Characterization of soil microbiome associated with soybean and soybean cyst nematode (*Heterodera glycines*) in Eastern Canada

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List of abbreviations

AAFC : Agriculture and AgriFood Canada

AMF: Arbuscular mycorrhizal fungi

CEC: Cation-exchange capacity

CHU: Corn Heat Units

db-RDA: distance-based redundancy analysis

GHG: Greenhouses gas

ISR: Induced systemic resistance

ITS: Internal Transcribed Spacer

ON: Ontario

PGPR: Plant Growth Promoting Rhizobacteria

PPN: Plant parasitic nematode

QC: Québec

QTL: Quantitative trait loci

SCN: Soybean cyst nematode

Abstract

Soybean is already well established in Canada and acreages are still increasing rapidly in many provinces, including Quebec. This crop offers many advantages to growers, obviously economically, but also because of the ecological services it renders such as nitrogen fixation. However, this expansion in cultivated area has also favored the spread of the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe (1952). This plant-parasitic nematode is present in Ontario since 1987 and has been discovered for the first time in Quebec in 2013.

Currently, the only viable way to manage SCN is the use of resistant soybean cultivars and rotation with non-host crops. This method has proven to be effective to reduce SCN populations to a low level but is in no way able to eradicate these nematodes. However, since the resistance is only partial, SCN populations quickly adapt and overcome the mechanism involved. Therefore, there is a need to find new ways to fight this pest before the current pest management approach become completely useless.

One of these possibilities is based on studies that have demonstrated the nematicidal properties of soils where soybean was cultivated in monoculture for a long period of time. These so-called suppressive soils were shown to contain microorganisms, including bacteria and fungi that demonstrated antagonistic effects on SCN.

Using a metagenomic approach, the present study characterized for the first time the soil microbiome associated with soybean in Eastern Canada. Additionally, the internal microbiome of SCN cysts (cystobiomes) was studied. Results seem to indicate that soybean strongly influence the soil microbiome on a short period of time and that the cystobiome is different from the soil microbiome. The data analysis made to fulfill the two primary objectives of this study also revealed possible new ways to manage SCN infestations. Amongst them, high aluminium concentrations in the soil and simultaneous presence of specific bacterial and fungal species seem to be negatively correlated to the viability of SCN.

Résumé

La culture du soya est très bien établie au Canada et elle ne cesse de prendre de l'ampleur en termes de superficies cultivées dans plusieurs provinces, incluant le Québec. Par contre, malgré les nombreux avantages que cette culture offre aux agriculteurs, que ce soit économiquement parlant ou encore grâce aux services écologiques qu'elle offre tel que la fixation d'azote, un problème est présent. Le nématode à kystes du soya (NKS), *Heterodera glycines* Ichinohe (1952), est présent en Ontario depuis 1987 et a été découvert pour la première fois au Québec en 2013.

Présentement, la seule façon viable de gérer le NKS est d'utiliser des cultivars de soya résistants en rotation avec des cultures non-hôtes. Cette méthode est efficace pour maintenir les populations de NKS à des niveaux modérés mais ne parvient en aucun cas à éradiquer ces nématodes des champs infectés. Cependant, comme la résistance des cultivars utilisés est partielle, les populations de NKS s'adaptent rapidement à cette dernière et contournent les mécanismes impliqués. Il y a donc un besoin de trouver de nouvelles façons de combattre ce ravageur avant que le présent système de rotation devienne complètement inutile.

Une des possibilités envisageables repose sur plusieurs études qui ont démontré les propriétés nématicides de sols où le soya a été cultivé en monoculture durant de longues périodes. Ces sols dits suppressifs sont connus pour contenir des microorganismes spécifiques, notamment des bactéries et des champignons, qui ont eux-mêmes des effets nématicides.

En utilisant une approche métagénomique, la présente étude a permis la caractérisation du microbiome des sols associés au soya dans l'est du Canada, ce qui à notre connaissance n'avait jamais été fait auparavant. Aussi, le microbiome de l'intérieur des kystes de NKS (cystobiomes) a été étudié. Les résultats obtenus semblent indiquer que le soya influence fortement le microbiome du sol sur une très courte période de temps et que le cystobiome est différent de celui du sol. L'analyse des données recueillies pour atteindre les deux objectifs principaux a également mis en lumière de possibles nouvelles façons de gérer les infestations de NKS. Parmi celles-ci, de fortes concentrations d'aluminium dans le sol et la présence simultanée de certaines bactéries et champignons dans celui-ci semblent être négativement corrélés à la viabilité du NKS.

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Contributions of authors

Many persons have contributed in several ways to the writing of the various sections of this thesis:

Chapter “2: Literature review”, was written by Guillaume Trépanier.

For chapter “4: Soil microbiome associated with soybean in Canada and its relation with soybean cyst nematode development”, Guillaume Trépanier actively participated to the complete manipulation process necessary to the realisation of this study, both in the field and in laboratory. He also performed the data analysis and wrote the manuscript, which was reviewed by Dr Benjamin Mimee and Dr Valérie Gravel. Both were also involved in supervising the project and provided support to the student. Etienne Lord and Pierre-Yves Véronneau, both research assistants at AAFC, helped with the data analysis.

1. Introduction

In recent years, more and more Canadian producers started incorporating soybean in their crop rotation systems. Ontario was the first province to grow this crop and still is to this date the biggest producer of the country (Statistics Canada, 2018). However, other provinces such as Quebec and more recently Manitoba and Saskatchewan have followed up and are showing a dramatic increase in term of production for this specific crop (Statistics Canada, 2018). There is nothing surprising about this considering all the benefits producers get from growing soybean, varying from enhancing the nitrogen fixation in soil, the versatility of its usages and its high value on the market (Trostle, 2010; Wright and Wimberly, 2013). On top of all, climate change is opening new regions for soybean and breeders are constantly developing new varieties that tolerate cold temperatures, thus allowing northern regions to grow it (Voldeng *et al.*, 1997; Egli and Bruening, 2000; Gendron St-Marseille *et al.*, 2019).

While the Canadian soybean market is thriving, the soybean cyst nematode (SCN) is casting a shadow on the industry. It is known to be the worst pest affecting soybean worldwide. It was first detected in the USA in 1954 and then quickly spread to all soybean growing areas of the country (Winstead *et al.*, 1955; Tylka and Marett, 2017). It was discovered in Ontario in 1987 (Anderson *et al.*, 1988) and spread to Quebec in 2013 (Mimee *et al.*, 2014). Damages caused by this pest are important and can reach 30% of yield losses, even before the first symptoms appear (Niblack, 2005). Once this nematode is present in a field, it is virtually impossible to get rid of it (Niblack and Tylka, 2012). Thus, managing this pest as early as possible is mandatory in order to limit the SCN population build up in an infected field. However, beside a crop rotation system with resistant soybean cultivars and non-host crop, there are not many effective options that are available for growers (Niblack and Tylka, 2012). SCN has been shown to overcome the resistance of cultivars in several instances and new ways to manage this pest are needed (Young, 1992; Niblack, 2005). One way to do so could be the use of antagonistic organisms. Several studies showed that some bacteria and fungi were effective to repress SCN development and some were already made commercially available, although their actual efficacy in the field and effect on the balance of the indigenous soil communities is not well described (Kerry, 2000; Xiang *et al.*, 2017).

The recent development of high throughput sequencing techniques allowed the gathering of huge amount of soil microbiome data. However, information about soybean soil microbiome is still scarce in Canada and very few studies have been conducted on the cyst microbiome of any species. Considering these premises and the opportunity to sample SCN infestations in two distinct areas, this thesis tried to deepen the knowledge on these subjects by fulfilling the following objectives.

2. Literature review

2.1 Soybean crop

The cultivated soybean, *Glycine max* (L.) Merrill, is part of the *Fabaceae* family, in the subfamily of the *Papilionoideae* and more precisely in the *Phaseoleae* tribe. It is a d tetraploid (2n=40) annual herbaceous and can reach as high as 1.5 meters under good conditions (Bernard and Weiss, 1973, Canadian Food Inspection Agency, 2012). Soybeans are harvested for their seeds, rich in oil and proteins, and are used by the food industry and the industrial sector. Soybean can be transformed into purified oil or used in a large variety of food products, the most known being tofu and soy sauce. It is also used in simulated milk and as a supplement in livestock feed rations. In the industrial sector, soybean can be used to produce disinfectants, soaps and biodiesel (Schmitt, 2004; Agriculture and Agri-Food Canada, 2015).

Soybean is produced all around the globe, from north to south (Fig. 1). However, this crop is produced in a much greater quantity in the Americas, with Brazil and USA being by far the biggest world producers, followed by Argentina. Three others American countries are part of the top 10, including Canada which is taking the 7th spot (United States Department of Agriculture, 2016). Although the soybean cultivation area in Canada is still significantly lower than the top producing countries, its increase is exponential and this trend will probably continue (Fig. 2). In 2018, the province of Ontario produced 57% of the Canadian soybean followed by Manitoba with 21% and Quebec with 16% (Statistics Canada, 2018).

