Viable microbial inoculation of granular fertilizer to improve row crop productivity in Southern Quebec

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July 2020

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

Abstract:

Seeding of row crops in Southern Quebec is often accompanied with a banded fertilizer to provide a rapidly available source of nutrients to seedlings. A portion of the banded fertilizer is not absorbed by plants or soil microorganisms, negatively affecting surrounding environments. Plant beneficial microbes found in soils play an active role in how plants absorb nutrients either by synthesizing plant-available molecules or by solubilizing nutrients unavailable to plants into plant-available forms. In the last few years, a multitude of products claiming to contain plant-beneficial microbes have been commercialized. The project aims to evaluate the effects of two commercial plant-beneficial microbial consortia, coated on granular fertilizer beads on plant growth and the dynamics of soil microbial communities. Responses of corn and potato were evaluated for two years, while the response of potato-associated soil microbial communities was evaluated during the second year. For both corn and potato, two different doses were used for each microbial consortium. In potato, a second application of fertilizer coated with microbial consortia was evaluated. Fertilizer inoculation with microbial inoculants resulted in a significant yield increase, up to 20.2% for corn for one year out of two, and an increase in the weight of corn seeds in both years. The lower microbial concentration had overall better performance for one product while results varied between years for the second product. In potato, tuber yield increased by up to 19.6% on inoculated plots, although no difference was significant in both years for all treatments investigated. Analysis of bulk soil and rhizospheric soil revealed that microbial inoculation did not have a significant effect on prokaryotic and fungal communities. Sampling date and block number had significant effects on both microbial communities. A significant difference could be observed between the fourth sampling and the first three in prokaryotic and fungal communities, most likely due to agricultural practices which included weekly pesticide application and a tillage operation. Microbial community composition was correlated with soil organic matter, phosphorus and many micronutrients contents likely explaining the difference in prokaryotic community composition between the first block and the three other blocks. Overall, the findings explain how microbial inoculants viability can be verified at different moments from production of inoculants up to the colonization of the rhizosphere, helping to explain the inconsistent plant growth-promotion observed in fields.

Résumé :

Dans le sud du Québec, le semis en rang est souvent accompagné d'un fertilisant appliqué en bande qui fournit une source de nutriments rapidement assimilables par les plantules. Une partie des fertilisants appliqués en bande n'est pas assimilée par les plantes ni les microorganismes du sol, affectant négativement les écosystèmes environnants. Les microbes bénéfiques aux plantes présents dans le sol jouent un rôle actif dans l'absorption des nutriments en les synthétisant ou en solubilisant des molécules non-disponibles aux plantes en formes facilement assimilable. Au cours des dernières années, une multitude de produits contenants des microbes bénéfiques aux plantes ont été commercialisés. Le but de ce projet vise à évaluer l'effet sur la croissance des plantes et la dynamique des communautés microbiennes de l'enrobage de deux consortia de microbes bénéfiques aux plantes sur les billes d'engrais granulaire. Le développement de maïs-grain et de pommes de terre fut suivi pendant 2 ans ainsi de l'évaluations des communautés microbiennes du sol produisant des pommes de terre durant la deuxième année. Les essaies sur le maïs et les pommes de terre utilisaient deux différentes doses d'inoculant microbiens pour chaque consortium. Dans la pomme de terre, un traitement additionnel ayant une deuxième application des consortia microbiens fut évalué. Les résultats montrent une augmentation significative du rendement jusqu'à 20.2% pour le maïs-grain pour une année sur deux, la masse de 100 grains de maïs était significativement augmentée pour les deux inoculants durant les deux années. La dose la plus faible procurait les meilleurs effets pour un produit tandis que pour l'autre produit, les meilleurs résultats variaient d'une année à l'autre entre les deux doses. Pour la pomme de terre, une hausse du rendement jusqu'à 19.6% fut mesurée, toutefois aucun traitement avait un effet significatif sur le traitement durant les deux années. L'analyse du sol en vrac et du sol rhizosphérique ne démontre pas d'effet provenant des consortia microbiens inoculés sur les communautés procaryotiques et fongiques du sol. La date d'échantillonnage et l'emplacement de l'échantillon avaient un effet significatif sur la composition des communautés microbiennes du sol. Une différence significative des communautés microbiennes était observée entre les trois premières campagnes échantillonnages et la dernière campagne d'échantillonnage. La grande période de temps entre le 3^{e} et le 4^{e} échantillonnage ainsi que des applications hebdomadaires de fongicides et un rehaussage des butons expliquent probablement la différence observée

entre les campagnes d'échantillonnages. La composition des communautés microbiennes était significativement corrélée avec la teneur du sol en matière organique, en phosphore et plusieurs micronutriments expliquant en partie la différence observée dans les communautés microbiennes entre le premier block de traitements et les trois autres blocks de traitements. De façon globale, les résultats de ce projet augmentent notre compréhension face à l'enrobage d'inoculants microbiens autour de l'engrais de synthèse et la vérification de la viabilité de ces inoculants à différents moments pendant la saison, de la production de l'inoculant jusqu'à sa colonisation de la rhizosphère, dans la perspective de mieux comprendre les effets inconsistants des inoculants observés au niveau des plantes

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List of abbreviations:

ARDRA: amplified ribosomal DNA restriction analysis

ARISA: Automatic ribosomal interspace spacer analysis

BNF: Biological N_2 fixation

CARD: Catalyzed reported deposition

CHU: Corn heat unit

DAPG: Diacetyl phloroglucinol

DGGE: Denaturing gel electrophoresis

DOPE: Double-tagged probes

ÉB: Éra boost ®

EVL: EVL Coating ®

FISH: Fluorescence *in situ* hybridization

GFP: Green fluorescent protein

GSF: Granular starter fertilizer

*Gus*a: β-D-glucoranidase

IAA: Indole-3-acetic acid

NO: Nitrous oxide

PGPR: Plant growth-promoting rhizobacteria

PMA: Propidium monoazide

qPCR: Real-time polymerase chain reaction

RCBD: Randomized complete block design

T-RFLP: Terminal restriction length polymorphisms

Acknowledgements:

This project involved many partners who each helped me in their own way. Without the support of any of these persons, this project would not have been possible to conduct. I want to first acknowledge Dr. Donald Smith, my supervisor, who gave me valuable help in the preparation, performance and analysis of my experiments. He also shared with me valuable insights on how agricultural research is conducted as well as gave me many academic opportunities. Secondly, I wish to thank my co-supervisor Dr. Philippe Séguin who was always there to support me with all my questions and issues. My thanks are also extended to all professors from the department of Plant Science who helped me throughout my progression as a graduate student, specially the ones who listened to my proposal and final seminars.

I would like to acknowledge the help of Dr. Richard Hogue team at Research and Development Institute for the Agri-Environment (IRDA) as I collaborated with them for the processing of soil samples and bioinformatic analysis done in this thesis. More specifically, I would like to thank Mr. Thomas Jeanne, Mr. Joël D'Astous-Pagé and Ms. Véronique Gagné who all helped me either by giving me laboratory tutorials, coordinating my visits to their laboratory and guiding me in my bioinformatic analysis.

I also offer my gratitude to the research team at Synagri who funded my project and gave me a part of their research plots so that I could perform the entirety of Chapter 3. More specifically, I would like to thank Mr. Pierre Pagé and Mr. Denis Lévesque for their generous help by showing me research plots at the beginning of each growing season as well as sharing all the data I requested from them. Moreover, Pierre helped me in analysing my numerous soil samples using Synagri laboratory ressources.

Furthermore, I would like to acknowledge the funding of NSERC and AAFC for the project I joined. For my personal funding during the last 4 semesters of my degree, I would like to thank FRQNT.

Finally, I would like to express my full gratitude toward my family who provided me with their encouragement, advice and access to a car big enough to transport hundreds of corn plants at a time.

Preface:

All experiments were designed by Dr. Donald Smith and William Overbeek with the help of the Synagri R&D team. Data collection was done by William Overbeek, laboratory technicians at IRDA, summer interns from Dr. Smith's laboratory and the Synagri R&D team. All data analysis was conducted by William Overbeek. William Overbeek wrote this thesis, except for figure 4.4, which comes from Thomas Jeanne.

Chapter 1: General Introduction

1.1 Introduction

The world population has been increasing for more than six centuries. The rate of population increase peaked between 1955 and 1975. imposing an immense pressure on our food production systems. To supply this demand, many agricultural innovations occurred during these years, included under the name "Green Revolution". Land intensification and the discovery of inorganic fertilizer revolutionized our agricultural systems, resulting in continuous yield increases, year after year. However, the yield increase of crops was far less compared to the increase in additional energy inputs applied to fields. In addition, many agricultural inputs come from non-renewable resources (e.g. fertilizers and pesticides) and often leads to environmental degradation. It is crucial to find alternatives to products currently in use, to lessen the impacts of agricultural operations on surrounding environments. One of the most promising avenues is the use of beneficial microbes, known to enhance plant growth.

Plants and microbes have coexisted in soil for about half a billion years, establishing intimate and synergistic relationships. Nitrogen-fixing rhizobacteria, arbuscular mycorrhizae and plant growth-promoting rhizobacteria are examples of synergistic relationships between plants and soil microorganisms. Our current understanding of those relationships has resulted in the development of inoculants that are now commercially available. Even if many of these products are known to enhance plant growth and yield in controlled conditions, their results are inconsistent at the field level (Nelson, 2004), indicating that we have more to learn in order to optimize the application of microbial inoculants in agricultural fields. Using multiple beneficial microbial strains together is a promising strategy to ensure consistent results as each species will occupy different niches present in soils. No academic research has examined the application of liquid microbial inoculants on inorganic fertilizer, while commercial formulations have already been designed for this purpose. The current project aims to testing inorganic fertilizer as a carrier for microbial inoculants to promote corn and potato growth and productivity.

1.2 Research hypotheses and objectives

Overall objective:

Understand the impacts of two microbial consortia inoculants on corn and potato growth and the dynamics of microbiome composition in soil used for potato cultivation.

Specific objectives:

1. Assess establishment and survival of microbial inoculants on fertilizer beads
2. Evaluate corn and potato development following the application of a microbial consortium to agricultural fields
3. Assess microbial diversity in bulk soil and in soil adhered to roots

Hypotheses:

1. Granular fertilizer can be used as a carrier for viable liquid microbial inoculants.
2. The addition of microbial inoculants to fertilizer promotes plant growth and yield.
3. Microbial inoculants coated on fertilizer shapes microbial communities of bulk and rhizospheric soil following soil inoculation.

Chapter 2: Literature review

Development of microbial inoculants for enhanced sustainability in agriculture

2.1 Bio-revolution

During the 1960's, the Green Revolution allowed food production to rise drastically, mainly due to enhanced fertilizer inputs coupled with the development of new plant genotypes, better adapted to high grain production. However, massive nutrient inputs in fields resulted in contamination of surrounding environments due to surface water runoff and leaching. With the advent of a changing climate, which will bring stressful conditions to crop production, a new agricultural revolution needs to happen. A bio-revolution must happen, comprised of increased usage of biologic inputs such as microbial inoculants to optimize the composition of microorganisms associated with plants, the phytomicrobiome (Backer et al., 2018). Various genera of bacteria have been shown to promote the growth of plants classified under the name of plant-growth promoting rhizobacteria (PGPR). *Azospirillum, Bacillus, Pseudomonas, Lactobacillus, Actinobacteria* have all been extensively studied for strains which are known PGPR (Borriss, 2011; Fukami, Cerezini, & Hungria, 2018; Lamont, Wilkins, Bywater-Ekegärd, & Smith, 2017; Santoyo, Orozco-Mosqueda, & Govindappa, 2012; Shivlata & Satyanarayana, 2017; Sivasakthi, Usharani, & Saranraj, 2014). Other strains found in genera such as *Acetobacter, Paenibacillus Serratia, Burkholderia, Herbaspirillum* and *Rhodococcus* have also shown plant growth-promotion properties (Babalola, 2010).

Individual strains of PGPR may promote plant growth under axenic conditions in laboratory and greenhouse conditions, however under field conditions, individual strains may not be able to compete with native communities and fail to establish in soil. Mixing several strains of PGPR together to form a consortium could be more effective than using only one strain, especially if members of the consortium are able to perform different metabolic processes (Baez-Rogelio, Morales-García, Quintero-Hernández, & Muñoz-Rojas, 2017). An ideal consortium would be efficient, versatile, practical, properly formulated, persistent in soil and be commercially viable while ensuring that there is no antagonism among included microbial strains (Parnell et al., 2016).

2.2 Plant growth-promoting rhizobacteria functions

PGPR are found mostly in the rhizosphere of plants. When plants secrete root exudates in the rhizosphere and a 10- to 100-fold increase in microbial diversity occurs in response to this source of carbon and nutrients (Weller & Thomashow, 1994). Microbes living in the rhizosphere can have positive, negative or neutral effects on the host plant. Rhizospheric microbes include bacteria, fungi, actinomycetes, protozoa and algae with bacteria being the most abundant (Kaymak, 2010). PGPR are the subset of bacteria which have positive effects on plants. Beneficial actions from PGPR can occur due to either 1) direct effects on plants, such as by increasing nutrient supply, by secreting phytohormones or by producing microbe-to-plant signals or 2) by indirect effects on plants in which PGPR interactions with other soil microbes result in net positive conditions for plants, such as antagonism toward plant pathogens, inducing plant systemic resistance, interfering with quorum sensing ability of detrimental microbes, or siderophore production (Pérez-Montaño et al., 2014).

2.2.1. Biofertilization:

Nutrients are often found in soil in forms which are not available to plants, bound to soil colloids or in microbial biomass. To acquire those nutrients, plants associate with various rhizobacteria. These bacteria are called biofertilizer since they help plants *via* nitrogen fixation and solubilization of minerals to increase nutrient availability or to promote root surface area development (Vessey, 2003).

Nitrogen-fixation

Plants can assimilate nitrogen in inorganic forms (i.e. nitrate and ammonium) and organic forms (e.g. urea, amino acids and peptides) using various mechanisms (Nacry, Bouguyon, & Gojon, 2013). However, about 90% of N found in soils is in unavailable forms. Mainly, plant-available N comes from soil microbial communities as a result of soil organic matter mineralization, biological N_2 fixation (BNF) and denitrification (Pii et al., 2015). Organic matter breakdown is achieved by hydrolytic enzymes secreted by soil microbes (Miransari, 2011), while BNF is carried out by diazotrophic microorganisms found in soils.

The most known diazotrophs are the *Rhizobiaceae* which associate with legumes to form a specialized structure called nodule. *Frankia* species are known to associate with woody, non-legume plants and produce N-fixing nodules along the roots of the host plant (Santi, Bogusz, & Franche, 2013). Inside the nodule, diazotrophs change from a free-living configuration to a branched bacteroid form, and fix dinitrogen (N_2) to ammonia (NH_4) under anoxic or near anoxic conditions (Oke & Long, 1999). Global fixation of nitrogen through legume-rhizobia symbioses is estimated to be from 20 to 40 Tg N per year (Galloway et al., 2008; Herridge, Peoples, & Boddey, 2008). Many rhizobial bacteria can establish symbioses with legumes, however rigorous research work on this symbiosis has selected for the best strains, making *Rhizobia* the most studied PGPR and biofertilizers (Pérez-Montaño et al., 2014).

Other diazotrophs are free-living in soil, and fix nitrogen without associating with specific plants. In contrast to *Rhizobiaceae*, free-living diazotrophs do not alter their cellular structure to fix nitrogen, nor do they communicate with plants to form specialized root structures. Therefore, their nitrogen production is far less efficient as they do not inhabit a protected anaerobic environment, such as nodules. Nonetheless, in corn, *Bacillus pumilus* S1r1 could fix 30.5 and 25.5 % of the total N requirement after 50 and 65 days, respectively (Kuan, Othman, Abdul Rahim, & Shamsuddin, 2016). Free-living diazotrophic species are found in genera such as *Azoarcus*, *Burkholderia*, *Gluconacetobacter*, *Diazotrophicus*, *Herbaspirillum*, *Azotobacter*, *Bacillus* (for example *B. polymyxa*), and especially *Azospirillum* (Vessey, 2003). Inoculation of crops with nitrogen-fixing strains from these genera is an attempt to provide biologically-fixed nitrogen to non-legume crops, to enhance their yield, while reducing the need for inorganic N fertilizer (Yoav Bashan, de-Bashan, Prabhu, & Hernandez, 2014).

Phosphorus-solubilization

Large amounts of the phosphorus are found in soils, however, the vast majority is either in inorganic forms complexed with Ca, Fe, or Al, or organic forms, predominantly phytic acid; both inorganic and organic forms are unavailable to plants (Pii et al., 2015). To increase the availability of plant-available phosphate in soil, plants secrete root exudates which microbial communities metabolize into various low molecular weight organic acids

(e.g. acetic, lactic, malic, succinic, tartaric and oxalic) (Goswami, Thakker, & Dhandhukia, 2016). Those acids can act as chelators for the cations associated with phosphate, solubilizing inorganic P. Beneficial microbes can also solubilize inorganic phosphorus using HCN or excretion of H^+ ions. (Backer et al., 2018; Rijavec & Lapanje, 2016).

Phosphorus can be solubilized from its organic form through chelation or through the activity of enzymes produced by microbes such as phytase and nuclease (Hameeda, Harini, Rupela, Wani, & Reddy, 2008; Singh, Kumar, & Agrawal, 2014). About 20-40 % of the culturable bulk soil microbial populations have shown ability to solubilize phosphorus; the percentage of rhizosphere microbes that can solubilize phosphorus is even higher (Chabot, Antoun, & Cescas, 1993). *Azospirillum*, *Bacillus*, *Burkholderia*, *Ewinia*, *Pseudomonas*, *Rhizobium* and *Serratia*are are all reported to have P-solubilization abilities (Mehnaz & Lazarovits, 2006; Sudhakar, Chattopadhyay, Gangwar, & Ghosh, 2000).

K-solubilization

K content in soil ranges from 0.04 to 3 %, although only 1 or 2 % of total soil K is available to plants (Sparks & Huang, 1985). The rest is bound to minerals rendering it inaccessible to plants. K-solubilizing bacteria can solubilize K from silicate rocks into biologically available forms. Although only few reviews discuss the potential of PGPR to solubilize K, species such as *Bacillus mucilaginosus*, *B. circulanscan*, *B. edaphicus, Burkholderia* sp.*, A. ferrooxidans*, *Arthrobacter* sp., *Enterobacter hormaechei*, *Paenibacillus mucilaginosus*, *P. frequentans*, *Cladosporium* sp., *Aminobacter* sp.*, Sphingomonas* sp., and *Paenibacillus glucanolyticus* have been reported to solubilize K (Meena, Maurya, Verma, & Meena, 2016). K-solubilizers have been shown to significantly increase germination rate, seedling vigour, plant growth, K-uptake and yield under controlled and field conditions, possibly due to additional effects other than K-solubilization (Anjanadevi, John, John, Jeeva, & Misra, 2016; Awasthi, Tewari, & Nayyar, 2011; Lynn, Swe, Kyaw, Latt, & Yu, 2013)

2.2.2 Phytohormone production

Phytohormones that affect plant growth and metabolism are not synthesized exclusively by plants. Microbes, especially those living in the soil, can also produce various

phytohormones such as auxins, cytokinins and abscisic acid, or regulate production of ethylene.

Auxins

Auxin is a category of phytohormone that controls several aspects of plant development such as cell elongation, cell division, tissue differentiation and apical dominance (Goswami, Thakker, & Dhandhukia, 2016). In roots, auxin is associated with formation of lateral roots, enhancing root surface area and therefore contact with soil, and leading to enhanced accessibility to nutrients present in soils (Ahmed & Hasnain, 2014). The best characterized auxin is indole-3-acetic acid (IAA), however other molecules such as diacetyl phloroglucinol (DAPG) and nitrous oxide (NO) are known to interact with the auxin pathway (J. Vacheron et al., 2013). About 80% of rhizospheric micro-organisms can synthesize IAA, likely because IAA has been linked to increased root exudates by plants, which are the main carbon source for rhizospheric communities (Etesami, Alikhani, & Mirseyed Hosseini, 2015; Khalid, Arshad, & Zahir, 2004). As an auxin, IAA stimulates root growth through enhanced cell elongation and division rate, effectively increasing root surface area. Therefore, the production of IAA from precursors such as L-tryptophan, found in roots exudates, is often tested when screening for PGPR candidates (Etesami, Alikhani, & Hosseini, 2015). DAPG is known to have antimicrobial properties, however it can also interfere with plant auxin production (Brazelton, Pfeufer, Sweat, Gardener, & Coenen, 2008). NO mediates IAA signaling pathways, influencing the root architecture of plants. NO is synthesized through nitrite reductase, an activity performed by nitrogen-fixing species such as *Azospirillum*. Inoculation of tomato seeds with *Azospirillum brasiliense,* which can aerobically produce NO, increased root branching even in strains with mutations in IAA production-related genes (Molina-Favero, Creus, Simontacchi, Puntarulo, & Lamattina, 2008).

Cytokinin

Cytokinin acts in concert with auxins to regulate various plant functions. Cytokinin is involved in cell division, tissue differentiation and apical dominance. In shoots, cytokinin increases the rate of cell division and promotes shoot growth (Kieber & Schaller, 2018). In roots, cytokinin inhibits growth by increasing cell differentiation in apical meristems and

decreasing both root branching and primary root elongation (Chang, Ramireddy, & Schmülling, 2013). Many rhizospheric microorganisms have been shown to synthesize molecules which act as cytokinins (Maheshwari, Dheeman, & Agarwal, 2015; Ortíz-Castro, Valencia-Cantero, & López-Bucio, 2008)). Inoculation with microorganisms able to synthesize cytokinins has been shown to promote shoot growth while reducing the root: shoot ratio (Arkhipova et al., 2007). In *Platycladus orientalis*, inoculation with a *Bacillus* strain able to synthesize cytokinin reduced drought stress of seedlings while increasing the root: shoot ratio (F. Liu, Xing, Ma, Du, & Ma, 2013).

Ethylene

Ethylene is a signaling compound produced by plants. It is produced in response to abiotic stresses such as salt or drought (Etesami, Alikhani, & Mirseyed Hosseini, 2015). Negative effects are linked to high ethylene concentrations, such as leaf senescence, leaf abscission, chlorosis and flower wilting (Goswami et al., 2016). The enzyme 1-aminocyclopropane-1-carboxylic (ACC) deaminase binds the precursor of ethylene, ACC, and breaks it down into ammonia and α-ketobutyrate (Honma & Shimomura, 1978). Therefore, ACC deaminase can reduce ethylene synthesis in roots, reducing the negative consequences of ethylene production in roots. Glick et al. proposed a model in 1998 (Glick, Penrose, & Li, 1998) by which a portion of bacteria present in the rhizosphere can produce ACC deaminase, resulting in reduced levels of ethylene present in roots. Reduced ethylene concentration leads to enhanced root proliferation. Various abiotic stresses upregulate the production of ACC inside plant roots, resulting in ethylene production in the absence of ACC deaminase enzymes. Inoculation of plants with bacteria able to synthesize ACC deaminase could effectively increase abiotic stress tolerance in plants as bacterial production of this enzyme would inhibit ethylene formation (Glick, 2014). Various abiotic stresses, such as drought, flooding, salt and heavy metals have been used to confirm enhanced plant tolerance following inoculation with ACC deaminase-producing bacteria (Barnawal, Bharti, Maji, Chanotiya, & Kalra, 2012; Burd, Dixon, & Glick, 1998; Shakir, Bano, & Arshad, 2012; Siddikee, Glick, Chauhan, Yim, & Sa, 2011).

