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Soil fungal communities are primarily influenced by vegetation rather than fertilizers and fungicides in a lowbush blueberry production system.

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ASBTRACT

Fertilizer and fungicide applications are commonly used in lowbush blueberry cropping systems (*Vaccinium angustifolium* Aiton and *Vaccinium myrtilloides* Michaux) to enhance fruit yield. Such crop inputs can significantly affect soil fungi, a topic that is not well documented, even though these organisms impact the development and survival of lowbush blueberries in natural forest ecosystems. We evaluated soil fungal biomass and community structure in a commercial lowbush blueberry field located in Lac-Saint-Jean, Québec, Canada. Since 2017, mineral fertilizers and fungicide (Proline 480 SC®) have been applied once every two-years. In-growth sandbags were incubated for 90 days during the 2019 and 2020 growing seasons to collect fungal hyphae biomass in vegetated and unvegetated areas. Soil samples were also collected to analyze the structure of the fungal community using next-generation sequencing. Our results showed that applying fungicide alone increased hyphal biomass in sandbags by 40%, whereas adding both fungicide and fertilizer or fertilizer alone did not change hyphal biomass compared to the control. The structure of the fungal community was only slightly affected by the applications of fungicide and fertilizer, with fungicide decreasing the relative abundances of plant pathogens and fertilizer negatively influencing saprotrophs. Low doses and infrequent applications could explain such weak effects. Among the 33 amplicon sequence variants that were positively associated with the presence of lowbush blueberry plants, eight *Penicillium* species, four Clavariaceae, two *Serendipita* species, and one ericoid mycorrhizal fungi (EMF) (*Oidiodendron maius*) were identified.

INTRODUCTION

Wild lowbush blueberry (*Vaccinium angustifolium* Aiton; *Vaccinium myrtilloides* Michaux) is a major fruit production in Eastern Canada. This crop has a significant economic impact, especially in the Saguenay–Lac-Saint-Jean region, which accounts for about 80% of Quebec's lowbush blueberry production, with 336 growers managing nearly 30,000 hectares (MAPAQ, 2022).

Lowbush blueberry is part of the Ericaceae plant family and grows in acidic, nutrient-poor soils where nutrients are locked in recalcitrant soil organic matter (OM) (Vander Kloet, 1978). A symbiotic association with ericoid mycorrhizal fungi (EMFs) is a survival strategy of Ericaceae in such harsh soil environments (Smith and Read, 2008). EMFs supply nutrients to the ericoid plants in return for plant photosynthates (Smith and Read, 2008). By mobilizing nitrogen (N) and carbon (C) from OM, EMFs participate in OM decomposition and soil nutrient cycling processes (Ward et al., 2022). Besides EMFs, saprotrophic fungi (SAP) also play an important role in OM decomposition (Moore et al., 2020). SAPs are the most abundant guild in soil, and by decomposing OM like litter, dead plants, and roots, they return essential nutrients to the soil for plant development (Moore et al., 2020).

Soil fungi are affected by environmental factors, especially vegetation, which sustains fungal growth through plant exudates and litter production (Shi et al., 2019). Vegetation not only drives the production of fungal biomass but also shapes the composition of fungal communities (Tedersoo et al., 2016). Moreover, vegetation has a different impact on soil fungi according to their guild. As symbionts, mycorrhizal fungi, such as EMFs, should be positively influenced by the presence of blueberry, which may competitively displace non-mycorrhizal fungi via competitive interactions (Ward et al., 2022).

Lowbush blueberry growers prune shoots every two years to enhance the number of flowers per stem and improve fruit yield per hectare. The season after mowing is called the pruning year, during which new shoots grow from buds located on rhizomes. The second year is known as the harvesting year, during which flower buds open in spring and plants produce fruits in summer that are harvested at the end of the growing season. In addition to pruning, growers add fertilizers and fungicides to increase or maintain fruit productivity (Percival and Beaton, 2011; Lafond and Ziadi, 2011). However, the use of agricultural inputs might have a negative impact on soil fungi. Meta-analyses from various crops and ecosystems have demonstrated that mineral nitrogen can decrease fungal biomass and abundance (Zhang et al., 2018), a finding that is aligned with observations made for fungicide applications (Wang et al., 2025).

Fungicide applications have become a common management practice in commercial lowbush blueberry fields since this management practice may control leaf and fruit diseases such as sclerotinia rot (*Monilinia vaccinii-corymbosi*), leaf spots (e.g., *Valdensinia heterodoxa*), and stem canker (*Sphaerulina vacciniae* sp.). In Quebec, growers primarily use the fungicide Proline©, a broad-spectrum triazole fungicide containing prothioconazole as its active ingredient. Prothioconazole is known to inhibit the synthesis of sterols in fungi, specifically ergosterol, which is essential for maintaining fungal cell integrity, ultimately leading to the death of the fungus (Bayer, 2021). Since prothioconazole can harm or eliminate non-target soil fungi, including beneficial root endophytes (Salhi et al., 2022), its utilization may also affect other beneficial soil fungi such as EMFs and SAPs. A recent study from a lowbush blueberry field in Nova Scotia, Canada, revealed a decline in soil fungal diversity and an increase in *Clavaria sphagnicola* after three repeated applications of prothioconazole during

the same year (Lloyd et al., 2021), a beneficial Basidiomycota fungus likely participating in the formation of ericoid mycorrhizal associations with *Vaccinium* spp. (Yang et al., 2018). However, such a conclusion has never been reported in Quebec, where only one application of prothioconazole is performed once every two years (i.e., pruning year only).

Because EMFs and SAPs are beneficial to ericoid plant development and since fertilizers and fungicides are frequently used by lowbush blueberry growers, it is essential to better understand their impacts on soil fungal biomass and community structure. Thus, our study was conducted to assess the effect of mineral fertilizer and foliar fungicide applications on the growth and abundance of EMFs and SAPs in a lowbush blueberry field. The assessment was conducted both in the presence of and absence of the host plant to determine if agricultural inputs differentially affect the two fungal guilds. Our study aimed to quantify fungal biomass according to fertilizer and foliar fungicide applications alone or combined, cropping phase (pruning vs harvesting phase), and vegetation (with vs without vegetation). Our study also aimed to characterize fungal community structure according to fertilizer and fungicide applications (with vs without) and vegetation (with vs without vegetation) and determine if fungal guilds are differentially affected by these treatments. We hypothesized that (1) fertilizer and fungicide applications would reduce fungal biomass and diversity, particularly when combined, (2) fungal biomass and diversity would be lower during the pruning year, and (3) the vegetation exclusion would significantly reduce EMFs community structure.

