



Phosphite indirectly mediates protection against root rot disease via altering soil fungal community in *Rhododendron* species

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Abstract

Background and Aims The soil-borne pathogen *Phytophthora cinnamomi* causes a deadly plant disease. Phosphite is widely used as an effective treatment to protect plants from *Phytophthora cinnamomi*. Phosphite as a common fungicide might influence the composition of soil fungal communities. However, whether the belowground effects of phosphite-mediated protections are direct or indirectly mediated through soil biota are unknown. Therefore, exploring belowground effects could contribute to the evaluation of the sustainability of phosphite use and tests hypotheses about direct versus indirect effects in pathogen response.

Methods Our greenhouse pot experiment on *Rhododendron* species had either an after-pathogen or a before-pathogen use of phosphite to compare and evaluate plant and soil fungal responses to phosphite

and the presence of an oomycete pathogen *Phytophthora cinnamomi*. The factorial experiment also included with and without pathogen and soil biota treatments, for a test of interactive effects. High throughput sequencing analyzed the soil fungal communities, and we measured the diversity, evenness and richness of soil fungi.

Results Phosphite effectively increased survival of *Rhododendron* species. It altered the composition of soil fungal communities, and the timing of using phosphite determined the way in which the fungal communities changed. *Trichoderma* taxa also responded to soil phosphite and *Phytophthora cinnamomi*.

Conclusions The benefits of antagonistic fungi such as *Trichoderma* are context-dependent, suggesting protection against pathogens depends on the timing of phosphite application. This study provides evidence that phosphite-mediated pathogen protection includes both direct benefits to plants and indirect effects mediated through the soil fungal community.

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Introduction

The effects of soil-borne pathogens are likely to depend on both ecological context and plant-soil microbial

interactions. *Rhododendron* root rot, caused by an oomycete soil-borne pathogen *Phytophthora cinnamomi* (Hardham 2005), is leading to an enormous economic loss in horticultural practice. *Phytophthora cinnamomi* could spread as water flows and cause wilted plants. An effective treatment in the horticultural trade is to add phosphite fungicide to symptomatic plants by either a foliar spray or a soil drench. Phosphite can directly influence the oomycete pathogens (i.e. *Phytophthora*, *Pythium* and *Plasmopora*) by interfering with the phosphorylation process in pathogens by competing for the binding sites of phosphorylating enzymes (Lim et al. 2013). Phosphite can also induce plant defense responses and activate the expression of protective molecules like phytoalexin and pathogen-related proteins (e.g. proteinase inhibitors) (Lim et al. 2013). Studying transcriptomes also demonstrated that phosphite both directly exerted toxicity to pathogens and indirectly induce the disease resistance among susceptible plant species (Kasuga et al. 2021). In addition to direct protection against pathogens, some studies report phosphite as a potential choice of fertilizer or growth stimulant (Gómez-Merino and Trejo-Téllez 2015), even though most soil microbes and plants cannot directly utilize phosphite as a nutrient source. Phosphite can improve nutrient assimilation and increase the productivity and yield of crops (Gómez-Merino and Trejo-Téllez 2015), even though phosphite is likely to have phytotoxicity to plants at relatively high concentrations (Barrett et al. 2004; Pilbeam et al. 2000). However, whether and how phosphite interacts with the soil fungal community, including mutualists and antagonists, is not known.

Soil biota are important for plant growth performance and plant defense. It is well documented that mycorrhizal fungi are critical in promoting plant performance by enhancing nutrient uptake from soil and protecting plants from pathogens (Abdel-Fattah et al. 2011; Mohammadi et al. 2011; Diagne et al. 2020; Pozo et al. 2002). The plants provide both the space to colonize and the nutrients for the growth of mycorrhizal fungi. Some soil fungi can act as antagonists to pathogens and thus might promote plant growth performance. These fungal antagonists are known to comprise several genera, including *Trichoderma*, which can form coiling structures on soil fungal pathogens and compete for nutrients with their host pathogens (Benítez et al. 2004). In addition, *Trichoderma* can exude secondary metabolites to interfere with the growth of their host

pathogens (Benítez et al. 2004; Ghisalberti and Sivasithamparam 1991; Reino et al. 2008). *Trichoderma* contains the most identified biocontrol agents (Thambugala et al. 2020), and therefore, *Trichoderma* are used as biocontrol agents to control several soil-borne pathogens (reviewed in Sood et al. 2020).

Advances in high throughput sequencing technology are improving our ability to understand complex interactions in the soil microbial community, including how those interactions might influence plant responses to pathogens. Through a utilization of high throughput sequencing, experimental addition of phosphite is found to lead to a more disease suppressive soil by an accumulation of antagonistic bacterial taxa (Su et al. 2022a, b; Farooq et al. 2022). For example, soil phosphite can increase *Streptomyces coelicoflavus* and *Paenibacillus favisporus*, which are antagonistic to the soil-borne bacterial pathogen *Ralstonia solanacearum* (Su et al. 2022a, b). However, we are not aware of any study to evaluate the effect of phosphite on soil fungi, including critically important plant mutualists such as mycorrhizal fungi and pathogen antagonist fungi such as *Trichoderma*.