2.3 Commercial microbial inoculants

Microbial inoculants can have several beneficial effects on plant health and growth, as indicated in the previous section. To guarantee the maximum effect of an inoculant under field conditions, the product cannot be simply cultured on media and then applied to crops. The cost of media used in a laboratory setting becomes prohibitive for commercial production of microbial inoculants. Moreover, various abiotic stresses including as heat, oxidation, desiccation and ultraviolet light can reduce the number of viable cells found in a product. To be of sufficient quality, inoculants should have between 10^7 to 10^9 colony forming units per gram or per milliliter (CFU g^{-1} or CFU mL^{-1}) (Malusá & Vassilev, 2014). Some poorly-formulated inoculants will not contain viable organisms or be contaminated with other strains by the time they are used by farmers, resulting in an absence of plant growth-promotion following inoculation (Yadav & Chandra, 2014). Therefore, to ensure that the positive results demonstrated under laboratory conditions translate to field condition, adequate microbial biomass production in a bioreactor is necessary and development of a formulation that ensures a long shelf-life is required. Dozens of companies have commercialise microbial inoculants beneficial to plant growth, each inoculant optimized to accomplish specific functions.

2.3.1 Production and formulation of microbial inoculants

When growing PGPR for commercial purposes, the materials traditionally used as an energy supply in a laboratory environment, such as ammonium mineral salt (AMS) broth and nutrient broth, cannot be the utilized, largely due to the high costs of such products. Instead, waste materials such as animal manure, soybean meal, whey, fruit and vegetable wastes, corn flour, or corn bran can be acquired in large quantities at low prices and used as carbon or protein sources for PGPR growth (H. Liu et al., 2016; Pastor-Bueis, Mulas, Gómez, & González-Andrés, 2017; Peng, He, Wu, Lu, & Li, 2014; Zhang, Liu, & Wang, 2018). Once a mixture of waste materials is developed that supports microbial growth, various factors such as pH, temperature, oxygen/air levels and agitation need to be optimized (Posada-Uribe, Romero-Tabarez, & Villegas-Escobar, 2015; Xu, Bai, Jin, Xiao, & Zhuang, 2014). Most importantly, during the biomass production phase of inoculants preparation, sterile materials should be used as much as possible to ensure that the desired

beneficial microbes propagate, instead of contaminants, even though sterilization will result in increased cost of production.

Once sufficient biomass is produced, PGPR need to be collected and maintained in a way that prolongs viability as much as possible. To do so, formulations, mostly developed by companies, ensure proper shelf-life of microbial inoculants. Shelf life can vary from two months up to two years under controlled temperature (Lobo, Juárez Tomás, Viruel, Ferrero, & Lucca, 2019). Either liquid or dry formulations can be used to extend microorganism longevity. Most formulations commercially available are in liquid forms as they are generally cheaper and easier to produce than solid formulations (Kumaresan & Reetha, 2011). Liquid formulations contain compounds such as water and oil or polymers that increase adhesion, dispersion, stability, and longevity of microbial inoculants (Lee et al., 2016). Natural polymers used in formulations include carrageenan, arabic gum, xantham gum, gelatin and alginate while synthetic polymers include polyvinyl alcohol and polyvinylpyrrolidone (Lobo et al., 2019). Formulations can also be developed to protect microbial inoculants from abiotic stresses using glycerol and lactose mixed and dissolved in water; these formulations contain several hydroxide groups which protect against free radicals, and act as a carbon source for the longevity of microbes (Lobo et al., 2019).

Solid formulations can either be wet or dry, depending on the water content of the original material and whether the product is eventually dried. Wet formulations include alginate (Liffourrena & Lucchesi, 2018), clay (Schoebitz, Mengual, & Roldán, 2014), peat (Oliveira et al., 2017) and biochar (Tripti, Kumar, Usmani, Kumar, & Anshumali, 2017). In solid wet formulations, microbial inoculant must be immobilized by adhesion onto carriers or by microbial cell entrapment using beads or nanofibers (De Gregorio et al., 2017). Dry formulations, which require drying of the formulation, can extend the shelf life of microbial inoculants by lowering the water potential (Lobo et al., 2019). To dry the inoculum, air-drying or shade drying are both low-input techniques, however each requires a long period of time. Freeze-drying or spray drying are two fast drying techniques that require substantially more energy and special considerations to ensure bacterial viability. Alternatively, dry materials like talc, peat, kaolinite, lignite and vermiculite can be used as microbial carriers. For instance, beneficial strains of *Pseudomonas fluorescens* have been

tested in combination with several powdered carriers (Rabindran & Vidhyasekaran, 1996). Prior to use, dry carriers can be rehydrated to create a cell suspension which can be used to inoculate seeds, seedlings transplants or roots (Malusá, Sas-Paszt, & Ciesielska, 2012).

2.3.2 Overview of commercial microbial inoculants

Use of microbial inoculants in agriculture has been increasing steadily in recent years. In 2019, microbial inoculants commercialized as biofertilizers had a global market value of 1.0 billion dollars USD, with an estimated compound annual growth rate of 12.8% from 2020 to 2027 (Grand View Research 2020). Globally, more than sixty companies now offer a variety of microbial inoculants designed as inputs for plant production (Wood, 2019).

2.3.2.1 Single-strain and microbial consortia inoculants

Microbial inoculants can consist of one or more microbial strains. For decades, the microbial inoculant market was dominated by single strain formulations. These inoculants often had one precise function, such as nitrogen fixation (Yoav Bashan, 1998). However, soil is heterogeneous by nature, making it is impossible to predict whether or not an inoculated microbe will find suitable niche space in a given soil (van Elsas et al., 1992). The lack of consistent results observed with single-strain inoculants along with the discovery of synergistic interactions among co-inoculated microbial strains (Rajendran, Sing, Desai, & Archana, 2008) pushed companies to design microbial consortia, which contain multiple strains of plant-beneficial microbes. As each strain thrives in an unique ecological niche, the consortia has the potential capacity to colonize many soil niches, allowing for successful colonization under a wide range of soil conditions and a variety of environmental circumstances (Pandey et al., 2012).

Today, microbial consortia are offered by a wide range of agricultural companies, with some local examples being: Agri Life, BASF, Bayer Crop Science, Biomax, EVL Inc., Fertibio, Lallemand, Novozymes, Symborg and Ulysse Biotech; it is now that a substantial diversity of microbial-based commercial products are available worldwide (Grand View Research 2020; Sekar, Raj, & Vaiyapuri, 2016).

2.3.2.2 Field inoculation of microbial inoculants

Typical field inoculation of PGPR occurs during the seeding operation, to ensure that microbial inoculants are well positioned to colonise seedling roots (O'Callaghan, 2016). During seeding, microbial inoculants are often coated around seeds to ensure colonization of the emerging roots (Ma, 2019). As of 2019, in North America, 73.2% of all biofertilizers are offered as seed treatments, while 26.8% were offered as soil treatments (Grand View Research 2020).

To supplement seedling with sufficient nutrients, fertilizers are often applied before or during seeding. When applied in granular form, fertilizers can act as carriers for microbial inoculants. Using granular fertilizer as a microbial inoculant carrier comes with issues. Granular fertilizers have a high salt index, not suitable for long-term microbial viability. To resolve this problem, companies have designed formulations that keep microbial inoculants viable once coated around granular fertilizer. EVL Inc. (Saint-Hyacinthe, Qc) has commercialized such a product with the brand-name EVL Coating ®. Alternatively, microbial inoculants can consist of microbes that are able to sporulate and survive harsh environments. Ulysse Biotech (Trois-Rivière, Qc) has commercialized a microbial inoculant named Éra Boost ® composed of five *Bacillus* strains found as spores in a liquid formulation. These special features in the EVL Coating ® and Éra Boost ® products makes these two microbial inoculants best candidates for microbial inoculation with granular fertilizer as the effective carrier.

2.4 Fate of PGPR

Once a microbial inoculant has been proven to promote plant growth under controlled environment conditions, efficient microbial biomass production methods have been developed and a formulation that provides a long shelf-life has been developed, field-tests of the final product must be performed. As opposed to controlled environments, field experiments are conducted in non-sterile conditions in which the inoculant has to compete for a space in the rhizosphere to produce its beneficial effects on plant growth. Initial root colonization by the inoculant, followed by its persistence during the growing season, are crucial. To ensure these criteria are met, a range of monitoring strategies such as reporter genes and molecular markers have been developed (Rilling et al., 2019).

2.4.1 Root colonization

The most important characteristics for PGPR are their ability to establish on extant host roots and disperse along growing roots (Benizri, Baudoin, & Guckert, 2001; Podile et al., 2014). Soils are inhabited by a vast array of microbes. When plants secrete roots exudates only microbes which are best adapted to break-down these exudates will be able to thrive in the rhizosphere (Ahmad, Husain, & Ahmad, 2011). As root exudate composition changes from one plant genotype to another, the associated rhizomicrobiome will also be different (Berg & Smalla, 2009). For instance, bacteria isolated from various crops showed the greatest growth promotion on the same crop species (Chaudhry et al., 2016). Additionally, bacteria isolated from monocots performed well on other crops with a similar fibrous root system but performed poorly on plants with a tap-root system, showcasing specific plant-microbe preferences.

Inoculating seeds with a desired microbial inoculant can ensure that initial root formation will be accompanied by colonization by this microbe. Microbial inoculants not used as seed treatments should ensure that their initial dispersal on the ground and their dissemination through the soil will allow them to reach the rhizosphere of plants and outcompete native microbial communities for niche space along plant roots. To travel through soils, microorganisms have flagella and pili that act as propellers (Fenchel, 2002). During root colonization, microbes can use their flagella to adhere to roots. *Azospirilum brasilense* first binds to roots *via* a weak bond between its polar flagella and root surfaces (W. Michiels, L. Croes, & Vanderleyden, 1991). This is followed by the secretion of polysaccharides, which firmly bind the bacteria to roots, and incorporate surrounding free-living bacteria into this association. The importance of flagella in microbial motility has been proven in numerous research efforts using mutants that lack flagella in comparison with wild-type strains. Non-motile mutants cannot move along plant roots, which reduces their colonization ability while their wild-type counterparts successfully colonized different regions of roots (Y. Bashan & Holguin, 1995). However, the presence of percolating water is essential for the dispersion of motile bacteria. Non-flagellated *Pseudomonas* had the same colonization ability as flagellated *Pseudomonas* in the absence of percolating water, while both strains had enhanced presence along plant roots under sufficient irrigation (Bowers & Parke, 1993).

2.4.2 Monitoring of PGPR

Techniques to track specific microbial strains following inoculation with a microbial product can be developed. The most used approach identifies a unique characteristic of the microbial inoculant and develops a test which indicates the presence of these unique characteristics. As our understanding of molecular biology has been refined over the years, many techniques have been developed. Initially such techniques were based on plate-counts while more recently, various PCR-dependent methods have been developed.

Reporter genes

A reporter gene is a gene inserted in an organism between a regulatory sequence and a target gene (Rilling et al., 2019). This insertion allows for the identification of the organism when this target gene is actively transcribed. The reporter gene should allow for easy visualization of the organism(s) using as little disturbance as possible. Reporter genes include genes which will confer luminescence or fluorescence to organisms. However, horizontal gene transfer from the tagged organisms to nearby organisms in the rhizosphere could induce false positive results, while the loss of the inserted plasmid from the tagged organism after inoculation would lead to false negative results. Using *lux* as a reporter gene, De Weger et al. (De Weger, Dunbar, Mahafee, Lugtenberg, & Sayler, 1991) showed that about 80 % of the vector inserted in *Pseudomonas fluorescens* WCS374 were lost. Additionally, producing genetically modified organisms for *in situ* tracking in soil would result in biosafety risks that need to be considered when dealing with field experiments (Glick, 2015). Hence, reporter genes are often used in controlled environment settings, while other techniques are used for field experiments.

The *lacZ* and β-D-glucoranidase (*gus*A) are two reporter genes that have been used for decades to track PGPR. By inserting either of these genes into a potential PGPR, quantification of the modified bacteria is possible using proper selective media (X-gal for *lacZ* and X-glu for β-glu). For instance, the presence of a P-solubilizing *Pseudomonas striata* strain was shown to decrease from 10^8 to 10^4 CFU g^{-1} of soil in the four months following inoculation using *lacZ* tagging (Mourya & Jauhri, 2002). Another experiment, using *gus*A showed that *P. fluorescens* population increased from 10^6 to 10^8 CFU g^{-1} of

fresh root biomass when comparing colonization at 14 and 30 days post-inoculation (Villegas & Paterno, 2008). The main drawback of those two reporter genes is the ability of the indigenous bacteria to transcribe those genes, resulting in false positives, especially if horizontal gene transfer occurs (Rilling et al., 2019).

Bioluminescence and fluorescence by microorganisms can be used with reporter genes such as *lux* and green fluorescent protein (GFP). Both techniques do not require plate counting, unlike *lacZ* and *gus*A, allowing for *in situ* visualization of the root colonization. Using luminometry, it is possible to semi-quantitively assess colonization using the *lux* gene (Rilling et al., 2019). The detection of *lux*-tagged *Pseudomonas fluorescens* KT2440 was performed for both corn and bean rhizospheres, in which 10^7 and 10^5 CFU g^{-1} of soil were detected, respectively (Molina et al., 2000). Unlike *lux* which requires the formation of an aldehyde, GFP techniques do not require any substrate and have almost no background expression from native soil organisms or plants. Therefore, it is possible to quantify GFP-tagged bacteria which live as endophytes, while it is not reasonable to perform such analysis using *lux*. Inoculation of muskmelon with GFP-tagged *B. subtilis* showed 10^8 CFU g^{-1} of soil in the rhizosphere with 10^5 CFU g^{-1} living as endophytes one month after inoculation (Zhao et al., 2011). Combining more than one technique is a promising strategy to ensure that false positives and false negatives from one reporter gene are compared with those of other reporter genes. For instance, *Burkholderia tropica* MTo-293 was transformed with *lacZ* and *gfp* in different strains. The two resulting strains were used to quantitively assess root colonization by *B. tropica* MTo-293 and provided two sets of *in situ* visualization (Bernabeu et al., 2015). Recent discoveries of alternative versions of GFP have led to the development of reporter genes with fluorescent properties with different colours such as cyan, yellow, blue, and red (Bloemberg, 2007). By tagging the *P. fluorescens* WCS365 strain with different colours of fluorescent protein Bloemberg et al. (Bloemberg, Wijfjes, Lamers, Stuurman, & Lugtenberg, 2000) visualized several root colonization patterns resulting from inoculation with different populations of the same bacterial strain emitting different fluorescent colours in the same rhizosphere.

Nucleic acid markers

With the advent of DNA extraction and sequencing and the development of various PCR techniques, approaches have been established to identify specific microbes in soil. Nucleic acid techniques aim to find fingerprint patterns in either DNA or RNA expression of microbes allowing for their identification in samples containing multiple organisms such as soil or rhizospheric samples.

Techniques not reliant on PCR to detect microbes have also been developed, such as fluorescence *in situ* hybridization (FISH) and more specialized techniques including catalyzed reported deposition (CARD)-(FISH) and double-tagged probes (DOPE)-(FISH) (Eickhorst & Schmidt, 2014; Stoecker, Dorninger, Daims, & Wagner, 2010). Those techniques consist of tagging ribosomal RNA sequences, such as 16S, with small probes that emit fluorescence. Following sample fixation, probe hybridization and washing, root samples can be analyzed under a microscope to visualize root colonization and quantify fluorescent-tagged microbes (Moter & Göbel, 2000).

PCR-based techniques have been used to track specific microbes in soil communities, including denaturing gel electrophoresis (DGGE), terminal restriction length polymorphisms (T-RFLP), automatic ribosomal interspace spacer analysis (ARISA) and amplified ribosomal DNA restriction analysis (ARDRA) (Gamalero, Lingua, Berta, & Lemanceau, 2009). Those techniques can allow for detection of a bacterial strain within a community; however, quantification is not possible (Rilling et al., 2019). In order to quantify strains within a community, real-time PCR (qPCR) can be used. For this approach, strain-specific primers are first designed based on known sequences of the strains of interest. Strain-specific primers were designed for *Bacillus amyloliquefaciens* QST713 and for *B. firmus* I-1582, which are the strains found in the commercial products Serenade® and VOTiVO®. Once a strain genome is sequenced, its genome can be aligned to closely related genomes available in DNA libraries and unique regions can be identified to develop strain-specific qPCR primers (Mendis et al., 2018).

2.5 Conclusion

Microbial inoculants are key alternatives to current agricultural inputs which are depleting non-renewable resources and contaminating environments in areas surrounding agricultural production. The positive effects of PGPR on plants are often shown to hold

great promises under controlled experiments but fail to deliver under field conditions, where a multitude of factors cannot be optimized. To improve the efficacy of microbial inoculants under field conditions, microbial biomass production and effective formulations must be optimized for each microbial product. Such an approach will ensure that the viability of fickle microorganisms is maintained under the various stresses involved faced by a commercial inoculant in the field and during storage. Once a product is properly formulated, its persistence in a field environment, in the rhizosphere more precisely, is crucial to obtain positive effects of the microbial inoculant, or conversely, to observe an absence of positive effects when the inoculant is not present in the rhizosphere. Collaboration amongst experts in microbiology, molecular biology, bioreactors and chemistry is needed to design high-quality microbial inoculants. Agronomists, plant scientists, and soil scientists must unravel why inoculants work in some experiments and fail in others. With the advent of various tools such as strain-specific qPCR primers and metagenomics, answers to these puzzling questions should be revealed in the coming years.

Connecting statements between chapter 2 and chapter 3

To evaluate the potential of microbial inoculants coated onto granular starter fertilizer (GSF), the first and second objectives of the thesis are addressed in chapter 3. First, survival of microbial consortia coated on GSF was investigated and quantified. Plant growth promotion due to application of microbial inoculation was measured in corn and potato field trials. In-season growth and harvest quantity and quality were measured.

Chapter 3: Granular starter fertilizer can be used as a carrier of viable microbial consortia inoculants for corn and potato production in Southern Quebec

Abstract

In the conventional row crop production used in Quebec, banding "starter" fertilizer during seeding is commonly used to provide readily available nutrients for emerging seedlings. However, nutrient use efficiency of fertilizer is at most of 50 %, while a portion of the lost fertilizer damages soil and surrounding environments. Plant beneficial soil microbes can associate with plants to increase the availability of nutrients to plants and to provide other beneficial effects to plants. Addition of beneficial soil microbes is usually conducted using peat-based materials or using direct inoculation onto seeds. The objective of this study was to evaluate growth promotion and yield quantity and quality of corn and potato (plant height, leaf area and dry weight) in response to the application of two commercial microbial inoculants coated onto granular fertilizer banded into soil at seeding. The two-year field experiment was conducted at one field-site for each crop, using commercial inoculants Éra Boost® and EVL Coating®. In 2018, corn development was significantly promoted during vegetative growth stages with no significant effect on grain yield. Potato plant growth was not significantly affected in-season, while tuber yield was increased up to 19.6 %, with an average increase of 8.9 %. In 2019, corn in-season development was generally not promoted while grain yield was significantly increased up to 20.2 %, with an average increase of 13.0 %. Potato growth was smaller on inoculated plots during the first sampling while later sampling showed no difference in growth. Microbial inoculants did not affect tuber yields and nutritional quality. These results suggest that microbial inoculation can improve crop growth and yield but not consistently. Further research investigating critical factors for the success of microbial inoculum coated on granular fertilizer is needed.

3.1 Introduction

The Green Revolution introduced the application of chemical fertilizer to crop production with the aim of increasing soil nutrients to levels necessary to fulfill plant yield potential. Nutrient use efficiency of chemical fertilizers is generally 30-50 % for N, 15-25 % for P and 30-50 % for K (Fixen et al., 2015; Ladha, Pathak, J. Krupnik, Six, & van Kessel, 2005; Syers, Johnston, & Curtin, 2008) depending on soil fertility, soil management strategies and the quantity of fertilizer applied. Overuse of fertilizer can cause significant damage to soil and the surrounding environment by acidifying the soil and leaching into surface and ground waters (Leggett et al., 2015). For example, it is estimated that 20 % of N in fertilizer leaches out of a field (Jones & Downing, 2009). With a continuing increase in world food demand and a need to reduce the negative environmental impacts of human activity, fertilizer efficiency must increase to produce higher-yielding crops with less fertilizer.

Plants absorb most nutrients through their root systems. Roots secrete various compounds into the soil and these are consumed by soil microorganisms who provide key functions for plant development, such as nutrient acquisition and resistance to biotic and abiotic stresses (Mendes, Garbeva, & Raaijmakers, 2013). Through the production of root exudates, a plant shapes the microbial community associated with its roots, in an attempt to attract beneficial soil microbes (Rudrappa, Czymmek, Paré, & Bais, 2008). Soil microbial communities vary substantially within a small area due to the heterogeneous nature of soils (Berg & Smalla, 2009). The use of biological agents to modify rhizospheric microbial communities and increase nutrient acquisition by plants is gaining significant attention. A meta-analysis showed that microbial inoculants increase yield in all regions, with the strongest effects reported in dry environments, and show an average yield increase of 16.2 ± 1.0 % across all crops (Schütz et al., 2018). Nitrogen use efficiency was increased by 5.8 ± 0.6 kg yield per kg N while phosphorus use efficiency was increased by 7.5 ± 0.8 kg yield per kg P, with the largest increases reported in legumes, and the lowest increases reported in root crops.

Microbial inoculant utilization in corn production results in increased above-ground biomass production, increased grain yield and increased nutrient uptake (Calvo, Watts,

Kloepper, & Torbert, 2017). An experiment investigated corn growth following the application of a commercial microbial product, SoilBuilder^{TM} in conjunction with different forms of inorganic fertilizer. For all fertilizer types (urea, CAN and UAN), the microbial product promoted corn growth at V2, V4, V6 and VT, with the greatest effect occurring at VT. In addition, nutrient concentration in corn shoots were significantly increased. In another trial, 50 % of normal inorganic P input, combined with phosphate-solubilizing microbes, did not change corn yield compared to standard fertilization (Yazdani, Bahmanyar, Pirdashti, & Esmaili, 2009). A seven-year study showed an average corn yield increase of 3.5 % associated with *P. bilaiae* (JumpStart) seed inoculation on large plots (Leggett et al., 2015). Hence, corn production seems to benefit from the addition of beneficial soil microbes.

Different strains of possible beneficial microbes have been tested for growth promotion of potato plants. *Azospirillum* TN10 strains produced the greatest growth promotion, with 50 % more biomass than the uninoculated treatment (Naqqash et al., 2016). Field trials conducted on potato in Peru showed a yield increase up to 60 %, however growth-promotion was inconsistent, with further research needed to properly use microbial inoculant in fields (Calvo Velez & Oswald, 2009). In India, other research has shown that tuber inoculation with N-fixing and P-solubilizing bacteria significantly increased potato yield with only 75 % of the recommended rate of NP fertilizer (Dash & Jena, 2015). Few experiments have investigated the effects of microbial inoculant using multiple applications during crop growth (Naqqash et al., 2016), whereas other research evaluated inoculant application only at seeding (Dash & Jena, 2015). However, no research has investigated the effect of repeated microbial inoculant application within one season of field potato production.

