



VITICULTURE ORIGINAL RESEARCH ARTICLES

Experimental field trials model how the climate crisis will alter phyllosphere and carposphere fungal communities of *Vitis* sp. L'Acadie blanc across growth stages

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Article number: 8363



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Associate editor:

Gerardo Puopolo



Received:

24 October 2024

Accepted:

27 June 2025

Published:

12 September 2025



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ABSTRACT

The climate crisis is changing temperature regimes worldwide, threatening global viticulture and wine production, as temperature is a primary driver of grape development. In Atlantic Canada, temperatures are projected to increase, inducing premature grape ripening. This can impact their biochemical profiles and, consequently, the quality of the vines, in terms of plant health and fruit yield, and therefore the quality of the wines produced. Temperature is also a key factor in determining the composition fungal communities on the leaves (phyllosphere) and fruits (carposphere) of grape vines. Therefore, to better understand how these communities might change under potential future temperature regimes, we experimentally manipulated grapevines (*Vitis* sp. cv. L'Acadie blanc) in the field. We used on-the-row mini-greenhouses to increase the temperature at different developmental or phenological stages of the fruits, and across the whole season. Phyllosphere and carposphere were sampled at four developmental stages, their DNA was extracted, and the fungal communities were identified *via* ITS metabarcoding. We found that phyllosphere and carposphere had significantly different community composition, which remained relatively stable throughout plant development. Increased temperature treatments had the most significant effect on fungal phyllosphere communities; we observed that phyllosphere samples exposed to higher temperatures before the onset of ripening maintained fungal communities with higher species richness throughout development. Our analysis showed that the increase in fungal diversity among phyllosphere communities corresponds to enrichments in potential phytopathogenic fungal taxa. However, this increase in phyllosphere fungal diversity was not conserved at other growth stages when the leaves developed at higher temperatures for the whole season. The results of this study will contribute to better understanding the impact of the climate crisis on grapevine phyllosphere and carposphere fungal community composition and assembly. This will allow producers to better adapt to climate variability and to better understand the role that these communities could play on grapevine health.

KEYWORDS: fungi, wine grapes, climate crisis, phyllosphere, carposphere, grapevine phenology, metabarcoding

INTRODUCTION

The Intergovernmental Panel on Climate Change (IPCC) conservatively estimates that global temperatures will increase by 1.4–4.4 °C by 2100 depending on greenhouse gas emissions (Masson-Delmotte *et al.*, 2021). In Atlantic Canada, temperatures are predicted to increase from 2 to 4 °C in the summer and 1.5 to 6 °C during the winter (Vasseur & Catto, 2007). As a result, growing seasons will be longer and hotter, with shorter, warmer, and damper winters. These temperature changes will impact local agriculture by decreasing yields through heat stress, drought, frequent heavy rainfalls, and the increased populations of phytopathogens (Looby & Treseder, 2018; Jobin Poirier *et al.*, 2020; Lesk *et al.*, 2022; Singh *et al.*, 2023).

Temperature is a key driver of grape development, and climate-induced temperature changes are threatening global viticulture and wine production (Jobin Poirier *et al.*, 2020; Ausseil *et al.*, 2021). In Atlantic Canada, these increased temperatures could affect grapevine phenology (*i.e.*, developmental stages) and grape ripening, increase disease pressure and, ultimately, affect fruit quality. Grape varieties grown in the region are interspecific crosses between *Vitis vinifera* and *Vitis* species indigenous to North America (*e.g.*, *V. labrusca*, *V. riparia*), which have been selected for strong cold resistance due to a unique biochemical profile and an early maturity (Pedneault *et al.*, 2013). Typically in the region, berry fruit-set occurs in late June, with growth continuing until the end of August and the onset of veraison, the ripening stage. Berries will continue to ripen until harvested in mid to late October (Campos-Arguedas *et al.*, 2022). During the entirety of berry development, including the berry growth phase, temperature affects the development of flavour, aroma, sugar, and alcohol content of berries (van Leeuwen & Seguin, 2006; Campos-Arguedas *et al.*, 2022). For example, early development of berries is characterised by the accumulation of malic and tartaric acids, and proanthocyanidins. During late development (*i.e.*, ripening), malic acid breaks down, while sugars and various secondary metabolites accumulate (Bonada *et al.*, 2015; Campos-Arguedas *et al.*, 2022). Our previous work has further experimentally confirmed in field experiments that increasing the temperature during early berry development changes their biochemistry at harvest (Campos-Arguedas *et al.*, 2022). Unexpected changes to the biochemical profile of the grapes due to climate-induced temperature variation may thus impact the quality of the grapes and wine.

Temperature, a key factor in the “*terroir*” (van Leeuwen & Seguin, 2006; Di Paola *et al.*, 2023; Johnston-Monje *et al.*, 2023), also helps determine the composition and structure of resident microbial communities (Romero-Olivares *et al.*, 2017; Looby & Treseder, 2018; Nottingham *et al.*, 2018; Frindte *et al.*, 2019; Malik *et al.*, 2019; Jansson & Hofmockel, 2020; Tiedje *et al.*, 2022). In soils, bacterial and fungal diversity generally tracks global abiotic patterns of temperature and precipitation (Kivlin *et al.*, 2014; Leff *et al.*, 2018;

Nottingham *et al.*, 2018; Malik *et al.*, 2019; He *et al.*, 2020; Koskella, 2020; Zhu *et al.*, 2021; Tiedje *et al.*, 2022). However, fungi do tend to make up more of the soil biomass across the boreal forests (50° N–70° N, Treseder *et al.*, 2014; He *et al.*, 2020). This appears to be due to the slower rates of decomposition, largely performed by free-living filamentous fungi (Treseder *et al.*, 2014; Treseder & Lennon, 2015). Despite this, many fungal lineages have been shown to quickly adapt to warming soils. This is particularly true of phylogenetically younger lineages and those more adept at budding, such as yeasts, unlike filamentous fungi (Treseder *et al.*, 2014; Romero-Olivares *et al.*, 2017; Looby & Treseder, 2018). Although soils may be the reservoir for many microbes in the aerial portions of plants (phyllosphere; hereafter used to specify the leaves), important differences can still accrue between above- and below-ground communities over the development of the plant host (Jansson & Hofmockel, 2020; Koskella, 2020; Blakney *et al.*, 2024b).

In general, microbes that colonise aerial portions of plants survive under difficult conditions of limited nutrients, exposure to UV radiation, and fluctuations in pH, humidity, and temperature (Lindow & Brandl, 2003; Koskella, 2020). The composition and structure of above-ground plant microbial communities has also been shown to be impacted by temperature (Cordier *et al.*, 2012; Koskella, 2020; Zhu *et al.*, 2021; Perreault & Laforest-Lapointe, 2022). Among fungi, for example, increased temperatures have been seen to increase the diversity of yeasts, which are thought to be more stress resistant, as well as ascomycetes, such as *Cladosporium* sp. (Treseder & Lennon, 2015; Looby & Treseder, 2018; Cureau *et al.*, 2021). However, other studies have highlighted decreases in phyllosphere fungal diversity due to warming (Zhu *et al.*, 2021), as well as across growth stages as communities stabilise (Boutin *et al.*, 2024). Foliar microbes also play important roles for their plant hosts by providing nutrients (Moyes *et al.*, 2016; Laforest-Lapointe *et al.*, 2017), as well as by disrupting the invasion and colonisation of potential phytopathogens (Ritpitakphong *et al.*, 2016; Laforest-Lapointe *et al.*, 2017). This can be particularly true of fungal communities, since they can disperse aerially, and are not necessarily dispersal limited (McGuire *et al.*, 2011; Abdelfattah *et al.*, 2019; Wang *et al.*, 2021; Johnston-Monje *et al.*, 2023).

In freshly developing foliar plant organs, like leaves and fruits, the order of colonisation is a key driver of microbial community dynamics (Fukami, 2015; Hiscox *et al.*, 2015; Mori *et al.*, 2017; Debray *et al.*, 2021). The priority effects of arrival order are generally described by niche pre-emption or niche modification (Fukami, 2015; Debray *et al.*, 2021). In the former, available niches are filled on a first-come first-served basis, leading to the exclusion or inhibition of late arrivals into the environment (Fukami, 2015; Debray *et al.*, 2021). In the latter, early-arrivals change the local environment to create new niche opportunities for later arrivals (Fukami, 2015; Debray *et al.*, 2021). Several factors can modulate the strength of priority effects, including phylogenetic relatedness, niche overlap, and

environmental stability, (Fukami, 2015; Hiscox *et al.*, 2015; Mori *et al.*, 2017; Debray *et al.*, 2021). All of these elements could contribute to the emergence and spread of new phytopathogens, as the climate crisis creates more variable temperatures across geographic regions (Malik *et al.*, 2019; Jansson & Hofmockel, 2020; Koskella, 2020; Zhu *et al.*, 2021; Tiedje *et al.*, 2022; Singh *et al.*, 2023). Therefore, there is a pressing need to document microbial community composition and assembly dynamics through time in different plant-associated environments; *e.g.*, soil, leaves, fruits, flowers, and stems.