This study used a manipulative, factorial experiment combined with high throughput sequencing to determine direct and indirect effects of soil phosphite on plant response to the plant pathogen *Phytophthora cinnamomi*. The application of high throughput sequencing enables us to identify the soil fungal community and explain the belowground effects of phosphite-mediated protection. We expect that phosphite increases the overall survival proportion of *Rhododendron* species. We also expect that phosphite might shape the fungal community in a way that makes soil more suppressive to pathogens, because of a more diverse fungal community. In addition, fungal antagonists to pathogen like *Trichoderma* might be accumulated. For example, *Trichoderma* might be more abundant with higher richness, evenness or diversity in the presence of phosphite and pathogens, which contributes to plant defense or soil suppressiveness.

Method

Experimental design

A greenhouse experiment on 8 *Rhododendron* species from 4 taxonomic sections (Table S1) was conducted

at Case Western Reserve University Farm (Squire Valleevue Farm, Hunting Valley, OH). Species-true seeds were collected in Fall 2020 from hand-pollinated crosses made on plants growing in the Holden Arboretum collections and germinated in growth chambers in Spring 2021 (see details below in “Seed collection and germination”). Conspecific soils were collected from the 8 *Rhododendron* species at Holden Arboretum (Table S1). A live versus sterile soil treatment was included. We used 3 genotypes of *Phytophthora cinnamomi* to infect the seedlings. Phosphite treatment was applied to plants as either a before-pathogen or after-pathogen use. Thus, we had 8 species $\times$ 2 soil inoculations (live and sterile) $\times$ 2 *Phytophthora* treatments (with and without) $\times$ 3 phosphite treatments (preventative: before-pathogen, curative: after-pathogen, and control) $\times$ 6 replicate pots, for 576 pots (1 seedling per pot) in total (Fig. 1).

Seed collection and germination

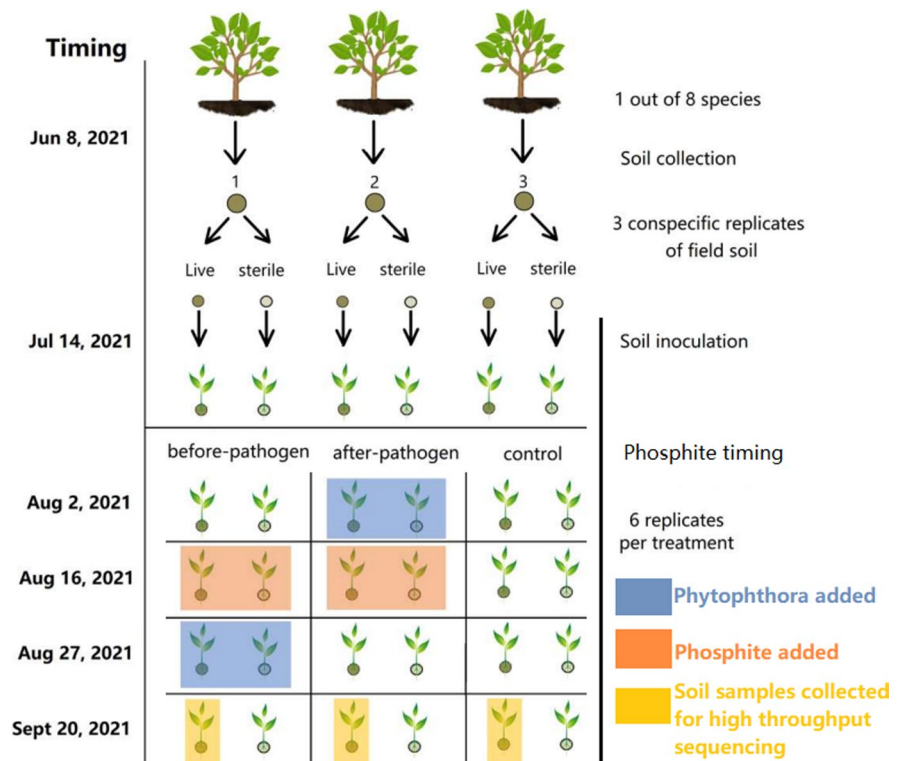
We generated species-true seeds of 8 *Rhododendron* species to avoid unknown paternal lineage from hybridization (Table S1). We controlled the

pollination of flowers on maternal *Rhododendron* species by bagging the buds prior to blooming in the collections of Holden Arboretum (Kirtland, OH) in Spring 2020. Flowers were hand pollinated with pollen from a different accession from the maternal plant and re-bagged. The seeds were collected in Fall 2020, and they were germinated in growth chambers with a diurnal program (Day (7:00AM–10:00PM): 25°C / Night: 22°C) at Case Western Reserve University Farm starting in April 2021. Seedlings of 8 species were transplanted to deep pots (6.4 cm diameter– 25.4 cm deep; Stuewe and Sons, Tangent, OR USA) filled with a 1:1 mixture of horticultural perlite and milled sphagnum moss in June 2021, with one seedling per pot.