Fertilizer placement in the field is a crucial factor for ensuring fertilizer assimilation by the target plants (i.e. crop) as opposed to undesirable plants (i.e. weeds). Banded fertilizer applications during seeding optimizes fertilizer placement for crop nutrient assimilation, leading to better nutrient use efficiency and higher yields under certain edaphic and environmental conditions (Randall & Hoeft, 1988). Consistent grain yield increases have been observed on soils with deficient P levels following banded fertilization

application (Wolkowski, 2000). Northern climates, where soil temperatures are still warming and microbial activity is at its annual minimum at planting time, have shown better responses to banded fertilizer application (Vetsch & Randall, 2002). Hence in regions like Southern Quebec, banded fertilizer application is often used at seeding in production of row crops, such as corn and potato, to provide readily-available nutrients to plants, until nutrient mobilization by soil microorganisms becomes the main source of nutrients for plants later in the growing season. No previous report has investigated the use of banded fertilizers, coated with plant beneficial microbes, in row crop production to enhance the availability of soil between seeding and subsequent fertilizer application. This new delivery method for beneficial microbes could enhance development of seedling root systems to further promote overall plant growth and final yield.

The objective of this study was to evaluate the suitability of inorganic fertilizer as a microbial inoculant carrier to increase corn and potato yield and quality in Southern Quebec. We hypothesised that inoculation of inorganic fertilizer with beneficial microbes would result in enhanced plant growth and final yield. Corn and potato were chosen due to their economic importance in the Quebec crop production sector and due to the considerable fertilizer requirements of both crops. In addition, these represent two contrasting crop types: corn is a C_4 monocot that produces seeds, while potato is a C_3 dicot that produces belowground tubers. A technology that is effective on both crops is likely to have wide application potential in crop production systems. Analysis of in-season plant growth as well as harvest quantity and quality were measured in inoculated and uninoculated plots. This research is one of the first to investigate inorganic fertilizer as a microbial inoculant carrier with the aim of increasing the use of biological products in conventional farming.

3.2 Materials and methods

3.2.1 Microbial inoculant characteristics and inoculation of granular fertilizer

Two commercial microbial inoculants were used, EVL Coating ® (EVL) and Éra Boost ® (ÉB). EVL is a microbial inoculant commercialized by the company EVL Inc. (Saint-Hyacinthe, Qc) that contains five different microbial strains, two *Bacillus*, one *Lactobacillus*, one *Pseudomonas* and one *Saccharomyces*, combined in a bioreactor

containing a variety of non-sterilized low-cost materials. ÉB is a microbial inoculant commercialized by Ulysse Biotech (Trois-Rivière, Qc) that contains five strains of *Bacillus* sp. capable of sporulation, stored together in a low pH liquid.

To confirm the suitability of inorganic fertilizer as a microbial inoculant, a small-scale inoculation was conducted on granular starter fertilizer (GSF) obtained from Synagri (Saint-Hyacinthe Qc), composed of a mixture of calcium ammonium nitrate (CAN), MicroEssential S10 (MES) and potash with an NPK equivalent to 16-20-8, commonly used as corn starter fertilizer. Fifty grams of GSF was inoculated with 0.175 mL of EVL, ÉB or sterile water for mock control, diluted in 2 mL of sterile water using a small sterile spray bottle. Inoculated fertilizer was air-dried for 1 h. Five beads of each fertilizer type (CAN, potash and MES) were rolled on a petri dish containing King's B agar (King, Ward, & Raney, 1954). Forty-eight h after inoculation, pictures were taken of the plates. A similar experiment was conducted with 0.0175 mL of ÉB instead of 0.175 mL used in the initial experiment. Twenty-four h after plate inoculation, colony number was counted.

For field work, GSF was inoculated in a cement mixer with undiluted microbial inoculants. For corn experiments, inoculants were sprayed at a rate of 3.5 and 2 L of inoculant GSF Mg^{-1} for ÉB and EVL, respectively. For potato, GSF was sprayed at a rate of 1 L inoculant GSF Mg^{-1} for ÉB and 2 L inoculant GSF Mg^{-1} for EVL, following the recommendations from each company.

3.2.2 Study sites and field characteristics

3.2.2.1 Corn trial

A field experiment was conducted in 2018 and 2019 at a Synagri research station located in St-Hyacinthe (45° 40' 43.4" N, 73° 00' 10.6" W). The soil used was a Kierkoski loam on which the previous crop was corn. The soil was under a minimal tillage strategy with primary tillage conducted in Fall and two secondary tillage passes in the spring, prior to planting.

3.2.2.2 Potato trial

A similar field experiment was conducted in 2018 and 2019 at another Synagri research station located in St-Thomas de Joliette (46° 00' 12.1" N, 73° 20' 58.5" W in 2018

and 46° 00' 01.8" N ,73° 21' 02.2" W in 2019). The soil was a fine sand where cover crops were grown in the previous year.

3.2.3 Experimental setup and growing conditions

3.2.3.1 Corn trial

For each microbial inoculant, two different rates were investigated. The recommended rate (i.e. 3.5 L GSF Mg^{-1} for ÉB and 2 L GSF Mg^{-1} for EVL) and twice the recommended microbial inoculants rate (7 and 4 L GSF Mg^{-1} for ÉB and EVL, respectively) with an uninoculated control treatment, for a total of five treatments **(**Figure 3. 1). All treatments were replicated in a randomized complete block design (RCBD), with four blocks for hand-harvested measurements and five additional blocks for combine harvest measurements. Individual plots consisted of two rows, 75 cm apart, with a length of 5 m. Inoculated and uninoculated GSFs were applied at a rate of 315 kg ha^{-1} at seeding. At the mid-vegetative growth stage (~V6), 421 L ha^{-1} of urea ammonium nitrate (UAN) was applied between rows. A total of 220-63-25 kg ha^{-1} of NPK was applied to plots. For weed control, Broadstrike ® (flumetsulam) mixed with Dual ® (metolachlor) was applied pre-emergence and Crédit Xtrem ® (glyphosate), was applied post-emergence.

3.2.3.2 Potato trial

Potato trials included the same five treatments as the corn trial: two concentrations of ÉB, two concentrations of EVL and an uninoculated control. An additional treatment for each commercial inoculant was included, in which the microbial inoculant was coated onto fertilizer applied as a side-dress during the growing season at hilling, for a total of seven treatments (Figure 3**.** 1). Seeding microbial inoculants rates were 1 L GSF Mg^{-1} for ÉB and 2 L GSF Mg^{-1} for EVL; the inoculant rate at potato hilling was 2 L Mg^{-1} of side-dress fertilizer for both microbial inoculants. A GSF rate of 1050 kg ha^{-1} was applied as a 2×2×2 method, with banded strips on each side of the seed tuber. The rate of microbial-inoculated fertilizer applied as a side-dress was 491 kg ha^{-1}. Each plot received a total NPK equivalent of 250-75-205 kg ha^{-1}. The experimental layout followed an RCBD design with four blocks in 2018 for both hand sampling and machine harvest. In 2019, four blocks were used for hand sampling and seven blocks were used for machine harvest. Each plot consisted of six rows with a length of 20 m in 2018 and four rows with a length of 20 m in 2019. Pesticide

application was based on field monitoring during the growing season. In-season fungicide active ingredients were chlorothalonil (Echo 720 ®), a mix of fluopyram and pyrimethanil (Luna Tranquility ®), a mix of pyraclostrobin and metiram (Cabrio Plus ®), phosphites (Phostrol ®), azoxystrobin and difenoconazole (Quadris Top ®), fenamidon, (Reason ®) and cyazofamid (Ranman ®). Insecticide active ingredients were spinetoram (Delegate ®) and dimethoate (Lagon ®).

3.2.3 Plant development and harvest data collection

3.2.3.1 Corn trial

In-season measurements

Four sampling events, including harvest, were conducted during each growing season. The first sampling was carried out at the mid-vegetative stage (V6), the second sampling at mid-flowering (four days after tassel emergence, R2), the third sampling during grain filling (milk line is about half, R5-R6) and the final sampling at harvest. For the first three sampling timepoints, plant height (cm), dry weight of aboveground biomass (g per 5 plants) and leaf area (cm^2 $plant^{-1}$; Li3100C) were recorded for five consecutive plants in each plot.

At harvest, the aboveground biomass, including the cob, and a root sample were harvested for five consecutive plants. The dry weight of aboveground biomass (g per 5 plants), dry weight of grains (g per 5 plants), 100 seed weight (g), number of kernels per cob, cob length (cm) and harvest index (%) were recorded for each plot.

Preparation of corn grain harvest samples

Using dried corn kernels from 5 hand-collected corn plants, a sub-sample of about 100 kernels was collected. Those kernels were ground (Thomas-Wiley laboratory mill, Model 4) for 3 minutes to pass through a 1 mm sieve.

Estimation of total starch

Corn kernel starch content was estimated according to the protocol a) from a Total Starch Assay Kit (Megazyme 2017). About 100 mg of milled corn was placed in a glass test-tube; a positive corn starch control was included for every extraction. Ethanol (0.2 mL

at 80 % EtOH/20% H_2O) was added to each sample, followed immediately by the addition of 3 mL of thermostable alpha-amylase. Samples were incubated for 6 min in a boiling water bath, vortexing every 2 min. Next, 0.1 mL of amyloglucosidase was added to each tube, followed by incubation at 50 ºC for 30 min. The contents of the glass tubes were transferred to 50 mL Falcon tubes and the solution was brought up to 50 mL using distilled water to triple rinse the original glass tubes. The tubes were centrifuged at 3,000 rpm for 10 min.

For each sample, duplicate aliquots of 0.1 mL were added to 15 mL Falcon tubes containing 3 mL of GOPOD Reagent provided in the kit. The mixture was incubated for 20 min at 50 ºC. A blank of distilled water and a positive control of 0.1 mL D-glucose were included. Absorbance of the solution was observed at 510 nm, compared to a blank solution. Results were expressed as starch % of corn kernel, calculated according to Equation 2.

$$Starch\ \% = \Delta A * F * \frac{FV}{0.1} * \frac{1}{1000} * \frac{100}{W} * \frac{162}{180} \qquad \text{Equation 1}$$

$$Starch\ \% = \Delta A * \frac{F}{W} * FV * 0.9 \qquad \text{Equation 2}$$

Where: ΔA: absorbance read against the reagent blank

F: 100/absorbance for 100 µg of glucose

FV = Final volume (i.e. 50 mL)

W: the precise weight in milligrams of the flour analyzed (~100 mg)

3.2.3.2 Potato trial

For potatoes, sampling occurred at three stages during each growing season. The first sampling was at the mid-vegetative stage, about one week before hilling; the second sampling was at mid-flowering, 3 weeks after hilling; the final sampling was at harvest. For the first two sampling timepoints, plant height (cm), leaf area ($cm^2\ plant^{-1}$) and dry

weight (g plant^{-1}) were recorded for five consecutive plants in each plot. At the final sampling, tubers of five consecutive plants were harvested, graded and weighed. Grading was based on potato tuber diameter: 1) marketable size: between 3-6 cm, 2) below marketable size: under 3 cm and 3) above marketable size: greater than 6 cm.

Preparation of tuber samples

Using a sample of five tubers from each plot, tubers were washed, peeled and cut in longitudinal slices for a total of 10 g (Nordbotten et al. 2000). The prepared slices were immediately freeze dried and stored at -80 ºC until further analysis.

Starch estimation

The estimation of starch content inside the flesh of the tubers were done using the same methods as for corn.

3.2.4 Statistical analysis

Analysis of variance (ANOVA), using mixed models was performed. Microbial treatment was a fixed effect while block was a random effect. Bonferroni adjustments were used to determine significant differences and correct for multiple. For corn, pair-wise differences of interest were: each treatment compared to the control and the recommended concentration compared to twice the recommended concentration of each product for a total of 6 pair-wise differences. For potato, pair-wise differences were the same as corn with the addition of the pair-wise difference of one application compared to two applications and two applications compared to the control for both inoculants, for a total of 10 pair-wise comparisons. All statistical analyses were performed using Proc MIXED of SAS 9.4 (SAS institute, 2015) with a significance level of $\alpha = 0.05$. Heatmaps were produced using the R package “pheatmap” (Kolde, 2015).

3.3 Results

3.3.1 Microbial inoculation of granular starter fertilizer

Effects of microbial inoculation on culturable microbial species present on GSF was clear when a small quantity of GSF was inoculated (Figure 3. 2). The number of microbial colony forming unit (CFU) on agar plates 24 h following inoculation with ÉB was 5.82×10^5, 8.12×10^5 and 5.75×10^4 CFU per 100 g for CAN, MES and potash

respectively, with a standard error of 6.49×10^4. In the mock control, no colonies were observed on any plates.

3.3.2 Weather conditions in 2018 and 2019

Spring conditions in 2018 were close to optimal, seeding took place inside an optimum planting date window (May 13th to 24th) and temperatures were warm enough for corn and potato germination and growth. Summer 2018 was characterized by hot and dry conditions, leading to the occurrence of drought stress by the end of the summer. Fall 2018 was especially hot in September with slightly wet harvest conditions. In 2019, spring conditions were cold and humid, and seeding was carried out later than usual (June 1st), considerably reducing the growing season. However, environmental conditions were optimal for early plant growth. Similar to the 2018 growing season, drought stress occurred at the end of summer. Unlike 2018, 2019 fall conditions were cold and did not allow plants to accumulate heat units lost due to the late seeding. The end of autumn was characterized by a severe storm that lodged all corn plots, followed by snow in early November, which made combine harvesting of corn plots impossible.

3.3.3 Corn growth and harvest variables

3.3.3.1 In-season parameter responses to microbial inoculation

Corn height

In 2018, plant height was significantly increased for two treatments at the mid-vegetative and mid-flowering sampling timepoints. During the mid-vegetative period, ÉB 1× ($P < 0.0001$) and EVL 1× ($P = 0.0072$) plants were significantly taller than control plants. Both 1× treatments (ÉB and EVL) were significantly taller than 2× treatments ($P_{ÉB} = 0.0006$, $P_{EVL} = 0.0072$). At the mid-flowering stage plants treated with EVL 1× were significantly taller plants than control plants ($P = 0.0336$). At the grain filling stage, no significant difference could be detected between inoculated plants compared to control plants.

In 2019, plant height was significantly higher for ÉB 2× compared to the control, at the grain filling stage ($P = 0.0426$), while all other parameters for all other treatments were not significantly different from control at any sampling timepoints.

Leaf area

In 2018, leaf area was significantly higher at the mid-flowering stage for plants treated with EVL 1× compared to control plants (P = 0.0234) and compared to EVL 2× (P = 0.0402) whereas at grain filling, plants treated with ÉB 2× (P = 0.018) or EVL 2× (P = 0.0012) had significantly more leaf area than control plants.

In 2019, none of the treatments had significant effects on leaf area for any pair-wise comparisons that were evaluated.

Dry weight

Plant dry mass was significantly higher for those treated with ÉB 1× compared to ÉB 2× at the mid-vegetative stage in 2018 (P = 0.0114). At the mid-flowering stage in 2019, ÉB 1× significantly increased plant dry weight compared to the control (P = 0.0144). No other treatments effects were observed at the other sampling timepoints with respect to the control.

3.3.3.2 Harvest parameters in response to microbial inoculation

Grain yield

In 2018, there were no statistically significant grain yield differences observed between the control and any of the microbial treatments on hand-harvested or machine-harvested plots.

In 2019, extreme lodging of corn plants did not allow for machine harvesting, hence only hand harvesting was possible. Control treatments had the smallest grain yield of all treatments; ÉB 2× yield was significantly higher than the control (P = 0.0468).

Above-ground biomass at harvest

During both years, no statistical difference was observed in the non-grain above-ground biomass. With a shorter growing season in 2019 than 2018, the mean non-grain above-ground biomass was smaller in 2019 than 2018.

Seed weight

The weight of individual seeds is an indicator of grain quality as higher test weight generally represents a higher economic value of the grain while a lower test weight is of lower economic value. Similarly as for the non-grain above-ground biomass, the 2019 values were much lower than in 2018 as there was a difference of about 550 corn heat unit (CHU) between 2018 and 2019 in the St-Hyacinthe area (Agrométéo 2020; Figure 3. 3).

In 2018, ÉB 1× and EVL 1× seeds were 4.95 and 3.42 % heavier than control seeds (P = 0.0006 and P = 0.0396), respectively, while EVL 1× seeds were significantly heavier than EVL 2× seeds (P = 0.0018). In 2019, ÉB 2× and EVL 1× had heavier seeds than the control (P = 0.0126 and P = 0.0294, respectively), while EVL 1× seeds were significantly heavier than EVL 2× seeds (P = 0.0144).

Starch content

For both years, no statistical differences in starch content were observed between uninoculated and inoculated plots. When comparing 1× to 2× treatments, starch content was higher in 2× treatments for both inoculants for both years, even though no pair-wise differences were significant.

3.3.4 Potato growth and harvest variables

3.3.4.1 In-season parameters in response to microbial inoculation

Plant height

The height of potato plants was significantly affected by microbial inoculation at the mid-vegetative stage in both years, with different trends in each year. In 2018, plant height was significantly increased by ÉB 2× and EVL2(1×) treatments (P = 0.0448 and P = 0.0136, respectively), meanwhile in 2019 the same two treatments were significantly shorter than control (P = 0.013 and P = 0.003, respectively). However, by the 2019 mid-flowering stage, plant height of all treatments were similar with no significant differences when compared to the control treatment, which had the smallest height in both years.

Leaf area

The leaf area of potato was not significantly different for any pair-wise comparisons in 2018 at either sampling time. Leaf area values were extremely variable among treatments replicates, and there were no significant differences between treatments.

In 2019, no treatment had a significant effect on leaf area compared to the control, however most treatments had values smaller than control plants at the mid-vegetative stage. At the mid-flowering stage, no treatment was significantly higher than control, however all plants had leaf areas larger than control, meaning that many of the treatments "caught up" in the interval between the mid-vegetative and mid-flowering samplings.

Dry weight

In 2018, there were no significant differences amongst any pair-wise differences at both the mid-vegetative or mid-flowering samplings.

In 2019, there was a significant difference at both sampling times. At the mid-vegetative stage, ÉB 2× plants were significantly lighter than control plants ($P = 0.033$). At the mid-flowering stage there were no significant differences for any pair-wise comparisons.

3.3.4.2 Harvest parameters in response to microbial inoculation

Tuber yield

Both hand-harvested and machine harvested tuber weight was not significantly different in either year for any pair-wise comparisons. Although, ÉB 1× caused a yield increase of 27.9 % compared to control in 2018, which is considerable even if not significant.

Starch content

In both years, there was no significant difference observed in starch content between any of the treatments and the control, between rates of microbial inoculant application, or between one or two applications.

3.4 Discussion

3.4.1 Interaction of inoculum with its environment

Microbial inoculation to promote plant growth in the field has always given inconsistent results (Gange & Gadhave, 2018; Leggett et al., 2015; Niranjan Raj, Shetty, & Reddy, 2006). Between the production of the inoculant and its colonization of the rhizosphere, inoculants can lose their viability at many points, which would result in the absence of any potential plant growth promotion from the inoculum. On top of that, growing environment when the inoculant comes into contact with the plant is crucial. During adverse environmental conditions, inoculant should provide a higher level of growth promotion to plants compared to a plant facing minimal to no stress (Smith, Gravel, & Yergeau, 2017). In addition, plants in different soils will not react equally to microbial inoculations, resulting in variable plant growth promotion potential, even within a single field (Leggett et al., 2015). With all these uncertainties, varying degrees plant growth promoting results are to be expected with inoculation of beneficial microbes under field conditions.

In this experiment, trends of microbial inoculation effects on above-ground biomass were not consistent across the two years for corn (. There were important time differences between 2018 and 2019 for seeding and mid-vegetative sampling with a difference heat unit accumulation in 2019 compared to 2018 (Figure 3. 4). At every sampling time, a minimum of one parameter was significantly increased for at least one inoculated treatment, compared to the control. However, growth-promotion at an early stage did not necessarily translate to increase growth throughout the growing season. Calvo et al. (Calvo et al., 2017), reported that corn growth promotion due to microbial inoculants was clearer in later stages of vegetative growth than in earlier stages of plant growth. Here, we report significant differences observed at the later vegetative stages were often non-significant at the later reproductive stage (~R4). Similar trends occurred for potatoes, as significant differences in plant height and leaf area were observed at the mid-vegetative but not at mid-flowering samplings.

Corn grain yield was significantly increased by at least one microbial treatment one year out of two (Figure 3.4). One of the main causes for the difference in yield response

between the two years could be attributed to differences in the weather conditions of 2018 and 2019 (Figure 3. 3). The regular seeding time in 2018 meant that seeds were sown into a soil that was relatively cold and temperatures were not optimal for corn growth in early May. In 2019, seeds were sown in early June, with much higher temperatures compared to the early May seeding in 2018, leading to optimal conditions for seedling development. These optimal conditions in 2019 could inhibit potential microbial growth-promoting effects associated with the inoculants. Inter-year variability was also noted by Legget et al. (2015), in a six-year trial, where they observed that corn yield was significantly increased in two out of six years on small plots. Additionally, in our study the highest yield increase was observed for each crop in the year where the mean yield was low (2018 for potato and 2019 for corn). This observation suggests that microbial inoculation could help recover yield losses or boost yield in soil with low productivity, while high-yielding conditions benefit less from microbial inoculation.

3.4.2 Granular starter fertilizer as a carrier

The choice of carrier for a microbial inoculant plays a role in the dispersal and establishment of the applied microorganisms. In this research, GSF was tested as a carrier for inoculants, for which there are no previous reports. The most popular carrier is peat and other common carrier materials include talc or vermiculite (Yoav Bashan et al., 2014). Alternatively, coating microbial inoculants directly onto seeds guarantees that the emerging plant will be in contact with the inoculant (O'Callaghan, 2016). Many products for coating seeds with microbial strains are now commercially available (e.g. Acceleron from Monsanto).

The unique characteristic of coating microbial inoculants on GSF is its placement in the soil. As the fertilizer is banded 5 x 5 cm to the side and below the seeds, we hypothesized that the microbial inoculants would be in contact with emerging radicles. Additionally, beneficial microbes are thought to increase the availability of nutrients to plants by increasing lateral root branches and root hairs (Jordan Vacheron et al., 2013). Therefore, the placement of the microbial inoculant around the main source of early-season nutrients (i.e. banded fertilizer) could ensure optimal absorption of nutrients from the fertilizer. On the other hand, fertilizer granules have a high salt index and non-neutral pH,

which are not ideal conditions for microorganisms, possibly resulting in a poor dispersion through soil, where established microbial communities already thrive.

Quantification of bacterial colonies on agar plates showed CAN and MES as promising fertilizer carriers for microbial inoculants, as they were able to support more than 5 times the number of bacterial colonies found on potash fertilizer (Figure 3. 2). Targeting specific fertilizer forms which respond well to microbial inoculation may help in developing adequate strategies for microbial inoculation of granular fertilizers.

3.4.3 Optimal rate and number of applications

The dose of microbial inoculants applied to fields dictates the effects of the inoculation. Many experiments conducted in controlled settings use extremely high concentrations of inoculants in soil or coated onto seeds. Often those microbial inoculant rates would not be economically viable at the commercial field level. However, soils are inhabited by a diverse and highly competitive microbiome, resulting in more challenging conditions for inoculants as they try to effectively colonize the rhizosphere. Therefore, there is a critical need to identify a dose of inoculant that is high enough to have a significant effect on plant growth but low enough to ensure a positive economic return. The two concentrations of microbial inoculants used in this experiment gave different results for each inoculant. In corn and potato, the lower dose of EVL (EVL 1×) had a better overall growth promoting effect: all significant differences between the 1× and 2× treatments were larger for the 1x rate in both years. For ÉB in corn, the 1× treatment performed better than the 2× treatment in 2018, while in 2019, there were no significant differences between the two treatments, but ÉB 2× produced greater yields than 1×. For potato, there were no significant differences for any growth variables for ÉB. The lack of significant difference between the two microbial inoculant rates, coupled with significantly smaller values for the EVL 2× treatment compared to the control in 2019 indicated that the 1× rate would be preferable for both microbial inoculants for potato production.