To this end, bacterial and fungal communities of grapevines and vineyards have been well surveyed throughout time and space (Perazzolli *et al.*, 2014; Fort *et al.*, 2016; Kecskeméti *et al.*, 2016; Singh *et al.*, 2018; Abdelfattah *et al.*, 2019; Singh *et al.*, 2019; Deyett & Rolshausen, 2020; Perazzolli *et al.*, 2020; Cureau *et al.*, 2021; Liu & Howell, 2021; Steenworth *et al.*, 2021; Behrens & Fischer, 2022; Testempasis *et al.*, 2023; Wicaksono *et al.*, 2023; Cui *et al.*, 2024; Leal *et al.*, 2024; Teixeira *et al.*, 2024). However, to our knowledge, there have been no studies that capture the impact of temperature and phenology on the microbial communities of grapevines. Moreover, in Canada, there have been very few experiments exploring the relationship between grapevines and their microbial communities. Furthermore, to our knowledge, there have been no studies on the microbial communities of the hybrid grapevine cultivars grown in Atlantic Canada, nor of their response to the climate crisis. Therefore, experimentally modelling how fungal communities of grapevine phyllospheres and fruits (carposphere) change in response to increased temperatures at different phenologies will be useful for understanding i) fungal community dynamics over time, and ii) the impact of the climate crisis on the fungal communities.

Here, we report a field experiment used to test whether changes in temperature patterns at different stages of fruit development (pre-veraison and post-veraison) or a global rise in temperature (whole season) altered the fungal communities of grapevine phyllosphere and carposphere. Our hypothesis was that increased heat treatments would significantly increase fungal diversity of the phyllosphere and carposphere regardless of development stage. As such, we predicted that i) fungal diversity would increase over the growing season in untreated controls, ii) diversity would be higher among heat treated samples than the untreated controls, and iii) diversity would not be different between treated samples from different growth stages. We used on-the-row mini-greenhouses to increase the temperature at different fruit developmental stages of *Vitis* sp. cv. L'Acadie blanc; *i.e.*, pre-veraison, post-veraison, and throughout the growing season. Leaves and fruits were sampled at four developmental stages, and fungi were identified *via* ITS metabarcoding. We measured changes to fungal community composition and α - and β -diversity across different developmental stages to assess the impact of the increased temperature regimes to the grapevine phyllosphere and carposphere communities.

These results will allow us to evaluate the adaptability of the fungal community in the face of increasing temperatures in Atlantic Canada. Our data will also generate insights into the potential agroecological roles of grapevine fungal communities, such as pathogenicity and biological control, as well as the fungal components of the *terroir*.

MATERIALS AND METHODS

1. Site and experimental design

The field experiment, previously described in Campos-Arguedas *et al.*, 2022, was conducted at a commercial vineyard in the Gaspereau Valley in Wolfville, Nova Scotia (45° 4' 19" N 64° 17' 44" W) during the 2020 growing season. The experimental site was planted with an 11-year-old *Vitis* sp. cv. L'Acadie blanc (Cascade X Seyve-Villard 14-287). The experimental design was a randomised split-plot replicated in five complete blocks. Within each block, plots were split into four treatments, where polycarbonate mini-greenhouses were installed at specific growth stages to simulate increased temperatures. The control treatment lacked greenhouses throughout the season, and was therefore exposed to the ambient environmental conditions without any temperature treatment. The specific growth stages were determined by the modified Eichhorn-Lorenz (EL) system (Coombe, 1995). The whole season heat treatment maintained greenhouses from June the 3rd (developmental stage EL-15) to October the 9th (harvest, EL-38), with an increased growing degree (GDD) days of 197 relative to the control (as previously illustrated by Campos-Arguedas *et al.*, 2022). The pre-veraison heat treatment greenhouses were installed on June the 3rd until September the 10th (onset of veraison, EL-35), with an associated increase of 128 GDD, while in the post-veraison heat treatment the greenhouses were installed on September the 11th until October the 9th (harvest, EL-38), with an increase of 44 GDD (see Campos-Arguedas *et al.*, 2022 for further design details). These periods were selected based on the double sigmoid curve grape berries follow during their development. Note that due to climate variability in field trials, it is not possible to have a fixed temperature increase. Rather, the number of GDD are often reported in agricultural and viticultural field trials. GDD measure the number of days a temperature required for a particular biological process, like grapevine growth, occurred. Therefore, a treatment that received more GDD than the control in the same field trial will have received higher temperatures (Parker *et al.*, 2011; Gregorich *et al.*, 2016; Steenwerth *et al.*, 2021).

2. Crop management and sampling

Grapevines were grown and maintained according to standard management practices, as previously described by Campos-Arguedas *et al.* (2022). *Vitis* sp. leaves and berries were sampled at the following growth stages: EL-32 (bunch closure), EL-36 (intermediate fruit sugar levels), EL-37 (fruit not quite ripe), and EL-38 (ripe and harvest). At each of these growth stages, 6-8 berry clusters and their

adjacent leaves were randomly picked from all four vines in each treatment per block. Sampled material was divided into leaves and fruits and pooled, generating 160 samples (two compartments (*i.e.*, phyllosphere and carposphere) *4 growth stages *4 treatments *5 replicates). Samples were immediately frozen with liquid nitrogen, transported in dry ice, and stored at -80°C before being shipped to Université de Montréal's Biodiversity Centre (Montréal, QC, Canada) on dry ice for further processing (Delavaux *et al.*, 2020; Blakney *et al.*, 2022). As is typical for field experiments, we accounted for the use of the various agricultural management practices (*i.e.*, experimental treatments, fertilisers, pesticides, and management) in the downstream amplicon data by considering each sample and its management history as a unit; *i.e.*, the detection of a given fungal species is considered to be due to the total effect of the management and experimental treatments of the samples.

3. DNA extraction from *Vitis* sp. leaf and fruit samples

Total DNA was extracted from leaves and fruits after being ground separately in liquid nitrogen *via* sterile mortar and pestles. DNeasy Plant DNA Extraction Kits (Qiagen, Germany) were used following the manufacturer's instructions with ~ 160 mg of leaf material and ~ 225 mg of fruit material (Lay *et al.*, 2018; Blakney *et al.*, 2022). No-template extraction negative controls were included with each kit used in order to assess the influence of the extraction kits on our sequencing results, and the efficacy of our lab preparation. All extracted DNA samples were qualitatively evaluated by mixing ~ 2 μL of each sample with 1 μL of loading dye containing Gel Red (Biotium) and running it on a 0.7 % agarose gel for 50 minutes at 110 V. The no-template extraction negative controls were confirmed not to contain DNA after extraction.

4. Amplicon generation and sequencing to estimate fungal communities

To estimate the composition of the fungal communities in the phyllosphere and carposphere across the *Vitis* sp. growth stages, extracted DNA from all samples were used to prepare ITS amplicon libraries following Illumina's MiSeq protocols (Bell *et al.*, 2016; Lay *et al.*, 2018; Blakney *et al.*, 2022). First, all DNA samples were diluted 1:10 into 96-well plates. To assess potential bias caused by laboratory manipulations, sequencing and downstream bioinformatic processing, we also included the no-template extraction control samples.

The prepared plates of the *Vitis* sp. leaf and fruit DNA samples were submitted to Génome Québec (Montréal, Québec) for ITS amplicon generation and sequencing (Bell *et al.*, 2016; Lay *et al.*, 2018; Blakney *et al.*, 2023). Diluted DNA samples were used as templates for PCR amplification with the ITS3-KYO2 forward and ITS4-KYO3 reverse primers, which generated a 430 bp fragment from the ITS2 region, between the 5.8S and LSU regions (Toju *et al.*, 2012; Morvan *et al.*, 2020). Amplicons were then prepared for paired-end sequencing using Illumina's MiSeq

platform (Génome Québec, Montréal) (Bell *et al.*, 2016; Lay *et al.*, 2018; Blakney *et al.*, 2022). We estimated this would provide a mean of 40,000 ITS reads per sample, which is in line with previous studies that describe bacterial communities (Bell *et al.*, 2016; Lay *et al.*, 2018; Blakney *et al.*, 2022; Morvan *et al.*, 2020). Raw sequencing data and metadata are publicly available at NCBI Bioproject under accession number: PRA1046454.

5. Estimating ASV's from amplicon sequencing

The amplicons generated by Illumina MiSeq were used to estimate the diversity and composition of the fungal communities present in the leaves and fruits of *Vitis* sp. at different growth stages. The integrity and totality of the MiSeq data downloaded from Génome Québec was confirmed using their MD5 checksum protocol (Roy *et al.*, 2018). Subsequently, all data was managed and analysed in R (4.0.3 R Core Team, 2020), and plotted using ggplot2 (Wickham, 2016).

The raw ITS reads were processed to retain the highest quality reads before ASV inference and taxonomic assignment. Due to the variable length of the ITS region, we first used cutadapt (Martin, 2011) to carefully remove primer sequences from all 1,616,465 raw ITS reads generated from the control samples and the *Vitis* sp. samples, including any primer sequences generated due to read-through (Blakney *et al.*, 2023). The filtered and trimmed reads for the ITS region were then processed through DADA2 for ASV inference (Figure S1). The default settings were kept throughout the pipeline, except the `dada` inference function, which used the `pool = 'pseudo'` argument to increase the likelihood of identifying rare taxa. Consequently, the chimera removal function `removeBimeraDenovo` included the `method = 'pooled'` argument (Callahan *et al.*, 2016b).

Fungal amplicon sequence variants (ASVs) were assigned taxonomy following the default DADA2 pipeline (Callahan *et al.*, 2016b) and using the UNITE fungal database (Abarenkov *et al.*, 2022), as well as the UNITE database for all eukaryotes (Abarenkov *et al.*, 2020), in order to confirm fungal identities (Tedersoo *et al.*, 2018). Although this is the standard pipeline for fungal ITS2 ASV identification, it is worth noting that variability in the amplicon can bias taxonomy assignment (Yang *et al.*, 2018; Nilsson *et al.*, 2019; Sene *et al.*, 2025). The quality of the data was assessed using the included controls, and any off-target eukaryotic ASVs identified in the fungal data were removed. Rarefaction curves confirmed that we obtained sufficient coverage of the fungi present in both the phyllosphere and carposphere (Figure S2; Blakney *et al.*, 2024a).