Soil treatment

To conduct a live versus sterile soil treatment, we collected conspecific rhizosphere soils from 3 replicate locations under each focal species at Holden Arboretum. The soil samples were not pooled (i.e. each collection remained separate throughout the experiment) to avoid pseudo-replication (Reinhart and Rinella

Fig. 1 A schematic diagram for a greenhouse experiment using 8 *Rhododendron* species to evaluate the effects of phosphite to plant performance and soil fungal communities. Three soil samples were collected from the root zones of each species from Holden Arboretum, and soil inoculations were prepared following our previous study (Liu et al. 2021). Phosphite timing included before-pathogen, after-pathogen and control (Before-pathogen: plants treated with phosphite before the pathogens were inoculated. After-pathogen: plants treated with phosphite after the pathogens were inoculated. Control: plants treated with no phosphite during the experiment)



2016). All soil samples were dried in the greenhouse and sieved using Sieve #8 which has a mesh size of 2.36 mm (SARGENT-WELCH Scientific Company). Half of each replicate soil was autoclaved at 121 °C for 2 h (Market Forge Sterilematic, model STM-E) to produce sterile soil inocula. The other half of sieved soils were inoculated to pots under the live soil treatment. We inoculated each pot with 5 cm³ (5–6 g) live or sterile soil in July 2021.

Phosphite and disease treatments

We had several treatment combinations of phosphite and the pathogen *Phytophthora cinnamomi*. Phosphite treatments included before-pathogen treatment (phosphite treatment is prior to pathogen inoculation), after-pathogen treatment (phosphite treatment is after pathogen inoculation) and control (no phosphite). In each type of phosphite treatment, we either did or did not add *Phytophthora cinnamomi* (presence or absence of pathogen), for a fully factorial experimental design (Fig. 1).

The phosphite treatment in our experiment was using a commercial fungicide, Fosol Select Aliette/Aluminum Fungicide (Prime Source). This commercial product included 80% Aluminum tris (O-ethyl phosphonate) as the active ingredient. We applied phosphite solution (0.66 cm³ of product per liter of water) by a soil drench. We used half the recommended application dose because of a potential toxicity to plant seedlings according to our preliminary observations. We obtained 3 accessions (putative genotypes) of wild-isolated *Phytophthora cinnamomi* (LA-7, MAD-C, and BDW) obtained from Dr. Francesca Hand at Ohio State University (Columbus, OH). We cultivated these strains by using a lima bean culture and rice inoculum following the protocol from Krebs and Wilson 2002 (Krebs and Wilson 2002).

Starting in August 2021, we inoculated *Phytophthora* (1 month since cultivation) using the rice grain inoculum (3 rice grains per pot) to each pot for the after-pathogen treatment (Fig. 1). Each pot received one of the three *Phytophthora* accessions described above, haphazardly assigned to pot. After conducting phosphite treatment, we inoculated the *Phytophthora* to the pots for the before-pathogen treatment (Fig. 1).

The control pots were not inoculated with *Phytophthora* by inoculating sterile rice grains.

Survival count and plant harvesting

The number of leaves were counted as a measure of initial plant size after soil treatment, and again before *Phytophthora* inoculation. When harvesting plants, the number of surviving plants was counted. The susceptibility of each *Rhododendron* species was calculated as a log response ratio on survival data (Liu et al. 2021). The formula we used to calculate the susceptibility of each species was

$$\text{Susceptibility} = \ln(\overline{Y1}) - \ln(\overline{Y2})$$

where, $\overline{Y1}$ and $\overline{Y2}$ are the plant proportional survival without and with *Phytophthora cinnamomi* respectively (Liu et al. 2021). The variance of the susceptibility was calculated as

$$V_{\ln RR} = S_1^2 / (n_1 \times \overline{Y1}^2) + S_2^2 / (n_2 \times \overline{Y2}^2)$$

where, S_1^2 and S_2^2 are the variances of $\overline{Y1}$ and $\overline{Y2}$, and n_1 and n_2 are the sample size of $\overline{Y1}$ and $\overline{Y2}$ respectively (Liu et al. 2021). We pooled plants with and without phosphite when contrasting the survival of plants with and without *Phytophthora*, because *R. kaempferi* had a zero-survival proportion without phosphite presence.