Application of microbial inoculants can be carried out at different times during the growing season, from seed coating up to harvest. During the growing season, the ideal inoculant changes according to the current requirements of the plants. In this experiment, repeated application of two inoculants was investigated in potato production. There was no

significant difference in growth variables when EVL or ÉB was applied one or two times. These findings suggest that these two commercial inoculants do not provide any additional growth-promotion when applied twice compared to once. Therefore, the focus of future studies should be inoculant application at seeding since these treatments provided the highest tuber yield increases, compared to the control treatment.

3.4.4 Conclusions

Overall, this experiment reports the suitability of granular fertilizer to be a carrier for viable microorganisms one hour following inoculation. In crop years with a low average yield, inoculation resulted in higher yields than the uninoculated control, although the differences were not always statistically significant. The recommended inoculant dose resulted in better plant-growth promotion than application at twice recommended dose, while repeated application did not result in any additional plant growth. Growth promotion observed in-season did not translate into increased yield, although it increased the seed weight, which could translate into an increased value of the harvest, so that inoculation would generate an economic return to crop producers.

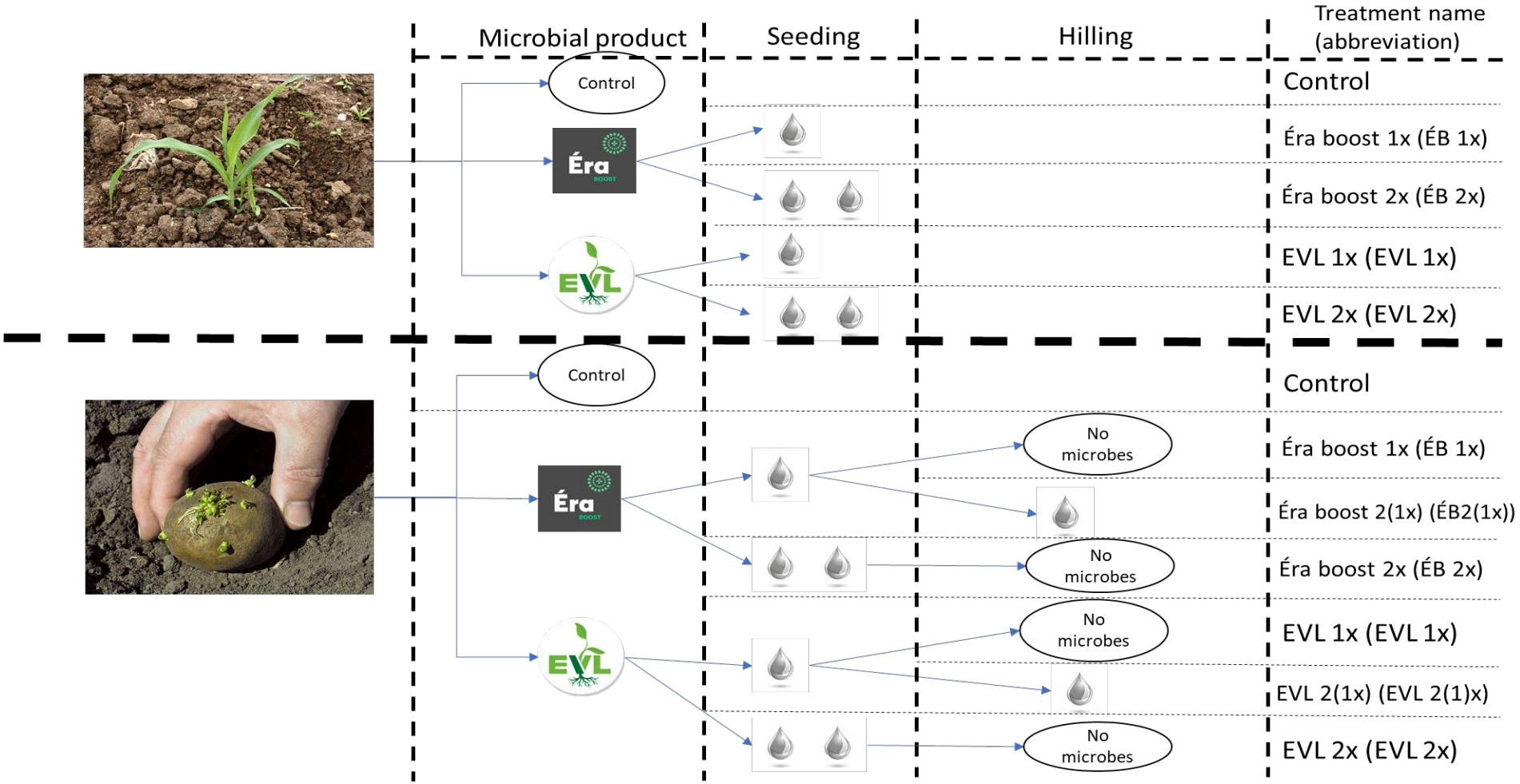

Figure 3. 1: Schematic diagram of corn and potato treatments with microbial application at seeding for corn and seeding and hilling for potato. One droplet represents the recommended rate applied while two droplets represents twice the recommended rate applied. Arrows represents each treatment composition. Each column is either a corn or potato treatment.

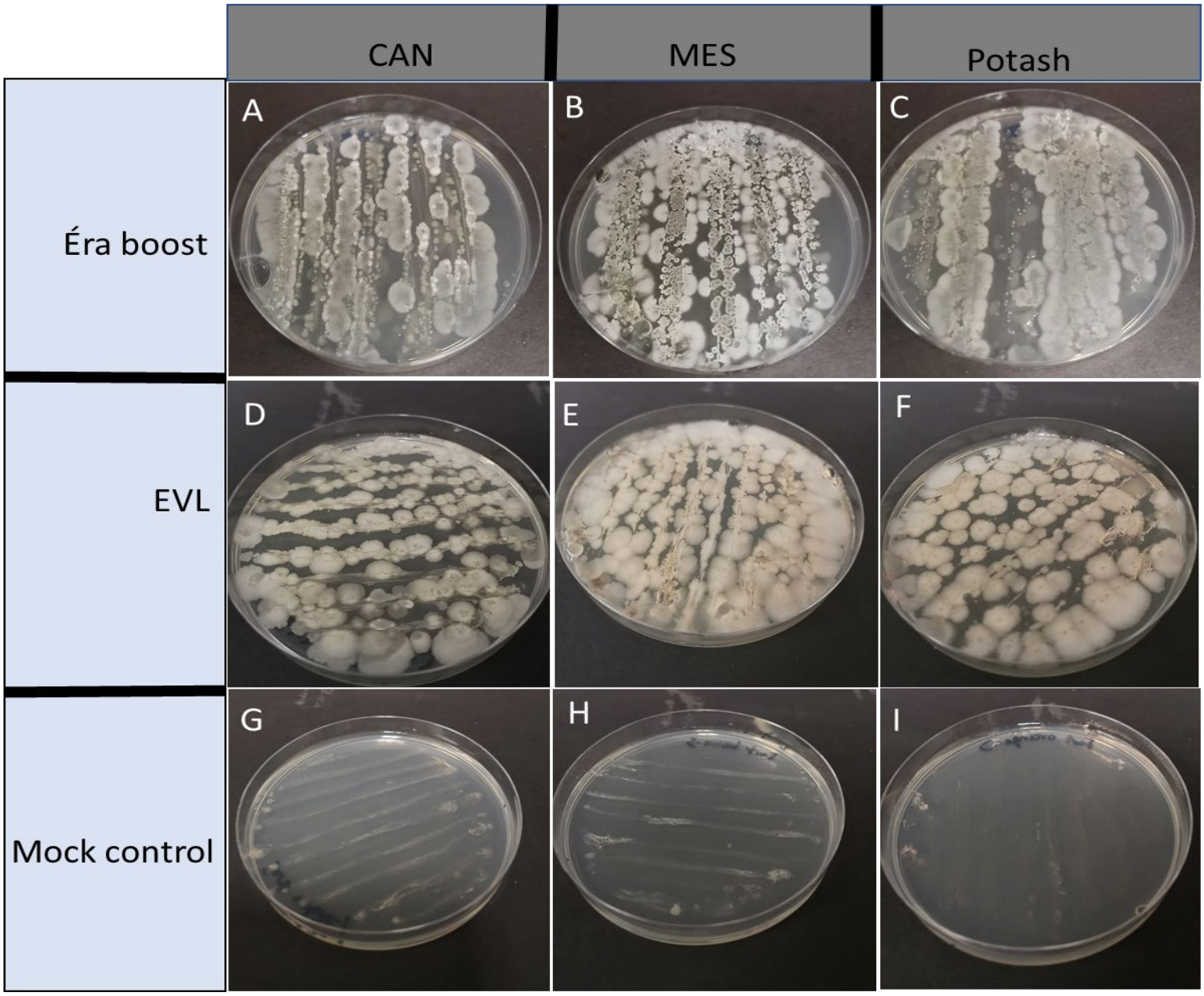

Figure 3. 2: Bacterial growth 48 hours following inoculation of granular fertilizer. A: Éra boost inoculation on CAN fertilizer; B: Éra boost inoculation on Micro Essential (MES) fertilizer; C: Éra boost inoculation on potash fertilizer; D: EVL inoculation on CAN fertilizer; E: EVL inoculation on MES fertilizer; F: EVL inoculation on potash fertilizer; G: Non-inoculated CAN fertilizer; H: Non-inoculated MES fertilizer; I: Non-inoculated Potash fertilizer

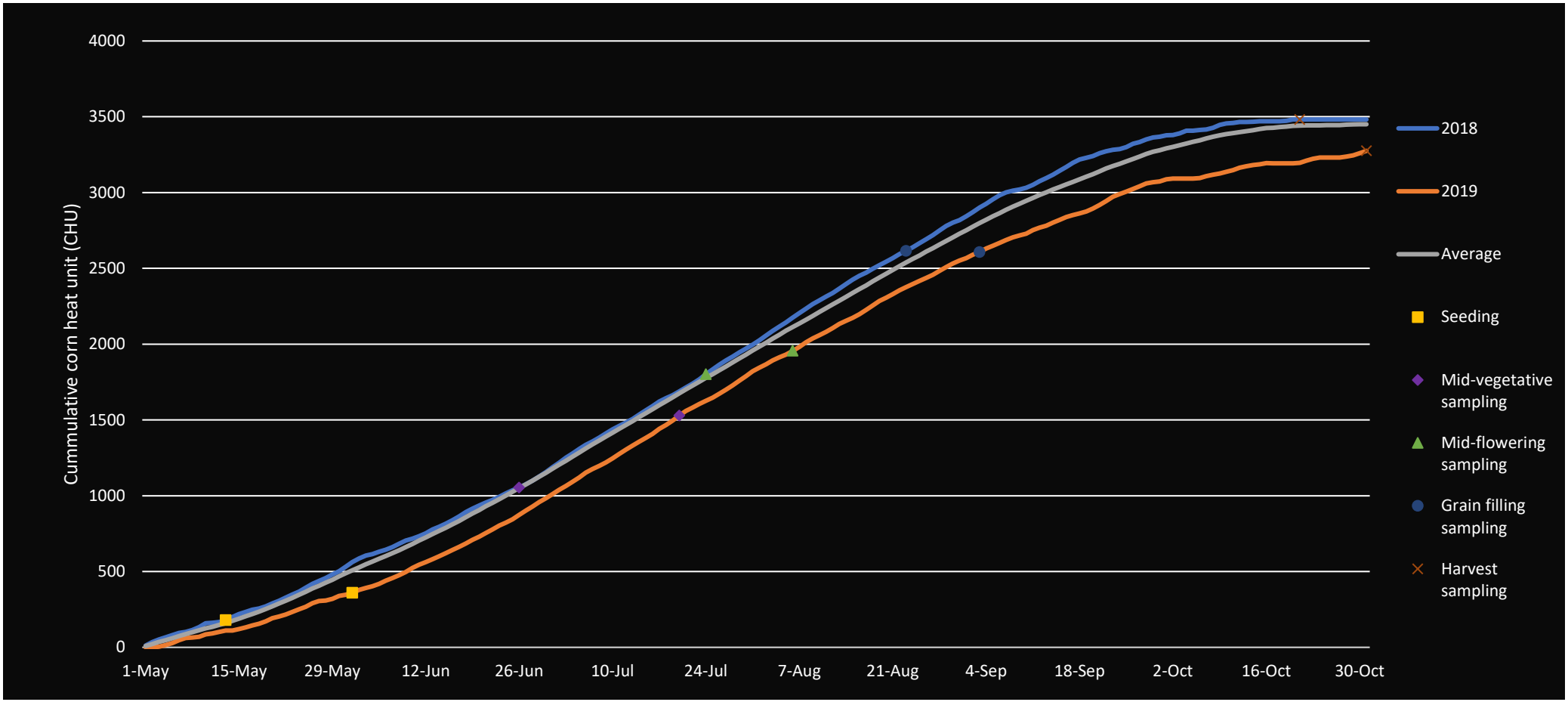

Figure 3. 3: Cumulative corn heat units (CHU) in 2018, 2019 and 10 years average at St-Hyacinthe with seeding and sampling dates. There were important time differences between 2018 and 2019 for seeding and mid-vegetative sampling with a difference heat unit accumulation in 2019 compared to 2018

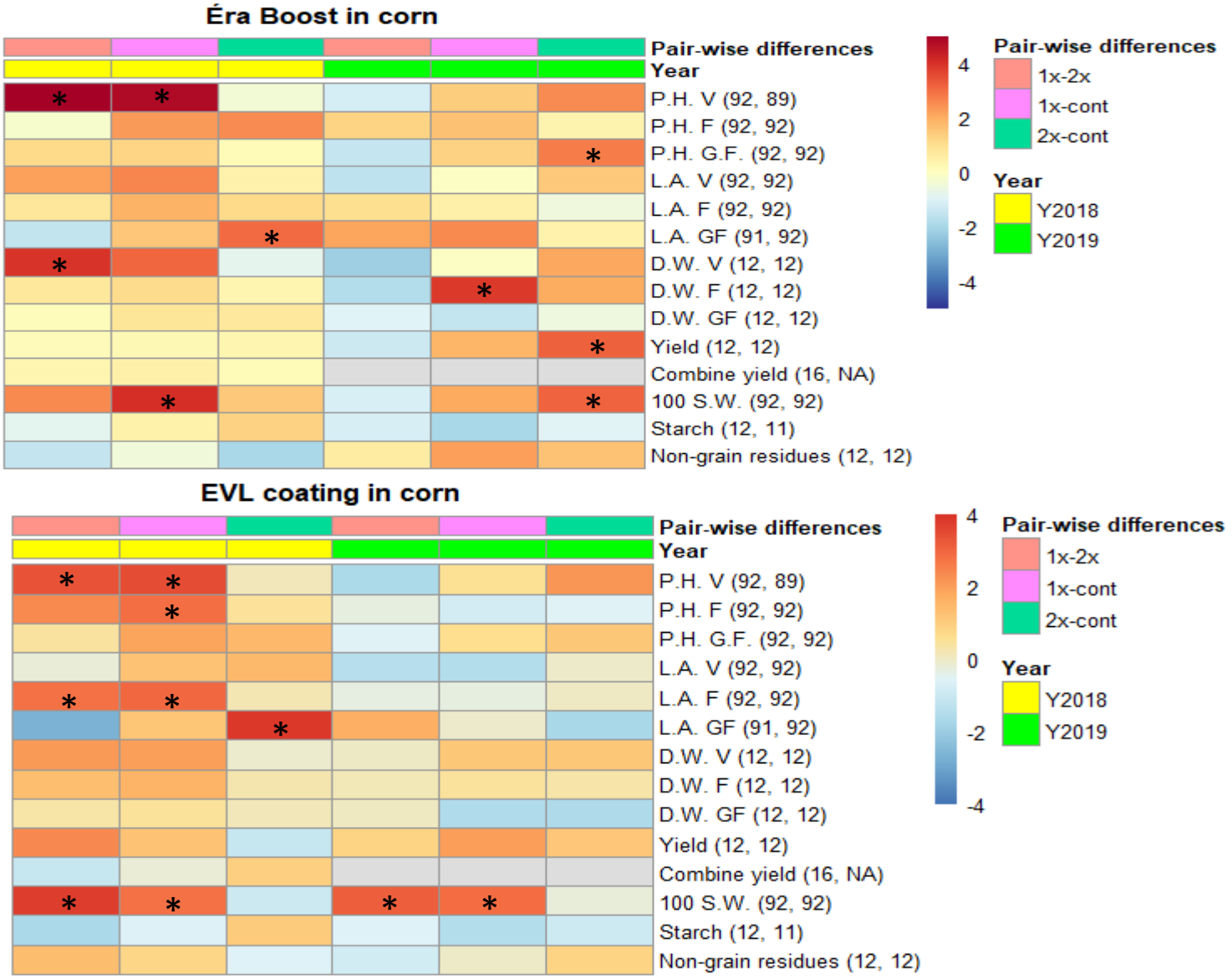

Figure 3. 4: Heatmaps of t-values of pair-wise differences of Era Boost® (top) and EVL coating® (bottom) treatments. Pair-wise differences from left to right: 1x vs. 2x; 1x vs. control; 2x vs. control; the first to third columns are for 2018 and the fourth to sixth columns are for 2019. Each row represents a growth trait measured during the growing season. Red indicates a larger value for the first treatment of the pair-wise comparison while blue indicates a larger value for the second treatment of the pair-wise comparison. Stars indicate a significant difference with a Bonferroni adjusted P-value = 0.05. Abbreviations: P.H.: plant height; L.A.: leaf area; D.W.: dry weight; 100 S.W.: 100-seed weight; V: mid-vegetative sampling; F: mid-flowering sampling; G.F.: grain filling sampling; (n1, n2): n1 is the number of degrees of freedom of T-values for 2018, n2 is the number of degrees of freedom of T-values for 2019

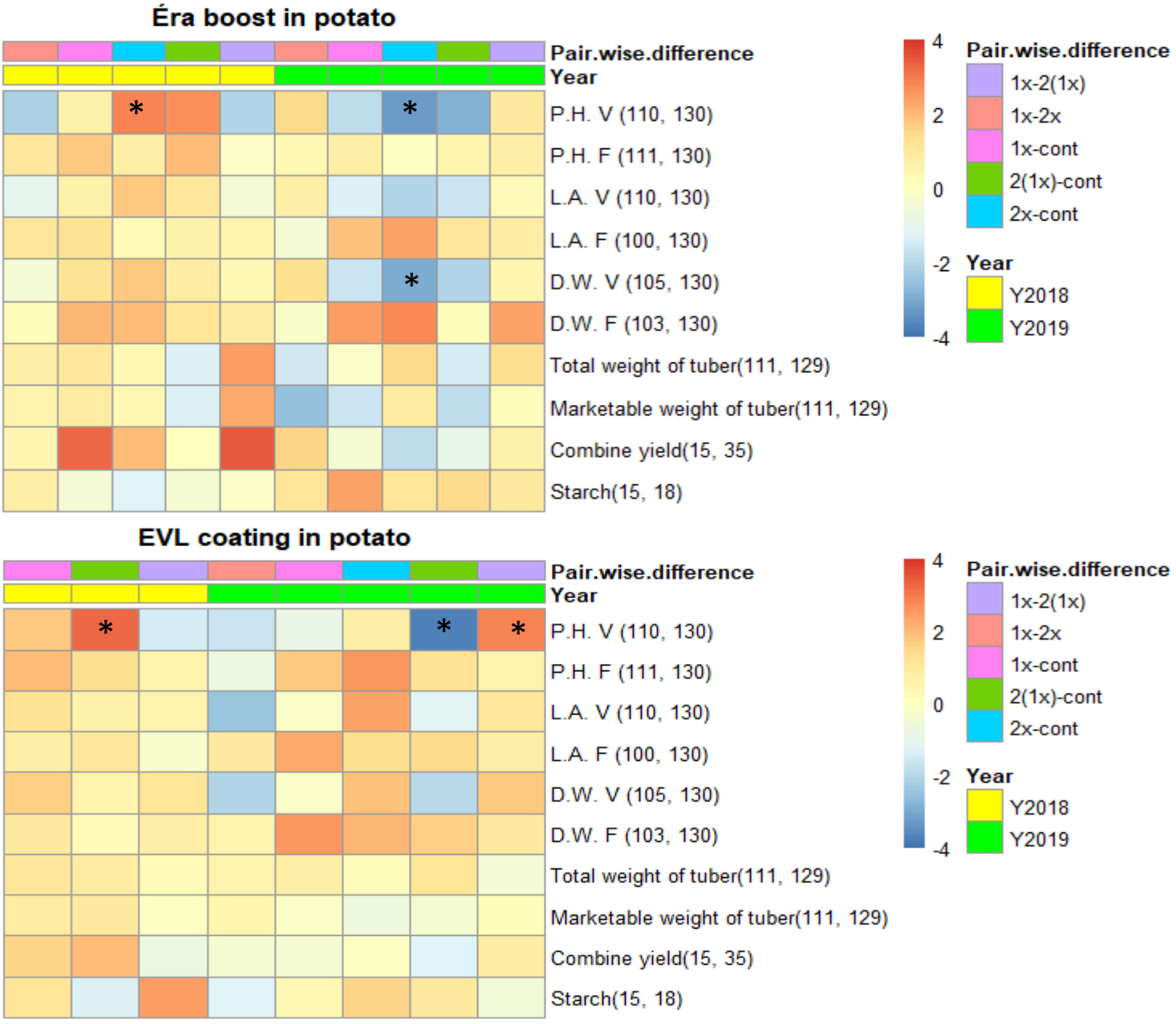

Figure 3. 5: Heatmaps of T-values of pair-wise differences of Era boost® (top) and EVL coating® (bottom) for potato. Columns are defined by a two-level colour legend. Each row represents a growth trait measured during the growing season. Red indicates a larger value for the first treatment of the pair-wise comparison while blue indicates a larger value for the second treatment of the pair-wise comparison. Stars indicate a significant difference with a Bonferroni adjusted P-value = 0.05. Abbreviations: P.H.: plant height; L.A.: leaf area; D.W.: dry weight; V: mid-vegetative sampling; F: mid-flowering sampling; (n1, n2): n1 is the number of degrees of freedom of T-values for 2018, n2 is the number of degrees of freedom of T-values for 2019

Table 3. 1: Corn growth variables at V6 and R1 growth stages

	Plant height / cm		**Leaf area** /cm^2		**Dry weight of five plants** / g	
	V6 stage					
Years	2018	2019	2018	2019	2018	2019
Control	91.7635±2.2479	174.37±2.5207	1853.86±94.2095	3759.7±131.75	75±5.0179	253.45±24.2959
Éra boost 1x (ÉB1x)	98.4575±2.2479	179.23±2.4569	2100.72±94.2095	3738.53±131.75	92±5.0179	250.05±24.2959
Éra boost 2x (ÉB2x)	91.2365±2.2479	183.42±2.5207	1893.07±94.2095	4026.52±131.75	70.5±5.0179	318.84±24.2959
EVL 1x	96.578±2.2479	176.15±2.4569	1971.34±94.2095	3486.53±131.75	85.75±5.0179	289.27±24.2959
EVL 2x	91.917±2.2479	181.95±2.5207	1991.34±94.2095	3748.21±131.75	74.5±5.0179	289.5±24.2959
P values	<0.0001	0.0656	0.0898	0.0701	0.01	0.2185
	R1 stage					
Years	2018	2019	2018	2019	2018	2019
Control	257.56±5.7846	299.6±3.582	4547.27±124.64	4215.99±127.07	427.5±20.1631	649.38±21.8271
Éra boost 1x (ÉB1x)	265.7±5.7846	307.8±3.582	4881.32±124.64	4294.39±127.07	458±20.1631	767.9±21.8271
Éra boost 2x (ÉB2x)	266.48±5.7846	301.45±3.582	4743.23±124.64	4133.17±127.07	436.5±20.1631	711.64±21.8271
EVL 1x	267.53±5.7846	295.3±3.582	5069.1±124.64	4165.82±127.07	472.5±20.1631	663.08±21.8271
EVL 2x	259.26±5.7846	296.8±3.582	4579.83±124.64	4218.13±127.07	434±20.1631	658.58±21.8271
P values	0.013	0.1249	0.0215	0.8945	0.5073	0.0116

