**The relationship between cover crop species and soil fungal communities in Okanagan vineyards**

Erika Y. Lin1,2, Daniel Rosa1,2, Mehdi Sharifi3, Selina Spence1,2, Michael J. Noonan1,2,4, Miranda Hart1,2,\*

1. Department of Biology, The University of British Columbia Okanagan, Kelowna, British Columbia, Canada.
2. Okanagan Institute for Biodiversity, Resilience, and Ecosystem Services, The University of British Columbia Okanagan, Kelowna, British Columbia, Canada.
3. Summerland Research and Development Centre, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada.
4. Department of Computer Science, Math, Physics, and Statistics, The University of British Columbia Okanagan, Kelowna, British Columbia, Canada.

\*Corresponding Author: miranda.hart@ubc.ca

Words in abstract: 175

Words in main text: XX

Figures: 5

Tables: 2

References: XX

Appendices: XX

# Abstract

As important as grapevine (*Vitis vinifera*) productivity is to the wine industry in British Columbia (BC), Canada, grape growers face many challenges, such as disease, which can devastate crop harvests. In response to vine pathogens, viticulturalists employ non-crop groundcover vegetation as a natural alternative to chemical pesticides, however different cover crop species may have different plant-soil feedback effects associated with its microbial rhizosphere community. To investigate the relationship between cover crop identity, location, and soil microbial species composition, and to determine potential positive or negative impacts that cover crops may have on vines via soil microbes, we characterized the fungal communities in soil sampled from nine cover crop species used for vine row groundcover at three separate Okanagan vineyards. We found that within sites, regardless of cover crop identity, soil fungal communities did not vary greatly in composition and measures of community structure, however soil fungal communities were distinct across sites, indicating that site-specific conditions may play a larger role in shaping soil fungal communities in BC vineyards than the type of cover crop planted.

# Keywords:

Soil fungi, Viticulture, Vineyards, Cover crop, Microbial community

# Introduction

The Canadian wine industry is of growing economic importance, contributing to commercial products, employment opportunities, and tourism (Wine Growers Canada, 2023). Specifically, in mainland British Columbia (BC), unique conditions—intense intra-annual temperature variation, limited precipitation due to mountain rain shadows, and long daylight periods during the growing season—have made the BC interior a valuable location for wineries and grape vineyards, greatly supporting the BC economy with the revenue generated (Snoek & Elbourne, 2022). Ultimately, success of the wine industry depends on the health and crop yield of grapevines (*Vitis vinifera*), and thus to produce high-quality products, grape growers in the Okanagan Valley must contend with the increasingly extreme environmental challenges that plague their crops. These include harsher winters, invasive pests, drought, severe wildfire seasons, and prolonged smoke exposure from fires (Favell et al., 2019; Marín et al., 2021).

Disease control is a major issue for grapevine growers, particularly during establishment. Soil-borne root diseases such as Blackfoot and young vine decline cause significant losses to the viticulture industry in Canada (Úrbez-Torres et al., 2014). These diseases are difficult to control and treat because they are considered “disease complexes” and tend to accumulate over time (Mazzola & Manici, 2012).

Conventional viticulture employs plant protection products such as pesticides, herbicides, and fungicides for disease prevention (Peña et al., 2018). However, high biocide inputs have been shown to negatively impact biodiversity of arthropods and soil organisms, affect soil and water ecotoxicity, and possibly reduce biological pest control (Möth et al., 2021; Peña et al., 2018). Although pesticides are used for pathogen control, local soil biodiversity, including both pathogens and beneficials, is decreased en masse, leading to significant repercussions for soil function and associated vine performance (Karimi et al., 2020). As such, there has been a shift towards exploring organic viticulture practices, such as implementing groundcover vegetation in vine rows and inter-rows, which could prove to be more environmentally friendly and sustainable (Karimi et al., 2020; Möth et al., 2021).

Annual crops use crop rotation to decrease pathogen build up, by exploiting associations between plants and soil microbial communities. For long-lived perennials, however, this is not an option. The manipulation of non-crop vegetation (i.e. groundcover in vineyards) may provide similar benefit, by decreasing the incidence of crop specific pathogens. While the role of groundcover on vine performance and nutrition is well studied (Guerra & Steenwerth, 2012), its role in reducing root and trunk disease is not well understood.

There is good reason to believe that cover crop identity may lead to disease suppressive soils. There is some evidence from natural systems showing that pathogens are reduced with increasing plant diversity (Latz et al., 2016). Increased plant diversity leads to increased soil microbial diversity (Berg & Smalla, 2009), and this relationship is based on the ability of plant species (or even varieties) to ‘culture’ specific rhizosphere communities based on exudation composition, and root architecture (Aleklett & Hart, 2013; Rosa et al., 2022).

Specific cover crop taxa may also confer protective benefits to crop species. Many plants are known to produce biocidal compounds which inhibit soil borne pathogens. For example, members of the Brassicaceae and Capparidaceae produce glucosinolates, whose derivatives have been shown to have strong anti-fungal activity (Berlanas et al., 2018; Bleach, 2013; Richards et al., 2020). Plants in the Fabaceae, Alliaceae, Asteraceae, Polygonaceae, Polygalaceae and Agavaceae produce saponins and triterpene glycosides, which inhibit both herbivores and fungi (XX). Essential oils, particularly those in the Lamiaceae have similarly been shown to have strong antifungal activity in the soil (Mohammadi et al., 2015). Taken together, these results suggest that judicious choice of cover crops could control common, soilborne vine pathogens.

To identify cover crops that create a favourable soil fungal microbiome, we performed a field study manipulating cover crop identity in vine rows in three vineyards in the British Columbia. We sequenced soils growing and vine row treatments and looked for changes in fungal community structure and presence of key fungal taxa, like Cylindrocarpon and arbuscular mycorrhizal (AM) fungi.

# Methods

To understand the role of the cover crops species on soil fungal communities, in three fields we conducted field trials of 9 cover crops in three vineyards in the Okanagan Valley – BC,

1. Vineyard situated in Oliver BC, Canada (49°14'39.8"N 119°32'42.7"W), Covert Farms Family Estate. The vineyard contains 13 years old Merlot in Loamy Sand soil texture.
2. Vineyard situated in Summerland BC, Canada (49 ° 33'49.2" N - 119°38'19.0" W), experimental farm *Agriculture and Agri-food Canada’s Summerland Research and Development Centre* (SuRDC). The vineyard contains 2 years old Riesling in Loamy Sand soil texture.
3. Vineyard situated in West Bank BC, Canada (49°50'31.2"N 119°38'42"W), Kalala Organic Estate Winery. The vineyard contains 10 years old Zwiegeld in Silty loam soil texture.
4. Greenhouse located in Summerland BC, Canada (49 ° 33'49.2" N - 119°38'19.0" W), experimental farm *Agriculture and Agri-food Canada’s Summerland Research and Development Centre* (SuRDC). The pots were filled with Skaha sandy soil texture.

