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Compost suppression of a fungal pathogen, *Rhizoctonia solani,* and its impact on root microbiomes

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ABSTRACT
Composts are not only beneficial to plants as available nutrients but for their suppression against pathogens such as the global fungal plant pathogen, *Rhizoctonia solani*. First, a plate competition assay and a suppressive colony assay were used to investigate the role of the soil microbiota in the suppression of *R. solani* by two different management techniques, anaerobic soil disinfestation (ASD) and vermicompost. *R. solani* grew more on agar plates with sterile soil than on plates with non-sterile soil, indicating the importance of the ASD and vermicompost microbiomes in *R. solani* suppression. *R. solani* was suppressed—indicated by zones of inhibition around colony forming units—by more bacteria than fungi. ASD soil contained more suppressive bacteria than vermicompost or untreated soil. Suppressive colonies of Actinobacteria from ASD and untreated soil were phenotyped for carbon utilization using Biolog EcoPlates™ and showed greater phenotypic diversity in ASD soils. Secondly, I characterize how incorporation of vermicompost and *R. solani* infestation of soil affects composition of soil and rhizosphere microbiomes and health status of radish (*Raphanus sativus*) plants. The composition of microbial communities in vermicompost and untreated bulk soil and rhizospheres were sequenced using 16S amplicons. Microbial community composition was influenced by sampling location (root vs. soil), amendment, presence of *R. solani,* and plant health status.

KEY WORDS: *Rhizoctonia solani*, anaerobic soil disinfestation, vermicompost, soilborne disease suppression, organic amendments, Bray-Curtis similarity, soil microbiome

INTRODUCTION

Rhizoctonia solani is a ubiquitous soilborne pathogenic fungus that inflicts a variety of disease states (e.g., damping-off, root and crown rot, and sheath blight) in many economically important crops globally. Most management techniques against fungal pathogens are resistant plant varieties and synthetic chemicals that are often toxic, unreliable, or lead to pathogen resistance (McDonald and Linde 2002; Bonanomi et al. 2007). The harmful methods are not suitable for organic farming and those that are acceptable, such as resistant plant varieties, are not available for *R. solani*. In response to undesirable, harsh, and inconsistent fungicides, there have been great efforts in using and studying organic amendments to both improve plant health and manage soilborne diseases (Bonanomi et al. 2007).

Organic amendments are a class of soil additives including composts, peats, and manures. Many amendments promote the growth of beneficial microorganisms for the plant health through successive populations of microbes breaking down organic matter. From these successions, degradable substrates become available for consumption by the dominant soil microflora (e.g., fungi, gram-negative bacteria, and Actinobacteria) (Hadar and Papadopoulou 2012). The volume of soil surrounding a plant's roots (rhizosphere) is a biological 'hotspot' of microbial activity and biodiversity that is important to the plant's fitness. Plants recruit microbes to form a plant-microbe symbiosis by producing small molecules such as sugars and ions for the microorganisms to consume (Hartmann et al. 2008; Bais et al. 2006; Jacoby and Kopriva 2019). The result is the root and rhizosphere microbiomes—consortiums of microorganisms that associate with the roots of a plant—that have key roles in the plant's nutrient uptake and defense against pests and pathogens.

Within the rhizosphere microbiomes, organisms can be detected and identified using amplicon sequencing e.g.,16S or ITS marker gene sequencing (Bulgarelli et al. 2013). Such methods suggest that γ- and β-Proteobacteria, Firmicutes, and Actinobacteria are relatively abundant in suppressive soils against *R. solani* (Mendes et al. 2011). Suppression is the reduction of disease in presence of the pathogen, generally by metabolically active microbiomes, or specifically by individual microbial species acting against the pathogen. General suppression can be identified and measured by plate competition assays, in which agar containing soil with a living microbiome limits or inhibits the growth of a pathogen (Neher et al. 2017).

The ability of compost-resident microbes to suppress fungal pathogens is variable, with upwards of 50% suppression of multiple composts against multiple fungi, and between 3-20% significant increase in disease incidence (Bonanomi et al. 2007; Termorshuizen et al. 2006). These inconsistencies are at least partly due to insufficient standards for feedstock material and maturity as well as compost application rates (Scheuerell et al. 2005). With further insight into the mechanisms of suppression within organic amendments, reliable standards can be developed to manage soilborne fungal pathogens more effectively without using fungicides.

Anaerobic Soil Disinfestation (ASD) and vermicompost are two organic amendments/processes that have been studied for their suppression against a variety of soilborne plant pathogens, including *R. solani* (Asciutto et al. 2006; Sarma et al. 2010; Butler et al. 2012; Shennan et al. 2014; Strauss and Kluepfel 2015). Vermicompost presented greater suppression against *R. solani* compared to anaerobic static pile (ASP) or windrow amendments (Neher et al. 2017). Little, however, is known about the microbial composition of these amendments or their suppressive qualities,

and ASD has never been studied in Vermont. This experiment investigates the suppression of *R. solani* by various soil amendments (ASD and vermicompost) to better understand the soil microbiomes associated with plants and how the composition and diversity influences *R. solani* suppression. Additionally, the aim is to identify which approach can be effectively used to manage soilborne fungal pathogens.