Table 3. 2:Corn growth variable at grain filling (R4) growth stage

	Plant height / cm		**Leaf area** /cm^2		**Dry weight of five plants** / g	
	R4 stage					
Years	2018	2019	2018	2019	2018	2019
Control	265.78±2.768	308.55±2.2797	4208.18±108.03	4322.47±157.13	1065.25±59.8661	980.38±33.4524
Éra boost 1x (ÉB1x)	269.8±2.768	312.1±2.2797	4438.37±108.03	4734.48±157.13	1133.5±59.8661	913.26±33.4524
Éra boost 2x (ÉB2x)	266.17±2.768	316.3±2.2797	4674.13±108.03	4388.43±157.13	1126±59.8661	955.95±33.4524
EVL 1x	271.99±2.768	310.25±2.2797	4390.14±108.03	4311.94±157.13	1103±59.8661	910.75±33.4524
EVL 2x	270.64±2.768	311.8±2.2797	4804.02±108.03	4042.83±157.13	1076.5±59.8661	909.16±33.4524
P values	0.2376	0.0869	0.0017	0.0017	0.9024	0.4053

Table 3. 3 Corn harvest variable

	Harvest					
	Hand-harvest yield of 5 plants / g		**Combine-harvest yield** / kg ha^{-1}		**Non-grain aboveground biomass of five plants** / g	
Years	2018	2019	2018	2019	2018	2019
Control	91.7635±2.2479	174.37±2.5207	1853.86±94.2095	3759.7±131.75	75±5.0179	253.45±24.2959
Éra boost 1x (ÉB1x)	98.4575±2.2479	179.23±2.4569	2100.72±94.2095	3738.53±131.75	92±5.0179	250.05±24.2959
Éra boost 2x (ÉB2x)	91.2365±2.2479	183.42±2.5207	1893.07±94.2095	4026.52±131.75	70.5±5.0179	318.84±24.2959
EVL 1x	96.578±2.2479	176.15±2.4569	1971.34±94.2095	3486.53±131.75	85.75±5.0179	289.27±24.2959
EVL 2x	91.917±2.2479	181.95±2.5207	1991.34±94.2095	3748.21±131.75	74.5±5.0179	289.5±24.2959
P values	<0.0001	0.0656	0.0898	0.0701	0.01	0.2185

	100 seed weight / g		**Starch content** / %	
Years	2018	2019	2018	2019
Control	35.1005±0.377	27.4355±0.5092	61.1738±1.2312	64.9513±0.6786
Éra boost 1x (ÉB1x)	36.838±0.377	28.4625±0.5092	61.9151±1.2312	63.4212±0.7456
Éra boost 2x (ÉB2x)	35.736±0.377	29.0185±0.5092	63.383±1.2312	64.3003±0.6786
EVL 1x	36.2995±0.377	28.8785±0.5092	60.1113±1.2312	63.9029±0.6786
EVL 2x	34.678±0.377	27.316±0.5092	63.0258±1.2312	64.2966±0.6786
P values	<.0001	0.0009	0.3659	0.3873

Table 3. 4: Potato growth variables at mid-vegetative and mid-flowering growth stage

	Plant height / cm		**Leaf area** /cm^2		**Dry weight of five plants** / g	
	Mid-vegetative stage					
Years	2018	2019	2018	2019	2018	2019
Control	37.8±2.4551	40.85±0.9777	2186.76±312.31	1607.79±120.31	23.2109±3.5191	23.927±1.479
Éra boost 1x (ÉB1x)	38.8±2.4551	38.3±0.9777	2443.78±312.31	1377.62±120.31	28.6±3.4509	20.492±1.479
Éra boost 2x (ÉB2x)	42.2±2.4551	36.3±0.9777	2892.2±312.31	1252.97±120.31	30.6±3.4509	17.666±1.479
Éra boost 2(1x) (ÉB2(1x))	41.9747±2.4551	36.95±0.9777	2640.01±319.4	1328.2±120.31	27.0783±3.8821	19.6125±1.479
EVL 1x	40.525±2.4551	39.65±0.9777	2681.62±312.31	1585.83±120.31	30.25±3.4509	23.6047±1.479
EVL 2x	NA	41.9±0.9777	NA	2005.33±120.31	NA	27.8535±1.479
EVL2(1x)	42.8±2.4551	35.65±0.9777	2469.33±312.31	1411.99±120.31	25.4±3.4509	19.92±1.479
P values	0.0076	<0.0001	0.628	0.0004	0.4769	<0.0001
	Mid-flowering stage					
Years	2018	2019	2018	2019	2018	2019
Control	76.9±2.5193	55.85±1.5002	10176±967.28	4592.11±532.62	77.6667±6.885	65.8479±6.7383
Éra boost 1x (ÉB1x)	81.9±2.5193	57.4±1.5002	11607±967.28	5525.32±532.62	97.8889±6.885	84.2107±6.7383
Éra boost 2x (ÉB2x)	78.9±2.5193	56.75±1.5002	10419±967.28	5761.2±532.62	96.4±6.5317	86.2432±6.7383
Éra boost 2(1x) (ÉB2(1x))	82.3±2.5193	55.85±1.5002	10931±967.28	5114.05±532.62	89.25±7.3026	66.8046±6.7383
EVL 1x	82.2±2.5193	59.45±1.5002	11064±967.28	5749.33±3.582	86.9±6.5317	85.1521±6.7383
EVL 2x	NA	60.95±1.5002	NA	5275.6±532.62	NA	80.8371±6.7383
EVL2(1x)	80.6±2.5193	58.35±1.5002	11369±967.28	5351.68±532.62	80±6.5317	78.1938±6.7383
P values	0.2998	0.1046	0.8017	0.2668	0.1946	0.0189

Table 3. 5: Potato harvest variables

	Harvest							
	Marketable tuber weight of one plant / g		**Total tuber weight of one plant**/ g		**Machine harvest yield** / Mg ha^{-1}		**Starch content** / %	
Years	2018	2019	2018	2019	2018	2019	2018	2019
Control	1111.75±115.93	1471.2±78.3304	1151.95±121.86	1576.5±92.5782	28.6195±2.7491	53.5719±2.4822	53.3309±0.8952	57.9059±1.34
Éra boost 1x (ÉB1x)	1237±115.93	1287.07±78.3304	1308.25±121.86	1561.45±92.5782	36.6162±2.7491	52.9848±2.4822	52.7081±0.8952	61.5072±1.34
Éra boost 2x (ÉB2x)	1161.2±115.93	1568.8±78.3304	1195.85±121.86	1734.9±92.5782	31.1448±2.7491	49.9515±2.4822	51.7057±0.8952	59.6832±1.34
Éra boost 2(1x) (ÉB2(1x))	917.8±115.93	1273.9±78.3304	955.65±121.86	1408.2±92.5782	32.4074±2.7491	51.7365±2.4822	52.9049±0.8952	60.0969±1.34
EVL 1x	1234.95±115.93	1450.9±78.3304	1304.85±121.86	1658.3±92.5782	34.0909±2.7491	52.9359±2.4822	54.7355±0.8952	58.4162±1.34
EVL 2x	NA	1401.7±78.3304	NA	1593.3±92.5782	NA	53.5230±2.4822	NA	60.3341±1.34
EVL 2(1x)	1239.8±115.93	1433.8±78.3304	1268.65±121.86	1703.4±92.5782	31.5657±2.7491	51.1746±2.4822	51.6146±0.8952	59.3176±1.34
P values	0.1706	0.1051	0.1353	1576.5±92.5782	0.2318	0.4625	0.2054	0.3285

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Connecting statements between chapter 3 and chapter 4:

Chapter 3 focused on plant responses following the addition of a microbial inoculant, to determine if microbes coated onto fertilizer could promote plant growth and increase crop productivity. However, plants will probably not be the only organisms in a field ecosystem that will be impacted by microbial inoculation of agricultural soils. In fact, for the microbial inoculant to produce plant growth-promoting effects, the inoculated microbial species should alter plant-microbe interactions resulting in stronger beneficial effects on plants. Therefore, we hypothesized that soil bacterial and fungal community compositions would be impacted by microbial inoculation. This led us to conduct a side-experiment with a portion of the 2019 potato trial. In Chapter 4, we analyzed bacterial and fungal community compositions in bulk soil at four time-points during the growing season and in rhizospheric soil at one time-point for inoculated and non-inoculated plots.

Chapter 4: Bacterial and fungal soil communities are not affected by microbial inoculation of soil but are instead shaped by sampling location and date

Abstract

The use of biological input (e.g. bio-stimulants) as complements to synthetic inputs (e.g. pesticides and fertilizers), is gaining popularity in the agricultural industry due to increasing consumer demand for environmentally friendly agriculture. Inoculation of fields with beneficial microbes, to promote plant growth, has been used on a broad scale for over a century. The aim of inoculant use is to increase plant growth and resistance to biotic and abiotic stresses, although at the field-level, inconsistent results are commonly observed. The response of soil microbial communities to inoculation with beneficial microbes has been under-examined and could shed light on the inconsistencies underlying plant responses to microbial inoculants. The aim of this research was to investigate microbial community responses to two commercial microbial inoculants coated onto fertilizer granules. Bulk soil samples were collected before and after inoculation and rhizospheric soil was collected during the last bulk soil sampling. Pyrosequencing of prokaryotic and fungal DNA revealed that microbial inoculation did not affect bulk soil or mid-season rhizospheric microbial communities. Significant effects of sample location and sampling date revealed that microbial community composition and diversity were different for the first treatment block compared to the other three blocks. Microbial community composition was correlated with many soil properties, most importantly CEC while organic matter and P content significantly varied between blocks. The time between the third sampling date (June 21) and the final sampling date (July 26) significantly affected microbial community composition, especially of prokaryotes. Pesticide applications and the potato hilling operation occurred during this time interval and could explain changes to the microbial community composition over time. Rhizospheric samples had more abundant but less diverse microbial communities than bulk soil samples. Pre-treatment of bulk soil samples with propidium monoazide revealed significant changes in relative abundance of prokaryotic and fungal phyla with a significant increase in *Firmicutes* abundance, confirming that relic DNA perturbs analyses of soil microbial communities.

4.1 Introduction

Roots exude various compounds into the soil in close vicinity to roots which are consumed by soil microorganisms. In turn, soil microorganisms provide key functions for plant development, such as nutrient acquisition and resistance to biotic and abiotic stresses (Mendes et al., 2013). Root exudates are composed of primary metabolites such as saccharides, amino acids and organic acids, and secondary metabolites such as phenolics and terpenoids (Gargallo-Garriga et al., 2018). Oxalic acid and other organic acids produced by roots reduce soil pH, enhancing microbial mobilization of nutrients into plant-available forms, while limiting the adverse effects of toxic elements (Ma, Ryan, & Delhaize, 2001). Through the production of root exudates, a plant shapes the microbial community associated with its roots, aiming to attract beneficial soil microbes (Rudrappa et al., 2008).

Plant growth-promoting rhizobacteria (PGPR) are a category of bacteria that have beneficial effects on plant development. Plant growth promotion occurs through various direct modes of actions, such as nutrient synthesis (e.g. N_2 fixation), enhanced nutrient uptake (e.g. phosphate solubilisation), phytohormone production (auxins, gibberellins and cytokinins) and abiotic stress alleviation (Pérez-Montaño et al., 2014). PGPR can also improve plant growth by interfering with other microbes in the rhizosphere. Examples of these indirect benefits include the production of anti-microbial compounds or interference with microbial quorum sensing mechanisms. Application of PGPR as inoculants is a potential mechanism for increasing plant productivity while reducing environmental damage (Kloepper, Lifshitz, & Zablotowicz, 1989). Many commercial product formulations include PGPR which, when applied as a seed treatment or as a soil drench, boost plant productivity. Most research dealing with the application of PGPR has examined plant variables exclusively, overlooking the persistence of inoculants in soil and their impacts on native microbial communities (Rilling et al., 2019). As the native microbial community present in bulk soil is much larger than the quantity of microbes in the applied inoculants, the effect of microbial inoculants should be localized within the soil.

There are several limitations to current knowledge of how microbial inoculants alter agricultural soil microbial community, including soil sampling strategy and background

noise generated by relic DNA in soil. To date, most experiments have investigated rhizospheric microbial communities as opposed to bulk soil microbial communities (Assainar et al., 2018; Deng et al., 2019) assuming that microbial inoculants proliferate in the vicinity of plant roots allowing them to have beneficial effects on plant growth, and benefit from root exudates. Our study analyzed samples taken from bulk and rhizosphere soil to broaden our understanding of the impact of microbial inoculants on the soil microbial community. Additionally, the soil is filled with extra-cellular DNA which can adsorb onto humic and clay particles, obscuring the analysis of soil microbial communities. To limit the background noise generated by this relic DNA, adding propidium monoazide (PMA) to soil samples denatures DNA not contained in viable cells, removing all relic DNA and improving assessment of the microbial community composition (Carini et al., 2016).

In conventional agriculture, a portion of the fertilization of row crops comes from fertilizer banded 5 x 5 cm (below and to one side) away from the seed, applied at the same time as seed sowing. In regions where seeding is done following harsh and cold winters, this banded fertilizer is the main nutrient source for plants until the soil warms enough for nutrient mineralization to become an important source of nutrients for plants. Chapter 3 of this thesis hypothesized that coating banded fertilizers with PGPR could enhance the availability of these nutrients in the rhizosphere and increase the efficacy of this fertilizer application with a focus on the improvement of plant growth variables such as above-ground biomass production and final crop yield.

In this study, we examined the soil microbial community of a potato field following the banded application of granular fertilizer coated with one of two commercial microbial inoculants at seeding. We sampled bulk and rhizospheric soils with the aim to understand the impacts of coating granular fertilizer with a microbial inoculant on soil/plant-associated bacterial and fungal microbiomes and to determine if the microbial species present in the inoculants were enriched in the soil. The results will be the first academic report regarding the use of granular fertilizer as a carrier for microbial inoculants, and its potential impacts on bulk and rhizospheric soil ecosystems.

4.2 Materials and methods

4.2.1 Experimental design and sampling methods

A field experiment was conducted on a fine sand soil located in St-Thomas de Joliette (46°00'01.8"N 73°21'02.2"W) during 2019. Three treatments were investigated: 1) uninoculated control (fertilizer only) 2) Éra boost® (ÉB) or 3) EVL coating® (EVL). Either product was coated onto granular starter fertilizer (GSF) applied at seeding. For all treatments, the rate of GSF was 1050 kg ha^{-1} with an NPK equivalent of 12-14-10. The microbial inoculant rate was of 1 L Mg^{1} of fertilizer for ÉB and 2 L Mg^{-1} of fertilizer for EVL, following company recommendations. The fertilizer was banded as a 2 x 2 x 2 application, meaning that fertilizer was placed at a distance of 5 cm from each side of the seed tuber and both strips were 5 cm below the seed tuber. All treatments were replicated seven times in a randomized complete block design in the field; four of the seven replicates were used to sample bulk soil and potato roots with closely adhering soil.

Each bulk soil sample consisted of four quadrants of a 2 x 2 m^2 plot, pooled together. Bulk soil samples were collected directly on the potato row, between the seed tuber and the fertilizer rows Within each quadrant, a 2.5 cm-diameter corer in was used to sample soil to a depth of 30 cm. Samples were collected at four time points: 1) four days before seeding (May 27th), 2) seven days after seeding (June 7th), 3) 21 days after seeding (June 21st), 4) 56 days after seeding (July 26th) (Figure 4.11). At the fourth time point root samples were also collected. Each root sample consisted of fine roots and closely adhering soil from three plants randomly selected from each plot. Immediately following sampling, soil and roots were placed on ice and transferred to storage at -20 °C within 2 h of sampling.

For each soil sample, a representative subsample of about 100 grams was prepared from the original sample. From these 100 g samples, around 400 mg were collected and stored in 2 mL collection tubes for DNA extraction. For each root sample, a subsample of 1 g was ground with liquid nitrogen and 200 mg were collected in 2 mL collection tubes for DNA extraction. For propodium-monoazide (PMA) extraction, 100 mg of soil was collected in a 2 mL collection tube.

4.2.2 Soil physicochemical analysis

Analysis of soil chemical properties was done by Laboratoire Géosol (St-Hyacinthe, Canada). P, K, Mg, Ca, Al, Zn, Mn, Cu, Fe and B were extracted with Melich III and quantified by plasma spectrometry. Soil pH, buffer pH and organic matter content were estimated with a 1:1 dilution with water, SMP and Walkley-Black solutions, respectively.

4.2.3 Propodium-monoazide (PMA) pre-treatment

Soil collected at the second time point from uninoculated and EVL-treated plots were used to perform a viable cell experiment, following the protocol of Carini et al. (Carini et al., 2016). One hundred mg of soil was diluted in 10 mL of PBS (pH = 7.4). Every sample was prepared in duplicate; one sample received PMA and one did not receive PMA. In each tube, 20 µL of PMaxx (Biotium Inc.) was added in the dark to achieve a final concentration of 40 µM of PMA. Following PMA application, tubes were incubated for 10 min and vortexed for 4 min during the incubation time. After incubation, samples were subjected to light in a growth chamber for 30 min, shaking the tubes every 10 min. After light exposure, 1 mL of the PBS soil slurry was used for DNA extraction.

4.2.4 DNA extraction and sequencing

All DNA extractions were performed using FastDNA Spin Kit for Soil (MPBio), which shares similarities to the protocol of Jeanne et al. (Jeanne, Parent, & Hogue, 2019). The soil or root subsample was added to tubes containing 1.4 g of the bead matrix E and 1 mL of the lysis buffer supplied with the kit. DNA extraction was performed according to the manufacturer's instruction. The resulting DNA pellet was suspended in 100 µL of sterile molecular-grade water.

The quality and quantity of the DNA extracts were evaluated by spectrophotometry using a Biophotometer (Eppendorf, Mississauga, ON, Canada) using a G1.0 µCuvette (Eppendorf, Mississauga, ON, Canada) with readings at 260, 280, 230 and 320 nm. The V4 region of prokaryota (archaea and bacteria) *rRNA 16S* gene was amplified using 515FB and 806RB primers (Apprill, McNally, Parsons, & Weber, 2015; Parada, Needham, & Fuhrman, 2016). For the fungi, the eukaryotic (fungal) *ITS1* gene was amplified (Bokulich & Mills, 2013). Both genes were amplified in a two-step dual approach PCR designed for

Illumina instruments by *Plateforme d'analyses génomiques* (IBIS, Universite´ Laval, Quebec City, QC, Canada).

DNA sequencing was performed by IBIS on an Illumina MiSeq platform, following the methods of Jeanne et al. (Jeanne et al., 2019). The procedure used for fungal DNA amplification and sequencing was similar to the procedure used for prokaryotic DNA amplification.

Obtained sequences were demultiplexed based on the tag used. Sequence quality control and feature table construction was performed using QIIME 2 (Caporaso et al., 2011) and dada2 plugin (Callahan et al., 2016). Reference databases Greengenes 13.8 (DeSantis et al., 2006) and UNITE version 8 (Kõljalg et al., 2013) were used for taxonomic identification of amplicon sequence variants (Callahan, McMurdie, & Holmes, 2017).

4.2.5 Quantitative PCR of bacterial and fungal DNA

From the isolated DNA, 4 µL of DNA diluted at 1:20 in molecular-grade water was mixed with 6 µL of a master mix. Fluorescence detection was performed on a CFX96 (Biorad, Herclues, CA, USA), master mix consisted of SYBR green qPCR mix (Qiagen, Toronto, ON, Canada). Standard curves were generated from a known quantity of amplified DNA fragments, diluted over a 4-log range (efficiency of 89.1 % and $r^2 = 0.99$ for prokaryotes; efficiency of 91.7 % and $r^2 = 1$ for fungi). Samples were prepared on 96-well plates with four wells acting as blanks to which water was added instead of DNA. PCR conditions were 15 min at 95 ºC, followed by 40 cycles of 95 ºC for 1 min, 30 s at the annealing temperature and 72 ºC for 1 min. For prokaryotic DNA, the primer pair EUB-338/518 was used with an annealing temperature of 53 ºC (Fierer, Jackson, Vilgalys, & Jackson, 2005) with a threshold of quantification of 32. For fungal DNA, the primer pair FF390/FR1 was used with an annealing temperature of 51 ºC (Emerson et al., 2015) with a threshold of quantification of 42. All samples were replicated at least twice. If the standard deviation between the two CT values for a sample was more than 0.5, a third replicate was analyzed. Prior to quantification of total bacteria and total fungi, DNA extracts were tested for the presence of PCR inhibitors using a M13-assa that amplifies a cloned M13 sequence made with the TOPO PCR cloning kit (Thermo Fisher Scintific). No inhibition of the amplification process was detected during the M13-assay.

Amplification units (AU) could be derived from the average CT values of samples using linear regressions designed by the Microbial Ecology Laboratory of IRDA for prokaryotic and fungal DNA. AU g^{-1} of dry soil were obtained using moisture content and the exact weight of soil used during DNA extraction.

4.2.6 Downstream data analysis

From feature and taxonomical tables created with QIIME 2, the R package "phyloseq" (McMurdie & Holmes, 2014) was used to measure the alpha-diversity through Shannon and Chao1 indices, based on evenness and number of observed ASVs. ANOVA was performed on alpha-diversity measurements of soil samples that received the microbial inoculation treatment; time was a fixed effect and block was a random effect. A second ANOVA was performed on roots with treatment as a fixed effect and block as random effect. The Bonferroni adjustment was used to determine significant differences. To compare alpha-diversity measurements between roots and soil at the same sampling date (July 26th) a paired T-test of roots vs. soil samples was performed for all diversity indices.

Beta-diversity was calculated using Bray-Curtis dissimilarity matrices with the ordinate function from the phyloseq package. Ordination of soil and roots samples was observed using principal coordinate analysis (PCoA). Distance matrices were computed using the distance function from phyloseq. PERMANOVA was performed on these distance matrices using the Adonis function from the Vegan package with the explanatory variables set as microbial treatment, sampling date and treatment block number and 999 permutations. A post-hoc test was conducted using the pairwiseAdonis package with p-values adjusted using a Bonferroni adjustment.

A distance-based redundancy analysis (dbRDA) was performed using the "capscale" function from Vegan package. Prokaryotic and fungal ASV feature table was used with all soil chemical variables measured. PERMANOVA of soil chemical variables revealed statistically significant variables. These variables were represented as vectors on a constrained ordination for both prokaryotic and fungal communities.