The vineyards consisted of in-row and inter-row spacing was 1.2 x 2.7m. The experimental design was a randomized complete block design (RCBD) with 5 replications. Cover crop species were selected according to regional studies (Olmstead, 2001), literature (Guerra & Steenwerth, 2012); Tompkins, 2010), their function within the agroecological landscape, and in consultation with experts. Each in-row plot consisted of 5-7 vines between two panels with a guard vine at each end of the plot. The irrigation system consisted of a drip irrigation system. The experiments were conducted in the 2018-2019 crop year, beginning in May for the field studies.

The field experiments design consisted of in-row cultivation of 9 cover crops species: Buckwheat (*Fagopyrum esculentum*), Buffalo Grass (*Bouteloua dactyloides*), Crescendo Ladino White Clover (*Trifolium repens cv. Ladino*), Field Pea (*Pisum sativum*), White Mustard (*Sinapis alba*), Phacelia (*Phacelia tanacetifolia*), Spring Lentil (*Lens culinaris*), Turnip (*Brassica rapa subsp. Rapa*), and Winfred Brassica (*Brassica napus cv. Winfred*).

**Table 1**. Summary of selected experimental sites’ description and management history in Okanagan Valley, British Columbia - CA.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site Location | Latitude and Longitude (oN, oW) | Growing season rainfall† (mm) | Mean growing season air temperature† (0C) | Crop Heat Unit (CHU) ‡ | Previous Crop | Rates applied (kg N ha-1) | Starter N applied (kg N ha-1) | Grape variety | Soil type\texture | Seeding rate | Management practices |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Covert | 49°14'39.8"  119°32'42.7" |  |  |  |  |  |  |  |  |  |  |
| SuRDC | 49 ° 33'49.2"  119°38'19.0" |  |  |  |  |  |  |  |  |  |  |
| Kalala | 49°50'31.2"  119°38'42 |  |  |  |  |  |  |  |  |  |  |

† From May to October: [http://climate.weather.gc.ca/prods servs/cdnclimate summary e.html](http://climate.weather.gc.ca/prods%20servs/cdnclimate%20summary%20e.html)

‡ OMAFRA Factsheet: Crop Heat Units for Corn and other Warm Season Crops in Okanagan Valey, BC – CA.

§ UAN = urea ammonium nitrate fertilizer (28% N).

¶ N/A = not applicable.

## Soil Chemistry

The concentration of total C and total N in each finely ground plant tissue and soil sample was determined by dry combustion and Dumas methods, respectively using a LECO CHN 628 analyzer (LECO Corporation, 3000, Lakeview Avenue. St. Joseph, MI). Soil mineral N was extracted from moist soil using 2 mol L-1 KCl (1:5 soil to extractant ratio) and the NO3–N plus ammonium (NH4–N) concentrations were determined colorimetrically using Segmented Flow AutoAnalyzer 3 (Keeney & Nelson, 1982).

Cations and micronutrients were extracted using Mehlich III solution (Mehlich ,1984) with a 1:10 soil to solution ratio and analyzed by ICP-OES (Teledyne Leeman Labs, USA). Sulfates were extracted with a Ca(H2PO4)2 solution containing 500 ppm of phosphorus and determined turbidimetrically (Blanchar, 1986) using a flow injection analyzer (QUIKCHEM 8500 series 2. Lachat instruments, Germany). Soil pH was measured in 1:2 soil:water ratio after 15 min steering using a pH electrode.

## Soil Sampling DNA extractions and sequencing

Field soil samples were taken in August 2019, close to the grape harvest, from the 0-20 cm layer. Each soil sample was composed of five sub-samples mixed and immediately stored in ice at -20oC and then frozen at -80oC until DNA extractions. Greenhouse soil samples were taken at the end of the plant cultivation after homogenizing and were immediately frozen until DNA extractions.

DNA from soil samples (0.25 g) was then extracted using PowerSoil 96 well extraction kit (MoBio, Inc., acquired by Qiagen in 2017) following the manufacturer's instructions. Extracted DNA in concentration >10 ng/µL, volume of 10 µL of each sample was sent ITS amplification by Microbiome research by microbiome researchers, Dalhousie University in Halifax, Nova Scotia, Canada.

## Data Processing

Sequences were returned in fastq files which were filtered and denoised using the dada2 package (v1.28.0; Callahan, McMurdie, et al., 2016) in R (v4.3.0; R Core Team, 2023), following the workflow of Callahan, Sankaran, et al. (2016). Samples that returned weak or failed sequencing reads were excluded from subsequent processing and analyses. Successful sequence reads were trimmed at nucleotide position 240 for forward reads and position 160 for reverse reads due to a decrease in average sequencing quality towards the ends of reads (Callahan, Sankaran, et al., 2016). The first ten nucleotide for each read were also removed as they likely contained pathological errors (Callahan, Sankaran, et al., 2016). Sequencing reads were subsequently filtered for a maximum of 2 expected errors per read and dereplicated to remove redundant comparisons for a quicker computation time when inferring amplicon sequence variants (ASVs; (Callahan, Sankaran, et al., 2016). Using ASVs instead of operational taxonomic units (OTUs) allows sequence variants to be differentiated by single-nucleotide differences rather than dictating an arbitrary threshold used to group sequences into OTUs (Callahan et al., 2017). Substitution errors were distinguished from errors in sequencing via the DADA2 method, which involves using a parameterized learning model for independent inference by sample, with non-pooled sequencing reads, to identify unique ASVs (Callahan, Sankaran, et al., 2016). As the DADA2 output includes chimeras, sequence pairs were merged before removing the chimeric sequences from the resulting sequence table to produce the final ASVs and abundance counts for each.