MATERIALS AND METHODS

Field sites

This study was conducted from June 2019 to June 2021 at the *Bleuetière d'Enseignement et de Recherche* (BER) located in Normandin, Québec, Canada (48°49'35"N; 72°39'35"W). The soil at the BER is a Ferro-Humic Podzol originating from glaciofluvial coarse sand sediments (Raymond et al., 1965). This well-drained soil is characterized by acidic conditions (i.e., pH = 4.0) and low nutrient content (Table 1). The region has a cold and humid climate with an average annual temperature and precipitation of 2.5°C and 829 mm, respectively (Environment Canada, 2024), and more specifically, an average air temperature of 14.9°C and 409 mm of rainfall from May to September (Table 2). The research plots were established at the BER in 2017.

Experimental design

An experimental design was established on two sites, with each site includes 32 experimental units (EU). Site 1 was under pruning phase for years 2019 and 2021, and under harvesting phase for year 2020. Conversely, site 2 was under harvesting phase for years 2019 and 2021, and under pruning phase for year 2020. Pruning was performed in fall by both mechanical and thermal methods. First, plots were pruned mechanically during the plant dormancy period in the fall with a commercial mower (model TB-1072, JR Tardif, Rivière-du-Loup, Canada). Second, thermal pruning occurred immediately after mechanical pruning using a homemade propane gas burner towed by a tractor. The gas burner has four individual propane burners at a height of 10 cm above the soil surface and burns the vegetation with an intensity of 6 580 MJ ha⁻¹. Thermal pruning increased the soil temperature <10°C for several minutes after burning (Paré et al., 2022).

A combination of eight treatments was established at each site, organized in a four-block (replicates) split-split plot design, with fungicide (either applied or not) as the main plot factor, fertilizer (either applied or not) as the subplot factor, and vegetation (either present or not) as the sub-subplot factor (Table 3). Fertilizer and fungicide treatments were applied in June and July of each pruning year, respectively. Fungicide was sprayed on the leaves on July 24, 2019, and July 22, 2020, according to the manufacturer's recommendations (0.4 L ha^{-1} of proline© [$192 \text{ g of prothioconazole ha}^{-1}$] dissolved with 0.125 L ha^{-1} of AG Surf adjuvant) using a commercial sprayer (P950E, MSG MS Gregson, Drummondville, QC, Canada). The sprayer had a 15 m wide spray boom with a 900 L tank capacity. Applications were done at 235 L ha^{-1} using ultra lo-drift sprayer nozzles (Hypro-ULD120-03) set at 35 psi. Granular formulation of mineral fertilizers was broadcast on the following dates: June 6, 2019; June 4, 2020; and May 28, 2021. Fifty kg of N ha^{-1} as ammonium sulfate, 30 kg of $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ as super triple phosphate, 20 kg of $\text{K}_2\text{O ha}^{-1}$ as potassium sulfate, and 0,7 kg of boron (B) ha^{-1} as borate were applied. Each EU were delimited by plastic cylinders (diameter 30 cm) inserted in the ground to their full height (30 cm) to isolate plant roots from the rest of the field. Cylinders were arranged in plots measuring $15 \times 22 \text{ m}$ (330 m^2) that received the same fungicide and fertilizer treatments. Cylinders were inserted 1 m from the transverse border and 5 m apart from each other and the longitudinal border. Each EU was manually weeded. For EUs without vegetation, all plants, including lowbush blueberry shoots, were cut with shears at the beginning of summer and throughout the growing season.

Sampling of fungal biomass

Fungal hyphal biomass production was monitored using in-growth sandbags ($6 \times 10 \text{ cm}$) made from $50 \mu\text{m}$ mesh nylon (Wallander et al., 2001). Sandbags were filled

with 50 g of autoclaved silica sand (99.6% SiO₂) (VWR, Mississauga, Canada). On June 20, 2019, sandbags were inserted under the soil organic horizon (5 cm) near the inner edge of the cylinder. On June 25, 2020, sandbags with vegetation were placed outside cylinders according to the same layout due to a high mortality rate of blueberry shoots that occurred inside the cylinders. Every year, sandbags were harvested 90 days after insertion in the soil on Sept. 16, 2019, and Sept. 21, 2020. Harvested sandbags were transported on ice before being stored at -20°C the same day.

Soil sampling

On June 11, 2021, 10 to 12 core samples of soil organic horizon (5-0 cm) were collected from each 330 m² plot using a tubular probe (dia 22 mm). The sub-samples were homogenized to form a composite sample for each EU. For treatment without vegetation, a handful of organic soil was collected from the center of the cylinder. In both cases, soil samples were mixed, and then 60 to 80 mL were transferred into a 100 mL container. Soil samples were transported on ice and stored at -20 °C upon arrival at the laboratory the same day. Sterile containers and nitrile gloves were utilized to prevent contamination of the samples. Moreover, between each plot or experimental unit, nitrile gloves were sterilized with isopropyl alcohol 70%, and the sampling material was water cleaned, sterilized with a sodium hypochlorite solution of 10% (Prince and Andrus, 1992), and then rinsed with water before resampling another location.

Measured variables

Fungal biomass in sand bags

Fungal biomass was assessed in sandbags via both (1) observing hyphae on gridded membranes under a compound microscope and (2) measuring organic matter (OM) content with the loss on ignition method (Wallander et al., 2013). Hyphal length measurement was modified from the microscopy approach of Bukovská et al. (2016). Mycelium extraction from sand started by stirring 10 g of sand from each in-growth bag in 100 mL of sodium citrate solution (20 mM) for 30 minutes on a magnetic stirrer plate (Thermix® Stirring Hot Plate Model 210T, Fisher Scientific). Then, 900 ml of ultrapure water was added and mixed to full speed for one minute. While full speed was maintained, two 10 mL aliquots were taken along the side of the beaker in the middle of the water volume, rinsed over a 125 µm sieve to remove coarse sand particles, and then filtered through a 0.45 µm pore size mixed cellulose esters gridded membrane. The wet membrane was mounted with glycerin on microscope slides for observation at G=200X. Every contact point between a fungal hyphae and the vertical grids was recorded. We translated these numbers of contact points into fungal hyphal length following Newman (1966):

$$R = \frac{\pi NA}{2H} \quad (1)$$

where R is the hyphal length, N is the number of contact points between hyphae and the vertical grids, A is the membrane area, and H is the total vertical grid length of the membrane. Finally, OM content was determined in triplicates using the loss-on-ignition method (Ball, 1964).