MATERIALS AND METHODS

Model pathogen system

Pearl radishes (*Raphanus sativus*; Johnny Selected Seeds 2019) were used to study the *in vivo* effect of soil treatments on *R. solani* disease suppression. Radishes were chosen for their short generation time and clear *R. solani* disease symptoms. One *R. solani* inoculum, Riverside (Riverside Farm, East Hardwick, VT), served as the culture used in the plate competition assay and suppressive colony assay. *R. solani* from three different sources were used to determine the inoculum with the desired pathogenicity for Experiment 3 disease severity assay. The *R. solani* inoculums used were from different Vermont farms: Riverside, VT Youth (Vermont Youth Conservation Core, Richmond, VT), and Pete's Greens (Pete's Greens Farm, Craftsbury, VT). From the three sources, the Riverside inoculum was used in the disease severity assay.

To prepare inoculum, radish seedlings were grown in soil with a history of *R. solani* disease. One-centimeter diseased segments of the hypocotyl root were cut from the seedlings, soaked in 10% bleach for 1 minute, and rinsed in sterile water. The root segments were blotted dry before being placed on a potato dextrose agar (PDA) plate at room temperature. *R. solani* isolates were stored and maintained on PDA plates at room temperature.

Soil Sampling

Three soil treatments were used in this experiment: soil treated with anaerobic soil disinfestation (ASD), vermicompost, and a non-amended soil to act as a field control. The ASD soil was produced at the Intervale Community Farm in Burlington, VT by adding a labile carbon (molasses) slurry to the soil and covering with an impermeable tarp to produce anaerobic conditions. The untreated soil is a Hinesburg B fine sandy loam from a field that has a pH of 6.4 and 2.9% organic matter (Neher et al. 2019). The vermicompost was produced at Black Dirt Farm in Greensboro Bend, VT. Vermicompost is produced by feeding a thermophilically treated compost containing dairy manure to earthworms (*Eisenia fetida*) for the curing and maturation phase (Neher et al. 2013).

Sample collection

Experiment 1: Plate Competition Assay

This assay compares the general suppression, i.e., the growth of *R. solani* with and without living microbes, of media containing ASD soil, vermicompost, and untreated soil. For each sample, two tubes of 0.5g of soil in 10ml of autoclaved (sterile) water were prepared and shaken overnight. The following day, two flasks of 1.5g agar and 90ml distilled water were autoclaved for 30 minutes and cooled to 45$_{o}$C in a water bath. One tube of soil was mixed into the agar flask before autoclaving (killing the microbes in the soil; control) and the other was added to the agar flask after autoclaving. Each flask was poured into 5 petri dishes and cooled. Plugs of an established *R. solani* culture on PDA were transferred onto the center of each agar plate and incubated at room temperature for 1 to 2 days. The radius of *R. solani* mycelial growth to the nearest 1mm was measured using Zen (Version 2.3, Carl Zeiss Microscopy 2011) video imaging software at 6.3x magnification. Suppressive soils were determined by computing the mean difference of mycelial growth on autoclaved and non-autoclaved plates such that negative values represent suppression.

Experiment 2: Suppressive Colony Assay

The assay was used identify the presence of suppressive bacteria in the soils and isolate suppressive colonies to investigate their metabolic diversity. Suppressive colonies were defined by creating a zone of *R. solani* inhibition around the colony. This procedure was adapted from Bakker et al.'s (2013a) use on the antagonistic behavior of *Streptomyces.*

Briefly, 5g samples of ASD soil, untreated soil, and vermicompost were dried overnight in a cheesecloth-covered dish and then added to 50ml autoclaved water. The mixture was shaken to ensure uniformity before diluting the mixture to 10$_{-6}$, as this dilution produced the best level of growth of the slow-growing Actinobacteria while limiting other microbial growth. A thin layer of 0.1ml soil-water mixture was added onto 5 water agar plates for each treatment and smoothed with sterile disposable hockey sticks. The plates were allowed to dry before pouring a layer of 5ml prepared, molten Starch Casein Agar (SCA). After 4 to 5 days at room temperature in ambient air, five *R. solani* plugs were transferred onto the SCA layer and allowed to grow for 2 days before counting the number and type of suppressive colonies (i.e., Actinobacteria, bacteria, or fungi). Actinobacteria were distinguished from non-filamentous bacteria based on colony morphology. The abundances of colonies per plate were used to calculate the number colony forming units (CFUs) present in each treatment.

This procedure of 5 plates per replication of each treatment was replicated until 25 suppressive Actinobacteria were isolated from each treatment to be phenotyped using

Biolog EcoPlates™. This resulted in 3 replications of ASD plates, 2 replications of untreated soil plates, and 2 replications of vermicompost plates. From the ASD and untreated soil treatments, 40 Actinobacteria colonies were isolated and grown in pure culture. For each treatment, 30 isolates which were randomly selected for phenotyping on Biolog EcoPlates™ for utilization of 31 carbon types. Procedure was performed as instructed by Biolog™. Positives were determined by being 2 standard deviations over the negative control, water.

Experiment 3: Virulence Assessment, Disease Severity Assay

Inoculum Preparation.

For the following two experiments, *R. solani* was incorporated into radish pots via inoculated bottles of sand commensal culture media. The first experiment used two concentrations of *R. solani* by incorporating either one or two inoculated media bottles into the radish growth medium. Each level was replicated 3 times for 3 strains of *R. solani* for a total of 27 bottles of sand commensal culture media. The second experiment used 24 bottles of sand commensal culture media; 9 bottles were not inoculated with *R. solani* to serve as controls.

