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Abondance et croissance des champignons saprotrophes et à mycorhizes éricoïdes sous culture de bleuets sauvages (*Vaccinium angustifolium* Aiton et *Vaccinium myrtilloides* Michaux) soumise à des applications de fertilisants et de fongicides

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RÉSUMÉ

Le Québec est un des plus grands producteurs de bleuets sauvages (*Vaccinium angustifolium* Aiton and *Vaccinium myrtilloides* Michaux) au monde. Par conséquent, la culture du bleuet sauvage a un impact significatif dans l'économie de cette province et particulièrement dans la région du Saguenay–Lac-Saint-Jean, qui regroupe à elle seule les trois-quarts des producteurs de bleuets sauvages québécois. Afin d'optimiser la productivité, différents travaux de recherche ont été entrepris sur la production du bleuet sauvage au Québec au cours des dernières années. L'étude des pratiques agricoles et de leurs effets sur la qualité des sols, dont notamment les champignons du sol, figure parmi les sujets prioritaires. Du fait que le bleuet sauvage prolifère sur des sols acides où les nutriments sont rares et la matière organique récalcitrante, les champignons saprotrophes (SAP) et les champignons à mycorhizes éricoïdes (CME) sont essentiels à son développement et à sa productivité par leurs rôles de décomposeurs mais aussi de symbiotes dans le cas des CME. Parallèlement à ceci, l'application de fertilisants et de fongicides sont des pratiques courantes dans la culture du bleuet sauvage afin d'améliorer les rendements en fruits et de contrôler plusieurs maladies fongiques. Ces intrants peuvent toutefois avoir un impact négatif sur les champignons du sol et le sujet n'est pas bien documenté. Le présent projet a donc pour objectif principal d'évaluer l'effet des applications de fertilisant et de fongicide sur l'abondance et la croissance des CME et des SAP dans la culture du bleuet sauvage. L'hypothèse vérifiée est que les traitements de fertilisant et de fongicide auront un impact négatif sur ces champignons.

Nous avons évalué la biomasse et l'abondance fongiques du sol dans une bleuetière commerciale au Lac-Saint-Jean, Québec (Canada) dans laquelle un fertilisant minéral (50 N kg ha^{-1}) et un fongicide (0,315 L ha^{-1} Proline 480 SC) ont été appliqués depuis 2017 durant la phase végétative, c.-à-d. à tous les deux ans. Cette régie correspond à celle appliquée par les producteurs de bleuets sauvages locaux. Des sacs de sable en nylon (maille de 50 µm) ont été incubés dans le sol pendant 30 et 90 jours durant deux saisons de croissance afin de récolter des hyphes fongiques. La biomasse fongique a été évaluée par des mesures de longueur des hyphes et de contenu en matière organique dans les sacs de sable. Des échantillons de sol ont également été prélevés pour effectuer des analyses moléculaires, afin d'évaluer la structure des communautés fongiques. Nos résultats ont démontré que l'application de fongicide a fait augmenter la longueur des hyphes d'environ 25% en comparaison au contrôle et que le fertilisant n'a pas eu d'impact sur la longueur des hyphes. La structure de la communauté fongique n'a pas été influencée par l'application de fertilisant et de fongicide. Nos résultats suggèrent que les pratiques agricoles extensives dans la culture du bleuet sauvage n'affectent pas négativement les communautés fongiques du sol, ce qui infirme notre hypothèse initiale. Toutefois, des recherches à long terme et dans différentes bleuetières sont encore nécessaires pour évaluer l'effet cumulatif de ces pratiques après plusieurs années et dans des contextes pédoclimatiques différents.

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CHAPITRE 1

INTRODUCTION GÉNÉRALE

1.1 Importance de la culture du bleuet sauvage

Le bleuet sauvage est l'un des fruits les plus riches en anthocyanes dont l'effet antioxydant est considéré bénéfique pour la santé humaine (Kalt *et al.* 2019). La popularité grandissante du bleuet, en raison de ses bienfaits, a permis à l'industrie de croître pendant plusieurs années (MAPAQ 2016; IBO 2017). Au niveau mondial, le Canada est le plus grand producteur de bleuets sauvages. Parmi les provinces canadiennes qui cultivent ce petit fruit, c'est au Québec que les plus grandes superficies sont exploitées et que les volumes les plus importants sont récoltés (MAPAQ 2022). Au Québec, la région du Saguenay–Lac-Saint-Jean regroupe à elle seule 77% des producteurs de bleuets sauvages de la province et 80% des superficies cultivées (MAPAQ 2022; Gouvernement du Québec 2023). De ce fait, la culture du bleuet sauvage a un impact significatif dans l'économie de cette région (MAPAQ 2022). Afin d'optimiser la productivité à l'hectare, plusieurs travaux de recherche sur la production du bleuet sauvage ont été entrepris au Québec au cours des dernières années. L'étude des pratiques agricoles et de leurs effets sur la qualité des sols, dont notamment les champignons du sol, figure parmi les sujets prioritaires (MAPAQ 2016; MAPAQ 2022).

1.2 Bleuet sauvage et champignons du sol

La culture du bleuet sauvage en Amérique du Nord compte deux espèces, *Vaccinium angustifolium* Aiton et *Vaccinium myrtilloides* Michaux (Barker *et al.* 1964), dont le genre

appartient à la famille des Éricacées (Kloet 1988). Les Éricacées sont des végétaux qui prolifèrent dans des environnements caractérisés par des sols acides, pauvres en éléments nutritifs et où la matière organique est récalcitrante, donc difficilement minéralisable (Read *et al.* 2004; Smith et Read 2008). La survie des Éricacées dans un milieu aussi inhospitalier dépend notamment d'une association symbiotique avec des champignons mycorhiziens (Watkinson 2016). De façon générale, les champignons mycorhiziens (CM) sont présents chez la majorité des végétaux terrestres. La principale fonction des CM est de fournir des éléments nutritifs aux végétaux en échange de sucres, mais les CM contribuent également à augmenter la résistance à la sécheresse et aux parasites (Smith et Read 2008). Différents types de mycorhizes existent et chaque type occupe un habitat préférentiel (Figure 1). Les plus communs sont les champignons à mycorhizes arbusculaires (CMA). Les CMA sont des symbiotes obligés appartenant au sous-phylum des Glomeromycotina (phylum des Mucoromycota) qui compte 342 espèces (Bonfante et Venice 2020; Soudzilovskaia *et al.* 2020; International Culture Collection of Glomeromycota 2021). Les CMA sont en association avec près de 80% de tous les végétaux vasculaires, principalement des herbacées (55%) et des arbres (20%) mais aussi des arbustes, des épiphytes, des ptéridophytes et des hépatiques (Brundrett 2009). Les CMA sont surtout présents dans les prairies et les forêts de feuillus qui sont caractérisées par un sol au pH légèrement acide ou neutre dans lequel la minéralisation de la matière organique est rapide (Read 1991). Les champignons à ectomycorhizes (CEM) sont beaucoup moins communs que les CMA. Ils se retrouvent chez seulement 2% des plantes vasculaires (Brundrett 2009). Ils colonisent essentiellement des arbres (75%) et des arbustes (15%) et la plupart ont évolué à partir de champignons décomposeurs d'humus et de bois (Tedersoo *et al.* 2010; Soudzilovskaia *et al.* 2020). De ce fait, la majorité des CEM ne sont pas des symbiotes obligés puisqu'ils sécrètent des enzymes

qui permettent la dégradation de composés organiques (Koide *et al.* 2008; Field *et al.* 2017). Les CEM comptent entre 5 000 et 6 000 espèces mais certains estiment que leur nombre serait supérieur à 20 000 (Kariman *et al.* 2018; Moore *et al.* 2020). Les CEM appartiennent principalement aux phyla des Basidiomycota (~2/3) et des Ascomycota (~1/3) avec quelques espèces associées au phylum des Mucoromycota (sous-phylum des Mucoromycotina) (Moore *et al.* 2020). Les CEM se retrouvent principalement dans les forêts mixtes et boréales où le sol présente un pH acide et un taux intermédiaire de minéralisation de la matière organique (Read 1991). Enfin, les champignons à mycorhizes éricoïdes (CME) ne sont présents que chez une faible proportion de plantes vasculaires (1,3%) (Brundrett 2009). Les végétaux qu'ils colonisent font exclusivement partie de l'ordre des Éricales (familles des Éricacées et des Diapensiacées) et la plupart sont des arbustes (85%) (Brundrett et Tedersoo 2018; Soudzilovskaia *et al.* 2020). Les CME ont été peu étudiés et le faible nombre d'espèces connues appartient aux phyla des Ascomycota et des Basiodiomycota (Vohník 2020). Les CME ne sont pas des symbiotes obligés étant donné qu'ils produisent une gamme relativement vaste d'enzymes qui leur confèrent des facultés de décomposeurs (Smith et Read 2008; Martino et al. 2018). Les CME colonisent notamment les landes de la forêt boréale dont le sol est très acide et où la minéralisation de la matière organique est relativement lente (Read 1991).

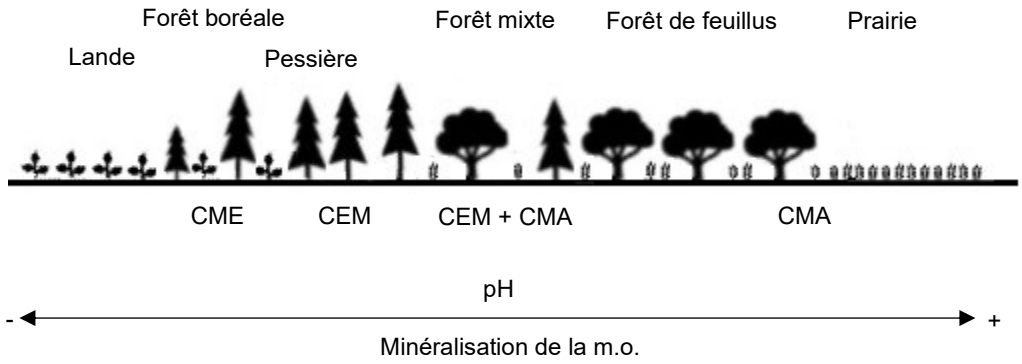

Figure 1: Relation entre les habitats, les mycorhizes, le pH et la minéralisation de la matière organique. CME = champignons à mycorhizes éricoïdes. CEM = champignons à ectomycorhizes. CMA = champignons à mycorhizes arbusculaires. m.o. = matière organique. Adaptée de Smith et Read (2008) et Fortin *et al.* (2015).

