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Cover Crop Diversity as a Tool to Mitigate Vine Decline and Reduce Pathogens in Vineyard Soils

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Abstract: Wine grape production is an important economic asset in many nations; however, a significant proportion of vines succumb to grapevine trunk pathogens, reducing yields and causing economic losses. Cover crops, plants that are grown in addition to main crops in order to maintain and enhance soil composition, may also serve as a line of defense against these fungal pathogens by producing volatile root exudates and/or harboring suppressive microbes. We tested whether cover crop diversity reduced disease symptoms and pathogen abundance. In two greenhouse experiments, we inoculated soil with a 10^6 conidia suspension of *Ilyonectria liriodendri*, a pathogenic fungus, then conditioned soil with cover crops for several months to investigate changes in pathogen abundance and fungal communities. After removal of cover crops, Chardonnay cuttings were grown in the same soil to assess disease symptoms. When grown alone, white mustard was the only cover crop associated with reductions in necrotic root damage and abundance of *Ilyonectria.* The suppressive effects of white mustard largely disappeared when paired with other cover crops. In this study, plant identity was more important than diversity when controlling for fungal pathogens in vineyards. This research aligns with other literature describing the suppressive potential of white mustard in vineyards.

Keywords: grapevine trunk disease; cover crops; biofumigant; young vine decline; plant-microbe interactions

1. Introduction

Grapevines (*Vitis vinifera* L.) are one of the most widely grown crops worldwide and an important economic commodity, especially in British Columbia where vineyards account for a total of 9652 hectares [1]. Grapevines experience multiple challenges, including competition with weeds [2], nutrient leeching [3], root lesion nematodes [4], viral infections [5], and especially fungal diseases [6] that reduce profit for growers. Although historic reports of fungal diseases exist [7], this problem has gained a considerable amount of attention in the 1990s [6] as wine grape production increased in Australia, Canada, the United States, and South Africa, among other countries [8–10].

Young vine decline (YVD) is a type of grapevine trunk disease that results in stunted growth, reduced yield, delayed fruiting, root necrosis, and eventually death in young vineyards 5–7 years old [11]. YVD occurs in British Columbia and other major wine grape regions around the world [6,12], resulting in significant economic losses [13]. YVD is considered a disease complex whereby the physical

symptoms observed are a result of abiotic and biotic factors. Among many of the biotic stressors is *Ilyonectria*, a genus of soil-borne fungi and a causal agent of YVD [14]. Moreover, these fungi are generalist pathogens and are known to infect the roots of certain apple and cherry cultivars [15]. *Ilyonectria* is not only confined to vineyard soil, but also found in nurseries all over the world that often serve as breeding grounds for the pathogen [12,16,17].

In Canada, there are no commercially available fungicides or fumigants for managing young vine decline and other grapevine trunk diseases (GTDs) [18]. Methyl bromide, a once popular soil fumigant, has been phased out due to its toxicity and ozone depletion [19], and has been shown to reduce arbuscular mycorrhizal (AM) fungi and other beneficial organisms [20]. Available fumigants such as 1,3-dichloropropene and chloropicrin do not protect against the full spectrum of fungal pathogens including *Phytophthora* and *Fusarium* [21]. In addition, fungicides that are applied directly to plants as a liquid or powder coating can enter soil and accumulate overtime, reducing microbial diversity and activity [22]. Other approaches for controlling grapevine trunk pathogens include hot water treatment, in which propagation material is soaked in hot water (~50 °C) for a specified time [23,24]. This approach carries risks however, as improper procedures can damage propagation material and reduce vigor [25].

Cover cropping is a potential tool to mitigate GTDs in vineyards. Traditionally, cover crops have been grown to reduce soil erosion [26], increase available nitrogen for grapevines [27], control pests [28], and suppress weeds via allelopathy [29]. Growers also use cover crops to decrease vegetative growth in high vigor situations, which reduces canopy cover and improves the microclimates for ripening fruits [30]. Although cover crops have a long history of use in vineyards, their potential to mitigate soil-borne diseases has not been fully explored.

Existing literature highlights the biofumigant effects of brassicaceous crops (mustards/crucifers) that have exhibited suppression of soil-borne pathogens in vineyards and nurseries [31,32]. Other cover crops including forbs, legumes, and grasses may help reduce soil-borne diseases by harboring beneficial and antagonistic microbes [33,34], or via host dilution in which the risk of infection decreases with increasing host diversity [35]. The different mechanisms of suppression though these plants provide an incentive to implement cover crop diversity in vineyards as a management strategy for soil-borne diseases.

A diverse plant community can increase soil microbial diversity [36], biomass [37], and activity [38] via root exudation, rhizodeposition, and plant litter [39,40], which can improve ecosystem services. Soils from long term grasslands and forests contain plant growth-promoting rhizobacteria (PGPR), which can suppress pathogens when added to agricultural soil [41,42]. Implementation of cover crops in vineyards can increase the activity of PGPR [43,44], which are commonly found in soil [45,46]. If beneficial microbes can be isolated from nearby soil and used to reduce disease symptoms in agricultural plots, it is possible that cover crops can provide similar soil inputs and encourage proliferation of microbes that suppress fungal pathogens.

To date, most cover crop experiments use commercial rather than native plants [47,48], and the efficacy of native cover crops has not been studied extensively in vineyards [49–52]. Plant provenance may be as important as diversity due to local adaptation and coevolution between native plants and their microbial counterparts [53]. This is observed in highly specific legume-rhizobia interactions [54,55] and could hold true for other plant–microbe interactions.

In many "home vs away" studies, plants perform better when grown with soil from the same region as the plant [56–58]. Moreover, decomposition rate is increased when a plant litter is sympatric to the soil compared to allopatric soils [59,60]. Since plant–microbe interactions heavily depend on genotypic differences [61] and resource availability [40,62], microbial communities under native plants may differ compared to common cultivar cover crops, leading to differences in ecosystem services and possibly the suppression of pathogens. Given these circumstances, native cover crops may stimulate and harbor local microbial communities through more-efficient interactions based on root exudation,

litter decomposition, and chemical signaling that has been subject to selective forces over many generations [63].

To evaluate the effect of cover crop diversity on GTD symptoms and the abundance of pathogenic fungi, the effects of single cover crops grown on their own were compared to the same cover crops grown together. Using native and common cover crops, we hypothesized that mixtures of cover crops would result in fewer disease symptoms, reduce pathogen abundance, and increase fungal diversity more than any plant on its own. The present study provides insight into cover crop management in vineyards, primarily in the context of disease mitigation.

2. Materials and Methods

2.1. Establishment of Experiments

In order to understand the effects of cover crop diversity on GTD symptoms, we established two separate greenhouse experiments at the Summerland Research and Development Centre (SuRDC) in Summerland, BC, Canada:

"Cultivar Study". This experiment used four cover crops that are commonly used in vineyards (Table 1). Crimson clover (*Trifolium incarnatum* L.) and buckwheat (*Fagopyrum esculentum* Moench) were purchased from a local supplier (WestCoastSeeds, Vancouver, Canada), while white mustard (*Sinapis alba* L.) and wheatgrass (*Triticum aestivum* L.) were purchased from a commercial seed supplier (Richters, Ontario, Canada).

Table 1. Selected cover crops for native study and cultivar study greenhouse experiments. Native plants were collected in the Okanagan Valley, while seeds of cultivar plants were purchased from seed suppliers. N/A = not applicable.

Treatment	Latin Binomial	Study	Diversity	Group	Life Cycle
Crimson clover	*Trifolium incarnatum*	Cultivar	1	Legume	Annual
Wheat	*Triticum aestivum*	Cultivar	1	Grass	Annual
Buckwheat	*Fagopyrum esculentum*	Cultivar	1	Forb	Annual
White mustard	*Sinapis alba*	Cultivar	1	Brassica	Annual
All cultivar		Cultivar	4		Annual
Fallow	N/A	Cultivar	0	N/A	N/A
Silky Lupine	*Lupinus sericeus*	Native	1	Legume	Perennial
Bluebunch wheatgrass	*Pseudoroegneria spicata*	Native	1	Grass	Perennial
White yarrow	*Achillea millefolium*	Native	1	Forb	Perennial
Holboell's rockcress	*Bochera hoellbelii*	Native	1	Brassica	Perennial
All native		Native	4		Perennial

"Native Study". This experiment used four plants native to the southern interior British Columbia as cover crops. We used white yarrow (*Achillea millefolium* L.) and silky lupine (*Lupinus sericeus* Pursh), which were sourced from a local supplier (Xeriscape Endemic Nursery, West Kelowna, BC, Canada) along with bluebunch wheatgrass (*Pseudoroegneria spicata* Pursh, Löve), which was collected in Summerland, BC. Holboell's rockcress (*Bochera hoellbelii* Hornem, Löve) seeds were donated by SeedsCo Community Conservation, a local native plant supplier (Table 1).

2.2. Effect of Cover Crop Diversity on Disease Symptoms

In order to observe the effect of cover crop diversity on incidence of disease, each species for the native and cultivar studies was grown on its own (monoculture) as well as with all other plants (all native or all cultivar), totaling five treatments and 10 replicates per treatment for each study (Table 1). In addition to the cover crop treatments, the cultivar study had an additional "fallow" treatment in which the soil was kept bare. This treatment was not seeded with cover crops to determine the

incidence of disease and grapevine growth without the addition of inoculant or cover crop. Both experiments consisted of a randomized block design with 10 blocks (five blocks per table) to account for environmental variation inside the greenhouse and the rectangular shape of the tables. Each treatment was assigned to its block via random number generation. Treatments were standardized to four plants per pot such that monoculture pots consisted of four plants of the same species while all native and all cultivar pots consisted of one individual for each species totaling four plants (Table 1).

2.3. Location and Greenhouse Conditions

Plants were grown in a greenhouse at SuRDC (49°33'57.8" N 119°38'10.0" W) from 27 April 2018 to 4 March 2019. To reduce stress during warm summer months, the room was cooled by a fog system that turned on when temperatures rose above 28 °C and shade curtains were activated from 12:30 p.m. until sunset. During the spring and fall, daytime and nighttime temperatures were kept at 20 and 15 °C, respectively, with supplementary lights to maintain 15-hour days.

2.4. Soil

Soil was collected at SuRDC on 21 March 2018 from a small cherry block. This soil is described as a Skaha loamy sand which had previously harbored apples (Braeburn grafted to M.26 rootstock) until it was replanted with sweet cherry during the 2014 growing season (Table A1) [64,65]. *Fusarium*, *Ilyonectria*, and *Rhizoctonia* species (which are known to infect grapevine roots) were previously isolated from this site [64], increasing the likelihood of resident pathogens already in the soil. Soil was collected from the northwest guard zone, which consisted of a sweet cherry row that separated treatments from the access road. A trench (250 × 40 × 25 cm) was dug, keeping as close to the row as possible. Soil was thoroughly homogenized by hand on a large tarp and stones were removed before the soil was transferred into 3-liter nursery pots that were filled, leaving a gap of 4 cm from the top to prevent water overflow. Nursery pots were placed in SuRDC greenhouse facilities for the duration of the study.