Sand commensal culture media was prepared from 192g sand, 4g cornmeal, and 20ml acidified water (1ml lactic acid/liter water) and was autoclaved 1 hour on two consecutive days. The media was allowed to cool before infesting with two plugs of a *R. solan*i strain from a PDA source and incubated for approximately 2 weeks at room temperature.

Assessing Strain Pathogenicity and Virulence

The objective was to test the pathogenicity of 3 *R. solani* strains in order to identify the *R. solani* strain causing pre-emergence damping off in half of the plant population. This strain and concentration were chosen to ensure disease while allowing diseased and healthy germinated plants to be examined in the next stage of the experiment. The experimental design was three isolates and two pathogen concentrations. Each combination was replicated three times, for a total 18 experimental units. Isolates were collected from three locations in Vermont: Riverside Farm, Vermont Youth Conservation Core, and Pete’s Greens.

Each isolate was prepared at two inoculum concentrations (1% and 2% of final soil mixture weight). Upon assembly, each pot was filled halfway with rocks for drainage, covered in a layer of landscaping fabric, and topped with a mixture of 1-liter of soil mixed with inoculum. Twenty-five radish seeds were planted in a 5 by 5 grid and grown in a greenhouse (10-hour daylength with temperatures at 21°C during day and 18°C at night) for 10 days (radish germination period), when the number of emerged radishes

were counted. The number of non-germinating radishes were assumed to be attributed to pre-emergence damping-off.

Disease Severity Assay

The goal was to examine the effects of the soil amendment, presence of *R. solani*, and the plant's disease status on the soil- and root microbiome. Three amendments (vermicompost, poultry pellets, and untreated control soil) were combined with and without *R. solani* inoculum. Inoculated amendments were replicated five times and uninoculated amendments were replicated three times for a total of 24 experimental units. The *R. solani* isolate causing closest to 50% pre-emergence damping-off from Experiment 1 was chosen for this experiment. Specifically, the isolate collected from Riverside Farm in a 1% concentration was chosen because it caused 50.7% pre-emergence damping-off (Fig.4). This level of virulence would allow for both healthy and diseased plants to be sampled.

The pots were assembled as described in Experiment 1, but the soil mixture contained one bottle (~200g) of sand commensal culture with or without *R. solani*, 1-liter of soil, and 100ml of either vermicompost, poultry pellets, or more soil. Initial soil sampling took place before 25 radish seeds were plants and grown in a greenhouse (see Experiment 1). After 10 days, the number of emerged radishes were counted. On day 28 (radish generation time), final soil samples were taken, and root samples were harvested.

<u>Soil sample collection</u>: For both the initial and final soil sample collection, three soil subsamples (1650mm^3 each) were taken from each pot with a corer and pooled. The corer was dipped in ethanol and flamed once between each soil sample and twice between each treatment to avoid cross-contamination.

<u>Plant samples:</u> From each treatment pot, 2-3 random pairs of diseased and healthy plants were extracted and compared to 3 random healthy plants extracted from each uninoculated pot. Approximately 5mm of the lesions on the diseased hypocotyl were excised and the same size and location of the hypocotyl was removed from the corresponding healthy treatment plant. The uninoculated plants had 5mm of their hypocotyls removed in a similar manner. Hypocotyls were washed with water and photographed for comparison. Scissors and forceps were dipped in ethanol and flamed before and in between each excision to control for contamination. The hypocotyls were washed again and set in a micro tube with a 0.2μm filtered PBS buffer, sonicated for 2 minutes to remove contaminants, and placed into DNA extraction bead tubes for temporary storage at -80°C.

Genomic Sequence

Soil samples were treated with the photoreactive DNA-intercalating dye propidium monoazide (PMA) directly after collection and then frozen until DNA extraction. Three subsamples from each pot were treated and sequenced to obtain better representation of the soil microbiome of each pot. For the PMA treatment, 3.5µl stock solution and 1.4ml PBS pH7.4 buffer (8g NaCl, 1.44g Na_2HPO_4, and 0.24g NaH_2PO_4 in 1-liter of water) filtered with a 0.1µm sterile filter were added to a micro tube with 30-40mg of soil. The soil tubes were treated with light in a PMA-Lite™ Photolysis Device on a shaker set to 60% for 5 minutes, vortexed, and treated with light for 5 minutes at 60% again (Carini et al. 2016).

DNA from the soil and hypocotyl tissue was extracted using the DNeasy PowerSoil Kit™ (QIAGEN, USA). The subsamples of the extracted soil DNA were pooled into a single tube to represent their treatment pot. Amplicon sequencing of 16S markers in the isolated DNA were performed in Dr. Noah Fierer's lab at University of Colorado at Boulder. Briefly, the samples were amplified via Polymerase Chain Reaction (PCR) using 515F/806R primers targeted for the V4 region of the 16S rRNA gene in bacteria and archaea. PCR was performed in triplicate for each sample and pooled in equimolar amounts. Amplicons were sequenced on an Illumina MiSeq at the University of Colorado's Next Generation Sequencing Facility. Fierer's lab cleaned the data and matched the sample sequences with microbial taxa in current databases. The number of sequences per OTU were returned to me to further clean and analyze. Dr. Neher assisted me with rarefaction to 11,059 sequences per sample to assure equal sampling effort. I removed singletons and compared community composition among treatments.