Les CME permettent aux Éricacées de proliférer dans les milieux qu'elles colonisent, tout en participant à la décomposition de la matière organique et au recyclage des éléments nutritifs du sol (Read *et al.* 2004; Martino *et al.* 2018). Toutefois, les CME ne sont pas les seuls à effectuer cette fonction indispensable pour la croissance des végétaux puisque les champignons saprotrophes (SAP) sont les principaux décomposeurs de la matière organique dans le sol (Deacon 2013; Moore *et al.* 2020). Les SAP constituent la guilde la plus importante parmi les champignons et sont abondants dans les phyla des Blastocladiomycota, des Chytridiomycota, des Ascomycota et des Basiodiomycota, On les trouve également dans les phyla des Mucoromycota et des Zoopagomycota mais en moins grand nombre que dans les phyla précédents (Naranjo-Ortiz et Gabaldon 2019). Les SAP existent dans un éventail allant du simple épiphyte et parasite bénin, s'alimentant respectivement de substances excrétées et produites par leur hôte, au décomposeur spécialisé dans la dégradation de matière organique récalcitrante (Deacon 2013). En décomposant les résidus organiques comme la litière et les tissus végétaux morts, les SAP restituent les éléments nutritifs dans le sol et les rendent disponibles pour la croissance des végétaux (Moore *et al.* 2020). Par ailleurs, un

même champignon peut posséder plus d'un mode trophique. C'est le cas entre autres de *Hyaloscypha variabilis* qui peut être soit saprotrophe, endophyte ou mycorhizien (Vohník *et al.* 2013; Peltoniemi *et al.* 2021)

Quelques études ont mis en évidence différents effets bénéfiques des CME et des SAP sur le bleuet sauvage et le bleuet en corymbe (*Vaccinium corymbosum* Linné), une espèce apparentée au bleuet sauvage. Par exemple, il a été démontré que certaines espèces de CME augmentent la biomasse de même que la résistance à la sécheresse et à la salinité pour *V. myrtilloides* (Fadaei *et al.* 2020; Mu *et al.* 2021). Chez le bleuet en corymbe, il a été démontré que les CME augmentent la croissance végétative (Koron et Gogala 2000; Ważny *et al.* 2022) et reproductive (Brody *et al.* 2019), la biomasse ainsi que l'absorption et/ou l'efficacité d'utilisation des nutriments (Scagel 2005). Les résultats de Montalba *et al.* (2010) suggèrent même que les CME pourraient contribuer à réduire l'incidence de maladies telle que de la fusariose chez *V. corymbosum*. Enfin, les travaux de Vohník *et al.* (2012) ont pour leur part révélé que la présence d'*Agrocybe praecox* (Pers.), une espèce de SAP, pouvait non seulement augmenter la hauteur des tiges et la biomasse de *V. corymbosum*, mais également améliorer l'absorption des nutriments et accélérer la production de fruits. Par conséquent, dans beaucoup de cas, la présence des CME et des SAP est bénéfique au développement et à la productivité du bleuet.

1.3 Écologie des champignons du sol

Les champignons du sol sont affectés par différents facteurs environnementaux, dont notamment les conditions météorologiques. Les précipitations et la température de l'air

influencent le contenu en humidité et la température du sol qui, à leur tour, ont un effet sur le métabolisme et le développement des champignons (Zheng *et al.* 1993; Pietikäinen *et al.* 2005; Feng et Liu 2015; Borowik et Wyszkowska 2016). La croissance des champignons dépend également de la fertilité du sol (Treseder 2004; Tedersoo *et al.* 2016) et du contenu en carbone dans le sol qui est fortement impacté par les végétaux contribuant aux ajouts de carbone via notamment la biomasse (racinaire et végétative) et les exsudats racinaires (Eisenhauer *et al.* 2017; Shi *et al.* 2018; Prommer *et al.* 2020). Les facteurs environnementaux n'agissent pas uniquement sur la biomasse fongique mais aussi sur les espèces de champignons présentes dans le sol. Parmi les facteurs qui déterminent la diversité et la structure des communautés fongiques du sol se trouvent le pH, la texture, le contenu en azote, en phosphore et en carbone du sol tout comme la litière et les espèces végétales (Wubet *et al.* 2012; Tedersoo *et al.* 2016; Shi *et al.* 2018; Zhang *et al.* 2019). Par ailleurs, l'impact de la végétation diffère selon les guildes de champignons du sol. Ainsi, en tant que symbiotes, les CM sont défavorisés comparativement aux SAP lorsqu'il n'y a pas de transfert de sucres dans les racines des plantes (Shi *et al.* 2018).

1.4 Pratiques agricoles dans la culture du bleuet sauvage

Le bleuet sauvage possède certains traits qui sont exploités par les producteurs. Lorsqu'une graine de bleuet sauvage germe au sol, il y a développement d'un plant-mère à partir duquel émergent des rhizomes (Chiasson et Argall 1996). Ces rhizomes s'étendent pour former un important réseau sur lequel apparaissent plusieurs tiges aériennes (Chiasson et Argall 1996). Quand une nouvelle tige émerge, le plant-mère fait un investissement maximal de ressources dans son développement, entraînant généralement une grande

production de bourgeons floraux dès la première année et une production de fruits conséquente durant la deuxième année (Eaton et McIsaac 1997). Afin de tirer profit de ce trait, les producteurs taillent et coupent les plants de bleuets sauvage à tous les deux ans (suite à la récolte des fruits) afin d'optimiser les rendements (Eaton et McIsaac 1997). La taille s'effectue par une fauche mécanique et/ou thermique à l'automne ou au printemps avant le débourrement (Eaton et McIsaac 1997). La saison suivant la taille, les plants sont en croissance végétative, appelée phase végétative, et l'année suivante, les bleuetiers sont en phase de production de fruits, appelée phase productive (Chiasson et Argall 1996).

La culture du bleuet sauvage s'effectuant généralement sur un sol peu fertile, l'utilisation de fertilisants permet d'augmenter la productivité (Eaton et Nams 2006; Lafond et Ziadi 2011; Fournier 2020). Toutefois, l'ajout de fertilisants peut avoir une incidence négative sur la biomasse et l'abondance des champignons du sol (Treseder 2004; Zhang *et al.* 2018). Également, le bleuet sauvage est sujet à différentes maladies fongiques et certaines ont un impact considérable sur la vigueur des bleuetiers (AAC 2016). Ainsi, l'application de fongicides est devenue une pratique courante dans les bleuetières commerciales pour enrayer les infections les plus sévères ou les plus communes, telles que la tache septorienne (*Septoria* spp.), la tache valdensinéenne (*Valdensinia heterodoxa*) et la rouille des feuilles (*Thekopsora minima*) (AAC 2016). L'utilisation des fongicides aide à maintenir un bon niveau de productivité (Percival et Dawson 2009; Percival et Beaton 2012; Esau *et al.* 2014). Cependant, comme pour les fertilisants, l'utilisation de fongicides peut réduire la biomasse fongique du sol et impacter significativement la structure des communautés fongiques du sol (Ullah et Dijkstra 2019; Ma *et al.* 2021). L'impact des fertilisants et des fongicides n'est pas nécessairement le même pour tous les champignons du sol. Par exemple, les CEM associés

aux conifères sont plus sensibles aux ajouts d'azote que les autres CEM (Lilleskov *et al.* 2019) et différents CEM ont différents degrés de sensibilité à divers fongicides (Laatikainen et Heinonen-Tanski 2002). Par ailleurs, les champignons en relation avec les racines des plantes semblent plus affectés par l'ajout d'azote et certains fongicides que les champignons rhizosphériques ou les champignons non-mycorhiziens (Callaway *et al.* 2003; Marupakula *et al.* 2021). Enfin, une étude rapporte une plus grande sensibilité pour les CEM et les pathotrophes que pour les CMA et les SAP pour un même fongicide (Yang *et al.* 2021) alors qu'une autre mentionne que les CMA sont plus sensibles que les CME aux ajouts d'azote (Turnau *et al.* 1992).

Puisque l'activité des CME et des SAP est souvent bénéfique pour la survie et le développement du bleuet sauvage en contextes naturels et que l'utilisation des fertilisants et des fongicides est une pratique fréquente dans les bleuetières commerciales, il importe de documenter les effets seuls et combinés de ces pratiques sur la communauté fongique du sol. Dans le chapitre qui suit, nous avons évalué l'effet d'un fertilisant et d'un fongicide sur la croissance et l'abondance des CME et des SAP dans une bleuetière commerciale au Lac-Saint-Jean. L'évaluation a été faite en présence et en absence de plants de bleuet pour déterminer si les pratiques agricoles impactent différemment les deux guildes fongiques. Ainsi, nous avons échantillonné des hyphes fongiques avec des sacs de nylon remplis de sable que nous avons incubés dans le sol. Les hyphes récoltés ont servi à quantifier la biomasse fongique. Nous avons également prélevé des échantillons de sol, desquels l'ADN, extrait et séquencé, a permis d'identifier les espèces fongiques présentes dans la bleuetière.