Fungal community structure in soil samples

The structure of the fungal community in soil samples was evaluated using fungal ITS metabarcoding. The DNA was extracted from 250 mg of fresh soil using QIAGEN PowerSoil PowerLyzer kits, following the manufacturer's instructions. DNA extracts were amplified

using the ITS1-ITS2 primer pair (White et al., 1990) and the following thermocycling conditions: 94°C for 5 min, 30 cycles with 94°C for 30 s, annealing at 52°C for 30 s, elongation at 70°C for 30 s, and final elongation at 70°C for 5 min. Triplicate PCR reactions were conducted for each extract, and amplicons were pooled to prepare libraries for amplicon sequencing on Illumina MiSeq (PE250bp), conducted at the Center of Excellence in Research on Orphan Diseases (CERMO) facilities (Montreal, Québec, Canada). Sequences were demultiplexed, primers were removed, and we visualized sequence quality profiles using the R package *dada2* v.1.18, R 4.0.1 (Callahan et al., 2016). Forward reads were trimmed at 210bp and reverse reads at 200bp, and sequences with any expected errors or ambiguous bases (i.e., N's) were filtered out (using the 'filterAndTrim' function in *dada2*). Paired reads were merged, and potential chimeras were removed to define our pool of non-chimeric amplicon sequence variants (ASVs) to which we would assign taxonomy (Table S1 – Supplementary material). We used a naïve Bayesian classifier with the UNITE reference database (version 8.3) using the R package *dada2*. This provided us with a samples × ASVs table and a taxonomy assigned for each column. The table had a total of 797 ASVs, from which 749 were assigned to Fungi kingdom. From those fungal ASVs, 39% were assigned at the species level, 17% at the genus level only, 11% at the order level only, and 12% were unknown fungi.

Statistical analyses

Fungal biomass

Before conducting statistical analysis, normality was assessed graphically and using the Shapiro-Wilk test, while homoscedasticity was evaluated through residue analysis and

confirmed with Levene's test. Only hyphal length needed to be log-transformed. The treatment effect on fungal biomass was assessed with a linear mixed model (i.e., mixed model procedure) in SPSS (IBM Corp., 2016). Four fixed factors (i.e., cropping phase, fungicide, fertilizer, and vegetation) and two random factors (i.e., sampling year and block nested within site) were included in the model. For significant factors, the mean comparison was made with Tukey's post hoc test at a significant level of $\alpha=0.05$.

Fungal community

We evaluated the response of fungal α -diversity using the exponential form of Shannon's diversity (Hill numbers, $q = 1$) (Hill, 1973), which was modelled against treatments and random factors (block and site) using a generalized linear mixed model (GLMM), as implemented in the R package *glmmTMB* (Brooks et al., 2017). Gaussian models were selected after proper validation (i.e., normality, homoscedasticity and independence of residuals being visually inspected) (Zuur et al., 2009). The treatment effect on fungal community structure was assessed by permutational multivariate analysis of variance (PERMANOVA) with three fixed factors (i.e., fungicide, fertilizer, vegetation) and two random factors (i.e., site and block nested within site) using the function *adonis* in *vegan* v.2.6-4 (Oksanen et al., 2015). Comparison between samples was performed using Hellinger distance as a weighed community dissimilarity metric. The assumption of homogeneity of multivariate dispersions was assessed by running permutation tests on group-level dispersions using the function *betadisper* in *vegan* v.2.6-4. Communities were visually represented with a principal component analysis computed on Hellinger-transformed data as implemented in the function *rda* in *vegan* v.2.6-4. We also looked at the differential relative abundance of fungal taxa individually across treatments using the *zicoseq* function in

GUniFrac v.1.6 (Chen et al., 2022), using false discovery rate to adjust *P*-values, in order to correct for multiple comparisons. Fungal taxa with a significant response to one or several experimental treatments were assigned a putative guild using the FunGuild database, using the *funguild_assign* function in FUNGuildR package *v.0.2.0.9000* (Furneaux et al., 2021). This allowed us to determine if the fungi affected by treatments were mostly mycorrhizal or non-mycorrhizal fungi, which includes potential saprotrophs and pathogens. We also used FunGuild assignments to assess the overall effect of treatments on the cumulative relative abundances of (1) EMF, (2) plant endophytes, (3) plant pathogens and (4) saprotrophs, and modelled such cumulative abundances using beta-regressions (with logit link function) given the bounded nature (0-100%) of the input data. We computed models and performed validation as described above, using *glmmTMB*.

RESULTS

Fungal biomass

Hyphal length indicated that fungal biomass in sandbags was significantly affected by the fungicide $\times$ fertilizer interaction (Table 4). In comparison to the control, using fungicide alone resulted in a 40% increase in hyphal length in sandbags (Figure 1). Adding fertilizer did not significantly change hyphal length compared to the control; however, when combined with fungicide, it reduced hyphal length by approximately 43% compared to using fungicide alone (Figure 1). Although not statistically significant ($P=0.054$), the organic matter content displayed patterns similar to those of hyphal length (Figure 1).

The mixed ANOVA model reveals that the content of OM in sandbags was significantly ($P=0.020$) affected by the fungicide $\times$ vegetation interaction (Table 4). However, Tukey's post hoc test did not find any significant differences among the treatments (Figure 1).