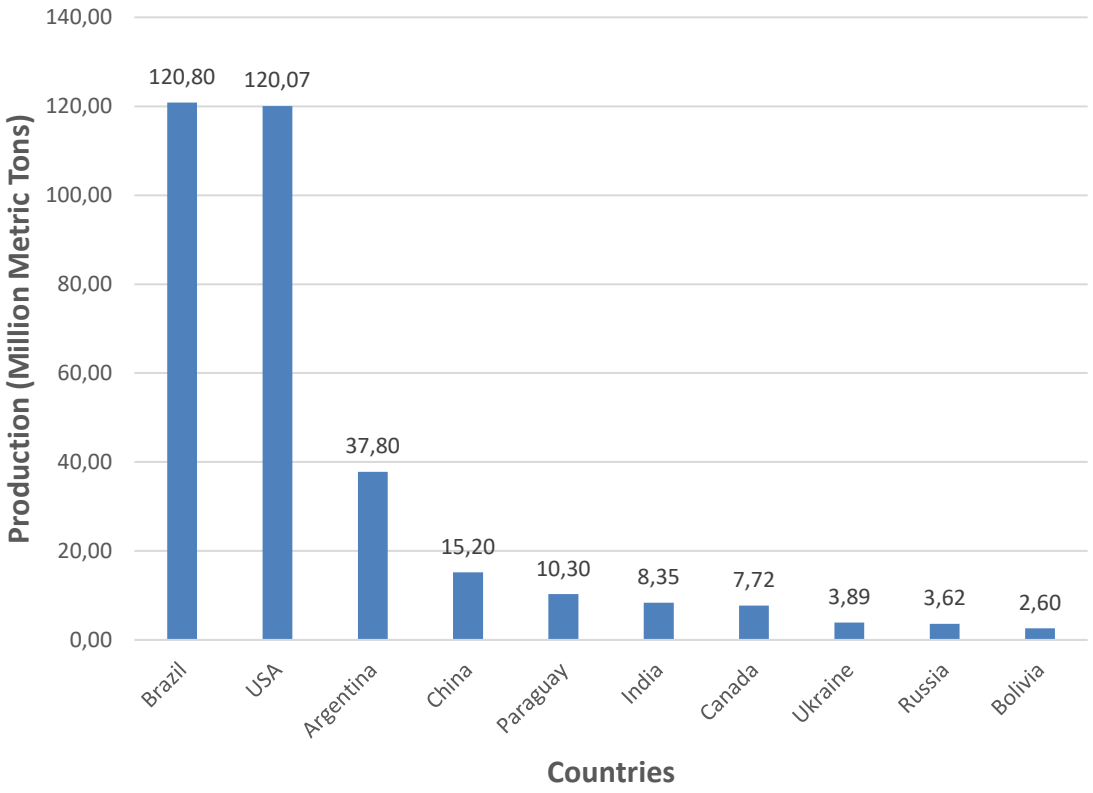

Fig. 1: Soybean production data (compiled in August 2016) of the top 10 producer countries for the 2015-2016 year (United States Department of Agriculture, 2016).

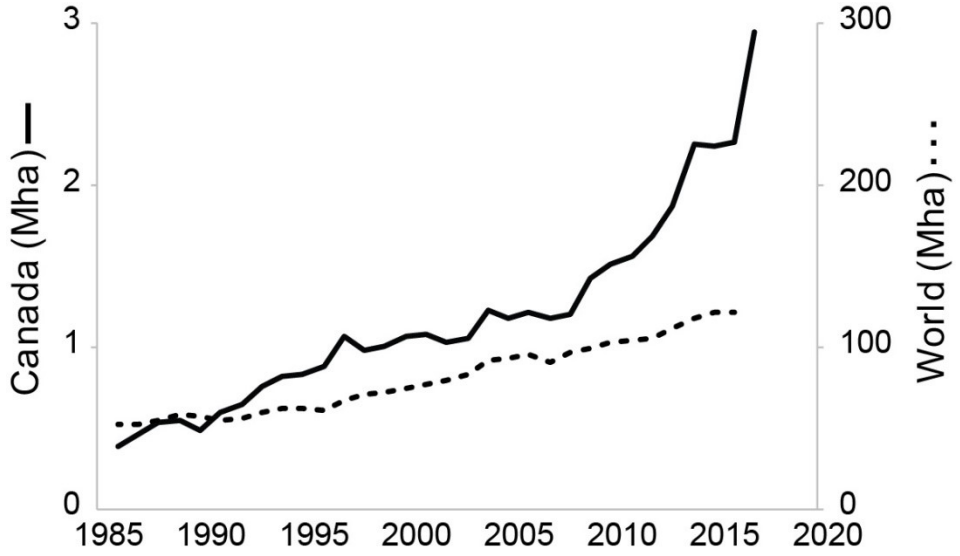

Fig. 2: Soybean cultivation area (in Mha) around the world (dotted line) and in Canada (full line) since 1985 (Statistics Canada, 2018; FAO. Food and Agriculture Organization of the United Nations, 2019).

The advantages of growing soybean are numerous, the main one being the enhanced nitrogen fixation. While on the short term, chemicals fertilizers can be more effective to deliver nitrogen to the plant, incorporating soybean in crop rotations for several years can lead to a significant increase in nitrogen fixation (Peoples *et al.*, 1995). The reason for this high effectiveness lies in the symbiotic relationship *G. max* has with *Bradyrhizobium japonicum*, an endosymbiont Gram-negative bacterium that lives in soil. *B. japonicum* colonizes root cells and then induces the formation of a structure called nodule. This structure allows the plants to assimilate the NH_2 by transforming it into NH_3 (People *et al.*, 1995). Nodules also catalyze the nitrogenase system and therefore cause an increase of the dinitrogen (N_2) to ammonium (NH_4^+) fixation efficiency. Both of those forms can be easily assimilated and retained by the plants. After harvest, decomposed soybean roots left in the soil are bringing available nitrogen to the next crop (Hennecke, 1990).

On top of helping farmers to have a healthy soil and great yields by naturally enriching the field, growing soybean allows them to get a considerable monetary income at the end of the summer. Soybean price has remained high for several years and exportation alone generated revenues of $CAN 3.2 billion for growers in 2015 (SOY Canada, 2018). A report conducted by

MNP (2016) for Soy Canada concluded that the soybean industry has a major economic impact in the country. Activities related to this industry have created a direct output of $CAN 5.8 billion in 2014. By including the $CAN 6.9 billion of indirect output, this industry generated a total of $CAN 12.7 billion. Soybean industry also provided many jobs with 34 996 employments in Ontario and 11 569 in Quebec (MNP, 2016). On top of that, simulations of future climate demonstrate that the length of the growing season will increase in all regions of Quebec, allowing growers to plant soybean in new areas in the coming years (Gendron-St-Marseille, 2013). This will also be facilitated by the early-maturing soybean lines developed by Canadian breeders that are more adapted to the northern latitudes (Egli, 1993; Rincker *et al.*, 2014). Considering all this, it is clear that soybean is an important crop in Canada that will continue to be for a long time.

2.2 Soybean cyst nematode

2.2.1 Life cycle

Heterodera glycines Ichinohe (1952), also known as the soybean cyst nematode (SCN), is part of the phylum *Nematoda*. It is in the *Tylenchida* order and in the *Heteroderidae* family (CABI. Invasive Species Compendium, 2008). SCN is an obligatory sedentary endoparasite of plant roots (Niblack *et al.*, 2006). When the larvae infect a root, they move in it, undergo two molts and then create a giant feeding cell called the syncytium, providing them all the energy needed at this specific site. The larvae do not move afterward. As the development continues, the female body will become large enough that it emerges from the root and get exposed to the soil. Then, after mating with males, females lay a part of their eggs in a gelatinous matrix in contact with soil and keep another part in their body (Maggenti, 1981; Niblack *et al.*, 2006). In average, a single female can produce 200 eggs, being split between the matrix and the cyst (Niblack, 2005). The body of the dried dead female will form a cyst protecting the eggs and will eventually detach from the plant root. Thus, at least a fraction of the offspring will be protected inside the cyst and will only hatch after a latency period when environmental conditions will be favorable again (Niblack *et al.*, 2006). SCN eggs have been shown to survive in soil as long as eleven years inside the cyst (Riggs *et al.*, 1993).

During its life cycle, SCN needs to complete four molts in order to reach the adult stage. Three of them are between juvenile stages (J1 to J4) and the first one is taking place in the egg (Fig. 3). Sexual differentiation begins in the J3 stage as the female slowly begin to become more and more swollen while the male remains vermiform. At J4, distinction between both genders is clear (Plant Health Australia, 2013). The temperature drastically affects the life cycle of this species, making it shorter when it is hotter, taking only 18 days to complete a cycle at 31 ºC. Eggs do not hatch anymore when soil temperature is below 16ºC or over 36ºC. These values mean that in Canada, SCN is inactive during the colder months of the year, restraining the number of generations it is able to complete in a single year but also that there is virtually no way that the eggs die of heat. Finally, the optimum temperature for egg emergence is 24ºC (Alston and Schmitt, 1988). Noteworthy, climate models simulations of 2050 conditions made by Gendron St-Marseille *et al.* (2015) showed that SCN populations would benefit from the warmer temperature. In Quebec southern regions, SCN populations could be able to complete as much as 5 generations a year. This would be possible by allowing the completion of life cycle in less time and by lengthening the soybean growing season (Gendron-St-Marseille, 2013).