6. α -diversity of the *Vitis* sp. fungal communities in the phyllosphere and carposphere

First, to visualise the taxonomic diversity, ASVs from the phyllosphere and carposphere were plotted separately as taxa cluster maps using the `heat_tree` function from the `metadecoder` package (Foster *et al.*, 2017), where nodes represent phyla to genera: node colours represent the

abundance of 16S rRNA reads, while node size indicates the number of unique taxa. Taxa cluster maps facilitate the visualisation of abundance, as well as diversity across taxonomic hierarchies (Foster *et al.*, 2017).

Second, we compared species richness using Simpson's α -diversity index calculated from the phyloseq object (McMurdie & Holmes, 2013). We assessed differences in the mean indices for each heat treatment between growth stages, and their interactions using a multi-factor ANOVA and Tukey's post-hoc test for significant groups that respected the assumptions of normality (Blakney *et al.*, 2022; Blakney *et al.*, 2023). Normality of the residuals was established with a Shapiro-Wilk test using the `shapiro.test` function, while the heteroscedasticity of residuals was confirmed with using a Bartlett test, `bartlett.test` function. For significant ANOVAs, a post-hoc Tukey's Honest Significant Difference test, TukeyHSD, was used to determine which groups were statistically different.

7. Identification of differently abundant ASV's and specific indicator species

To refine our understanding of the abundance and composition of the *Vitis* sp. fungal communities, we used two complementary methods to identify taxa specific to growth stages and treatments. First, taxa cluster maps were used to calculate the differential abundance of ASVs between experimental groups using the `heat_tree_matrix` function from the `metadecoder` package (Foster *et al.*, 2017). Second, indicator species analysis was used to detect ASVs that were preferentially abundant in pre-defined environmental groups (compartments, growth stages, treatments) using the `multiplatt` function from the `indicspecies` package (De Cáceres & Legendre, 2009), with an FDR correction. A significant indicator value is obtained if an ASV has a large mean abundance within a group compared to another group (specificity), and if it has a presence in most samples of that group (fidelity) (De Cáceres & Legendre, 2009; Legendre & Legendre, 2012). The fidelity component complements the differential abundance approach between taxa clusters, which only considers abundance.

8. β -diversity of the *Vitis* sp. fungal communities in the phyllosphere and carposphere

To test for significant differences between the *Vitis* sp. fungal communities at different growth stages and heat treatments, we used the non-parametric permutational multivariate ANOVA (PERMANOVA), where any variation in the ordinated data distance matrix is divided among all the pairs of specified experimental factors. The PERMANOVA was calculated using the `adonis`, `adonis` function, from the `vegan` package (Oksanen *et al.*, 2020), with a distance matrix calculated using the Bray-Curtis formula, with 9999 permutations, and the experimental blocks were included as "strata". We used Bray-Curtis distances because it is appropriately sensitive for microbial community data, and the frequent "zero" counts of ASVs common in these datasets (Jeganathan & Holmes, 2021; Kers & Saccenti, 2022).

We confirmed that our data met the assumption of homogeneity using the `betadispr` function, and applied an ANOVA and Tukey's Honest Significant Difference post-hoc test to determine that none of the groups were statistically different.

Similarity between communities was also tested and visualised using principal co-ordinate analysis (PCoA, Legendre & Legendre, 2012) using the Bray-Curtis distance matrix. Singleton ASVs were removed before the phyloseq data were transformed using Hellinger's transformation, such that ASVs with high abundances and few zeros are treated equivalently to those with low abundances and many zeros (Legendre & De Cáceres, 2013).

To further characterise the ecological mechanisms that may be responsible for changes to β -diversity, we partitioned β -diversity into two components: turnover (*i.e.*, species replacement) and nestedness (*i.e.*, loss/gains that result in poor species richness being a subset of richer sites; Blakney *et al.*, 2024b). We compared the β -diversity components for each heat-treated plot at each growth stage with its cognate plot at the following growth stage (*e.g.*, control phyllosphere at EL-32 was compared to control phyllosphere at EL-36), using the `betapart.core.abund` function from the `betapart` package (Baselga *et al.*, 2023). Within the phyllosphere and the carposphere, we separately identified any significant differences among the means of each component between growth stages for each heat treatment and their interactions, with a multi-factor ANOVA, as described above for α -diversity, as normality was respected.

RESULTS

1. Illumina MiSeq yielded similar numbers of fungal ASVs from both *Vitis* sp. phyllosphere and carposphere

Illumina's MiSeq produced 1,616,465 raw reads for the whole fungal ITS dataset, which were processed through cutadapt and DADA2 (Callahan *et al.*, 2016a; Callahan *et al.*, 2016b), where 1,160,088 reads were retained from all the experimental samples (Figure S1). The number of ITS reads were similar between both leaf and fruit samples, and across growth stages, with a mean of $8,044 \pm 2,328$ reads among leaves, and $7,865 \pm 2,281$ reads among fruit (Table 1). From this, a total of 655 distinct fungal ASVs were inferred. The majority of reads from across the dataset were assigned to class *Dothideomycetes* (phylum *Ascomycota*), where the percentage of *Dothideomycetes* reads ranged from 43 % to 99 %, with an average of 93 %, across all 146 samples (Figures 1 and S3). We also observed a consistent trend concerning the number of unique ASVs across the dataset, with a mean of 29 ± 8 unique ASVs among leaf samples and a mean of 22 ± 6 ASVs among fruit samples (Table 1).

TABLE 1. The leaves and fruits of *Vitis* sp. cv. L'Acadie blanc, sampled throughout the 2020 growing season in Wolfville, Nova Scotia, yielded 1,616,465 raw reads for the fungal ITS data via Illumina's MiSeq platform at Génome Québec. The raw reads were processed through DADA2 to retain 1,160,088 reads (ITS Reads reported here) for ASV inference. A total of 655 fungal ASVs were identified across the dataset.

Growth stage	Compartment	ITS reads	Fungal ASV occurrence
EL-32	Leaf n = 13	6,628 ± ,858	26 ± 9
	Fruit n = 20	7,225 ± 1,967	20 ± 7
EL-36	Leaf n = 19	7,860 ± 1,901	28 ± 5
	Fruit n = 20	7,470 ± 1,751	22 ± 7
EL-37	Leaf n = 18	9,367 ± 2,644	30 ± 6
	Fruit n = 20	8,980 ± 2,978	24 ± 5
EL-38	Leaf n = 16	7,922 ± 2,137	33 ± 9
	Fruit n = 20	7,786 ± 1,982	21 ± 5

2. Fungal communities were significantly different between phyllosphere and carposphere

Overall, the PERMANOVA supported that the fungal communities from the phyllosphere and carposphere of *Vitis* sp. were significantly different, though the effect size was small (PERM $R^2 = 0.01461$, $p = 0.044$; Table 2). The interaction between growth stage and compartments (*i.e.*, phyllosphere and carposphere) was also significant (Table 2). Several ASVs were identified as specific to the phyllosphere, though weakly significant (Table 3); the ascomycetes *Nigrospora* spp. (*Trichosphaeriaceae*), *Sphaerulina* spp. (*Mycosphaerellaceae*), *Alternaria* spp. (*Pleosporaceae*), an unknown *Didymellaceae* (*Pleosporales*), and a sole basidiomycete, *Piptoporus* spp. (*Fomitopsidaceae*, Table 3). Only ASVs identified as *Cladosporium* spp. (*Cladosporiaceae*) were enriched (*i.e.*, more reads) in the phyllosphere compared to the carposphere ($p < 0.05$; Figure S4). In the carposphere, only the *Polyporales* and *Hymenochaetales* orders were enriched compared to the phyllosphere ($p < 0.05$; Figure S4), but no family or genus was found to be significant. Two ASVs were identified as specific to the carposphere, the basidiomycetes *Naganishia* spp. (*Filobasidiaceae*), and *Tilletiopsis* spp. (*Entylomatales*; Table 3).