4.3 Results

4.3.1 Effect of microbial inoculants on soil physical and chemical properties and tuber yield

The physico-chemical properties of plots prior to seeding and the final yield of plots were collected (Table 4. 1 and Table 4.2). There was a significant difference in mean yield across blocks. PERMANOVA following a dbRDA of prokaryotic and fungal community composition and soil chemical variables showed a significant effect of organic matter, CEC, Mg, P and Buffer pH measurements on prokaryotic communities and CEC on fungal communities (Figure 4.10).

4.3.2 Microbial inoculant compositions

Pure inoculants were analyzed for their microbial community composition. The EVL-treated samples had 17110 and 16311 reads for prokaryotic and fungal communities while ÉB-treated samples had 21950 and 4932 reads for prokaryotic and fungal communities, respectively. Figure 4.1 shows the composition of prokaryotic and fungal communities in the inoculants. ÉB was predominantly *Bacillus*; all other prokaryotic genera and all fungi were contaminants. The EVL inoculant was more diverse: the major prokaryotic genera were *Lactobacillus* and *Clostridium* and the major fungal genus was identified as *Saccharomyces*.

4.3.2 Soil prokaryotic and fungal community dynamics

The average number of reads for soil samples were 30067.57 ± 1905.309 for prokaryotes and 22716.98 ± 2341.46 for fungi. The two dominant bacterial phyla were *Proteobacteria* and *Actinobacteria* across all sampling time points, while the dominant fungal classes were *Sordariomycetes*, *Leotiomycetes* and *Eurotiomycetes*, with the latter having a noticeable enrichment at the final sample date (July 26th).

Microbial inoculation of fertilizer did not have a significant effect on any diversity indices, observed ASVs or evenness of soils at any time point. Sampling date had a significant effect on Shannon (P = 0.0226, P = 0.0017) and Chao1 (P = 0.0033, P = 0.0004) indices, observed ASVs (P = 0.0035, P = 0.0006) and evenness (P = 0.0099, P = 0.015) for prokaryote and fungal populations, respectively. Significant differences were observed in prokaryotic diversity between May 27th and June 21st for Shannon and Chao1 indices and

number of observed ASVs and between May 27th and July 26th for Chao1 index and evenness. Fungal community composition and diversity were significantly different between July 26th and all other sampling dates for Shannon and Chao1 indices and number of observed ASVs, while there were no significant differences for evenness for May 27th and July 26th.

A principal coordinate analysis (PCoA) was used to visualize the community assemblages at the ASV level using Bray-Curtis dissimilarities. Prokaryote communities displayed a strong clustering of samples along the first PCoA axis, which explained 17 % of the variation, with strong loading of sampling time points: the fourth sampling time point (July 26th) was segregated from the first three. The second axis, which explained 6.9 % of the variation, showed a clear clustering of physical location indicated as block number (Figure 4.3).

Fungal communities displayed a weak clustering effect of sampling time point along the first axis, which explained 16.1 % of the variation. The second axis does not represent any interesting biological variables and explained 9.4 % of the variation. Microbial inoculants did not have any clustering effect on prokaryotic or fungal communities (Figure 4.3).

PERMANOVA results indicated that microbial inoculants did not produce a significant effect while sampling date and physical location (block number) significantly affected prokaryotic and fungal communities. The pairwise.adonis command revealed that the May 27th prokaryotic composition was significantly different from that of June 21st ($P = 0.012$), and the July 26th prokaryotic composition was significantly different from the three other sampling dates ($P = 0.006$). Fungal composition was significantly different on July 26th from the three other sampling times ($P = 0.006$). The first block had a significantly different prokaryotic composition from the three other blocks, as observed in the PCoAs. Fungal community composition was significantly different between blocks 1 and 2.

Both alpha-diversity and beta-diversity indices showed a significant shift in prokaryotic and fungal community composition between the third and fourth sampling dates. Many ASVs had differential expression between June 21st and July 26th with the

majority belonging to the classes *Gammaproteobacteria*, *Actinobacteria* and *Betaproteobacteria* (Figure 4.4).

The number of amplification units g^{-1} dry soil was not significantly impacted by microbial inoculation or sampling date for either prokaryotic or fungal communities. Average values were 3.57×10^9 and 3.39×10^7 for prokaryotes and fungi, respectively.

Prokaryotic and fungal genera present in EVL and ÉB were not significantly enriched following microbial inoculation, or in comparison to uninoculated plots. The genus *Bacillus* was less prevalent in ÉB plots than control plots, while the predominant prokaryotic genus in the EVL consortium, *Lactobacillus*, was not detected in soil samples. Similar results were observed for fungal genera in soils inoculated with the EVL consortium, in that *Saccharomyces* and *Wickerhamomyces* were not observed in soil samples (Figure 4. 5).

4.3.3 Roots bacterial and fungal diversity and quantity

Root samples had an average of 18822.58 ± 1382 and 14718.75 ± 1720.549 reads for prokaryotes and fungi, respectively. The potato root prokaryotic community consisted mostly of the phyla *Proteobacteria*, *Cyanobacteria* and *Actinobacteria*, while the most abundant fungal classes were *Sordariomycetes*, *Leotiomycetes* and *Eurotiomycetes* (Figure 4.6). No significant difference from microbial inoculation could be observed for Shannon and Chao1 indices, evenness and number of observed ASVs for either prokaryotic or fungal communities. The number of amplification units g^{-1} dry roots was not significantly impacted by microbial inoculation for either prokaryotic or fungal communities. Average values were 1.27×10^{10} and 9.17×10^7 reads for prokaryotes and fungi, respectively.

Soil samples collected on the same date as root samples (July 26th) were combined for analysis as paired T-tests. Prokaryotic Shannon and Chao1 indices, evenness and number of observed prokaryotic ASVs were significantly different between root and soil samples. Fungal Chao1 index and number of observed fungal ASVs were significantly different between root and soil samples. The number of AU g^{-1} of dry roots was significantly greater than the number AU g^{-1} dry soil for both prokaryotes and fungi. Community composition revealed clear clustering between bulk and rhizospheric soil samples (Figure 4.7)

PERMANOVA analysis of root and soil samples collected on July 26th showed a significant effect ($P < 0.001$) of sample type (roots or soil) for prokaryotic and fungal communities. Microbial inoculation and block did not have significant effects for prokaryotic communities ($P_{treatment} = 0.434$ and $P_{block} = 0.15$) or fungal communities ($P_{treatment} = 0.757$ and $P_{block} = 0.357$). Analysis of root samples alone showed a significant effect of block ($P = 0.019$) for prokaryotic communities, but this was not significant ($P = 0.415$) for fungal communities.

Similarly as for soil samples, potato roots were not enriched in genera present in microbial inoculants. *Lactobacillus, Saccharomyces* and *Wickerhamomyces* genera, all present in the EVL inoculant, were not observed in roots samples (Figure 4.6).

4.3.4 Propodium-monoazide addition

To investigate the viability of microbial organisms present in soil following inoculation, a small side-experiment used control and EVL soil samples from the second sampling date (June 7th). Those soil samples were diluted in PBS and incubated in the presence or absense of propodium-monoazide (PMA), a chemical used for viability tests. These soil slurries were compared to the results of the corresponding soil samples analyzed conventionally (i.e., without the addition of PBS or PMA).

The addition of a pre-extraction method had clear effects on the relative abundance of some prokaryotic phyla and fungi classes (Figure 4.8). For prokaryotes, the most important change was for the phylum *Firmicutes*, which increased in relative abundance in PBS and PBS+PMA samples. For fungi, the greatest increase was for the class *Leotiomycetes*.

Diversity and composition indices were significantly impacted by PMA addition to the soil/PBS slurry. For all indices no significant effect was observed due to the addition of the microbial inoculant, although there was a significant interaction between PMA addition and microbial inoculation for Shannon index ($P = 0.0196$) and evenness ($P = 0.0126$) of fungi. Quantification of microbial DNA revealed a reduction in the amount of DNA present in samples with PMA ($P_{prok} < 0.0001$ and $P_{fung} = 0.007$) of 0.4855 ± 0.09395 and 0.4974 ± 0.06127 AU log g^{-1} dry soil, respectively.

Prokaryotic and fungal community compositions were significantly affected by PBS and PBS+PMA addition to soil, with clear clusters for the composition of each communities and stronger differences for prokaryotic community composition (Figure 4.9). PERMANOVA analysis showed a significant effect of pre-extraction method ($P < 0.001$) for both microbial communities. Neither microbial inoculation treatment ($P_{prok} = 0.176$, $P_{fung} = 0.605$) nor blocks ($P_{prok} = 0.088$ and $P_{fung} = 0.151$) had significant effects on community composition when all pre-extraction methods were combined. Pairwise comparisons revealed significant differences between all three pre-extraction methods. When analyzing PBS+PMA samples alone (n = 8) the effects of microbial inoculation ($P_{prok} = 0.079$ and $P_{fung} = 0.214$) and blocks ($P_{prok} = 0.122$ and $P_{fung} = 0.418$) were not significant.

4.4 Discussion

4.4.1 Microbial inoculation impacts on soil microbial communities

We hypothesized that microbial inoculation of granular fertilizer would influence soil microbial communities. However, neither soil nor root samples were significantly affected by microbial treatments. Banded fertilizers are applied as narrow strips across the field, making their effect extremely localized within the soil. In this experiment, soil cores were collected up to a depth of 30 cm, much deeper than the range of action of the banded fertilizer, making it difficult to see a significant effect from a one-time microbial inoculation. Most research looks at rhizospheric soil samples instead of bulk soil. In this experiment, rhizospheric samples were only taken in concordance with the final bulk soil sampling, because at the first two bulk soil sampling time points, no living root systems were present in the soil. An experiment on *Fragaria* × *ananassa* (strawberry) showed that a soil amendment, applied at a rate of 75 L ha^{-1} every month for 5 months, had an effect on the soil microbial community (Deng et al., 2019). Inoculation of *P. ginseng* as a biofertilizer significantly impacted bulk soil microbial community with a one time-application of 90-270 L ha^{-1} of microbial inoculant diluted in manure (Dong et al., 2019). An inoculation of 10^9 spores L^{-1} of soil did have an effect on the rhizospheric prokaryotic community of *Cucumis sativus* (cucumber) 80 days after inoculation (Tian & Gao, 2014). In comparison, our research added 1 and 2 L ha^{-1} (about 4-8 x 10^{11} cells ha^{-1}) of microbial

inoculant as a one-time application at seeding in a banded application, which possibly explains the lack of effect on bulk soil microbial communities.

4.4.2 Sampling time and sample physical location effects on soil microbial community

Variables other than microbial inoculation had significant effects on microbial community composition, namely sampling date. Changes in bacterial and fungal communities over the course of a growing season have been observed repeatedly on agricultural lands (Degrune et al., 2017; Houlden, Timms-Wilson, Day, & Bailey, 2008; Lauber, Ramirez, Aanderud, Lennon, & Fierer, 2013). Even with minimal human inputs, microbial community composition changes drastically, often in correlation with changes in soil temperature and/or humidity (Lauber et al., 2013). In addition to changes in environmental conditions, application of pesticides and in-season tillage activities influence the presence of microbial species in certain layers (strata) of the soil, as well as their relative abundance (Degrune et al., 2017; Lo, 2010; Sun et al., 2018). In this experiment, a significant shift could be observed for both prokaryote and fungal communities between June 21 and July 26, much more than between May 27 and June 21. In fact, between mid-June and mid-July, fungicides were applied at a weekly interval and soil was tilled once around potato rows to promote tuber production and apply additional fertilizer. In comparison, between the end of May and mid-June when the first three sampling time points occurred, only seeding and application of banded fertilizer were carried out; there was no pesticide application or tillage.

Since this experiment followed a RCBD design, we had physical treatment replicates pooled into seven blocks across a field of less than 1 ha. Our hypothesis that microbial inoculation would have a stronger effect than the block effect on microbial community composition was rejected. Block effect was significant for prokaryotic bulk soil and rhizospheric compositions and fungal bulk soil composition. The prokaryotic bulk soil community present in the first block was significantly different from that of the three other sampled blocks, while the fungal bulk soil community present in the first block was significantly different only from that of the second block. Prokaryotic community composition and soil organic matter, P, Ca, Mn, Fe and B contents were significantly

different between the first block and other blocks. Db-RDA revealed P content significantly explained prokaryotic community composition. The C:N:P ratio was found to be a strong predictor of bacterial diversity in previous research (Delgado-Baquerizo et al., 2017), further indicating that P content in bulk soil drives soil bacterial community composition. In addition, mean yield of each block was highly variable, with lower yields observed in block 1 than in the other six blocks (Table 4.2). Complex interactions among soil properties, environmental conditions and microbial community composition contribute to determination of tuber yield at a given location and has been investigated in other research (Jeanne et al., 2019; Xue, Christenson, Genger, Gevens, & Lankau, 2018).

4.4.3 Roots-associated microbial communities and relic-DNA free microbial communities

Rhizospheric microbial community composition differed substantially from bulk soil microbial community composition (Figure 4.6). Such differences have been reported in numerous other studies (Berendsen, Pieterse, & Bakker, 2012; Smalla et al., 2001; Xue et al., 2018). How plants shape the rhizsopheric microbial community composition from the bulk soil microbial community remains a puzzle to scientists. More thorough analysis of root exudates and how microbes influence plant roots exudate is needed (Huang et al., 2014). With such knowledge, samples of bulk soil could be much more informative and could guide the prediction of the soil potential for various functions, such as the ability to promote plant growth, plant yield potential and nutrient flux rates.

The soil matrix can conserve dead microbial DNA for many years, affects the interpretation of soil microbial community data collected *via* DNA extraction and sequencing (Carini et al., 2016). The addition of PMA to soil samples denatures relic DNA with only the DNA contained in viable cells remaining. The difference in the composition of relic DNA and viable DNA dictates the impact of relic DNA removal (Lennon, Muscarella, Placella, & Lehmkuhl, 2018). In this study, PMA addition resulted in less diverse communities and lower quantities of DNA than soil extracted without PMA. The effect of microbial inoculation was stronger in PMA-treated soil than conventional soil extraction, suggesting that the viable organisms are more affected by the addition of inoculants than the relic-DNA community.

4.4.4 Conclusion and future directions

This experiment did not confirm its original hypothesis; this might have been due to several aspects of the work which could be improved in future experiments. First, a more localized soil sampling (proximal to the site of inoculant application) could be conducted to assess the effect of microbial inoculation associated with banded fertilizer. Laboratory experiments showed that microbial inoculation of fertilizer results in addition of viable cells to the soil, which grow under favourable conditions. In the soil, microbial growth must happen in the few centimeters surrounding the applied fertilizer, the exact location which would be affected by water movement in the soil. Hence sampling directly at the site of fertilizer placement, to a depth of 7.5-12.5 cm would ensure that soil analyzed would have, at the very least, been affected by fertilizer solubilization. Additionally, sampling of the rhizosphere during the first few weeks of potato root development, instead of after eight weeks, is crucial as microbial inoculants applied at seeding are most likely to be observed in the rhizosphere during early root development and may disappear rapidly as the plant develops the ability to modulate its root-associated microbial community. Finally, the removal of relic DNA from soil samples would help by removing unwanted DNA during extraction and provide a clearer assessment of microbial inoculant effects.

The combination of a viability test of inoculant coated onto fertilizer conducted during seeding, proper soil and root sampling times and location, and the removal of relic DNA would allow a thorough assessment of inoculants fate through time and determine if it enhanced plant/crop growth.

Table 4. 1 Chemical properties of soil for all soil sampling dates (n=4)

Soil sampling date	Treatment	CEC	OM	pH	Buffer pH	P	Al	K	Mg	Ca	Zn	Mn	Cu	Fe	B
		meq $100g^{-1}$	%			Mg kg^{-1}	Mg kg^{-1}	Mg kg^{-1}	Mg kg^{-1}	Mg kg^{-1}	Mg kg^{-1}	Mg kg^{-1}	Mg kg^{-1}	Mg kg^{-1}	Mg kg^{-1}
May 27	Control	5.2	2.025	5.375	6.5	825.5	1837.5	168.75	78.25	550	7.125	16.75	2.4	209.5	0.4425
	Éra Boost	5.55	1.975	5.325	6.475	792.5	1830	196.75	92.25	600	7.6	16	2.5	195.25	0.4025
	EVL Coating	5.775	2.1	5.2	6.4	782.25	1812.5	204	85.5	550	7.475	16.75	2.45	197.5	0.3925
June 7	Control	7.2	2.05	4.925	6.225	962.25	1772.5	190.5	130.75	725	9.125	20.25	2.875	210	0.4525
	Éra Boost	5.1	1.975	5.4	6.55	779.25	1832.5	171.5	87.5	600	7.35	16.5	2.525	198	0.41
	EVL Coating	5.275	1.9	5.325	6.5	701.75	1840	164.5	80	575	6.75	14.75	2.425	188.5	0.37
June 21	Control	5.675	1.975	5.2	6.425	700.5	1837.5	192	80	575	7.1	15	2.5	186.25	0.4025
	Éra Boost	7.55	2.1	4.975	6.2	907.75	1775	240.25	151.25	750	7.775	21	2.95	206	0.4475
	EVL Coating	5.525	2.125	5.35	6.475	809	1842.5	174.25	85.75	625	7.125	16.25	2.5	207	0.425
July 26	Control	5.475	2.025	5.45	6.5	733.25	1825	322	93	575	7.4	18	2.475	197.5	0.4225
	Éra Boost	5.675	2.15	5.2	6.425	771.25	1817.5	193.75	79	575	7.4	15.75	2.725	202.5	0.4725
	EVL Coating	7.25	2.175	5.025	6.225	924.75	1760	192	132	750	8.725	19.25	2.975	206.75	0.4325

Table 4.2: Tuber yield in sampled plots and the overall mean of all harvested plots (n = 7) blocks not sampled for their soil and roots are not shown, but are included in treatment means.

Treatment	**Block**	**Tuber yield** / Mg ha^{-1}
Control	1	49.32
	2	59.59
	3	55.14
	4	59.59
	Mean	53.57±2.48
Éra boost	1	49.32
	2	58.90
	3	57.53
	4	47.50
	Mean	52.98±2.48
EVL Coating	1	52.74
	2	55.82
	3	59.90
	4	55.48
	Mean	52.94±2.4822

Table 4. 3: Alpha-diversity measurements for all samples grouped by tissue type and extraction method PBS: Phosphate-buffered saline; PMA: Propidium mono-azide; AU: Amplification units

Sample type	Date	Treatment	Shannon	Chao1	Evenness	Observed ASV	log(AU)
			Prokaryotes				
Soil	May 27	Control	5.915163	575.6218	0.93403	564	9.559764
	May 27	EVL	5.960742	596.4814	0.935371	586	9.581787
	May 27	Era Boost	5.955646	600.483	0.934385	586.75	9.568863
	June 7	Control	5.966598	607.8344	0.93389	595.75	9.525545
	June 7	EVL	6.000563	634.8419	0.933494	619	9.554599
	June 7	Era Boost	5.937789	597.7586	0.932445	583.25	9.516707
	June 21	Control	6.030225	646.2383	0.935043	632.75	9.557091
	June 21	EVL	5.998486	636.2008	0.933348	619.75	9.560179
	June 21	Era Boost	6.004566	641.5222	0.932478	626.5	9.461811
	July 26	Control	5.996197	644.1263	0.930892	628	9.584194
	July 26	EVL	6.014049	652.2131	0.931217	638	9.578724
	July 26	Era Boost	5.894099	593.9126	0.926498	579.5	9.543526
Roots	July 26	Control	3.955185	246.3299	0.718955	243.5	10.13304
	July 26	EVL	3.96585	239.7864	0.725782	236.5	9.997353
	July 26	Era Boost	4.111531	245.9417	0.748302	243	10.13701
Soil + PBS	June 7	Control	6.224377	825.9453	0.928479	816.25	7.544986
	June 7	EVL	6.247637	845.2437	0.92853	837	7.510014
Soil + PBS + PMA	June 7	Control	5.927118	528.9712	0.946811	528	6.968157
	June 7	EVL	6.00383	599	0.940899	595	7.092024
			Fungi				
Soil	May 27	Control	4.612076	265.1049	0.829528	262	7.461886
	May 27	EVL	4.903385	316.5836	0.854429	314	7.433783
	May 27	Era Boost	5.043333	302.0866	0.885619	298	7.490462
	June 7	Control	4.934525	292.2828	0.870807	289.75	7.413932
	June 7	EVL	4.906701	276.1255	0.87526	274.3333	7.551113
	June 7	Era Boost	4.803848	276.8295	0.85592	275.5	7.55152
	June 21	Control	4.845417	281.1987	0.860997	278	7.483912
	June 21	EVL	4.924463	319.5682	0.856175	315.25	7.432601
	June 21	Era Boost	5.085527	315.8651	0.885403	312.75	7.413578
	July 26	Control	4.494773	229.7807	0.83064	228.5	7.672568
	July 26	EVL	4.525951	230.7003	0.832761	229.75	7.629515
	July 26	Era Boost	4.275442	210.5881	0.801276	209.25	7.548529
Roots	July 26	Control	4.236626	166.1083	0.829014	165.5	7.87477
	July 26	EVL	4.07541	154.9	0.808679	154.75	7.706849
	July 26	Era Boost	4.453727	186.4821	0.854156	184.25	7.96453
Soil + PBS	June 7	Control	4.468065	170.25	0.86972	170.25	5.146972
	June 7	EVL	4.601419	169.75	0.896492	169.75	5.236798
Soil + PBS + PMA	June 7	Control	4.054878	131.625	0.831303	131.5	4.71968
	June 7	EVL	3.7115	123.75	0.772185	122.75	4.692992

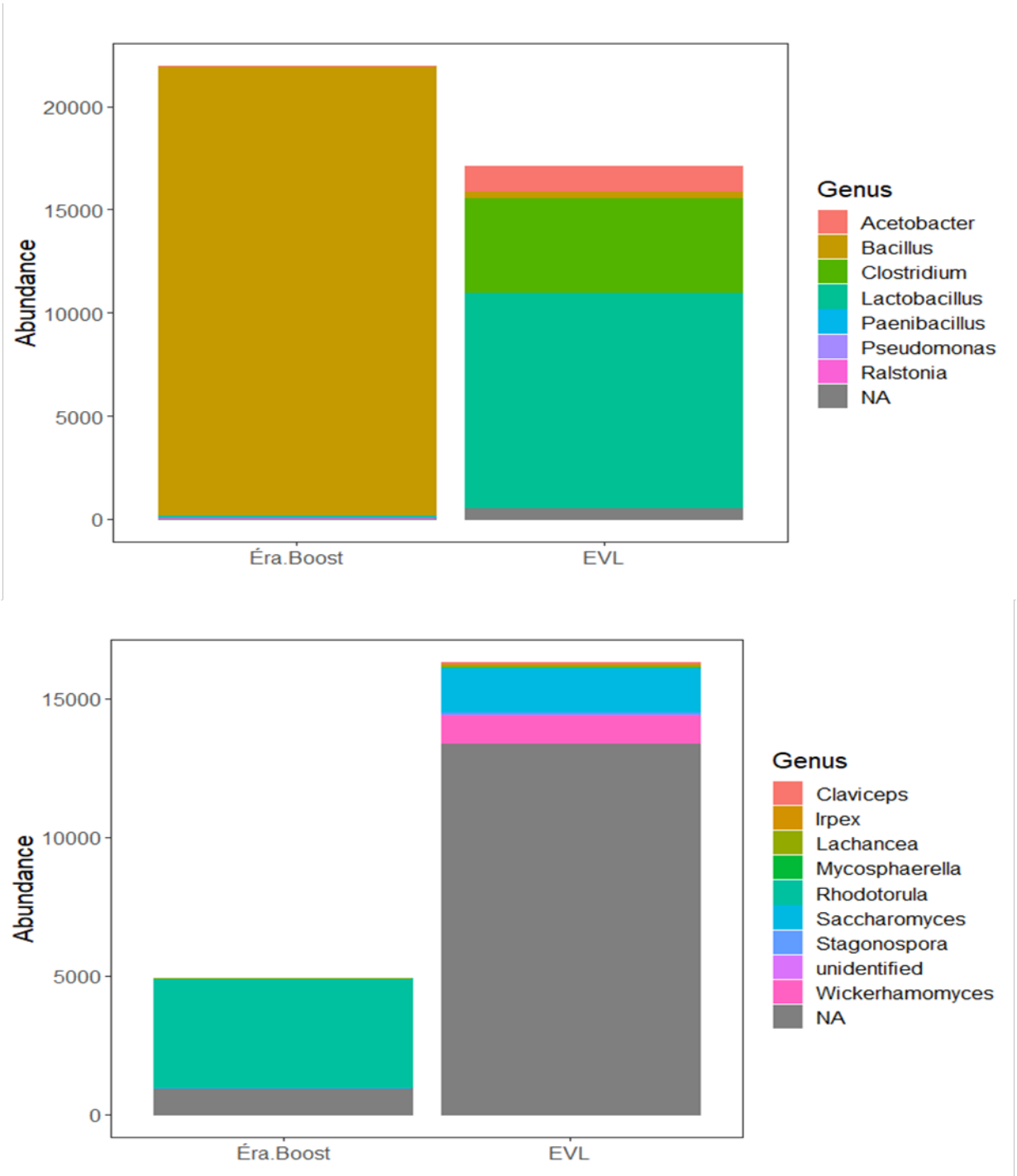


Figure 4.1: Abundance of prokaryotic and fungal genera in Éra Boost and EVL coating microbial inoculants. The top panel represents prokaryotic abundance, the bottom panel represents fungal abundance. NA represents ASV that could not be identified at the genus level.