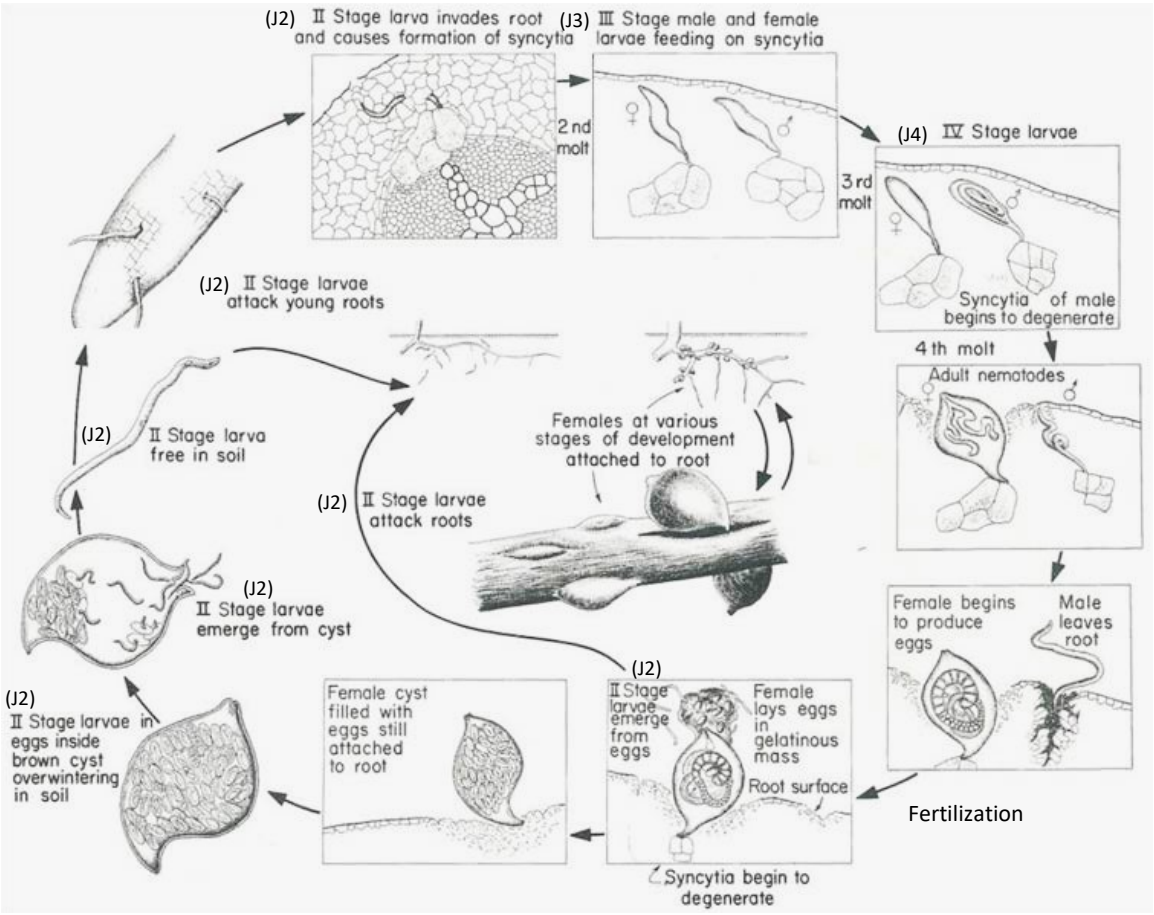

Fig. 3: Life cycle of SCN, modified from Agrios (1978).

2.2.2 Dissemination

The first official mention of SCN was made in 1915 in Japan by Hori (Riggs, 2004). However, SCN probably co-evolved with soybean, its principal host, which originate from central and northern regions of China. Some recent information suggests that as soon as in 1899, SCN damages on soybean were unofficially reported in northeastern China, the phenomenon being known at this time as the "fire-burned seedlings disease" by the farmers, giving even more credits to the Chinese origin hypothesis (Liu *et al.*, 1997). After Hori's publication, SCN has been detected in numerous countries, including Korea (1936), Manchuria (1938) and the United States of America (1954), proving that this pest had spread almost all around the world (Riggs, 1977). Its spreading never stopped in the USA (Fig. 4) and more locally, this organism has been detected in

1987 for the first time in Canada, in the Kent County in Ontario (Anderson *et al.*, 1988). SCN eventually reached Quebec, being found in 2013 in a field located in Saint-Anicet (Mimee *et al.*, 2014). Later on, SCN has been detected in 54 out of 171 fields all around the province, proving once again that it spreads very quickly (Mimee *et al.*, 2014; Mimee *et al.*, 2016). Surprisingly, the population density of cysts in the positive fields was relatively low, with a provincial average of 4.72 [1-25] cysts per 1000 cm^3 of soil. Also, the quality of cysts that were found was generally poor and they were containing a limited number of viable eggs. Cysts with a lot of viable eggs were found only in a few samples, indicating that active reproduction probably did not occur often in infected fields. Ultimately, no explanations were brought up to explain these low numbers and further investigations are needed to find what refrains SCN development in Quebec.

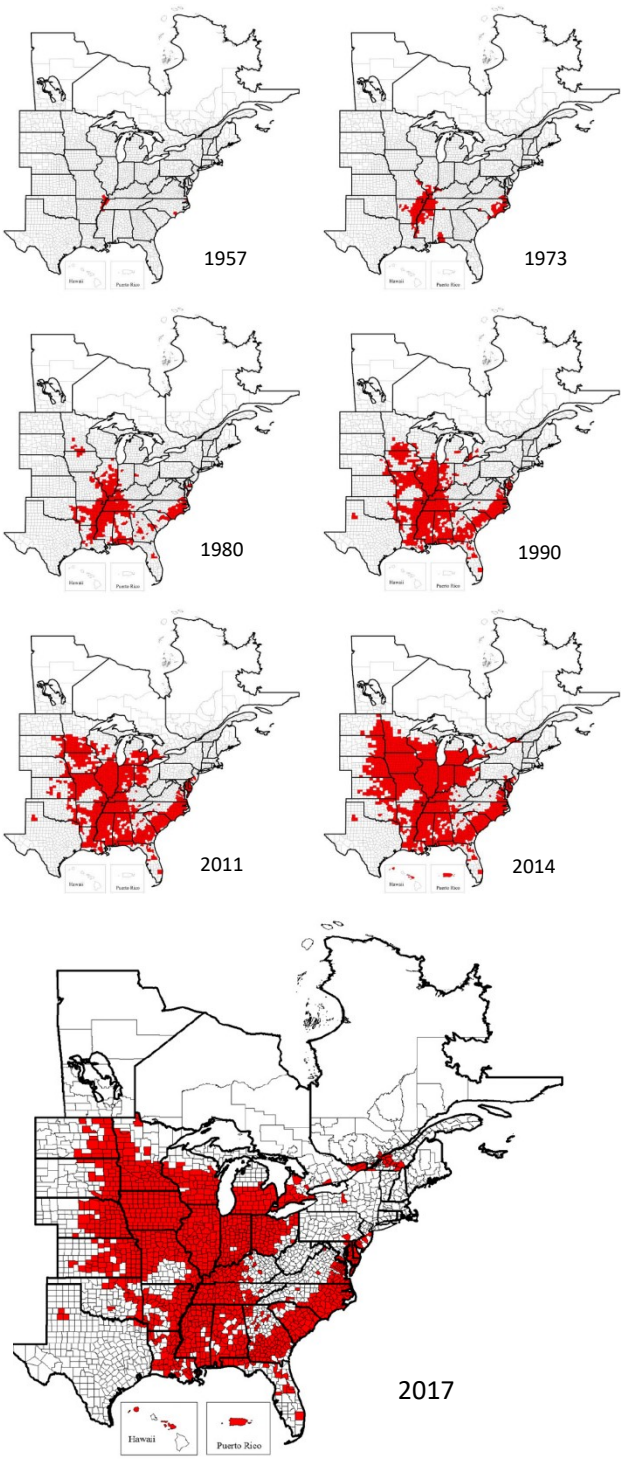

Fig. 4: Evolution of the known distributions (counties in red are infected) of the SCN for selected years between 1957 and 2017 in the U.S. and Canada (modified) (Tylka and Marett, 2014; 2017).

2.2.3 SCN damage

Yield losses caused by SCN are important and were evaluated at more than $US 1 billion per year in the USA (Koenning and Wrather, 2010). In fact, by evaluating the cumulative losses registered by the top ten countries in terms of soybean production, SCN has been determined as the biggest cause of yield losses, above all other diseases or organisms that could affect this plant (Wrather *et al.*, 1997). Moreover, yield losses happen before aboveground symptoms are present, sometimes reducing the yield by up to 30% before symptoms appear (Wang *et al.*, 2003; Niblack, 2005). Also, classic symptoms associated with SCN such as stunting and chlorosis are not specific and thus can be confused with other common diseases. For example, chlorosis can look like a potassium or a nitrogen deficiency, leading to SCN symptoms being unnoticed, which results in no action being taken against this pest (Noel, 2004).