CHAPITRE 2

FERTILIZERS AND FUNGICIDES HAVE VERY LITTLE INFLUENCE ON FUNGAL COMMUNITY STRUCTURE IN EXTENSIVE WILD BLUEBERRY PRODUCTION

2.1 ASBTRACT

Fertilizer and fungicide applications are common in wild blueberry cropping systems (*Vaccinium angustifolium* Aiton and *Vaccinium myrtilloides* Michaux) for improving fruit yield. These crop inputs may significantly impact soil fungi, and the topic is not well documented despite these organisms being essential for wild blueberry development and survival in natural environments. Therefore, we assessed soil fungal biomass and abundance in a commercial wild blueberry field from Lac-Saint-Jean, Québec (Canada) in which mineral fertilizer (50 N kg ha^{-1}) and fungicide (0,315 L ha^{-1} Proline 480 SC) were applied since 2017 during pruning years, i.e., every two years. In-growth sandbags (50 µm mesh) were incubated for 30 and 90 days during two growing seasons in order to collect fungal hyphae. Soil samples were also collected to characterize fungal community structure using next-generation sequencing PLC. Compared to no fungicide application, our results revealed that fungicide application increased hyphal length in sandbags by 25% and that fertilizer had no effect on hyphal length. Fungal community structure, however, was neither influenced by fertilizer, nor by fungicide application. Our findings suggest that extensive management in wild blueberry cropping systems does not negatively impact soil fungal communities. However, long-term research is still needed to evaluate the cumulative impacts of such management practices over several years and under different pedoclimatic conditions.

2.2 INTRODUCTION

Wild blueberry (*Vaccinium angustifolium* Aiton and *Vaccinium myrtilloides* Michaux) is a major fruit production in Quebec (MAPAQ 2022). Therefore, wild blueberry production has a significant economic impact in that province and especially in the Saguenay–Lac-Saint-Jean region, which accounts for about 80% of the wild blueberry Quebec production with 336 growers exploiting 29 874 ha (MAPAQ 2022; Gouvernement du Québec 2023). Several research studies have been launched in Quebec during the last few years to maximize per hectare production. The study of agricultural practices and their effects on soil quality, including soil fungi, are among priority topics (MAPAQ 2016; MAPAQ 2022).

Wild blueberry belongs to the Ericaceae plant family and thrives in acidic and nutrient poor soils in which soil nutrients are locked in recalcitrant soil organic matter (SOM) (Kloet 1988; Watkinson 2016). A symbiotic association with ericoid mycorrhizal fungi (EMF) is a survival strategy in such harsh soil environments (Watkinson 2016). EMF supply nutrients to the ericoid plants in return for plant photosynthates (Smith and Read 2008). By mobilizing nitrogen (N) and phosphorus (P) from SOM, EMF participate in SOM decomposition and soil nutrient cycling processes (Smith and Read 2008; Watkinson 2016; Martino *et al.* 2018). Besides EMF, saprotrophic fungi (SAP) play an important role in SOM decomposition (Deacon 2013). In fact, SAP are considered the most abundant guild in soil and by decomposing organic residues such as litter, dead plants and roots, SAP return nutrients to soil which are essential for plant development (Moore *et al.* 2020).

Soil fungi are affected by environmental factors, especially vegetation, which sustains fungal growth through root biomass, plant exudates, and litter production (Eisenhauer *et al.* 2017; Shi *et al.* 2018). Vegetation not only influences fungal biomass production but also determines fungal community species (Wubet *et al.* 2012; Tedersoo *et al.* 2016; Shi *et al.* 2018; Zhang *et al.* 2019). Moreover, vegetation has a different impact on soil fungi according to their guild. Thus, as symbionts, mycorrhizal fungi are disadvantaged comparatively to SAP when they no longer receive sugars from plant roots (Shi *et al.* 2018).

Growers prune wild blueberry shoots every two years to increase the number of flowers per stem and fruit yield per hectare (Eaton and McIsaac 1997). The season after mowing is called the pruning year, during which new shoots grow from buds located on rhizomes. The second year is called the harvesting year, as flower buds open in spring, and plants produce fruits during summer that are harvested at the end of the growing season (Chiasson and Argall 1996). In addition to pruning, growers add fertilizers and fungicides to increase or maintain fruit productivity (Eaton and Nams 2006; Percival and Dawson 2009; Lafond and Ziadi 2011; Percival and Beaton 2012; Esau *et al.* 2014). However, adding fertilizers and fungicides can reduce soil fungal biomass and abundance, as shown for other crops (cereals, legumes, vegetables, orchards) and in other ecosystems (forests, grasslands, wetlands, tundra) (Zhang *et al.* 2018; Ullah and Dijkstra 2019; Ma *et al.* 2021).

Because EMF and SAP are beneficial to wild blueberry development and since fertilizers and fungicides are frequently used by wild blueberry growers, it is essential to understand

their effects on soil mycobiota. Thus, our study was conducted to assess the effect of fertilizer and fungicide applications on the growth and the abundance of EMF and SAP in a wild blueberry field. The assessment was done in the presence and absence of blueberry plants to see if the agricultural inputs differentially impact the two fungal guilds. Our study aimed to quantify fungal biomass according to fertilizer and fungicide applications (with vs without), cropping phase (pruning vs harvesting phase), vegetation (with vs without vegetation), and incubation time (30 vs 90 days). Our study also aimed to characterize fungal community structure according to fertilizer and fungicide applications (with vs without) and vegetation (with vs without vegetation). We hypothesized that fertilizer and fungicide applications would reduce fungal biomass and diversity. We also hypothesized that the cropping phase, vegetation, and incubation time would significantly affect EMF and SAP.

2.3 MATERIAL AND METHODS

2.3.1 Study site

The 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; IRDA 2009). This well-drained soil is characterized by acidic conditions (i.e., pH<4.5) and low nutrient contents (Table 1) (Raymond *et al.* 1965; IRDA 2009). The region has a cold and humid climate with an average annual temperature and precipitations of 2,5˚C and 828,8 mm (Table 2) (Gouvernement du Québec 2020). Fertilizers and fungicides have been used in the BER since 2017.

Table 1: Edaphic conditions observed during the study (2019-2021).

Variable	Units	Site 1		Site 2	
		5-0 cm	**0-15 cm**	**5-0 cm**	**0-15 cm**
pH		4.02 ± 0.25	4.86 ± 0.18	4.00 ± 0.24	4.91 ± 0.26
P	mg/kg	25.03 ± 11.25	18.65 ± 7.45	22.83 ± 8.64	18.98 ± 6.62
K	mg/kg	4.31 ± 2.76	4.20 ± 2.88	4.75 ± 2.65	4.29 ± 2.60
Ca	mg/kg	155.30 ± 96.46	22.44 ± 83.52	152.70 ± 75.99	11.35 ± 12.23
Mg	mg/kg	1046.56 ± 541.03	41.23 ± 30.70	1168.99 ± 575.62	44.50 ± 27.37
NO_3	mg/kg	145.92 ± 71.93	5.62 ± 3.45	170.72 ± 80.53	9.16 ± 5.20
NH_4	mg/kg	0.01 ± 0.01	0.00 ± 0.01	0.01 ± 0.02	0.01 ± 0.01

Note: Means ± standard error

Table 2: Monthly mean temperature (°C) and total rain (mm) from May to September for the two studied sites during the study (2019-2021).

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

2.3.2 Experimental design

An experimental design was established on two sites, each with 32 experimental units. Site 1 was in pruning years in 2019 and 2021, and in harvesting year in 2020. Site 2 was in harvesting years in 2019 and 2021, and in pruning year in 2020 (Table 3). Pruning was performed by mechanical and thermal methods.

Table 3: Cropping phases calendar for the studied sites

Year	Site 1	Site 2
2019	Pruning year	Harvesting year
2020	Harvesting year	Pruning year
2021	Pruning year	Harvesting year

The experimental units received a combination of eight treatments settled as fungicide (with and without), fertilizer (with and without), and vegetation (with and without) organized in a four-block (replicates) in a split–split plot experimental design (Table 4).

Table 4: Treatment combinations applied to 64 experimental units

Treatment	Fungicide	Fertilizer	Vegetation	Abbreviation
1	With	With	With	Fu×Fe×Vg
2	With	With	Without	Fu×Fe
3	With	Without	With	Fu×Vg
4	With	Without	Without	Fu
5	Without	With	With	Fe×Vg
6	Without	With	Without	Fe
7	Without	Without	With	Vg
8	Without	Without	Without	C

Note: 4 plot replicates. 32 plots per site. Fu = fungicide. Fe = fertilizer. Vg = vegetation. C = control.

Fungicide and fertilizer treatments were applied respectively in July and June of every pruning year. Fungicide was used according to manufacturer recommendations as 0.315 L ha^{-1} of Proline 480 SC (active ingredient: prothioconazole) mixed with 0.250 L ha^{-1} of AG Surf adjuvant on July 24, 2019 and July 22, 2020. Proline 480 SC is a systemic foliar broad-spectrum fungicide that is largely used in agriculture for the control of fungal diseases (Bayer CropScience Inc. 2017). Fertilizer was applied as 50 kg ha^{-1} of nitrogen (N) in the form of ammonium sulfate, 30 kg ha^{-1} of phosphorus (P) in the form of super triple phosphate, 20 kg ha^{-1} of potassium (K) in the form of potassium sulfate, and 0,7 kg ha^{-1} of boron (B) in the form of borate on June 6, 2019, June 4, 2020, and May 28, 2021. Experimental units were delimited by plastic cylinders (diameter 30 cm) inserted in the ground to their full height (30 cm) to isolate blueberry plant roots from the rest of the field. Cylinders were paired in 15 x 22 m (330 m^2) plots treated with the same fungicide and fertilizer treatments. To insure spatial independency (Pelletier *et al.* 2023), there was a 3 m zone between each plot where no treatment was applied. Cylinders were inserted 1 m from the transverse border and 5 m apart from each other and the longitudinal border (Figure 2). Each experimental unit was manually weeded, and for experimental units without vegetation, blueberry shoots were cut with shears once at the beginning of the summer.

