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MICROBIAL RECRUITMENT AS A WAY TO IMPROVE DROUGHT RESISTANCE OF WHEAT PLANTS

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RÉSUMÉ

Une augmentation de la fréquence des événements de sècheresse est à prévoir en raison des changements climatiques et il serait bénéfique pour le secteur agricole de trouver des méthodes novatrices afin de rapidement venir en aide aux plants en manque d'eau. Une solution possible serait, par exemple, l'inoculation de communauté microbienne complexe dans la rhizosphère de la plante. Le but du projet était de tester, au moyen d'une expérience multifactorielle menée en serre, si des plants de blé en situation de stress hydrique s'associeraient avec de nouveaux microorganismes extraits d'un sol avec un historique d'exposition à la sècheresse. L'amplification et le séquençage du gène bactérien ARNr 16S et de la région fongique ITS1 de l'ADN environnemental extrait du sol de la rhizosphère des plants a permis d'observer les changements rapides dans la communauté microbienne causés par le stress hydrique. Quelques UTO fongiques furent recrutés par la plante, indépendamment du type de sol ou de la présence d'un stress hydrique. Règle générale, les changements dans la communauté microbienne de la rhizosphère étaient plus au niveau de l'amplification et ou la réduction d'espèces déjà présentes plutôt que le recrutement de nouvelles espèces. En ce sens, les plants ont fortement réagi au type de sol initial et au stress hydrique mais de façon plutôt faible et inconsistante à l'inoculation. Ces résultats illustrent que des plants de blé en condition de sècheresse peuvent former de nouvelles associations avec quelques bactéries et champignons, mais que cette réponse ne représente pas la réponse principale de l'holobiont au stress.

Mot clés : Sècheresse, blé, holobionte, inoculation, séquençage d'amplicon

ABSTRACT

In the context of climate change, finding ways to rapidly adapt crops to abiotic stress would be a great asset for the agricultural sector. One potential solution is to modify the plant associated microorganisms through, for instance, the inoculation of complex pre-adapted microbial communities. Here, using a multifactorial greenhouse experiment, we tested if wheat plants under water stress would associate with the microorganisms extracted from a soil that had a long-term history of exposure to water stress. Through bacterial 16S rRNA gene and fungal ITS region amplicon sequencing we observed that the rhizosphere microbiota responded rapidly to the water stress. A few fungal OTUs were recruited, independently of the soil type or the water stress. Generally, changes in the microbial community structure across inoculum treatments were more due amplification/reduction of already present microorganisms rather than recruitment of novel species. Similarly, the plant responded strongly to water stress and to initial soil diversity, but only weakly and inconsistently to the inoculations. Our results highlight that wheat plants under water stress do form new associations with fungi and bacteria, and that these new associations do not constitute the bulk of the response of the wheat holobiont.

Keywords: drought, wheat, holobiont, inoculation, amplicon sequencing

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THE PLANT HOLOBIONT UNDER DROUGHT

1.1 The holobiont concept

Center to the science of ecology is the idea that all life form within an environment interact with each other. This was first theorized by Karl Möbius in 1877, who named all the interactions and interdependences between the different organisms of a same niche the biocenosis concept (Bosch & McFall-Ngai, 2011; Glaubrecht, 2008). The thought was pushed further by the entomologist William Morton Wheeler in 1911 who studied the closely knitted ant colonies. This intra-species community was described as a superorganism by the myrmecologist (Gordon, 2013). The concept was disregarded for decades before it made a comeback. In her 1991 book, Lynn Margulis who was interested in cases of symbiosis involving individuals of different species was the first to use the term holobiont (Wenseleers, 2009). She described the holobiont as the tight interspecies associations between individuals (or bionts) forming an entity, with *holos* meaning whole in Greek (Bordenstein & Theis, 2015). It is the coral-zooxanthella algae-microbes symbiosis that was one of the first to be described as a holobiont (Gordon, 2013). Since then, the holobiont concept is used to describe a host and all its associated microbial community that are either obligate or facultative symbionts and that have either a harmful, neutral or beneficial interaction with the host (Theis et al., 2016). The holobiont concept has created mind shifts in many life science fields and with the blooming of microbial ecology, we now know that microorganisms are essential for the fitness of many organisms (Rosenberg & Zilber-Rosenberg, 2016).

Some scientists also took the holobiont concept and pushed it one step further, enunciating the hologenome theory of evolution, in which the holobiont is described as an evolutive unit and the genetic information of all members of the holobiont forms a whole, the hologenome (Zilber-Rosenberg & Rosenberg, 2008). This implies that modifications of the hologenome can lead to phenotypical modifications of the holobiont (Bordenstein & Theis, 2015). Consequently, when trying to rapidly modify a plant holobiont, for example,

the microbiome (genomes of the microbiota) could be targeted (e.g. Shlaeppi & Bulgarelli, 2015; Vandenkoornhuyse et al., 2015). Zilber-Rosenberg & Rosenberg (2008) stated three ways that the microbiome component of a hologenome could be modified: either by amplifying or reducing the abundance of already present microbial species, by recruiting new microbial species, or by recruiting new microbial genes through horizontal gene transfer.

In plant science, many scientists have argued in favor of the holobiont as an ecologically sound concept (e.g. Hacquard, 2016; Hassani et al., 2018; Nogales et al., 2016; Theis et al., 2016). Still, the holobiont approach challenges our understanding of biology and the hologenome theory is debated and not unanimously adopted within the scientific community. As an example, some argue that considering the hologenome as an evolutive entity overstates the importance of co-evolution between a host and its symbionts (e.g. Douglas & Werren, 2016; Foster et al., 2017; Moran & Sloan, 2015) and understates the importance of horizontally recruited microbes (Doolittle, 2016). More research is needed to really understand the level of interdependence and co-evolution between a host and its microbiota. For this thesis, the term holobiont is used to describe the plant and its microbiota, without any implied evolutionary meaning.

1.2 The wheat holobiont

Wheat (*Triticum aestivum*) is one of the pillar crops in today's food system and accounts for about 20% of the world protein consumption (Shewry, 2009). In 2016, wheat production in Canada reached 31.7 million tons, a 15% increase compared to the previous year (Statistics Canada, 2016). The wild ancestors of wheat originated from Turkey 10,000 years ago and had either diploid or hexaploid genomes. Now, about 95% of the cultivated wheat world-wide is hexaploid (Shewry, 2009). Domestication of wheat was mainly driven by the mutation of two essential traits: non-dispersion of the seeds and free-hulled forms of grain (Heun et al., 1997). Since then, many generations of breeding selection have created the high-yielding different types of wheat cultivars that we know today. The fully annotated genome of a bread wheat species, containing 16 billion base pairs, was published for the first time in 2018 (IWGSC, 2018). The core microbiota of the

wheat holobiont (if there is one) is yet to be unraveled. Multiple studies have tested the effect of different environmental parameters on the taxonomic composition and abundances of wheat microbiota and some of the influencing factors identified were: fertilization (Robinson et al., 2016), field cropping history (Vujavonic et al., 2012), cultivar (Larran et al., 2002), precipitation regime (Bankina et al., 2017), abiotic and biotic stresses and phytohormone production (Liu et al., 2017) as well as growth stage (Qin et al., 2016), type of plant tissue (Vujavonic et al., 2012) and spatial variability in the rhizosphere (Don et al., 2015). This high variability limits the possibility to make general assumptions about which specific microbial species is generally associated to wheat plants. Still, many studies have used *Triticum aestivum* as a model crop to study the plant microbiota.

When looking at the endophytic community associated with the above versus below ground compartments, Robinson and colleagues (2016) found that the *Proteobacteria* phylum was the most prominent one in the roots whereas *Actinobacteria* and *Firmicutes* were the most abundant one in the leaves. A study conducted by Vujavonic et al. (2012) specifically looked at fungal species colonizing the different plant tissues of durum wheat plants and found that with a few exceptions, most species were able to colonize more than one plant organ. They also observed that the most prominent seed fungal endophyte was *Pyrenophora triticirepentis*. Majeed et al. (2015) found that *Erwinia* and *Rhizobiales* bacteria were present in all tested seeds and sprouts and *Acetobacter diazotrophicus* was present in all analysed root endospheres. One study found through isolation techniques that the *Emericella* and *Aspergillus* were the most frequently observed fungal genera in all inner compartments (Huang et al., 2016) whereas another study found that *Alternatia alternata*, *Cladosporium herbarum*, *Epicoccum nigrum* and *Fusarium graminearum* were the most abundant fungal species (Larran et al., 2006). When looking at the external microbiota, by comparing seed epiphytes of wheat plants with *Brassica* sp., out of a total of 5,477 OTUs (bacterial and fungal) 578 were shared between both plant species (Links et al., 2014). Another group looked at the rhizosphere microbial profiles in relation to shoot biomass production of wheat plants and identified *Duganella, Rhizobium, Janthinobacterium, Acidobacteria* Gp6 and *Cellvibrio* as the five bacterial taxa with the strongest positive association with plant biomass (Anderson & Abiger, 2011).

1.3 Drought: definition and context

Drought can have many definitions depending on if you study it from a meteorological, agricultural or economic standpoint. But for the purpose of this thesis, drought events are defined from the agricultural perspective as the absence of precipitation during a long enough period that non-irrigated crops will suffer from water stress (Ngumbi et al., 2016). It is important to note that although drought often leads to water-stress in agricultural plants, water-stress is not always caused by drought as it can also be due to high soil salinity for example. For simplicity's sake, "water-stress" is used in this thesis as the consequence of a drought event on a plant.

One third of the land on earth is situated on arid or semi-arid zones, with limited amounts of rainfall (Schimel et al., 2007) and these areas are expanding (Schimel et al., 2018). In Canada, the southern part of British Columbia as well as the Prairies are semi-arid areas with a total annual precipitation level of 300-500 mm (McGinn, 2010). With ongoing climate change and global temperature rise, leading to cascading events like increased evapotranspiration rates, many have predicted an increase in the frequency and length of drought events (Cayan et al., 2010; Cook et al., 2015). It is said that by 2050, more than 50% of the world's arable land will be exposed to annual episodes of drought (IPCC, 2013). In Brazil, the world second largest producer of soybean, 20% of the production was lost due to drought during the 2004 and 2005 growing seasons (Politzel et al., 2011). In the Canadian prairies, some predicted through modelling that a water crisis is likely to happen in the near future, due to decreasing flow levels in the main streams, increasing drought events and increasing water demand (Schindler & Donahue, 2006).

1.4 Effect of drought on the plant host

When a plant is subjected to drought, three main strategies exist as to how it can fight off the stress. First, by completing its life cycle before the arrival of dry conditions, the plant can escape drought. It can also avoid drought by, for example, increasing its

root system to reach deeper horizons where moisture is still present. Lastly, the plant is said to tolerate drought if it can keep on growing as if there was no drought (Fang & Xiong, 2015). Sometimes grouped together, avoidance and tolerance mechanisms are the ones targeted when trying to improve crops.

1.4.1 Physiological changes

Changes in the leaf and root configuration are the main physiological modifications in response to hydric stress (Fang & Xiong, 2015). Loss of turgidity leads to the wilting of the leaf, or rolling, which helps stopping excessive water consumption (Fischer & Maurer, 1978). Some plants can also orient their leaves in a specific direction to avoid frontal exposition to sun (Valliyodan & Nguyen, 2006). To prevent water from leaving the leaf, plants can also close their stomates. This will in turn also reduce the amount of carbon dioxide entering the cell (van den Boogaard et al., 1996). Plants can as well extend their root system as a deeper and more widespread rhizosphere will have a better access to water. In general, a reduction of the shoot to root ratio is observed on water-stressed plants, with the produced carbohydrates directed to the water searching roots instead of to the water consuming aboveground parts (Verslues et al., 2006). All these mechanisms generally come at the cost of reduced photosynthesis rates and CO2 assimilation (Fang & Xiong, 2015).

1.4.2 Metabolic changes

When under water stress, some plants accumulate inorganic compounds in order to lower their osmotic potential (Ngumbi et al., 2016). Indeed, in dry conditions water will tend to move from an area of high osmotic potential (high water content or low solute concentration), often the plant, to an area of lower osmotic potential, in this case the soil. Proline, glycine or mannitol are examples of osmolytes (solutes) capable of helping the plant to maintain its cell turgor pressure (Mwadzingeni et al., 2016). Another problem faced by plants under water stress is the increased amounts of reactive oxygen species (ROS), which, if present in high enough concentrations, can damage cell structures and

cause cell death (Cruz de Carvalho, 2008). In fact, ROS naturally occur in plant cells because they are a byproduct of photosynthesis, produced through the photorespiration process. Photorespiration is what happens during photosynthesis when, instead of reducing CO2, other compounds such as O2 are reduced. Under drought conditions, lower levels of CO2 due in part to stomatal closure cause the rate of photorespiration to increase leading to increased levels of ROS (such as superoxide radicals, hydroxy radicals, perhydroxyl radicals, etc.) in the cells (Apel & Hirt, 2004; Gill & Tuteja, 2010). In some drought-tolerant plants, increased activity of antioxidative enzymes that can scavenge ROS (e.g. such as superoxide dismutase, catalase, etc.) has been observed (Caverzan et al., 2016; Chakraborty et al., 2012) and can help lower the amount of ROS within the plant (Luna et al., 2005).