2.2.4 SCN management

Currently, the use of resistant cultivars and rotation with non-host crop such as corn is the best way to manage SCN (Niblack *et al.*, 2008; Cook *et al.*, 2012). Several genomic regions were associated with SCN resistance in soybean; the rhg1, rhg2 and rhg3 (recessive) as well as Rhg4 and Rhg5 (dominant) loci were thought to be the most determinant in SCN resistance. However, subsequent studies revealed that only two of them were involved (Vuong *et al.*, 2015). These two regions are rhg1 and Rhg4 and are both related to quantitative trait loci (QTL) that have been mapped several times (Melito *et al.*, 2010). Located on the chromosome 18, the QTL rhg1 has been repeatedly observed as the locus with the greatest impact on the SCN resistance and explains the totality of the PI88788 parental line resistance (Melito *et al.*, 2010; Cook *et al.*, 2012). This QTL is also involved in the Peking parental line resistance mechanism. Although, in order to exhibit its resistant phenotype, Peking line's resistance need to be completed with the second QTL, Rhg4 (Melito *et al.*, 2010).

The downfall of this approach is that SCN populations can adapt to these lines and will eventually overcome resistance (Young, 1992; Niblack, 2005). Recently, an evaluation of SCN virulence made in Missouri showed that PI88788, the main source of resistance was overcomed in

100% of the fields (Howland *et al.*, 2018). The same authors also demonstrated that the second source of resistance (PI548402) was also overcomed in 43% of the fields. Since most of the resistant cultivars are derived from PI88788 and to a lesser extent PI548402, the need for new alternatives is pressing (Niblack *et al.*, 2008). Although PI437654 is getting more popular and that 4 other resistance genes are being worked on in breeding programs to be used as SCN management options, there is still a long way to go before having a satisfying choice of varieties in term of available resistant cultivars to effectively control SCN without worrying about the shifting issue (Niblack *et al.*, 2008).

Considering this fact, finding new ways to manage SCN populations in fields is important and manipulating soil microbiota by enriching or inoculating soil with various organisms would be an enticing way to look at this problem, complementing or even totally replacing the current approach (Sikora, 1992).

2.3 Possible effects of microbiota on plants

Rhizosphere microbiota can interact with plants in a lot of way and therefore affect their growth and reproduction, either positively or negatively and directly or indirectly (Fig. 5). Because synergetic and antagonistic relations take place between the different components of the microbiota, it is very difficult to bring out the net effect of the whole community on plants and therefore the individual effect of every single species present in the soil, since removing or adding one will completely change the outcome of the situation (Bever *et al.*, 1997).

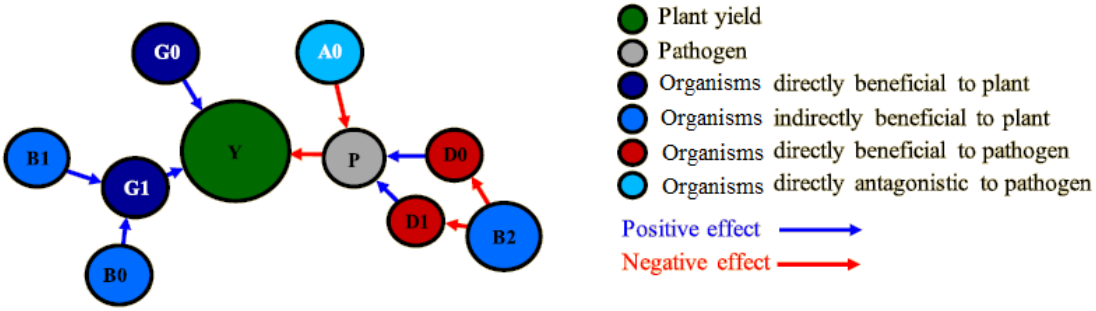

Fig. 5: Schematic representation of an hypothetical situation where only a few organisms are involved, modified from Poudel *et al.* (2016)

Of course, effects are easier to determinate for some microorganisms, like for root pathogens such as SCN, directly causing clear and often massive damage to plants. In addition, those can also benefits from pathogenic soil bacteria and fungi that weaken plants defenses, leading to increased effects due to synergy (Burdon, 1987). Conversely, some organisms are direct antagonists to root pathogens and will be described more in section 2.5, as their effects on phytopathogenic nematodes (PPN) are very clear and that numerous researches have already been done in order to use them as biological control agents. On the other hand, others microorganisms can contribute to plants welfare in other more or less direct ways: by transforming nutrients and facilitating their uptake by plants, by contributing to soil organic matter formation, by producing secondary metabolites (plant growth regulators and hormones) and even by lessening or completely preventing the damages or diseases caused by the phytopathogenic organisms (Höflich *et al.*, 1994; Zahir *et al.*, 2003; Zhu *et al.*, 2013b). These beneficial organisms can either be plant growth promoting rhizobacteria (PGPR) or mycorrhizae (Kloepper, 1994; Schouteden *et al.*, 2015). For soybean PGPR, the classical example is the symbiotic relation with *B. japonicum*, allowing nitrogen fixation (Peoples *et al.*, 1995). Non-pathogenic rhizobacteria can also induce the Induced Systemic Resistance (ISR), a state of enhanced defense that is developed when appropriate stimulation is done to a plant, *Pseudomonas* and *Bacillus* spp being the most studied in the ISR triggering field (Van Loon *et al.*, 1998; Beneduzi *et al.*, 2012). Plants exposed to more diverse bacteria often show higher level of defense compounds (Gera Hol *et al.*, 2010).

Arbuscular mycorrhizal fungi (AMF), contrary to rhizobacteria, are obligate root symbionts which colonize more than 80% of plant species (Schouteden *et al.*, 2015). In return of the photosynthetic carbon given by the plant host, AMF improve plant growth by increasing the nutrient uptake (Smith *et al.*, 2010). They also relieve plant from stress caused by pathogen or other biotic factors (Singh *et al.*, 2011). Pinochet *et al.* (1996) even found that they can directly affect some PPN by suppressing them. The mutualism relation between AMF and the host presumably relies on a combination of multiple mechanisms. Some are direct like competition for space and for resources (Schouteden *et al.*, 2015) while others are more indirect like ISR and the modification of root morphology (Schouteden *et al.*, 2015). Rhizosphere interactions can also be altered through changes in plant root exudation which could influence PPN attractiveness and reduce the hatching stimulation (Jones *et al.*, 2004; Schouteden *et al.*, 2015).

2.4 Possible effects of cystobiome on SCN

Microorganisms undeniably have an effect on plants and thus probably also have some on SCN directly. Almost no research was previously made about the inside microbiome of SCN cysts and even less about the effect the microorganisms could have on SCN development. By using deep sequencing, a team recently found that the cyst bacterial community was more similar to the root endosphere community than to the rizhosphere one (Hussain *et al.*, 2018). This group identified a consortium of various taxa, including genera such as *Pasteuria*, *Pseudomonas* and *Rhizobium* that were consistently more abundant when SCN was present in soil (Hussain *et al.*, 2018). These taxa were also over-represented inside the SCN cysts when compared to bulk soil (Hussain *et al.*, 2018). Another study showed that some bacterial taxa were shared among all the SCN samples of the study (Nour *et al.*, 2003). These findings suggest the existence of a core cystobiome but the effect of it on SCN development is still unknown.

2.5 Known SCN antagonistic organisms

2.5.1 Bacteria

Little is known about the bacteria that have an antagonistic effect on SCN. The first study on that subject was conducted by Kloepper *et al.* (1992) and is still considered to be a reference in that field of study. Kloepper and his team identified the rhizosphere bacteria communities isolated from the roots of plants with well-known antagonistic effects for nematodes. These communities were then compared to other rhizosphere bacterial isolates, this time coming from soybean, which does not have known antagonism. Without any surprise, microbiota of both types of isolates were completely different, with significantly more Gram-negative (especially *Pseudomonas cepacia* and *Pseudomonas gladioli*) and coryneform genera in the antagonistic plants group. Soybean rhizospheres communities were dominated by *Bacillus* spp. The results suggested that rhizosphere of these plants could potentially be used as biological control agents for pathogenic nematodes (Kloepper *et al.*, 1992). Putting aside this study, some genera of bacteria that live in the rhizosphere have been found to reduce diverse nematode species populations and include *Actinobacteria*, *Agrobaterium*, *Alcaligenes*, *Alphaproteobacteria*, *Bacillus*, *Clostridium*, *Desulfovibrio*,

Pseudomonas, *Serratia* and *Streptomyces* (Siddiqui and Mahmood, 1999; Zhu *et al.*, 2013b). Also, in conjunction with a chitin amendment in soil as first introduced by Mankau and Das (1969, 1974), five chitinolytic bacterial isolates were found to have consistent suppressive effects on SCN, with four of them enhancing the effectiveness of chitin amendment treatment (Tian *et al.*, 2000). These five organisms are *Comamonas acidovorans*, *Flavobacterium johnsoniae*, *Methylobacterium zatmanii*, *Streptomyces cyaneaus chartreusis* and *Streptomyces* sp. (Tian *et al.*, 2000).

Finally, Zhu *et al.* (2013a) demonstrated that soybean monoculture for an extensive period (over 8 years in this study) led to shift in bacterial communities and a decline in SCN populations and they hypothesized that monoculture soils could have suppressive effects on SCN.