The ASVs for each soil sample were assigned taxonomic classification with reference to the most recent (November 29, 2022) UNITE General FASTA release for Fungi at the time of analysis (Abarenkov et al., 2022). The DECIPHER package (v2.28.0; Wright, 2016) was used for the multiple alignment of sequences prior to generating a neighbour-joining phylogenetic tree using the phangorn package (v2.11.1; Schliep, 2011). The neighbour-joining tree served as a base structure to which a general time-reversible nucleotide substitution model with a Gamma rate variation (GTR+G+I) and stochastic tree rearrangement. The model was adjusted to according to the parameters set by (Callahan, Sankaran, et al., 2016), where the number of intervals of the discrete gamma distribution (k) was set to 4 and a 0.2 proportion of invariable sites (inv) was allowed.

Using the phyloseq package (v1.44.0; McMurdie & Holmes, 2013), an individual co-occurrence network was constructed for each sample, where each vertex represents a unique ASV and edges connecting vertices represent the association between two co-occurring ASVs. To build the networks, Jaccard’s dissimilarity index (*J*) was calculated for each pair of ASVs to determine the distance between pairs, forming edges when less than 0.35, the maximum ecological distance set for Jaccard’s dissimilarity by McMurdie & Holmes (2013).

All sample sequence tables, taxonomic classifications, phylogenetic trees, and metadata were stored in a list of phyloseq-class() objects to facilitate data retrieval and handling in subsequent analyses (Callahan, Sankaran, et al., 2016). Individual phyloseq-class() objects were merged to combine all sample data into a single object for global analyses, while the data was also restructured to form site-specific objects. Phylogenetic trees were excluded from the composite objects, as it is not possible to merge networks with different numbers of nodes, representing branching events and ASVs.

## Statistical Analyses

For an overview of the fungal communities across sites, a Venn diagram was generated using the ggvenn package (v0.1.10; Yan, 2023). This initial comparison distinguished the number of unique species found in each of the three sampling sites: Covert, Kalala, and the Summerland Research and Development Centre (SuRDC), and the species shared between two or all sites. Pathogens of concern for vine health, including fungi in the Nectriaceae, Diatrypaceae, and Botryosphaeriaceae families have been previously highlighted by XX. Key vine pathogens were identified from the taxonomy table at decreasing order of taxonomic level, from family to species, to visualize the differences in the relative and absolute abundance of pathogenic fungi across cover crops and sites.

To understand the possible differences in the mycorrhizal communities between and within cover crop species, measures of community structure were extracted from the ASV abundances and phylogenetic trees for each sample. Alpha species richness was calculated as the total species count per sample after agglomerating ASV abundances by species. The total ASV count and number of unique ASVs were summed per sample. Species alpha diversity within each sample were estimated using Shannon’s and Simpson’s indices (Fedor & Zvaríková, 2019), calculated using the phyloseq package (McMurdie & Holmes, 2013). Phylogenetic diversity (PD) was calculated as the sum of branch lengths derived from the phylogenetic tree corresponding to each sample (Safi et al., 2011). These measures of community ecology were visualized in boxplots to compare mean and variance values across cover crop species. To describe the fungal community composition of each soil sample at a higher-order taxonomic rank, relative ASV abundances were agglomerated by fungal class to construct a stacked bar plot, with samples grouped by cover crop species. To determine any significant differences in the mean and variance of each richness and diversity measure between cover crops regardless of the site location, a generalized additive model was generated for each measure as a function of cover crop species, with random effects on the site variable. A gamma location scale distribution was specified for all measures, except for Simpson’s index, for which a beta location scale distribution was employed, because it was calculated as a proportion with values ranging from 0 to 1.

Measures of network topology were gleaned from the co-occurrence network built for each sample: the number of vertices, equal to the number of unique ASVs; the total number of edges connecting vertices in the graphical model, indicating co-occurrence of ASVs; network connectivity; connectance; and the degree of each vertex per sample. The number of vertices (v) and edges (e) was provided by the vcount() and ecount() functions in the igraph package (v1.4.2; Csárdi et al., 2006), while the degree, representing the number of edges extending from each individual vertex, was calculated using the same package. Network connectivity is the average number of associations between ASVs, calculated as the number of edges in the graphical model divided by the total number of vertices (e/v; Lurgi, 2016). Network connectance is the proportion of edges inferred at the predetermined maximum distance threshold out all possible edges, calculated as the number of edges divided by the square of the total number of vertices (e/v2; Lurgi, 2016). A generalized additive model was also generated for measures of network topology, extracted at the sample level, across cover crop species, with random effects on the site variable. All models employed a gamma location scale distribution, with the exception of network connectance, calculated as a proportion, for which a beta location scale distribution was specified.

As an alternative intercommunity statistic to beta diversity, co-occurrence affinity (α; Mainali et al., 2022) was calculated to compare the tendency of mycorrhizal species to co-occur across cover crop species. The co-occurrence affinity statistic was newly proposed by Mainali et al. (2022) as an improved index used measure co-occurrence of species between pairs of sites; comparisons between α, Jaccard’s index (*J*), and Sørensen-Dice index (*S-D*) revealed α to be insensitive to prevalences, making it a better estimator of intercommunity diversity than traditional beta diversity indices. The corresponding CooccurrenceAffinity package (v1.0; Mainali et al., 2022), developed by the authors, was used to verify the structure of the species occurrence matrices fed into the analysis, and to calculate α for each unique pair of cover crop species (Mainali et al., 2022). A lower triangle heat map of pair-wise cover crop α values was also generated to better reveal cover crop species of interest.

Three different gamma location scale generalized linear models (GLM) were constructed using the nlme package (v3.1-162; Pinheiro et al., 2012) to visualize trends in both the mean and variance in α for pairings made by each cover crop and the overall trend of all pairs: α as a function of the summed occurrences for each cover crop in a pair with no random effects, added random effects for cover crop, and added random effects for cover crop using the summed occurrences as the intercepts. The support for these models was then evaluated via AICc.

We complemented these statistical analyses with a within-sample random forest classification analysis. Random forest models were used to classify soil samples (n = 123) by cover crop and site, and to determine sorting success upon comparing model categorization results to the actual cover crops and sites to which each sample belongs. A species abundance matrix was prepared by summing the species abundances for each sample to be sorted by the random forest. The possible categories were set by two separate single-column data frames which tabulated the actual cover crops each sample was taken from: buckwheat (n = 15), buffalo grass (n = 14), crescendo ladino clover (n = 13), field pea (n = 15), white mustard (n = 15), phacelia (n = 15), spring lentil (n = 15), turnip (n = 9), and winfred brassica (n = 12), and the actual site of each sample: Covert (n = 43), Kalala (n = 44), and SuRDC (n = 36). A model for cover crop categorization and a model for site categorization were built using the randomForest package (v4.7-1.1; Breiman, 2001). The species abundance matrix and the corresponding data frame of actual cover crops or sites was fed into the models for analysis. For each model, a forest of 2,000 trees was specified, with 5 variables allowed at each branching split. Variable importance plots were produced for the two random forest model outputs.