1.4.3 Phytohormones

Phytohormones are also involved in drought tolerance mechanisms. Abscisic acid (ABA) is an important plant signaling molecule (Finkelstein et al., 2002). In water limiting conditions, it will accumulate in the plant and, after reaching a certain threshold, can induce stomatal closure and activate other drought-related genes (Daskowska-Golec & Szarejko, 2013). For example, ABA is involved in the production of proline, an osmolyte and membrane stabilizer. Still, control of the two guard cells surrounding the stomatal pores is not exclusively done by ABA as other phytohormones such as jasmonic acid, brassinosteroids, cytokinin and ethylene are also involved in this complex signaling network (Huang et al., 2008). Auxin is another major phytohormone, as it influences the morphology and structure of the roots, important during water shortages (Bielach et al., 2017). When produced locally in the roots, cytokinin hormones can also lead to modifications in the root architecture, whereas when present in the leaves it can promote the ROS scavenging activity (Bielach et al., 2017). Ethylene production will also often increase, leading to an increase in the root to shoot ratio to improve water uptake and decrease the rate of evapotranspiration (Marasco et al., 2013).

1.4.4 Pleiotropy

Responses of the plant holobiont to drought are complex. Conventional plant breeders have tried for many years to improve drought tolerance of plants through genetic selection of the host, but with limited success (Eisenstein, 2013). This is partly due to the complexity of the drought-signaling pathways (Nakashima et al., 2014). Drought tolerance, as detailed above, involves many mechanisms and the genes involved in characteristics of interest, like proline production, are often pleiotropic (i.e. the gene affects more than one trait) (Ashraf, 2010; Coleman-Derr & Tringe, 2014). Furthermore, a complete understanding of the drought-signaling pathways and of the links between the drought tolerance genotypes and phenotypes is lacking due to the complexity of the plant response (Nogales et al., 2016; Budak et al., 2015). In addition, the level of phenotypic plasticity can vary quite a lot, even amongst the same species and cultivars (Franks, 2011). Even though genotype x environment interactions are more likely to occur in perennial plants with longer life cycles, it was proven that significant variation in drought tolerance exists even within one lineage of *Arabidopsis thaliana* (Juenger et al., 2013). The challenges and limits faced by plant breeding when trying to improve plant drought tolerance could be because this research area underestimates one major component of the plant holobiont, the microbiota (Ngumbi et al., 2016).

1.5 Effect of drought on the rhizosphere microbiota

Decreasing water levels leads to oligotrophic conditions, meaning oxygen-rich environment with lower nutrient availability (Hartmann et al., 2017; Naylor et al., 2018). When facing drought, soil microorganisms must compose with osmotic stress and increased resource competition (Ngumbi et al., 2016). Generally, one of the main effects of drought is an overall decrease in microbial respiration (Birch, 1958; Manzoni et al., 2012; Azarbad et al., 2018). Bacteria are also known to be typically more sensitive than fungi to dehydration and a higher fungal to bacterial ratio is often observed with highly variable responses across phyla, going from opportunistic to sensitive (Bapiri et al., 2010). Various physiological and metabolic strategies are associated both to drought-resistant

fungal and bacterial microorganisms. These include the production of solutes or osmolytes to reduce the unfavorable water potential that forces water out of the cell and leads to desiccation, and the production of drought-resistant structures like spores (Yang et al., 2009).

1.5.1 The bacterial community

Because bacterial soil niches generally are smaller soil pores, it might take longer for the bacterial communities to feel the effect of drought compared to other soil inhabitants (Fuschlueger et al., 2014). Still, at the community level, when a soil is under drought the total bacterial biomass decreases (Meisner et al., 2018). The alpha diversity is often not significantly affected, but there are shifts in the relative abundance of different taxa (Naylor et al., 2018). One strategy adopted by bacteria under hydric stress is to aggregate and form biofilms, a multicellular structure mainly composed of exopolysaccharides. This microenvironment enables water attraction and retention, therefore protecting the cells from extreme environmental conditions (Bérard et al, 2015). Because of the oligotrophic conditions in dry soils, bacteria with slower growth strategies are favored, and consequently the abundance of genes related to the degradation of complex plant polysaccharides was reported to increase as compared to genes involved in the degradation of oligosaccharides (Bouskill et al., 2016). Dormancy and spore or cyst production are also commonly used strategies for some bacteria. For these reasons, *Actinobacteria* and *Firmicutes* are often relatively more abundant under drought (Barnard et al., 2013; Naylor et al., 2017; Meisner et al., 2018). Because gram-positive bacteria monoderm peptidoglycan outer layer is generally thicker (Xu et al., 2018) than the gram-negative membrane, contrasting drought tolerance are often observed between the two groups. Many gram-positive bacteria are also known oligotrophs with spore-forming abilities (Naylor et al., 2018). Some examples of gram-positive bacteria include most *Actinobacteria*, *Firmicutes* and some *Proteobacteria* whereas some examples of gram-negative bacteria for which drought can have harmful effects include the *Verrucomicrobia* and *Bacteroidetes* phyla (Fuchslueger et al., 2014; Xu et al., 2018). It is important to note

also that phylum-level susceptibility to drought is context dependent and the responses are not always consistent in the literature.

1.5.2 The fungal community

Many studies concluded that drought had very little to no significant effect on the fungal community (e.g. Fuchslueger et al., 2016; Yuste et al., 2011). Here again there are contrasting findings in the literature, with the fungal biomass sometimes increasing, decreasing or remaining constant under drought (Meisner et al., 2018). Fungal microorganisms are generally more resistant to hydric stress (Barnard et al., 2013) partly due to the extended hyphal systems of several fungi, enabling them to better reach limited water resources. General fungal drought resistance is also due to their lower nutrient requirements as compared to bacteria (Strickland and Rousk, 2010), making fungi better suited for the oligotrophic conditions induced by the limited water resources (Naylor et al., 2018). During drought events, fungi will remain more active than bacteria (Meisner et al., 2018) sometimes exhibiting higher diversity then under moist conditions (Stefano et al., 2012). Increased stability of the fungal co-occurrence network during drought was also observed (de Vries et al., 2018). Hawkes et al. (2011) described the fungal community response to drought events as plastic, meaning it can reversibly and quickly adapt to and recover from changes in water content. It was even suggested that the expected increase in drought events and the drier conditions in general would lead to soil microbial communities dominated by fungal species (Yuske et al., 2011).

1.5.3 The rest of the soil community

Very few studies have specifically looked at the effect of drought on the archaeal soil community, but it seems like soil archaea, similarly to bacteria, are generally quite vulnerable to drought (Santos-Medellin et al., 2017; Tian et al., 2012) although they can also form biofilms as a protective structure against abiotic stress (Bérard et al., 2015).

As for protists, it was observed that abundance generally decreases with decreasing soil water content levels, with different responses amongst taxa and with the

Stenamoeba genus being the most affected one (Geisen et al., 2014). Indeed, protists are aquatic microorganisms that depend on water films to move, eat and reproduce. Here again very few studies specifically looked at the effect of drought on the protist community and more research is needed.

When looking at the effect of different soil water content treatment on the nematode community, Yan et al., (2018) found significantly lower populations of nematodes in the drought treatments compared to the control. A total of 32 genera was identified in all the treatments and the *Encephalobus* as well as the *Helicotylencus* genera were the most abundant ones in the pots under drought conditions. The bacterial-feeding nematodes were the ones most affected by the lack of water.

1.5.4 Soil memory

Drought is an important driver of adaption for the soil microbial community and it can have long-lasting effects (Meisner et al., 2018). Indeed, iterative exposition of a soil microbial community to abiotic stress can impact the way it faces subsequent stress and this concept was coined as the “soil memory” (Lapsansky et al., 2016). Concerning drought, there is compelling evidences that precipitation history alters microbial community responses to contemporary water regime (e.g. Bouskill et al., 2013; Evans & Wallenstein, 2012). More interestingly, it was also shown that this soil memory can in return alter the plant host (Kaisermann et al., 2017). Indeed, plants grown in a soil that was pre-exposed to drought have shown phenotypic signs of improved drought resistance when subjected to contemporary drought, like greater fruit production (Lau & Lennon, 2012) or larger root biomass (Azarbad et al., 2018).

1.6 Plant-microbe interactions

As the holobiont concept puts forward, the interactions between a plant and the microorganisms living in its surroundings are important to consider when trying to understand how plants adapt to their environment and to different stresses. There are multiple ways that plant and microorganisms can interact and communicate. One of the

pillars of plant-microbe interactions in the rhizosphere is the production of root exudates (Shi et al., 2011). Rhizodeposits serve as chemo-attractants or growth inhibitors, helping the plant to select for specific microorganisms and to influence the assembly of the rhizosphere microbiota (Steinauer et al., 2016). As soon as water enters the seed and restarts the biological activity, exudates are produced, attracting microbial life in the spermosphere (area around the seed) (Nelson, 2004). When the plant starts photosynthesizing, 10-40% of the photosynthesized carbon compounds are transferred to the rhizosphere (Zhalnina et al., 2018). The composition and quantity of root exudates produced is not constant and can vary depending on factors such as growth stage, environmental condition or nutrition (Sasse et al., 2018). Exudates can therefore also act as indicators of environmental change between the plant and the microbiota.

Many types of rhizodeposits exist, such as ions (H+), oxygen, water, sugars, amino acids, organic acids, mucilage and proteins (Badri et al., 2009). The volatile organic compounds (VOCs), such as alcohol, ketones or aldehydes are of special interest because with their diffusive capabilities, they can impact soil activity over longer distances (Schulz-Bohm et al., 2018). It was even suggested that VOCs produced by the plant could serve as the main source of carbon for certain types of fungi like basidiomycotas in soils with low nutrient levels (Gramss & Bergmann, 2008). Some bacteria living in the rhizosphere of pine trees were also able to survive on a strict diet, with only pinene (a VOC produced by pine trees) as a source of carbon (Penuelas et al., 2014).

The communication between a plant and its microbiota is not unidirectional (Hassani et al., 2018). Interestingly, Schenkel et al. (2015) found that a large fraction of the volatiles produced by the plants are also produced by either soil bacteria or fungi, supporting the idea of a common language between the plant and its microbiota. It was also shown that the different phytohormonal pathways were activated when the plant was provided a mixture of VOCs produced by *Bacillus subtilis* (Ping & Boland, 2004). Some VOCs produced by rhizosphere bacteria are also known to activate the induced systemic resistance response, related to biotic stress defense (Ryu et al., 2004).

Phytohormones such as ethylene, giberellin, cytokinin and auxin are also central to the interactions between plants and microorganisms. Understanding the pathway and

effects of plant hormones on the plant holobiont can be challenging because they are produced both by the plant and some microorganisms, implying a lot of cross-talking (Calvo et al., 2014). Rudrappa et al. (2008) demonstrated that under pathogen attack *Arabidopsis thaliana* secrete malic acid which stimulates biofilm formation of *Bacillus subtilis*, leading to increased plant resistance against pathogens. It was also shown that increased levels of the phytohormone jasmonic acid shifted the composition of the root endophytic community, with most of the microbial species stimulated by jasmonic acid being antagonist to pathogens or able to promote plant (Liu et al., 2017). Salicylic acid produced by the plant was also shown to influence the composition of the rhizosphere microbiota (Lebeis et al., 2015), and plants produce strigolactones that attract mycorrhizae and stimulate spore germination (Lopez-Raez et al., 2017). Plants are also known to regulate bacterial root colonization and biofilm formation by producing compounds that can interact positively or negatively with bacterial receptors for quorum sensing molecules (acyl homoserine lactones, AHL) (Gaeiro et al., 2013). Interestingly, it was shown that, in return, root morphology can be affected by these AHL (Friesen, 2013).

Microbially-mediated production of phytohormones is also important to consider. Microorganisms can have an impact on the overall phytohormonal balance within the plant holobiont. It is estimated that 80% of bacteria living in the rhizosphere of plants can produce auxin (Dodd et al., 2010). Some bacteria can produce IAA, jasmonic acid, giberellins and abscisic acid (Gaiero et al., 2013; Leach et al., 2017). Arbuscular mycorrhizae (AM) can produce some phytohormones such as auxins and cytokinins (Lopez-Raez et al., 2017). These hormones not only help plants grow but also increase mycelium development (Basu et al., 2018). Even some nematodes can produce phytohormones such as cytokinin (Leach et al., 2017). Interestingly, bacteria and fungi do not only produce some of the phytohormones, they can also degrade some and use them as a carbon or nitrogen source (Berg et al., 2009). Bacteria-mediated degradation of the ethylene precursor ACC probably is the most studied example of this and will be described in more details below. Some bacteria, such as *Pseudomonas*, *Rhodococcus* and *Bulkholderia*, also can degrade other plant hormones such as salicylic acid, abscisic acid and indole acetic acid (Dodd et al., 2010).

1.6.1 Effect of drought on plant-microbe interactions

Water stress also induces changes in the interactions between the plant host and the microbiota. For instance, the amount of rhizodeposits varies according to water availability, but whether it is increasing, or decreasing is still a debate, mainly because variation in the measurement and normalization methods can lead to contradictory results (Preece and Peñuelas, 2016). Changes in both the quantity and composition of the rhizodeposits can in turn alter the microbial community living in the vicinity of the plant roots (Shi et al., 2011; Zhalnina et al., 2018). Interestingly, it was observed through a labelled 13CO2 experiment that during drought, plants will reduce the amount of photosynthesized carbon provided to bacteria, but not to fungi (Fuchslueger et al., 2014). It was also shown that certain VOCs produced by rhizosphere microbial species can trigger drought resistance trait like stomatal closure in the plant (Liu et al., 2015). Although more research is needed to unravel the underlying mechanisms, this again supports the idea of bidirectional communication within the holobiont network.