2.5. Pathogen Incubation and Inoculation

We inoculated each pot with three isolates of *Ilyonectria liriodendri* (SuRDC 340, 60, 393) to increase the likelihood of infection. This pathogen was previously isolated from vineyards in British Columbia [6] and the isolates were selected for their ability to grow and sporulate. The addition of inoculum also ensured the presence of YVD pathogens that could infect grapevine cuttings. Single cultures of each isolate were incubated for one week (22 °C) using 5% potato dextrose agar (PDA) solution (autoclaved at 121 °C for 30 minutes). Cultures were propagated by cutting a 1 cm^2 slice of colonized agar and placing it upside down on new PDA until enough material was available for inoculation of all pots. Plates were examined under a compound light microscope to observe sporulation before inoculum preparation. A 10^6 conidia spore suspension was created for each isolate by flooding the agar plates with 1% tween solution and disturbing the surface with a metal utensil. The liquid was then passed through double-layer cheese cloth to form the stock solution. A hemocytometer was used to count conidia spores and make the specified concentration. Soil was inoculated on 24 April 2018 by pouring 45 mL of inoculum in a circle near the center of the pot.

2.6. Germination and Growth of Plants

Seeds were germinated in starter trays with an equal mixture of field soil and Sunshine Mix #4 (Sun Gro) peat/perlite mix (autoclaved at 121 °C, 1.5 hours) before transplantation into 3-liter pots on 27 April 2018. Due to the lower germination of native plants, pots were re-seeded following transplantation so that the number of plants in each pot was equal to four. Pots were watered by hand with no additional supplements and allowed to dry before subsequent watering. During the summer months, pots were watered more frequency to prevent drought and heat stress. Cultivar study plants were grown in the greenhouse until 30 July 2018, while native study plants were grown for an additional month until 3 September 2018 due to the perennial nature of the native plants. At harvest,

soil was removed from roots followed by a thorough rinsing to remove as much soil as possible. Plants were bagged and taken to University of British Columbia (UBC) Okanagan where they were dried and weighed.

Vitis vinifera (Chardonnay) cuttings were collected from SuRDC on 15 February 2018 (49°33′56.2″ N 119°37′46.7″ W) and placed in a cold storage room at 2 °C until propagation. Cuttings were taken out of cold storage in June 2018 and cut into smaller pieces containing two nodes (30 cm) with a pruning tool. Canes with visible signs of mold on the surface were discarded and the remaining canes were put in a plastic container filled to a 3-cm depth of water then placed in the experimental greenhouse until the appearance of roots. Chardonnay cuttings were transplanted on 31 July 2018 (cultivar study) and 1 August 2018 (native study). Cultivar study vines were grown for approximately four and a half months while native study vines were grown for seven months to maximize exposure to pathogens. During the first week any vines that died were removed.

Initially, grapevines were given 150 mL of Miracle Gro© (20-20-20) fertilizer on a weekly basis according to manufacturer's instructions. Nutrients were reduced to (15-15-18) after six weeks followed by dilutions to induce stressful conditions (Table A2). Cultivar study grapevines were harvested on 12 December 2018. At harvest, soil was removed from roots, followed by a thorough rinsing with reverse osmosis water. Samples were placed into paper bags and held at 4 °C until January 2019. Cuttings grown in soil conditioned by native cover crop treatments were left without fertilizer from 7 December 2018 to 6 January 2019 to further induce nutrient stress. On 8 January 2019, leaves were removed from each vine to further stress the plants and increase susceptibility to pathogens. Grapevine cuttings were removed from the greenhouse on February 12 and put into cold storage for two weeks until they were destructively harvested on 4 March 2019.

2.7. Incidence of Disease

To determine the extent of necrotic tissue in Chardonnay cuttings, a cross section was cut 1 cm from the basal end of the cane and placed on a scanner (Epson Expression 1680). Images were created with Adobe Photoshop© CS2 and analyzed with WinRhizo Pro (©2013) by defining color classes representing necrotic and healthy tissue. Percent necrosis was determined by dividing the area of necrotic tissue by the total analyzed area. For native treatments an additional measurement was performed. After imagery analysis, the progression of necrosis from the basal end to the top was determined by cutting the cane into 1-cm sections and looking for signs of necrotic tissue under a dissecting microscope (VWR Bioimager BRC-1600). Disease progression was rounded to the nearest centimeter.

2.8. Molecular Data

To determine the effects of cover crop diversity and provenance on the abundance of *I. liriodendri*, we assayed the abundance of DNA extracted from soil. Soil samples were also used to measure fungal community composition and species richness. Soil samples were taken from each nursery pot after inoculation with *I. liriodendri* before seeding with cover crops (starting soil), and again before removal of cover crops (conditioned soil). Root samples were collected after four and five months of growth for the cultivar study and native study experiments, respectively. We used a digital droplet (dd) PCR assay to observe changes in the abundance of *I. liriodendri* and Illumina sequencing of the internal transcribed spacer (ITS) 2 region to uncover fungal community composition.

2.9. DNA Extraction

On 26 April 2018, three rhizosphere core samples (1 cm diameter) totaling approximately 20 g were collected from the center of each pot at a depth of five centimeters. On 30 July 2018, another set of soil samples from cultivar treatments was collected before commercial cover crops were removed using the same method described above. Soil samples from native cover crop treatments were collected on

5 September 2018 before removal of cover crops. Soil cores were homogenized and kept at −20 °C at UBC Okanagan laboratories until DNA extraction.

Soil was dried at 60 °C for 24 hours to remove water from soil, allowing a higher DNA concentration during the final elution step [66,67]. Half a gram from each sample was used for DNA isolation. DNA was extracted using the FastDNA Spin Kit for Soil (MPBio ©2018, Irvine, CA, USA) according to the manufacturer's instructions. This resulted in approximately 90 µL of eluded DNA per sample, with an average concentration of 30 ng/µL (nanodrop 1000c ©2009, Thermo Fisher Scientific, Wilmington, NC, USA). DNA was stored at −80 °C until PCR and Illumina sequencing.

After surface sterilizing, 1 gram of root subsamples was placed in a 15-mL falcon tube and frozen at −20 °C until DNA extraction. Roots were then broken down in a mortar and ground up with liquid nitrogen until very small root fragments remained. Half a gram of ground-up roots was put into lysing tubes and the rest was put back into their original falcon tubes and frozen at −20 °C. DNA extractions performed using the FastDNA Spin Kit for Soil (MPBio ©2018) with a few modifications. Lysing was performed at an intensity of 6.5 m/s instead of the standard 6.0 m/s, and initial centrifugation was extended to 10 min to promote complete separation of root tissues and nucleic acids.

2.10. Droplet Digital Assay

In order to detect the *Ilyonectria* isolates used in the inoculum, a specific primer/probe assay that targets the beta-tubulin region was designed [68]. The primer, forward 5′-CGAGGGACATACTTGTTTCCAGAG-3′ (Tm 61, GC 60%), reverse 5′-TCAACGAGGTACGCGAAATC-3′-R (Tm 62, GC 50%), and probe TGTCAAACTCACACCACGTAGGCC amplify beta-tubulin, a highly conserved region and single-copy gene, making it ideal for the quantification or spores and/or septate hyphae in soil and roots.

Reactions consisted of 10 µL Supermix (Supermix for probes no dUTP by Bio-Rad Inc., Hercules, CA, USA), 7 µL DNAse free water, 1 µL primer/probe, and 2 µL DNA, for a total volume of 20 µL. Droplets were created using the Bio-Rad QX100 Droplet Generator using the total reaction volume per sample and 70 µL of Bio-Rad Droplet Generator Oil for Probes. PCR runs were completed in the C1000 Thermal Cycler (Bio-Rad) with the following conditions: initial heating at 95 °C for 10 min, 94 °C for 1 min, and annealing at 59 °C for 2 min × 44 cycles. Fluorescence was measured using the QX 100 Droplet Reader (Bio-Rad) and Quantalife software (version 1.7.4. Bio-Rad) by selecting FAM-HEX as the fluorescence setting. The threshold was set manually at 3000 using a pure positive and environmental positive controls as a reference. For analysis, the copy number of each sample was back calculated to represent the number of copies per gram of soil and root using a formula described in Kokkoris et al. (2019) [69].

2.11. Illumina Sequencing and Bioinformatics

Illumina sequencing was completed at the Centre for Comparative Genomics and Evolutionary Bioinformatics (Dalhousie University, Halifax Nova Scotia). Amplicon sequencing of the ITS2 sub-region was performed for each treatment (n = 5 for cover crops, n = 10 for starting soil) using primers ITS86F 5′-GTGAATCATCGAATCTTTGAA-3′ and ITS4R 5′-TCCTCCGCTTATTGATATGC-3′. Samples were demultiplexed, and barcodes were removed and returned as individual per-sample fastq files from the sequencing facility.

Initial quality control and amplicon filtering was performed using the Divisive Amplicon Denoising Algorithm (DADA2 package 1.12.1) in R statistical software (R version 3.6.1, 2019) by following the DADA2 ITS Pipeline Workflow 1.9 [70]. Primers, their reverse orientation, and complements were removed from reads using cutadapt (version 2.3). Sequence reads were filtered and trimmed using filterAndTrim (DADA2) by setting standard parameters (maxN = 0, truncQ = 2, rm.phix = TRUE, and maxEE = 2). Forward and reverse reads were dereplicated using derepFastq before applying the DADA algorithm [70]. Denoising was done by pooling samples (pool = TRUE). Sharing information across

samples makes it easier for singletons appearing multiple times across samples to be resolved. Paired reads were merged and an amplicon sequence variant (ASV) table was created. Finally, chimeras were removed using removeBimeraDenovo (method = "consensus") resulting in high-quality, filtered reads. The number of reads retained at each DADA2 step is shown in Table A3.

Beta diversity analyses were performed in QIIME2 (version 2019.10, https://qiime2.org) [71] and completed separately for native and cultivar studies. First, a phylogenetic tree was constructed using the q2-phylogeny plugin for QIIME2. To assign taxonomy, a reference classifier from UNITE (version 8.0) was used [72] and applied to the representative sequences from DADA2 (see above).

Native study samples were analyzed using the q2 diversity core-metrics-phylogenetic plugin. First, the ASV table containing all samples was filtered to contain only native samples. A sampling depth of 3316 was chosen based on sample B3-3 (silky lupine) because it excluded only three samples while maximizing the sampling depth. Weighted UniFrac dissimilarity [73] was used to create a distance matrix and beta diversity results were viewed via Principal Coordinates Analysis (PCoA).