To expose any sample clustering from the random forest models, principal component analyses (PCA) were conducted for each, formed from the proximity coordinates of each sample across each dimension of the PCA. The two PCA were separately plotted using the FactoMineR package (v2.8; Lê et al., 2008) to show any overlap in the clusters. Density plots made with ggridges (v0.5.4; Wilke, 2022) allowed for closer examination of the expression of the top two species from each PCA. The abundances of the top 50 species ranked by importance from the cover crop random forest model were displayed in a heat map built using the ComplexHeatmap package (v2.16.0; Gu et al., 2016). Heat map columns were annotated by true cover crop and site, and dendrograms were added for both samples in columns and species in rows.

All statistical analyses were performed in R (v4.3.0; R Core Team, 2023) and the scripts required to reproduce these analyses can be found in the GitHub repository at <https://github.com/ErikaYLin/Cover-Crop-Study>.

# Results

## Overall soil chemistry and fungal communities

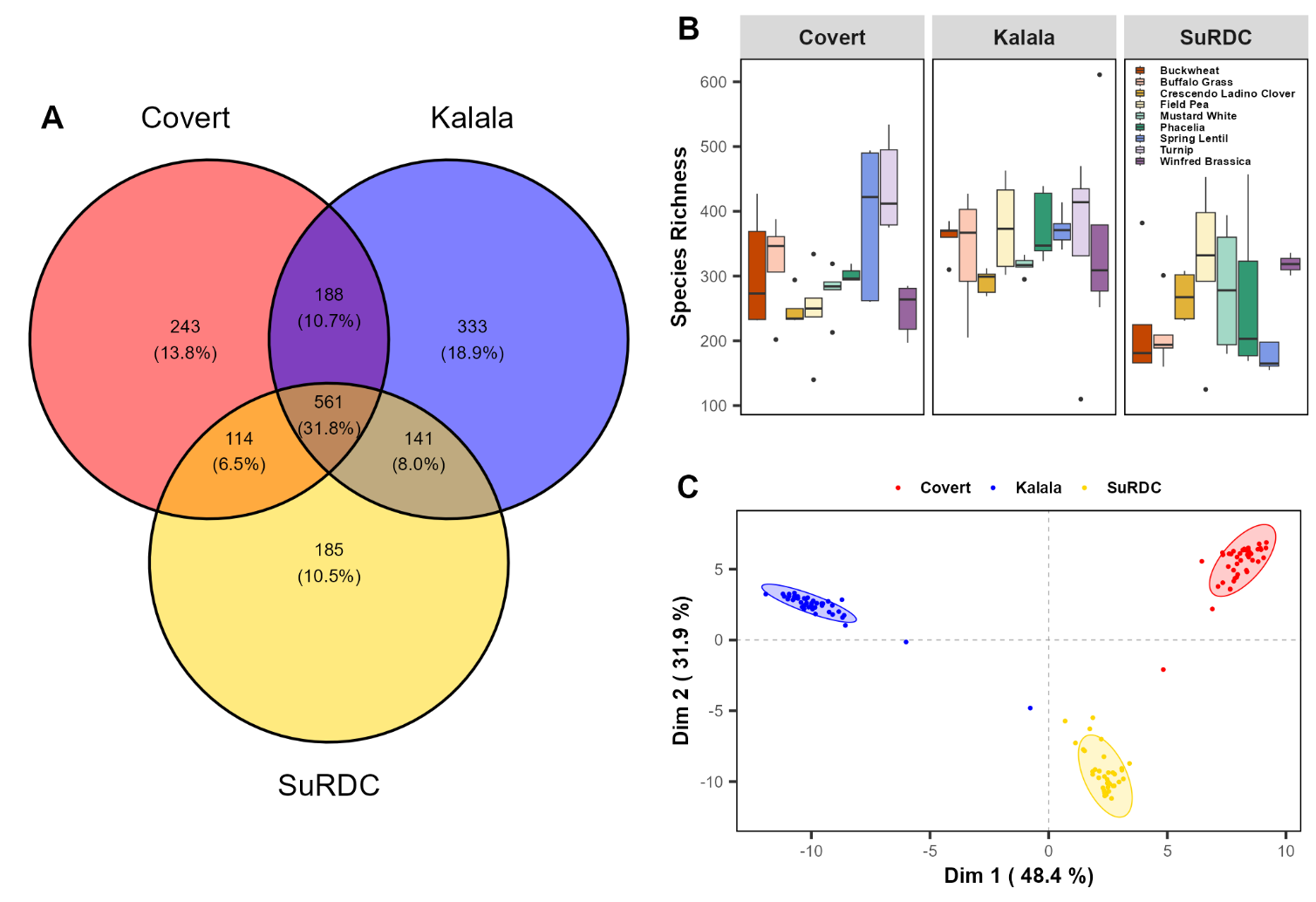
The soil pH did not vary greatly with depth and within each site. The pH of soil sampled at Covert and Kalala was comparable, averaging a pH of 6.39 at Covert, 6.32 at Kalala. However, soil samples from SuRDC were found to be more acidic with an average pH of 3.95. The phosphorus content in the soil decreased with sample depth and was consistent for all cover crops within each site. The amount of phosphorus measured across sites differed, with SuRDC having on average significantly less phosphorus (8.9 ppm) than Covert (115.7 ppm) and Kalala (247.0). The same trend could be observed for several other nutrients as well, including potassium, calcium, and sulfur (Table 2). Overall, although site-wide soil chemistry was homogenous, the soil at SuRDC was characteristically dissimilar to the other two sites, which may have either contributed to or resulted from any differences in the soil microbial communities between the three sites. Full site-wide soil chemistry data can be found in Supplementary Materials (Table SXX).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Site** | **Depth (cm)** | **pH** | **P (ppm)** | **K (ppm)** | **Ca (ppm)** | **Mg (ppm)** | **S (ppm)** |
| Covert | 0-15 | 6.34 (0.10) | 140.1 (70.3) | 249 (72) | 1084 (515) | 143 (58) | 14.2 (7.5) |
| 15-30 | 6.44 (0.16) | 91.2 (17.4) | 285 (20) | 830 (109) | 110 (5) | 8.9 (1.4) |
| Kalala | 0-15 | 6.31 (0.60) | 276.2 (13.6) | 484 (40) | 2349 (599) | 198 (32) | 27.5 (2.0) |
| 15-30 | 6.33 (0.44) | 217.7 (21.2) | 354 (8) | 1933 (638) | 161 (26) | 20.6 (2.8) |
| SuRDC | 0-15 | 4.14 (1.06) | 7.1 (5.0) | 127 (40) | 2.93 (1.16) | 105.2 (31.7) | 11.5 (4.2) |
| 15-30 | 3.75 (0.81) | 10.7 (145.6) | 138 (339) | 56.57 (2151.05) | 102.1 (197.7) | 8.5 (21.1) |

**Table 2:** Mean and range (bracketed) of soil chemistry measures sampled from 0-15 cm and 15-30 cm in depth within each site. Results for pH, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) content are reported on an oven dry (105C) weight basis.