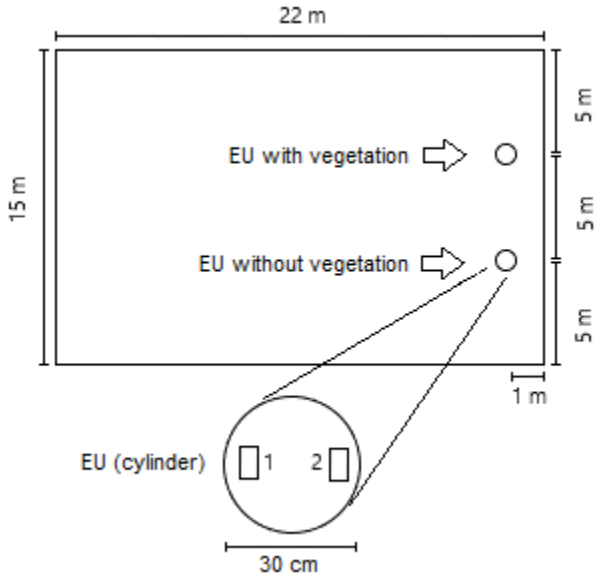

Figure 2: Diagram of one plot with two experimental units (cylinders) and diagram of the layout of in-growth sandbags inside one experimental unit. Cylinders were used to isolate blueberry plant roots from the rest of the field

2.3.3 Sampling

2.3.3.1 Fungal hyphae

Fungal hyphae were sampled with in-growth sandbags (6 x 10 cm) made from 50 µm mesh nylon (Wallander *et al.* 2001). Sandbags were filled with 50 g of autoclaved silica sand (99.6% SiO_2), and the edges were sealed with hot glue. On June 20, 2019, and June 19, 2020, two sandbags were oppositely inserted under the soil organic horizon close to the cylinder's inner border (Figure 2). On June 25, 2020, due to a high mortality rate of blueberry shoots that occurred inside cylinders in 2019, sandbags for experimental units with vegetation were placed outside cylinders under a bunch of shoots belonging to *V. angustifolium* according to the same layout as inside cylinders. Every year, sandbags were harvested 30 and 90 days after insertion in the ground, respectively, on July 22, 2019, July 23, 2020, Sept. 16, 2019, and Sept. 21, 2020. Harvested sandbags were transported on ice before being stored at -20˚C.

In-growth sandbags were thawed for analysis, and sand was poured into 100 ml containers. Then, the containers were put into a mechanical mixer for 30 minutes to allow sand homogenization. After sand homogenization, containers were stored at -20˚C until sample processing.

2.3.3.2 Soil samples

On June 11, 2021, soil samples were collected. In each plot, 10 to 12 core samples of organic soil were collected with a tubular probe (diameter 22 mm, length 5-0 cm). Core samples from plots were homogenized to form a composite sample. For experimental units without vegetation, a handful of organic soil was collected from the center of the experimental unit. 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. To prevent samples from contamination, sterile containers and nitrile gloves were used. Moreover, between each plot or experimental unit, nitrile gloves were sterilized with isopropylic alcohol 70% and the sampling material was water cleaned, sterilized with a sodium hypochlorite solution of 10% (Prince and Andrus 1992), and rinsed with water.

2.3.4 Measured variables

2.3.4.1 Fungal biomass

Fungal biomass was assessed in sandbags by hyphal length and organic matter (OM) content measurements. Hyphal length measurement was modified from Bukovská *et al.* (2016) and started with mycelium extraction from sand by stirring 10 g of sand from each in-

growth bag in 100 mL of sodium citrate solution (20 mM) for 30 minutes. Then, 900 ml of ultrapure water was added and stirring was brought to full speed for one minute. As full speed was maintained, two 10 mL aliquots were taken along the side of the beaker in the middle of the water volume, poured on a 125 µm sieve, and then filtered through a 0.45 µm pore size mixed cellulose esters membrane with grid. Membranes were mounted with glycerin on microscope slides without a cover slip. Every contact point between hyphae and the vertical grids were counted at 200X magnification. The mean value for both membranes was used to calculate hyphal length (m g^{-1} of dry sand) for each in-growth bag with Newman's formula (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.

Organic matter content determination was done using the loss-on-ignition method based on protocols from *Centre d'expertise en analyse environnementale du Québec* (CEAEQ 2003; CEAEQ 2017). Thus, 3 x 5 g of sand from each in-growth bag were dried overnight (16 h) at 105˚C in aluminum dishes. After drying, sand was poured into porcelain crucibles and covered with lids. The sand was then incinerated in a muffle furnace overnight (16 h) at 550˚C. Dry and incinerated weight means for every in-growth bag (accurate 0.01 mg) were measured for the set of triplicates. Organic matter content for each in-growth bag was calculated with the following formula (CEAEQ 2003) :

$$\% \, O.M. = \frac{\text{dry soil weight (g)} - \text{incinerated soil weight (g)}}{\text{dry soil weight (g)}} \times 100 \quad (2)$$

where *% O.M.* is soil organic matter content in percentage.

2.3.4.2 Fungal community structure

The fungal community structure was assessed in soil samples from the blueberry field using fungal DNA sequencing. DNA extraction was carried out by the *Centre sur la biodiversité de l'Université de Montréal* (Montréal, Québec, Canada). Briefly, DNA was extracted from 250 mg of fresh soil using QIAGEN PowerSoil PowerLyzer kits, following the manufacturer's instructions. DNA extracts were sent to *Centre d'Excellence en Recherche sur les Maladies Orphelines – Fondation Courtois* established at the *Université du Québec à Montréal* (Canada) for PCR amplification and amplicon sequencing. Fungal ITS was amplified with PCR master mix from New England Biolabs, containing $MgCl_2$, High Fidelity DNA Polymerase and dNTPs, mixed with 1 µL of DNA extract and 0.5 mM of each primer of the following pair : ITS1f (AATGATACGGCGACCACCGAGATCTACAC GG CTTGGTCATTTAGAGGAAGTAA) - ITS2 (CAAGCAGAAGACGGCATACGAGAT NNNNNNNNNN CG GCTGCGTTCTTCATCGATGC) (White *et al.* 1990), using the following thermocycler 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 DNA extract, and amplicons were pooled to prepare libraries for amplicon sequencing on Illumina MiSeq (PE250bp). 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*). The chosen values of 210bp and 200 bp provided scores between 30 and 35. 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.

2.3.5 Statistical analyses

The treatment effect on fungal biomass was assessed by a mixed variance analysis (ANOVA) model (JMP Pro 14.3.0, SAS Institute Inc., Cary, NC, USA), which included five fixed factors (cropping phase, fungicide, fertilizer, vegetation, incubation time) and three random factors (sampling year, site, and block nested within site). Post-hoc Tukey HSD tests were used to test for significant pairwise differences among treatments. Only hyphal length data needed to be log-transformed to improve normality and homoscedasticity of model residuals. These assumptions were verified upon visual inspection of residuals. We tested the model for hyphal length with and without shoot density (data not shown) as a covariable, and it did not change the statistical significance. We thus excluded shoot density from downstream models.

The treatment effect on fungal community structure was assessed by permutational multivariate analysis of variance (PERMANOVA) with three fixed factors (fungicide, fertilizer, vegetation) and two random factors (site and block nested within site) using the function *adonis* in *vegan v.2.6-4* (Oksanen *et al.* 2022). 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 graph created with the function *rda* in *vegan v.2.6-4*.

We also looked at differential abundance of fungal taxa individually using the *zicoseq* function in *GUniFrac v.1.6* (Chen *et al.* 2022; Yang and Chen 2022), and correcting with the FDR method. Fungal taxa with a significant response to one or several experimental treatments were represented graphically with a forestplot. Their guild was also assigned using the FunGuild database (Nguyen *et al.* 2016), using the *funguild_assign* function in FUNGuildR package *v.0.2.0.9000* (Furneaux 2021). This allowed to determine whether fungi influenced by treatments were predominantly mycorrhizal or non-mycorrhizal fungi (including putative saprotrophs and pathogens).

2.4 RESULTS

2.4.1 Fungal biomass

Hyphal length measurements and OM content indicated that fungal biomass in sandbags was significantly affected by the incubation time (Table 5). After an incubation period of 90 days, the hyphal length (Figure 3A) increased by 150%, and the OM content (Figure 3B) increased by 30% compared to the 30-day incubation period.

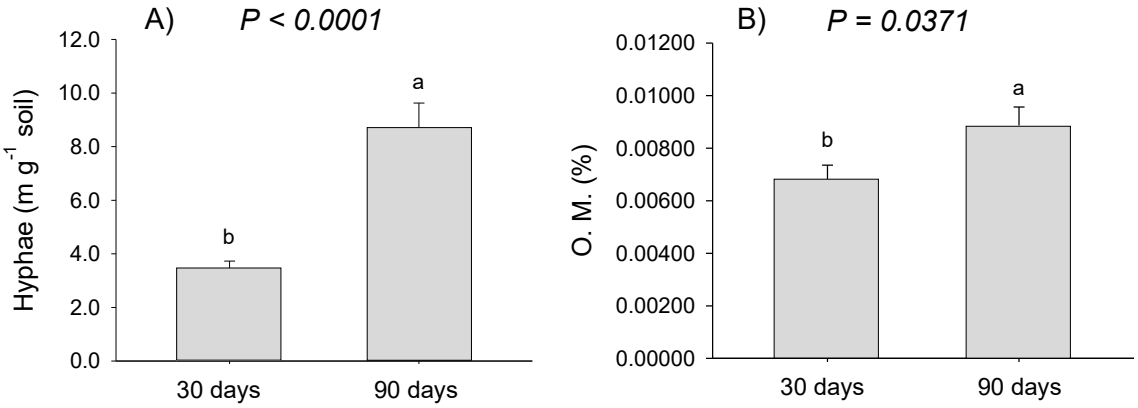

Figure 3: Hyphal length (A) and organic matter content (B) inside in-growth sandbags [means (± standard error)] per incubation time. Letters show significant differences between means using a least significant difference (LSD) post-hoc test ($P < 0.05$).