Due to non-normal distribution of features and appearance of horseshoe distributions with Weighted UniFrac distance, beta diversity for cultivar study samples was performed using the DEICODE plugin (version 0.1.5) for QIIME2 [74], which creates a Robust Aitchison principal component analysis (PCA) distance matrix that handles sparse and/or non-normal datasets. A new ASV table with only cultivar samples was created. A sampling depth of 3146 was chosen, as it compromised sample exclusion and maximal sampling depth for beta diversity (see nonchim, Table A3). As with the native study, beta diversity results were viewed via PCoA.

2.12. Statistical Analyses

Data for root necrosis were transformed by taking the square root of (k-x), where k is the maximum value for percent necrotic tissue plus 1 and x is percent necrotic tissue for each sample. Disease progression of native study grapevines was normalized by taking the natural logarithm of 1+x, where x is the vertical progression of the disease, measured in centimeters.

For native study treatments, copy number per gram of root was square-root transformed to satisfy normality. After transformation, two outliers were removed from the copy number values before modelling and subsequent statistical analyses using Tukey's interquartile range (IQR). According to this method, values that are more or less than 1.5 times the IQR are removed. In the cultivar study, the copy number from root samples was cube-root transformed to meet normality assumptions. All statistical analyses were performed by fitting a linear mixed-effects model in R (R version 3.6.1, 2019, open source, http://www.r-project.org/) using the lme4 package (1.1.21). Normality was assessed using a Shapiro–Wilk normality test (stats package 3.6.1), and variance homoscedasticity was tested using Levene's test (car package 3.0.6). For each analysis, treatment was tested as a fixed factor and block as a random factor. Post hoc comparisons were completed using Tukey's honest significant difference test [75] within the emmeans package (1.4.1).

Alpha diversity of native and cultivar study samples was compared in QIIME2 with q2 diversity alpha-group-significance using Shannon evenness vectors from the q2 diversity core-metrics-phylogenetic. Overall and pairwise interactions were determined with the Kruskal–Wallis test by ranks [76] at a significance of 0.05. Beta diversity of native samples was determined via PERMANOVA in the q2 diversity beta-group-significance plugin using the Weighted UniFrac distance matrix created from the q2 diversity core-metrics-phylogenetic plugin, as it incorporates sequence abundance and phylogeny in community composition and distance between samples. Distances for native cover crop treatments were visualized using principal coordinate analysis (PCoA) with the q2-emperor plugin. For cultivar study samples, Robust Aitchison distance matrices were used from the DEICODE plugin to determine beta diversity (version 0.2.3). All PERMANOVA tests used 999 permutations and pseudo-F as the test statistic. Dispersion of native and cultivar study samples was determined with q2 diversity beta-group-significance by setting –p-method to permdisp. Dispersion tests were executed with 999 permutations and the F-value as the test statistic.

3. Results

3.1. Cover Crop Growth

Yarrow, bluebunch wheatgrass, and rockcress germinated after two to three weeks (Table A4). Silky lupine experienced lower germination rates likely due to lack of appropriate rhizobia and/or high temperatures. When grown separately, above- and below-ground biomass of bluebunch wheatgrass and white yarrow were similar to each other and significantly higher than silky lupine and rockcress. When all cover crops were grown together, above- and below-ground biomass was not different than bluebunch wheatgrass or yarrow. Cultivar monocultures varied in biomass. When grown separately, wheat yielded the highest root biomass, followed by buckwheat and clover. Crimson clover yielded the most biomass above ground followed by buckwheat then wheat. The lowest biomass measurements were observed for white mustard, in which below- and above-ground were significantly different from all other cover crop treatments. When cultivar crops were grown together, below-ground biomass was greater than all but wheat monocultures and the highest above-ground biomass.

3.2. Effect of Cover Crops on Incidence of Disease in Vines

Contrary to our hypothesis, grapevines grown in native study monocultures did not have higher rates of necrosis when compared to all plants growing together (Figure A1). Necrotic progression (evidence of necrosis from the basal to distal end) was near significant among monocultures ($p = 0.057$), with rockcress yielding the lowest average necrotic progression (Figure A2). Contrary to predictions, white mustard yielded the lowest percent necrotic tissue and was significantly different than fallow and crimson clover treatments in the cultivar study ($p = 0.035$), as seen in Figure 1. The lower necrotic damage found in grapevines growing in white mustard soil increased slightly when white mustard was grown with other cover crops.

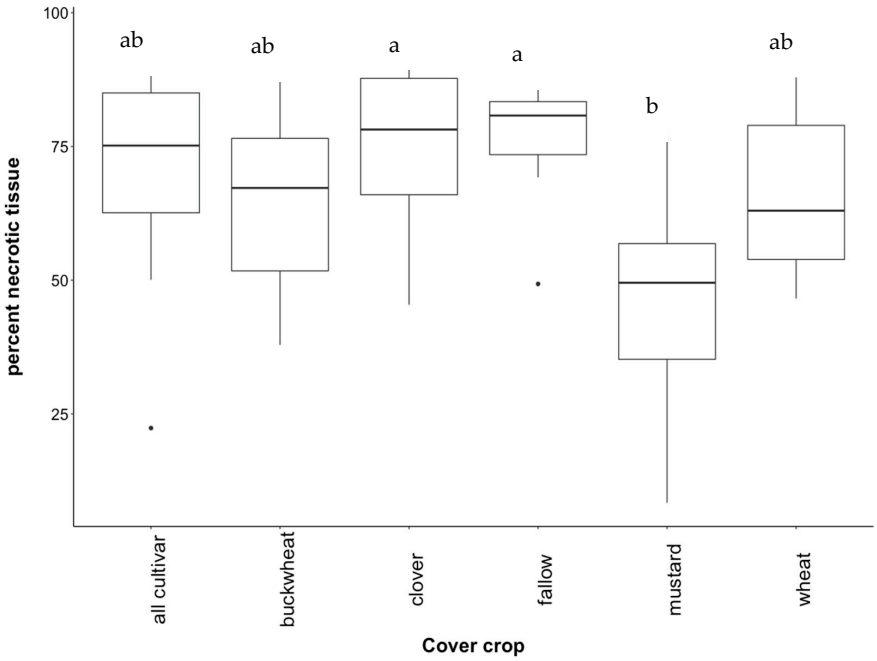

Figure 1. Percent necrotic tissue of grapevines growing in soil conditioned by cultivar cover crops. Treatments include a mixture of all plants ("all cultivar"), buckwheat ("buckwheat"), crimson clover ("clover"), uninoculated fallow ("fallow"), white mustard ("mustard"), and wheatgrass ("wheat"). Boxplots show the first and third quartile, median (middle line), range (whiskers), and circles (outliers). Letters represent statistical significance at $p < 0.05$. This section may be divided by subheadings and should provide a concise and precise depiction of the experimental results, their interpretation, and the experimental conclusions that can be drawn.

3.3. Recovery of Ilyonectria from Soil

In the native study, *I. liriodendri* was recovered from all treatments; however, its abundance varied highly between samples. Contrary to predictions, there was no significant variation between individual cover crops and when plants were grown together (Figure A3). Abundance of *I. liriodendri* was lowest in white yarrow soil while bluebunch wheatgrass yielded the highest abundance (Figure A3). In the cultivar study, abundance of *Ilyonectria* did not vary significantly between cover crop treatments (Figure 2) except for fallow, which was expected ($p < 0.001$). White mustard yielded an average of 1326 copies of *I. liriodendri* target DNA per gram of soil, the highest average copy number of all treatments, which was inconsistent with percent necrotic tissue.

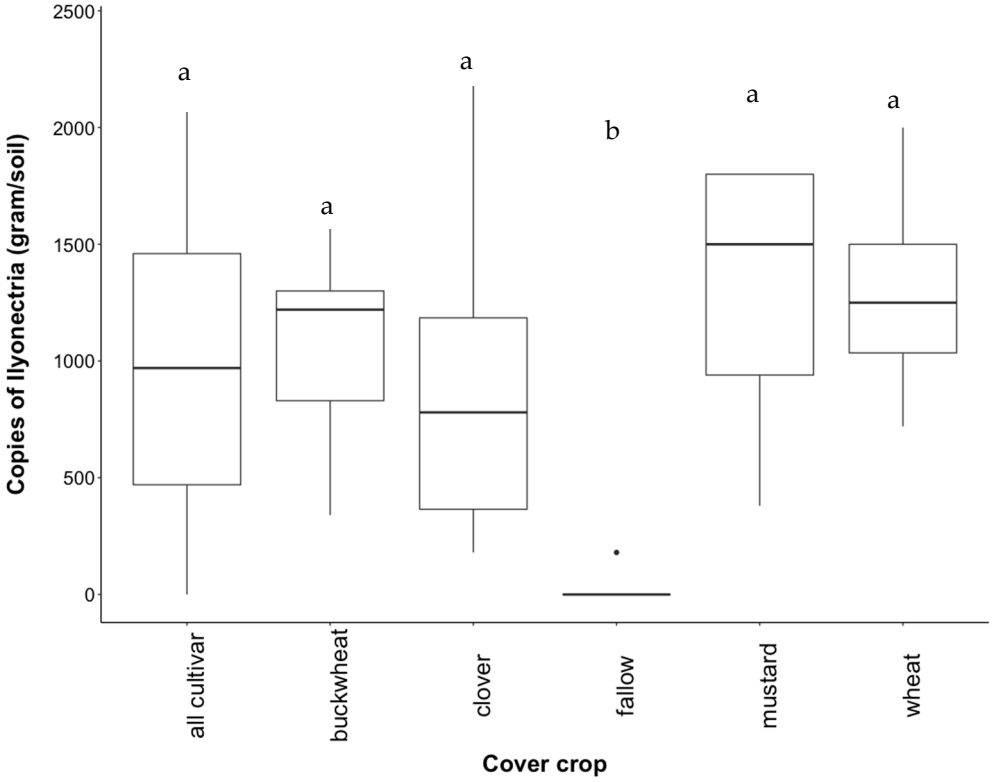

Figure 2. Recovery of *I. liriodendri* DNA from soil conditioned by cultivar cover crops. Treatments are a mixture of all plants ("all cultivar"), buckwheat ("buckwheat"), crimson clover ("clover"), uninoculated fallow ("fallow"), white mustard ("mustard"), and wheatgrass ("wheat"). Letters above boxplots represent statistical significance at $p < 0.05$.

3.4. Recovery of Ilyonectria from Roots

Contrary to predictions, *I. liriodendri* abundance did not change significantly in the native study (Figure A4). *Ilyonectria* abundance from grapevine roots was extremely variable in monocultures, with silky lupine displaying the most variability. Roots from rockcress and bluebunch wheatgrass showed the highest abundance of *I. liriodendri*, followed by white yarrow and all native (Figure A4).