1.7 Roles of the microbiota

Microbial members of plant holobionts are responsible for many important functions, which is not surprising because the microbiome can hold up to ten times more genes then the host genome (Mueller & Sachs, 2015). One of the main microbiota functions of interest is what is often vaguely call “Plant Growth Promotion” (Wei & Jousset, 2017) which can occur from the seed germination all the way to seed production. Seed endophytes and epiphytes are already intimately interacting with the host and involved in seed germination, plant cell expansion and disease suppression (Goggin et al., 2015; Nelson et al., 2018; Pitzschke, 2018; White et al., 2018). Once the seedling starts growing, contrasting microbiotas are observed for the different plant areas and amongst the commonly accepted divisions we find the phyllosphere (microbiota associated to the leaves), endosphere (microbiota found inside the plant tissues), caulosphere (stem), carposphere (fruits), anthosphere (flowers), rhizosphere (area surrounding the roots) and the rhizoplane (root surface) (Shade et al., 2017). The spectrum of action of the

microbiotas is very broad and beyond the scope of this chapter. The rhizosphere microbiota is generally involved in many aspects of aboveground and belowground disease and pest suppression, nutrient and water acquisition, plant biomass accumulation (e.g. Berendsen et al., 2012; Friesen et al., 2013, Gopal et al., 2013; Mendes et al., 2013; Schlaeppi & Bulgarelli, 2017; Vimal et al., 2017) and abiotic stress tolerance (Marasco et al., 2013), like water-stress tolerance for which the mechanisms are described in more details below.

1.7.1 Microbially-mediated plant drought resistance mechanisms

Many microorganisms have been isolated and identified as contributing to the drought tolerance in the plant host and up to now most of them were bacteria. Interestingly, although many microbes have been correlated to different physiological or hormonal changes in the plant, very few mechanisms have been detailed yet. Still, one example of a well-studied mechanism is the 1-aminocyclopropane 1-carboxylate (ACC) deaminase (ACCd) enzyme present in some bacterial species that can alter part of the plant hormonal response. The ACCd breaks down ACC, the precursor of ethylene, thereby reducing the growth inhibition caused by this hormone when a plant is facing abiotic stresses (Glick, 2014; Tiwari et al., 2018). This phenomenon has been observed, among others, in water stressed *Arabidopsis thaliana* inoculated with the bacteria *Achromobacter piechaudii* (Cho et al., 2013). Many other experiments conducted under laboratory conditions were successful at inoculating a single or a few bacterial isolates to improve plant drought tolerance. Amongst many, *Ochrobactrum pseudogregnonense* and *Bacillus safensis* previously isolated from saline environments were shown to delay wilting, increase antioxidative activity, increase plant height and yields in six wheat varieties (Chakraborty et al., 2013). Although more work is needed to provide a complete explanation, the presence of bacterial cytokinin was inversely proportional to the levels of ABA in a model plant inoculated with *Paenibacillus polymyxa* (Yang et al., 2009), suggesting that the bacteria cytokinin might affect ABA signaling pathways involved, among others, in stomatal closure. Drought-stressed wheat plants coming from seeds inoculated with *Bacillus amyloliquefaciens* 5113 and *Azospirillum brasilense* N040 had

better survival rates, higher fresh and dry aboveground biomass, higher water content as well as lower activity of multiple anti-oxidative enzymes, supporting the idea that bacteria priming can reduce oxidative stress (Kasim et al., 2013). The production of siderophores, which increases the bioavailability of certain minerals, like phosphorous, that are rendered unavailable when soil water content is low, could also be an interesting bacterial and fungal trait (Ngumbi et al., 2016). Rolli et al. (2015) supplemented the rhizosphere of different water-stressed pepper cultivars with bacterial strains screened from drought-adapted grapevine rootstocks and showed that the beneficial aspects of the association with the bacteria was a water dependent trait, meaning that it was observable in water-stressed plants but not in plants grown in optimal soil water content. Many bacteria regrouped under the plant growth promoting category show positive interactions with plants, but only when under water stress. Yang and colleagues (2009) suggested the term “induced stress tolerance (IST)” for all the beneficial physical and chemical changes that are activated in the plant host when interacting with these bacteria under abiotic stress (Figure 1). The term referred to induced systemic resistance that similarly describe bacterial-mediated plant resistance to biotic stress.

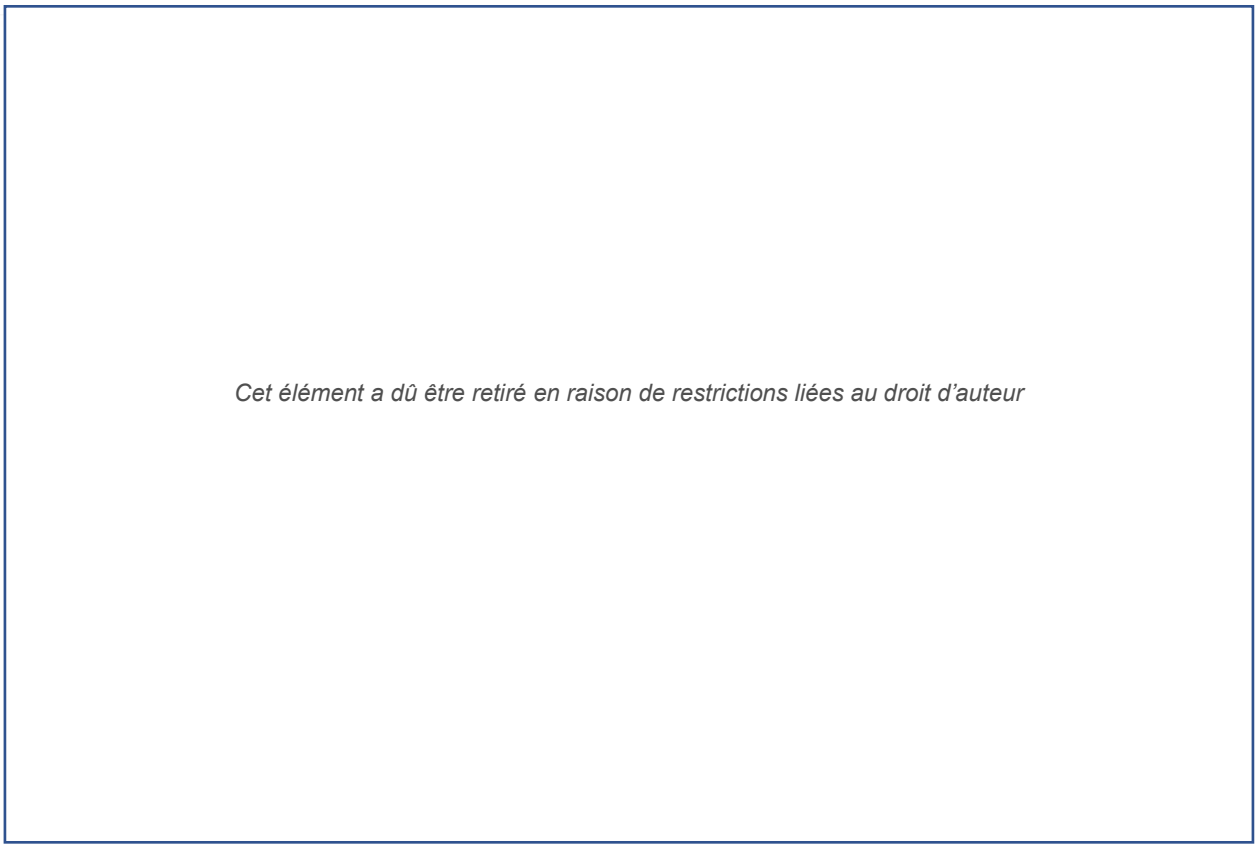

Fig. 1 Induced systemic tolerance (IST) initiated by beneficial bacteria (Yang et al., 2009)

This figure describes the different mechanisms (e.g. production of cytokinin, antioxidants or ACC deaminase enzymes) mediated by bacteria, involved in IST and helping plants tolerate abiotic stresses such as drought.

Arbuscular mycorrhizal fungi (AMF) are also known to improve plant tolerance to drought. AMF can facilitate the uptake of immobile nutrients, increase biomass and photosynthesis, modify transpiration rate and improve water uptake through an extended root system (Augé, 2001; Jayne & Quigley, 2014). A field experiment showed that wheat plants under drought stress inoculated with one of two different AMF strains (*Glomus mosseae* or *Glomus etunicatum*) were more colonized by the AMF than plants grown in optimal soil moisture. This association with AMF was also linked with higher plant biomass, grain yield as well as leaf phosphorus and iron concentration (Al-Karaki et al., 2004). By studying the proteome of wheat plants suffering from water stress and inoculated with AMF species, Bernardo and colleagues (2017) showed a possible interaction between the colonization of roots by AMF and the signaling pathway of

jasmonic acid, a phytohormone involved in abiotic stress tolerance. Association with AMF also affected the production of other proteins involved in sugar metabolism and cell wall integrity. Other fungi such as *Piriformospora indica* and *Trichoderma* species, were also correlated to drought resistance in plants (Cho et al., 2013). Research has also showed the beneficial impact of some endophytic fungal strains on the resistance of plants to drought stress (Singh et al., 2014). By testing six Ascomycota fungal isolates from the Saskatchewan Microbial Collection Database (SMCD), Hubbard et al. (2012) found that three out of the six endophytes significantly improved the hydrothermal time (a ratio based on seed temperature and water content measures compared to optimal values) and the energy of germination (germination rate) of drought-stressed wheat seeds. Fungal endophytic SMCD isolates were also shown to improve the photochemical efficiency, a proxy to assess how photosynthetic processes are affected in a plant, in inoculated wheat under water stress (Hubbard et al., 2014).

1.8 Rhizosphere microbiota engineering

It is the German scientist Lorenz Hiltner (1904) who first argued that plant health and resistance against pathogens relied on the presence of beneficial bacteria in the rhizosphere. By studying plant germination and growth and by using a microscope to look at plant tissues, Hiltner discovered the presence of bacteria, even in healthy plants, in the area he himself coined as the rhizosphere. He was also the first one to hold a patent on a *Rhizobium* inoculum (Hartmann et al., 2008). Since then, many researchers have tried to develop new techniques to engineer the microbiota of plants, most often the rhizosphere (Quiza et al., 2015). The rhizosphere is indeed a hotspot for both plant-microbe and microbe-microbe interactions (e.g. Aminov, 2011; Bakker et al., 2014). The potential of microbiota engineering has been extensively studied to improve disease resistance (e.g. Mendes et al., 2011; Santhanam et al., 2015), nutrient use efficiency and acquisition (Berg et al., 2014) and abiotic stress tolerance (e.g. Rodriguez et al., 2008; Marasco et al., 2012). Inspired by the holobiont approach, many new ideas on how to modify the microbiota have been brought up (Sánchez-Cañizares et al., 2017). Amongst the top-down approaches, some have successfully tried host-mediated selection of the microbiota

(Mueller & Sachs, 2015). One of the first experiments using host selection successfully increased or decreased plant shoot biomass of *Arabidopsis thaliana* by growing it in soil previously planted with A. thaliana with low or high shoot biomass (Swenson et al., 2000). Panke-Buisse and colleagues (2015) were able to shift flowering time of *Arabidopsis thaliana* by growing the plants in a soil iteratively planted with later-flowering cultivars. Others suggested plant-breeding to modify and shape the plant's recruiting pattern of microbes (Bakker et al., 2012). Inoculation of artificial root exudates or other prebiotic compounds has also been tried (Lebeis et al., 2015).

But still, the bottom-up approach of incorporating new microorganisms remains the most popular technique, in part because knowledge on the gene regulation of root exudates production and other mechanisms underlying microbial recruitment is still rudimentary and because plant breeding is time consuming (Mendes et al., 2013). Incorporation of new microorganisms can either happen through the inoculation of single microbial strains, synthetic microbial communities (cultivated in the lab), soil extracts or microbiota transfer (Foo et al., 2017; Gopal & Gupta., 2016). For the human microbiota, fecal microbiota transfer was shown to be a successful method when trying to cure patients suffering from *Clostridium difficile* infections. The method implies inoculating the gastro-intestinal tract with a healthy fecal matter microbial extract (Foo et al., 2017). In plants, mixing soil showing disease suppression ability against *Rhizoctonia solani* to soil vulnerable to the pathogen in a 1:9 ratio successfully improved the crop's disease resistance (Mendes et al., 2011). Still, this has limited applicability under field conditions. Until recently, researchers focused mainly on the use of single isolates or microbial consortiums of a few different strains to prime seeds (Calvo et al., 2014), inoculate soils (e.g. Marasco et al., 2013; Wang et al., 2012; Kohler et al., 2008) or flowers (Mitter et al., 2017). Although experiments were mostly successful in laboratory conditions they often resulted in failures in the field (Bakker et al., 2012). This is partly because microbial communities work in network and associations (van der Heijden & Hartmann, 2016) and because a desired ecological function will not necessarily be retrieved from a single microorganism but rather from a consortium of microorganisms (Brenner et al., 2008), as was observed for disease suppression in soils (Gopal et al., 2013).