Data Analysis

Plate competition assay data were analyzed as an analysis of variance using GraphPad Prism 8 software (GraphPad Prism 8 2020, Version 8.4.1). Phenotypic classification of the isolated suppressive Actinobacteria from the Biolog EcoPlates™ were transformed by a Bray-Curtis similarity index with Primer 7 software (PRIMER-E Ltd 2015). The Bray-Curtis results were visualized as a Principal Coordinates Analysis biplot to show the compositions of the suppressive species between the ASD and non-ASD soils. A Shannon Diversity Index (Hills N1) was calculated of the 29 functional Actinobacteria species between ASD and non-ASD soils to determine and compare the level of diversity of the soils.

Experiment 3: The number of sequences per exact sequence variant (ESV) from the 16S amplicon sequencings were transformed by a Bray-Curtis similarity index and illustrated as a Principal Coordinates biplot to visualize the community similarity of microbial communities among soil treatments and diseased and healthy root pairs. A

PERMANOVA was performed to test the null hypothesis that the similarity of microbial communities between treatments is equal. Analysis of variance was performed using the MIXED procedure in GraphPad Prism 8. Bray-Curtis transformations, principal coordinates analysis and PERMANOVA were performed using Primer 7 software.

RESULTS

Experiment 1: Plate Competition Assay

R. solani grew extensively on compost that was devoid of microbes (autoclaved) but minimally grow on non-sterile soil (*N*=3, *P*=0.0034 Fig. 1). The *R. solani* inoculated onto ASD soil lead to the most consistent and largest difference in growth (difference 23.13mm; Fig. 1) of *R. solani* on sterile versus non-sterile soil. The untreated soil and vermicompost had wider ranges in *R. solani* suppression and smaller differences in *R. solani* between sterile and non-sterile soils (median difference 15.86mm and 12.46mm respectively; Fig. 1).

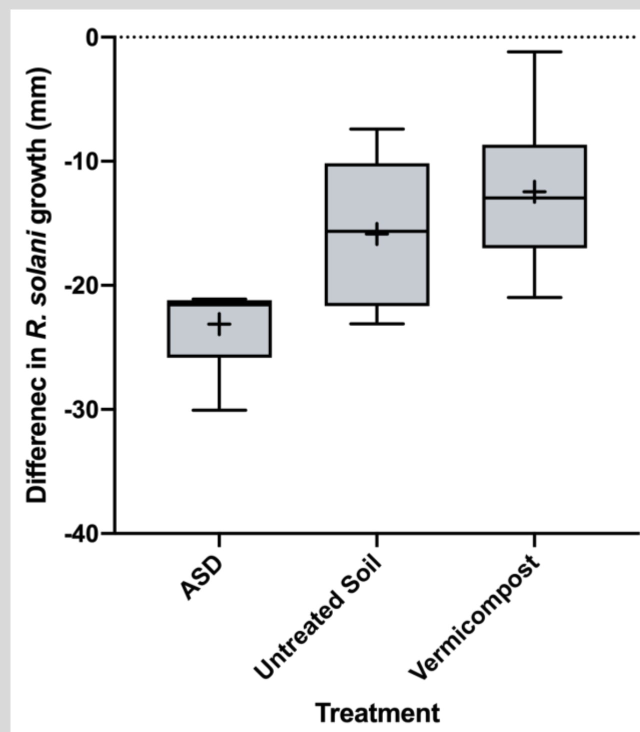

Fig. 1.
Difference in the radius of *Rhizoctonia solani* growth (mm) between autoclaved and non-autoclaved samples (autoclaved - non-autoclaved) of ASD soil, untreated soil, and vermicompost (*P*=0.0034).

Experiment 2: Suppressive Colony Assay

Suppressive colonies were identified as those within a zone of no *R. solani* growth and grouped based on colony morphology into Actinobacteria, non-filamentous bacteria, and fungi. Suppressive bacteria were most abundant in ASD soil and least abundant in vermicompost (*N*=3, *P*=0.0107; Fig. 2a). Suppressive Actinobacteria followed a similar trend but without statistical clarity (*N*=3, *P*=0.2071; Fig.2a). Suppressive bacteria and Actinobacteria had the greatest range of abundance in ASD soils compared to untreated soil and vermicompost. There were no significant differences in abundance of Actinobacteria and fungi among treatments (*N*=3, *P*=0.2071, *P*=0.6786 respectively; Fig. 2b,c). The abundance of fungi varied greatly in vermicompost.