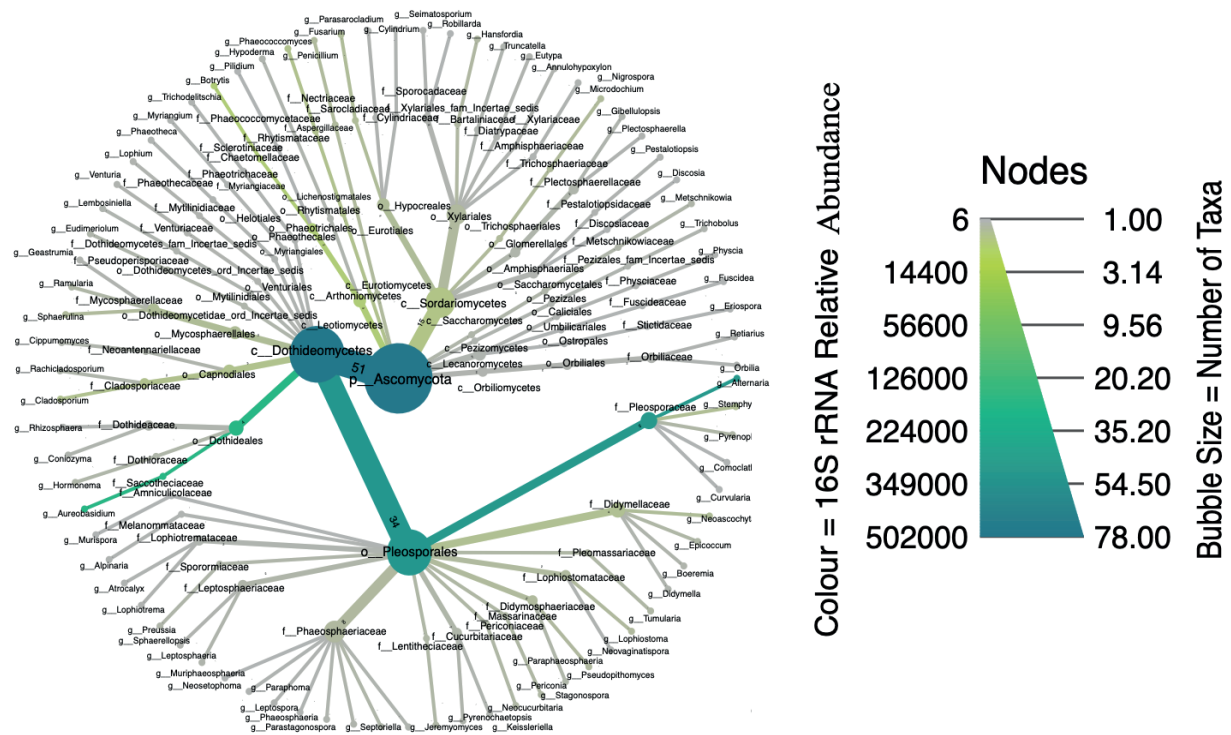
Contrary to our initial hypothesis, where we predicted that fungal diversity would increase over the growing season, we observed little influence of the different growth

stages on α - (Figure 2) and β -diversity (Figure 3). For example, although we did find that different growth stages had a significant effect on the fungal communities in the phyllosphere and carposphere, the effect size was small (PERM $R^2 = 0.03329$, $p = 0.027$; Table 2). We also found that species turnover across growth stages was more significant in explaining the β -diversity of phyllosphere and carposphere communities than species nestedness (p . adj < 0.001 ; Figure 3D). We did not detect any taxonomic enrichments or depletions according to growth stage (Figure 4), and the fungal communities remained largely taxonomically similar through time (Figure 3A).

3. Increased heat significantly altered fungal communities in the phyllosphere

The different heat treatments did significantly influence the fungal communities of the phyllosphere and carposphere of *Vitis* sp, and with a larger effect size than compartment or phenology (PERM $R^2 = 0.05794$, $p < 0.001$; Table 2). The interaction between treatment and growth stage was also significant (Table 2), though likely driven by the significant changes between the phyllosphere and carposphere communities, and the significant impact of the heat treatments. Although the structure and composition of the phyllosphere and carposphere communities across all treatments remained largely similar (Figure 3A) among the fungal phyllosphere communities, the heat treatments had significant effects on the α -diversity.

A Phyllosphere Ascomycota ASVs



B Phyllosphere Basidiomycota ASVs

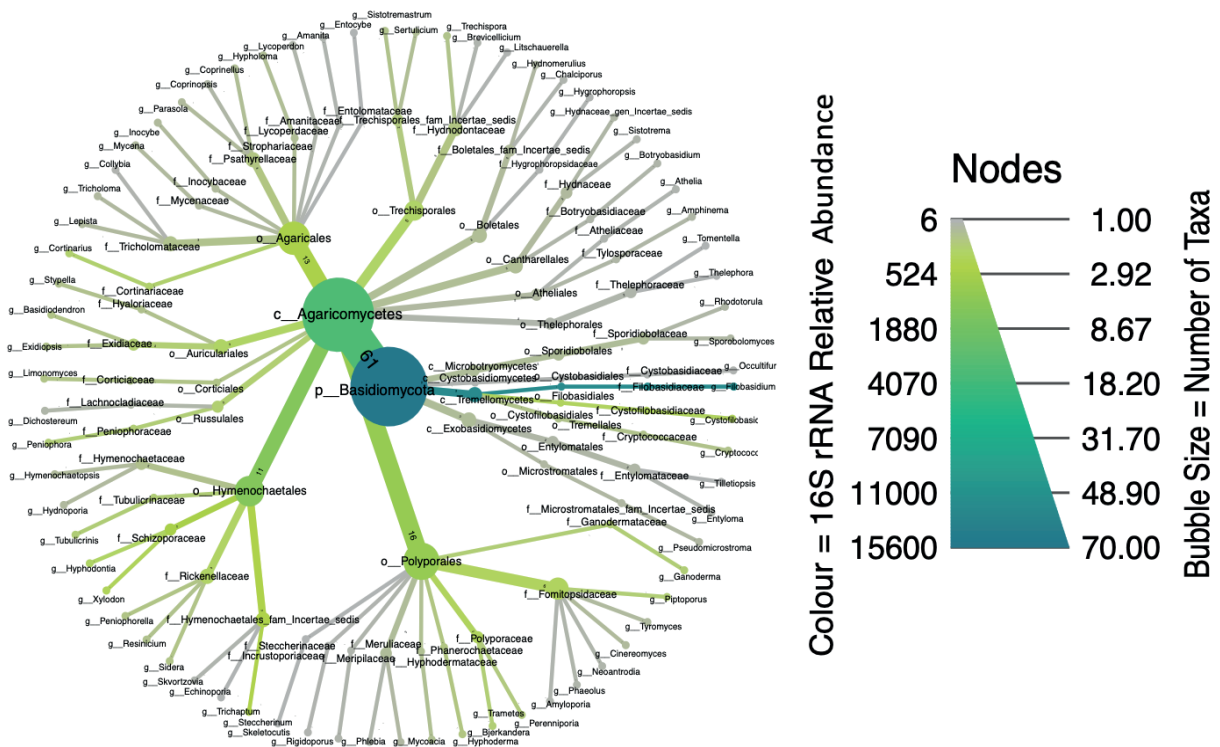
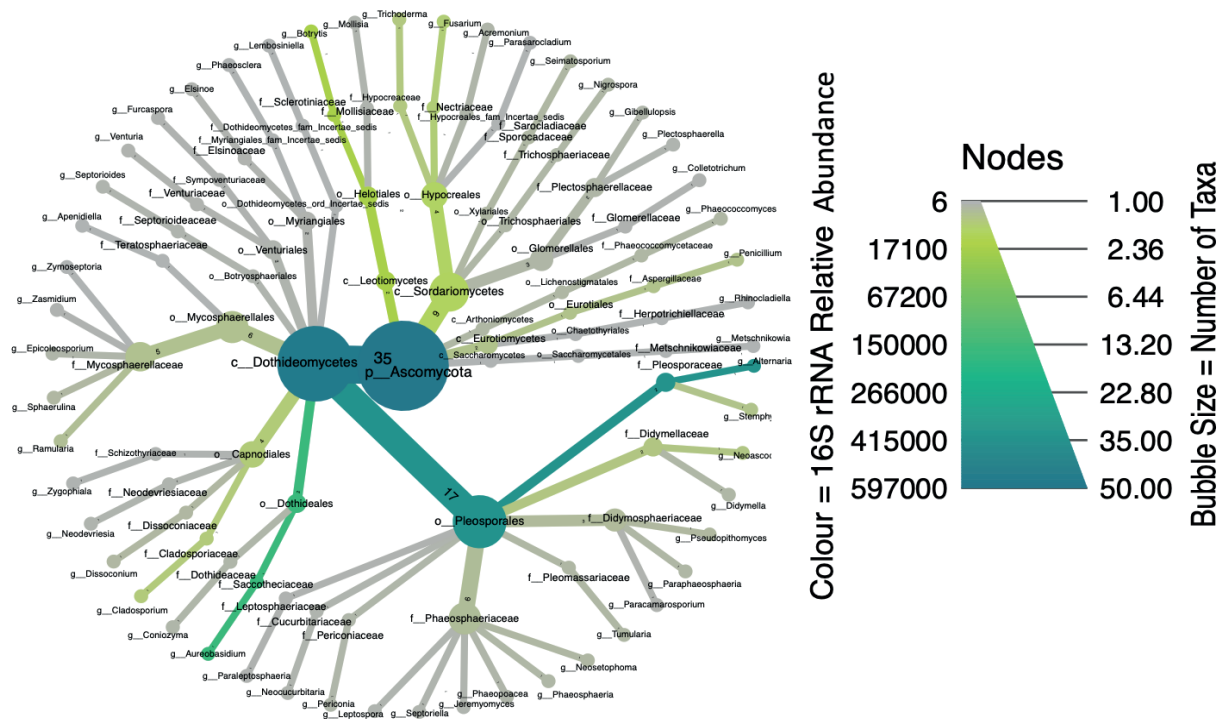


FIGURE 1. Fungal community composition from the phyllosphere (A and B) and carposphere (C and D) of *Vitis* sp. cv. L'Acadie blanc sampled across the 2020 season in Wolfville, Nova Scotia. The taxa clusters illustrate that the phyllosphere communities (A and B) had higher diversity than the carposphere (C and D). Communities were dominated by ascomycetes (A and C), of the class *Dothideomycetes*. The dominant *Dothideomycetes* were primarily *Alternaria* spp. and *Aureobasidium* spp. in both phyllosphere (A) and carposphere (C), while the dominant basidiomycetes in the phyllosphere (B) and carposphere (D) was *Filobasidium* spp. (part 1/2)