Fungal community structure

We identified 749 fungal ASVs from which 62% were assigned to Ascomycota phylum and 20% to Basidiomycota phylum. Other identified phyla were Mortierellomycota (3%), Mucoromycota (2%), Kickxellomycota (0.8%), Zoopagomycota (0.5%), Entorrhizomycota (0.1%), Chytridiomycota (0.1%), Calcarisporiellomycota (0.1%), Rozellomycota (0.1%), and 12% were unidentified fungi. Fungal α -diversity showed only a moderate response to treatments, being slightly higher in blueberry exclusion zones (Table 5, Figure S1), but not affected by either fertilizer or fungicide (Figure 2). The community structure showed an equally weak response to the treatments. At the guild level, some significant effects could be evidenced. Endophytes achieved higher relative abundances when blueberry was present, plant pathogens were also more abundant with blueberry, but less abundant when fungicide was applied, and saprotrophs were also more abundant with blueberry but less abundant when fertilizers were used (figure 3).

The differential abundance analysis revealed that 48 ASVs were significantly influenced by the presence of vegetation (Figure 4). From them, 33 ASVs are positively associated with the presence of lowbush blueberry plants and 15 ASVs are negatively associated with the presence of lowbush blueberry plants (Figure 4). Among the ASVs positively associated with the lowbush blueberry plants, eight *Penicillium* species, four members of the Clavariaceae family, two *Serendipita* species, and one recognized EMF (*Oidiodendron maius*). From the

15 ASVs negatively associated with the lowbush blueberry plants, three are basidiomycetous yeasts (*Rhodotorula*, *Solicoccozyma terricola*), three are from the Chaetothyriales order and two are *Hyaloscypha* species (*Meliniomyces* [= *Hyaloscypha*], *Hyaloscypha variabilis*).

DISCUSSION

The evaluation of agricultural practices and vegetation removal on the growth of EMFs and SAPs in a commercial lowbush blueberry field showed that soil fungal biomass was positively influenced by the fungicide application alone. Furthermore, the evaluation of agricultural practices and vegetation on EMFs and SAP revealed that the structure of the fungal community was primarily influenced by vegetation, and to a lesser extent by fertilizer and fungicide applications.

Fungal biomass

Fungicide application

Our results indicate that the application of fungicide alone increased the biomass of fungi. Our results are aligned with a recent study from Nova Scotia (Canada) that showed an increase of Clavariaceae (e.g., *Clavaria sphagnicola*) abundance in lowbush blueberry plots treated with prothioconazole (Lloyd et al., 2021). As reported in Yang et al. (2018), Clavariaceae are likely participating in the formation of EMF associations with *Vaccinium* plant species. However, these finding contrasts with previous conclusions regarding the harmful effects of fungicide on soil microbial biomass (Wang et al., 2025). In winter wheat, Bjørnlund et al. (2000) reported a decrease in saprotrophic fungal biomass with the use of fenpropimorph, while Elmholt (1991) reported a reduction in the colony-forming units (CFU) numbers of filamentous soil fungi when propiconazole was applied. Here, our results might

stem not from the fungicidal activity of prothioconazole, but from its physiological impact on the blueberry plants. In a related study at the same site, lowbush blueberry plants treated with fungicide during the pruning year had more leaves per shoot by mid-September (Paré et al., 2022). In a study conducted at the Nova Scotia Lowbush Blueberry Institute in Debert, the application of fungicide during the pruning year extended leaf retention by about three weeks at the end of the growing season (Percival and Burnham, 2006). Increasing the number and retention of leaves in late summer likely enhanced the photosynthetic rate and duration, which promoted the production of photosynthates in blueberry plants. In late summer, increased photosynthate production will likely lead to higher levels of soluble sugars in the roots as blueberry plants store reserves for the winter (Kaur et al., 2012). Increased sugar levels in roots likely provide more resources for the growth of EMFs and rhizospheric SAPs, as their development is linked to carbon allocation from the roots (Eisenhauer et al., 2017). Consequently, the impact of the foliar fungicide application on soil fungal biomass may be attributed to its effect on vegetation, which seems to be stronger than any potential harmful impact of the fungicide on soil fungi. Measuring the mycorrhizal rate in the roots of lowbush blueberry plants subjected to foliar fungicide applications would provide valuable information. In addition to the previous explanations, the increase in soil fungal biomass might also be induced to a decrease in phyllosphere epiphytic and endophytic fungi due to foliar application of the fungicide (Prior et al., 2017). A decrease in phyllosphere fungi would result in an increase of resources allocated to the EMFs and rhizospheric SAPs, favouring their development. This would be in line with several studies showing how fungal guilds co-colonizing a plant tend to compete for plant-derived carbon via exploitation competition (Zhou et al., 2022). In any case, all of these explanations deserve further investigation.

Fertilizer application

In our study, the use of mineral fertilization did not impact soil fungal biomass, contrasting with the findings of a meta-analysis on different terrestrial ecosystems (Zhang et al., 2018) and other studies from coniferous forests (Bäath et al., 1981; Maaroufi et al., 2019). One possible explanation for our results is the low amount of fertilizer used, specifically 50 kg of N ha⁻¹ once every two years. Our findings correspond with those recently reported by Morvan et al. (2025), who demonstrated that there were no significant differences in the relative abundance of known and potential EMF taxa between the control group and the fertilizer treatments in a lowbush blueberry field in Quebec, Canada. Our results also align with another study indicating that 60 kg N ha⁻¹ yr⁻¹ had no effect on mycorrhizal colonization in a lowbush blueberry field in Nova Scotia, Canada (Jeliazkova and Percival, 2003). Low fertilizer inputs, combined with high and rapid fertilizer assimilation (Eaton and Patriquin, 1990) by the crop, explain this no detrimental effect on fungi.

Fungal community structure

Vegetation

Our findings showed that the presence of vegetation significantly influenced the structure of the soil fungal community. Plants support soil fungi by supplying carbon through litter production, and root exudates (Shi et al., 2019). When blueberry was removed from our exclusion plots, this may have alleviated dominance by competitive soil fungi (including mycorrhizal fungi) that were most efficient at scavenging host-derived C (Chagnon and Bradley, 2013). This could explain the increase in fungal diversity upon blueberry exclusion. Plants can also influence fungal community structure not only by driving productivity and α -

diversity, but also by favoring some species over others, which is at the core of plant-soil feedback dynamics (Wardle et al., 2004). As an example, root exudates have been shown to promote symbiotic fungi and reduce saprotrophs and pathogens (Shi et al., 2019). Additionally, the morphological and functional traits of plants create unique habitats within their root systems, which are colonized by specific fungal species, thereby influencing the structure of soil fungal communities (Hugoni et al., 2018; Sietiö et al., 2018; Sheng et al., 2019). Our findings support a previous study that noted changes in mycorrhizal and saprotrophic community composition following the removal of ericaceous plant species (Fanin et al., 2022).