From amplicon sequencing we identified a total of 1765 unique fungal species across the 123 soil samples, with 1,004 (~56.9%) of these being identified at multiple sites, and a further 561 (~31.8%) being found in all three (Fig. 1A). The most abundant classes of soil fungi found in samples were Agaricomycetes, Dothideomycetes, Eurotiomycetes, Leotiomycetes, Mortierrellomycetes, Pezizomycetes, Sordariomycetes, Spizellomycetes, and Tremellomycetes (Fig. 2). An additional 4571 classes were identified that each represented less than 1% of the total abundance of fungi. These latter classes were grouped together in subsequent analyses.

Though a substantial proportion of fungal species were found across all sites, there were nonetheless clear differences in community composition between the sites. In particular, SuRDC samples showed lower species richness and diversity on average as compared to Covert and Kalala (Fig. 1B). We note though that all assessed indices of community diversity were highly correlated with one another, with the exception of Simpson’s diversity for which samples showed high diversity regardless of phylogenetic diversity (see Figs. SXX-SXX). The stark differences in community composition between sites was confirmed by our random forest model, which was able to classify the samples by site with an error rate of only 0.81%. Indeed, only a single sample from Kalala being sorted incorrectly into SuRDC and a PCA on the proximity matrix of the site-classification model showed three distinct clusters, one for each site, with no overlap (Fig. 1C).



**Figure 1:** (A) Venn diagram counting the unique fungal species found in soil samples from each site, as well as species shared across sites. (B) Boxplots grouped by site, showing the relationship between mycorrhizal communities for each cover crop species and species richness. All turnip (light purple) samples from SuRDC were excluded due to weak sequencing results. (C) PCA on the proximity matrix of the site-classification random forest model showing clusters formed from sorting results.

## Cover crop choice and soil fungal community structure

Although soil samples from the three different sites displayed clear differences in community structure and composition, we found no pronounced differences in the mean alpha diversity measures between cover crops (Fig. 2; see also supplementary material). We did, however, find that the variance in these measures differed significantly between cover crops (Fig. 1B; Table S1). In particular, we found significantly lower variance in both Shannon’s (*β* = 4.07, Z = 5.11, *p* = 3.2x10-7) and Simpson’s (*β* = 3.66, Z = 6.60, *p* = 4.05x10-11). diversity in soil fungal communities with Phacelia as a cover crop. The minimal difference in community composition between cover crop types was further confirmed by a random forest model that exhibited an estimated error rate of 98.37% when sorting samples between cover crops. Indeed, a PCA on the proximity matrix of the cover crop-classification model showed no clear separation, with the only structure being 3 clusters of points that ran parallel to each other along a diagonal, likely corresponding to the 3 sampling sites (Fig. 3A). The lack of any clear differences across cover crop types is exemplified via the abundances of *Pyrenochaetopsis indica* (Fig. 3B)and anUnclassified *Phaeosphaeriaceae* (Fig. 3C)*.* Though these two fungal species were identified by the random forest model as the primary predictors of cover crop difference, they exhibited near identical abundances. A heat map was made to visualize the abundances of the 50 species identified by the random forest model as the primary predictors for cover crop sorting (Fig. 3D). Unsurprisingly, abundances varied greatly by species, cover crop, and site, ranging from 0 (i.e., absent) to over a thousand sequences. Here again, there were no clear patterns for clustering important species by cover crop, though there was a tendency for samples to from the same site to cluster.

A screenshot of a computer screen

Description automatically generated

**Figure 2:** Bar plot describing the community composition in soil sampled under different cover crops. Relative abundances are grouped by cover crop species and coloured according to fungal class.

A heat map was made to visualize the abundances of the 50 species identified by the random forest model as the primary predictors for cover crop sorting (Fig. 4D). Unsurprisingly, abundances varied greatly by species, cover crop, and site, ranging from 0 (i.e., absent) to over a thousand. Here again, there were no clear patterns for clustering important species by cover crop, though there was a tendency for samples from the same site to be clustered, indicated by the larger blocks of red, blue, and yellow grouped together.

A screenshot of a computer screen

Description automatically generated

**Figure 3:** (A) Principal component analyses showing the clustering results from a random forest analysis of samples for each cover crop, with density plots (B) and (C) visualizing the abundance of the top two fungal species ranked by importance in the random forest classification. (D) Heat map of the abundances of the top 50 species ranked by importance in the random forest analysis of cover crop classification, annotated by site and cover crop.

Although there were few differences in community diversity across cover crops, trends in the community structure determined by co-occurrence network topology were more apparent (Table S1). For all measures of network topology modelled, including the total vertex and edge counted from network graphs, the connectivity, and connectance, fungal communities sampled under crescendo ladino clover were significantly different from buckwheat and from other cover crops (Fig. SXX; Table S1). Crescendo ladino clover communities had lower mean values and less variance than other cover crop communities in terms of the number of vertices (equal to the total ASV count), the number of edges, and connectivity, indicating lower network complexity and reduced co-occurrences among ASVs found in clover samples. Turnip soil samples were also found to have much greater variance in network topology; however, this may have resulted from having fewer samples for Turnips due to weak or failed sequencing reads.

A screenshot of a video game

Description automatically generated

**Figure 4:** (A) Generalized linear regression model with a Gamma distribution & log link showing co-occurrence affinity (α) of fungi between cover crop species pairs. The overall trend for α across all cover crop pairs is indicated by the solid black line. (B) Corresponding heat map of the α values for each pair of cover crop species in a matrix.

