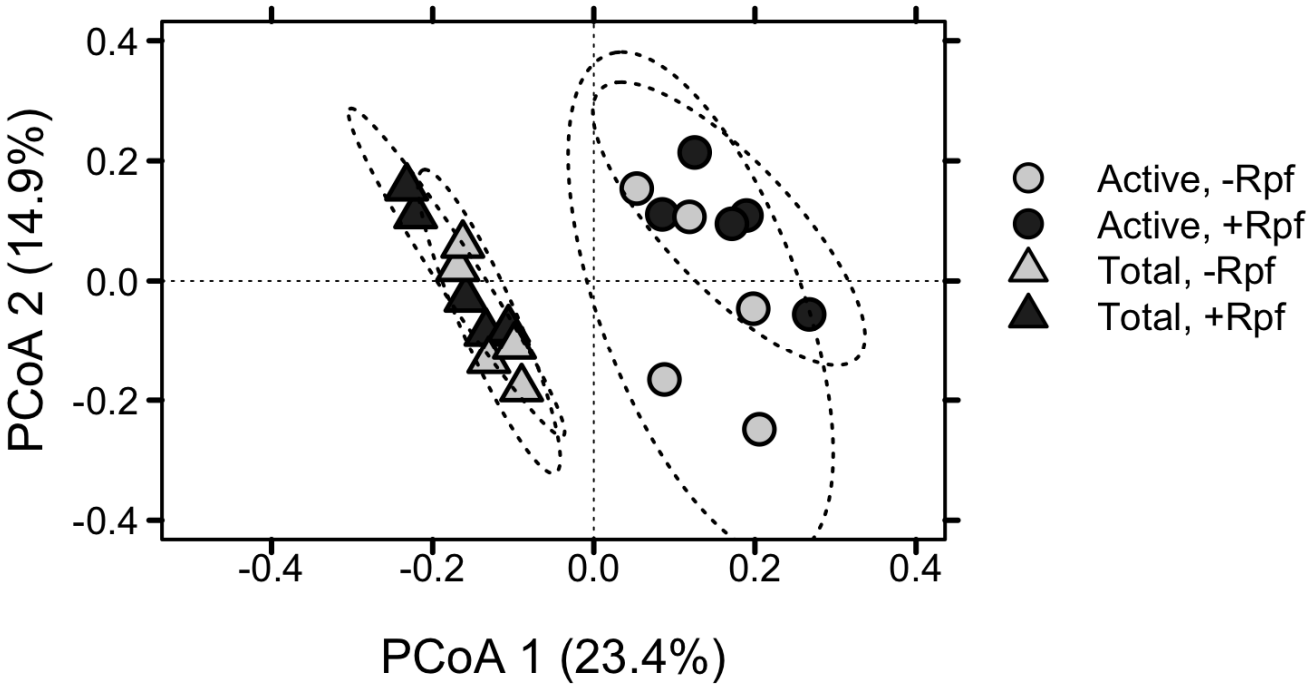

Rpf : P = 0.002
-Rpf
+Rpf
Respiration (ppm CO2 d−1 g−1 soil)
1000
750
500
250
0
1
2
3
4
5
6
Time (weeks)

Fig. 4


16S rRNA gene copy/ g soil
2.4E6
1.6E6
8.0E5
0.0E0
A
P = 0.016
-Rpf
+Rpf
ITS gene copy / g soil
1.5E6
1.0E6
5.0E5
0.0E0
B
P = 0.007
-Rpf
+Rpf
Fungal : bacterial ratio
1.5
1.0
0.5
0.0
C
P = 0.018
-Rpf
+Rpf

588

Fig. 5


0.4
0.2
0.0
-0.2
-0.4
PCoA 2 (14.9%)
-0.4
-0.2
0.0
0.2
0.4
PCoA 1 (23.4%)
Active, -Rpf
Active, +Rpf
Total, -Rpf
Total, +Rpf

590

592