Plants were harvested in September 2021, because our prior experiments suggested that most of the mortality due to *Phytophthora cinnamomi* should have occurred by this date. Plant performance was measured by the dry biomass of plant roots and shoots (Pinnacle Pi-314, Denver Instruments, Bohemia, NY, USA; 60 degrees C for 2 weeks).

High throughput sequencing data

To characterize the soil microbiome, subsamples from the field collections and the pot soils at the end of the greenhouse experiment were stored at -80°C. A total of 232 samples were sequenced including all alive plants in live soil treatment at the harvesting period. Soil fungal DNA was extracted from each sample using a bead-beating protocol as described

previously (Burke 2015). Extracted DNA from each sample was suspended in 100 µl Tris EDTA buffer and stored at -20 °C in 1.5-mL low retention centrifuge tubes (Fisher Scientific, Pittsburgh, PA). First round PCR was conducted on a S1000 thermal cycler (Bio-Rad, Hercules, CA, USA). The ITS2 region of the fungal genome was amplified with the primers 58A2F (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATCGATGAAGAACGCAG, Martin and Rygielwicz 2005) and ITS4 (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTCCGCTTATTGATATGC, White et al. 1990) containing Illumina overhang adapters (underlined). For each PCR reaction, 1.2 µl template DNA was added into a 25 µl PCR reaction mixture containing 1X buffer, 2 mM MgCl₂, 0.8 mM dNTPs, 0.2 µM of each primer, 0.5 µg/µl bovine serum albumin, and 1 unit of Fast Start Taq DNA Polymerase (MilliporeSigma, Saint Louis, MO, USA). The thermocycling conditions involved an initial denaturing step at 95 °C for 5 min, 30 cycles of 30 s at 95 °C, 60 s at 60 °C, 60 s at 72 °C, and a final extension of 5 min at 72 °C. First round PCR products were then indexed and sequenced on an Illumina high throughput sequencing platform at Case Western Reserve University's Genomics Core Facility with a 2×250 bp MiSeq run. The Genomics Core also performed a quality check prior to sequencing where the DNA concentration of the final libraries were determined with a Qubit Fluorometer, and fragment sizes were verified with agarose gel electrophoresis.

A total of 8,418,705 sequence reads for fungi were obtained from the Illumina MiSeq HTS platform. We excluded 2 samples with less than 1000 sequence reads from the analysis. Sequence reads from the remaining 230 samples were processed with the UNOISE3 pipeline (Edgar 2016a). We merged the forward and reverse sequence reads by conducting the fastq_mergepairs command in USEARCH, version 11.0.667 (Edgar 2010). To remove phiX reads, we used the filter_phiX command in USEARCH. Cut Adapt v2.8 (Martin 2011) was used to trim the PCR primer sequences from the reads, with 15% mismatches allowed during this process. Reads less than 250 bp and/or with one or more sequence error were excluded with the fastq_filter command. The unoise3 command was used for error correcting, removing chimeras, and generating zero radius OTUs (zOTUs)

(Edgar 2016b). Across all samples, 3583 zOTUs were reported. Merged reads with the primers trimmed and phiX reads removed for each sample were then mapped to the zOTUs using the otutab command. With the SINTAX algorithm (Edgar 2016c), each zOTU was assigned with fungal taxonomic information by matching to the UNITE database v 8.0 (UNITE Community 2019). The matrix of zOTUs was normalized through the function *estimateSizeFactorsForMatrix* in the 'DESeq2' package (Love et al. 2014) in R (Version 4.2.1) (Love et al. 2014). To solve the error of inflating zero entries, we added a pseudo count of 1 to all zOTU entries before the normalization.

Data analysis

Genomic data was analyzed through ordination and diversity indices on the 230 samples from live soil treatment that successfully returned sequence reads. Nonmetric multidimensional scaling was performed on the normalized fungal zOTU matrix with the function *metaMDS* in the 'vegan' package of R (Oksanen et al. 2022). We exported the ordination scores of fungal zOTUs (both "site scores" and "species scores") by using the function *scores*. To conduct an analysis of PERMANOVA (Permutational Multivariate Analysis of Variance Using Distance Matrices) with the R package 'vegan' (Oksanen et al. 2022), we imported the normalized zOTUs matrix and created a Bray–Curtis similarity matrix with the R function *vegdist*. The PERMANOVA was conducted with the function *adonis2* by modeling the similarity matrix as a response variable as the function of plant species, phosphite treatment and *Phytophthora* treatment.

We measured the diversity matrix of zOTUs in samples. The function of "specnumber" in package *vegan* (Oksanen et al. 2022) calculated the richness of zOTU matrix (S). To calculate evenness (J) of fungal zOTUs, Shannon Diversity Index (H) was calculated based on the zOTU matrix by the function of "diversity" in the same package. The formula was used to calculate the evenness.

$$J = H / \log(S)$$

The means of richness, evenness and Shannon diversity index were calculated by *Rhododendron*

species and incorporated into the dataset for further analysis.