Finally, in terms of patterns of co-occurrence affinity, α values did not differ greatly between pairs of cover crops and species were generally likely to be found in both cover crop species of a pair. For all combinations of cover crop pairs, we found a significant negative relationship between the total number of fungal species in the soil and co-occurrence affinity (*β* = -0.001282, t70 = -5.46, *p* < 0.001; Fig. 4A). This trend was consistent for all pairings of cover crops and α depreciated at a comparable rate for all, as seen by the slopes in figure 4. The only cover crop pairings that showed appreciable differences in co-occurrence affinity of fungal species were those that included turnips, which tended to have lower α values.

## Pathogens and Beneficials

The ratio of pathogenic to non-pathogenic fungal taxa was comparable across cover crop species and site, with less than 30% of taxa being pathogenic in each soil sample measured (Fig. 5B). Overall, Nectriaceae was the most abundant group of pathogens, identified based on a compiled list of taxa (Fig. 5A). This family of fungi was present in every sample, with the exception of SuRDC turnips, as these samples were excluded from the main analysis. Other taxa that were found include, in no particular order, Botryosphaeriaceae, Cadophora, Diaporthaceae, Kalmusia variispora, and Phaeoacremonium.

Of all cover crops at all sites, turnips and spring lentils at Covert had the highest abundance of pathogens, while spring lentils at SuRDC had the lowest abundance of pathogens (Fig 5). Turnips also had a greater abundance of Phaeocremonium than other cover crops for the two sites with viable samples (Covert and Kalala; Fig. 5A).

Glomeromycota, a known beneficial taxon which constitutes the fungal partner in AM symbioses, were not among the most abundant fungal taxa in the community compositions (Fig. 2). Due to their relatively rarer abundance, the Glomeromycota were grouped with other fungal classes that comprised <1% of the total fungal communities.

**A screenshot of a computer

Description automatically generated Figure 5:** (A) Stacked bar plot of the mean absolute abundance of fungal vine pathogens from various taxonomic levels (family, genus, species) for each cover crop, grouped by site. Turnip soils appear to have no pathogens at SuRDC, despite having the greatest abundance of pathogens at Covert and exhibiting pathogen presence at Kalala, due to weak or failed sequencing reads resulting in the exclusion of all SuRDC turnip samples from the analysis. (B) Bar plot comparing the relative abundances of pathogenic and non-pathogenic fungal species in each sample, grouped by cover crop.

# Discussion

We manipulated the identity of groundcover crops in vine rows of three vineyards in British Columbia, Canada, and sequenced DNA extracted from soils in the rhizosphere of vine row treatments to determine if cover crop species were associated with differing community structure. Specifically, we compared cover crop species that could potentially confer benefits to vine growth, either through mycorrhizal interactions (i.e. AM symbioses; XX), anti-microbial effects against soil-borne pathogens (i.e. Brassicaceae and Capparidaceae; Berlanas et al., 2018; Bleach, 2013), or protection against herbivory (i.e. Fabaceae, Alliaceae, Asteraceae, Polygonaceae, Polygalaceae and Agavaceae; XX), and looked for key fungal taxa, such as mutualists in AM symbioses and fungal pathogens that could negatively affect vine performance (i.e. Nectriaceae, XX). As such, the use of certain cover crops in viticulture may cultivate suppressive soils, promoting vine productivity.

Sequencing of soils and subsequent taxonomic classification revealed that despite substantial overlap in soil fungal species identified between the three sites, there were still distinct differences between the communities across the sites. However, the same cannot be said for different species grown for vine row groundcover within each site. To investigate soil fungal community composition and structure, we compared the abundances of soil fungi at both higher-order (class) and finer (species) taxonomic scales, modelled diagnostics of diversity and co-occurrence, and used a machine learning approach to determine any clustering of soil samples by site or cover crop. Overall, our analyses indicate that soil fungal communities differ significantly across sites but are similar for various cover crop species within each site.

As mean species richness and community diversity were found to be similar between cover crop species, but were distinct across sites, confirmed by random forest sorting, it is likely that site-specific differences account for variation in soil fungal communities. Indeed, this matches with what was observed for site-specific soil chemistry, where soil acidity and nutrients differed between sites. Specifically, SuRDC soil pH and other factors contrasted measurements at the other two sites; while it has been previously demonstrated that fungal community composition may be relatively insensitive to soil pH compared to bacteria (Wang et al., 2022), it is worth noting that differences in soil treatment (i.e. use of fertilizers, irrigation regime, vine treatment, and other variables) and soil sampling protocol (pooled or individual measurements) between the two commercial farms, Covert and Kalala, and the research centre, SuRDC, may have impacted the soil chemistry data and observed fungal communities. Furthermore, as strict controls were not implemented in the experimental design, this study served primarily to explore and characterize soil microbial communities as they would naturally occur in relation to vine-cover crop pairings.

Contrary to beta diversity, as co-occurrence affinity increases between two cover crops, the more likely for fungal species to be found in both cover crops (Mainali et al., 2022), and thus we would expect the soil fungal communities to be more similar. As expected, this indicates that the likelihood of fungal species to be found in the soil of two different cover crops is higher when there is less diversity within the communities, and lower when there is more diversity within communities. Fungal species were less likely to co-occur between turnips and other cover crops, meaning that the soil fungal community of turnips were more likely to be different than other cover crop species. This may not have been reflected in the abundance bar plot (Fig. 2) as the community composition was analyzed at a cruder taxonomic rank, fungal class, which likely did not capture differences between community composition at a finer scale, such as fungal genus. As a pairwise analysis of co-occurrence affinity between different cover crops showed similar slopes across most species, this indicated that soil fungal species were equally likely to co-occur in both cover crop species, apart from Turnip pairs that had lower tendencies for co-occurrence. This suggests a greater beta diversity between soil fungal communities promoted by Turnip growth and the soil communities of other cover crop species.

We note that there were certain samples, including all of SuRDC turnip soil samples, that were removed from the statistical analyses because of weak genetic sequencing outputs. The omission of certain samples and their associated fungal communities may have skewed model results and more importantly, the observed disjunction between turnips and other cover crops in terms of lower co-occurrence affinity, species richness, and pathogen abundances. It is unclear as to whether or not these contrasts were inflated by the exclusion of weak sequencing reads. Explicitly, while turnips and spring lentil had the most abundance of pathogens at Covert, this was not a trend exhibited at all sites as there was relatively less abundance at Kalala for both cover crop species and SuRDC for spring lentils (Fig. 5). Thus, differences in pathogen abundance may be more heavily attributed towards site environments. It seems that turnip soils may be associated with greater abundance of *Phaeocremonium spp.* (Fig. 5), potentially from the production of root exudates attractive to *Phaeocremonium spp.*, however due the missing SuRDC samples, this inference remains inconclusive. Regardless, as Nectriaceae were present and abundant across all cover crops and sites, it is possible that either all cover crops promote the proliferation of Nectriaceae or fungi in this family occur naturally in Okanagan soils at greater relative abundances.