C Carposphere Ascomycota ASVs



D Carposphere Basidiomycota ASVs

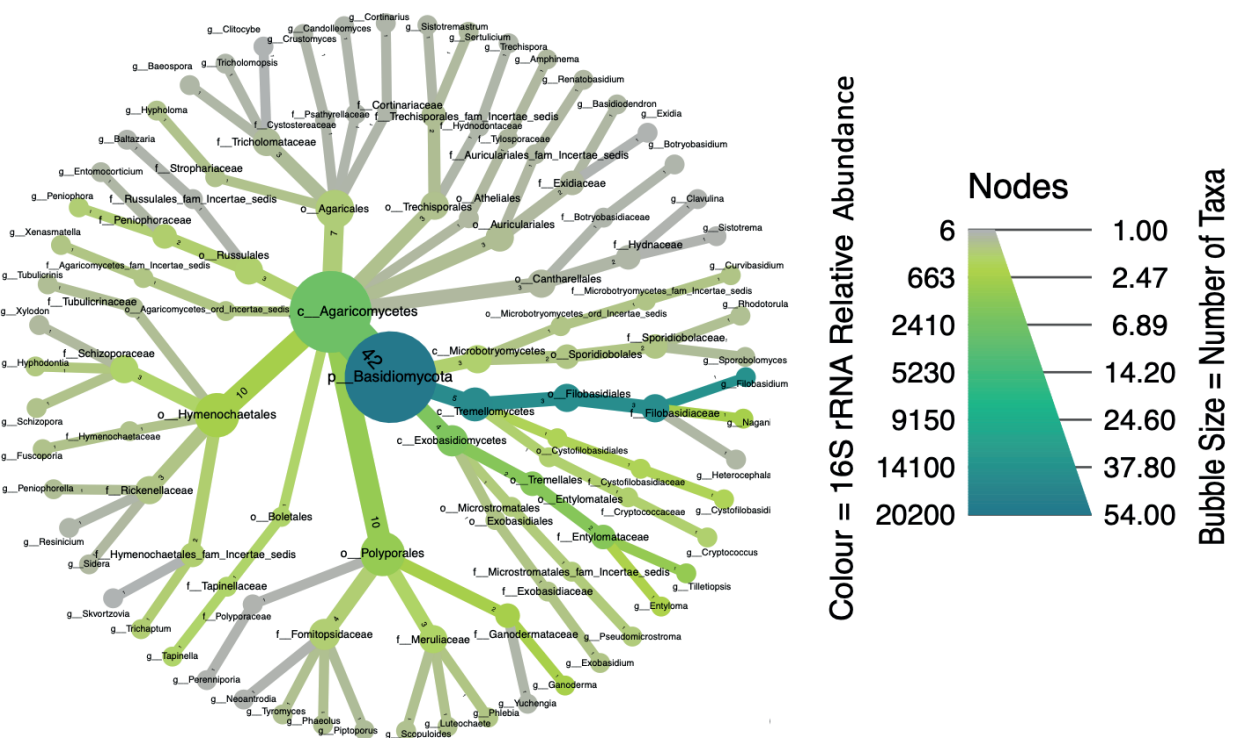


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TABLE 2. PERMANOVA identified compartments, growth stage and mini-greenhouse treatments as significant experimental factors for the fungal communities harvested in 2020 from *Vitis* sp. in Wolfville, Nova Scotia. Only significant interactions ($p \leq 0.05$) are presented (Pr (> F) in bold). PERMANOVA was calculated using a Bray-Curtis distance matrix, with 9999 permutations.

	ITS		
	F model	R ²	Pr (> F)
Compartment ^a	2.4943	0.01461	0.044
Growth stage ^b	1.8943	0.03329	0.027
Treatment ^c	3.2973	0.05794	0.001
Compartment ~ Growth stage	2.0629	0.03625	0.022
Compartment ~ Growth stage ~ Treatment	2.0613	0.10866	0.003

^a phyllosphere or carposphere communities.

^b growth stages: EL-32 (fruit bunching), EL-36 (intermediate fruit sugar levels), EL-37 (fruit not quite ripe), and EL-38 (ripe and harvest).

^c heat treatments applied pre-veraison (before ripening), post-veraison, all-season, or untreated control.

TABLE 3. Indicator fungal species were identified exclusively among the phyllosphere or carposphere of *Vitis* sp. grown during the 2020 season in Wolfville, Nova Scotia. Indicator species analysis relies on abundance and site specificity to statistically test each ASV, which we report here as a tendency ($p < 0.1$), with a FDR correction.

	Closest taxon	Compartment ^a ($p < 0.1$)
Ascomycetes	<i>Alternaria</i> spp.	Phyllosphere
	<i>Didymellaceae</i>	Phyllosphere
	<i>Nigrospora</i> spp.	Phyllosphere
	<i>Sphaerulina</i> spp.	Phyllosphere
Basidiomycetes	<i>Piptoporus</i> spp.	Phyllosphere
	<i>Naganishia</i> spp.	Carposphere
	<i>Tilletiopsis</i> spp.	Carposphere

^a phyllosphere or carposphere communities.

Simpson's index was higher for phyllosphere communities treated with increased heat pre-veraison at growth stages EL-32, 36, and 38, when compared to communities treated with increased heat post-veraison (p . adj = 0.04631; Figure 2A), in opposition to our initial prediction. Moreover, it is worth noting that the untreated control phyllosphere samples remained stable in terms of composition, relative abundance and diversity throughout the experiment (Figures 2A, 3B and 3D), contrary to our prediction. This further supports the relatively weak influence of the different growth stages on phyllosphere community structure (Figure 2A). In the β -diversity analyses of the phyllosphere, the PCoA captured 45.9 % of the variation and illustrated a shift in fungal

community similarity, where communities from the control and post-veraison heated communities were more similar in composition *versus* communities that were heated all season and pre-veraison (Figure 3B). The increased heat treatments also increased species turnover relative to the controls in the phyllosphere communities (p . adj < 0.05; Figure 3D).

A number of fungal taxa were also significantly enriched among the phyllosphere communities due to specific treatments ($p < 0.05$; Figure 4). ASVs belonging *Cladosporium* spp. and the *Mycosphaerellaceae* were over-represented in samples treated for the whole season with increased heat compared to samples from the control and post-veraison heat treatment ($p < 0.05$; Figure 4A).

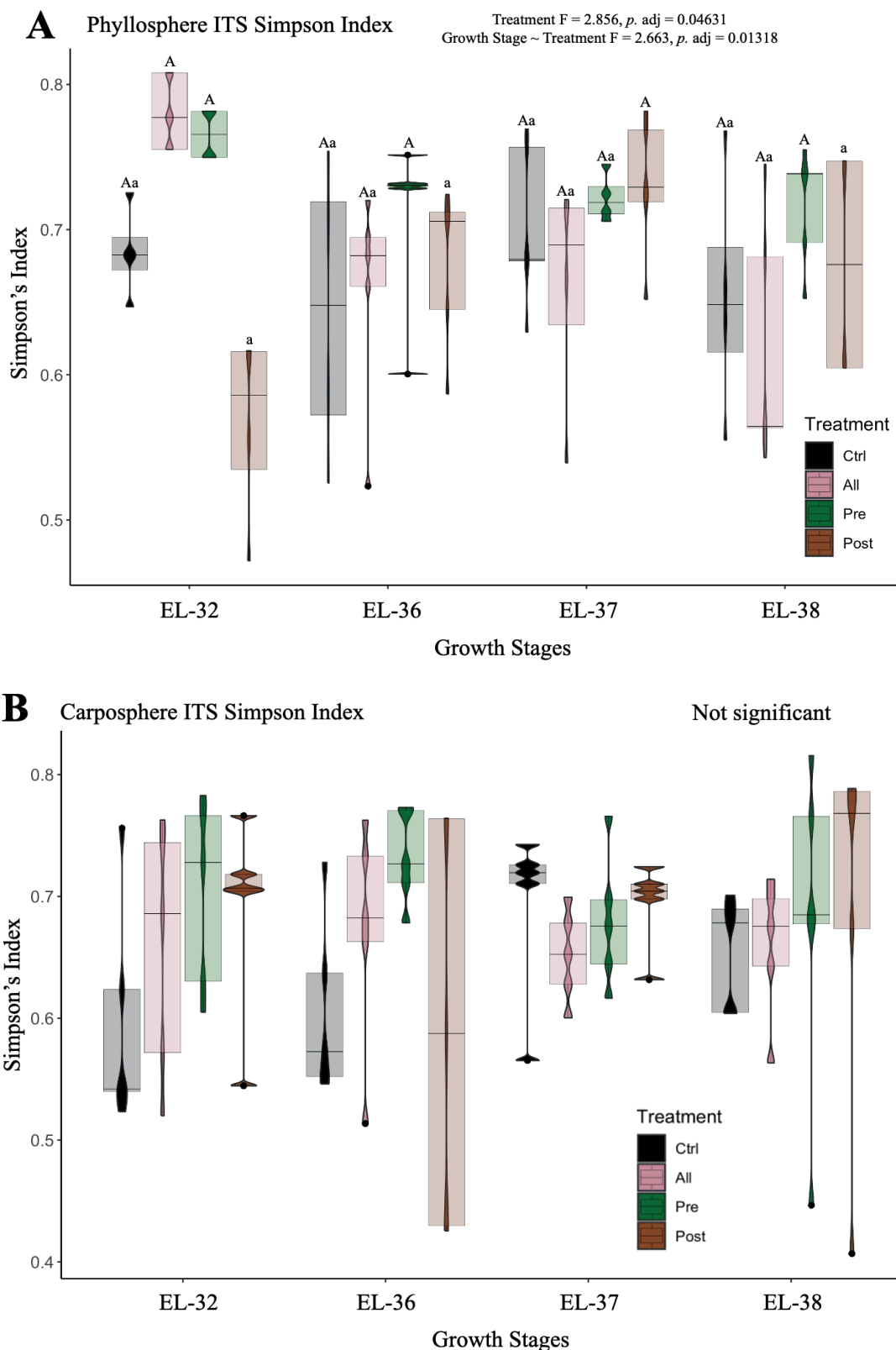


FIGURE 2. Fungal community diversity among *Vitis* sp. phyllospheres was significantly impacted by the different mini-greenhouse treatments (A, ANOVA *p* = 0.04631), while the carposphere communities remained stable (B) across the 2020 season in Wolfville, Nova Scotia. (A) Simpson’s diversity index was significantly higher for phyllosphere communities treated pre-veraison across the EL-32, 36, and 38 growth stages, when compared to communities treated post-veraison. Although the growth stages were not significant factors in the model, the growth stage ~ treatment interaction was (ANOVA *p* = 0.01318). (B) Diversity was not significantly impacted by growth stage or treatment in the carposphere fungal communities.