The phylogeny of 8 studied species was obtained from our previous project (Liu et al. 2021). All analyses were conducted in R program (Version 4.2.1) (R Core Team 2018). The analyses based on both greenhouse and high throughput sequencing data were conducted to answer the following questions. (1) Does phosphite enhance *Rhododendron* survival or growth in the presence of *Phytophthora cinnamomi*? (2) Do plant performance and the properties of the fungal community (i.e. diversity) respond to experimental treatments? (3) Do fungal community properties correspond to plant species pathogen susceptibility? (4) Does *Trichoderma* zOTU diversity correspond with phosphite treatment or protections against effects of the pathogen *Phytophthora cinnamomi*?

Does phosphite enhance *Rhododendron* survival or growth in the presence of *Phytophthora cinnamomi*?

A generalized linear mixed-effects model (glmer) from lme4 package (Bates et al. 2014) was used to estimate how plant survival responded to soil microbial community (live versus sterile) and phosphite treatments (before-pathogen, after-pathogen or control treatment) in the presence of *Phytophthora cinnamomi* (with- or without pathogen). The survival data was specified as a binomial response variable. We dropped the 3-way interaction because it was not significant ($P > 0.05$). The model included the main effect of soil treatment and the interaction between phosphite treatment and *Phytophthora* treatment. The random effects of the plant species and *Phytophthora* accessions were incorporated in this model. Statistical results were tested using the *Anova* function from 'car' R package (Fox et al. 2019). When significant interactions were detected, we used the *glht* function from 'multcomp' package (Hothorn et al. 2008) to conduct a posthoc comparison among the means of survival under various treatment combinations. This full contrast included a Holm adjustment to ameliorate the risk of inflated Type I error.

Do plant performance and the properties of the fungal community (i.e. diversity) respond to experimental treatments?

Generalized linear mixed-effects models were conducted to analyze the relationships between plant performance (plant total biomass, shoot biomass or root biomass) and experimental treatments including the soil (e.g. live versus sterile), phosphite and

Phytophthora treatments and their 3-way interactions, with the random effects of the plant species and *Phytophthora* accessions. All biomass data in model analyses were adjusted by a natural log transformation. Full contrast analyses with Holm adjustment were applied to significant treatments, and such analyses compared the biomass among treatments.

PERMANOVA was first conducted to detect whether the fungal community responded to phosphite, *Phytophthora* treatment and their interaction. We analyzed the diversity matrix (i.e. richness, Shannon diversity index and evenness) of plant-associated fungal communities through constructing generalized linear mixed-effects models including the main effects of phosphite, *Phytophthora* treatments and their two-way interaction, and we included the random effects of the plant species and *Phytophthora* genotypes. We used a natural log transformation for fungal richness. Note that soil treatment was not included in these analyses, because we didn't sample the sterile soils when studying the fungal community and the sequencing was not conducted for all sterile soil treatments. Full contrast analyses with Holm adjustment were used to compare the fungal community richness, Shannon diversity index and evenness among treatments.

Do fungal community properties correspond to plant species pathogen susceptibility?

We also predicted that the diversity of the fungal community in the soil might influence plant susceptibility to the pathogen, because live soil biota improves plant survival in the presence of *Phytophthora cinnamomi* (Liu et al. 2021). Thus, we used the *rma.mv* function, testing the main effect of the diversity matrix (i.e. richness, Shannon diversity index and evenness) of fungal community on plant susceptibility. Because plant susceptibility is a log-response ratio, we weighted the analysis by the inverse of the variance in its estimate. Phylogeny was again incorporated in the error structure because our susceptibility metric is a species-level measure (see Survival count and plant harvesting).

Does *Trichoderma* zOTU diversity correspond with phosphite treatment or protection against effects of the pathogen *Phytophthora cinnamomi*?

The subsets of data with detected *Trichoderma* zOTUs (only include the samples with *Trichoderma* zOTUs) were analyzed in linear mixed-effects models and phylogenetic meta-analysis models. Linear mixed-effects models tested the community diversity

(i.e. richness, Shannon diversity index and evenness) of *Trichoderma* zOTUs as a function of phosphite, *Phytophthora* treatments and their two-way interaction, in which plant species and *Phytophthora* genotypes were random effects. Full contrast analysis with Holm adjustment were conducted within significant main effects to compare *Trichoderma* zOTU diversity across treatments. Phylogenetic meta-analysis models were used to test the correlation between plant susceptibility and diversity matrix, using each of the richness, Shannon diversity index or evenness measures of *Trichoderma* zOTUs following methods detailed above.