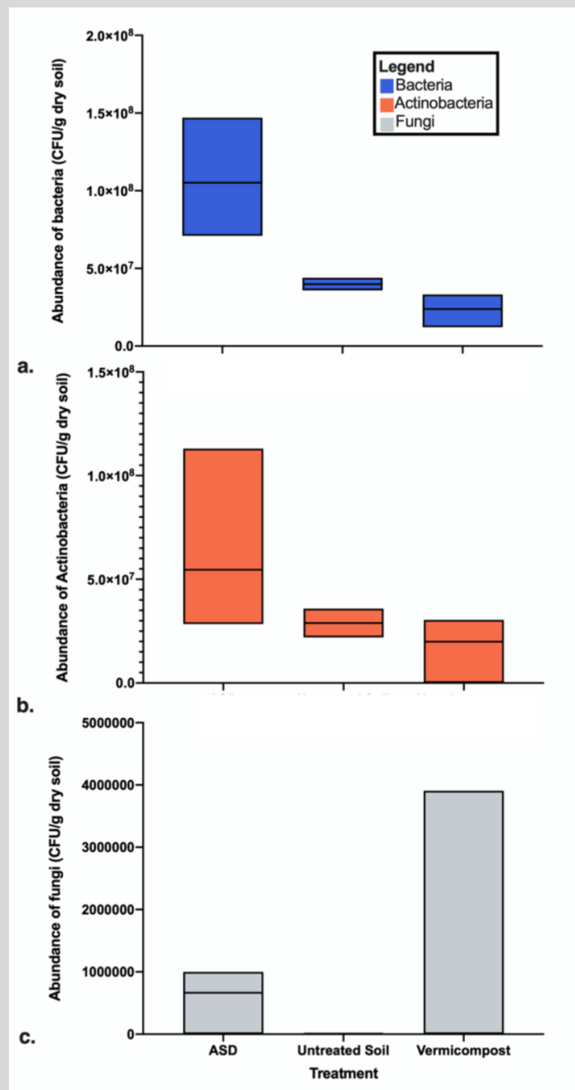

Fig. 2.
Distribution (CFU/g dry soil) of suppressive **(a)** non-filamentous bacteria (*P*=0.0207), **(b)** Actinobacteria (*P*=0.2097), and **(c)** fungi (*P*= 0.6471) in soil treated with Anaerobic Soil Disinfestation (ASD), unamended soil, and vermicompost.

Metabolic diversity of the Actinobacteria isolates comprised 29 functional species based on carbon sources, 5 of which contained multiple isolate members. The ASD and untreated soil Actinobacteria communities had Shannon diversity indices of 2.38 (*Hills N1*=10.82) and 2.27 (*Hills N1*=9.67) respectively. Four clusters of similar functional species formed within the 29 species (*N*=3, *N*=6, *N*=14, *N*=6 clockwise from bottom left) based on a PCO analysis (Fig.3). One cluster was solely comprised of ASD Actinobacteria (*N*=3), whereas the other three species clusters contained Actinobacteria found in both ASD and untreated soil (Fig. 3).

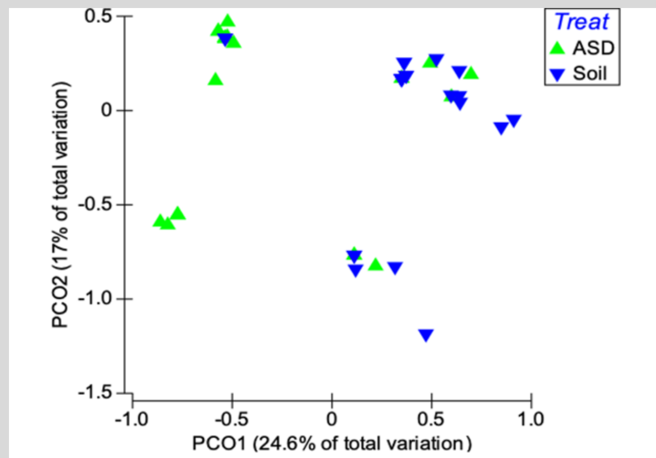

Fig. 3
Principal Coordinates Analysis Biplot of the Actinobacteria from soil treated with Anaerobic Soil Disinfestation (ASD; green triangles) and unamended soil (Soil; blue triangles) into 29 functional species based on relative similarity in utilization of 31 carbon sources.

Experiment 3:
Pathogenicity Assay

The VT Youth and Pete's Greens *R. solani* isolates were less virulent than the Riverside isolate, causing over 60% and over 80% germination in the planted radishes respectively (Fig. 4a). There was little to no difference between the percent germination of the radishes grown in 1% and 2% *R. solani* inoculum concentration in Pete's Greens and VT Youth. The Riverside *R. solani* isolate was the most virulent of the three *R. solani* isolates and had percent germinations ≤ 60% (Fig. 4a).

Disease Severity Assay

There was little to no difference in percent germination between radishes grown with and without *R. solani* inoculum for the untreated soil and vermicompost (Fig. 4b). For both the control (untreated soil) and vermicompost treatments, over 75% of the seeds germinated. No radishes planted in pots amended with poultry pellets germinated.