Contrary to our hypothesis, abundance of *I. liriodendri* did not decrease when cultivar plants were grown together (Figure 3). Abundance of *I. liriodendri* was lowest in white mustard roots, which was consistent with the lower necrotic damage observed in grapevine cross sections from the same treatment. Abundance of *I. liriodendri* in white mustard was significantly lower compared to wheatgrass ($p = 0.041$) (Figure 3). Consistent with the digital PCR results from soil samples, roots from uninoculated fallow treatment had either zero or very small copy numbers of target DNA.

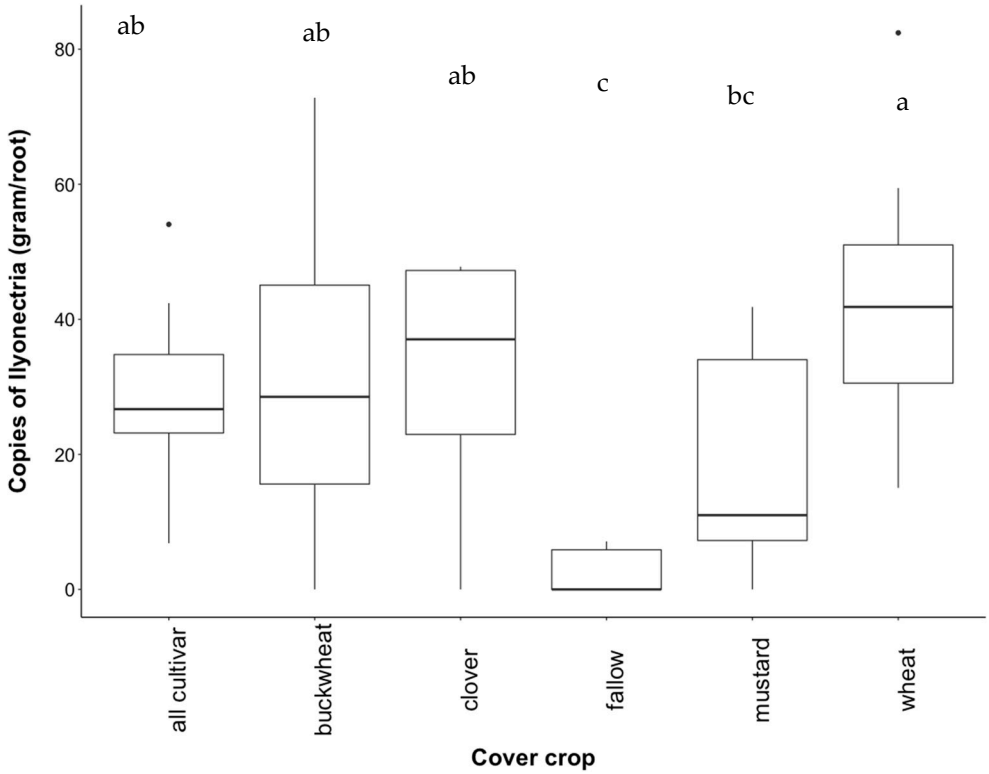

Figure 3. Abundance of *I. liriodendri* DNA from the cultivar study Chardonnay roots. The cube root of copy number was taken to normalize data. Treatments are a mixture of all plants ("all cultivar"), buckwheat ("buckwheat"), crimson clover ("clover"), uninoculated fallow ("fallow"), white mustard ("mustard"), and wheatgrass ("wheat"). Letters above boxplots represent significant differences at $p < 0.05$.

3.5. Sequence Results

A total of 2089 amplicon sequence variants (unique DNA sequences) with a combined frequency of 875,526 were present from the 111 soil samples after initial denoising and filtering. The minimum feature count per sample was 769 (bluebunch wheatgrass), while the maximum was 17,904 (soil before cover crop conditioning). The highest feature occurrence was 170,112 across all 111 samples while eight features occurred only once (0.004% of all features). A total of six phyla (one unidentified), 19 classes, 40 orders, 68 families, and 76 genera were recovered from all soil samples (Figures A5 and A6). Ascomycota yielded the highest relative frequency, followed by Basidiomycota, Mortierellomycota, and Chytridiomycota, which were present in all 111 samples. Glomeromycota was present in 78 samples, followed by an unidentified phylum that was observed in 94 samples.

3.6. Effect of Cover Crops on Fungal Diversity

3.6.1. Alpha Diversity

As predicted, alpha diversity of rhizosphere fungi increased with cover crop diversity in the native study. Silky lupine yielded the lowest fungal diversity followed by bluebunch wheatgrass. Fungal diversity was highest when all plants were grown together (Figure 4). Contrary to predictions, fungal diversity did not change with cultivar cover crops. Fungal communities were less diverse under crimson clover while buckwheat and wheatgrass were similar to the all species treatment. As expected, fallow soil contained the lowest diversity measurement, although no significant differences were detected between treatments (Figure A6).

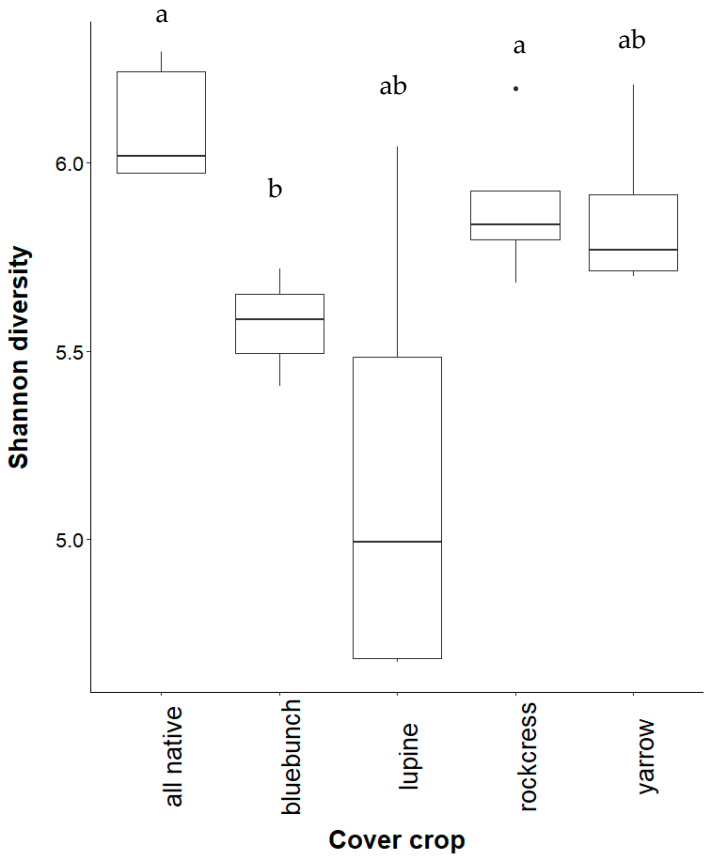

Figure 4. Shannon diversity of fungi in native study soils. Treatments are bluebunch wheatgrass ("bluebunch"), silky lupine ("lupine"), Holboell's rockcress ("rockcress"), and white yarrow ("yarrow"). Overall group significance was observed in monocultures ($p = 0.047$). Letters over treatments indicate pairwise differences at a significance level of 0.05.

3.6.2. Beta Diversity in Native and Cultivar Studies

Contrary to predictions, fungal community composition was similar among most native monocultures and when all plants were grown together ($p = 0.051$). However, community composition under bluebunch wheatgrass was distinct from white yarrow ($p = 0.036$) (Figure 5). Likewise, fungal community composition under silky lupine was different from white yarrow ($p = 0.056$). Dispersion of fungal communities (clustering) was similar under native monocultures ($p = 0.881$).

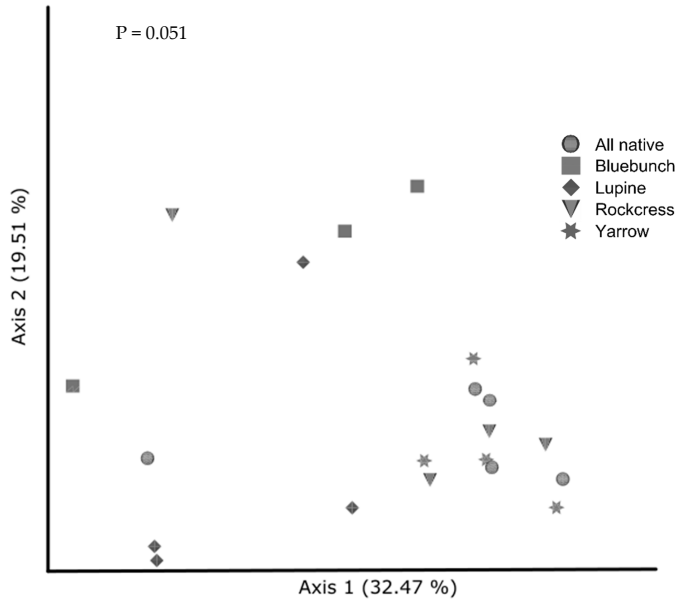

Figure 5. Principal coordinates analysis of fungal communities from native study cover crops visualized by Weighted UniFrac distance. Treatments are all species together ("All native"), bluebunch wheatgrass ("Bluebunch"), Silky lupine ("Lupine"), Holboell's rockcress ("Rockcress"), and white yarrow ("Yarrow"). Fungal communities show no significant clustering overall ($p = 0.051$); however, bluebunch wheatgrass (squares) and white yarrow (stars) reveal differences in beta diversity ($p = 0.036$, $q = 0.280$).

In the cultivar study, cover crop diversity changed community composition only in some treatments (Figure 6). All cultivar communities were distinct from fallow ($p = 0.005$) and wheatgrass ($p = 0.017$) but not others. Overall dispersion of fungal communities from monocultures was similar ($p = 0.183$).