Results

Phosphite increased the survival of plants in the presence of *Phytophthora cinnamomi*

Plant survival was analyzed as a function of soil treatment and the interaction of phosphite treatment and *Phytophthora* treatment. The two-way interaction of phosphite treatment and *Phytophthora* treatment was significant, while the main effect of soil was not significant (Table 1) and the survival measurement was high in both live (91.5%) and sterile (88.6%) soil treatments. The full contrast analysis on the two-way interaction between phosphite treatment and *Phytophthora* treatment indicated that phosphite treatment protected plants from *Phytophthora* by increasing survival (Table S2; $ya > yc$, $p < 0.001$; $yb > yc$, $p < 0.001$), but the protective effects were not different between before- and after-pathogen phosphite treatments (Table S2; contrast $yb - ya$: $p = 0.74$).

Table 1 Effects of soil treatment, phosphite, and *Phytophthora* treatment including the two-way interaction on plant survival

	Chisq	Df	Pr(> Chisq)
Soil treatment	2.67	1	0.1
Phosphite treatment	30.18	2	< 0.001*
<i>Phytophthora</i> treatment	11.88	1	< 0.001*
Phosphite x <i>Phytophthora</i> treatment	11.2	2	0.004*

Results with * are significant effects

We tested the main effect of the three *Phytophthora* accessions on plant survival including the random effect of plant species. The main effect of *Phytophthora* accession was insignificant, and the means of plant survival under these accessions were 81.4%, 79.5% and 84.7%. The plant overall survival without any pathogen (control) was 98.1%. If we subset the plants with phosphite treatment, the main effect of *Phytophthora* accessions was still insignificant, and the plant survival under these genotypes were 93%, 91.4% and 96.5%. We therefore excluded *Phytophthora* accession from analyses presented here.

Plants and their associated fungal community responded to experimental treatments

Plant performance

Plant performance significantly responded to experimental treatments. The main effect of soil treatments was significant for models testing total biomass and shoot biomass (Table 2). Plants growing in live soil had lower total biomass (contrast $1 < s$, $p = 0.01$) and lower shoot biomass (contrast $1 < s$, $p = 0.002$) (Table 2). The full contrast analysis indicated that phosphite treatment increased

Table 2 Effects of soil treatment, phosphite and *Phytophthora* treatment including significant interactions on plant (1) Total biomass, (2) Root biomass and (3) Shoot biomass

	Chisq	Df	Pr(> Chisq)
(1) Total biomass			
Soil treatment	6.42	1	0.01*
Phosphite treatment	37.27	2	< 0.001*
<i>Phytophthora</i> treatment	0.69	1	0.41
(2) Root biomass			
Soil treatment	3.71	1	0.05
Phosphite treatment	25.52	2	< 0.001*
<i>Phytophthora</i> treatment	0.95	1	0.33
(3) Shoot biomass			
Soil treatment	10.5	1	0.001*
Phosphite treatment	44.66	2	< 0.001*
<i>Phytophthora</i> treatment	1.78	1	0.18
Soil: Phosphite treatment	7.23	2	0.03*
Phosphite x <i>Phytophthora</i> treatment	6.02	2	0.049*

Results with * are significant effects

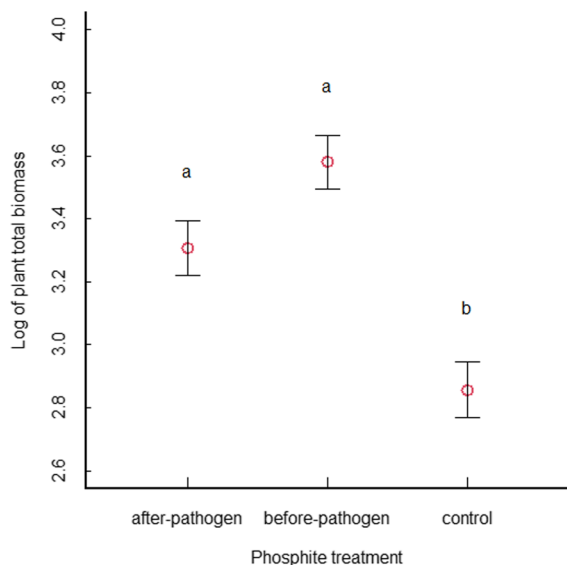


Fig. 2 Plant total biomass with natural log transformation as a function of the phosphite treatment. Sharing any letter represents insignificant comparison in a Holm adjusted contrast

the total biomass of plants (Fig. 2; contrast $a > n$, $p < 0.001$; contrast $b > n$, $p < 0.001$).

Fungal community

The PERMANOVA showed that the fungal community was significantly responsive to the phosphite treatment ($p < 0.001$) and *Phytophthora* ($p < 0.001$). Testing the properties of the fungal community indicated significant main effects of phosphite and *Phytophthora* treatment, and their significant two-way interaction was detected in analyzing fungal richness and evenness (Table 3). According to the full contrast analyses, a before-pathogen use of phosphite treatment reduced the fungal richness (contrast $b < c$, $p < 0.001$) and diversity (contrast $b < c$; $p = 0.045$), while an after-pathogen use of phosphite treatment increased fungal richness compared with control (contrast $a > c$, $p = 0.005$). *Phytophthora* treatment increased the fungal community richness (contrast $c > n$, $p < 0.001$) and Shannon's diversity (contrast $y > n$, $p = 0.01$) while reducing its evenness (contrast $c > n$, $p < 0.001$).