The differential abundance analysis highlighted the relationship between different fungal taxa in those with and without the presence of blueberry plants. There are eight species of *Penicillium* positively associated with lowbush blueberry plants. Interestingly, the trophic mode of the *Penicillium* genus is classified as saprotrophic in the FUNGuild database. Our results indicate that the genus may have an important relationship with lowbush blueberry as a root endophyte or rhizospheric fungus. *Penicillium* has been reported as a root endophyte of *Vaccinium virgatum* Aiton (Guo et al., 2021), *Vaccinium macrocarpon* Aiton PLC (Salhi et al., 2022) and *Vaccinium corymbosum* (Cai et al., 2021; Gomes et al., 2023) and in the rhizosphere of *Vaccinium darrowii* Camp (Li et al., 2020). This highlights the significant yet overlooked role of non-mycorrhizal fungi as endophytes in promoting the growth of lowbush blueberry plants and other ericoid species.

There are few recognized EMF species, and most belong to the Helotiales family in the phylum of Ascomycota (Vohník, 2020). *Oidiodendron* is one of the genus in which EMFs are observed, and one EMF belonging to this genus was identified in our study, namely

Oidiodendron maius (Leopold, 2016). In addition to recognized EMFs, we identified four Clavariaceae taxa and two *Serendipita* species associated with the lowbush blueberry plants. Those fungi are from the Basidiomycota phylum. Recent studies revealed that the genus *Clavaria*, and more generally the Clavariaceae family are abundant in wild blueberry fields (Morvan et al., 2020; Lloyd et al., 2021). Furthermore, *Clavaria* was reported to be very abundant in the rhizosphere and root tissues of highbush blueberry (*Vaccinium corymbosum*) (Li et al., 2020) and half-highbush blueberry (*Vaccinium corymbosum* x *Vaccinium angustifolium* hybrid) (Dong et al., 2022). More specifically, *Clavaria sphagnicola* has been identified in soil samples collected in a lowbush blueberry field (Lloyd et al., 2021) and in the hair roots of *Vaccinium uliginosum* Linné (Yang et al., 2018). Members of the genus *Serendipita* have also been found to be abundant in the rhizosphere or roots of different blueberry species, (i.e. *V. angustifolium*, *V. corymbosum* x *V. angustifolium*, *V. corymbosum*, *V. myrtillus* Linné and *V. uliginosum*) (Yang et al., 2018; Daghino et al., 2022; Dong et al., 2022; Gomes et al., 2023). Moreover, the species *Serendipita herbamans* has been identified in the roots of *V. myrtilloides* (Fadaei et al., 2020). Therefore, our results support that members of the genera *Clavaria* and *Serendipita* could likely be involved in close associations with blueberry tissues, without confirming any mycorrhizal status (Vohník, 2020). More broadly, it suggests that in the absence of an ericaceous host, fungal communities can shift rapidly to non-mycorrhizal taxa, which could potentially be attributed to putative antagonistic interactions between mycorrhizal and non-mycorrhizal fungi (Fernandez and Kennedy, 2016; Zhou et al., 2022). Our findings indicated that neither fertilizer nor fungicide applications affected the fungal community. This observation aligns well with the idea proposed by Wardle (2002) that fungal communities are primarily

regulated by bottom-up processes and competition, specifically here for vegetation-derived carbon.

The ASVs negatively associated with the lowbush blueberry plants included three basidiomycetous yeasts (*Rhodotorula* and *Solicoccozyma terricola*). Common yeasts found in boreal and temperate forest soils include *Solicoccozyma terricola* and some species of *Rhodotorula* (Yurkov, 2017). It is not surprising that our study found these species are associated with the absence of lowbush blueberry plants. The order Chaetothyriales belongs to the Ascomycota phylum and consists of melanized fungi that thrive in various habitats, including water, soil, rocks, lichens, and plant leaves (Geiser et al., 2006; Quan et al., 2020). Chaetothyrialean fungi exhibit diverse lifestyles, including pathotrophs (i.e., organisms that derive nutrients from a pathogen), saprotrophs (i.e., which feed on decaying organic matter), endophytes (i.e., that live within plant tissues without causing harm), and fungicolous species (i.e., which colonize other fungi) (Quan et al., 2020; Teixeira et al., 2017). The order is considered to contain putative EMFs (Leopold, 2016) and has been reported abundant in such lowbush blueberry habitats (Morvan et al., 2020) and in the roots or the rhizosphere of different *Vaccinium* species (Zhang et al., 2016; Yang et al., 2018; Li et al., 2020; Che et al., 2022). As for the Chaetothyriales, the ascomycetous *Hyaloscypha* species have different trophic modes and are known as saprotrophs, endophytes, mycorrhizal or bryophilous fungi (Fehrer et al., 2019). The genus is frequently reported in association with *Vaccinium*, or in its rhizosphere (Gorzelak et al., 2012; Baba et al., 2016; Yang et al., 2018; Li et al., 2020; Daghino et al., 2022). More specifically, *Hyaloscypha variabilis* is a possible EMF from the *Hyaloscypha s. str.*, formerly known as *Rhizoscyphus ericae* aggregate (Fehrer et al., 2019;

Vohník, 2020). Our results align with the multi-trophic characteristics of *Hyaloscypha* and Chaetothyriales, as they were found to thrive even without an ericaceous host.