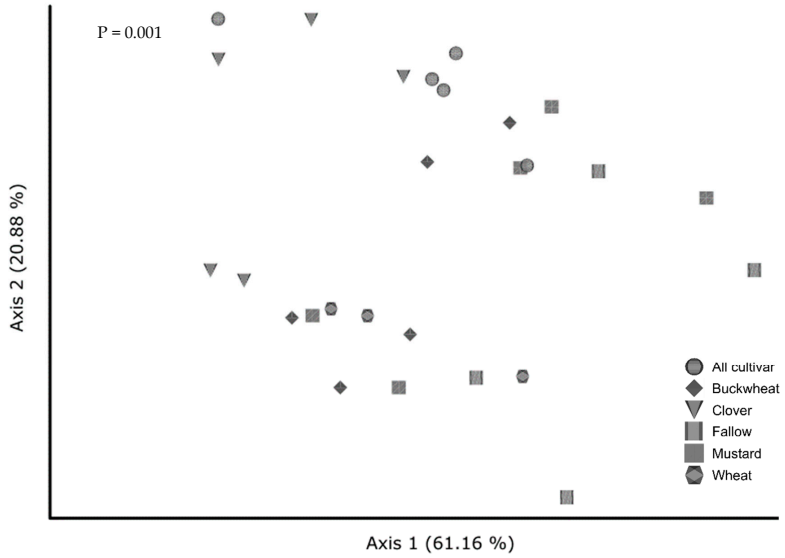

Figure 6. Principal coordinates analysis of fungal communities from cultivar cover crops visualized by Robust Aitchison distance. Treatments are all plants grown together ("All cultivar"), buckwheat ("buckwheat"), crimson clover ("Clover"), uninoculated fallow ("Fallow"), white mustard ("Mustard"), and wheatgrass ("Wheat")m with the following significant pairwise interactions: All cultivar and Fallow ($p = 0.005$, $q = 0.045$), All cultivar and wheatgrass ($p = 0.017$, $q = 0.084$), Clover and Fallow ($p = 0.006$, $q = 0.045$), clover and mustard ($p = 0.025$, $q = 0.084$), clover and wheatgrass ($p = 0.037$, $q = 0.092$).

4. Discussion

4.1. Effect of Cover Crop Diversity on Root Necrosis

Contrary to our hypothesis, cover crop diversity was not associated with necrotic root symptoms in self-rooted Chardonnay grapevines. This was true in both the native and cultivar study. One possible explanation is that the biotic properties of the soil did not change enough due to the short soil conditioning phase by cover crops. In these studies, cover crops were grown for three to four months, which translates to approximately half a growing season in the Okanagan Valley [77]. Since plant–soil feedback is not instantaneous [78], perhaps more time was needed to develop beneficial and/or antagonistic microbial communities, leading to a delay in their suppressive effects. Eisenhauer et al. (2012) [79] found that benefits from soil biota were more pronounced in long term grassland studies (four years) due to successional changes in soil microbial communities. Vogel et al. (2019) [80] further elucidated the effect of time on plant–soil feedback by showing that microbial biomass was greater in soil with a 14-year conditioning period by a specific plant community compared to new soil conditioned by the same plant community for only one year.

Another explanation could be the lack of cover crop incorporation into the soil, resulting in very little competition from other saprophytic fungi. Decomposers represent a significant group among soil microbial life and contribute to multiple ecosystem services [81]. In this experiment, we did not incorporate any litter at harvest. Instead, all cover crop material was removed including roots, which would have limited decomposer communities [82]. Most importantly, litter can contain symbiotic plant endophytes including *Trichoderma*, which are present during active plant growth and are also known to decompose litter [83,84]. The presence of *Trichoderma* could reduce GTD pathogens if they were surviving as saprophytes in plant litter, although further research is needed to determine whether stimulations of decomposer communities can reduce GTD pathogens.

In this study, the basal end of each cane was not covered with wax or another barrier, leaving a large area of vascular tissue exposed to pathogens. Such a large amount of exposed tissue in the soil would have facilitated infection even in the presence of antifungal exudates or antagonistic microbes, as below-ground wounds can serve as entry points in grapevines [85]. This is especially the case in pathogen transfer above ground in which pruning wounds left uncovered act as entry points for airborne spores [5,86].

White mustard, when grown as a monoculture, was the only cover crop that reduced necrotic tissue damage in grapevine roots. This plant matures quickly, is a high-biomass crop [87], and is known for its production of sulfur-containing glucosinolates including glucoerucin and glucoiberverin [88]. White mustard products have previously been associated with the suppression of grapevine and tree fruit pathogens [89–91]. The antifungal chemicals produced by white mustard are known to inhibit spore germination [92] and mycelial growth [93], which may have resulted in the lower incidence of necrosis observed. These results align with previous biofumigant studies that implement brassicaceous cover crops and their products [31,89,94,95].

In contrast, when white mustard was grown with other cover crops, necrotic damage was not reduced. Since each pot was standardized to four plants, only two white mustard plants grew in the soil, which would have reduced glucosinolate production. This likely reduced the concentration of antifungal compounds in the soil, allowing pathogens to proliferate more easily.

In the native study, Holboell's rockcress (a brassicaceous plant) was not associated with lower percentages of necrotic tissue. While the suppressive potential of Holboell's rockcress has not been studied in an agricultural setting, the plant matures slower due to its perennial nature, and has many natural predators including fungi [96]. Although rockcress did not show any signs of suppression in this short-term study, its persistence over multiple growing seasons and/or its degradation after maturity may contribute to the mitigation of soil-borne pathogens in vineyards.

4.2. Effect of Cover Crop Diversity on Abundance of Ilyonectria

In both studies, cover crops did not correlate with the abundance of *I. liriodendri* in the soil when grown by themselves or when grown together. This could partially be due to the absence of roots in the first centimeters of soil, where samples were taken. Overtime, the initial concentration of 1×10^6 conidia per milliliter would have diffused as pots were watered, causing spores to travel to deeper depths in the pot. Since the majority of root biomass was found below five centimeters, any effect of root exudation would have been more noticeable at lower soil depths but limited on the surface.

Consistent with percent necrotic tissue, abundance of *I. liriodendri* was lower in the white mustard monocultures. At the time of harvest, white mustard cover crops had gone to seed and had started to senesce, a period in which the metabolism of glucosinolates into antifungal isothiocyanates occurs. The breakdown and release of isothiocyanates from white mustard perhaps inhibited spore germination, reducing available inoculum during the grapevine growth stage. Antifungal compounds from brassicaceous crops can stay active for a period of 25–30 days [93,97] before they start to break down. Suppressive effects of white mustard may have been more pronounced had the plant been left to decompose in the soil [93,95,98].

In this study, *Ilyonectria* abundance in white mustard treatments was significantly lower than wheatgrass. Wheat is used in vineyards to manage soil erosion, prevent frost damage, and build organic matter [99]; however, wheat and other plants growing in a vineyard may act as off-target hosts, as has been observed in South African nurseries [48] and in Spanish vineyards [100]. In these studies, we did not examine cover crop roots for pathogens; however, it is possible that some acted as off-target hosts [48,100]. If cover crops can be colonized by *I. liriodendri* and/or other pathogens, this could maintain the spore bank and allow them to persist in soils, increasing the risk of infection. Creating a suppressive environment may require more than cover crop implementation. Changes to nutrient and watering regimes, pruning time [101], or inoculation with beneficial microbes and nearby soil may also reduce pathogens [102,103]. Indeed, there is a diverse array of fungal pathogens that infect grapevine tissues at various growth stages, which means further research is required to elucidate whether particular combinations of cover crops and pathogenic fungi can be problematic in vineyards and nurseries.

4.3. Effect of Cover Crop Diversity on Fungal Diversity

Alpha diversity of rhizosphere fungi increased with cover crop diversity in the native study but not cultivar study. The fact that microbial diversity changed under native but not cultivar cover crops perhaps implies that native plants are more dependent on resident fungi, and specifically mycorrhizal fungi, compared to plants introduced [104,105] through coevolutionary mechanisms [106]. Alternatively, carbon inputs and exudation of cultivar crops could have promoted specific fungi though positive plant–soil feedback, limiting diversity [107,108]. Since mycorrhizal fungi and resident bacteria can heavily influence functional traits—including nitrogen content, stress tolerance, morphology, leaf longevity, and pathogen resistance [109]—the presence of native plants may have stimulated these communities more than the cultivar varieties in order to maximize their fitness. Indeed, Klironomos (2003) [110] found that the frequency of positive responses from foreign plants was reduced when paired with resident AM fungi compared to the more-even distribution of responses observed when resident AM fungi and plants were paired. Alternatively, fungal diversity in the cultivar study may have been limited because the introduced plants increased the abundance of specific fungi. This has been observed in invasion studies in which the invasive plant experiences positive plant–soil feedback that allows it to outcompete native plants [107,108].

It is also possible that fungal diversity changed more under native cover crops due to a longer conditioning phase. Soil was conditioned by cultivar plants for three months whereas native plants were given four, allowing an additional month for fungi to respond to exudation, rhizodeposition [111], and root turnover [112]. In addition, root exudates and carbon deposits change as plants develop, which affects microbial communities [113]. The fact that cultivar plants matured quickly in our study

perhaps led to microbial turnover whilst inputs from native plants were more consistent, allowing communities to develop overtime.

Since soil fungi are saprophytic, diversity may have increased if cover crops were left to decompose [60]. This likely would have result in compositional differences in fungal communities, as decomposers are strongly affected by plant litter type [114]. However, despite the increase in fungal diversity in cover crop mixtures and when all plants were grown together, fungal diversity was not associated with incidence of root necrosis or abundance of *I. liriodendri* in soil or roots.

Regarding pairwise interactions between treatments, alpha diversity was significantly higher under rockcress compared to bluebunch wheatgrass. Brassicaceous crops can inhibit fungal activity due to hydrolysis products of the glucosinolates they produce [115]; however, this is often limited to fungal pathogens [97], and is not widely observed in symbiotic fungi [116,117]. The fact that fungal diversity under rockcress was comparable to that of white yarrow and all plants combined suggests that rockcress did not inhibit fungi as much as other brassicaceous crops. However, alpha diversity under white mustard was also similar to other cover crops, meaning factors other than glucosinolate content contributed to alpha diversity.

4.4. Effect of Cover Crops on Community Composition

In the native study, fungal communities were dissimilar only for bluebunch wheatgrass and yarrow. Historically, white yarrow has been used as a traditional medicine in many cultures because of the phenolic compounds it produces [118] and because its extracts are known to suppress the in vitro growth of pathogenic bacteria and fungi [119]. On the contrary, bluebunch wheatgrass is not as widespread as yarrow [120], and its competitiveness is more dependent on rhizosphere microbes [121]. Given the different life history strategies employed by these plants, it is not surprising that their soil microbial communities differ.

In the cultivar study, some cover crops appeared to be more influential than others. For example, fungal communities in clover soil were distinct from those in white mustard, wheatgrass, buckwheat, and fallow soil, but not when all plants were grown together. Crimson clover produced highly branched root systems with the most above-ground biomasses out of all cover crops. Legumes are known for their mycorrhizal attributes [122], and have previously been associated with increases in fungal diversity [123], abundance of AM fungi [124], and saprophytic fungi [125]. White mustard, on the other hand, typically reduces the abundance of soil fungi relative to controls [126], although in this experiment the community composition under mustard was similar to fallow, buckwheat, wheat, and all plants grown together. At the same time, this treatment was associated with a lower incidence of root necrosis, which suggests it reduced the overall abundance of fungi associated with disease [93,97].