The mixed ANOVA analysis revealed that hyphal length in sandbags was significantly affected by the interaction between fungicide and fertilizer treatments (Table 5). Fungicide application increased by 25% hyphal length compared to control and fertilizer treatment and about 40% compared to the combined treatment (Figure 4A). Although not significant, OM content showed similar patterns to hyphal length (Figure 4B).

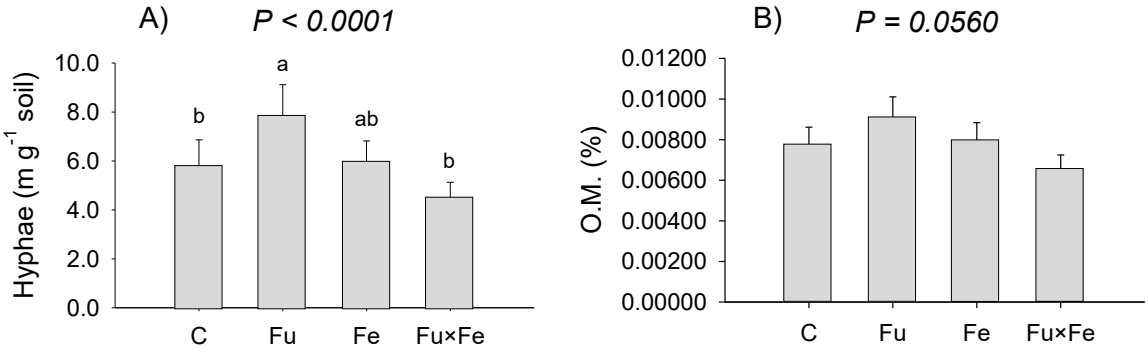

Figure 4: Hyphal length (A) and organic matter content (B) inside in-growth sandbags [means (± standard error)] per treatment. C = control. Fu = fungicide. Fe = fertilizer. Letters indicate significant differences between means using a least significant difference (LSD) post-hoc test ($P < 0.05$).

The mixed ANOVA analysis showed that OM content was significantly affected by the interaction between the cropping phase, fertilizer treatment, and incubation time (Table 5). OM content decreased by almost 60% after 30 days of incubation with the addition of fertilizer during the pruning year compared with no fertilizer addition during the harvesting year after 90 days of incubation (Figure S1B – Supplementary material). Although not significant, our results showed similar patterns as observed for hyphal length (Figure S1A – Supplementary material).

Table 5: Mixed ANOVA results for factor effects on hyphal length and organic matter inside in-growth sandbags

		Hyphal length		Organic matter content	
Factor	**df**	**F**	***P***	**F**	***P***
Cropping phase (Cr)	1	0.04	0.8405	1.19	0.2773
Fertilizer (Fe)	1	2.58	0.1095	1.57	0.2109
Fungicide (Fu)	1	1.33	0.2501	0.08	0.7718
Vegetation (Vg)	1	0.60	0.4399	0.25	0.6204
Incubation time (Ti)	**1**	**66.52**	**< 0.0001**	**4.40**	**0.0371**
Cr×Fe	1	0.06	0.8120	0.11	0.7446
Cr×Fu	1	0.74	0.3920	1.06	0.3053
Cr×Vg	1	0.54	0.4645	0.19	0.6624
Cr×Ti	1	0.23	0.6290	0.01	0.9353
Fe×Fu	**1**	**18.50**	**< 0.0001**	**3.69**	**0.0560**
Fe×Vg	1	0.40	0.5299	0.16	0.6883
Fe×Ti	1	0.10	0.7485	0.23	0.6286
Fu×Vg	1	0.66	0.4178	3.28	0.0715
Fu×Ti	1	0.37	0.5426	0.12	0.7327
Vg×Ti	1	0.83	0.3621	0.91	0.3411
Cr×Fe×Fu	1	0.02	0.8881	1.63	0.2033
Cr×Fe×Vg	1	1.92	0.1671	0.08	0.7764
Cr×Fe×Ti	**1**	**3.36**	**0.0683**	**6.35**	**0.0125**
Cr×Fu×Vg	1	0.00	0.9656	1.05	0.3076
Cr×Fu×Ti	1	0.18	0.6728	1.87	0.1731
Cr×Vg×Ti	1	1.46	0.2289	0.06	0.8068
Fe×Fu×Vg	1	3.82	0.0521	0.28	0.5998
Fe×Fu×Ti	1	0.46	0.4994	0.75	0.3863
Fe×Vg×Ti	1	1.05	0.3068	0.27	0.6068
Fu×Vg×Ti	1	0.00	0.9488	2.45	0.1189
Cr×Fe×Fu×Vg	1	0.04	0.8421	0.15	0.6948
Cr×Fe×Fu×Ti	1	0.17	0.6819	0.14	0.7060
Cr×Fe×Vg×Ti	1	0.98	0.3237	0.95	0.3316
Cr×Fu×Vg×Ti	1	0.11	0.7362	2.16	0.1434
Fe×Fu×Vg×Ti	1	1.71	0.1930	0.03	0.8721
Cr×Fe×Fu×Vg×Ti	1	0.22	0.6365	0.76	0.3837

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

2.4.2 Fungal community structure

The DNA sequencing resulted in the identification of 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.

The mixed PERMANOVA analysis performed on Euclidean distance-based metric revealed that the fertilizer and the fungicide treatments had no effect on the fungal community structure while the vegetation was significantly affecting it (Figure 5 & Table 6).

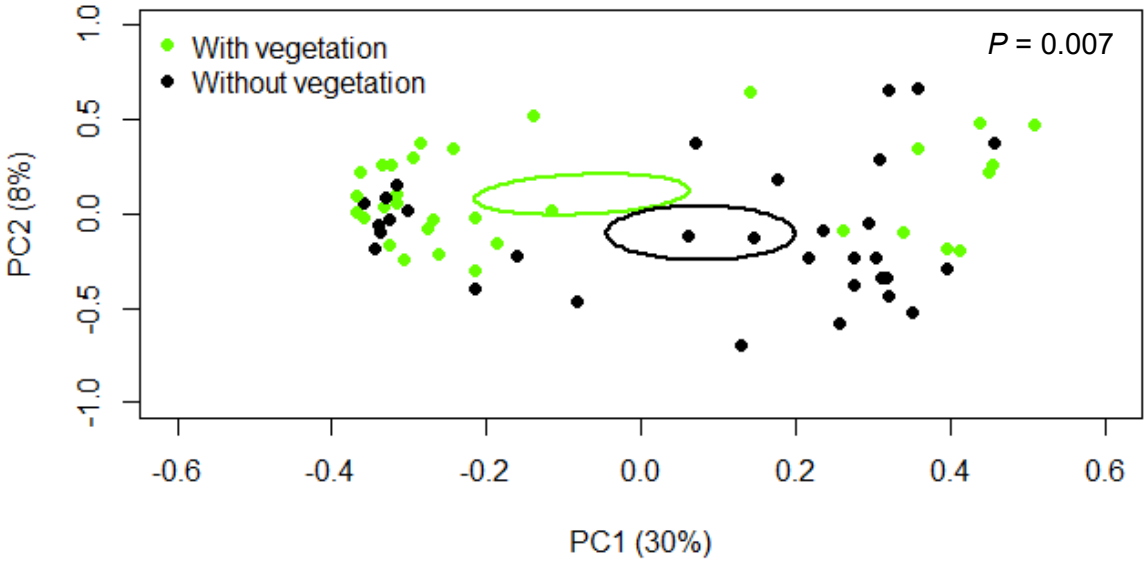

Figure 5: Principal component analysis (PCA) showing the effect of vegetation on fungal community structure after Hellinger transformation (ellipse = 95% confidence interval around the treatment centroid).

Table 6: Mixed PERMANOVA results for factor effects on fungal community structure

		Fungal community structure	
Factor	**df**	**F**	***P***
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. CG

The differential abundance analysis indicated that 48 ASVs were significantly affected by the presence of vegetation (Figure 6). From them, 33 ASVs are positively associated with the presence of wild blueberry plants and 15 ASVs are negatively associated with the presence of wild blueberry plants. Among the ASVs positively associated with the wild blueberry plants are 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 wild blueberry plants, three are basidiomycetous yeasts (*Rhodotorula*, *Solicoccozyma terricola*), three are from the Chaetothyriales order and two are *Hyaloscypha* species (*Meliniomyces* [= *Hyaloscypha*], *Hyaloscypha variabilis*).