Fertilizer application

Our findings indicated that fertilization had a moderate effect on the structure of the fungal community, with only a marginal decrease in the proportion of known fungal saprotrophs in fertilized plots. Previous research had shown a negative effect of N fertilization on soil fungal abundance (Treseder, 2004; Zhang et al., 2018), and other studies conducted specifically in boreal forests reported either negative (Allison et al., 2007), positive (Tahovská et al., 2020) or both effects (Jørgensen et al., 2022; Renaudin et al., 2023). However, our results agree with other observations made after a five-year application of 50 and 100 kg N ha⁻¹ yr⁻¹ in an Eastern Chinese boreal forest, reporting modest impacts of such fertilization rates (Liu et al., 2023). The absence of fertilization effects in our study may be due to the N-limited conditions typical of the experimental site, which is common in such agroecosystems (Lafond, 2018). Additionally, the production context plays a role, as lowbush blueberry plants are mowed and fertilized every two years. Research has demonstrated that lowbush blueberry plants treated with mineral fertilization show increased growth, higher fruit yields, and elevated N concentrations in their foliage (Smagula and Ismail, 1981; Lafond, 2010; Lafond and Ziadi, 2011). Since fertilization occurs only during the pruning year, the significant demand for nutrients from the production of new shoots could prevent the accumulation of N in the soil, thereby minimizing the fertilization impact on soil microorganisms. The combination of applying a low rate of nitrogen (50 kg ha⁻¹ every two years) and the rapid nitrogen uptake by lowbush blueberry plants likely explains our results.

Fungicide application

Like fertilizer, fungicide had a limited impact on fungal community structure. Putative fungal pathogens in soils tended to be less abundant in fungicide-treated plots; however, blueberry had a comparatively much more substantial (positive) impact on pathogen abundance. Thus, fungicide appears to exert a weaker control on soil fungal communities than aboveground vegetation (here, blueberry presence). Our findings contrast with the decline in soil fungal diversity observed in a commercial lowbush blueberry field (Lloyd et al., 2021) and in fungal root endophytes diversity of commercial cranberry plants (*V. macrocarpon*) (Salhi et al., 2022). In the study by Lloyd et al. (2021), a decrease was observed after three applications of Proline 480 SC at the same rate, conducted at ten-day intervals over one pruning year. Our results can be attributed to a reduced application of the fungicide, at 0.315 L ha⁻¹ every two years, compared to the findings of Lloyd et al. (2021). The persistence of prothioconazole may also explain our results since prothioconazole is rapidly degraded into metabolites, and its half-life in soil and plants has been assessed to be a maximum of 10 days (Dong et al., 2019; Zhai et al., 2022). However, prothioconazole main toxic metabolite, prothioconazole-desthio, is more persistent and has a longer half-life up to 51 days in soil (Wu et al., 2022). Nevertheless, prothioconazole-desthio concentrations in roots are two to three times lower than in leaves 14 days after foliar application according to Fang et al. (2023). Fungicide effect on soil fungi also depends on phyla, species, guild, and niches. Thus, a greater tolerance in Ascomycota unlike Basidiomycota fungi has been highlighted for some fungicides, including prothioconazole (dela Cruz et al., 2022). The low application rate of the fungicide (0.315 L ha⁻¹ every two years), along with the dominance of Ascomycota fungi, likely explains our findings.

CONCLUSION

In this study, we evaluated the effects of fungicide and fertilizer applications, as well as cropping phase and vegetation on soil fungi in a lowbush blueberry field. Our findings demonstrated that fungicide application alone positively influenced the soil fungi biomass production. Our results indicate that the fertilizer had no effect on the growth of soil fungi, likely due to the low N inputs in the lowbush blueberry field. Our results showed that removing vegetation affected the fungal community structure, while the applications of fertilizer and fungicide had relatively minor impacts. Such weak effect sizes from fertilizer and fungicide are likely due to rapid uptake of fertilizer by plants and infrequent applications (once every other year). However, further studies are needed to confirm these assumptions. Long-term research in various lowbush blueberry fields is also needed to evaluate practices over several years and in different pedoclimatic contexts.

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COMPETING INTERESTS

The authors declare there are no competing interests.

AUTHOR CONTRIBUTIONS

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Funding and resource acquisitions: MCP and JL

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Writing review & editing: GT, MCP, and PLC

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DATA AVAILABILITY

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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273

FIGURE CAPTIONS

274 **Figure 1.** Hyphal length (A, C) and organic matter content (B, D) inside in-growth sandbags
 275 [means ($\pm$ standard error)] per treatment. Graphs A and B display the interaction between
 276 fertilization and fungicide. Graphs C and D display the interaction between vegetation and
 277 fungicide. Letters indicate significant differences between means using a least significant
 278 difference (Tukey) post-hoc test ($P < 0.05$).

279 **Figure 2.** The effects of fertilizer, fungicide and vegetation on fungal alpha diversity. Bars
 280 represent means $\pm$ standard errors (SE). Faded bars represent non-significant results. P-
 281 value is associated with GLMM model coefficients for each experimental factor in the
 282 design.

283 **Figure 3.** The effects of fertilizer, fungicide and vegetation on fungal guilds. Bars represent
 284 means $\pm$ standard errors (SE). Faded bars represent non-significant results. For every panel,
 285 the y-axis represents the proportion of community reads that were assigned to the
 286 corresponding guild.

287 **Figure 4.** Forestplot diagram showing the ASVs with a significant response to vegetation as
 288 determined through differential abundance analysis. The color of the dots indicates the
 289 direction and the intensity of the association between the ASVs and the lowbush blueberry
 290 plants. Red dots = ASVs negatively associated with the lowbush blueberry plants. Green dots
 291 = ASVs positively associated with the lowbush blueberry plants. The x-axis coefficient was
 292 calculated in the generalized model built with the *zicoseq* function. $\square$ = known EMF. $\blacksquare$ =
 293 putative EMF. $\blacktriangle$ = possible EMF. $\bullet$ = possible root endophyte or rhizospheric fungi.

294

Table 1. Edaphic conditions (means $\pm$ standard error) measured during the study (2019-2021).

Variable □	Units	Site 1		Site 2	
		5-0 cm	0-15 cm	5-0 cm	0-15 cm
pH		4.0 $\pm$ 0.3	4.9 $\pm$ 0.2	4.0 $\pm$ 0.2	4.9 $\pm$ 0.3
P	mg/kg	25.0 $\pm$ 11.3	18.7 $\pm$ 7.5	22.8 $\pm$ 8.6	19.0 $\pm$ 6.6
K	mg/kg	4.3 $\pm$ 2.8	4.2 $\pm$ 2.9	4.8 $\pm$ 2.7	4.3 $\pm$ 2.6
Ca	mg/kg	155.3 $\pm$ 96.5	22.4 $\pm$ 83.5	152.7 $\pm$ 76.0	11.4 $\pm$ 12.2
Mg	mg/kg	1 046.6 $\pm$ 541.0	41.2 $\pm$ 30.7	1 169.0 $\pm$ 575.6	44.5 $\pm$ 27.4

□ Soil pH was measured in water (Hendershot et al., 2008). Other elements (P, K, Ca, and Mg) were extracted in a Mehlich-3 solution, P was determined by colorimetry (Murphy and Riley, 1962), where K, Ca, and Mg were determined by flame spectroscopy (Ziadi and Sen Tran, 2008).