This is also because traditional microbiota engineering largely minimizes the abiotic and biotic context dependency for colonization success (Wei & Jousset, 2017). The importance of considering the “social” context for successful modification of the microbiota is why many now argue, as for the example of fecal transplant to modify the human microbiota, that complex microbial community might be better at colonizing and modifying the plant rhizosphere microbiota (Gopal et al., 2013; Toju et al., 2018). The development of culture-independent “meta-omics” tools provided us with a broader understanding of microbial ecology. It is now becoming clearer that when trying to introduce new microorganisms in the rhizosphere, the priority effect of the native microbial community is far from negligible (Vanette & Fukami, 2014). The order in which microbial species are introduced largely determines the outcome of microbial community assembly. In soil, where the microbial diversity and abundance are usually very high, the resident community usually out competes newly introduced species, which is good to fight off pathogen invasion but challenging in the context of microbiota engineering. Still, the strength of the priority effect can vary quite a lot although the reasons for this variation are still obscure (Tucker & Fukami, 2014).

Recently, the microbial community coalescence concept was introduced to describe what happens when two microbial communities are merged together for the first time (Rillig et al., 2015). It is suggested that much knowledge could be gained by considering and studying the coalescence event, especially in the case of microbiota engineering (Rillig et al., 2016). Many variables can affect the outcome of a coalescence event and studies looking at, for example, the effect of the mixing ratio and of the incorporation methods are directly needed to optimize microbiota engineering methods. These experiments could also help answer pending interrogations, such as: can microbes adapt if repetitively exposed to coalescence events and what ability is needed for a microbe to be more adapted (Rillig et al., 2015)? In depth studies on the matter would help parametrize coalescence events up to the point where the outcome could be predicted.

CHAPTER 2:
HYPOTHESES AND OBJECTIVES

Water stress was shown to be a driver of adaption for all components of the plant holobiont: the plant host, the microbiota and the plant-microbe interactions. Water stress can also lead to long term modification of the soil microbial community, improving plant drought tolerance traits in the next generation. Finally, we know that the rhizosphere of a plant is a highly selective environment.

General hypothesis:

Inoculation of the rhizosphere with a microbial community with a previous history of water stress will improve plant tolerance to water stress.

Objectives:

1. Observe the effect of the soil type, the soil water content, the inoculation and their interactions on different parameters (aboveground biomass, leaf water content, total plant water content, anti-oxidative activity) of wheat plants.
2. Observe the effect of the soil type, the soil water content, the inoculation and their interactions on the microbial communities present in the rhizosphere of wheat plants through amplicon sequencing of the V4 region of the bacterial 16SrRNA gene and the fungal ITS1 region.

The purpose of this thesis was to test if, under water stress conditions, a drought-sensitive wheat cultivar can recruit and associate with an inoculated complex microbial community, extracted from a soil that was pre-exposed to drought. For that, a multi-factorial greenhouse experiment was designed. Wheat plants were grown in two soil types (irradiated or native soil) at two soil water contents (water stress or normal) and received one out of five inoculum options: the microbial community extracted from 1) an irrigated soil, 2) an irradiated version of the irrigated soil, 3) a non-irrigated soil, 4) an irradiated version of the non-irrigated soil or 5) water as a control.

CHAPTER 3:

A WATER STRESS-ADAPTED INOCULUM AFFECTS RHIZOSPHERE FUNGI, BUT NOT BACTERIA NOR WHEAT

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Author's contributions:

CGL and EY elaborated the experimental design. LB harvest the soils used for the inoculum. CGL ran the experiment. JT did the bioinformatics. HA acted as a mentor for all steps of the experiment. CGL ran the statistical analysis and CGL and EY wrote the article. The article was corrected by HA, LB and JT.

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3.1 Abstract

Here, we tested if inoculating microbial communities adapted to water stress would increase wheat resistance to water stress. Wheat plants were grown for four weeks in high and low diversity soils under well-watered conditions, after which they were subjected to a water stress. After another two weeks, the rhizospheres were inoculated with microbial communities extracted from soils with or without a history of water stress. The inoculations did not have significant effects on the plant growth, water content and catalase activity, and on the bacterial communities. However, the inoculation did successfully, though modestly, modify the fungal community, shifting the rhizosphere communities toward the inoculated communities. As hypothesized, these shifts were more pronounced and significant in the low diversity soil, and for the inoculum with a water stress history. Whereas the effects of inoculation were relatively subtle, the water stress resulted in large differences in the wheat phenotype and in both the bacterial and fungal communities. Generally, the microbial changes that followed the water stress were in large part due to shifts in the relative abundance of OTUs that were already present before the stress, rather than to the recruitment of microorganisms from the inoculum or the bulk soil.

Keywords: drought, wheat, rhizosphere microbiota, inoculation, amplicon sequencing

3.2 Introduction

It is estimated that by 2050, drought episodes will strike over 50% of the world's arable land, causing serious yield losses in major crops (Ngumbi and Kloepper, 2016). At the same time, the world demand for freshwater is rising, and 70% of it is used by agriculture (Fang et al., 2015). There is a need to find innovative approaches to make agricultural crops better at tolerating higher levels of hydric stress (Castiglioni et al., 2008). One such approach is to harness the potential of plant-associated microbes (Andreote & Silva, 2017; Wei & Jousset, 2017). Indeed, microbes are found on all plant parts and carry functions essential for plant fitness and survival (Friesen, 2013; Mendes et al., 2013). In the soil zone surrounding the roots (the rhizosphere) plants invest roughly 11 % of the net fixed carbon into the production of a broad range of root exudates (Jones et al., 2009), used to attract or deter specific microbial strains (Sasse et al., 2018).

Water stress is a driver of adaptation for both microorganisms (Evans & Wallenstein, 2014) and plants (Wu et al., 2011). When facing water stresses, the plant will modify both the abundance and composition of its root exudates (Preece & Peñuelas, 2016) which will then alter the rhizosphere microbiota (Zhalnina et al., 2018). Although evidences are still scarce, this was suggested as a mechanism the plants use to recruit microbial partners that might help them to tolerate water stress (Holz et al., 2017). In addition, water stress shapes soil microbial communities directly, decreasing the overall respiration rate (Birch, 1958; Azarbad et al., 2018) and richness (Meisner et al., 2018) and increasing the fungal: bacterial ratio (Bapiri et al., 2010). These changes often result in microbial communities being able to better tolerate subsequent water stresses (Evans & Wallenstein, 2012; Fierer et al., 2003), a phenomenon that was dubbed "soil memory" (Lapsansky et al., 2016). Interestingly, soil memory can affect plants (Kaisermann et al., 2017). For instance, *Brassica rapa* plants showed improved resistance to water stress when grown on soil that was pre-exposed to water stress (Lau & Lennon, 2012). Similarly, wheat growing in a soil with a long-term history of water stress produced more roots (Azarbad et al., 2018), which would improve the resistance to a subsequent water stress.

An interesting framework to study the adaptation of the host-associated microbiota to stressful events is the hologenome theory of evolution. This theory postulates that the complex interacting network involving a eukaryotic host and all its associated microorganisms (the holobiont) acts as one of the units of evolution (Zilber-Rosenberg & Rosenberg, 2008). As such, rapid adaptations of holobionts are hypothesized to be more likely related to the microbial partners via mechanisms such as the recruitment of new partners, amplification/reduction of the partners already present and horizontal gene transfer, suggesting a clear path to the improvement of plant resistance and resilience to stress (Berendsen et al., 2013; Duhamel & Vandenkoornhuyse, 2013; Nogales et al., 2016; Rosenberg & Zilber-Rosenberg, 2016). Most efforts up to date have focused on trying to direct the recruitment by the plant by providing selected microbial strains or communities. For instance, the priming of seed with single microorganisms has often been utilized to supplement the rhizosphere microbiota (Parnell et al., 2016). However, this technique can sometimes be of limited success, mainly because it underestimates the importance of the social context (microbe-microbe and plant-microbe interactions) for optimized colonization (Rivett et al., 2018). Some have suggested that complex communities targeting multiple niche spaces and exhibiting functional redundancies might be better at entering and surviving a new environment than single species (Yergeau et al., 2015). Knowledge on how microbial communities coalesce is still minimal (Rillig et al., 2016) but we know that the already established soil inhabitants often rule over the newcomers, a phenomenon referred to as the priority effect (Vanette & Fukami, 2014). This priority effect could be overcome when the native microbial community is weakened by environmental stressors (Calderon et al., 2017), like drought. The situation gets even more complex under the influence of a plant, which partly gets to choose which partners will stay (Mueller et al., 2015). The question of how to translate this theoretical framework into applied solutions to rapidly adapt crops to abiotic stresses remains unanswered.

Here, we asked the question: does an inoculum with a previous history of water stress improve plant tolerance to water stress? Specifically, we hypothesized that (1) inoculated microorganisms would better persist when added to a soil with a lower diversity (i.e. irradiated), (2) recruitment of novel microbial species by the plant would be enhanced

when under water stress, and (3) water stress adapted microorganisms would be more recruited than ones not adapted to this stress.

3.3 Materials and methods

3.3.1 Potting soil sampling

The potting soil was collected in May 2017 from our experimental field at the Institut national de la recherche scientifique (Laval, QC, Canada). The field was ploughed for the first time in 2016 with no agricultural crops cultivated in the area for over 20 years. Soil analyses carried were carried out in May 2016 by Maxxam (Montréal, QC) and revealed a total P concentration of 1,300 mg/kg, a total K concentration of 1,110 mg/kg, a pH of 7.30 and a total N concentration (Kjedldahl extraction) of 3,000 mg/kg. All the soil was dried, sieved with a 2 mm pore sieve and homogenized. Half of the soil was then gamma irradiated at a dose of 50 kGy by Nordion (Laval, QC, Canada) to reduce the microbial diversity and abundance (McNamara et al., 2003). Before the start of the experiment, the irradiated soil (thereafter referred as LD, low diversity) had a bacterial Shannon diversity index of 8.65 a fungal Shannon diversity of 3.67 and a DNA concentration of 15.1 ng/µl, as compared to a bacterial Shannon diversity index of 9.43, a fungal Shannon diversity of 5.64 and a DNA concentration of 82.8 ng/µl for the non-irradiated soil (thereafter referred as HD, high diversity). These differences were maintained throughout the experiment with the LD rhizospheres DNA concentration, bacterial and fungal Shannon diversity indices averaging at 13.7 ng/µl, 7.07 and 2.90, respectively whereas the HD rhizospheres DNA concentrations, bacterial and fungal Shannon diversity indices averaging at 44.2 ng/µl, 9.34 and 4.00, respectively.

3.3.2 Inoculum soil sampling

The soils used for the inocula came from two adjacent Agriculture and Agri-food Canada experimental fields in Swift Current, SK, Canada. These fields have been under a continuous wheat-fallow rotation to test newly developed wheat varieties for their resistance to water stress. Since 1981, the two fields have been managed similarly, except

that one was irrigated every second year (during the wheat phase of the rotation). The climate in this region is considered semiarid with limited rainfall events (Cutforth, 2000), meaning that the field under ambient conditions was continuously exposed to water stress for almost 40 years (see Azarbad et al. (2018) for more details about the sampling site). Both soils were sampled in April 2017 and sieved with a 2 mm pore size sieve. A part of each soil was irradiated with a dose of 50 kGy (Nordion). The inocula made from the irradiated soils were only used to contrast the effect of adding microorganisms vs. water-soluble nutrients on plant parameters.

3.3.3 Inoculum preparation

Even though it might suffer from some biases like all other extraction methods, a microbial water extraction method was used because of its simplicity and its successful use in previous studies (Wagner et al., 2014; Calderon et al., 2017). For each inoculum, the soil was mixed with sterile water for 20 minutes using a sterilized blender in a 150% W/V ratio. Resulting soil slurry was centrifuged at 5 000 × g for 20 minutes. This process was repeated 4 times per inoculum and the resulting supernatants were pooled and then split into 24 units so that each pot would receive 50 mL of its corresponding inoculum (equivalent to a water extract from 100 g of soil). 2 x 1 mL of each inoculum and a few grams of the precipitated soil slurry were stored at -20°C for microbial analysis while the rest of the water extracts was conserved at 4°C for 24 hours before use. On average, the water extracts contained 0.68 ng/µl of DNA as compared to 7.68 ng/µl for the soils before the microbial water extraction. The microbial communities present in the inocula were representative of the communities present in the soils before the microbial water extraction, as visualized by the similarity in their community compositions (Fig. S1a) and the tight grouping of the inocula with their respective soils and precipitated soil slurry (Fig. S1b). The inocula (200 µl) contained, on average, a total of 135 ng of DNA and the receiving soil (1.5 kg) contained, on average, a total of 174 µg of DNA, which mean that the inoculated microorganisms represented on average 0.078% of the recipient community.

3.3.4 Experimental design & sampling

To test our hypotheses, we designed a three-level factorial experiment with two potting soils (high-diversity (HD) and low-diversity (LD, irradiated) Quebec soil) x two soil water content (SWC, 15 or 50% soil water holding capacity) x five inoculation treatments (water extracts from native irrigated (NI), native ambient (NA), irradiated irrigated (II) or irradiated ambient (IA) Saskatchewan soils and a water control (CTRL)) (Fig. 1). The factorial combinations were replicated six times in a randomized complete block design, resulting in 120 pots. Each pot (1,000 cm^3) was filled with 1.5 kg (dry weight) of soil and sowed with 8 seeds of *Triticum aestivum* cv. AC Nass and placed in a greenhouse. For the first four weeks of the experiment, all the pots were maintained under optimal soil water content (50% SWHC), after which the water content of half the pots was reduced to 15% SWHC until the end of the experiment. Two weeks after adjusting the water content, the pots were inoculated with 50 mL of one of the four inocula or 50 mL of distilled water. After another three weeks, the experiment was terminated and sampled. Half a gram of the flag leaf material was taken, immediately flash frozen with liquid nitrogen and stored at -80°C before enzymatic measurements. To measure dry biomass and plant water content, the remaining upper biomass was sampled, weighed and dried at 80°C to constant weight. The plants were uprooted, and the soil still attached to the roots after vigorous shaking was considered as rhizosphere soil which was then stored at -20°C for microbial analysis.