2.5.1.1 Modes of action

Several modes of action have been demonstrated and can help to explain the mechanisms of these bacteria to suppress nematode populations. Those rhizobacteria are altering the root exudates and therefore affect the host-finding process by making roots less attractive or by directly making nematodes unable to find roots to infect (Sikora and Hoffman-Hergarten, 1993). Another possible mode of action is the production of nematicidal or "eggs-hatching-reducing" enzymes or metabolites that affect nematode's mobility and viability (Oka *et al.*, 1993; Kerry, 2000). Recently, Cao *et al.* (2015) studied the bacterial communities associated with the different life stages of *Meloidogyne incognita* and concluded that eggs were more negatively affected compared to juveniles stages.

2.5.2 Fungi

Fungi with antagonistic effects against SCN are known for a long time with the first report more than a century ago (Davies and Spiegel, 2011). The idea of using fungi as biological control agent for PPN was first introduced by Linford in 1937. Nevertheless, this concept did not get much attention before the 1980's, when the public start asking for to development of more environmentally friendly pesticides. With the hype caused by this phenomenon, scientists gathered

a lot of data and now, more than 200 nematophagous fungi are described. Song *et al.* (2016) discovered that there are shifts in fungal populations leading to a decrease in the number of cysts when long-term cropping in black soils is practiced. Similar results have been observed in potato monoculture, *Malassezia* spp. being frequently found in *Globodera pallida* cysts on healthy plants (Eberlein *et al.*, 2016). Because *Malassezia* have high affinities for highly collagenous substrates (like SCN shell), this species could be a possible biological control agent for SCN (Nour *et al.*, 2003).

2.5.2.1 Modes of actions

PPN-antagonistic fungi present three types of infection mechanisms (Davies and Spiegel, 2011): the nematode-trapping fungi, which capture nematodes using morphological structures specialized for this task, the endoparasitic fungi that infect nematodes using adhesive spores and finally the egg/cysts-parasitic fungi that infect nematode at those stages with their hyphal tips (Barron, 1977).

SCN females and egg masses are exposed to a large range of species of these fungi on the root surface (Kerry, 2000; Moosavi and Zare, 2012). No less than 150 species of fungi have been isolated from the cysts but only 10% of them have seen their parasitic status tested (Kerry, 1988). Stiles and Glawe (1989) found that normally, fungi isolated from SCN have the capacity to colonize soybean roots and proposed that they could colonize the nematode using the plant as an intermediate host rather than infecting it directly from the rhizosphere. Colonization would occur when the fungus infects the nematode's feeding cell and thus interrupt female's development (Kerry, 1988). Surprisingly, many of the fungi that have been isolated from cysts are also known as plant pathogens (Kerry, 2000). Interestingly, this non-specific parasitism strategy has been hypothesized as a way to keep a high number of spores and fungi in the soil when plants are absent of fields and therefore promote long-term survival (Kerry, 2000). Other reports have shown that some oomycetes are obligate parasites of females of cyst nematodes and are infecting the hosts by motile zoospores, such as *Nematophtora gynophila* or *Catenaria auxiliaris*, which is widely spread amongst several cyst nematode populations (Kerry and Crump, 1980). Those organisms seem to

be more fragile as they are dependent on good soil moisture but have resting spores that enable them to survive in soil in case there are no hosts available (Kerry, 2000).

Finally, some species are known to be effective directly against nematode or at least to decrease indirectly the plant infection by them. Among these species, *Pochonia* spp. that grow as endophytic fungi are known to have great chances to infect cyst nematodes such as SCN inside the roots and therefore decrease the infestation in a field (Moosavi and Zare, 2012).

2.5.3 Commercial biocontrol agents

Farmers have various options nowadays and can use some commercially available seeds treated with bacteria or fungi that have been proven to be effective to limit damages caused by SCN when used in combination with resistant varieties of soybean (Tenuta and Tenuta, 2015). Two of the bacterial possibilities are the following: VOTiVO® (Bayer Crop Science) and Clariva pn® (Syngenta). The former uses *Bacillus firmus* and creates a living barrier around the young roots of soy plants that limits the chance of nematode to find and infect them (SeedQuest, 2014). The latter one has *Pasteuria nishizawae* as an active component and the bacterium spores also create a protective zone around young roots, eventually infecting SCN that come into contact with roots and kill them by affecting their reproductive and feeding processes (Syngenta Canada, 2015). Two fungal species isolated from nematode eggs have been studied deeply and confirmed as good biological control agents: *Verticillium chlamydosporium* and *Paecilomyces lilacinus*. The strain 252 of the later has been commercialized under the names Bioact® (Bioact Corporation, Sydney, Australia) and PI Plus® (Biological Control Products, South Africa) to fight against root-knot and cyst nematodes (Kerry, 2000; Moosavi and Zare, 2012). Other frequently isolated fungi species from cysts and eggs and considered to be promising biocontrol agents are issued from the following genus: *Pochonia* spp., *Paecilomyces* spp., *Haptocillium* spp., and *Hirsutella* spp. (Moosavi and Zare, 2012).

3. Objectives and hypotheses

Considering all the known information mentionned in the literature review, the well-established presence of SCN in Ontario, its relatively new infestation in Québec and, above all, the scarcity of efficient ways to manage this pest, finding new alternatives to limit the SCN spreading is of the utmost importance. Numerous studies have demonstrated that some microorganisms negatively affect SCN and thus the scope of this thesis was primarly to study the microbiomes associated to the SCN and the soybean in Eastern Canada. Objectives and hypotheses are as follow:

Objectives

1. To characterize the fungal and bacterial soil microbiomes associated with soybean in Quebec and Ontario.

2. To characterize SCN cyst microbiome (cystobiome).

Hypotheses

1. Because of its recent history of soybean cultivation, Quebec soil microbiome will differ from Ontario.

2. Cystobiome will be composed of microorganisms that are part of the soil microbiome in the field where the cysts were collected.

4. Soil microbiome associated with soybean in Canada and its relation with soybean cyst nematode development

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4.1 Abstract

Soybean acreage has drastically increased in Canada over the past decade. This expansion has already favored the establishment of the soybean cyst nematode (SCN), one of the most damaging pest of soybean. This change in land use is also expected to influence soil microbial communities, with unpredictable impacts on soil ecosystem services. In this study, we characterized the soil microbiome associated with soybean in eastern Canada and investigated the possible correlations it could have with SCN development. Soil bacterial communities were very similar across all the fields, even after a single soybean crop and were dominated by nitrogen cycling phyla. In contrast, fungal communities were strongly dictated by their geographical origin. Interestingly, the microbial communities colonizing SCN cysts (cystobiome) did not originate from the associated soil and could be vertically transmitted by the female to the eggs. Specific taxa, including known SCN-antagonistic species, were found to be negatively correlated with SCN development. However, the presence of multiple microorganisms acting synergistically was required to observe this correlation. These results are in contradiction with the current commercial strategy of adding a single non-native biocontrol species to the system and suggest that the promotion of an assemblage of indigenous species could lead to a better management of SCN.

4.2 Introduction

Over the past two decades, grain prices have increased substantially due to a higher demand linked with meat consumption and biofuel production, among other factors (Trostle, 2010). These high prices and demand have created incentives for land conversion from grassland to cropland and for farmers to adopt grain crops. As a result, the United States has recorded in recent years, the greatest rate of land conversion of the last century (Wright and Wimberly, 2013), raising concerns about the resulting environmental impacts (Lark *et al.*, 2015). It was notably shown that such conversion had a negative effect on carbon sequestration and increased the emission of greenhouse gases (GHG) (Gelfand *et al.*, 2011). In Canada, it is anticipated that climate change will also lead to major changes in land use by opening new areas to grain cultivation (Gendron-St-Marseille *et al.*, 2019). This land conversion is also facilitated by breeding programs that generated early-maturing grain germplasms adapted to high latitudes (Voldeng *et al.*, 1997; Egli and Bruening, 2000). Consequently, the Canadian production of grain has already increased significantly since the beginning of the 21st century. For example, the soybean production rose from 2.7 million metric tons in 2000 to 7.7 million metric tons in 2015 (Statistics Canada, 2018). The area dedicated to soybean cultivation is increasing faster in Canada than in the rest of the World (Fig. 2; (Statistics Canada, 2018; FAO. Food and Agriculture Organization of the United Nations, 2019)). This massive change in land use is however associated with losses of biodiversity and a reduction of the ecosystems' capability to cope with biotic and abiotic stresses (Foley *et al.*, 2005).