Table 3 Effects of phosphite and *Phytophthora* treatment including their two-way interaction on fungal community (1) richness, (2) Shannon's diversity and (3) evenness

	Chisq	Df	Pr(> Chisq)
(1) Fungal richness (log transformed)			
Phosphite treatment	74.2	2	<0.001*
<i>Phytophthora</i> treatment	107.79	1	<0.001*
Phosphite x <i>Phytophthora</i> treatment	22.05	2	<0.001*
(2) Fungal diversity (Shannon Diversity Index)			
Phosphite treatment	17.16	2	<0.001*
<i>Phytophthora</i> treatment	7.36	1	0.01*
Phosphite x <i>Phytophthora</i> treatment	2.94	2	0.23
(3) Fungal evenness			
Phosphite treatment	14.63	2	<0.001*
<i>Phytophthora</i> treatment	18	1	<0.001*
Phosphite x <i>Phytophthora</i> treatment	6.73	2	0.04*

Results with * are significant effects

The diversity of the fungal community was correlated with plant susceptibility

Plant susceptibility was significantly positively correlated with fungal diversity and evenness (Table 4), while fungal richness was not correlated with plant susceptibility (Table 4). Our result indicated that more susceptible plants were associated with a more diverse fungal community.

Trichoderma community responded to soil phosphite and *Phytophthora*

Plant pathogen susceptibilities were negatively correlated with the evenness of the *Trichoderma* community, however, neither the richness nor diversity of *Trichoderma* zOTUs correlated with plant susceptibility (Table 4). Experimental treatments also influenced the *Trichoderma* community. The main effects of phosphite and *Phytophthora* treatments were significant for both richness (phosphite: Chisq (Df=2)=18.39, $p < 0.001$; *Phytophthora*: Chisq (Df=1)=22.89, $p < 0.001$) and diversity (phosphite: Chisq (Df=2)=21.59, $p < 0.001$; *Phytophthora*: Chisq (Df=2)=8.25, $p = 0.004$) of *Trichoderma*. An after-pathogen use of phosphite significantly increased the richness (contrast: $a > c$, $p = 0.001$) and Shannon

Table 4 Phylogenetic meta-analysis of the correlation between plant susceptibility and fungal diversity matrix within either the whole fungal community or *Trichoderma* community

Plant susceptibility ~ Diversity matrix	The fungal community		<i>Trichoderma</i> community	
	Estimate $\pm$ se	p-value	Estimate $\pm$ se	p-value
Richness	-0.0002 $\pm$ 0.0001	0.154	0.001 $\pm$ 0.006	0.898
Shannon diversity	0.14 $\pm$ 0.02	< 0.001*	0.02 $\pm$ 0.06	0.751
Evenness	1.41 $\pm$ 0.13	< 0.001*	-0.32 $\pm$ 0.04	< 0.001*

Results with * are significant effects

diversity (contrast: $a > c$, $p < 0.001$) of *Trichoderma* zOTUs. In the presence of *Phytophthora*, plants had a greater richness (contrast: $y > n$, $p < 0.001$) and Shannon diversity (contrast: $y > n$, $p = 0.005$) of *Trichoderma* in the soils. Phosphite treatment and the presence of *Phytophthora* did not influence the evenness of *Trichoderma* zOTUs (phosphite treatment: Chisq (Df=2)=2.69, $p = 0.26$; *Phytophthora* treatment: Chisq (Df=1)=2.27, $p = 0.13$).

Discussion

Our study demonstrated that *Rhododendron* species and their associated fungal communities responded to the presence of phosphite and *Phytophthora cinnamomi*, and below we discussed how this explains the effects of phosphite-mediated protection. We demonstrated that preventive-use of phosphite increased

both the survival of *Rhododendron* species and plant biomass performance as effective as a treatment-use. However, the fungal communities were shaped in different patterns due to the timing of adding phosphite (Fig. 3), which might indicate complex below-ground interactions. We also found that fungal diversity matrix correlated with plant susceptibility and plant performance. Our work deepened the understanding of plant-disease interactions and protecting plants from soil-borne pathogens through utilizing phosphite.