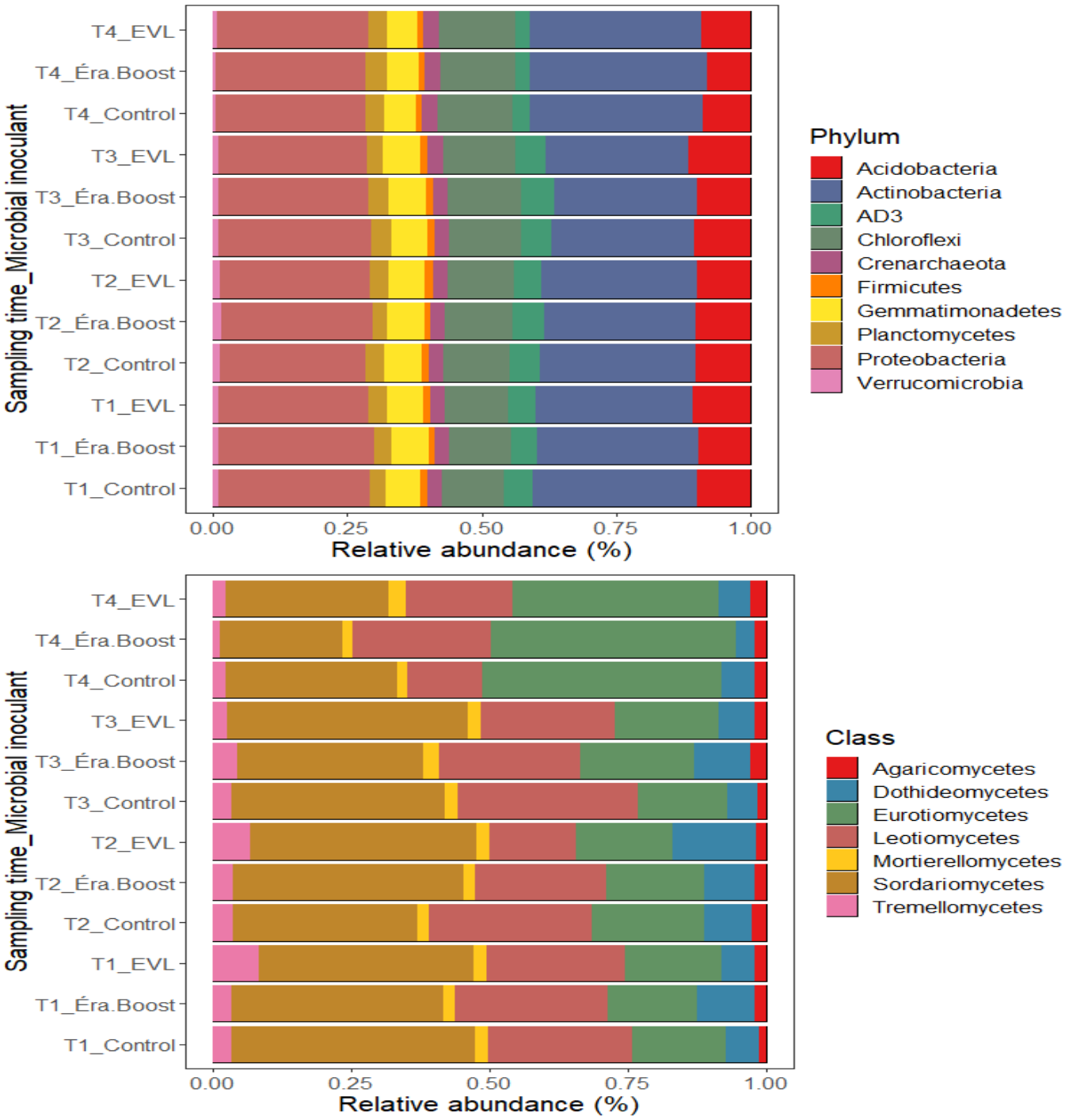


Figure 4.2: Relative abundance of prokaryote phyla and fungal classes in soil samples over time. T1: May 27th; T2: June 7th; T3: June 21st; T4: July 26th. Top panel represents prokaryotic relative abundance, bottom panel represents fungal relative abundance.

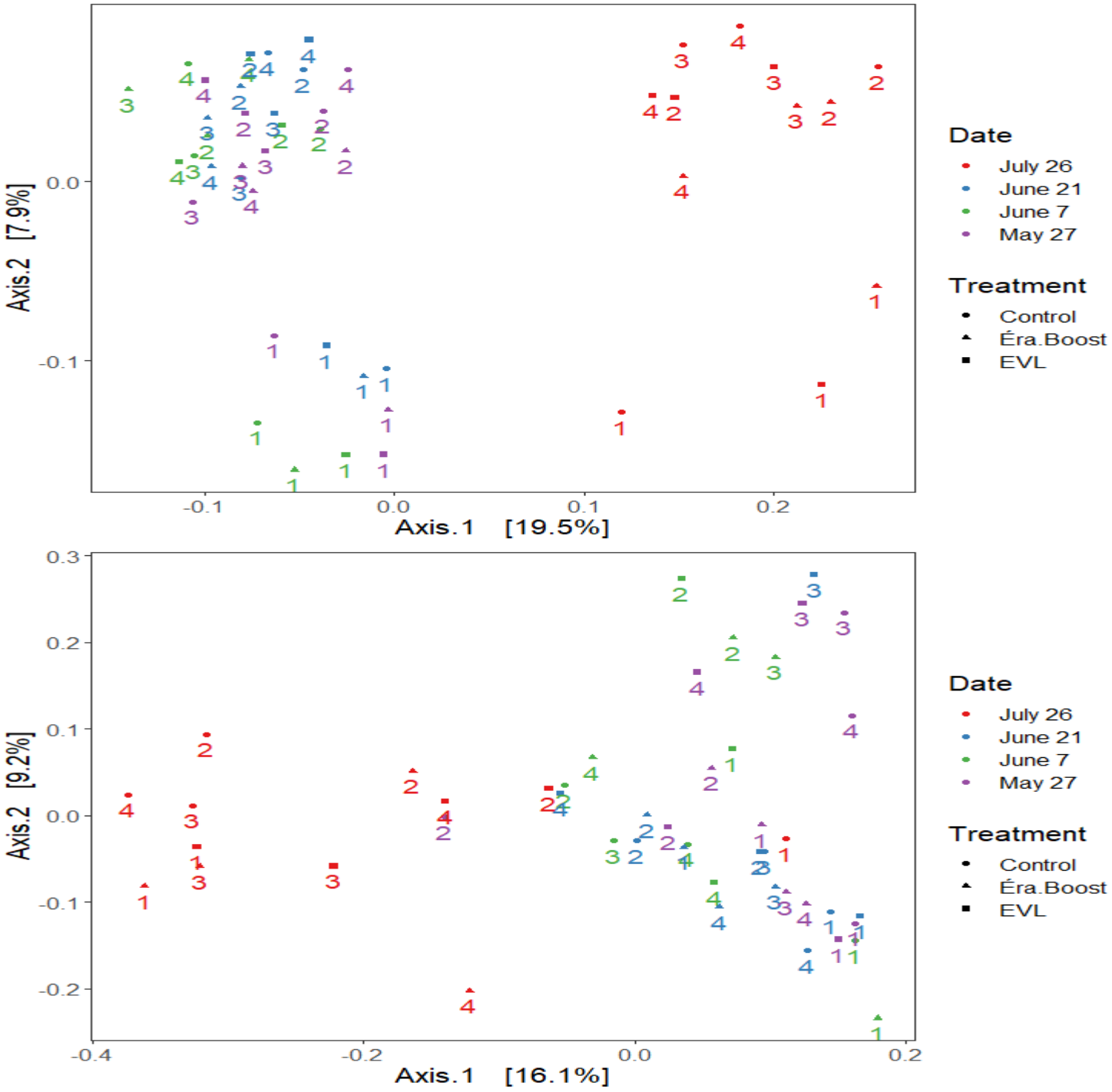


Figure 4.3: Principal coordinates analysis of prokaryotic and fungal communities in bulk soil samples. Blocks are represented as numerical subscripts. The top panel represents prokaryotic communities, the bottom panel represents fungal communities. PCoAs were based on Bray-Curtis distance at the ASV level.

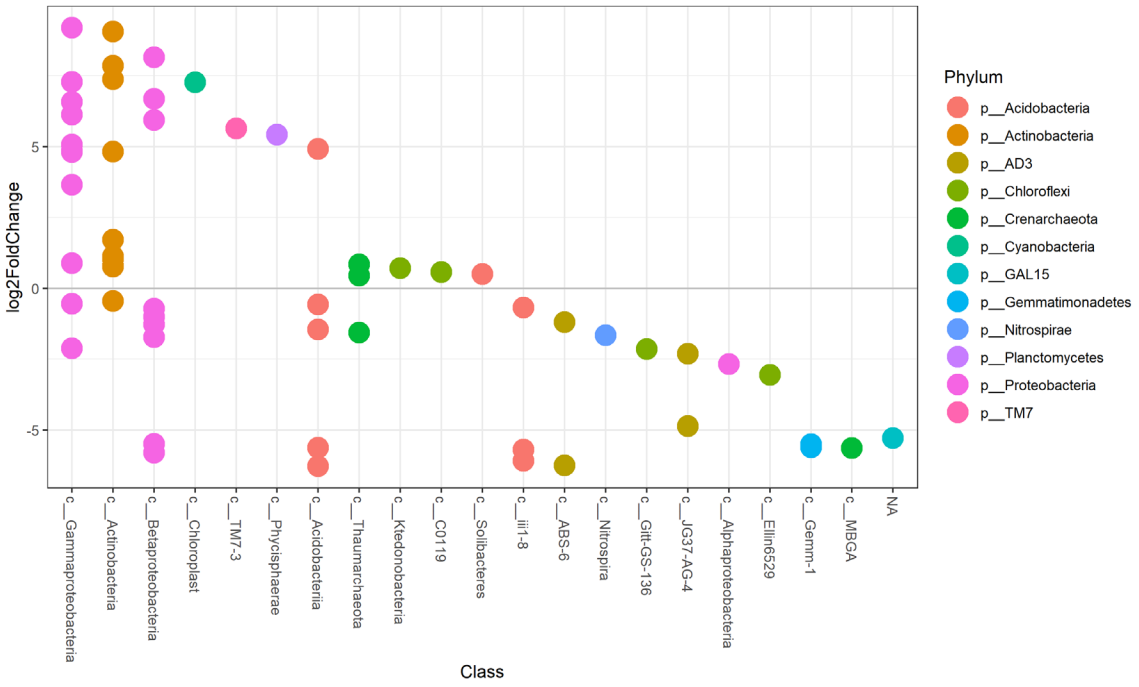


Figure 4.4: Prokaryotic ASVs differentially expressed (P<0.01) between June 21st and July 26th. Each point represents and ASV which relative abundance significantly changed between June 21st and July 26th, positive log2FoldChange numbers means the ASV abundance increased while negative numbers means the ASV abundance decreased

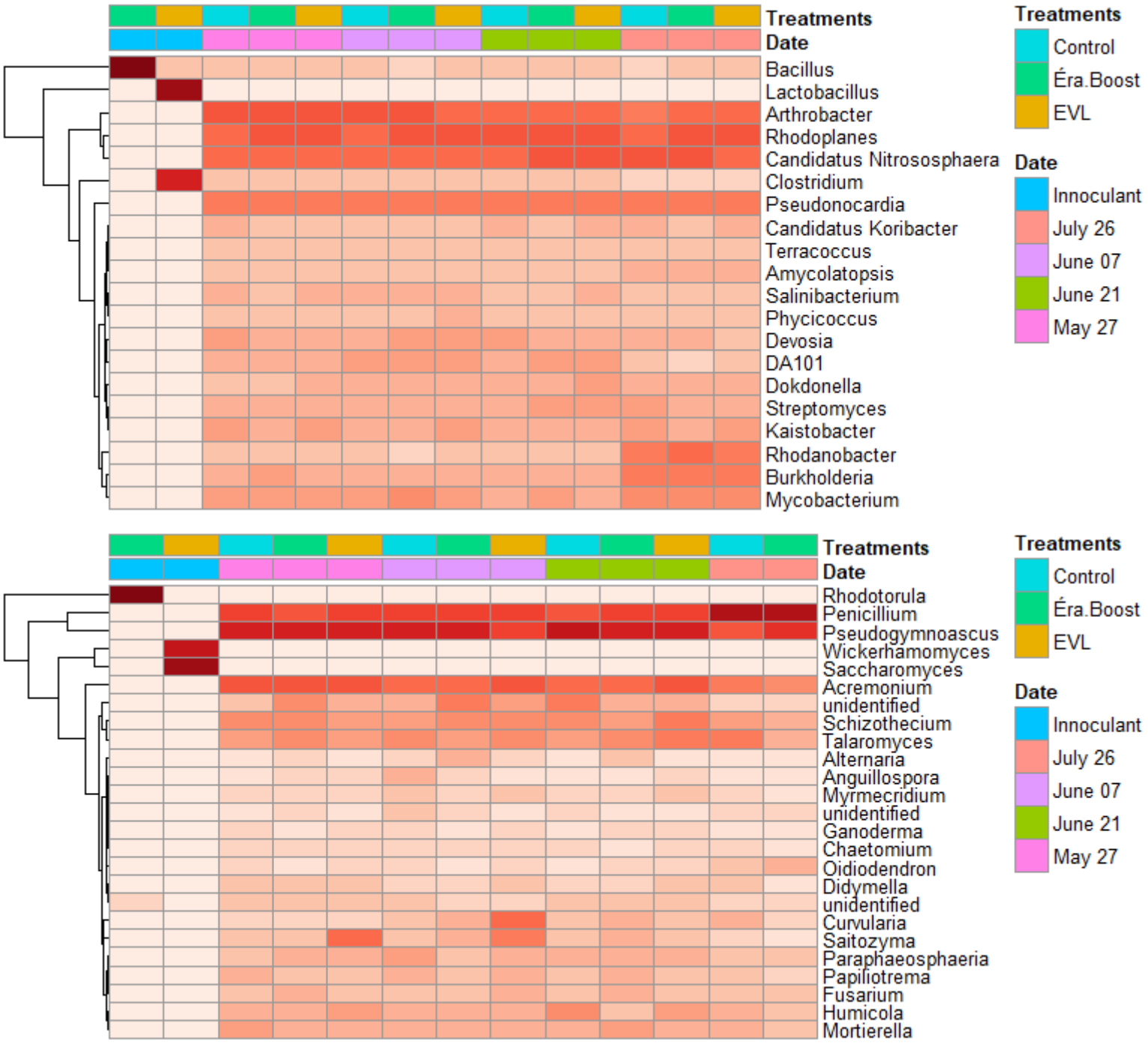
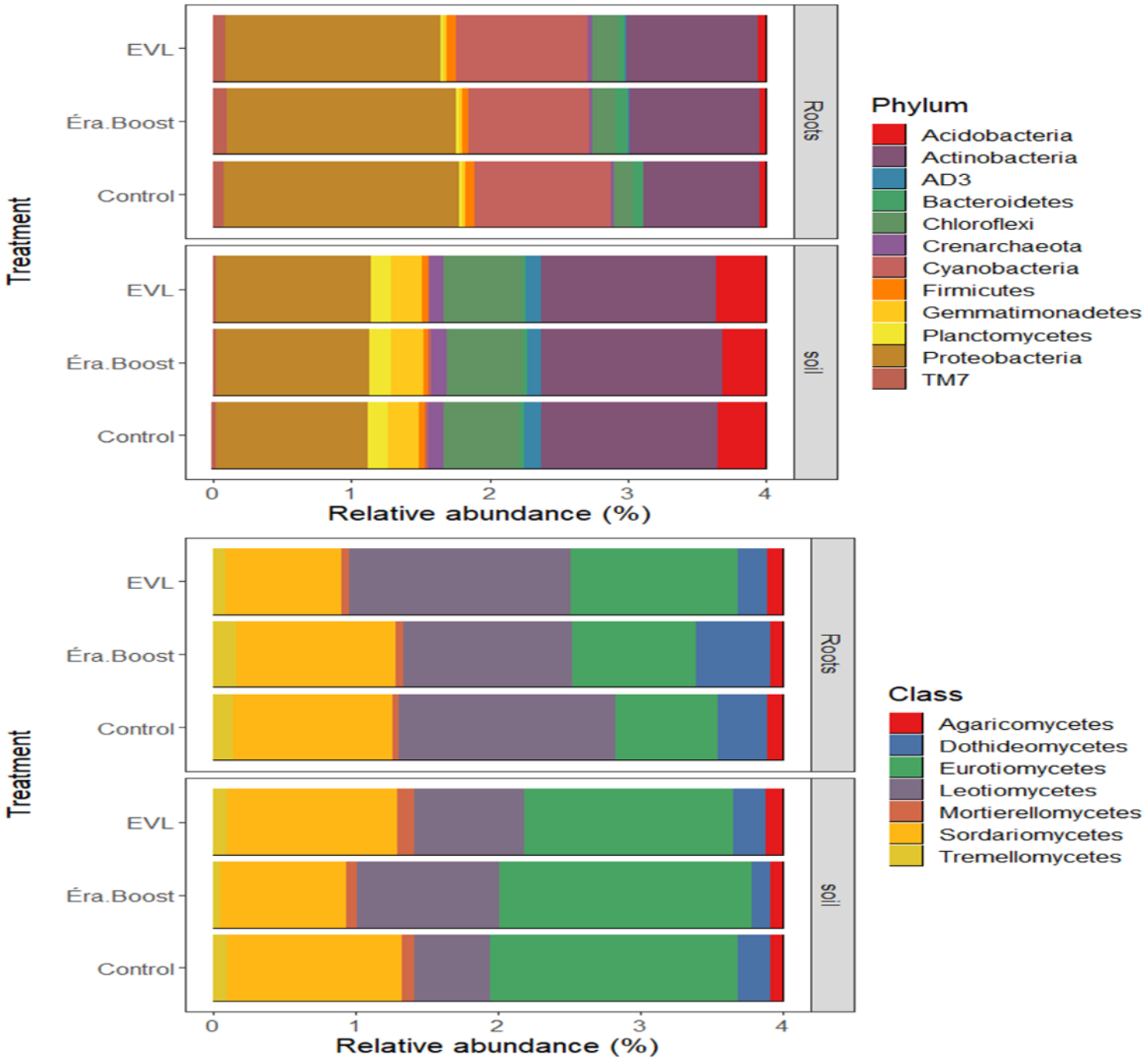


Figure 4. 5: Heat map of the 20 most abundant prokaryotic and fungal genera for all treatments in the first three sampling periods. Top panel represent



prokaryotic abundance, bottom panel represents fungal abundance.**Figure 4.6: Relative abundance of prokaryotic phyla and fungal classes with more than 1 % relative abundance for roots and soil samples collected on July 26th.** The top panel represent prokaryotic relative abundance, the bottom panel represents fungal relative abundance.

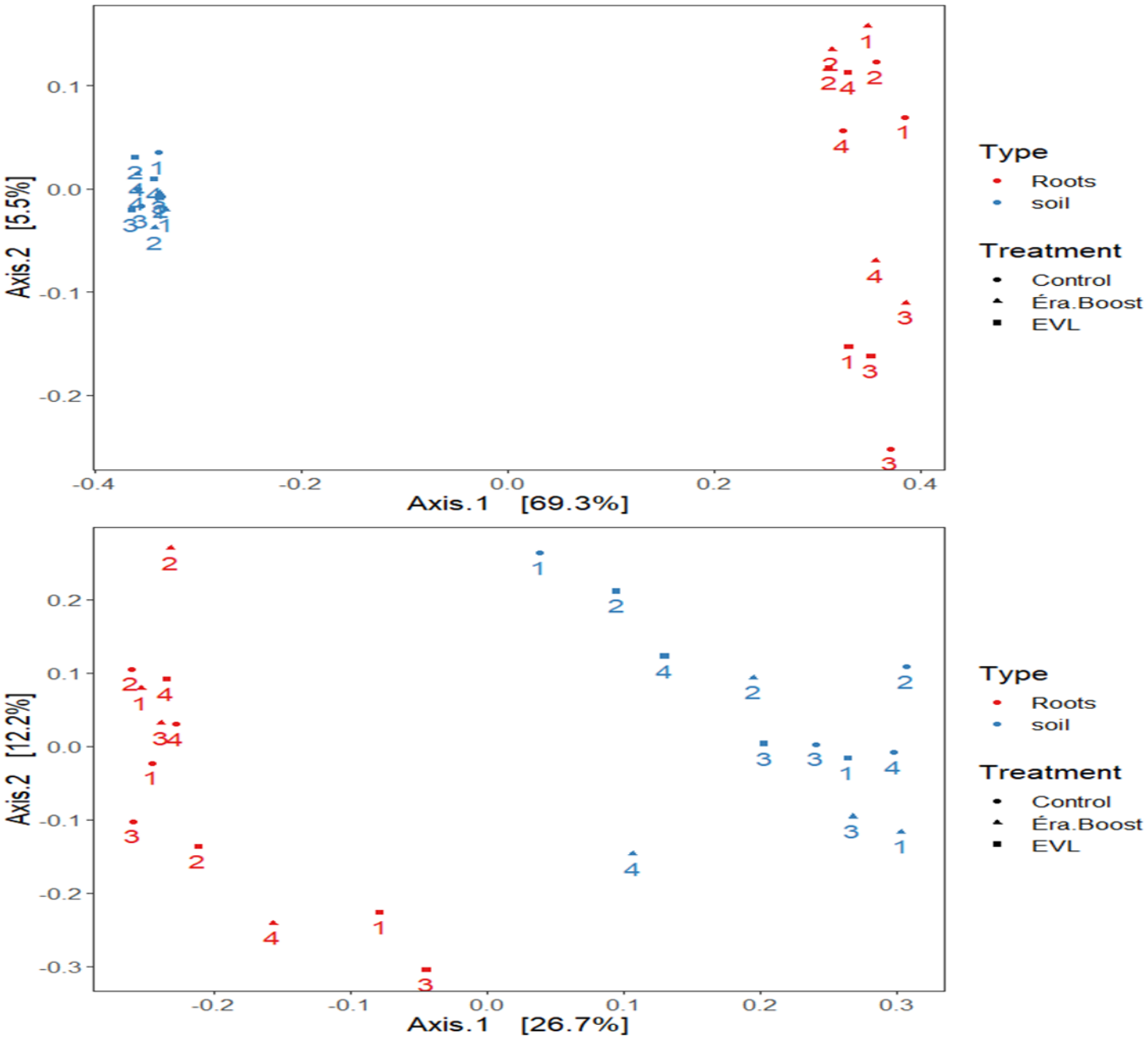


Figure 4.7: Principal coordinate analysis of root and soil samples collected on July 26. Blocks are represented as numerical subscripts. The top panel represents prokaryotic communities, the bottom panel represents fungal communities. PCoAs were based on Bray-Curtis distance at the ASV level.