In North America, the expansion of soybean cultivation has been followed rapidly by the spread of the soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe, 1952). This plant-parasitic nematode significantly reduces the yield even before the onset of symptoms on plants and is responsible for losses up to $US 1 billion annually in the United States (Koenning and Wrather, 2010). Introduced from Asia, SCN was first reported in North Carolina in 1954 (Winstead *et al.*, 1955) and is now present in all soybean-producing U.S. states (Tylka and Marett, 2017). In Canada, SCN was detected in southwestern Ontario in 1987 (Anderson *et al.*, 1988) and in Quebec in 2013 (Mimee *et al.*, 2014). The high genetic diversity that characterize SCN populations and the continuous gene flow in North America facilitate adaptation to diverse bioclimatic conditions and establishment in new soybean-producing area (Gendron-St-Marseille *et al.*, 2018). Warming

temperatures expected with climate change will also favor SCN development by increasing the number of generations per growing season (Gendron-St-Marseille *et al.*, 2019) and by increasing soybean susceptibility (Gendron St-Marseille *et al.*, 2015). Therefore, early detection of SCN is important in order to take phytosanitary measures as soon as possible. This includes crop rotation with non-host plant species and the use of SCN-resistant soybean cultivars. Unfortunately, selection pressure exerted by the continuous use of resistant cultivars has led to the selection of more virulent SCN (Niblack *et al.*, 2006). For example, a recent evaluation of SCN virulence in Missouri revealed that the main source of resistance (PI88788) was overcomed in 100% of the fields (Howland *et al.*, 2018). Even more concerning, the same authors revealed that aggressiveness was very high in half of the fields and that the second source of resistance (PI548402) was also overcomed in 43% of the fields. Thus, new sources of resistance and gene stacking are needed (Howland *et al.*, 2018) but we should also explore alternative strategies to limit SCN development and reduce the gene flow that facilitates its adaptation (Gendron-St-Marseille *et al.*, 2018).

Biological interactions with soil microorganisms have been shown to negatively affect the development of SCN in different parts of the world (Kerry, 2000). Isolated microorganisms have been proposed and sometimes commercialized for the biological control of SCN, including many plant growth-promoting rhizobacteria (PGPR) strains (Xiang *et al.*, 2017). However, this strategy often implies the addition of non-indigenous microorganisms to the system, with possible unwanted impacts on endemic species and low establishment success. A more sustainable approach would be to restore the natural soil biodiversity and to favor some soil biota to deliver specific functions like SCN suppression (Bender *et al.*, 2016). In regions with a very recent history of soybean cultivation, it was shown that even if SCN is detected in most of the fields, population densities remain very low and the recovered cysts are of poor quality with very few viable eggs inside (Mimee *et al.*, 2016). It is hypothesized that the decline of soil microbial diversity following intensive grain cultivation favors SCN establishment and development. This study aims to characterize soil and cyst microbiomes from regions infested with SCN but differing in their past history of soybean cultivation. The effect of cultural practices (tillage and crop rotation) as well as edaphic factors on soil microbial communities and SCN reproduction was evaluated. In addition, this work is the first characterization of soil microbiota in soybean fields in Eastern Canada.

4.3 Materials and methods

4.3.1 Soil and SCN sampling

Twenty-five soybean fields from Eastern Canada (12 in Ontario and 13 in Québec) were sampled in fall 2016 following harvest (Fig. S1). Crop history, tillage type and Corn Heat Units (CHU) information were graciously provided by OMAFRA, MAPAQ and producers. In each field, three different zones were sampled: field entrance, center of the field, and field margin. In each zone, a strip of 50 m x 2 m was identified and 64 soil cores from the 0-20 cm layer (approximately 20 liters of soil) were sampled in a zigzag pattern with a soil auger (Eijkelkamp; Giesbeek, Netherlands) to constitute one composite sample per zone. These composite samples were thoroughly mixed and separated in sub-samples for the different analysis. Fifty cm^3 of soil were stored at -80°C for metagenomics analysis, one liter was stored at -20°C for physical and chemical analysis and three sub-samples of 300 cm^3 were dried in paper bags at room temperature for SCN evaluation. After two weeks, SCN cysts were extracted from this dry soil using the Fenwick can procedure (Fenwick, 1940). Cysts were counted under a stereomicroscope and crushed to evaluate the number of eggs/100 g of soil and viability based on the internal morphology of both dead and living eggs and juveniles (Den Nijs, 2008; EPPO, 2013).

4.3.2 Edaphic factors analysis

Soil physico-chemical analyses were conducted at the Agriculture and Agri-Food Canada facilities in Saint-Jean-sur-Richelieu, QC, Canada. The pH and conductivity were measured in the supernatant of a 1:2 ratio of soil:H_20. The pH was also measured in a 1:2 ratio of soil:$CACl_2$ (0.01M) solution. Cation-exchange capacity (CEC) was determined from the following formula:

Total CEC ($cmol_c/kg$)=[(7.5 – buffer pH)] X 9 + K ($cmol_c/kg$) + Ca ($cmol_c/kg$) + Mg ($cmol_c/kg$)

Organic matter content (%) was determined by comparing soil weights before and after calcining samples at 420°C for one hour. A QuickChem 8500 serie II (Lachat Instruments, Milwaukee, Wisconsin, USA) was used to perform Isaac digestions (Kjedahl type) followed by

automated colorimetric analysis to measure total N (nitrogen) in samples (Isaac and Johnson, 1976, Lachat Instruments, 2003b). Colorimetric analyses were performed with the same instrument to quantify ammonium ($N\text{-}NH_4$) and nitrate ($N\text{-}NO_3$) concentrations in samples following a KCl (2M) extraction (Lachat Instruments, 2003a, 2007). The granulometry of each soil sample was determined using the hydrometer method (Kalra and Maynard, 1992). Finally, trace elements were analyzed using a iCAP 7400 ICP-OES Duo (Thermo Fisher Scientific, Waltham, Massasuchets, USA) following a Melich 3 extraction (Mehlich, 1984) to measure the concentration of potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), phosphorus (P) and aluminum (Al) in each soil sample.

4.3.3 DNA extraction

A small portion of every sample was screened using a stereomicroscope to separate SCN cysts from soil. DNA was extracted from 0.5 g of cyst-free soil using the PowerSoil DNA Isolation Kit (MO BIO, Carlsbad, CA, USA) following the manufacturer recommendations. Cysts removed from the same soil samples were washed five times by repeated up and down pipetting in nano-pure water to get rid of the surface contaminants. Then, 20 cysts (when available, minimum of 5) from each sample were crushed under the stereomicroscope to collect the eggs, which were transferred to microtubes. DNA extraction was carried out on the total quantity of eggs using the PowerSoil DNA Isolation kit following the manufacturer instructions.

4.3.4 Library preparation and sequencing

DNA extracts were then used for amplicon sequencing targeting the prokaryotic rRNA gene (16S) and the eukaryotic internal transcribed spacer (ITS) of the rRNA small-subunit. Cyanobacteria excluding primers 799F (5′-AACMGGATTAGATACCCKG-3′) and 1115R (5′-AGGGTTGCGCTCGTTG-3′) were used to analyze bacterial communities, targeting the V5-V6 regions of 16S rRNA while excluding the chloroplast DNA (Chelius and Triplett, 2001; Redford *et al.*, 2010). These primers were modified with the addition of a 13-19-bp barcode, a heterogeneity spacer and an Illumina (San Diego, CA, USA) adapter sequence, on the 5’ tail (Table S1). Amplification was done using the Phusion High-Fidelity DNA Polymerase Kit (Thermo Scientific,

Waltham, MA, USA). PCR conditions included an initial denaturation at 98°C for 30 seconds, followed by 30 cycles of denaturation (98°C for 10s), annealing (64°C for 30s) and elongation (72°C for 30s) and a final elongation at 72°C for 10 min (Kembel *et al.*, 2014). For the analysis of fungal communities, the primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′), targeting the ITS2 region of ITS fungal rRNA were used (White *et al.*, 1990; Nilsson *et al.*, 2018). These primers were also modified as described above (Table S2). PCR conditions included an initial denaturation at 98°C for 30 seconds, followed by 30 cycles of denaturation (98°C for 15s), annealing (56°C for 30s) and elongation (72°C for 30s) with a final elongation at 72°C for 10 min. For both analyses, the PCR reactions were carried out on a SureCycler 8800 (Agilent, Technologies, Santa Clara, CA, USA) in a volume of 25 µl containing 5 µl of HF buffer, 0.75 µl of DMSO, 0.5 µl of dNTPs (10 mM), 1 µl of each primer (5 µM), 0.25 µl of polymerase, 15.5 µl of nuclease free water (Fisher Scientific), and 1 µl of DNA template. Random verifications were done on gel to confirm amplification and amplicon size. The concentration of PCR products was then normalized using the SequalPrep Normalization Kit (Invitrogen, Carlsbad, CA, USA) and samples were pooled together using 5 µl of each. The solution was purified using Agencourt AMPure XP (Beckman, Coulter, Brea, CA, USA) to remove primers and contaminating sequences. The concentration and fragment sizes of each library were verified using the Qubit dsDNA HS Assay Kit on a Qubit Fluorometer (Life Technologies, Burlington, ON, Canada) and a BioAnalyzer 2100 with the High Sensitivity DNA Analysis Kit (Agilent). Libraries were denatured with NaOH, diluted to a final concentration of 9 pM and sequenced on a MiSeq instrument (Illumina, San Diego, CA, USA) using the reagent Kit v3 (600 cycles).

4.3.5 Bioinformatics and multivariate analysis

Sequences obtained from the MiSeq instrument were demultiplexed and filtered for quality using Mothur v.1.39.5 (Schloss *et al.*, 2009). Clean sequences were then transferred to QIIME v.1.9.1 (Caporaso *et al.*, 2010). Following the evaluation of rarefaction curves (Fig. S2), 16S sequences from soil samples were rarefied at 20 000 per sample and ITS at 5 000. Samples with less sequences than these thresholds were discarded and not included in the analyses.