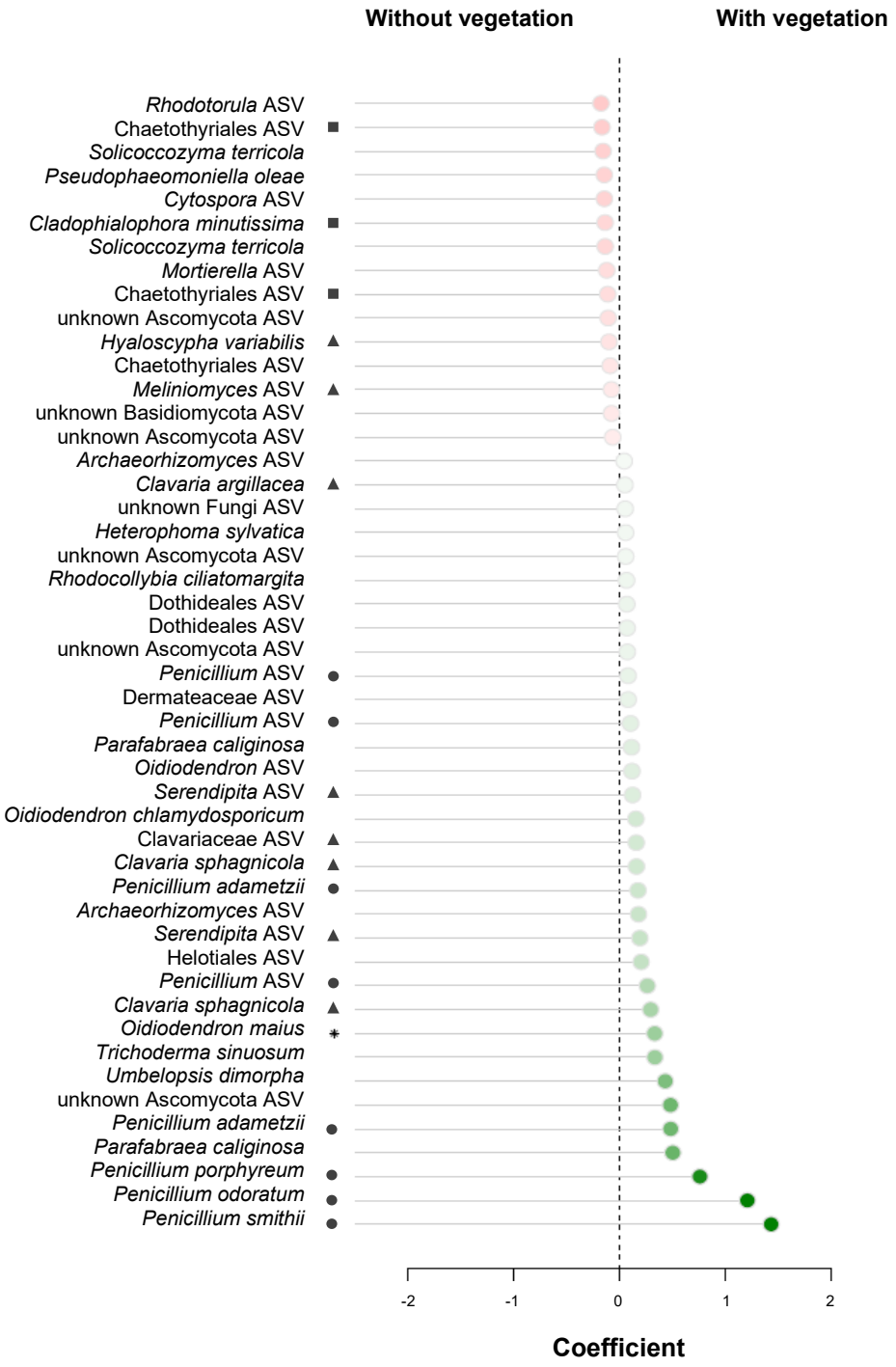

Figure 6: Forestplot diagram showing the ASVs with a significant response to vegetation as determined through differential abundance analysis. The color of the dots indicates the direction and the intensity of the association between the ASVs and the wild blueberry plants. Red dots = ASVs negatively associated with the wild blueberry plants. Green dots = ASVs positively associated with the wild 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.

2.5 DISCUSSION

The assessment of the effect of agricultural practices, incubation time, and vegetation on the growth of EMF and SAP in a commercial wild blueberry field revealed that soil fungal biomass was positively affected by incubation time and fungicide application. The assessment of the effect of agricultural practices and vegetation on the abundance of EMF and SAP highlighted that fungal community structure was only affected by the vegetation factor.

2.5.1 Fungal biomass

2.5.1.1 Incubation time

Hyphal length measurements and OM content in sandbags show that fungal biomass increased over the incubation time. These results are consistent with those of Wallander *et al.* (2013), Hagenbo *et al.* (2018) and Zuev *et al.* (2019), who reported larger fungal biomass inside in-growth sandbags according to the length of incubation of the sandbags in the ground. This pattern of growth is likely attributable to mycorrhizal fungi since, in mid-latitudes in the northern hemisphere, the growth increases through spring before achieving an optimum level during summer or fall (Stevens *et al.* 1997; McGonigle and Miller 1999; Wallander *et al.* 2001; Štursová *et al.* 2020). This phenomenon is due to plant productivity increases, which provide carbon to mycorrhizal fungi throughout the growing season (Olsrud *et al.* 2004; Ekblad *et al.* 2013; Voříšková *et al.* 2014; Shi *et al.* 2018). Conversely, for the same regions, saprotrophic fungal biomass is reported to peak in late winter or during spring

(Birgander *et al.* 2014; Voříšková *et al.* 2014; Santalahti *et al.* 2016). This is explained by increases in enzymatic activity of SAP related to litter decomposition during winter leading to a peak in biomass in the spring (Voříšková *et al.* 2014). Nevertheless, in some oak, hickory, and beech stands in south-central Indiana in the USA, saprotrophic fungal biomass has been found to peak in late summer from August to November (Midgley and Phillips 2019).

2.5.1.2 Fertilizer application

In our study, the application of mineral fertilization did not affect soil fungal biomass as opposed to the outcomes of the meta-analysis of Zhang *et al.* (2018) and the studies of Bääth *et al.* (1981) and Maaroufi *et al.* (2019) in pine forests. One possible explanation for our results may be the low inputs of fertilizer (50 kg N ha^{-1} every two years) since no effect on mycorrhizal colonization rate was observed even with higher inputs of fertilizer in a commercial lowbush blueberry field (60 kg N ha^{-1} yr^{-1}) (Jeliazkova and Percival 2003) or for other *Vaccinium* species (50-100 kg N ha^{-1} yr^{-1}) (Michelsen *et al.* 1999; Urcelay *et al.* 2003; Ishida and Nordin 2010). More generally, in studies assessing the effect of fertilization in temperate and boreal forests, fertilizer applications of 80 and 100 kg N ha^{-1} yr^{-1} did not affect soil fungal biomass (Sinsabaugh *et al.* 2002) nor soil microbial biomass (Liu *et al.* 2023), respectively.However, applications of 50 kg N ha^{-1} yr^{-1} during 14 years in a Scots pine forest (*Pinus sylvestris* Linné) in northern Sweden led to a decrease in soil fungal biomass (Maaroufi *et al.* 2019). Moreover, in 21 European ombrotrophic bogs, atmospheric nitrogen deposition varying from 5 kg to 30 kg N ha^{-1} yr^{-1} decreased fungal biomass in soil and in the roots of *Vaccinium oxycoccos* Linné (Boeraeve *et al.* 2022).

2.5.1.3 Fungicide application

The fungicide application had a positive effect on fungal biomass in our study. This result is in contrast with the conclusions about the detrimental effects of fungicides on soil microbial biomass provided by Ullah and Dijkstra (2019) and with the negative impact on soil fungi of two fungicides that are demethylation inhibitors as prothioconazole. Bjørnlund *et al.* (2000) reported a decrease in saprotrophic fungal biomass with the use of fenpropimorph, while Elmholt (1991) reported a reduction in the CFU (colony-forming units) numbers of filamentous soil fungi when propiconazole was applied. A physiological non-fungicidal effect of the fungicide on the blueberry plants could explain our results. In the context of another study conducted in the same study site in 2017 and 2018, wild blueberry plants that were treated with the fungicide during the pruning year had a higher number of leaves per shoot during mid-September in 2018 (Paré *et al.* 2022). In another study performed at the Nova Scotia Wild Blueberry Institute in Debert (Nova Scotia), fungicide application during the pruning year prolonged leaf retention by approximately three weeks at the end of the growing season (Percival and Burnham 2006). Therefore, increasing the number and the retention of leaves in late summer likely enhanced the photosynthetic rate and period, which promoted photosynthate production inside the blueberry plants. In late summer, more photosynthates will likely induce larger soluble sugar levels in roots as the blueberry plants are making reserves for overwintering (Kaur *et al.* 2012). More sugar in roots likely means more resources for EMF and rhizospheric SAP growth as their development is respectively linked to carbon allocations in roots (Hobbie and Hobbie 2006; Drigo *et al.* 2010) and root exudates (Eisenhauer *et al.* 2017). Moreover, other physiological non-fungicidal effects may have also enhanced carbon allocations in roots and rhizospheric soil. The application of

fungicides has also been reported to increase root biomass (Joshi *et al.* 2014) and the content of soluble sugars in root exudates (Jabaji-Hare and Kendrick 1985). Therefore, the effect of the fungicide on fungal biomass might be due to an effect on the vegetation which appears stronger than a possible detrimental effect of the fungicide on soil fungi. Consequently, the measurement of mycorrhizal rate in the roots of wild blueberry plants subject to fungicide applications would be informative. In addition to the previous explanations, the increase in soil fungal biomass might also be induced by a decrease in phylloplane epiphytic and endophytic fungi due to foliar application of the fungicide (Prior *et al.* 2017). A decrease in phylloplane fungi would result in an increase of resources allocated to the EMF and rhizospheric SAP favouring their development. In any event, all those explanations are worthy of further investigations.