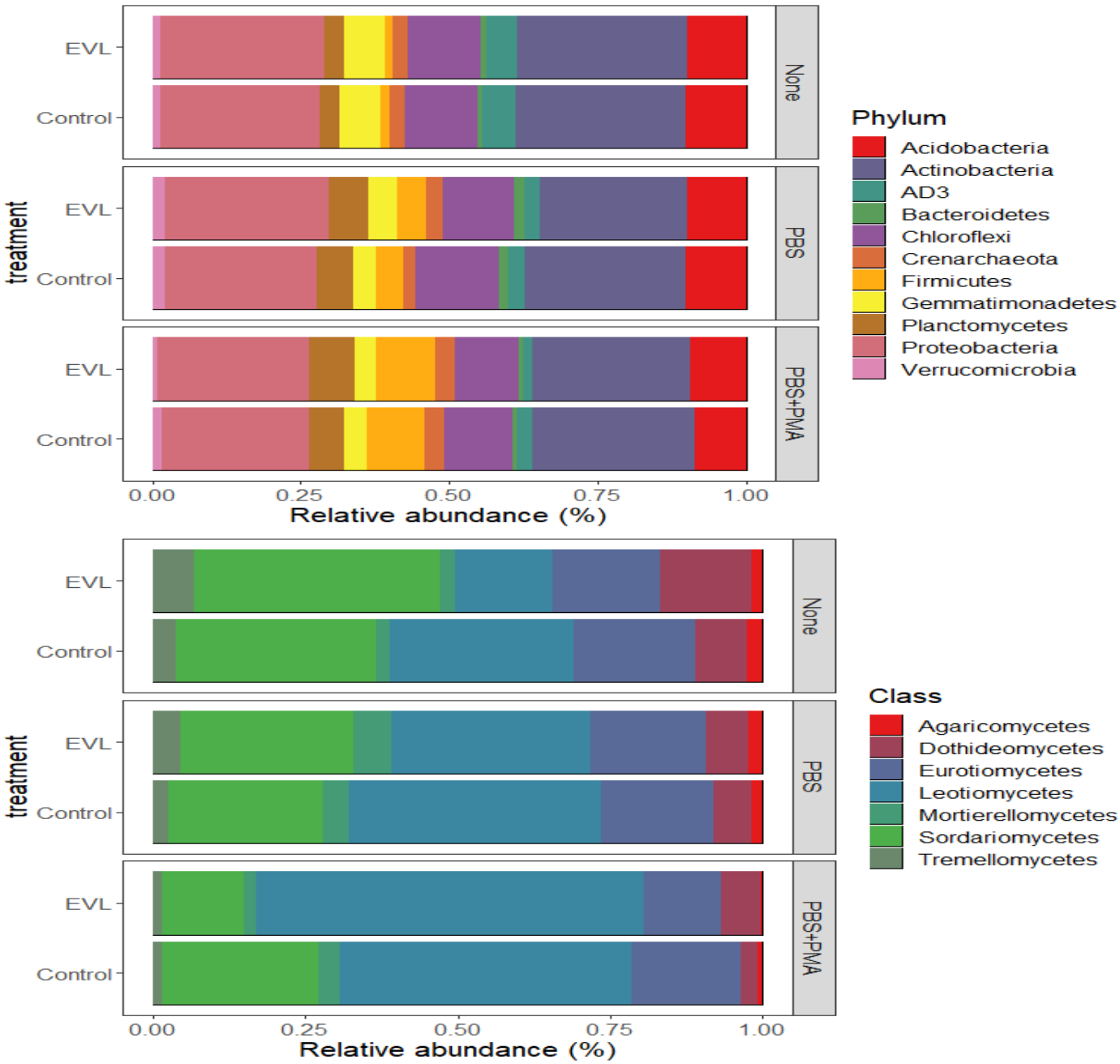


Figure 4.8: Relative abundance of prokaryote phyla and fungal classes in soil samples isolated with three pre-extraction solutions. None: no buffer added to soil; PBS: Phosphate buffer added to soil; PBS+PMA: Phosphate buffer and propidium-monoazide added to soil. The top panel represents prokaryotic relative abundance, the bottom panel represents fungal relative abundance.

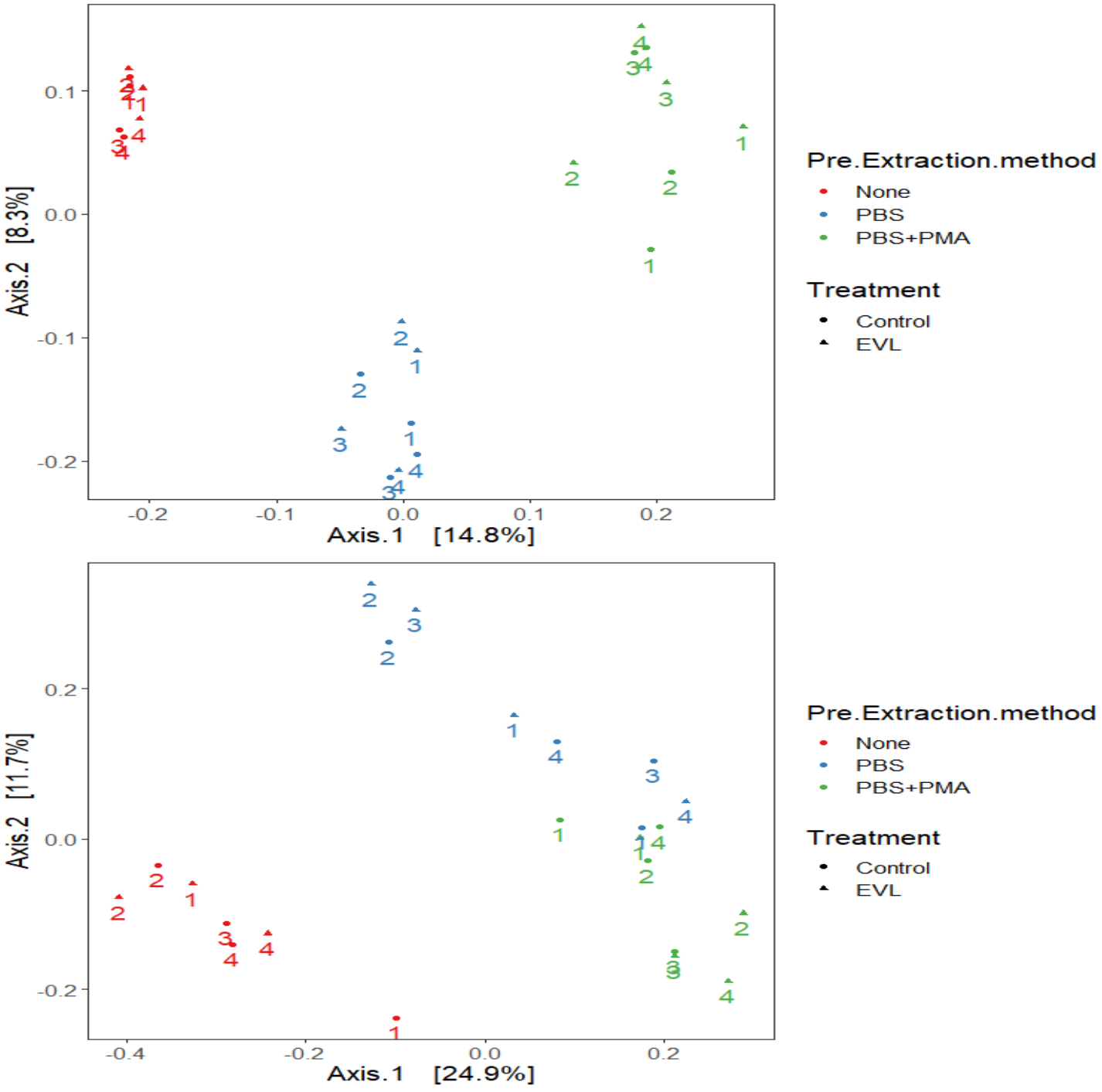


Figure 4.9: Principal coordinate analysis of soil samples extracted with a range of pre-extraction solutions. Blocks are represented as numerical subscripts. The top panel represents prokaryotic communities, the bottom panel represents fungal communities. PCoAs were based on Bray-Curtis distance at the ASV level.

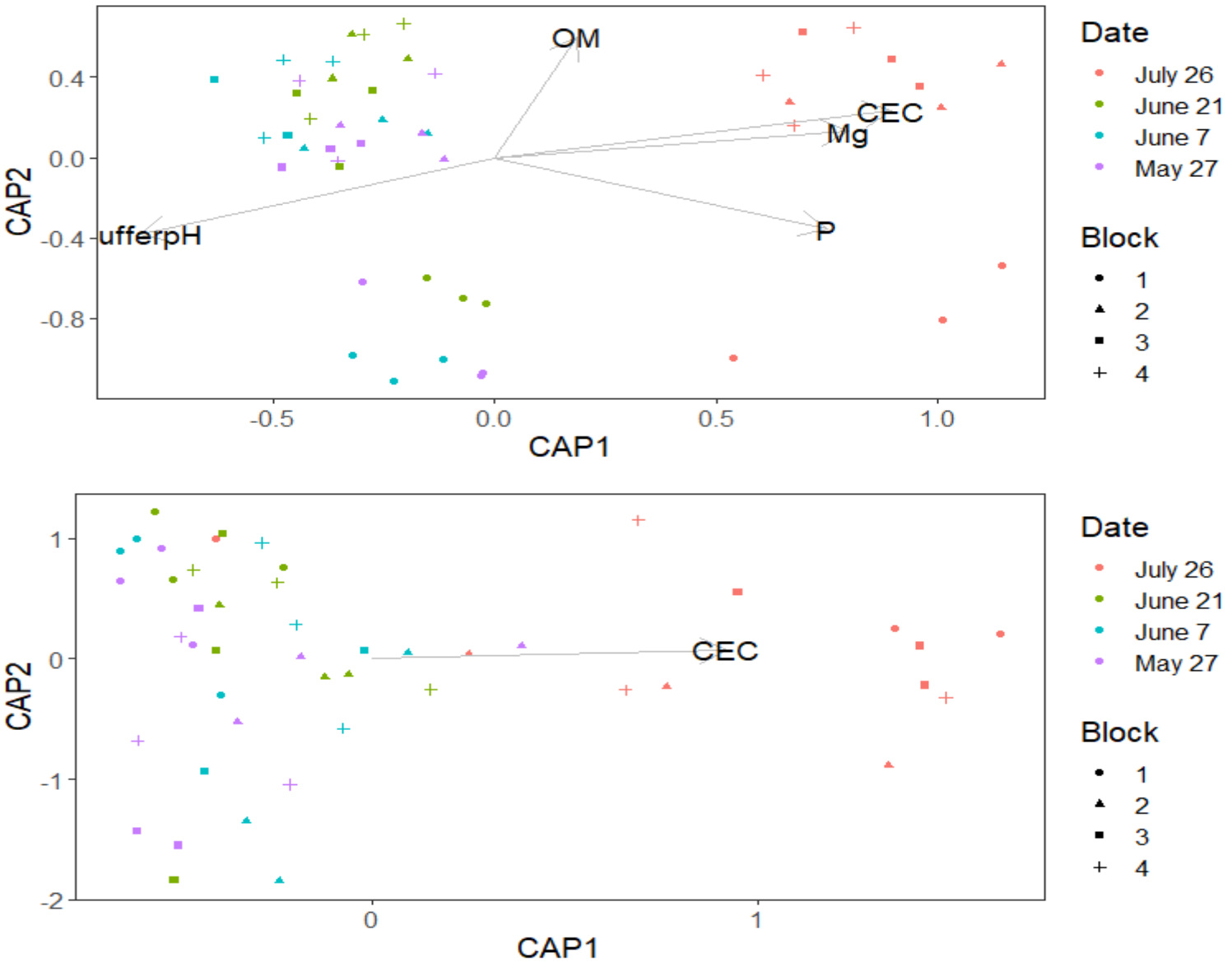


Figure 4.10 : Distance-based redundancy analysis (dbRDA) of prokaryotic and fungal community compositions with significantly correlated soil variables shown as vectors. Correlations between vectors and prokaryotic or fungal communities were tested using the function "capscale" from the "vegan" library of the R a package. Vector sizes are proportionate to the correlation between the soil parameter and prokaryotic or fungal communities' composition. The top panel represents prokaryotic communities, the bottom panel represents fungal communities.

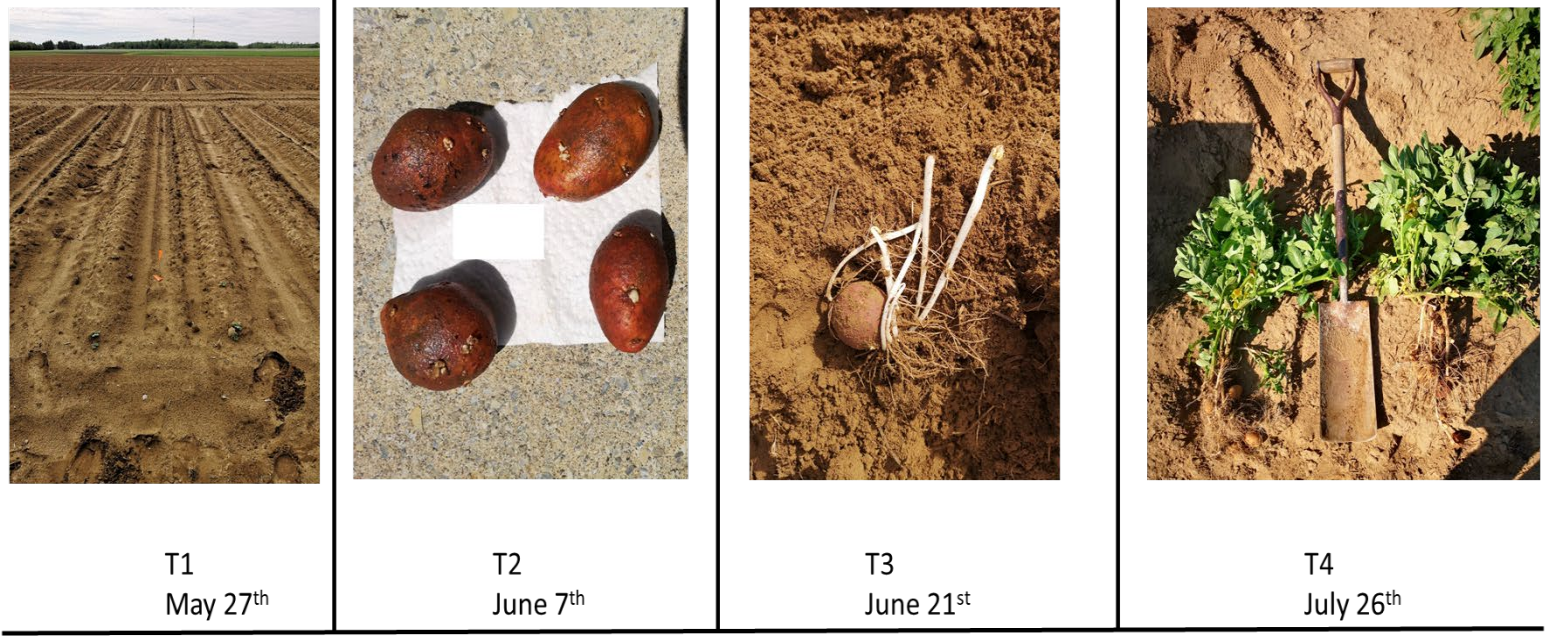


Figure 4.11: Potato growth stages during soil sampling. At T1, tubers were not yet seeded; at T2, radicles had started to emerge from seed tubers; at T3, small but well established root systems were present with no above-ground biomass; at T4 plants were at the mid-flowering stage.

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Chapter 5: General discussion and conclusions

Microbial inoculation of crops to increase crop nutrient assimilation is commonly used in agriculture. The most important inoculant is *Rhizobium* spp., which is inoculated onto legume crops to ensure biological nitrogen fixation in root nodules. More recently, other genera of bacteria, such as *Azospirillum, Bacillus*, *Lactobacillus* and *Pseudomonas* and mycorrhizal fungi have been studied as possible plant growth promoters, with varying degrees of success. When successful, microbial inoculation can reduce plant fertilizer requirements. Nitrogen fertilizer comes from energy-intensive processes and often uses non-renewable forms of energy, while other mineral fertilizers, such as P and K, are mined from non-renewable stocks. Therefore, sustainable agricultural practices aim to limit crop fertilizer requirements by 1) providing plants with access to nutrients already present in the soil in plant-available forms or 2) by providing access to unavailable forms of plant nutrients in soil *via* transformation by beneficial soil microbes; the latter has the potential to be fulfilled using microbial inoculants.

The potential of microbial inoculants to boost plant growth is not always achieved under field conditions. This lack of consistent effect has puzzled soil scientists, because there are a multitude of possible causes, such as environmental conditions, edaphic conditions, the native soil microbial community, the timing of inoculation, etc. The type of carrier used to apply the microbial inoculant shapes the effects of the inoculant. Usual carriers such as peat are often broadcast, requiring large quantity of inoculants to produce an effect on the desired crop. Seed inoculation of microbes ensures the presence of beneficial microbes on the emerging radicle and lowers the amount of inoculant required. Banded fertilizer is placed strategically so that it is easily accessible to the emerging plant root systems, which makes GSF an appropriate microbial carrier for row crop production systems. The general goal of this research was to test the benefits of microbial inoculants when coated on GSF, compared to uninoculated GSF. This study is the first to provide academic results on the efficacy of GSF coated with two commercial microbial inoculants to affect plant growth and the soil microbial community.

Revisiting hypotheses

The first objective of this study was to assess the viability of microbial cells following the application of ÉB and EVL inoculants onto GSF. Based on our results, inoculated GSF had viable cells on its surface, 1 h following inoculation, with colonies identical to those from the pure microbial inoculants. For ÉB, CAN and MES fertilizers had significantly more viable cells than potash fertilizer, while uninoculated fertilizer had no culturable cells. Due to the COVID-19 lockdown, no quantification was conducted for EVL-inoculated materials and no test was performed to quantify the viability of coated fertilizer several days following inoculation.

The second objective of this research was to assess corn and potato performance when GSF was coated with ÉB or EVL consortia under field conditions in 2018 and 2019. For both crops, yield was increased for most treatments one year out of two; the inoculant had positive effects on potato in 2018 and in 2019 for corn. Potato yield was increased by up to 27.9 % for the best performing treatment (ÉB 1x), although, the difference in tuber yield was not statistically significant. The high variability within blocks in both years resulted in few significant differences between potato treatments. Corn yield increased up to 20.2 % for the best performing treatment (ÉB 2x). Corn 100-seed weight was significantly increased in both years for both microbial inoculants. The differences between 2018 and 2019 results are likely due to the very marked differences in weather conditions resulting in different seeding dates and different CHU accumulation between years.

In the final objective, soil prokaryotic and fungal community compositions were assessed before and after the application of GSF coated with microbial inoculants. We hypothesized that microbial inoculants would affect the diversity and composition of the soil microbial community. Results showed that microbial inoculation did not affect bulk soil microbial communities one, three or eight weeks following inoculation. Eight weeks after seeding, root-associated microbial communities were not affected by microbial inoculation. Sampling date and block had significant effects on microbial communities. Microbial community changes due to sampling date were associated with pesticide and tillage application, both of which are known to alter microbial communities. Changes due

to block effects were associated with soil organic matter content, phosphorus content and all micronutrients contents.

Future directions

Future research in this area should target a more localized elements of the soil profile to assess the dispersal of GSF coated with microbial inoculants. Soil samples could be divided by soil layers centralized around the fertilizer depth, usually at 10 cm (e.g. 8-9, 9-11 and 11-12 cm layers). Root-associated soil communities should be analyzed much earlier in the growing season, ideally two or three weeks after seeding. In combination, these sampling strategies would target the precise locations where we expect the inoculum to be present, and therefore result in data collection in regions where changes are likely to be most detectable. To ensure inoculant quality, a portion of the fertilizer coated with microbes, should be assayed for viable cells on agar plates. Without this test, it is impossible to know if the lack of observed inoculant effects is due to field conditions or due to handling of coated fertilizer, leading to reduced microbial viability. The combination of localized soil testing and a test of coated fertilizer microbial viability immediately prior to seeding should allow for inoculant tracking through each region where microbial presence is expected, starting from the fertilizer granules, to bulk soil surrounding the fertilizer, and finally around roots of the emerging crop.

The application of beneficial microbes at seeding is gaining popularity in row crop production, as many seeders can band fertilizer or apply small quantities of liquid in-furrow, both of which are viable options for application of microbial inoculant products during seeding. Although many inoculants are currently on the market, an ideal inoculant should be adapted to the environment of its intended use; this would require a huge variety of products due to the heterogeneous nature of soils that occur even in a relatively small area. In Southern Quebec, our seeding and establishment season is characterized by soil temperatures below 20 °C. The minimum temperature for corn germination is 10 °C, however many microbes will not establish competitively at such low temperatures. Therefore, I suggest that microbial inoculants used at seeding in our province should be adapted to grow vigorously at low temperature (10 °C -15 °C) in order to provide beneficial

functions to plants. Future screening of inoculants should have such a temperature criterion, to further improve the local value of the next generation of inoculants.

Final words

As a final word, agricultural practices have changed tremendously over the past century with the discovery of chemical fertilizers, the development of synthetic pesticides, and the exponential rise of mechanical and technological advancements which together have revolutionized the average farm. These changes have led to an increase in agricultural productivity, allowing for a massive increase in the global human population. However, many of these discoveries came with drawbacks. Fertilizer use perturbs nutrient cycles, pesticides contaminate surrounding environments, 400 HP tractors compact the soil deeper than any tillage machine can plow, and so on. Agriculture today is now faced with a dilemma: how can it benefit from all these recent discoveries while limiting their potential negative consequences? The use of microbial inoculants to improve crop production and reduce fertilizer inputs is part of the solution. Yet, not enough is known about the capacity to leverage the benefits of plant-microbe interaction to produce consistent yield increases with limited use of less desirable inputs. While we have made progress in our understanding of plant-microbe interactions, techniques to improve soil health – such as the use of organic fertilizers, cover crops, maintaining living roots in soil at all times, limiting tillage, and reducing tractor passes within fields – should be the aims of all farms. These practices will ensure the resiliency of soils as increasingly frequent extreme environmental events associated with climate change challenge crop production over the coming years of this century.

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