OTU picking (de novo) was then made with default parameters using a 97% similarity threshold for 16S sequences (Stackebrandt and Goebel, 1994) and 99% for ITS (Hao *et al.*, 2017). OTUs were then annotated with the uclust algorithm using the SILVA database v.128 (Edgar, 2010; Quast *et al.*, 2012) for 16S and UNITE+INSDC database v.7.1 for ITS (Kõljalg *et al.*, 2013; Madden, 2013). Microbiome data were analysed using the phyloseq package v1.22.3 in R v3.4.0 (McMurdie and Holmes, 2013; R Core Team, 2016). Possible source of cross-sample contamination was checked using the SourceTracker package v1.0 (Knights *et al.*, 2011). The VEGAN package was used for the computation of dissimilarity distances (Bray-Curtis and Hellinger) between communities and to run adonis and betadisper tests (Oksanen *et al.*, 2015). These tests were used in conjunction to assess similarity between communities from the different areas. Finally, the “core” analysis from the microbiome package was used to determine the core microbiomes in each province and in the cysts (cystobiome) (Lahti *et al.*, 2017 (Version 1.5.28)).

4.4 Results

4.4.1 Soil and SCN microbiota in soybean fields

A total of 75 soil samples were harvested from soybean fields from regions with a long history of soybean cultivation (southwestern Ontario) or more recent fields (Quebec) in Canada (Fig. S1). Soil microbial communities as well as SCN cystobiome were analysed by amplicon-sequencing. After initial filtering, 5 512 917 high quality sequences were obtained for bacterial community profiling and 3 177 823 for fungi. Among samples, the number of sequences ranged from 8 082 to 140 088 for bacteria (Table S3) and from 1 to 139 234 for fungi (Table S4).

SourceTracker (v 1.0) analysis detected putative cross-contamination in only two samples that were thus removed from the dataset analyses.

In terms of absolute richness, there were 8 947 bacterial (QC: 8 706; ON: 8 577) and 1 538 fungal (QC: 928, ON: 1 291) OTUs identified across all soil samples. SCN cyst samples were much less diverse with only 359 bacterial (QC: 128; ON: 270) and 136 fungal (QC: 35; ON: 102) OTUs overall.

4.4.2 Effect of cropping history on soil microbiome

Following a rarefaction analysis (Fig. S2), 20 000 sequences per samples were randomly picked for bacterial community analysis and 5 000 for fungi. The comparisons of Shannon indexes computed at the OTUs level showed no significant differences between provinces for the bacterial communities (Fig. S3A, Wilcoxon test, $p = 0.53$, mean Shannon indexes: ON: 6.71; QC: 6.75) but revealed a significantly more diverse fungal communities in ON (Fig. S3C, Wilcoxon test, $p = 0.03$, mean Shannon indexes: ON: 3.54; QC: 2.87).

A PCoA based on the Bray-Curtis distances between individual samples showed a clustering of the soil bacterial communities by provinces (Fig. 6A). The adonis test ($p < 0,001$) and the betadisp graphical representation (Fig. 6B) confirmed that centroids of the two provinces were statistically different. Additionally, in most of the fields, the bacterial communities of the three independent composite samples, although from different locations (entrance, center and margin of the field) were extremely similar. For fungal communities, the distinction between the provinces was even stronger. When plotted on a PCoA (Fig. 6C), although major differences exist among the communities from ON on the first axis, the second axis clearly separated samples from ON and QC. This result was also confirmed by an adonis test ($p < 0.001$) and betadisp (Fig. 6D).

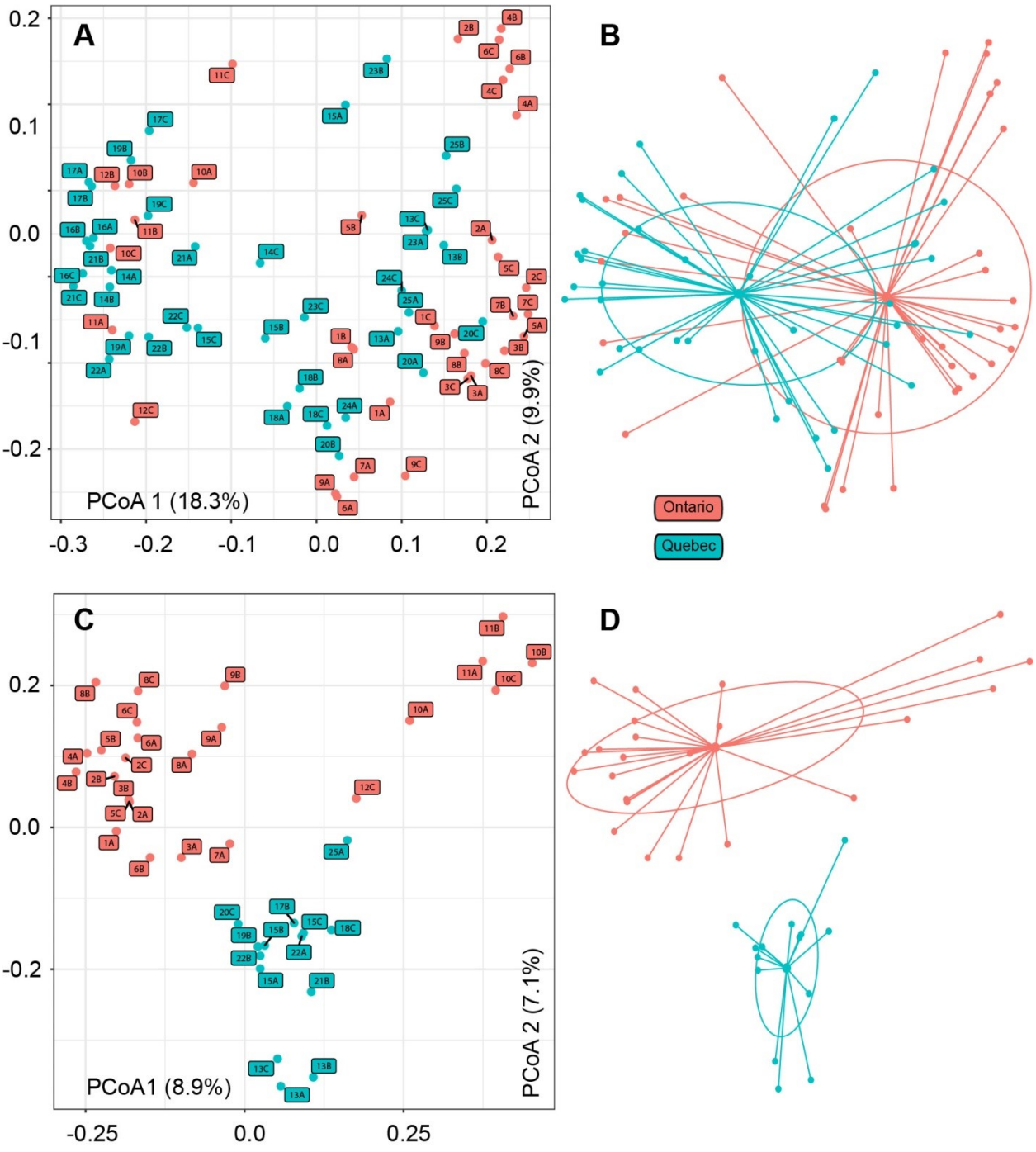

Fig. 6: PCoA based on Bray-Curtis distances of the bacterial (A) and fungal (C) soil communities from the Canadian provinces of Ontario (red) and Quebec (blue) and the graphical representation of the homogeneity of communities dispersion (B=bacteria, D=fungi) using the betadisper function. Labels identify the field number

followed by the area sampled in each field (a=field entrance, b=middle of the field and c=field margin).

Even if the microbial communities differed between provinces, their composition at the phylum level was very similar for soil bacteria (Fig. 7A). Proteobacteria, Chloroflexi and Actinobacteria were by far the most abundant phyla and their proportions were similar in each province. For the soil fungal communities, the dominant phyla plot revealed an important proportion of "Unidentified fungi", accounting for almost 50% of the sequences in both areas (Fig. 7B). Besides that, the phylum distribution was also similar between provinces. Ascomycota was the most dominant phylum, followed by Basidiomycota and Mortierellomycota.