2.5.2 Fungal community structure

2.5.2.1 Vegetation

The results of the PERMANOVA revealed that the vegetation significantly affected the fungal community structure in the soil. It is known that plants sustain the growth of soil fungi by allocating carbon to the microorganisms living in the soil through litter production along with root biomass and exudates (Eisenhauer *et al.* 2017; Shi *et al.* 2018; Prommer *et al.* 2020). Besides influencing fungal growth, plants can also influence fungal community structure, by favouring some species over others, which is at the very root of so-called plant-soil feedbacks (Wardle *et al.* 2004; Tedersoo *et al.* 2016). For example, root exudates have been found to promote symbiotic fungi species and decrease saprotrophs and pathogens (Shi *et al.* 2018). Furthermore, depending on their morphological/functional traits, plants create

distinct habitats in their root system, which are colonized by specific fungal species, thus influencing soil fungal community structure (Hugoni *et al.* 2018; Sietiö *et al.* 2018; Sheng *et al.* 2019). Therefore, our results align with these observations and the findings of Fanin *et al.* (2022), who reported a shift in mycorrhizal and saprotrophic community composition with the removal of ericaceous plant species.

The differential abundance analysis highlighted the relationship of different fungal taxa with the blueberry plants. From the species positively associated with the wild blueberry plants, there are eight *Penicillium* species. Interestingly, the trophic mode of the *Penicillium* genus is considered saprotrophic according to the FUNGuild database. Still, our results suggest that the genus may have a relationship of importance with wild blueberry as a root endophyte or rhizospheric fungi. *Penicillium* is the genus with the highest number of known endophytes (Rashmi *et al.* 2019). It has been reported as a root endophyte of *Vaccinium virgatum* Aiton (Guo *et al.* 2021), *Vaccinium macrocarpon* Aiton PLC (Salhi *et al.* 2022) and *V. corymbosum* (You *et al.* 2020; Cai *et al.* 2021; Gomes *et al.* 2023) and in the rhizosphere of *Vaccinium darrowii* Camp (Li *et al.* 2020). This could highlight the potentially important and underappreciated role of non-mycorrhizal fungi as endophytes influencing wild blueberry (and ericoid species in general) plant growth. The abundance of the genus in the soil of blueberry environment (Pinotti *et al.* 2011; Chroňáková *et al.* 2019) may also be because *Penicillium* is a phylloplane fungus of blueberry (Watanabe *et al.* 2011; Greco *et al.* 2012; Munitz *et al.* 2013; Elena *et al.* 2018) or because it is a pathogen causing rotting in postharvest blueberry fruits (Figueroa S. *et al.* 2010; Xiao and Saito 2017; Liu *et al.* 2018; Wu *et al.* 2022b).

There are few recognized EMF species, and most belong to the Heliotales family in the phylum of Ascomycota (Vohník 2020). *Oidiodendron* is one of the genus in which EMF are observed, and one EMF species belonging to this genus was identified in our study, namely *Oidiodendron maius* (Leopold 2016). In addition to recognized EMF, we identified four Clavariaceae taxa and two *Serendipita* species associated with the wild blueberry plants. Those fungi are from the Basidiomycota phylum and could also be EMF (Peterson *et al.* 1980; Vohník 2020). Recent studies revealed that Clavariaceae and *Clavaria* (Clavariaceae) are abundant in the soil of wild blueberry fields (Morvan *et al.* 2020; Lloyd *et al.* 2021) and in the rhizobiome of wild blueberry plants (Dong *et al.* 2022). Furthermore, *Clavaria* was reported to be very abundant in the rhizobiome of highbush blueberry (*V. 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 wild blueberry field (Lloyd *et al.* 2021) and in the hair roots of *Vaccinium uliginosum* Linné (Yang *et al.* 2018). For its part, *Serendipita* has been reported abundant either in the rhizosphere or in the roots of different blueberry species, i.e. *V. angustifolium*, *V. corymbosum* x *V. angustifolium*, *V. corymbosum*, *Vaccinium 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 2019). Therefore, our results support that EMF could include species from the *Clavaria* and *Serendipita* genera. More broadly, it suggests that in the absence of ericaceous host, fungal communities can shift rapidly to being dominated rather by non-mycorrhizal taxa, which supports putative antagonistic interactions between mycorrhizal and non-mycorrhizal (Fernandez and Kennedy 2016). Since we found no impact of our treatments on fungal productivity, this suggests that fungal communities are “zero-sum”

competitive arenas, which is in line with the predominantly bottom-up and competition-based regulation of fungal communities put forward by Wardle (2002).

The ASVs negatively associated with the wild blueberry plants included three basidiomycetous yeasts (*Rhodotorula* and *Solicoccozyma terricola*). Yeasts are distributed worldwide and found in numerous habitats, including soils (Péter *et al.* 2017; Boekhout *et al.* 2022). Among the common yeasts isolated from boreal and temperate forests soils are many *Rhodotorula* species and *Solicoccozyma terricola* (Yurkov 2017; Boekhout *et al.* 2022). Therefore, it is not surprising that in our study, these species are associated with the absence of wild blueberry plants. The Chaetothyriales order is part of the Ascomycota phylum and comprises melanized fungi which grow in diverse habitats like water, soils, rocks, lichens, or plant leaves (Geiser *et al.* 2006; Quan *et al.* 2020). Chaetothyrialean fungi have various lifestyles as pathotrophs, saprotrophs, endophytes or fungicolous (Teixeira *et al.* 2017; Quan *et al.* 2020). The order is considered to contain putative EMF (Leopold 2016; Watkinson 2016) and has been reported abundant in wild blueberry habitats (Morvan *et al.* 2020), 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) and in the roots of other Ericaceae (Walker *et al.* 2011; Toju *et al.* 2016). Among Chaetothyriales is the endophyte *Cladophialophora minutissima* (Herpotrichiellaceae) (Davey and Currah 2007), which has previously been isolated from the roots of Ericaceous plants *Kalmia angustifolia* and *Gaultheria hispidula* (Griffin 2019). 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 (REA) (Fehrer *et al.* 2019; Vohník 2020). Therefore, our results are in accordance with the multi-trophic nature of *Hyaloscypha* and Chaetothyriales since they were found here to thrive in the absence of an ericaceous host.

2.5.2.2 Fertilizer application

The PERMANOVA showed that fertilization did not affect fungal community structure. This result contrasts with the meta-analyses of Treseder (2004) and Zhang *et al.* (2018), which conclude that fertilization has a negative impact on soil fungal abundance, and with other studies conducted in boreal forests reporting either negative (Allison *et al.* 2007), positive (Tahovská *et al.* 2020) or both impacts (Maaroufi *et al.* 2019; Jörgensen *et al.* 2022; Renaudin *et al.* 2023) on soil fungal species. However, our results agree with the observations of Liu *et al.* (2023) after a five-year application of 50 and 100 kg N ha^{-1} yr^{-1} in an Eastern Chinese boreal forest. The absence of effect of fertilization in our study may be due to the N-limited conditions of the experimental site, which are typical in such boreal soil ecosystems (Bonan 1989; Lafond 2018), and to the production context, whereby wild blueberry plants are mowed every two years. It has already been shown that wild blueberry plants subjected to mineral fertilization exhibit higher growth, fruit yields and N foliar concentrations (Smagula and Ismail 1981; Lafond 2010; Lafond and Ziadi 2011; Lafond 2020; Schmitt 2023). Moreover, since fertilization is applied only during the pruning year, it is possible that the production of new shoots induces a significant demand for nutrients and prevents N accumulations in soil, thus avoiding impact on soil microorganisms. Another possible

explanation for our result is that soil fungi responses to N additions may be species, guild, niche or context-dependent. Hence, in the study of Maaroufi *et al.* (2019), some fungal species were positively affected by N additions, while others were negatively affected. For its part, Jörgensen *et al.* (2022) reported a positive effect on root-associated ascomycetes, a negative effect on ectomycorrhizal fungi (EcMF) and EMF and no effect on agaricomycete SAP. Marupakula *et al.* (2021) and Fu *et al.* (2022) highlighted divergent responses to N addition, whether fungi inhabit roots, rhizosphere or bulk soil. Finally, Turnau *et al.* (1992) showed that EMF were more tolerant to N additions than arbuscular mycorrhizal fungi (AMF) in an oak-pine forest, while Boeraeve *et al.* (2022) observed that EMF and AMF were not affected by N additions in ombrotrophic bogs, which are also N depleted soil ecosystems. Overall, the combination of a low rate of N application (50 kg ha^{-1} every two years) and a rapid N uptake by wild blueberry plants are likely to explain our results.

2.5.2.3 Fungicide application

As for the fertilizer, the fungicide did not affect fungal community structure. The topic is much less documented compared to N fertilization in boreal forests. Nonetheless, our result contrasts with the decrease in soil fungal diversity reported in a commercial wild blueberry field (Lloyd *et al.* 2021) and in fungal root endophytes diversity of commercial cranberry plants (*V. macrocarpon*) (Salhi *et al.* 2022). However, in the study of Lloyd *et al.* (2021), the decrease was observed after three applications of Proline 480 SC with the same dosage as in our study (0.315 L ha^{-1}) done at 10 day intervals during one pruning year. Therefore, our results can be explained by the lower applications of the fungicide (0.315 L ha^{-1} every two years) compared to Lloyd *et al.* (2021). Another possible explanation for our observations

might be related to the fungicide itself since fungicides have different effects on fungi and different behaviors in plants. For example, in a field study comparing three fungicides, Benomyl was more deleterious on soil fungal populations than procloraz and iprodione (West *et al.* 1993). This might be due to the mobility of the fungicide inside the plants since fungicides can be systemic or non systemic. Systemic fungicides are absorbed by the plant tissues and their mobility is either local, translaminar, upward (through the xylem) or upward and downward (through the xylem and the phloem – amphimobile). Non systemic or contact fungicides stay on the plant surfaces (Pérez-Rodríguez *et al.* 2018). However, even a theoretically non amphimobile fungicide can be partially transmitted to the plant roots (Fang *et al.* 2023). Thus, local mobile fungicides like prothioconazole, procloraz and iprodione might be less toxic to soil fungi than a xylem mobile fungicide like Benomyl (Mueller *et al.* 2013). Moreover, the fungicide effect on soil fungi depends on several factors such as host plant, fungal growth stage, resilience capacity, bacterial symbiosis as well as fungicide mode of action or application, dosage and physicochemical behaviour of the active agent in the soil (Uma *et al.* 2011; Hage-Ahmed *et al.* 2018). In consequence, persistence of prothioconzaole might also explain our results. Prothioconazole is rapidly degraded into metabolites and its half-life has been evaluated to maximum 10 days in soils and in plants (Dong *et al.* 2019; Zhai *et al.* 2022). Its main highly toxic metabolite, prothioconazole-desthio, is more persistent, its half-life has been assessed up to 51 days in soil (Wu *et al.* 2022a). However, according to Fang *et al.* (2023), prothioconazole-desthio concentration is two to three times lower in roots than in leaves 14 days after foliar application. Fungicide effect on soil fungi also depends on phyla, species, guild, or niches. Thus, a greater tolerance in Ascomycota unlike Basidiomycota fungi has been highlighted for some fungicides, including prothioconazole (Ma *et al.* 2021; dela Cruz *et al.* 2022). For their part, Laatikainen and