5. Conclusions

After a short conditioning period, we found that cover crop diversity was not associated with incidence of disease in grapevine roots. Incidence of disease was instead associated with white mustard, a common brassica cover crop. Although this apparent biofumigant effect was not observed in Holboell's rockcress (the native brassica), the results from the cultivar study align with the biofumigant literature of white mustard and other brassicaceous crops. Consistent with necrotic tissue damage, we found that white mustard was associated with a lower abundance of *I. liriodendri* in the roots of Chardonnay cuttings. However, this effect was reduced when white mustard was paired with other cover crops, and was not observed in any other monoculture.

Cover crop diversity increased fungal diversity, but only in the native study. Fungal diversity was higher in cultivar cover crops compared to fallow soil; however, there was no additive effect when all cover crops were grown together. Although not observed in this study, cover crop diversity could play a major role in the long term, especially if more diverse plant communities support diverse microbes with suppressive properties.

In summary, cover crop identity was more important than diversity for controlling fungal pathogens in grapevines. Results from the cultivar study align with other literature, which highlights the suppressive effect of brassicas. We found that when grown from seed, a brassica cover crop could offer traditional benefits such as erosion control or weed suppression as well as partially suppressing soil-borne fungi.

These results provide evidence that disease symptoms and pathogen abundance can be reduced by growing a cover crop that produces antifungal compounds. While seeding multiple cover crops confers a wide range of benefits, certain cover crops may act as vectors for fungal pathogens, thus maintaining the inoculum load. To further unveil how fungal pathogens persist in vineyard soils, future studies should focus on whether native or commercial cover crops act as vectors for GTD pathogens.

Author Contributions: Conceptualization, A.R., M.H., and J.R.Ú.-T.; methodology, A.R.; validation, P.B., T.L., and J.R.Ú.-T.; formal analysis, A.R. and M.E.; writing—original draft preparation, A.R.; writing—review and editing, M.H. and A.R.; supervision, M.H.; funding acquisition, M.H. and J.R.Ú.-T. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors of this paper declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A

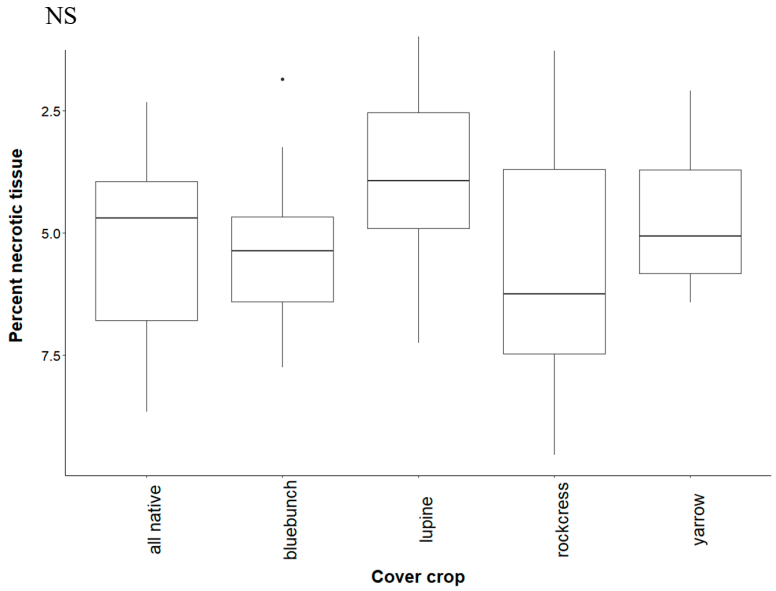

Figure A1. Percent necrotic tissue of grapevines grown in soil conditioned by native cover crops. The extent of necrosis is measured from the basal end up. Treatments are all plants grown together ("all native"), bluebunch wheatgrass ("bluebunch"), silky lupine ("lupine"), Holboell's rockcress ("rockcress"), and white yarrow ("yarrow"). Boxplots show the first and third quartile, median (middle line), range (whiskers), and circles (outliers). Data were normalized by taking the square root of the reciprocal (100.975 – x) where x is the value for percent necrosis. There was no significant difference (NS) between cover crop treatments ($p = 0.407$).

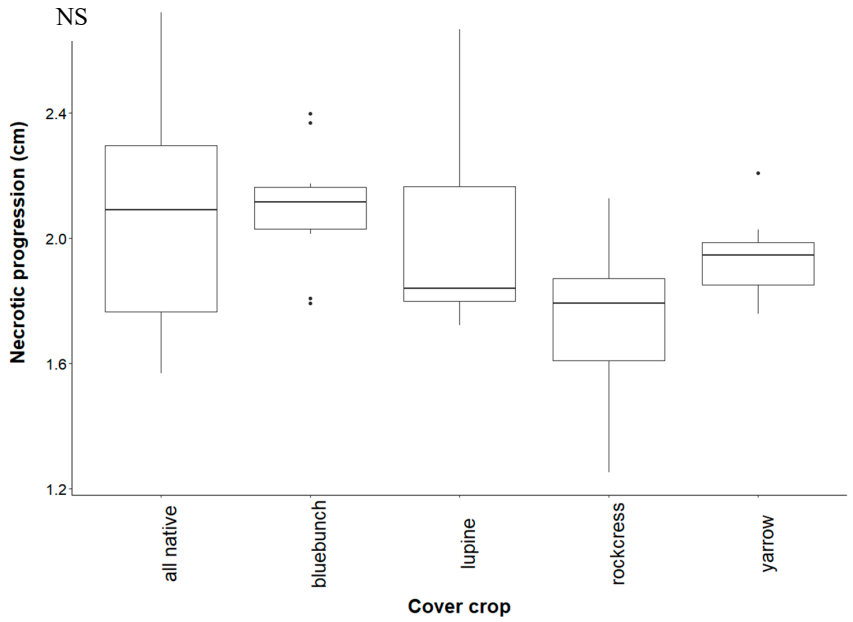

Figure A2. Progression of necrotic damage in grapevines grown in soil conditioned by native cover crops. The extent of necrosis is measured from the basal end. Treatments are all plants grown together ("all native"), bluebunch wheatgrass ("bluebunch"), silky lupine ("lupine"), Holboell's rockcress ("rockcress"), and white yarrow ("yarrow"). Boxplots show the first and third quartile, median (middle line), range (whiskers), and circles (outliers). Data were normalized by taking the natural logarithm plus 1 (log1p) of necrotic progression values. There was no significant difference (NS) between cover crop treatments.

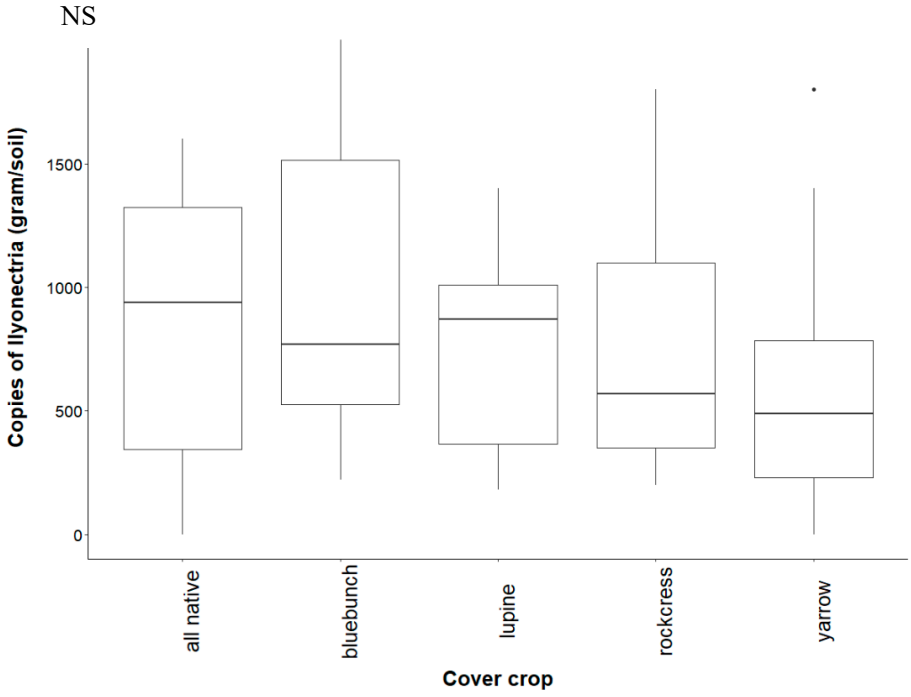

Figure A3. Recovery of *I. liriodendri* DNA from soil after conditioning with native cover crops. Treatments are all plants grown together ("all native"), bluebunch wheatgrass ("bluebunch"), silky lupine ("lupine"), Holboell's rockcress ("rockcress"), and white yarrow ("yarrow"). Copy number did not differ significantly (NS) among treatments ($p = 0.731$).

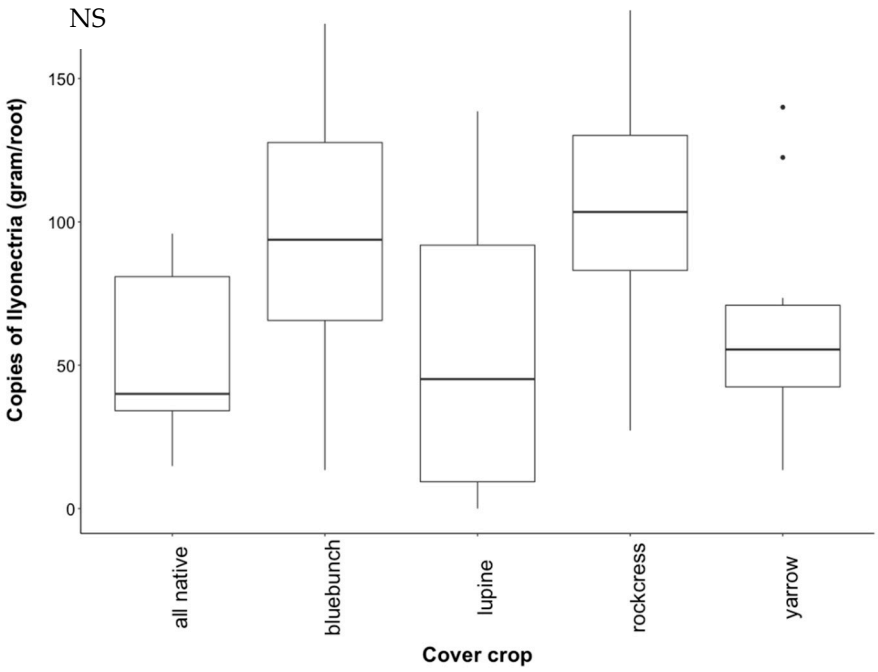

Figure A4. Recovery of *I. liriodendri* DNA from native study Chardonnay roots. Treatments are all plants grown together ("all native"), bluebunch wheatgrass ("bluebunch"), silky lupine ("lupine"), Holboell's rockcress ("rockcress"), and white yarrow ("yarrow"). Data were normalized by taking the square root of the copy number then removing outliers using Tukey's interquartile range (IQR). Copy number did not differ significantly (NS) between cover crop treatments ($p = 0.109$).