The marginal error rate of the random forest classification shows that the fungal communities differed significantly and sufficiently between sites, regardless of the cover crop species, such that the random forest model was able to classify samples by site with high accuracy. The model’s poor ability in accurately sorting samples by cover crop species further supports the findings for co-occurrence affinity, that is fungal communities across cover crops were very similar. In figure 3, the clear overlap of sample clusters in the PCA plot, defined by cover crop species, emphasizes once more that within each site, no soil fungal community of a cover crop species was any more different from the fungal community of any other cover crop species, however, as indicated by the three nondelineated clusters running parallel to each other, soil communities are distinguishable by the three sites. This finding is also confirmed by the samples clustering by site in the column annotation of the corresponding heatmap generated for the top 50 most important species in the random forest model (Fig 3C). Therefore, soil fungal communities differed greatly across sites, but communities resembled each other across cover crop species within each site.

While we cannot definitively conclude that any specific cover crop species fostered soil microbiomes that promoted or harmed vine growth within the experimental constraints of our study, we can extract certain patterns, or the lack thereof, in soil fungal communities with respect to site and cover crop species which allow us to form predictions on the potential plant-soil feedback of certain cover crops on grapevines. Increasing the biodiversity of Canadian vineyards will have downstream affects in addition to pathogen suppression, including improved nutrient retention, improved soil structure, reduced herbivory (Faucon et al., 2017). These ecosystem services will become increasingly important as growers experience the effects of future climate regimes.

# Conclusion

As stressors to plant performance become increasingly problematic in the face of global climate change and anthropogenic activity, grape growers and wine makers that form the backbone for the Canadian wine industry depend on the development of sustainable solutions to increase grapevine crop yields and quality (Favell et al., 2019; Marín et al., 2021; Möth et al., 2021). Disease suppression and protection is an area of continual concern for which various viticulture practices have been adopted, including the use of groundcover crops in vine and inter-vine rows (Möth et al., 2021). This organic technique demands knowledge of the cover crop-soil effects on below-ground microbial communities which impact vine growth. Our results suggest that soil fungal communities in Okanagan vineyards are not heavily impacted by cover crop identity, however as shown by site-specific differences in fungal community composition, the environmental conditions dictated by vineyard location may explain the soil fungal community observed. These findings contribute to the expanding body of research on sustainable agricultural practices (Karimi et al., 2020), here specifically addressing the question of cover crop choice, and while it appears that no specific cover crop species that we studied was especially outstanding in the soil fungi it cultured, further investigation is necessary to elucidate the effects of these fungal communities on vine productivity.

# References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R. H., & Kõljalg, U. (2022). UNITE general FASTA release for Fungi. *UNITE Community*. https://doi.org/https://dx.doi.org/10.15156/BIO/2483911

Aleklett, K., & Hart, M. (2013). The root microbiota—a fingerprint in the soil? *Plant and Soil*, *370*(1–2), 671–686. https://doi.org/10.1007/s11104-013-1647-7

Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, *68*(1), 1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x

Berlanas, C., Andrés‐Sodupe, M., López‐Manzanares, B., Maldonado‐González, M. M., & Gramaje, D. (2018). Effect of white mustard cover crop residue, soil chemical fumigation and *Trichoderma* spp. root treatment on black‐foot disease control in grapevine. *Pest Management Science*, *74*(12), 2864–2873. https://doi.org/10.1002/ps.5078

Bleach, C. M. (2013). *Management of Cylindrocarpon Black Foot Disease in New Zealand nurseries and vineyards* [Lincoln University]. https://researcharchive.lincoln.ac.nz/server/api/core/bitstreams/a2ed689e-8314-4bde-839d-e0da700b70b7/content#:~:text=In%20field%20trials%2C%20HWT%20protocols,nursery%20grapevines%20in%20New%20Zealand.

Breiman, L. (2001). Random forests. *Machine Learning*, *45*(1), 5–32. https://doi.org/10.1023/A:1010933404324

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal*, *11*(12), 2639–2643. https://doi.org/10.1038/ismej.2017.119

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Callahan, B. J., Sankaran, K., Fukuyama, J. A., McMurdie, P. J., & Holmes, S. P. (2016). Bioconductor Workflow for Microbiome Data Analysis: from raw reads to community analyses [version 2; peer review: 3 approved]. *F1000Research*, *5*, 1492. https://doi.org/10.12688/f1000research.8986.2

Csárdi, G., Nepusz, T., Traag, V., Horvát, S., Zanini, F., Noom, D., & Müller, K. (2006). The igraph software package for complex network research. *InterJournal*, *Complex Systems*, 1695. https://doi.org/https://doi.org/10.5281/zenodo.7682609

Faucon, M.-P., Houben, D., & Lambers, H. (2017). Plant functional traits: Soil and ecosystem services. *Trends in Plant Science*, *22*(5), 385–394. https://doi.org/10.1016/j.tplants.2017.01.005

Favell, J. W., Noestheden, M., Lyons, S. M., & Zandberg, W. F. (2019). Development and Evaluation of a Vineyard-Based Strategy To Mitigate Smoke-Taint in Wine Grapes. *Journal of Agricultural and Food Chemistry*, *67*(51), 14137–14142. https://doi.org/10.1021/acs.jafc.9b05859

Fedor, P., & Zvaríková, M. (2019). Biodiversity Indices. In *Encyclopedia of Ecology* (2nd ed., Vol. 1, pp. 337–346). Elsevier. https://doi.org/10.1016/B978-0-12-409548-9.10558-5

Gu, Z., Eils, R., & Schlesner, M. (2016). Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics*, *32*(18), 2847–2849. https://doi.org/10.1093/bioinformatics/btw313

Guerra, B., & Steenwerth, K. (2012). Influence of floor management technique on grapevine growth, disease pressure, and juice and wine composition: A review. *American Journal of Enology and Viticulture*, *63*(2), 149–164. https://doi.org/10.5344/ajev.2011.10001