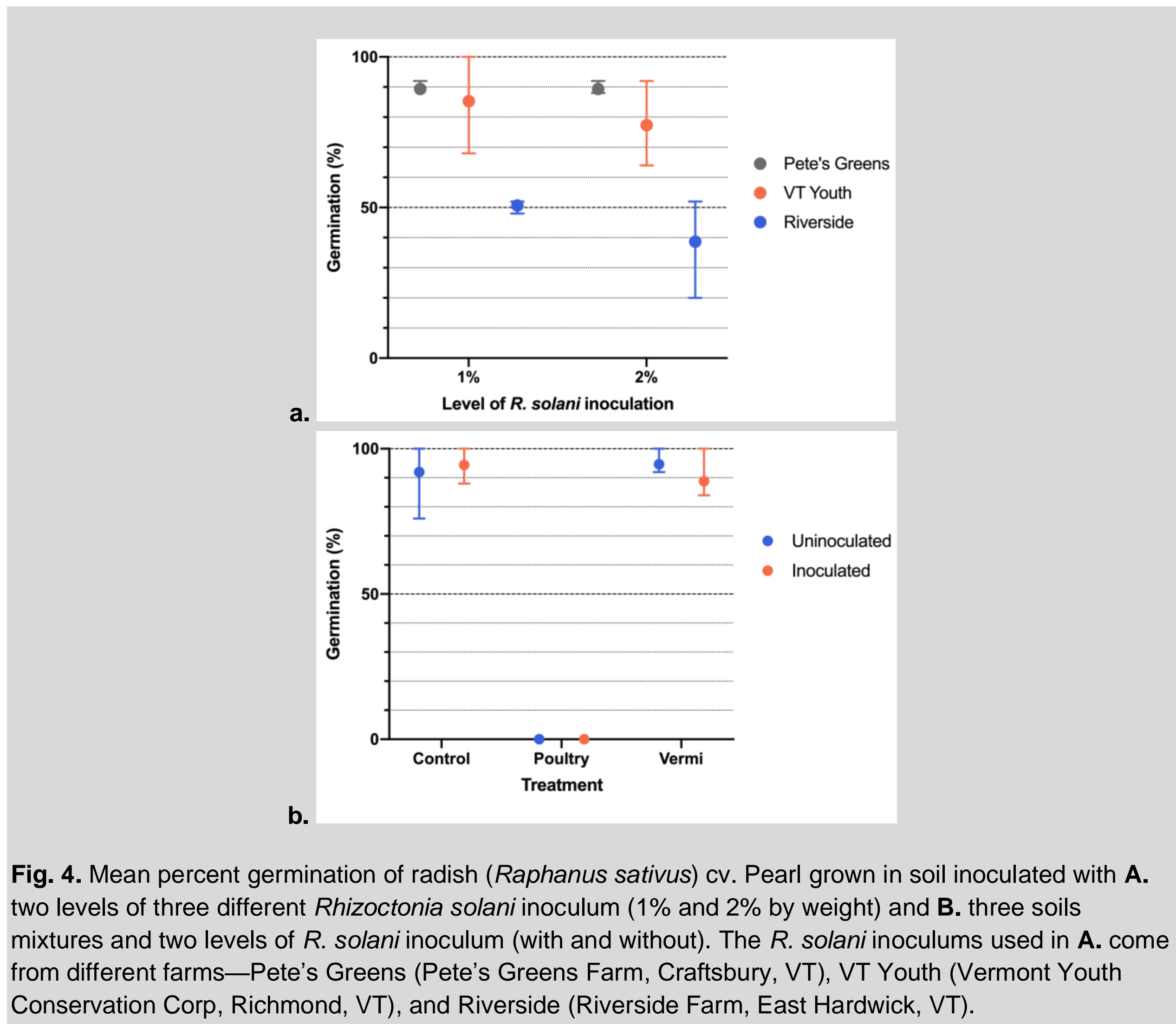

Fig. 4. Mean percent germination of radish (*Raphanus sativus*) cv. Pearl grown in soil inoculated with **A.** two levels of three different *Rhizoctonia solani* inoculum (1% and 2% by weight) and **B.** three soils mixtures and two levels of *R. solani* inoculum (with and without). The *R. solani* inoculums used in **A.** come from different farms—Pete's Greens (Pete's Greens Farm, Craftsbury, VT), VT Youth (Vermont Youth Conservation Corp, Richmond, VT), and Riverside (Riverside Farm, East Hardwick, VT).

From both root and soil samples, 1490 archaea and bacteria exact sequence variants (ESV) were identified. Root and soil samples were compositionally different from each other (Fig. 5a), as were the initial and final soil samples (Fig. 5a,b). There was greater difference between clusters based on treatment in the final soil samples than in the initial soil samples (Fig. 5b). The presence of *R. solani* lead to greater clustering in vermicompost—particularly in the final sample—than in the control soil (Fig. 5b). PERMANOVA supported the PCoA results, showing significant differences based on sample location (DF=1, F= 8.8761, P= 0.017), soil sampling round (DF=1, F=8.1062, P=0.001), and the treatment within rounds (DF=2, F=2.2021, P= 0.006)(Table 1).

The greatest divergence of microbial composition was between roots and bulk soil. Within soil, microbial composition was most dissimilar between vermicompost and control treatments in soil (Fig.5b,c). Nonetheless, there were also clear dissimilarities in

root microbial composition based on treatments (*DF*=1, *F*=4.7997, *P*= 0.001)(Table 1). Microbial communities associated with root samples clustered along the vertical axis based on the presence or absence of *R. solani.* Uninoculated control roots had more similar microbial compositions with vermicompost roots than inoculated control roots (Fig.5c). There is some clustering between diseased and healthy roots along the horizontal axis, but only the control roots have significance divergence based on health status (*T*=1.5162, *P*=0.008) (Fig.5d, Table 1). Most healthy roots were compositionally different from their diseased pair (Fig.5e,d).

TABLE 1 Permutational multivariate analysis of variation (PERMANOVA) and pair-wise T-test of Bray-Curtis distances between samples

TABLE 1
Permutational multivariate analysis of variation (PERMANOVA) and pairwise T-test of Bray-Curtis distances between samples[a]

Factors	DF	F	t	p
Sample location	1	8.8761	-	**0.017**
Soil sampling round	1	8.1062	-	**0.001**
Treatment x soil sampling rounds	2	2.2021	-	**0.006**
Treatment on roots with *R. solani*	1	4.7997	-	**0.001**
Root health status x Treatment x Pot[b]	1	1.3225	-	**0.025**
Control root health status	-	-	1.5162	**0.008**
Vermicompost root health status	-	-	1.1086	0.218

[a]Type III PERMANOVAs were conducted on sampling location and rounds, treatments, and root health status using a total of 999 permutations. Degrees of freedom (DF), pseudo-F value (F), and p-value (p) were reported. Pair-wise T-tests were conducted on root health status for both treatments. T-value (t) and p-value (p) were reported

[b]Pot refers to the replications for treatments and *R.* solani inoculation. Only pots without *R. solani* were involved in this factor.