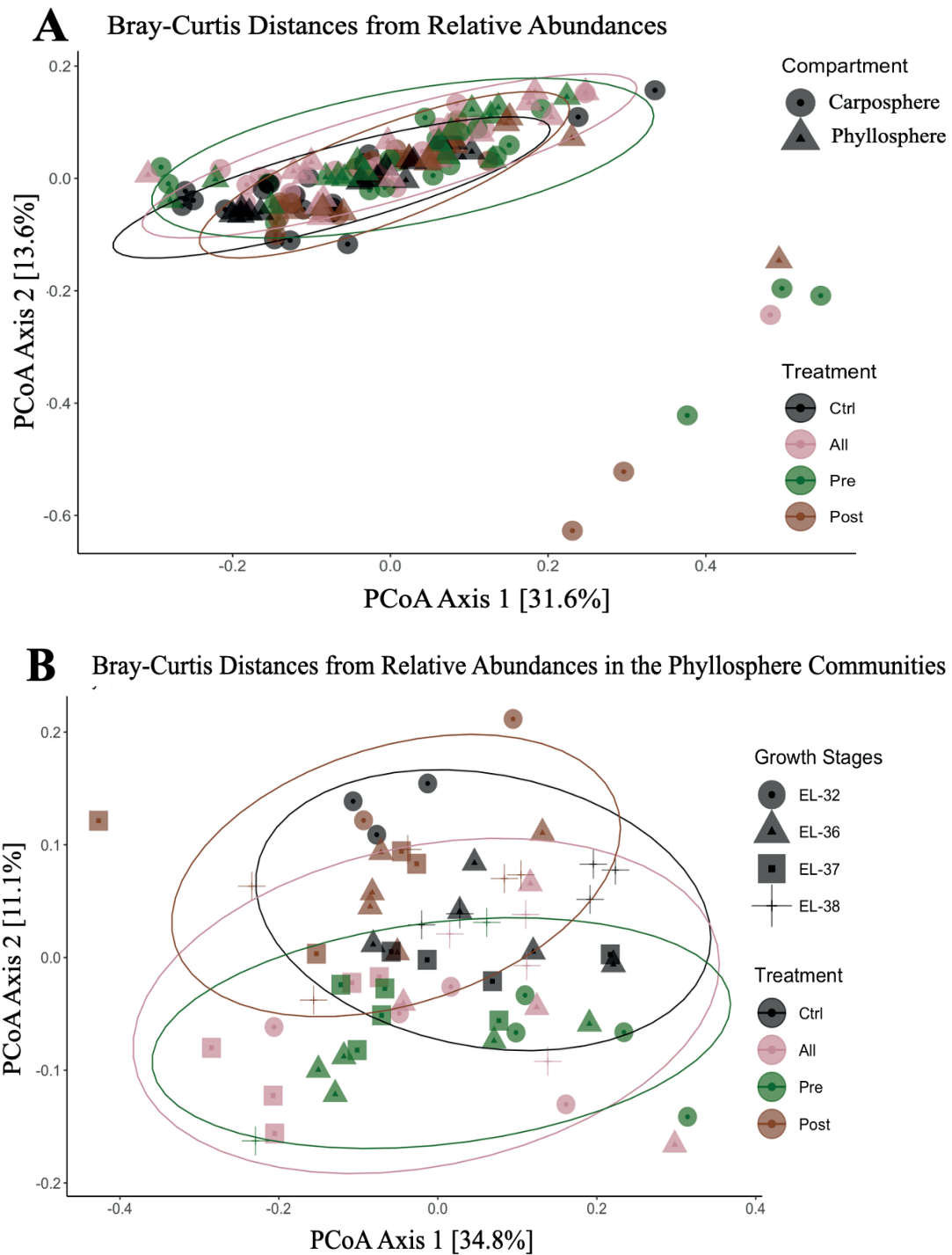


FIGURE 3. β -diversity of fungal communities identified from the phyllosphere and carposphere of *Vitis* sp. illustrates similar composition and structure between compartments and growth stages, primarily explained by species turnover. Samples were harvested throughout the 2020 growing season in Wolfville, Nova Scotia. (A) Principal co-ordinate analysis captured 45.2 % of the variability among the phyllosphere and carposphere communities, which remained similar in composition and diversity between compartments and across temperature treatments. (B) Principal co-ordinate analysis captured 45.9 % of the variability among the phyllosphere communities, where samples from the control and post-veraison treatments were more compositionally similar compared to communities from other treatments. Phyllosphere communities from the EL-38 growth stage were also more compositionally similar, regardless of treatment, compared to communities from other growth stages. (C) Principal co-ordinate analysis captured 50.8 % of the variability among the carposphere communities, which remained similar in composition and diversity across growth stages and between temperature treatments. (D) Species turnover was significantly higher than nestedness in both phyllosphere and carposphere communities (ANOVA p . adj < 0.001), with turnover being significantly higher among treated phyllosphere communities compared to control communities (p . adj < 0.05). (part 1/2)