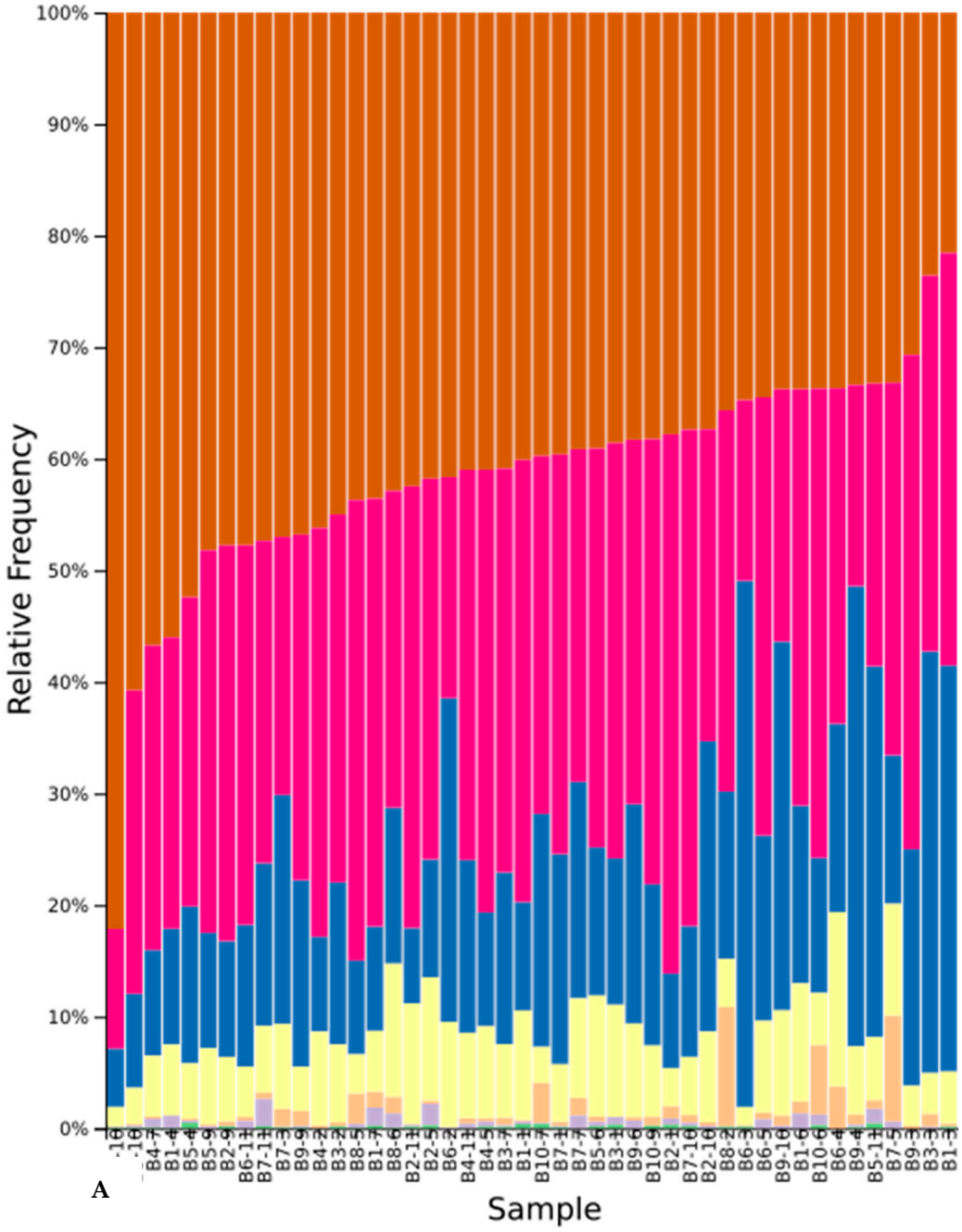

Figure A5. *Cont.*

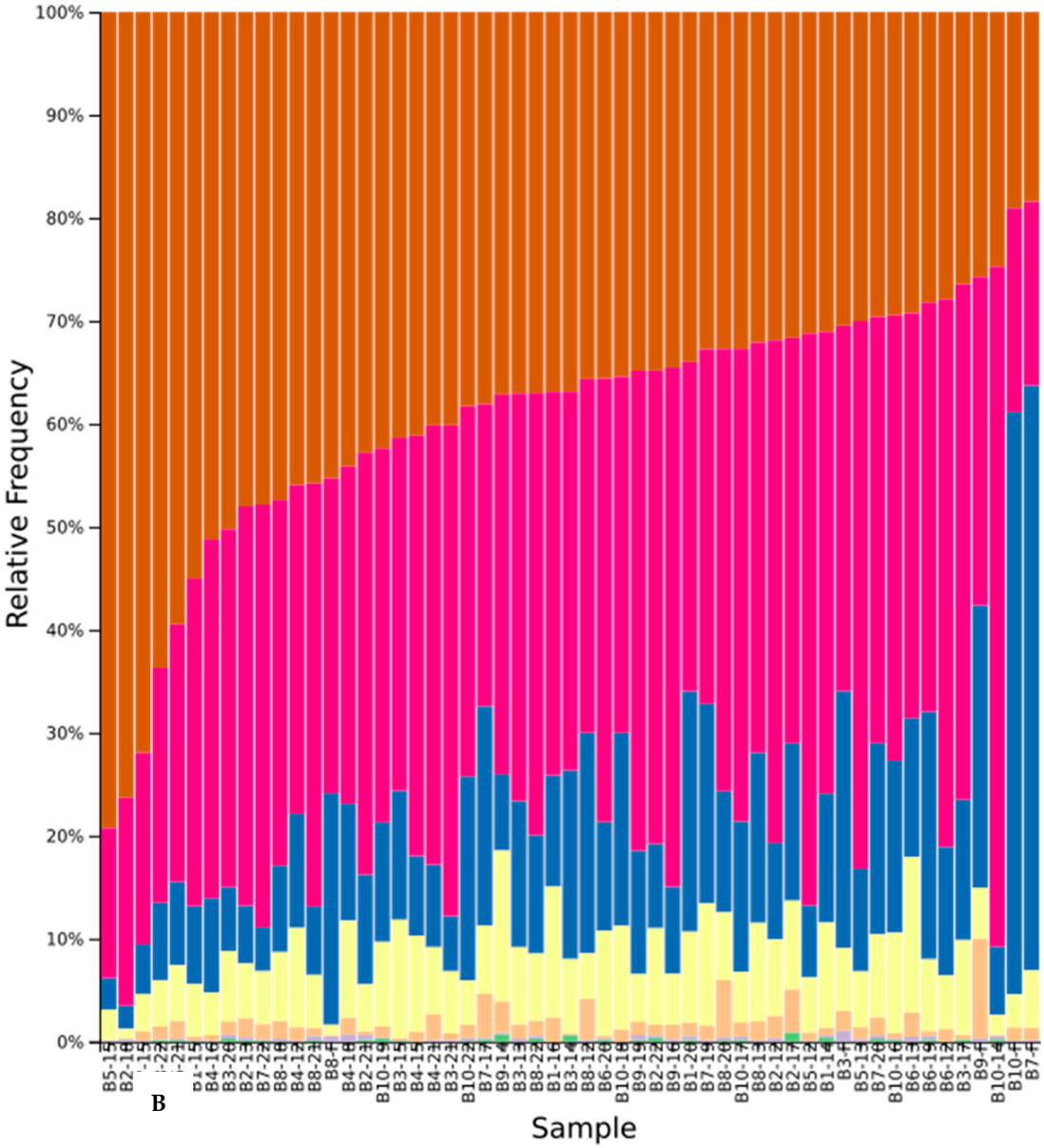

Figure A5. Relative abundance of phyla across native study samples (**A**) and cultivar study samples (**B**) after initial denoising and filtering. Samples are shown with block number followed by treatment number. Numbers 1–4 are white yarrow, Holboell's rockcress, silky lupine, and bluebunch wheatgrass, respectively while 11 is all native. Numbers 12–15 are white mustard, buckwheat, wheatgrass, and crimson clover, respectively, while 22 and 23 are all cultivar and fallow, respectively. Phyla are Ascomycota (brown), Basidiomycota (pink), Mortierellomycota (yellow), Chytridiomycota (orange), Glomeromycota (purple), and unidentified (blue and green).

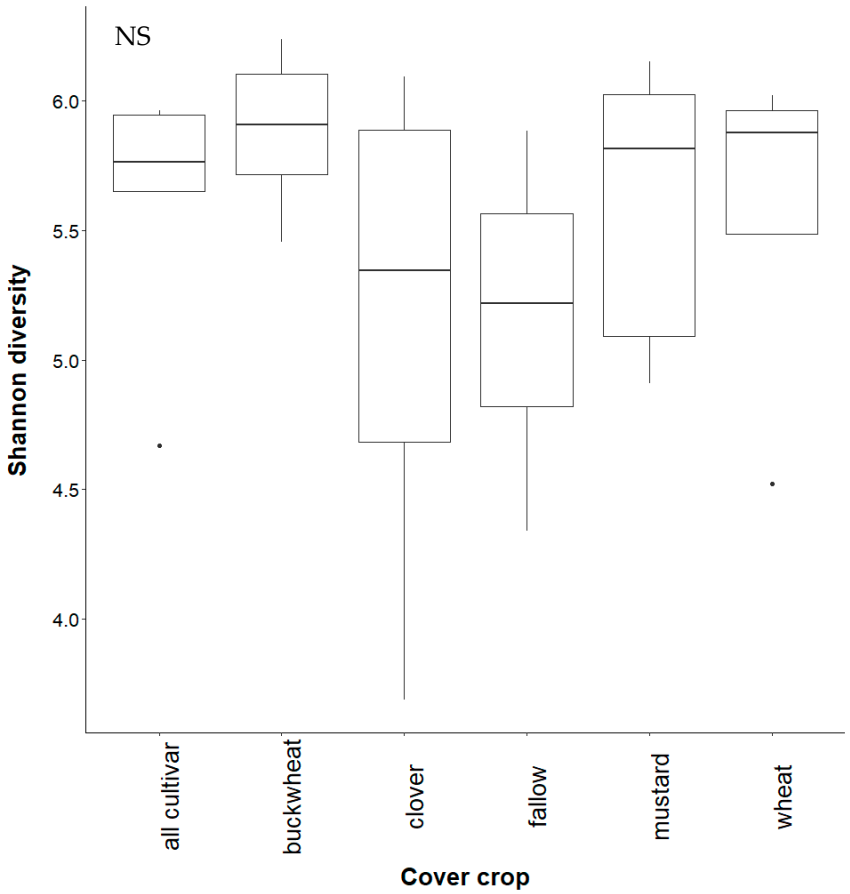

Figure A6. Shannon diversity of fungi in cultivar study soil. Treatments are all plants ("all cultivar"), buckwheat ("buckwheat"), crimson clover ("clover"), uninoculated fallow ("fallow"), white mustard ("mustard"), and wheatgrass ("wheat"). Fungal diversity did not vary significantly (NS) between treatments ($p = 0.531$).