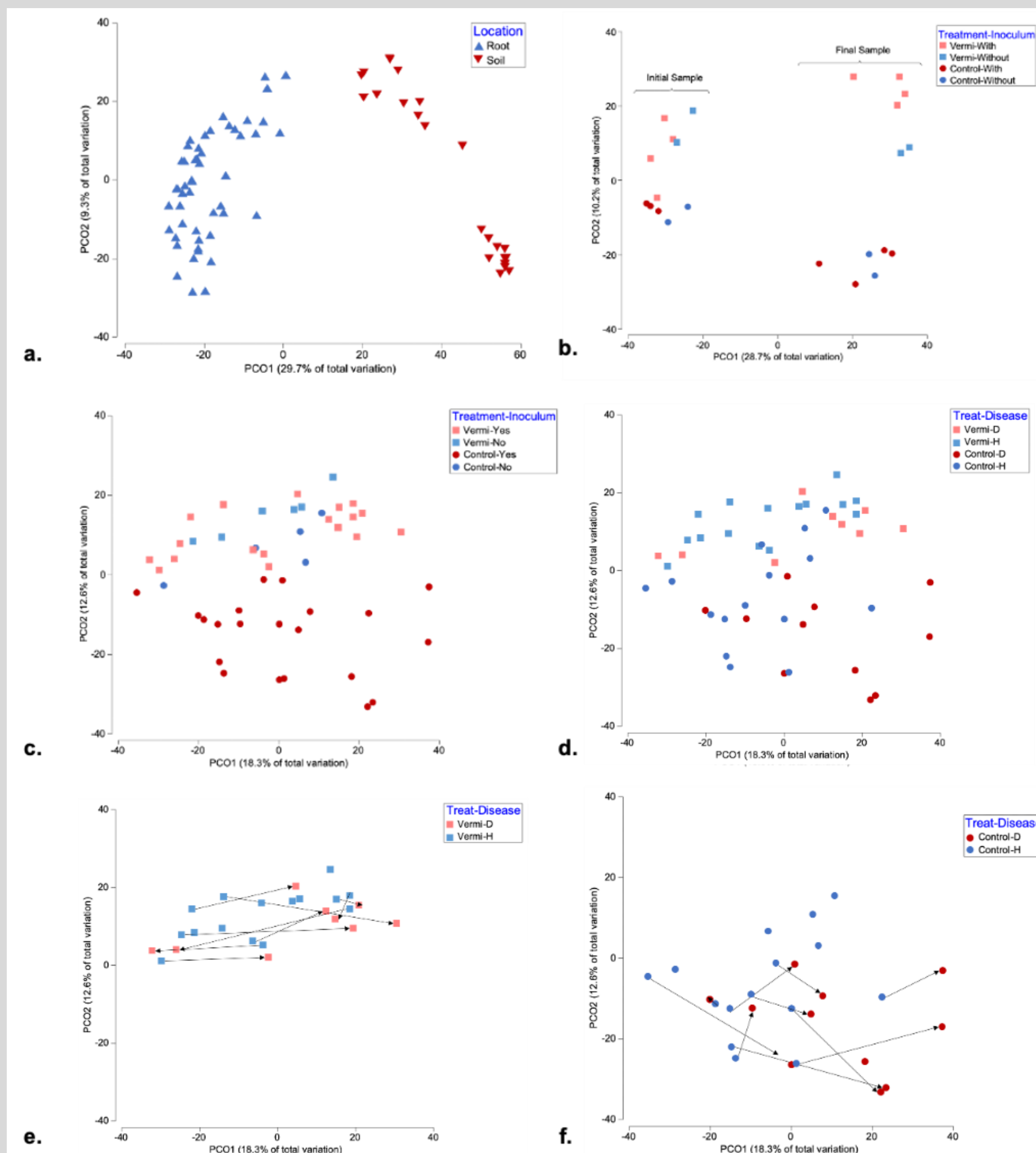

Fig. 5

Principal Coordinates Analysis (PCoA) Biplots for the Bray-Curtis similarity of 16S amplicons for root and/or soil samples from radishes (*Raphanus sativus*) grown in vermicompost (Vermi; squares) and untreated soil (Control; circles). **A.** Similarity between the soil (red) and radish root (blue) samples. **B.** Bray-Curtis PCoA of initial and final soil samples. Red indicates the soil was inoculated with *R. solani* while blue indicates it was not. **C-F.** Ordination of root samples with **C.** showing whether the treatments were inoculated with *R. solani* (red) or not (blue) and **D.** showing the health status of the roots in which healthy roots are blue and diseased roots are red. The random diseased and healthy pairs in the vermicompost (**E.**) and control (**F.**) pots are connected by arrows. The unconnected healthy roots were uninoculated by *R. solani* and the unconnected diseased control root was eliminated during rarefaction.

DISCUSSION

Through a series of assays and 16S amplicon sequencing, the soil treated with ASD appears to be highly suppressive and contain many diverse Actinobacteria species as hypothesized. Actinobacteria are among the taxa commonly found in soils suppressive to fungal pathogens and are the most dynamic of these taxa (Mendes et al. 2011). Within members of this large microbial group, many biological control mechanisms exist such as antibiotics, volatile organic compounds, and competition mechanisms that suppress the growth of pathogens (Palaniyandi et al. 2013). ASD-treated soils fostered a greater diversity of suppressive Actinobacteria compared to untreated soils, including a cluster that was not present in the untreated soils (Fig. 3). I hypothesize that the more diverse phenotypic composition of Actinobacteria promoted by ASD provided the ASD soils with a greater repertoire to antagonize *R. solani*. Further research should identify the taxonomic groups that represent the suppressive Actinobacteria in organic amendments.