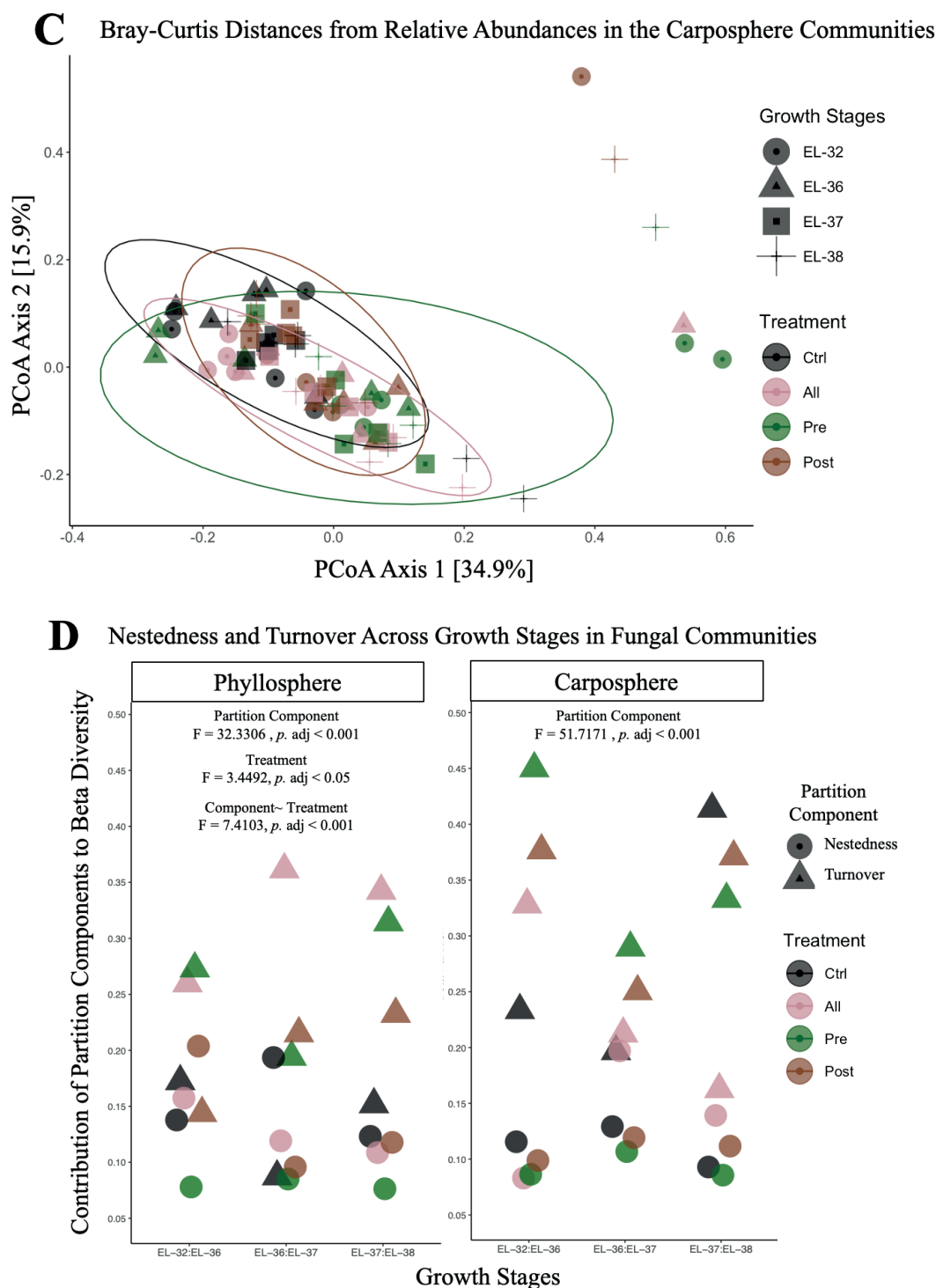


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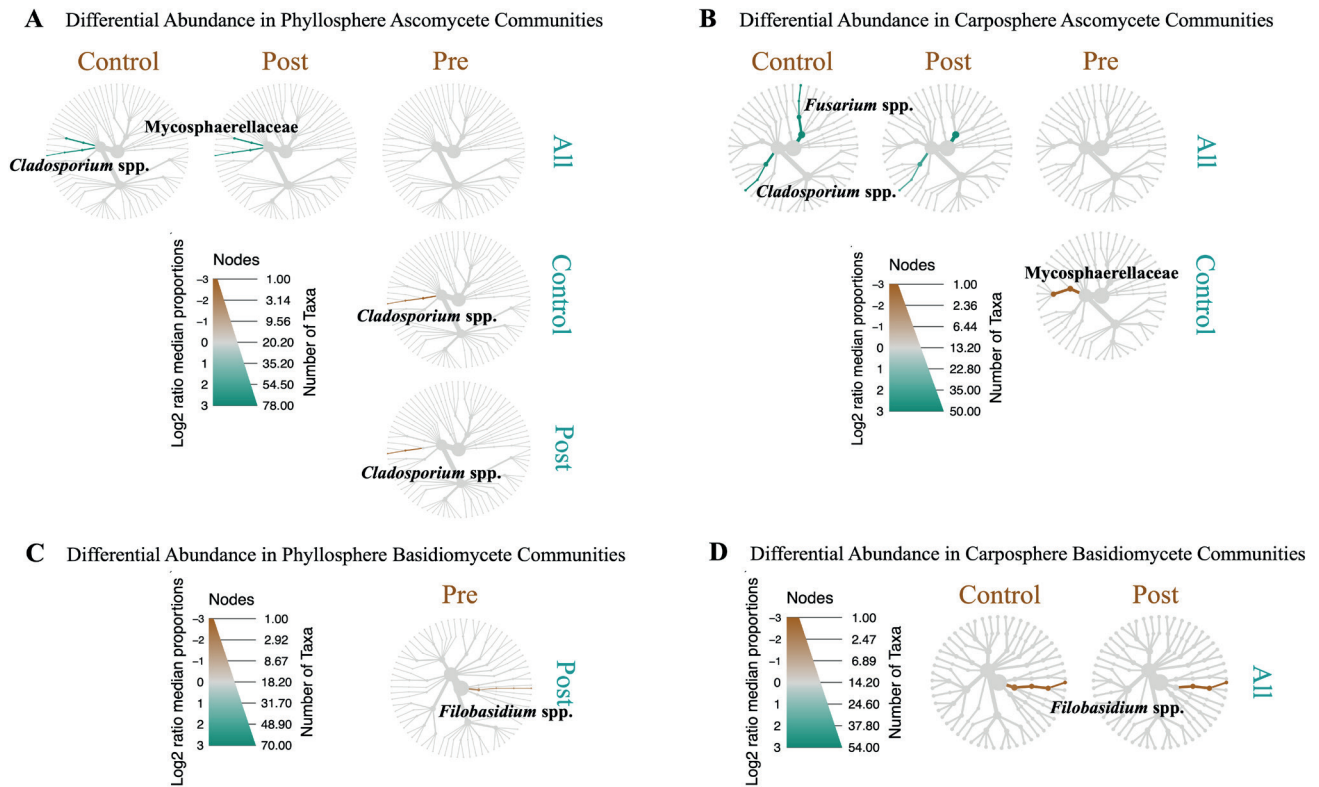


FIGURE 4. Certain fungal ASVs were significantly enriched (labelled in bold, Kruskal $p < 0.05$) in the phyllosphere (A and C) and carposphere (B and D) due to the increased temperature treatments, but not due to growth stage of *Vitis* sp. grown during the 2020 season in Wolfville, Nova Scotia. (A) Among the phyllosphere ascomycete communities, ASVs identified as *Cladosporium* spp. and *Mycosphaerellaceae* were over-represented in samples treated all season compared to both control and post-veraison samples. Similarly, samples treated with increased heat during pre-veraison were also enriched in *Cladosporium* spp., compared to control and post-veraison samples. (B) Among the carposphere ascomycete communities, ASVs for *Cladosporium* and *Fusarium* spp. were enriched in samples treated all season compared to both control and post-veraison samples. Pre-veraison carposphere samples were also enriched in the *Mycosphaerellaceae* compared to the control samples. Among the basidiomycete communities, phyllosphere samples treated pre-veraison were over-abundant in *Filobasidium* spp. compared to the samples treated post-veraison (C), while *Filobasidium* spp. were also enriched in the carposphere control and post-veraison samples compared to those treated with increased heat all season (D). Taxa that were significantly more abundant are highlighted brown or green, following the labels for each compared host.

Samples treated with increased heat pre-veraison were also over-abundant in *Cladosporium* spp. compared to control samples or those treated with increased heat all post-veraison ($p < 0.05$; Figure 4A). The pre-veraison samples were also enriched in *Filobasidium* spp. compared to the samples treated with increased heat post-veraison ($p < 0.05$; Figure 4C).

In the *Vitis* sp. carposphere communities, the α - (Figure 2B) and β -diversities (Figure 3) remained stable across the growing season regardless of heat treatments, contrary to our predictions. However, there were slight variations among taxa in the carposphere communities: the samples treated with increased heat all season were enriched in *Cladosporium* and *Fusarium* spp., relative to both the control and post-veraison samples ($p < 0.05$; Figure 4B), while the pre-veraison fruit samples were enriched in the *Mycosphaerellaceae* compared to the controls ($p < 0.05$; Figure 4B). Finally, the control samples and those treated with increased heat pre-veraison were enriched in *Filobasidium* spp. compared to samples treated all season ($p < 0.05$; Figure 4D).

DISCUSSION

Microbial communities are influenced by temperature and are expected to be impacted globally due to increased temperature variation induced by the climate crisis (Malik *et al.*, 2019; Jansson & Hofmöckel, 2020; Koskella, 2020; Zhu *et al.*, 2021; Perreault & Laforest-Lapointe, 2022; Tiedje *et al.*, 2022). This will have significant consequences on productivity and the quality of most agricultural crops, including grapevines, as microbial communities are tightly related to the *terroir* characteristics of wine (Bokulich *et al.*, 2014; Bokulich *et al.*, 2016; Belda *et al.*, 2017; Liu *et al.*, 2020; Zhou *et al.*, 2021; Gobbi *et al.*, 2022; Johnston-Monje *et al.*, 2023). Therefore, changes in the fungal community of the phyllosphere or carposphere could have negative effects by favouring pathogen populations. Changes in these fungal communities could also yield positive effects by benefitting fungi with a role in biological control or the regulation of pathogen populations. However, despite their important role in grapevine

health and disease, the impact of temperature and phenology on the fungal community in the grapevine phyllosphere has been understudied (Perazzolli *et al.*, 2014; Fort *et al.*, 2016; Kecskeméti *et al.*, 2016; Singh *et al.*, 2018; Abdelfattah *et al.*, 2019; Singh *et al.*, 2019; Deyett & Rolshausen, 2020; Perazzolli *et al.*, 2020; Cureau *et al.*, 2021; Liu & Howell, 2021; Steenworth *et al.*, 2021; Behrens & Fischer, 2022; Testempasis *et al.*, 2023; Wicaksono *et al.*, 2023; Cui *et al.*, 2024; Leal *et al.*, 2024; Teixeira *et al.*, 2024), even more so for the carposphere (Kecskeméti *et al.*, 2016; Deyett & Rolshausen, 2020; Testempasis *et al.*, 2023). Here, our field experiment used on-the-row mini-greenhouses to increase the temperature of *Vitis* sp. cv. L'Acadie blanc across four different growth stages. We then tested how increased temperatures significantly altered the fungal communities of the phyllosphere and carposphere of *Vitis* sp. throughout the growing season. We identified the fungal communities of the phyllosphere and carposphere at each growth stage using ITS metabarcoding and measured fungal community composition and α - and β -diversity to assess the impact of the increased temperature treatments to the phyllosphere and carposphere communities.

Our results showed typical fungal communities for the phyllosphere, dominated by *Ascomycota*, with high abundance of *Dothideomycetes* (Figures 1 and S3), primarily *Alternaria* spp. and *Aureobasidium* spp. (Kraus *et al.*, 2019; Molnár *et al.*, 2023). Common phyllosphere fungi, including *Alternaria* and *Nigrospora* spp. were also identified as indicator species (Table 3). We observed that the increased temperature treatments had more significant effect on fungal communities than the different growth stages (Table 2, Figures 2, 3 and 4). Although the PERMANOVA supported the significant differences between the fungal communities across growth stages (Table 2), we did not observe any clear causes in terms of composition (Figures 1, 3 and 4). This could be attributed to fungal communities being more stable through time, unlike bacterial communities (Blakney *et al.*, 2022; Blakney *et al.*, 2023; Blakney *et al.*, 2024b). Indeed, previous studies have also reported limited temporal effects on fungal communities (Duchicela *et al.*, 2013; Gschwend *et al.*, 2021). Unlike bacterial communities, which grow and shift relatively rapidly, fungal communities tend to remain more stable over time and are less affected by their hosts (Hannula *et al.*, 2019; Hannula *et al.*, 2021; Zhang *et al.*, 2023; Li *et al.*, 2024). This may be due to fungi having slower growth rates and being more mobile than bacteria. Thus, in our experiment it would be reasonable not to detect vastly different fungal communities across different developmental stages.