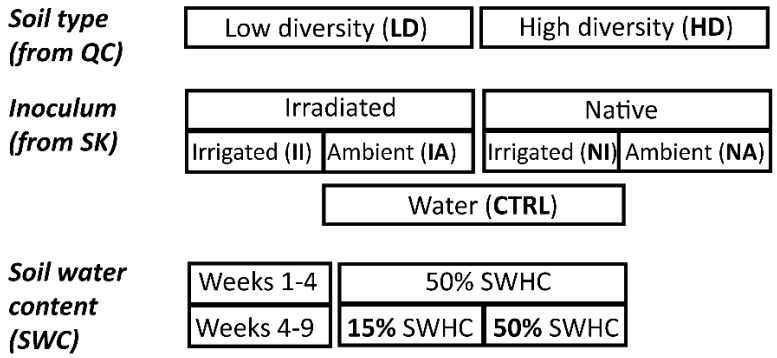


Figure 3.1 Experimental design.

3.3.5 Catalase enzymatic assay

Catalase activity was measured as an indicator of plant water stress (Luna et al., 2005; Cruz de Carvalho, 2008). The 0.5 g leaf material was first homogenized in liquid nitrogen using a mortar and pestle, then mixed with 1 mL of the extraction buffer composed of 100 mM of phosphate buffer (pH 7.5), 1 mM EDTA, 5% W/V PVPP and 1 mM of ascorbic acid (Lester et al., 2004), as well as 1 µL of protease cocktail inhibitor (Sigma P9599). The mix was vortexed and centrifuged at 10,000 × g for 20 minutes at 4°C. The supernatant was stored at -80°C before quantification of the catalase activity using a chemical catalase assay kit (Cayman, Michigan, USA). Results were normalized according to the total protein content measured with the Bradford assay method (Bradford, 1976) and the Bio-Rad protein assay dye kit (Bio-Rad Laboratories, Hercules, CA, USA).

3.3.6 DNA extraction, library preparation and Illumina MiSeq sequencing

Rhizosphere soil DNA was extracted using the PowerLyzer soil extraction kit (Qiagen, Ontario, Canada). Amplicon libraries were prepared for the v4 region of the bacterial 16S rRNA gene using primers 515F and 806R (Caporaso et al., 2012) and for the fungal ITS1 region using primers ITS1F and 58A2R (Martin & Rygiewicz, 2005) following the “16S Metagenomic Sequencing Library preparation” Illumina guide (Part #15044223 Rev. B). The two pools (one for the 16S rRNA gene and one for the ITS region) were sent for 2 × 250 bp paired-end sequencing on an Illumina MiSeq apparatus at the McGill University and Genome Québec Innovation Center (Montréal, Canada). A total of 20,847,162 16S rRNA gene reads and 22,299,878 ITS region reads were produced. The raw datasets and associated metadata are available through NCBI BioProject accession PRJNA508533

3.3.7 Bioinformatics

Sequence data were treated following our in-house pipeline as already described elsewhere (Tremblay et al., 2015). Briefly, both 16S v4 rRNA gene and ITS1 region

sequences were separately filtered, assembled, trimmed and controlled for quality. Quality controlled reads were dereplicated at 100% identity and denoised at 99% identity using dnaclust v3 (Ghodsi et al., 2011). Clusters of less than three reads were discarded and remaining clusters were scanned for chimera using UCHIME in denovo and reference modes consecutively (Edgar et al., 2011) using the Broad Institute's 16S rRNA gene Gold reference database (Broad Institute, Microbiome Utilities - http://microbiomeutil.sourceforge.net/). Remaining clusters were clustered at 97% identity. (dnaclust). Resulting clusters or OTUs were assigned a taxonomic lineage using the RDP classifier with training sets constructed from the Silva database (release 128) (Quast et al., 2012) for the 16S rRNA gene and the ITS Unite database (Kõljalg et al., 2013) for the ITS region. In total, 20,477 bacterial OTUs and 2,798 fungal OTUs were identified. Alpha diversity indexes (Simpson, Shannon, Chao1 and Species Observed) were calculated with the QIIME v1.9.1 software (Caporaso et al., 2010) with the datasets rarefied to 1,000 sequences.

3.3.8 Data analysis

All statistical analyses were performed in R version 3.3.0 (R core team, 2013). Fresh/dry upper biomass weight and plant water content values were normalized by the number of plants per pots before analyses. Most of the plant variables did not follow the assumptions for parametric testing and Kruskal Wallis test and post-hoc Dunn's test with Bonferroni type of *p* value correction for multiple testing were used instead.

For the analysis of beta diversity, OTU abundances were normalized (so that the sum of all OTU abundances within one sample equals one) before calculating the Bray-Curtis dissimilarity. The square root of Bray-Curtis dissimilarity was used as the input for Principal Coordinate Analysis (PCoA). Because the intra-group variances between the LD and HD soils were not equal according to the BETADISP multivariate test, the beta diversity profiles were analysed separately for the two soils. The impact of soil water content (SWC) and inoculation, their interaction and the block effect on the similarity of the microbial community profile between samples was tested for significance through Permutational Multivariate Analysis of Variance (PERMANOVA) with 1,000 permutations.

For alpha diversity, the effects of SWC and inoculation, and their interaction on Shannon diversity and the number of observed OTUs (S_{obs}) were tested using two-way ANOVAs for LD and HD soils separately.

The effect of the inoculation on the microbial community was tested by comparing the Bray-Curtis dissimilarity between the microbial communities of the inoculated rhizosphere and their respective inoculum to the dissimilarity between the control rhizosphere (CTRL, water) and the same inoculum. The significance of these differences was tested for all subgroups using the Wilcoxon ranked test, with Bonferroni correction for multiple testing. Similarly, using the rarefied OTU tables, the number of shared OTUs shared between the microbial communities of the inoculated rhizosphere soil samples and their respective inoculum was compared to the number of shared OTUs between the communities of the control rhizosphere (CTRL, water) and the same inoculum using Wilcoxon ranked tests, with Bonferroni correction for multiple testing. To have enough statistical power, the tests were only carried out on the OTUs that were found in at least four of the six blocks for each sub-category.

Finally, the likely origin of the OTUs found in the rhizosphere of the water-stressed inoculated plants (15% SWHC) was determined by comparing the OTUs present in their rhizosphere to the ones detected in 1) the potting soil before seeding, 2) the rhizosphere of the 50% SWHC - inoculated plants and 3) the inoculum. Venn diagrams were created to visualize the data using Venny (http://bioinfogp.cnb.csic.es/tools/venny/index.html). The analyses were based on the OTU tables rarefied at 1000 sequences and an OTU was deemed present when observed in either one of the six replicates.

3.4 Results

3.4.1 Plant biomass, water content and catalase activity

The plant aboveground dry weight differed significantly according to soil type (HD vs. LD) and SWC (15% SWHC vs. 50% SWHC) and was on average 0.469 g for LD-15%, 0.685 g for LD-50%, 0.237 g for HD-15% and 0.276 g for HD-50% (Table 1).

Table 3.1 Average values for plant dry weight, plant water content and leaf catalase activity and associated Kruskal Wallis tests for the effect of the experimental treatments. Results in boldface type are significant at *P*<0.05.

Soil	SWC	Inoculum	Dry weight	PWC	Catalase
LD	15%	II	0.463	0.780	1.940
		IA	0.475	0.795	1.547
		NI	0.465	0.776	1.844
		NA	0.468	0.785	1.564
		CTRL	0.475	0.783	1.400
LD	50%	II	0.705	0.875	1.434
		IA	0.694	0.875	1.875
		NI	0.694	0.875	1.366
		NA	0.700	0.868	1.790
		CTRL	0.660	0.870	1.790
HD	15%	II	0.237	0.824	2.269
		IA	0.247	0.816	1.977
		NI	0.231	0.822	2.030
		NA	0.236	0.802	1.793
		CTRL	0.243	0.809	2.032
HD	50%	II	0.304	0.858	1.768
		IA	0.247	0.866	1.440
		NI	0.275	0.860	2.299
		NA	0.282	0.851	2.288
		CTRL	0.271	0.853	2.640

		Chi^2	P	Chi^2	P	Chi^2	P
--	Soil type	88.7	**<0.001**	0.1	0.706	1.9	0.170
--	Block	2.6	0.754	1.2	0.940	18.4	**0.002**
LD	SWC	39.5	**<0.001**	44.3	**<0.001**	3.5	0.062
HD		9.4	**0.002**	42.6	**<0.001**	1.0	0.317
LD-15%	Inoculum	0.3	0.989	2.3	0.68	2.3	0.675
LD-50%		1.0	0.913	2.6	0.64	12.0	**0.017**
HD-15%		1.6	0.816	8.0	0.09	2.2	0.691
HD-50%		2.8	0.596	6.1	0.20	2.1	0.712

The effect of the water regime on the dry weight of the plants was more prominent for plants grown in LD soil then in the HD soil (higher chi-square). The inoculation did not significantly influence the plant dry weight. The plant water content (PWC) was not affected by soil type or inoculation but differed significantly according to SWC. The average PWC was 79.64 % for plants grown in 15% SWHC and 86.28% for plants grown in 50% SWHC. The effect of SWC on the catalase activity in the flag leaves was almost significant (*P*=0.06) for plants grown in the LD soil but not for those grown in the HD soil. The inoculum treatment had a significant effect on catalase activity within the LD-50%

subgroup, with the leaves of plants inoculated with the II and NI inocula showing lower catalase activity (Table 1).

3.4.2 Bacterial and fungal community structure and diversity

As expected, irradiation had a very large impact on fungal and bacterial community structure, with LD and HD soil types clustering separately on each side of the first axis of the principal coordinates analysis (PCoA) ordination plots (Fig. 2). The effect of the SWC can be observed on the second axis, but only for bacteria in the HD soil. To confirm these visual trends, PERMANOVAs were performed for each soil type (LD and HD) separately (Table 2). These analyses showed a significant effect of the SWC on the bacterial community for both soil types. For the fungal community structure, the SWC treatment had an effect only within the HD soil, where a significant effect of the SWC x inoculum interaction term was also observed (Table 2). The shifts induced by the SWC treatment were also visible in the microbial community composition (Fig. 3), with, for example, a clear increase in the relative abundance of the *Actinobacteria* concomitant to a decrease in the relative abundance of the *Acidobacteria* when comparing the HD-15% to the HD-50% samples.

For bacteria, both the observed number of OTUs (S_{obs}) and the Shannon diversity index were affected by soil water content except for the Shannon index in the LD soil (Table 3). No inoculum effect was observed on the alpha diversity for the bacterial community. In contrast, for fungi, soil water content did not affect significantly any of the diversity indices. However, the inoculum almost significantly affected ($p=0.051$) the fungal S_{obs} in the LD soils, whereas the interaction term SWC x inoculum significantly affected fungal Shannon diversity for the HD soil.

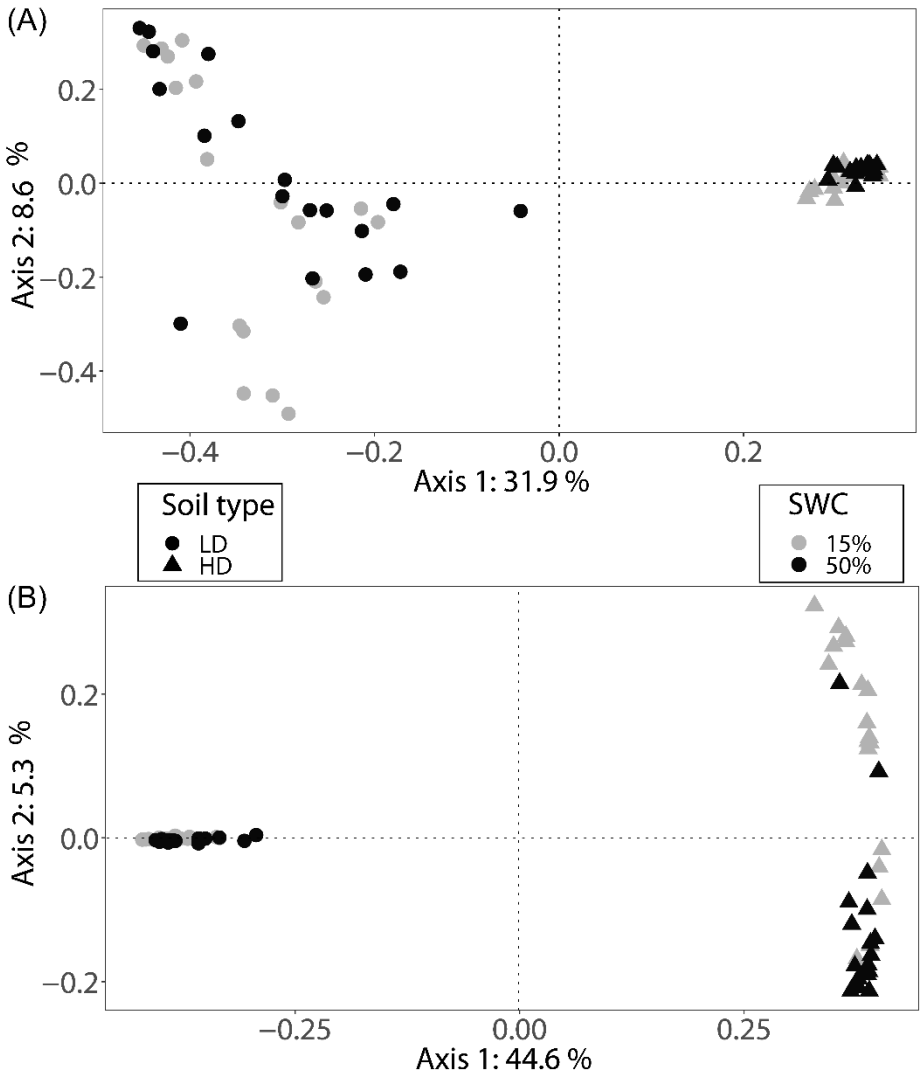


Figure 3.2 Principal Coordinate Analysis of the Bray-Curtis dissimilarity for all rhizosphere samples of the bacterial 16S rRNA gene (A) and the fungal ITS1 region (B) datasets.