Variation in the soil and root microbiome composition related more to sampling location and treatment than the infestation of soil with *R. solani*. The location of sampling (root vs. bulk soil) had a great influence on the microbial composition due to the role of the plant in selecting the community living around it (Doornbos et al. 2012; Bakker et al. 2013b; Hartmann et al. 2008). After the radishes grew in the soils, there was a major shift in the bulk soil microbial composition and a stronger distinction between the communities of plants grown in different amendments. This shift suggests the role of the plant selection and nutrient utilization extending past the rhizosphere. Whether or not the soil was inoculated with *R. solani* only lead to a clear compositional change in vermicompost's bulk soil microbiome. Mendes et al. (2011) found compositional changes in suppressive and conducive soils depending on inoculation status. Such results are consistent with the vermicompost but not with the untreated soil in this study despite it being suppressive to *R. solani* in previous parts of this study (Fig. 1,2,4). The inoculum status did distinguish the ordination of the roots in untreated soil, indicating that the suppression-induced microbial composition changes in the untreated soil remain close to the root rather than influencing the bulk soil.

The absence of suppression in autoclaved soil indicated that the presence of a soil microbiome is crucial for *R. solani* suppression (Weller et al. 2002) in ASD-treated soil, untreated soil, and vermicompost. ASD-treated soil provided the highest level of *in vitro* suppression and diversity in suppressive colonies in this study, showing promise as a useful organic amendment for *R. solani*. Other studies report ASD suppressing a variety of pathogens *in vivo* (Shennan et al. 2014; Hewavitharana and Mazzola 2016), including *R. solani*, which occurs in a variety crops (Strauss and Kluepfel 2015). The level and

consistency of suppression, however, can vary with carbon sources used in ASD (Hewavitharana and Mazzola 2016).

The suppressive effects of vermicompost in this experiment were less pronounced than vermicompost in previous plate competition assays with *R. solani* (Neher et al. 2017). This difference may be influenced by the source and feedstock of the vermicompost, or by the level of maturity, as additional months of maturation can improve suppression (Tuitert et al. 1998). Said parameters and the compost application rate, as studied in cucumbers, tomatoes, and *Impatiens wallerana,* are important to successfully suppressing *R. solani* (Asciutto et al. 2006; Ersahin et al. 2009; Rivera et al. 2004). Therefore, there is a need to optimize the parameters to yield improved suppression against *R. solani* by vermicompost.

Within the *R. solani*-infested pots, the microbial composition of the diseased and healthy roots differed, suggesting a role in the infection activating changes in rhizosphere microbiome (Bakker et al. 2013b; Zhang et al. 2011; Trivedi et al. 2012). In vermicompost, the differences in diseased and healthy root communities were insignificant, perhaps due to vermicompost acting as a biological buffer to avoid changes in the community when the plant is diseased. Whether the change in community led to suppression in the healthy plants or were a response to infection in the diseased plants is not known. These differences may be partly influenced by individual plant genotypes dictating the rhizosphere community (Bulgarelli et al. 2012; Berendsen et al. 2012). The presence of healthy plants in the *R. solani*-infested soils could have been a result of suppression, the virulence of the *R. solani* strain, or the probability of soilborne pathogens being spatially oriented to infect each host (Gilligan 1985).

The limitation of this study's small sample size was enhanced by the lack of germination in the disease severity assay's poultry pellet treatment. While the poultry pellets were included for being conducive to *R. solani*, the uninoculated poultry pots similarly did not germinate. Due to high ammonium levels, poultry manure can be phytotoxic in higher concentrations and was the likely cause of the complete non-germination in this experiment's poultry treatment (Minchinton et al. 1973; Delgado et al. 2010).

One of the biggest limitations in the study of soil ecology is that the majority of soil microorganisms are still unable to be cultured or are still unknown to science, making it difficult to determine accurate compositions of the soil communities (Bakker et al. 2013b; Doornbos et al. 2012). Although the use of metagenomic studies are incredibly useful in generating a fuller picture of the microbiome composition (Bakker et al. 2013b), it is only a snapshot and does not capture the complexity of the system (Hadar and

Papadopoulou 2012). There is a need for a better understanding of the ecology and the niches within the soil microbiome such as the rhizosphere, rhizoplane, and seeds, rather than just the bulk soil (Hadar and Papadopoulou 2012). Furthermore, future research on *R. solani* suppression can determine the presence of specific suppression in ASD-treated soil and vermicompost to understand whether their suppression can be transferred to other soils (Weller et al. 2002) and their mechanisms of suppression.

Most methods of preventing soilborne-fungal plant pathogens involve toxic and unreliable fungicides (Bonanomi et al. 2007; Weller et al. 2002) that do not follow organic farming guidelines. Compost and other organic amendments can promote the growth of microorganisms that are suppressive against fungal pathogens like *R. solani* (Bonanomi et al. 2007) without the use of harsh chemicals. This study found greater suppression using ASD soil and differences in bulk and rhizosphere soil microbiome diversity when infected with *R. solani*. Being able to identify the type and microbial composition of amendments that provide effective and reliable suppression can further improve the maintenance of the pathogens in agriculture.

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