Karimi, B., Cahurel, J.-Y., Gontier, L., Charlier, L., Chovelon, M., Mahé, H., & Ranjard, L. (2020). A meta-analysis of the ecotoxicological impact of viticultural practices on soil biodiversity. *Environmental Chemistry Letters*, *18*(6), 1947–1966. https://doi.org/10.1007/s10311-020-01050-5

Keeney, D. R., & Nelson, D. W. (1982). Nitrogen-Inorganic Forms. In A. L. Page (Ed.), *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties* (2nd ed., pp. 643–698). American Society of Agronomy, Inc., Soil Science Society of America, Inc. https://doi.org/10.2134/agronmonogr9.2.2ed.c33

Latz, E., Eisenhauer, N., Rall, B. C., Scheu, S., & Jousset, A. (2016). Unravelling linkages between plant community composition and the pathogen-suppressive potential of soils. *Scientific Reports*, *6*(1), 23584. https://doi.org/10.1038/srep23584

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*, *25*(1), 1–18. https://doi.org/10.18637/jss.v025.i01

Lurgi, M. (2016). *Networks as Graphs*. Ecological Networks in R. https://mlurgi.github.io/networks\_for\_r/lesson-4.html

Mainali, K. P., Slud, E., Singer, M. C., & Fagan, W. F. (2022). A better index for analysis of co-occurrence and similarity. *Science Advances*, *8*(4), eabj9204. https://doi.org/10.1126/sciadv.abj9204

Marín, D., Armengol, J., Carbonell‐Bejerano, P., Escalona, J. M., Gramaje, D., Hernández‐Montes, E., Intrigliolo, D. S., Martínez‐Zapater, J. M., Medrano, H., Mirás‐Avalos, J. M., Palomares‐Rius, J. E., Romero‐Azorín, P., Savé, R., Santesteban, L. G., & de Herralde, F. (2021). Challenges of viticulture adaptation to global change: tackling the issue from the roots. *Australian Journal of Grape and Wine Research*, *27*(1), 8–25. https://doi.org/10.1111/ajgw.12463

Mazzola, M., & Manici, L. M. (2012). Apple replant disease: Role of microbial ecology in cause and control. *Annual Review of Phytopathology*, *50*(1), 45–65. https://doi.org/10.1146/annurev-phyto-081211-173005

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Mohammadi, A., Hashemi, M., & Hosseini, S. M. (2015). Comparison of antifungal activities of various essential oils on the Phytophthora drechsleri, the causal agent of fruit decay. *Iranian Journal of Microbiology*, *7*(1), 31–37.

Möth, S., Walzer, A., Redl, M., Petrović, B., Hoffmann, C., & Winter, S. (2021). Unexpected Effects of Local Management and Landscape Composition on Predatory Mites and Their Food Resources in Vineyards. *Insects*, *12*(2), 180. https://doi.org/10.3390/insects12020180

Peña, N., Antón, A., Kamilaris, A., & Fantke, P. (2018). Modeling ecotoxicity impacts in vineyard production: Addressing spatial differentiation for copper fungicides. *Science of The Total Environment*, *616–617*, 796–804. https://doi.org/10.1016/j.scitotenv.2017.10.243

Pinheiro, J. C., Bates, D. J., DebRoy, S., & Sakar, D. (2012). The Nlme Package: Linear and Nonlinear Mixed Effects Models, R Version 3. In *R package version* (Vol. 6). https://www.researchgate.net/publication/272475067\_The\_Nlme\_Package\_Linear\_and\_Nonlinear\_Mixed\_Effects\_Models\_R\_Version\_3

R Core Team. (2023). *R: A Language and Environment for Statistical Computing* (4.3.0). R Foundation for Statistical Computing. https://www.R-project.org

Richards, A., Estaki, M., Úrbez-Torres, J. R., Bowen, P., Lowery, T., & Hart, M. (2020). Cover Crop Diversity as a Tool to Mitigate Vine Decline and Reduce Pathogens in Vineyard Soils. *Diversity*, *12*(4), 128. https://doi.org/10.3390/d12040128

Rosa, D., Sharifi, M., & Hart, M. M. (2022). Cover Crops as Reservoirs for Young Vine Decline Pathogens. *Agronomy*, *12*(10), 2422. https://doi.org/10.3390/agronomy12102422

Safi, K., Cianciaruso, M. V., Loyola, R. D., Brito, D., Armour-Marshall, K., & Diniz-Filho, J. A. F. (2011). Understanding global patterns of mammalian functional and phylogenetic diversity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *366*(1577), 2536–2544. https://doi.org/10.1098/rstb.2011.0024

Schliep, K. P. (2011). phangorn: Phylogenetic analysis in R. *Bioinformatics*, *27*(4), 592–593. https://doi.org/10.1093/bioinformatics/btq706

Snoek, J., & Elbourne, L. (2022). *BC Vineyard Resiliency Project*. https://www2.gov.bc.ca/assets/gov/farming-natural-resources-and-industry/agriculture-and-seafood/animal-and-crops/crop-production/bc\_vineyard\_resilience\_study\_july\_18\_2022.pdf

Úrbez-Torres, J. R., Haag, P., Bowen, P., & O’Gorman, D. T. (2014). Grapevine trunk diseases in British Columbia: Incidence and characterization of the fungal pathogens associated with black foot disease of grapevine. *Plant Disease*, *98*(4), 456–468. https://doi.org/10.1094/PDIS-05-13-0524-RE

Wang, T., Cao, X., Chen, M., Lou, Y., Wang, H., Yang, Q., Pan, H., & Zhuge, Y. (2022). Effects of Soil Acidification on Bacterial and Fungal Communities in the Jiaodong Peninsula, Northern China. *Agronomy*, *12*(4), 927. https://doi.org/10.3390/agronomy12040927

Wilke, C. O. (2022, September 26). *ggridges: Ridgeline plots in ggplot2*. CRAN. https://wilkelab.org/ggridges/

Wine Growers Canada. (2023). *Industry Economic Impact*. Wine Growers Canada. https://www.winegrowerscanada.ca/canadian-wines/economic-impact/

Wright, E. S. (2016). Using DECIPHER v2.0 to Analyze Big Biological Sequence Data in R. *The R Journal*, *8*(1), 352–359. https://doi.org/https://doi.org/doi:10.18129/B9.bioc.DECIPHER

Yan, L. (2023, March 31). *ggvenn: Draw Venn Diagram by `ggplot2`*. CRAN. https://cran.r-project.org/package=ggvenn