Table 2. Monthly average temperature (°C) and total precipitation (mm) from May to September for the years 2019 to 2021.

Month	Temperature (°C)	Rain (mm)
2019		
May	7.5	87.6
June	14.2	102.2
July	19.9	44.2
August	16.6	61.4
September	11.0	70.6
2020		
May	8.6	63.8
June	17.2	62.2
July	20.1	179.2
August	17.0	119.0
September	10.9	113.8
2021		
May	10.5	41.4
June	17.3	68.6
July	17.1	68.4
August	20.3	16.4
September	12.9	124.6

Table 3. Treatment combinations established on two (2) sites of 32 experimental units (EU) each.

Treatment	Fungicide	Fertilizer	Vegetation
1	With	With	With
2	With	With	Without
3	With	Without	With
4	With	Without	Without
5	Without	With	With
6	Without	With	Without
7	Without	Without	With
8	Without	Without	Without

Note: Four (4) plot replicates, 32 plots per site.

Table 4. Mixed ANOVA examining the effects of different factors on fungal biomass within in-growth sandbags.

Factor	Hyphal length	OM content
Cropping phase (Cr)	ns	ns
Fertilizer (Fe)	ns	ns
Fungicide (Fu)	ns	ns
Vegetation (Vg)	ns	ns
Cr×Fe	ns	ns
Cr×Fu	ns	ns
Cr×Vg	ns	ns
Fe×Fu	***	ns
Fe×Vg	ns	ns
Fu×Vg	ns	*
Cr×Fe×Fu	ns	ns
Cr×Fe×Vg	ns	ns
Cr×Fu×Vg	ns	ns
Fe×Fu×Vg	ns	ns
Cr×Fe×Fu×Vg	ns	ns

***: <0.001

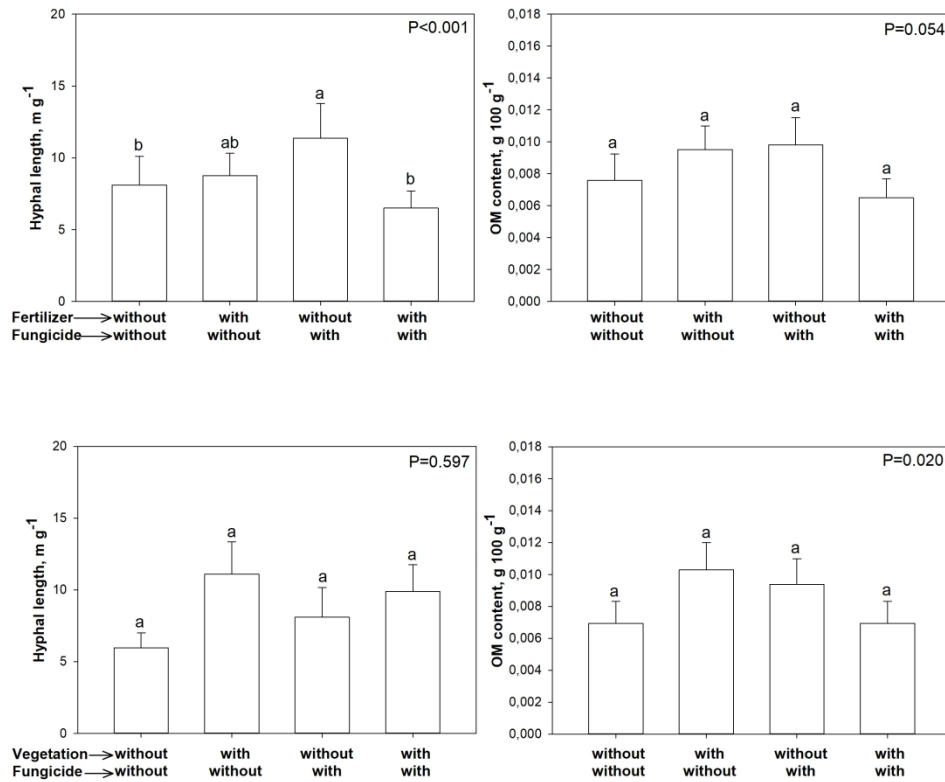
*: <0.05

ns: non-significant

Table 5. Mixed PERMANOVA results for factor effects on fungal community structure.

Factor	df	Fungal community structure	
		F	<i>P</i>
Fertilizer (Fe)	1	0.86	0.380
Fungicide (Fu)	1	0.85	0.394
Vegetation (Vg)	1	2.55	0.007
Fe×Fu	1	0.82	0.448
Fe×Vg	1	0.65	0.758
Fu×Vg	1	0.46	0.990
Fe×Fu×Vg	1	1.15	0.164

Note: Significant differences ($P < 0.05$) are in bold. df = degree of freedom. F = F-statistic. P = P-value.



Hyphal length (A, C) and organic matter content (B, D) inside in-growth sandbags [means ($\pm$ standard error)] per treatment. Graphs A and B display the interaction between fertilization and fungicide. Graphs C and D display the interaction between vegetation and fungicide. Letters indicate significant differences between means using a least significant difference (Tukey) post-hoc test ($P < 0.05$).