Table 3.2 Two-way PERMANOVAs performed within each soil type subgroups for both bacteria and fungi based on Bray-Curtis dissimilarities. Results in boldface type are significant at *P*<0.05.

Soil		Bacteria		Fungi	
		F	P	F	P
LD	**SWC**	3.276	**0.001**	1.349	0.167
	Block	2.515	**0.001**	1.581	**0.011**
	Inoculum	0.939	0.563	0.761	0.815
	SWC*Inoculum	1.031	0.375	1.080	0.317
HD	**SWC**	10.092	**0.001**	4.585	**0.003**
	Block	1.574	0.062	2.307	**0.003**
	Inoculum	0.882	0.480	0.671	0.787
	SWC*Inoculum	1.122	0.301	2.396	**0.014**

Table 3.3 Two-way ANOVA tests conducted within each soil type subgroup for the species observed (s_{obs}) indicator of richness and the Shannon alpha diversity index. Results in boldface type are significant at *P*<0.05.

Soil	Effect	Bacteria				Fungi			
		S_{obs}		Shannon		S_{obs}		Shannon	
		F	P	F	P	F	P	F	P
LD	**SWC**	6.503	**0.018**	1.005	0.327	0.692	0.415	0.756	0.395
	Block	5.226	**0.003**	3.560	**0.017**	5.288	**0.003**	4.063	**0.010**
	Inoculum	0.638	0.538	0.363	0.699	3.450	0.051	2.786	0.086
	SWC*Inoculum	0.526	0.598	0.215	0.808	0.320	0.730	0.384	0.686
HD	**SWC**	23.915	**<0.001**	32.380	**<0.001**	0.650	0.428	3.241	0.084
	Block	2.380	0.069	3.700	**0.013**	1.312	0.291	1.802	0.149
	Inoculum	1.455	0.253	1.346	0.279	0.294	0.748	0.526	0.597
	SWC*Inoculum	1.411	0.264	2.323	0.120	2.829	0.078	4.620	**0.020**

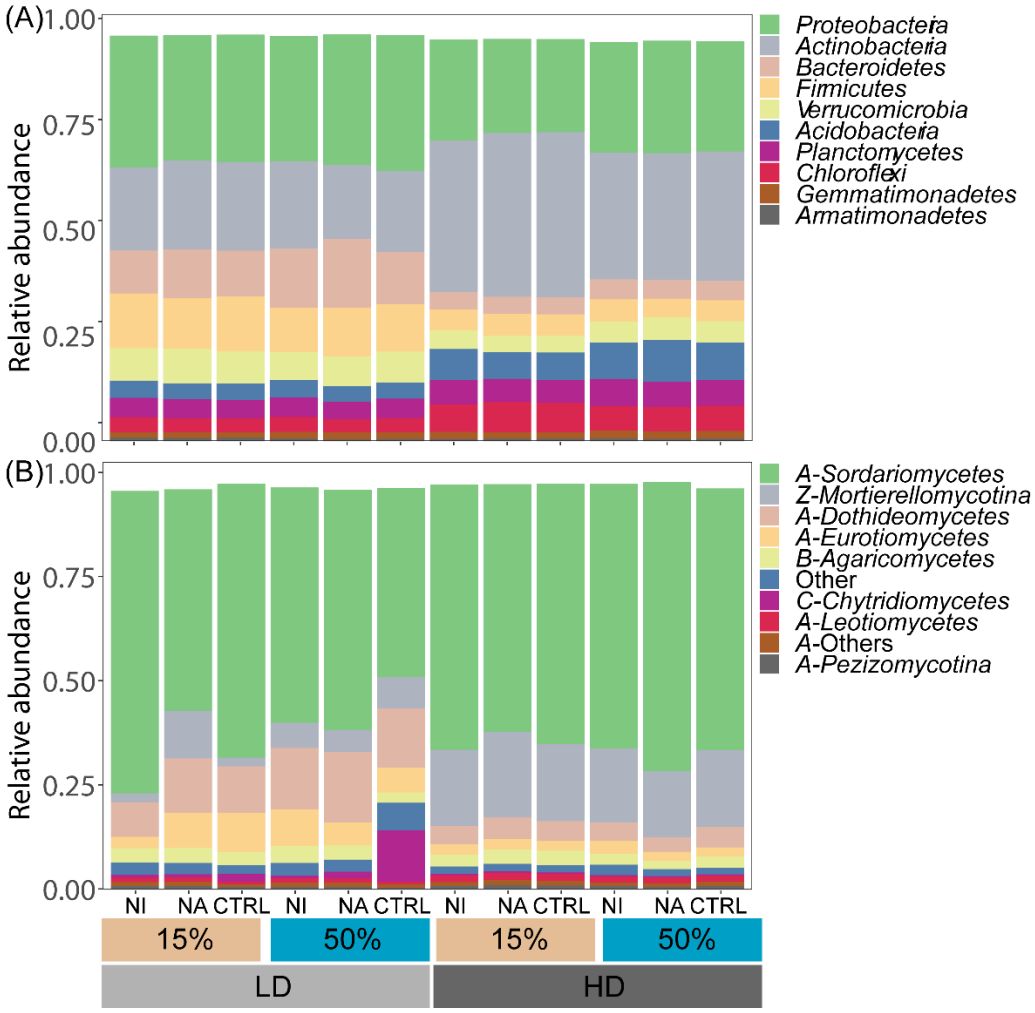


Figure 3.3 Relative abundance of the (A) 10 most abundant bacterial phyla and the (B) 10 most abundant fungal classes according to the different treatments (soil type, SWC, inoculum). Values represent the average of six replicates. In (B), A=*Ascomycota*, B=*Basidiomycota*, C=*Chytridiomycota* and Z=*Zygomycota*.

3.4.3 Similarity and shared OTUs between inoculated soils and their inocula

To uncover small shifts in the microbial communities that would have been missed using total community analyses, we tested if the inoculum and the rhizosphere they inoculated were more similar and shared more OTUs than it would be expected by chance. Bray-Curtis dissimilarity was calculated for each inoculum-inoculated rhizosphere pairs and compared to the values obtained when comparing the inoculum to the control rhizospheres. Significant negative values indicate that the community compositions shifted toward their inocula. Significant results were only observed for the fungal

communities in the LD soil inoculated with the NA inoculum (Table 4). The average shift in dissimilarity was of -0.04 (15% SWHC) and -0.06 (50% SWHC), representing 0.05-0.08% of the total dissimilarity between the samples. This probably explains why these shifts were not observable in the PCoA plots (Fig. 2) and in the PERMANOVA tests (Table 2). No significant shifts were found for bacteria across all treatments, and for fungi in the HD soils and for the NI inocula. This result indicates that, in low diversity (LD) soils, the fungal communities shifted toward the NA inoculum, but bacteria did not.

Similarly, the number of OTUs shared between each inoculum-inoculated rhizosphere pairs were compared to the values obtained when comparing the inoculum to the control rhizospheres. Bacterial communities from the inoculated rhizospheres shared more OTUs with their inoculum than the fungal communities, but this was never significantly different from number of OTUs shared between the control rhizosphere and the inoculum (Table 4). For fungi in the LD soil, in contrast, significantly more fungal OTUs were shared between the inoculated rhizosphere and their respective inocula as compared to the control rhizosphere and the inocula (except for the NI inoculum under 50% SWC; Table 4). This indicates that, in the low diversity (LD) soils, some members of the fungal communities were likely recruited from the inoculum, whereas this did not happen for bacteria.

Table 3.4 Bray-Curtis dissimilarity and number of shared OTUs between inoculated rhizosphere soil samples and their inoculum. Results in boldface type are significant at *P*<0.05.

			Dissimilarity Comparison		Shared OTUs	
Soil	**SWC**	**Inoculum**	**Bacteria**	**Fungi**	**Bacteria**	**Fungi**
LD	**15%**	**NI**	-0.001	-0.010	16.83	**13.00**
LD	**15%**	**NA**	0.006	**-0.042**	31.33	**16.60**
LD	**50%**	**NI**	0.002	-0.016	14.60	12.40
LD	**50%**	**NA**	-0.004	**-0.06**	34.83	**17.67**
HD	**15%**	**NI**	-0.002	0.002	31.17	16.50
HD	**15%**	**NA**	-0.002	-0.002	72.50	18.67
HD	**50%**	**NI**	0.005	0.001	32.83	14.67
HD	**50%**	**NA**	0.002	0.014	75.00	18.50

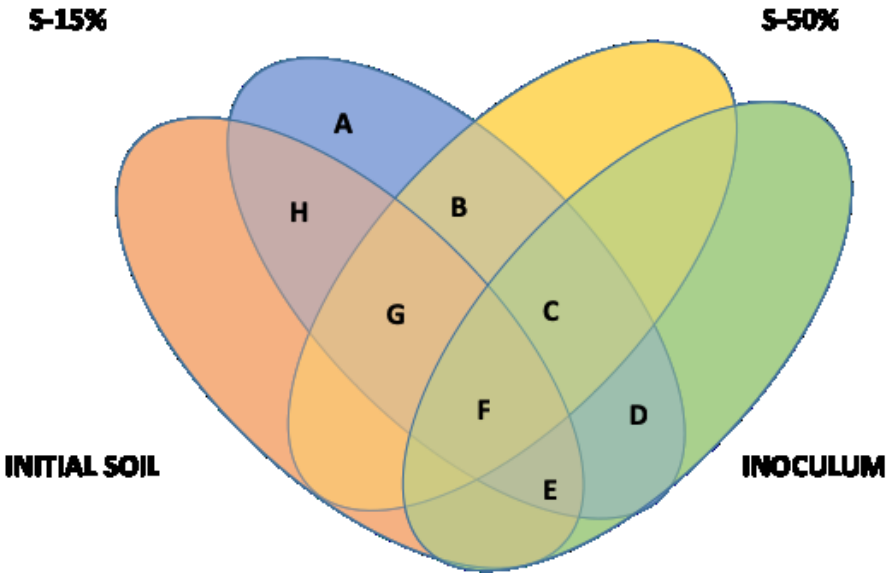
3.4.4 Source of the OTUs in the rhizosphere of wheat under water stress

As our results showed significant shifts in community composition and diversity with water stress, we sought to determine what proportion of the community in the rhizosphere of the water stressed plants was 1) already present in the rhizosphere before the stress, 2) recruited from the inoculum during the stress, 3) recruited from the bulk soil during the stress. Therefore, within each soil-inoculum combination, we compared the OTUs present in the rhizosphere of the 15% SWHC plants to 1) the ones in the rhizosphere of the 50% SWHC plants, 2) the ones in the inoculum, and 3) the ones in the potting soil at the beginning of the experiment. The relevant fractions are highlighted in the bottom panel of Table 5. In the rhizosphere of the 15% SWHC plants, 35-46% (bacteria) and 25-33% (fungi) of the OTUs were unique to the stressed plants (fraction A), with a larger proportion when plants were grown in the HD soil. These OTUs probably represent relatively rare undetectable taxa under normal condition that were relatively more abundant in the plant rhizosphere following water stress to a point where they could be detected. Not surprisingly, the more diverse HD soil had more such OTUs. The fractions B+C+F+G (43-56% for bacteria and 47-63% for fungi) represent the OTUs present in the rhizosphere of

wheat plants, regardless of the SWC. The relative abundance of these OTUs could have shifted positively or negatively during the water stress (amplification/reduction), leading to the patterns observed in Fig. 2 and 3. Fraction H (4-6% for bacteria and 1-9% for fungi) represents OTUs that were detectable in the original soil and were probably only recruited from the bulk soil by the plants under 15% SWHC. Fraction C (2-9% for bacteria and 4-10% for fungi) represents OTUs that were probably recruited from the inoculum both by 15% and 50% SWHC plants. Finally, fraction D (1-3% for bacteria and 5-7% for fungi) represents the OTUs probably recruited from the inoculum only by the 15% plants. Taken together, these results suggest that most changes in the rhizosphere communities of wheat plants under the 15% SWC treatment occurs through shifts in the OTUs already present in the rhizosphere before the stress (amplification/reduction). A small proportion of the OTUs were recruited from the soil and an even smaller part from the inocula, and this proportion was consistently larger for fungi as compared to bacteria.

Table 3.5 Proportion of shared OTUs between the 15% SWHC inoculated rhizospheres, their inocula, the 50% SWHC rhizospheres and the original potting soil. Letters refer to the area designated in the lower panel.