Table A1. Site information and soil physiochemical properties adopted from Watson et al. (2018) [65].

Site Properties	Response
Fruit tree	Sweet cherry
Soil texture	Loamy sand
pH	6.9
Organic matter (%)	2.3
C/N ratio	8.5
Phosphorous (ppm)	66
Potassium (ppm)	360
Magnesium (ppm)	170
Calcium (ppm)	1330
Sodium (ppm)	25
Aluminum (ppm)	13
Sulfur (ppm)	9
Nitrate (ppm)	23

Table A2. Fertilizer application for *Vitis vinifera* cuttings in native and cultivar studies. Miracle-Gro fertilizer was used to prepare solutions of varying concentrations. Enough fertilizer was applied to cover the soil and soak through.

Date	Fertilizer Type	Amount Applied	Dilution
October 11 2018	15-15-18	150 mL	50%
October 25 2018	15-15-18	150 mL	No
November 1 2018	15-15-18	150 mL	50%
November 22 2018	15-15-18	150 mL	33%
November 29 2018	15-15-18	150 mL	40%
December 7 2018	15-15-18	150 mL	40%

Table A3. Number of reads retained at each step in the DADA2 pipeline. Samples are shown with the block number first followed by the treatment number separated by a hyphen. Numbers 1–4 are white yarrow, Holboell's rockcress, silky lupine, and bluebunch wheatgrass, respectively, while 11 is all native. Numbers 12–15 are white mustard, buckwheat, wheatgrass, and crimson clover, respectively, while 22 and 23 are all cultivar and fallow, respectively. From left to right are the initial read counts (input), reads after filtering (filtered), forward reads after denoising (denoisedF), reverse reads after denoising (denoisedR), number of reads after merging (merged), and number of reads after chimera removal (nonchim). Denoising and filtering were completed with R statistical software.

Sample	Input	filtered	DenoisedF	DenoisedR	Merged	Nonchim
B1-1	12,251	6665	6644	6644	6184	6176
B1-10	14,589	8155	8133	8127	7909	7907
B1-14	14,193	8257	8222	8183	7307	7290
B1-15	16,920	9042	9020	9013	8424	8409
B1-16	16,354	8844	8808	8789	8057	8053
B1-20	21,936	9842	9807	9799	9040	9028
B1-3	9439	4599	4589	4590	4276	4268
B1-4	13,175	6559	6524	6520	6091	6086
B1-6	10,873	6568	6514	6530	5964	5962
B1-7	10,950	6140	6113	6094	5469	5451
B10-14	6011	3446	3429	3431	3150	3146
B10-16	13,831	7968	7941	7918	7248	7233
B10-17	10,982	4927	4898	4896	4548	4506
B10-18	10,059	5622	5605	5607	5053	5029
B10-19	12,876	7437	7419	7397	6604	6579
B10-22	21,342	11,584	11,551	11,564	10,118	10,041
B10-6	29,911	15,434	15,381	15,404	14,299	14,200
B10-7	8427	4811	4787	4784	4364	4352
B10-9	13,366	6560	6530	6534	5964	5957
B10-fal	24,131	12,374	12,348	12,349	11,735	11,671
B2-1	12,412	6563	6522	6512	6117	6079
B2-10	19,272	11,002	10,973	10,965	10,166	10,161
B2-11	8626	4766	4754	4751	4461	4400
B2-12	17,980	9804	9768	9770	8801	8729
B2-13	10,050	5568	5552	5539	5178	5158
B2-17	18,591	10,111	10,080	10,067	8978	8935
B2-18	6488	3754	3734	3735	3644	3640
B2-21	20,710	10,897	10,864	10,863	10,170	10,086
B2-22	20,429	10,227	10,190	10,178	9386	9305
B2-5	12,175	7021	6998	6981	6355	6338
B2-9	15,943	8738	8696	8686	8171	8105
B3-1	14,605	8041	8015	8012	7281	7259
B3-13	8386	4782	4765	4758	4303	4296

Table A3. *Cont.*

Sample	Input	filtered	DenoisedF	DenoisedR	Merged	Nonchim
B3-14	17,549	7704	7666	7648	7118	7107
B3-15	13,922	5942	5919	5924	5398	5398
B3-17	7866	4491	4473	4469	4090	4069
B3-2	22,018	11,332	11,274	11,270	10,318	10,247
B3-20	19,794	10,473	10,436	10,427	9681	9618
B3-21	24,717	12,958	12,908	12,923	12,068	11,915
B3-22	19,204	10,218	10,182	10,191	9640	9581
B3-3	6693	3643	3631	3622	3322	3316
B3-7	20,743	10,079	10,010	10,007	9315	9237
B3-fal	18,855	10,320	10,296	10,251	9587	9503
B4-11	10,411	6031	6007	5994	5049	5049
B4-12	14,153	7003	6958	6956	6417	6403
B4-15	16,611	8708	8658	8662	7915	7840
B4-16	19,340	10,285	10,240	10,241	9650	9558
B4-18	8515	5131	5124	5122	4708	4697
B4-2	25,973	13,688	13,617	13,625	12,315	12,203
B4-21	23,882	12,563	12,498	12,473	11,638	11,530
B4-5	23,443	12,027	11,955	11,961	11,003	10,927
B4-7	20,209	10,317	10,270	10,265	9600	9531
B5-10	12,457	6726	6689	6707	6415	6378
B5-11	35,399	18,215	18,142	18,138	16,671	16,523
B5-12	24,203	13,410	13,350	13,349	12,385	12,216
B5-13	23,829	12,481	12,429	12,414	11,449	11,327
B5-15	29,538	17,066	17,035	17,022	16,486	16,416
B5-4	23,032	9630	9594	9580	8953	8943
B5-6	7570	3983	3967	3965	3565	3562
B5-9	11,398	4582	4558	4557	4269	4268
B6-11	11,204	6595	6572	6555	6071	6052
B6-12	8155	4512	4484	4491	4028	4021
B6-13	10,779	5783	5755	5744	5194	5178
B6-19	29,866	16,427	16,367	16,360	15,012	14,867
B6-2	17,688	9582	9411	9366	8683	8677
B6-20	8519	4795	4783	4782	4331	4317
B6-21	22,819	12,354	12,305	12,295	11,632	11,567
B6-3	5341	2866	2855	2851	2574	2573
B6-4	6254	847	832	835	771	769
B6-5	5377	2209	2198	2187	2000	1995
B7-1	24,124	12,565	12,508	12,504	11,635	11,547
B7-10	25,220	13,851	13,789	13,798	13,062	12,847
B7-11	9586	5557	5530	5516	5131	5111
B7-15	26,469	14,189	14,156	14,150	13,580	13,489
B7-17	16,917	8897	8856	8842	8204	8161
B7-19	13,208	8050	7998	7990	7187	7167
B7-20	10,609	6169	6151	6140	5595	5589
B7-22	29,654	16,540	16,492	16,503	15,391	15,259
B7-3	19,382	10,255	10,220	10,216	9450	9450
B7-5	11,105	5371	5353	5342	4865	4863
B7-7	12,032	6861	6824	6819	6298	6291
B7-fal	24,746	13,168	13,147	13116	12,135	12,111
B8-12	20,947	10,878	10,833	10,825	9907	9825
B8-13	14,151	7813	7771	7781	7010	7009
B8-18	13,899	6928	6888	6886	6373	6342
B8-2	26,482	14,400	14,337	14,317	13,350	13,228
B8-20	18,913	9475	9447	9451	8770	8695

Table A3. *Cont.*

Sample	Input	filtered	DenoisedF	DenoisedR	Merged	Nonchim
B8-21	14,129	7922	7901	7893	7391	7332
B8-22	18,843	9856	9801	9807	9007	8966
B8-5	20,503	10,688	10,648	10,648	10,081	10,023
B8-6	13,991	7014	6976	6985	6394	6378
B8-fal	4603	2518	2478	2466	2362	2358
B9-10	11,953	5229	5209	5202	4679	4670
B9-14	6549	3955	3938	3941	3602	3599
B9-16	11,558	6775	6752	6755	6119	6111
B9-19	11,146	5144	5116	5115	4804	4744
B9-3	10,354	4664	4648	4644	4081	4076
B9-4	13,701	6974	6945	6933	6440	6422
B9-6	9487	4444	4414	4411	4093	4089
B9-9	19,158	10,136	10,094	10,088	9287	9246
B9-fal	7037	4131	4117	4116	3850	3836
PRE-1	9227	5446	5423	5416	4963	4954
PRE-10	41,280	20,182	20,115	20,095	18,033	17,904
PRE-2	10,046	5575	5566	5559	5085	5084
PRE-3	16,164	7724	7665	7679	7081	7074
PRE-4	25,025	11,935	11,915	11,903	11,141	11,055
PRE-5	21,858	11,157	11,125	11,117	10,273	10,187
PRE-6	31,867	17,222	17,163	17,145	15,889	15,758
PRE-7	29,499	14,842	14,816	14,809	13,730	13,649
PRE-8	17,772	9465	9443	9436	8714	8689
PRE-9	22,917	12,095	12,053	12,062	11,220	11,125

Table A4. Average biomass (grams) for each cover crop treatment in native and cultivar studies. Cover crops are bluebunch wheatgrass ("bluebunch"), silky lupine ("lupine"), Holboell's rockcress ("rockcress"), white yarrow ("yarrow"), buckwheat ("buckwheat"), crimson clover ("clover"), white mustard ("mustard"), and wheatgrass ("wheat"). Letters to the right of values indicate significance at $p \leq 0.05$.

Experiment	Cover Crop Treatment	Below-Ground Biomass	Above-Ground Biomass
Native study	All native	0.885 ^{a}	2.715 ^{a}
	Bluebunch	0.795 ^{a}	3.041 ^{a}
	Lupine	0.319 ^{b}	0.730 ^{b}
	Rockcress	0.138 ^{b}	1.380 ^{b}
	Yarrow	1.056 ^{a}	2.822 ^{a}
Cultivar study	All cultivar	1.238 ^{a}	6.917 ^{a}
	Buckwheat	0.694 ^{b}	4.771 ^{b}
	Clover	0.527 ^{b}	7.483 ^{a}
	Mustard	0.121 ^{c}	1.834 ^{c}
	Wheat	2.272 ^{d}	3.663 ^{d}

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