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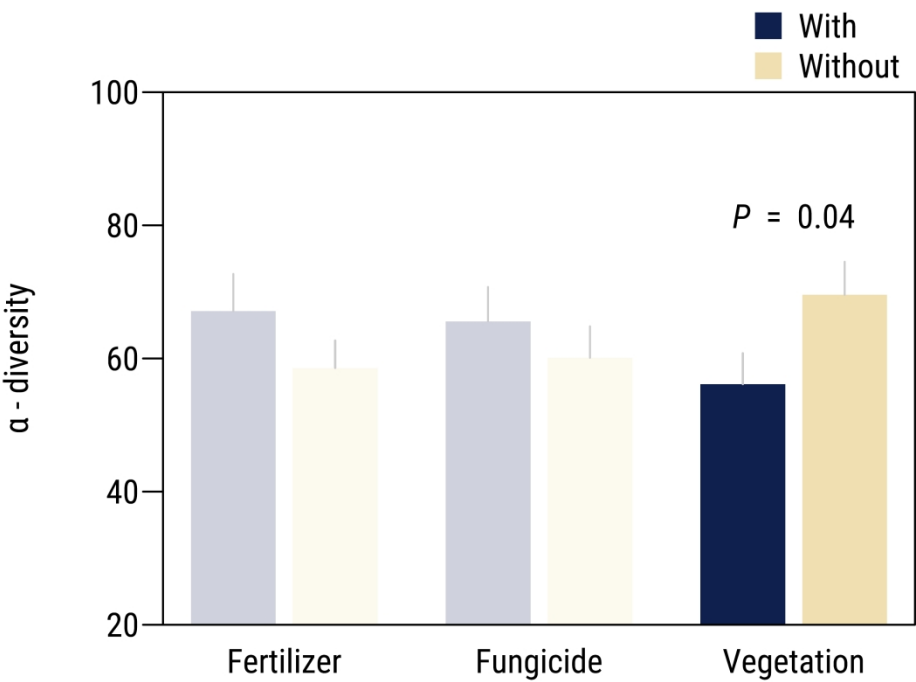


Figure 2. The effects of fertilizer, fungicide and vegetation on fungal alpha diversity. Bars represent means +/- standard errors (SE). Faded bars represent non-significant results. P-value is associated with GLMM model coefficients for each experimental factor in the design.

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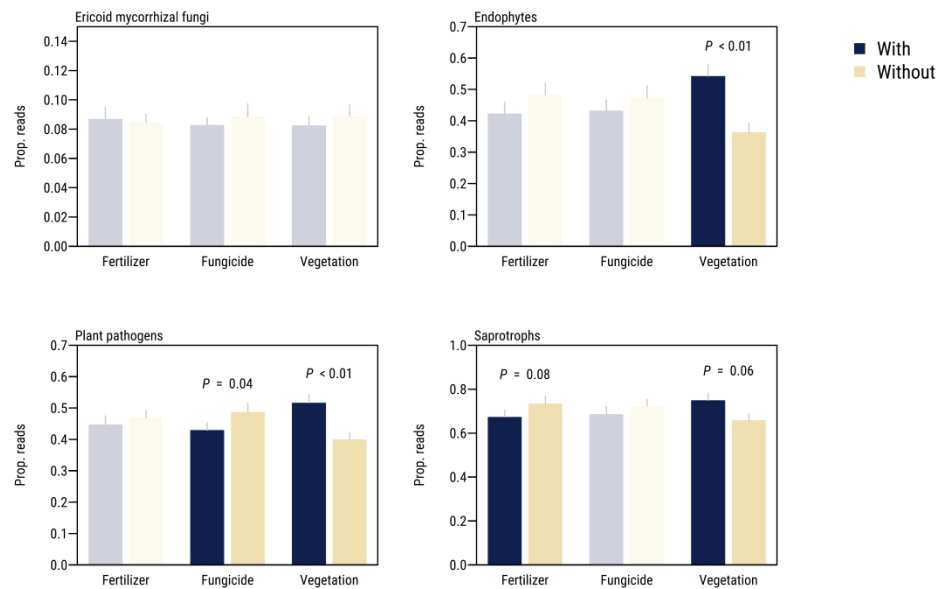


Figure 3. The effects of fertilizer, fungicide and vegetation on fungal guilds. Bars represent means $\pm$ standard errors (SE). Faded bars represent non-significant results. For every panel, the y-axis represents the proportion of community reads that were assigned to the corresponding guild.

516x329mm (236 x 236 DPI)

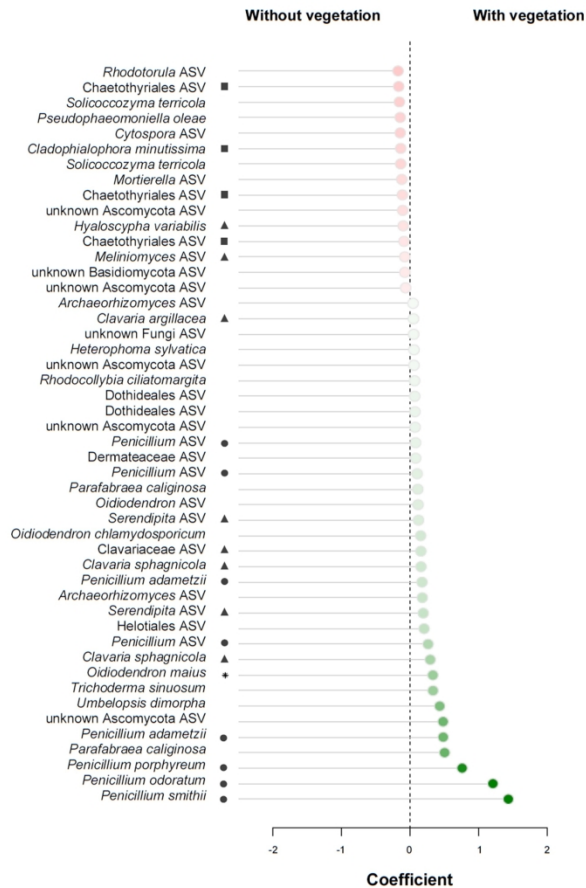


Figure 4. Forestplot diagram showing the ASVs with a significant response to vegetation as determined through differential abundance analysis. The color of the dots indicates the direction and the intensity of the association between the ASVs and the lowbush blueberry plants. Red dots = ASVs negatively associated with the lowbush blueberry plants. Green dots = ASVs positively associated with the lowbush blueberry plants. The x-axis coefficient was calculated in the generalized model built with the zicoseq function. □ = known EMF. ■ = putative EMF. ▲ = possible EMF. ● = possible root endophyte or rhizospheric fungi.

190x338mm (300 x 300 DPI)