Kingdom	Soil	Inoculum	A	B	C	D	E	F	G	H
Bacteria	LD	NI	0.38	0.40	0.04	0.02	0.01	0.03	0.06	0.06
		NA	0.35	0.37	0.09	0.03	0.02	0.05	0.05	0.05
	HD	NI	0.42	0.23	0.02	0.01	0.00	0.05	0.23	0.04
		NA	0.46	0.17	0.04	0.03	0.01	0.09	0.15	0.05
Fungi	LD	NI	0.33	0.10	0.04	0.07	0.05	0.19	0.14	0.09
		NA	0.25	0.12	0.10	0.05	0.04	0.23	0.18	0.03
	HD	NI	0.32	0.19	0.05	0.07	0.01	0.15	0.18	0.01
		NA	0.33	0.19	0.05	0.05	0.02	0.11	0.23	0.03



3.5 Discussion

The overarching question of our study was: does an inoculum with a previous history of water stress improves plant tolerance to water stress? Within the parameters of our study, the answer to this question is no. The underlying assumption was that wheat plants subjected to water stress would recruit new beneficial microorganisms that had a previous exposure to water stress. Although it was not reflected on the plant phenotype, we did observe significant shifts in the microbial community following inoculation, but only for fungi and not for bacteria. The difference between fungi and bacteria could be due to their different biology, but also to the fact that fungi were significantly less diverse than bacteria. This would reduce the competition the fungal fraction of the inoculum has to face and reduce the potential taxonomical, functional and ecological overlap between the native and invasive fungal communities, which was shown to favor invasion success (Kinnunen et al., 2018). Alternatively, the higher bacterial turnover rates compared to fungi (Gunina et al., 2017), could potentially lead to a quicker extermination. Finally, bacteria-fungi competitive interactions can often be antagonistic (de Boer et al., 2005) and could also have negatively affected bacterial establishment.

We had further hypothesized that the microbial recruitment would be more successful (1) in soils with a low diversity (i.e. irradiated), (2) under water stress conditions, and (3) for water stress-adapted microorganisms. In this study, inoculation success was measured by a significant shift of the community toward its inoculum, either at the level of community similarity or shared OTUs, as reported in Table 4. The first hypothesis was confirmed, as the significant shifts of the rhizosphere fungal communities toward their inocula were only observed for the LD soil (Table 4). It is already well known in the field of community ecology that the level of resistance to biological invasion increases with the diversity of the local community (Shea & Chesson, 2002). In contrast, the second hypothesis was not supported by our data, as shifts were observed in the rhizosphere of both 15% and 50% plants (Table 4). This is surprising since the priority effect (Vanette & Fukami, 2014) was reported to be weakened by environmental stressors (Calderon et al., 2017), and we therefore thought that the water stress would increase the chances of the microorganisms of the inoculum to colonize the rhizosphere. It could be

that the magnitude of the water stress imposed here was not high enough to significantly alter the priority effect. The third hypothesis was partly proven, as the shifts of the rhizosphere fungal communities toward their inocula were of a larger magnitude and more often significant for the NA inoculum (Table 4). The underlying assumption behind this hypothesis was that the microorganisms from the water stress-adapted soil would be more beneficial for the plant under water stress, and therefore, preferentially recruited. Alternatively, traits for the adaptation to water stress might co-occur with traits related to rapid and aggressive growth in the plant environment.

In addition to the main results about inoculation, many other interesting trends were observed in the dataset. For instance, as compared to the high diversity (HD) soil, the rhizosphere microbial communities in the low diversity (irradiated, LD) soil showed a reduced response to the water stress, both for fungi and bacteria (lower F-ratio for the SWC effect, Tables 2 and 3). The irradiation of the LD soil probably favored the presence of opportunistic colonizers, spore-forming bacteria and other environmental hardy organisms that are known to resist to water stress (e.g. spore-forming; Ngumbi & Kloepper, 2016, *Firmicutes*; Xu et al., 2018). In fact, the *Firmicutes*, that contain many spore-formers, were relatively more abundant in the rhizosphere of plants growing in the LD soil as compared to the HD soil (Fig. 3). As previously reported, the fungal community was in general less disrupted than bacteria by the water stress (Hawkes et al., 2011; Barnard et al., 2013; de Vries et al., 2018) but, here again, the fungal community in the HD soil was more affected than in the LD soil. Interestingly, the interaction between the effects of soil diversity and soil water content was also visible for the plant phenotype but in reverse, with a much stronger decrease in the aboveground biomass and plant water content in the LD soil when exposed to water stress (Table 1). Many microorganisms can have a beneficial effect on plants when exposed to water stress (Yang et al., 2009; Chakraborty & Pradhan, 2012; Marasco et al., 2012), and the reduced diversity of the LD soils could have limited the choice of potentially beneficial partners the plant could associate with. Alternatively, irradiation results in massive cell death that increases nitrogen mineralisation (McNamara et al., 2003) which could explain the increased wheat biomass observed in the LD soils. The drought susceptibility of plant communities was

reported to increase with biomass (Wang et al., 2007), which could explain the larger decreases in biomass and water content for the larger plants growing in the LD soil.

The hologenome theory of evolution posits that holobionts can rapidly adapt/evolve through changes in the microbial partners, such as 1) amplification/reduction of the microorganisms already present, 2) recruitment of new partners from the environment and 3) recruitment of new genes from the environment through horizontal gene transfer (HGT) (Zilber-Rosenberg & Rosenberg, 2008). Our experiment was conceived as a potential hologenome-inspired approach to “rescue” plants when under abiotic stress. The wheat holobiont rapidly shifted in response to the decreased water content, with the microorganisms in the rhizosphere of the plants subjected to 15% SWHC being significantly different from the ones subjected to 50% SWHC. This difference was mostly due to shifts in the relative abundance of the associated microorganisms (amplification/reduction) as most microorganisms were present in the 15% and 50% rhizospheres. A small part of the rhizosphere community of the 15% SWHC plants was apparently recruited from the bulk soil (microorganisms present in the original potting soil but not seen in the rhizosphere of the 50% SWHC plants) and another small part appear to have been recruited from the applied inoculum. This suggests that the microbial response of well-established wheat holobionts to short-term water stress is mainly through amplification/reduction, i.e. shifts in the relative abundance of microbial partners that were already present before the occurrence of the stress. Interventions to improve plant resistance to water stress might therefore be more efficient if focused on the initial plant-soil microbial diversity. The most active period for plant microbial recruitment within the soil is indeed during the seedling phase (Micallef et al., 2009; Edwards et al., 2018) when the diversity is low, with the later stages being associated to an increased production of defense proteins (De la Peña et al., 2010) and a stable and more diversified rhizosphere microbial community. Accordingly, some studies have shown that exposing plants to different complex microbial communities early in their development leads to lasting effects on the plant phenotype (Yergeau et al., 2015; Lau & Lennon, 2011). However, the explicit goal of this study was to “rescue” plants subjected to water stress and these are often unpredictable and often happen when plants are well-established. Even under these unfavourable conditions, between 1-3% of the bacterial OTUs and between 5-7% of the

fungal OTUs detected in the rhizosphere of stressed plants were probably recruited from the inoculum.

3.6 Conclusion

The goal of this study was to test if an inoculum with a water stress history would improve plant tolerance to water stress. Generally, the inoculations did not modify the plant phenotype, nor the bacterial communities, but it did significantly alter the fungal communities. The quantity of inoculum and the timing of its application could be optimized, but ample theoretical and empirical evidence suggest that resident microbial communities will probably have the upper hand. Since 1) the bulk of the plant-associated microbial response to water stress was due to shifts in already present members of the rhizosphere community and 2) the soil initial diversity strongly affected the response of the rhizosphere microbial communities to water stress and modulated the plant phenotypic response, then "rescuing" plants suffering from abiotic stress using microbially-driven strategies could be targeted at designing the initial soil communities. This would in turn affect which organisms would be amplified/reduced during stressful events. Alternatively, harnessing naturally evolved HGT mechanisms to modify the resident community could be a promising approach.

3.7 Acknowledgements

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CONCLUSION

The goal of this thesis was to test a microbial-based method to help drought sensitive wheat plants under water stress conditions. Specifically, we tried supplementing the plant's rhizosphere with a microbial community extracted from a soil with a long-term history of exposure to drought. For that purpose we designed a pot experiment with three factors: initial potting soil type (normal or with a reduced microbial diversity and richness), soil water content (normal or water stressed) and inoculum treatment (the microbial community extracted from a soil with a history of water stress or with no history of water stress). The main hypothesis was that water-stressed plants would be more receptive to the inoculation compared to the not-water-stressed plants and that it would be more prone to recruit the drought-adapted microbial community compared to the other non-drought-adapted microbial community. The other hypothesis was that the reduced initial microbial diversity and richness caused by the irradiation of the potting soil and by the water stress would create an optimal environment for the colonization of the exogeneous microbial community from the inoculum.

From our results, we observed that a small portion of the fungal species from the inoculums successfully and consistently persisted in the rhizosphere and that this translated in a small but significant shift in the fungal community structure. The inoculum effect was spread inconsistently amongst treatments, meaning it was not associated to a specific potting soil or soil water content. When looking at the distribution of the water-stressed and inoculated rhizosphere microbiota as compared to the not-water-stressed inoculated plants, we found that, although the community structure was significantly different, most microorganisms were either present in both or shared with the bulk soil, meaning that the community shift was more related to amplification and or reduction of already present species rather than recruitment of novel species. Still, a small fraction of both the bacterial and fungal community originated from the inoculum. The small inoculum effect observed on the microbial community did not translate into changes in the measured plant phenotype variables.

The method tested in this experiment did not succeed at helping wheat plants tolerate the water stress. These results could largely be attributable to the chosen methodology and many of the methods used in the experiment could be refined, modified and tested again. One important aspect that could be reconsidered is the soil used to prepare the inoculum. It would be interesting to redo the experiment but instead pre-expose a soil to drought iteratively but in controlled greenhouse condition. Another important aspect of the experiment was the extraction protocol used to prepare the inoculum. Extracting most of the living microbial community from one soil is a challenge and different methods could be used (Wagner et al., 2014), such as sonication and extraction buffers to try and optimize the amount and diversity retrieved. It would also have been interesting to quantify the number of living cells in each inoculum after the extraction process. The inoculation protocol is also an important factor that could have influenced the success of the colonization by the exogeneous microbial community (Parnell et al., 2016). It would be interesting to test different timing (relative to the plant growth stage), different amount of inoculum (relative to the living cell count) and maybe test repeated inoculation. An improved experiment could include measures of an extended amount of plant parameters, including root morphology and biomass, osmolyte concentration and stomatal conductance to have a refined portrait of the plant phenotype under drought. Another important aspect is the timing of the sampling. One fastidious but interesting thing to do would be to sample the rhizosphere microbiota at multiple time points, which would provide us with a more detailed picture of what happened after the inoculation event. Letting some of the plants produce seed would have also enabled us to measure the most important agronomic variable, yields. Lastly, an important aspect largely minimized when studying the coalescence of two microbial communities such as in an inoculation event, is the possibility of horizontal gene transfer (HGT) (Rillig et al., 2016). The rhizosphere area is known to be a hotspot for HGT, because of the high microbial activity and nutrient content (Aminov, 2011). As a side project to this thesis, a second very similar experiment was performed, but using DNA extracts from the same soils as the inocula. Metagenomics was performed to observe any pattern of HGT within the rhizosphere microbiota and analyses are ongoing.

Due to climate change, Canadian wheat crops will increasingly suffer from drought episodes in the future (Schindler & Donahue, 2006). We know from previous experiments that crops grown on land exposed repetitively to drought receive help from their rhizosphere microbiota (Azarbad et al., 2018). Still, more work is needed to try and find ways to improve plants drought resistance abilities. The omics era completely changed our way of perceiving soil life and plant-microbe interactions. We are now able, with only a few grams of soil, to dress a detailed portrait of the microbial community. At the same time new methods are constantly proposed such as metabolomics and even metaphenomics (Jansson et al., 2018). In our case, a finer scale or an interdisciplinary approach might indeed be needed to better understand the tenants of the priority effects and of microbial community coalescence, so we can overcome it. To reach the goal of predictable microbiota engineering in the field, there is also a need to develop modeling tools that can incorporate variables such as regionality in soil characteristics (Toju et al., 2018). Until then, empirical methods such as the use of cover crops to promote beneficial microbiota are investigated (Coleman et al., 2014) and might have better chances of success.