Here, we found phosphite is effective in protecting plants from *Phytophthora cinnamomi*, consistent with a large body of literature (Burra et al. 2014; Carmona et al. 2018; Machinandiarena et al. 2012; Miller et al. 2006; Smillie et al. 1989). More importantly, we explored the possibly indirect mechanism of phosphite protecting plants through incorporating a high throughput sequencing analysis. *Trichoderma*

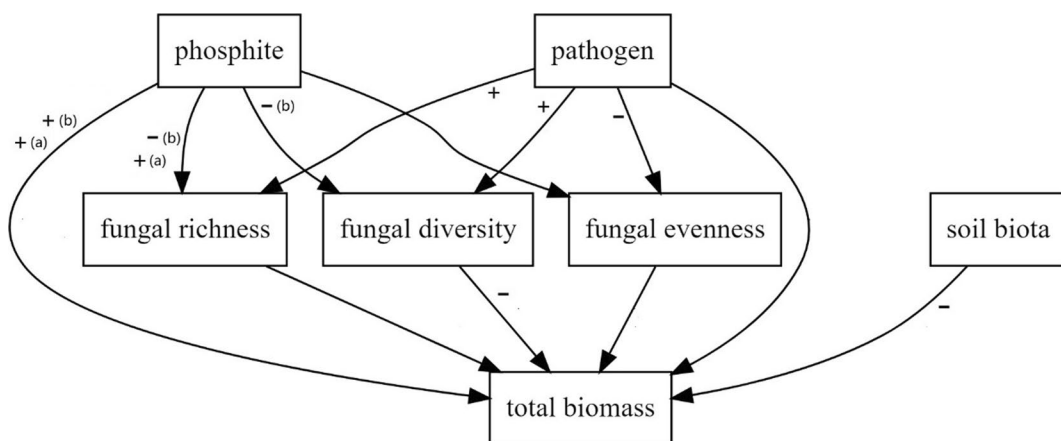


Fig. 3 The summary of (1) direct effects of inoculated soil biota, phosphite ((a): after-pathogen use; (b): before-pathogen use) and pathogen (*Phytophthora cinnamomi*) to plant total biomass; (2) their impacts to soil fungal diversity matrix (rich-

ness, diversity and evenness); and (3) direct effects of fungal diversity matrix on plant total biomass. The positive and negative correlations are presented in arrows marked as “+” and “-”, and the insignificant effects have arrows only

are strongly antagonistic to soil-borne pathogens, so they are widely utilized in biological control applications (Akladios and Abbas 2012; Begum et al. 2010; Blaszczyk et al. 2014; Lombardi et al. 2020; Mbarga et al. 2012). Our study explored the relationships between phosphite use and the *Trichoderma* community. Below we summarized the evidence that the effects of phosphite might be mediated by indirect effects on the fungal community, a novel finding. Plants with an after-pathogen use of phosphite had a more diverse assemblage of *Trichoderma*. The diversity matrix of *Trichoderma* was strongly correlated with the application of phosphite and *Phytophthora* presence, which implied that plants might recruit a more diverse *Trichoderma* community as a response to both phosphite and *Phytophthora cinnamomi*. Here, we offered a possibility of considering both phosphite and *Trichoderma* taxa as a potential solution to plant infections by *Phytophthora* species, however, more studies are still urgently needed before extensive applications in planting practices. For example, different *Phytophthora* genotypes might vary in their evolving sensitivity to phosphite (Hunter et al. 2022; Wilkinson et al. 2001). In addition, the mobility of zoospores is one important factor to determine the outcome of infections (Bassani et al. 2020), while we still need to further understand how zoospore density and movement correlate with the pathogen responses to soil fungal taxa including *Trichoderma* species.

Phosphite-induced protection against *Phytophthora* was likely a result of both direct and indirect mechanisms, depending on the timing of phosphite-use. This study demonstrated that phosphite protects plants against pathogens via both direct and indirect effects. Phosphite can exert a direct pressure to the growth of *Phytophthora*, well supported by existing knowledge (Havlin and Schlegel 2021; Hunter et al. 2022). In addition, phosphite can indirectly mediate protection by influencing soil fungal community through recruiting soil *Trichoderma*. A preventative use of phosphite reduced the diversity and richness of the whole fungal community, while a curative use of phosphite increased the fungal diversity and accumulated more diverse *Trichoderma* species. This work further suggests the hypothesis that plants exposed to pathogens are primed to recruit *Trichoderma*, though further tests are still needed to demonstrate how our

knowledge can benefit agricultural or planting applications under natural conditions.

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Author contributions YL and JHB contributed to the proposal submission. YL, JHB, DJB and JSM contributed to the experimental design. Material preparation and data collection were performed by YL, collaborating with JHB, DJB and SRK. Data analyses were conducted by YL collaborating with JHB and SRK. The first draft of the manuscript was written by YL, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The data is archived in Open Science Framework (OSF): OSF | Phosphite indirectly mediates protection against root rot disease via altering soil fungal community in *Rhododendron* species. <https://doi.org/10.17605/OSF.IO/HU35A>.

The sequencing data is archived in Sequence Read Archive under BioProject number PRJNA964695 | Phosphite indirectly mediates protection against root rot disease via altering soil fungal community in *Rhododendron* species, at the National Science Center for Biotechnology Information site.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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