Our most significant finding was that the increased temperature during the pre-veraison stage significantly increased the α -diversity of the phyllosphere fungal communities, and that this effect was maintained throughout the growing season (Figure 2A). This suggests that the phyllosphere fungal communities may be more prone to changes earlier in development, nearer the time microbial communities begin colonisation. For instance, the increased

temperature treatment could promote the establishment of primary colonisers or pioneer species, or create other kinds of niche space as a priority effect, which continues to structure the community throughout the season (Chase *et al.*, 2010; Hiscox *et al.*, 2015; Kraus *et al.*, 2019; Smets *et al.*, 2022). Interestingly, increasing the temperature all-season did not yield a similar increase in α -diversity of the phyllosphere communities as the pre-veraison treatment did (Figure 2A). Both treatments shared a similar temperature increase at a similar developmental stage for the leaf, but we did not observe a consistent impact on the diversity of the fungal communities. This could suggest that any priority effect or additional niche space created in the phyllosphere by the pre-veraison temperature treatment may not be sustained by the prolonged all-season heat treatment. Instead, the longer all-season heat treatment may provide more time for the fungal communities in the phyllosphere to recalibrate.

The potential pre-veraison priority effect could also be related to the significant enrichment of ASVs belonging to the *Cladosporium*, or *Filobasidium* spp. in the phyllosphere communities (Figure 4). Both groups have the potential to be antagonistic toward the plant: *Filobasidium* spp. are common basidiomycetes and tend to be poor saprotrophs, favouring environments where they can parasitize other fungi, or plants (Weiss *et al.*, 2014; Detheridge *et al.*, 2020), while *Cladosporium* spp. are also well-known in the phyllosphere and diverse plant pathogens (Kraus *et al.*, 2019; Cosseboom & Hu, 2023). We also identified a number of ASVs specific to the phyllosphere as indicator species (Table 3) from among the *Dothideomycetes* class, which dominated our communities by relative abundance (Figure 1), including *Sphaerulina* and *Alternaria* spp. (Table 3). A number of *Sphaerulina* species are known as causative agents of leaf spot disease among other plants (Ali *et al.*, 2021). Equally diverse among grapevine phyllosphere communities are *Alternaria* spp., which range from saprotrophs to pathogens (Molnár *et al.*, 2023). *Alternaria* spp. produce a wide range of metabolites and depending on their host and environmental factors can contribute to anti-microbial activities or virulence (Molnár *et al.*, 2023). Any of these taxa could contribute to the development of the community through priority effects (Fukami, 2015; Debray *et al.*, 2021).

The pre-veraison priority effect showed increased fungal diversity after increased heat early in fruit development and that higher diversity was maintained at each subsequent growth stage. Fungi arriving later during leaf development could depend on the earlier microbes i) occupying all available niche space (niche pre-emption), or ii) altering the niches that exist (niche modification; Fukami, 2015; Debray *et al.*, 2021). Soil fungi have been shown to extensively partition available niche space, allowing for increased diversity (Cho *et al.*, 2017). Other studies have also suggested that early-arrivals are less specialised and less able to manage microbe-microbe interactions, which encourages species turnover (Debray *et al.*, 2021; Wang *et al.*, 2023; Bai *et al.*, 2024). We also observed significantly increased species turnover in the pre-veraison

heat treated communities compared to the untreated controls (Figure 3C). Fungal turnover in the phyllosphere has been observed previously, suggesting dispersal-limited communities, subject to strong environmental filtering occurring on the leaf and fruits (Wang *et al.*, 2023; Bai *et al.*, 2024).

Our data suggests that primary colonisers could be used in biological control or even in agroecological protection, specifically by applying a cocktail of organisms early in the season composed of good colonisers or pioneer species in order to limit possible colonisation by phytopathogenic organisms (Kraus *et al.*, 2019). Biological disease control can be achieved by applying registered biological control agents, but also by promoting populations of biological control agents naturally present in the vineyard; an approach widely used in agroecology (Kraus *et al.*, 2019; IPPC, 2024). Microbes – essentially bacteria, fungi and nematodes (Lindow & Brandl, 2003; Perazzolli *et al.*, 2014; Sapkota *et al.*, 2015; Laforest-Lapointe *et al.*, 2017; Koskella, 2020) – that colonise the phyllosphere or carposphere (Vorholt, 2012) can act as biocontrol agents insofar as they compete for space and nutrients, *i.e.*, niche pre-emption (Fukami, 2015; Debray *et al.*, 2021). For example, *Aureobasidium pullulans* and *Trichoderma* spp. compete with the grapevine pathogen *Botrytis cinerea* (Fedele *et al.*, 2020); ASVs for both *Aureobasidium* and *Trichoderma* spp. were detected in our data (Figure 1).

The most common diseases present in northern climates are downy mildew (*Plasmopara viticola*), powdery mildew (*Erysiphe necator*), anthracnose (*Elsinoe ampelina*), and botrytis bunch rot (*Botrytis cinerea*), but there are more than 50 diseases listed in the American Compendium of Grape Disease (Carisse *et al.*, 2006; Wilcox *et al.*, 2015). Among the common phytopathogens identified in our data *Botrytis*, *Cladosporium*, and *Fusarium* spp., were all detected in the phyllosphere, and in higher abundance in the carposphere (Figure 1; Lorenzini & Zapparoli, 2015; Cosseboom & Hu, 2023; Bustamante *et al.*, 2024). These diseases not only affect yields, but also the quality of the grapes and, therefore, the wine they produce (Lorenzini & Zapparoli, 2015). Currently, these diseases can be controlled by the application of synthetic fungicides, which are still a key input in viticulture, because most traditional wine grape varieties (*V. vinifera* varieties) are highly sensitive to them (Pedneault & Provost, 2016; Provost & Pedneault, 2016). Synthetic fungicides can also change or damage the resident fungal community (Fournier *et al.*, 2020). As such, more and more winegrowers are seeking to reduce the use of these products in favour of biological disease control along with the introduction of resistant grape varieties (Pedneault & Provost, 2016; Provost & Pedneault, 2016; Zhang *et al.*, 2020; Candel *et al.*, 2023; Oliver *et al.*, 2024).

The advantage of using organisms naturally occurring in the vineyard is their redundancy, as several can either act in concert or individually if conditions favour one species

over another. Fungal communities can therefore play an important role in the resilience of agrosystems – including viticulture – to climate change, and be a tool for the agroecological control of grapevine diseases. Our study provides a robust baseline for the succession of fungal communities across the development of the phyllosphere and carposphere that future experiments can use, particularly in determining the ecological roles of the fungal ASVs we report.

CONCLUSION

Temperature variations induced by the climate crisis are a significant threat to global agriculture, including viticulture. Canadian viticulture has been reported to be the least prepared to adapt to future temperature variation (Jobin Poirier *et al.*, 2020). Moreover, climate changes also have important consequences for the resident above-ground fungal communities. To date, there have been very few experiments exploring the relationship between grapevines and their microbial communities. To our knowledge, there have been no observations concerning the microbial communities of the hybrid grapevine cultivars grown in Atlantic Canada, nor of their microbial response to the climate crisis. We hypothesised that increased heat treatments would significantly increase fungal diversity of the phyllosphere and carposphere regardless of development stage of the host, *Vitis* sp. cv. L'Acadie blanc. We found specific phyllosphere and carposphere communities that were statistically different, with each compartment having unique indicator taxa, despite both compartments having similar composition overall. Both phyllosphere and carposphere fungal communities tended to remain stable across grapevine development. However, we did observe significant changes in the phyllosphere communities pre-veraison due to increased temperatures. These findings suggest that early-season warming fosters fungal diversity, which could influence microbial interactions relevant to disease suppression. The on-the-row mini-greenhouses or tunnels could be deployed early in the growing season to foster more diverse fungal communities, as more diverse communities tend to be more resilient to phytopathogens. This could allow producers to avoid having to resort to more costly options, such as additional fungicides or external heaters. Further research is needed to assess the potential role in phytopathogen control of this approach. This knowledge may also be integrated into decision-making processes for selecting sites for new vineyards, where historic meteorological data could be used to identify sites with warmer conditions prevalent early in the season. Finally, our study provides a standard reference for future work to determine the precise ecological roles of the fungal ASVs that were enriched due to the increased temperature, and more broadly for the adaptation of Atlantic Canadian grapevines and their fungal communities to future climates.

ACKNOWLEDGEMENTS

AJCB performed the DNA extraction and sequencing prep, analysed the data, and drafted the manuscript with input from all co-authors. This work was supported by a Mitacs Acceleration Grant to AJCB & KP (Grant Number IT31632), which we gratefully acknowledge. We thank graduate students Francisco Campos Arguedas and Guillaume Sarrailhé who performed the greenhouse experiment as part of their projects, and Paméla Nicolle at Université du Québec en Outaouais for her technical help.

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