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ANNEXE I: SUPPLEMENTARY FIGURE

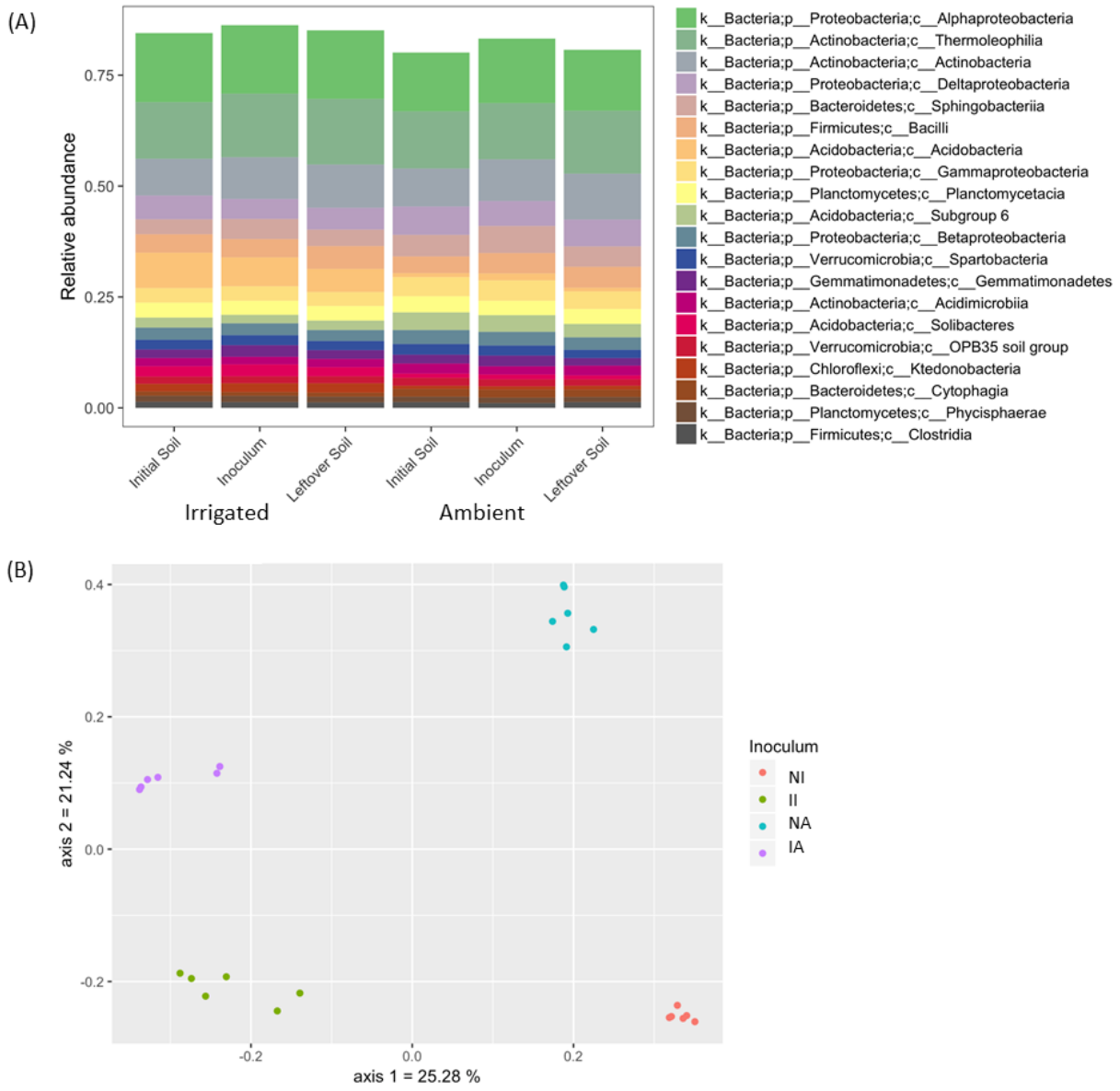

Figure S1. Comparison of the inocula with the soil used to make them. (A) Relative abundance of the 20 most abundant classes for the NI (irrigated) and NA (ambient) inocula, the soils used to make them, and the leftover soil/sludge after the water extraction process. (B) Principal Coordinate Analysis of the Bray-Curtis dissimilarity for the four inocula (II, IA, NI, NA), the soils used to make them, and the leftover soil/sludge after the water extraction process. Each sample was sequenced in duplicate, resulting in 6 samples per inoculum

ANNEXE II: SOMMAIRE RÉCAPITULATIF

Revue de littérature

Le concept de l'holobionte est utilisé pour décrire un organisme complexe (exemple : un humain ou une plante) et toute la communauté microbienne qui y est associée (le microbiote). En microbiologie végétale, le terme holobionte est de plus en plus utilisé depuis sa première apparition dans la littérature scientifique en 1991 dans le livre de Lynn Margulis. Pour les fins de ce mémoire, le concept d'holobionte est utilisé pour décrire la plante et son microbiote, sans aucun sens évolutif implicite.

Le blé est une composante essentielle à l'alimentation humaine et représente environ 20% des protéines consommées mondialement. Bien que plusieurs scientifiques aient étudié le microbiote du blé, l'identification d'un microbiote commun aux différentes variétés de blé reste à faire, advenant qu'un tel microbiote existe. Plusieurs études ont en revanche identifié de multiples paramètres environnementaux, dont la sécheresse, influençant la composition taxonomique du microbiote de plants de blé.

Il existe plusieurs définitions de ce qu'est une sècheresse, en fonction de si celle-ci est étudiée d'un point de vue météorologique, agricole ou économique. Dans le contexte de cette mémoire, la sècheresse est définie du point de vue agricole comme l'absence de précipitation pendant une période de temps suffisamment longue pour que les cultures non irriguées souffrent de stress hydrique. On dit que d'ici 2050, plus de 50% des terres arables du monde seront exposés à des épisodes annuels de sècheresse. Dans les prairies canadiennes, lieu central de la production de grain au Canada, certains ont prédit à l'aide d'outils de modélisation qu'une crise de l'eau était susceptible de se produire dans un avenir proche, en raison de la baisse des niveaux d'écoulement dans les principaux cours d'eau, l'augmentation des épisodes de sècheresse et l'augmentation de la demande en eau. Il serait donc bénéfique pour le secteur agricole de trouver des méthodes novatrices afin de rapidement venir en aide aux plants en manque d'eau.

Lorsqu'une plante est soumise à la sècheresse, celle-ci peut tenter de résister au stress hydrique en résultant au moyen de différents mécanismes d'ordre physiologique, métabolique ou hormonal. Les changements dans la configuration des feuilles et racines sont les principales modifications physiologiques observées en réponse au stress hydrique. Au niveau métabolique, certaines plantes peuvent accumuler des composés organiques dans le but de réduire leur potentiel osmotique ou augmenter l'activité enzymatique antioxydante afin de réduire la présence des espèces réactives d'oxygène. Certaines phytohormones, telles que l'auxine, l'éthylène, l'acide abscissique et les cytokinines sont également impliquées dans les mécanismes de tolérance à la sècheresse.

La sècheresse affecte aussi le microbiote de la rhizosphère de la plante. La diminution des niveaux d'eau entraîne une baisse de la disponibilité des nutriments et des conditions aérobiques dans les sols, un environnement qualifié d'oligotrophe. Face à la sècheresse, les microorganismes du sol doivent composer avec le stress osmotique, des dommages au niveau de l'ADN et une concurrence accrue pour les ressources. Règle générale, les bactéries sont plus sensibles que les champignons à la déshydratation et un ratio plus élevé de champignons sur bactéries est souvent observé lors de la sécheresse. Diverses stratégies physiologiques et métaboliques permettant aux champignons et aux bactéries de résister à la sècheresse, telles que la production d'osmolytes et la formation de structures résistantes à la sècheresse comme les spores.

La sècheresse est un moteur important d'adaptation pour la communauté microbienne du sol et peut avoir des effets à long terme sur celle-ci. En effet, l'exposition itérative d'une communauté microbienne du sol à un même stress abiotique peut influer la façon dont cette communauté fait face à un stress ultérieur, un concept appelé « mémoire du sol ». En ce qui concerne la sècheresse, quelques études ont démontré que l'historique des précipitations d'un sol a un impact sur la réponse des communautés microbiennes au régime d'eau contemporain. Il a même été démontré que cette mémoire du sol pouvait améliorer la résistance à la sècheresse de plantes cultivées dans un sol avec un historique de sècheresse.

Afin de bien comprendre l'effet d'un stress abiotique comme la sècheresse sur des plants de blé, il est aussi important de prendre en compte les interactions entre la plante et son microbiote. En effet, plusieurs études ont démontré l'impact de la sècheresse sur les interactions à l'intérieur de l'holobionte de la plante. La quantité d'exsudats produits par la plante peut par exemple varier en fonction de la disponibilité de l'eau, menant à des changements dans le microbiote de la rhizosphère. Les membres microbiens de l'holobionte de la plante sont aussi responsables de nombreuses fonctions importantes reliées à la capacité d'une plante à résister à la sècheresse. Toutefois bien que beaucoup de microbes ont été corrélés à différents changements physiologiques ou hormonaux dans la plante, très peu de mécanismes ont été expliqués en détail. Tout de même, plusieurs chercheurs étudient le potentiel de l'ingénierie du microbiote, entre autres pour améliorer la résistance des plantes à la sècheresse. Jusqu'à récemment, les chercheurs ont principalement utilisé des isolats ou des consortiums microbiens de quelques souches différentes pour inoculer des semences, du sol ou des fleurs. Bien que plusieurs expériences aient donné les résultats escomptés en conditions de laboratoire, les résultats ont souvent été décevants lors d'expériences menées au champ. Ceci est en partie dû au fait que les communautés microbiennes travaillent en réseau et en associations et parce qu'une fonction écologique désirée, tel que par exemple la suppression des pathogènes, ne se retrouve pas nécessairement dans un seul microorganisme, mais plutôt dans un consortium de microorganismes.

Hypothèses et objectifs

En résumé, il a déjà été démontré que les épisodes de sècheresse représentaient un moteur d'adaptation pour toutes les composantes de l'holobionte d'une plante: la plante hôte, le microbiote et les interactions plante-microbes. La sècheresse peut aussi mener à une modification à long terme de la communauté microbienne du sol, menant à son tour à l'amélioration de la résistance à la sècheresse des plantes cultivées dans ce sol. Enfin, nous savons que la rhizosphère d'une plante est un environnement très sélectif.

L'hypothèse principale était que les plantes maintenues en condition de stress hydrique seraient plus réceptives à l'inoculation par rapport aux plantes cultivées avec

une teneur en eau du sol optimale, et que celles-ci seraient plus enclines à recruter une communauté microbienne préadaptée à la sècheresse comparée à une communauté microbienne non préadaptée à la sècheresse.

L'objectif de ce mémoire était de tester une méthode basée sur l'inoculation microbienne afin d'aider des plants de blé en condition de stress hydrique. Plus précisément, nous avons essayé de bonifier la rhizosphère de la plante avec une communauté microbienne extraite d'un sol ayant un historique de sècheresse.

Méthodologie

Une expérience multifactorielle en pot a été conçue. Les trois facteurs étaient 1) le type de terreau initial utilisé (normal ou avec une diversité et une richesse microbienne réduite), 2) la teneur en eau du sol (condition optimale pour la plante ou condition de stress hydrique) et 3) l'inoculum (la communauté microbienne extraite d'un sol avec un historique de sècheresse ou sans antécédents de sècheresse). Les plants de blé ont été cultivés dans une serre pour un total de neuf semaines. Sur la base des résultats de nos expériences antérieures, nous avons établi la condition de stress hydrique à 15% de la capacité de rétention d'eau du sol et le régime optimal de l'eau à 50%. À la sixième semaine, les pots ont été inoculés. À la neuvième semaine, l'expérience a été terminée et échantillonnée. Différents paramètres ont été mesurés sur la plante. Afin d'étudier le microbiote de la rhizosphère, un échantillon de sol a été récupéré dans chaque pot. L'ADN total a été extrait et la région V4 de l'ARNr 16S pour les bactéries et la région ITS pour les champignons ont été séquencées avec la technologie Illumina MiSeq.

De nos résultats, nous avons observé qu'une petite partie des espèces fongiques provenant des inoculations avaient persisté dans la rhizosphère des plants et que cela s'était traduit en un changement faible, mais significatif, dans la structure de la communauté fongique. Selon nos analyses, l'effet de l'inoculation n'était pas associé à un terreau spécifique ou à la teneur en eau du sol. Lorsqu'on regarde la composition du microbiote de la rhizosphère de plants inoculés et en condition de stress hydrique et qu'on le compare au microbiote des plants inoculés sans stress hydrique, nous avons constaté

que, bien que la structure de la communauté microbienne fût significativement différente, la plupart des microorganismes étaient soit présents dans les deux ou partagés avec la communauté microbienne du terreau initial. Cela signifie que les changements dans la communauté microbienne étaient plus liés à l'amplification et la réduction d'espèces déjà présentes plutôt qu'au recrutement de nouvelles espèces. Une petite fraction de la communauté bactérienne et fongique provenait tout de même de l'inoculum. Le petit effet de l'inoculum observé sur la communauté microbienne ne s'est toutefois pas traduit par des changements au niveau du phénotype de la plante. La méthode testée dans cette expérience n'a donc pas réussi à aider les plants de blé tolérer le stress hydrique. Ces résultats pourraient être en grande partie attribuables à la méthodologie choisie et bon nombre des méthodes utilisées dans l'expérience pourraient être affinées, modifiées et testées à nouveau.

En raison du changement climatique, il y à prévoir que de plus en plus d'épisodes de sècheresse affecteront la culture du blé au Canada. Des expériences précédentes ont déjà démontré que les plantes cultivées sur des terres répétitivement exposées à la sècheresse reçoivent de l'aide de leur microbiote. Pourtant, plus de travail est nécessaire afin d'essayer de trouver des méthodes applicables au champ qui permettraient d'améliorer la résistance à la sècheresse des plants. Les technologies « omiques » ont complètement changé notre façon de percevoir les interactions plante-microbe et la vie du sol. Nous sommes maintenant en mesure, avec seulement quelques grammes de sol, d'avoir un portrait détaillé de la communauté microbienne. En même temps, de nouvelles méthodes utilisant des échelles d'observations de plus en plus précises et détaillées sont constamment proposées telles que la métabolomique et même la metaphénomique. Afin d'être capable de prédire les effets de l'ingénierie du microbiote, il est également nécessaire de développer des outils de modélisation intégrant les multiples caractéristiques d'un sol. D'ici là, les méthodes plus générales telles que l'utilisation de cultures de couverture pour promouvoir une communauté microbienne bénéfique sont étudiées et pourraient avoir de meilleures chances de succès.