Heinonen-Tanski (2002) reported differential sensitivity of several EcMF species or strains to different fungicides. Callaway *et al.* (2003) observed that non-mycorrhizal fungi were less sensitive to Benomyl than mycorrhizal fungi. Yang *et al.* (2021) reported that the richness of EcMF and pathotrophic fungi was negatively affected by Benomyl, whereas AMF and SAP were not. Finally, Uma *et al.* (2011) highlight that intraradical hyphae respond differently to fungicides compared to extraradical hyphae. Overall, the low application of the fungicide (0.315 L ha^{-1} every two years), combined to its low mobility and the short half-life of its main toxic metabolite as well as the predominance of Ascomycota fungi are likely to explain our results.

2.6 CONCLUSION

In this study, we assessed the impact of fertilizer and fungicide applications and cropping phase, vegetation, and incubation time on soil fungi in a wild blueberry field. Our results showed a positive effect of the incubation time and the fungicide application on the growth of soil fungi. On the other hand, our results highlight the absence of the effect of the fertilizer on the growth of soil fungi, possibly due to the low input of N in the wild blueberry field. Moreover, our results revealed that the removal vegetation affected fungal community structure, whereas fertilizer and fungicide applications gave rise to no effect on this variable. This absence of effect of the fertilizer and the fungicide is plausibly due to the rapid uptake of N by the wild blueberry plants, the low input and the low mobility of the fungicide as well as the short half-life of its main metabolite and the predominance of Ascomycetes, but further studies are required to support these assumptions. Similarly, long-term research in different

wild blueberry fields is still needed to evaluate the practices over several years and under different pedoclimatic contexts.

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CHAPITRE 3

CONCLUSION GÉNÉRALE

Les fertilisants et les fongicides sont couramment utilisés dans la culture du bleuet sauvage (Eaton et Nams 2006; Percival et Beaton 2012). Bien qu'avantageux pour la productivité, ces intrants sont susceptibles d'impacter les microorganismes présents dans le sol (Zhang *et al.* 2018; Ullah et Dijkstra 2019; Ma *et al.* 2021). Comme les champignons du sol sont souvent bénéfiques pour le développement et la survie du bleuet sauvage dans son habitat naturel (Watkinson 2016; Coleman *et al.* 2017) et qu'ils peuvent être avantageux pour la productivité du bleuet cultivé (Vohník *et al.* 2012; Mu *et al.* 2021), il est important de documenter l'impact des intrants agricoles sur le mycobiote du sol.

3.1 Croissance des champignons du sol

Dans notre étude, les ajouts de fertilisant et du fongicide Proline 480 SC n'ont pas eu d'impact négatif sur la croissance des champignons du sol. Les mesures de biomasse fongique ont même montré une augmentation de la croissance des champignons du sol à la suite de l'application du fongicide. Cette augmentation serait possiblement causée par une hausse des ressources disponibles pour le développement des champignons du sol en raison d'une plus grande allocation de carbone dans les racines. Cette allocation de carbone accrue serait due à une plus grande production et une plus longue rétention de feuilles à la fin de l'été sur les plants de bleuets traités avec le fongicide. Les mesures de biomasse fongique ont également montré que le temps d'incubation des sacs de sable dans le sol avait un impact significatif et positif sur le développement des hyphes fongiques dans le sol.

3.2 Structure des communautés fongiques

Les résultats de notre étude ont montré que les ajouts de fertilisant et de fongicide n'ont pas eu d'impact significatif sur la structure des communautés fongiques du sol. Toutefois, la suppression de la végétation a eu un effet significatif sur plusieurs espèces fongiques dont quelques espèces de *Penicillium*, des Clavariacées, deux espèces de *Serendipita* et une espèce de CME connue, soit *Oidiodendron maius*. L'effet de la phase de production et du temps d'incubation sur la structure des communautés fongiques n'a pu être évalué comme prévu initialement. En effet, la quantité d'ADN fongique étant trop faible dans les sacs de sable, des échantillons de sol ont été prélevés pour effectuer les analyses moléculaires. Comme les échantillons n'ont été prélevés qu'à une seule date, il a été impossible de prendre en compte la phase de production et le temps d'incubation des sacs de sable dans nos analyses.

3.3 Limites de la recherche

L'évaluation de la biomasse fongique par la mesure de longueurs d'hyphes sous microscope et par la technique de la perte au feu sont des méthodes peu coûteuses qui ont fait leurs preuves. Toutefois, l'utilisation d'autres méthodes de mesures pour la biomasse fongique, comme l'amplification en chaîne par polymérase quantitative (qPCR) ou la technique par analyse des acides gras phospholipidiques (PLFA), et pour la structure de la communauté fongique, comme la diversité alpha, auraient pu ou pourraient être utiles pour confirmer ou compléter les résultats obtenus. Par ailleurs, le fait de n'avoir pu faire qu'un seul échantillonnage de sol pour les analyses moléculaires a empêché l'évaluation de l'effet de certains paramètres sur la structure de la communauté fongique.

3.3 Conclusions

L'objectif principal de notre étude qui était de quantifier la biomasse fongique du sol et de caractériser la structure de la communauté fongique dans le sol d'une bleuetière commerciale a été atteint. Les mesures de biomasse fongique et de diversité spécifique infirment notre hypothèse première concernant l'effet du fertilisant et du fongicide sur les champignons du sol. Toutefois, l'effet significatif du temps d'incubation sur la biomasse fongique et du retrait de la végétation sur la composition spécifique confirme partiellement deux de nos hypothèses secondaires. Par conséquent, nos résultats suggèrent que les pratiques agricoles peu intensives dans la culture du bleuet sauvage n'impactent pas négativement les communautés fongiques du sol. Cependant, des recherches à long terme et dans différents contextes pédoclimatiques demeurent nécessaires pour évaluer et comprendre les effets potentiellement cumulatifs de ces pratiques sur le mycobiote du sol du bleuet sauvage cultivé. De surcroît, de nouvelles études mesurant la biomasse racinaire, le contenu en sucres solubles des exsudats racinaires, le taux de mycorhization des racines et la mobilité réelle du fongicide pourraient permettre de déterminer les causes de l'augmentation de la croissance des champignons du sol dans les bleuetières commerciales lors de l'application du fongicide Proline 480 SC.

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ANNEXE 1 SUPPLEMENTARY MATERIAL

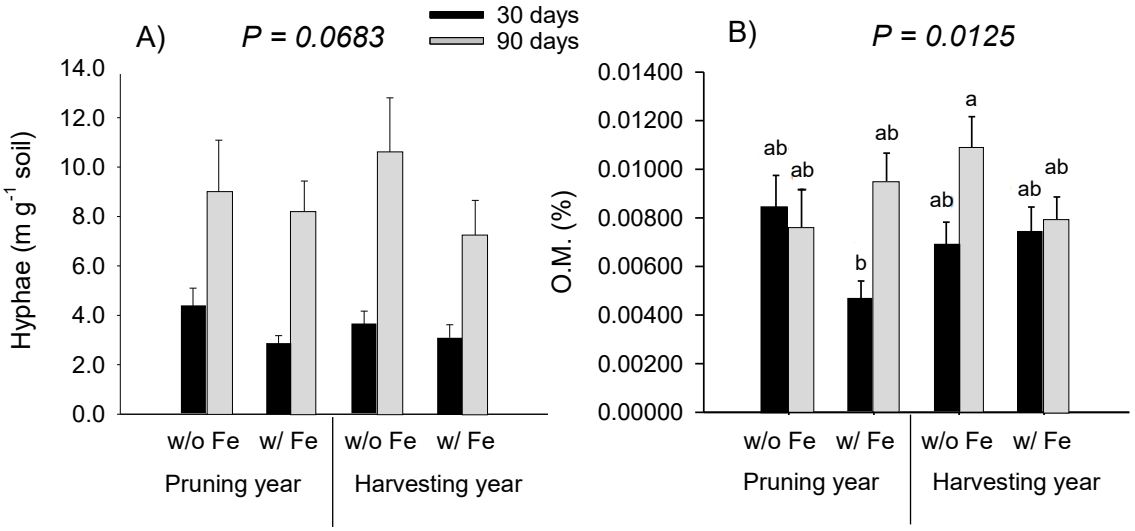

Figure S1: Hyphal length (A) and organic matter content (B) inside in-growth sandbags [means (± standard error)] per cropping phase, fertilizer treatment, and incubation time. Fe = fertilizer. w/ = with. w/o = without. Letters indicate significant differences between means using a least significant difference (LSD) post-hoc test ($P < 0.05$).

Table S1: Percentage of DNA sequences lost during fusion process.

Step	**%**
Initial	0
filter_And_trim	~ 5
Merge	~ 5
RemoveBimeraDenovo	~ 15
Total	~ **25**