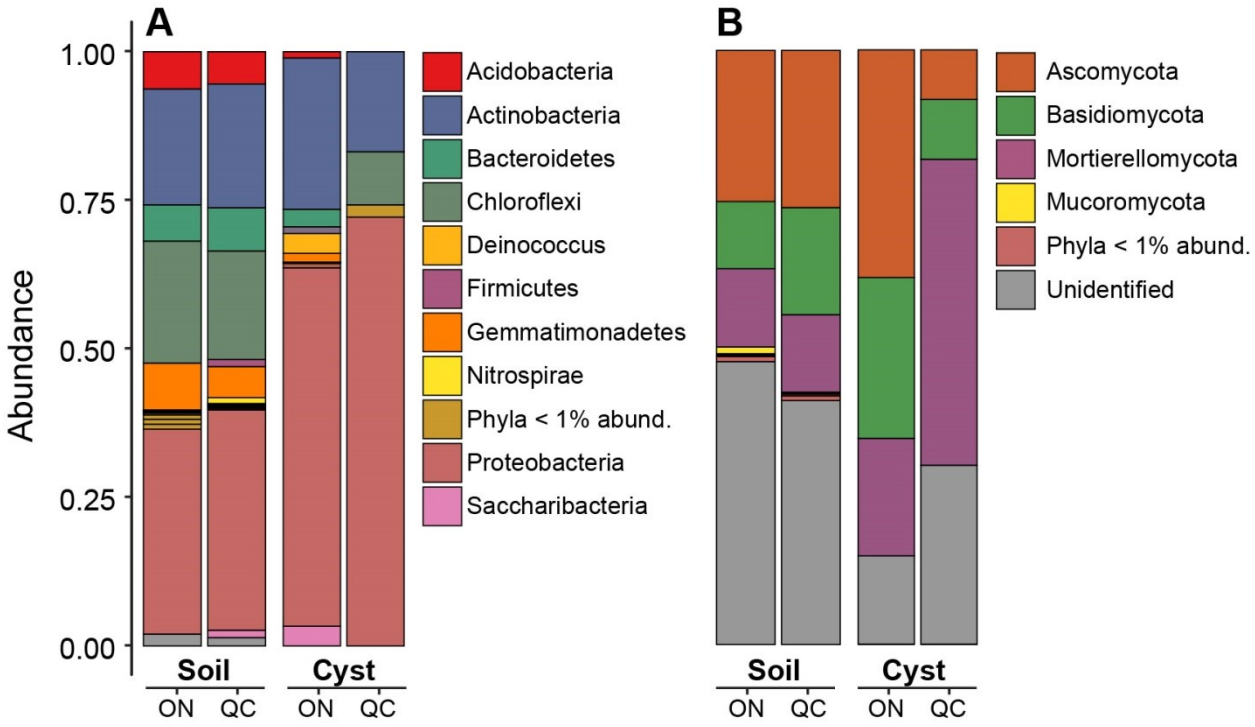

Fig. 7: Relative abundance of the most dominant phyla (representing over 1% of the sequences) in soil and cysts of the soybean cyst nematode from the Canadian provinces of Ontario (ON) and Quebec (QC). Communities were identified by amplicon-sequencing of rRNA targeting the 16S region for bacteria (A) and ITS for fungi (B).

The analysis of the core soil bacterial microbiome showed that the two areas shared 12 out of 17 most dominant taxa (Table 1). For the remaining five taxa, two (genus *Roseiflexus* and family Xanthobacteraceae) were found in all the ON fields while three others were only systematically found in fields from QC (genera *Variibacter* and *Arenimonas* as well as the order SC-I-84 from the Betaproteobacteria class).

Table 1: List of bacterial OTUs detected in the soil core microbiome of soybean fields from the Canadian provinces of Ontario and Quebec. Taxa highlighted in grey were not shared between the provinces.

Ontario	**Quebec**
Bradyrhizobium	Bradyrhizobium
Acidimicrobiales	Acidimicrobiales
Gaiellales	Gaiellales
Chloroflexi S085	Chloroflexi S085
Chloroflexi TK10	Chloroflexi TK10
Gemmatimonadaceae	Gemmatimonadaceae
Nitrospira	Nitrospira
Rhodobiaceae	Rhodobiaceae
Sphingomonas	Sphingomonas
Comamonadaceae	Comamonadaceae
Nitrosomonadaceae	Nitrosomonadaceae
Incertae Sedis Acidibacter	Incertae Sedis Acidibacter
Roseiflexus	Variibacter
Xanthobacteraceae	Betaproteobacteria SC-I-84
	Arenimonas

Distance based RDAs revealed that Aluminium (Al) and nitrate (NO_3) concentrations, as well as longitude were correlated with bacterial communities composition (Adjusted R^2=0.057, *p*

< 0.001, Fig. S4) while no significant model was found for the fungal communities. Pearson correlations were computed independently for Al and NO_3-N to remove the effect of longitude and identified eight OTUs that were significantly correlated ($p < 0.05$) to one of these factors (Table 2).

Table 2: Significant Pearson correlations between individual OTUs abundance and edaphic factors (concentrations of aluminum and nitrate).

Factor	OTUs	Pearson's R value	p-value
Al	Chloroflexi JG30-KF-CM66	0.47	1.557e-04
Al	Rhizobiales A0839	0.49	6.734e-05
Al	Hyphomicrobiaceae	0.51	2.931e-05
Al	Rhodospirillales MND8	0.54	6.723e-06
NO_3-N	Erythrobacteraceae	0.47	1.493e-04
NO_3-N	Betaproteobacteria TRA3-20	0.46	2.075e-04
NO_3-N	Deltaproteobacteria NB1-j	0.55	5.545e-06
NO_3-N	Arenimonas	0.50	4.534e-05

4.4.3 Biotic and edaphic factors influencing SCN development

SCN infestation in the fields under investigation was significantly different between the two provinces with a mean number of eggs per 100g of 8 271 for ON compared to 19 for QC ($p <$ 0.001) (Fig. 8A). Egg viability was not statistically different at the provincial level ($p = 0.12$) but appeared reduced in QC and showed high variability between and among fields (Fig. 8B).

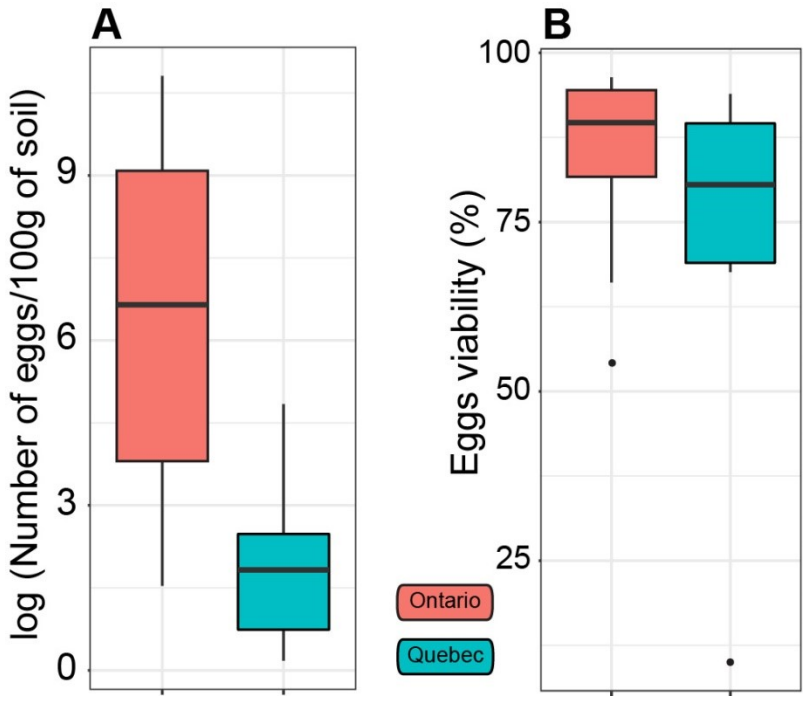

Fig. 8: Comparison of soybean cyst nematode populations in the Canadian provinces of Ontario (red) and Quebec (blue) for the number of eggs per 100g of soil (A) and egg viability (B).

Cystobiomes were considerably less diverse than soil microbiomes and Proteobacteria dominated their composition with over 50% of the sequences (Fig. 7A). No significant difference was observed between ON and QC for bacterial (Wilcoxon test, $p = 0.26$, mean Shannon indexes: ON: 1.56, QC:1.15) nor fungal (Wilcoxon test, $p = 0.64$, mean Shannon indexes: ON:1.59, QC: 1.15) communities. However, Chloroflexi were abundant in QC and almost absent in ON while several phyla were present in ON (Acidobacteria, Bacteroidetes, Chlamydiae, Deinococcus-Thermus and Saccharibacteria) and absent from QC cystobiome. Surprisingly, only one OTU (*Shewanella* sp.) was identified in the core bacterial cystobiome of both provinces while absent from their core soil microbiomes.

Egg numbers and viability were correlated with edaphic factors and the presence of specific OTUs in soil (Fig. 9A). Aluminium concentration was found to be linked with lower SCN egg viability while soil class and phosphorus concentration were shown to be correlated to higher numbers of eggs per 100g of soil (Adjusted R^2=0.567, p <0.001). Loamy sand soils and sandy clay

loam to a lesser extent, were more likely to have a high number of SCN eggs. Two bacterial soil OTUs were also significantly associated with a change in SCN eggs (Adjusted R^2=0.181, $p < 0.02$, Fig. 9B). The rhizobacteria genus *Nordella* seemed to be linked to lower egg viability while an OTU from Bacillales order was associated with lower numbers of eggs. When tested individually however, only the *Nordella* OTU showed a significant negative Pearson correlations with egg viability (Fig. S5). Five fungal OTUs were also strongly associated with variations in SCN egg number and viability (Adjusted R^2=0.69, $p < 0.001$, Fig. 9C). The presence of a member of the genus *Olpitrichum* was directly opposed to egg viability while *Arthrobotrys oligospora* was negatively correlated to egg number. However, when assessed individually using regression analysis, only Pyronemataceae sp. was significantly correlated with the number of eggs per 100 g of soil (Fig. S6). Altogether, these results suggest that the potential negative effect of the microbiome on SCN eggs is the result of an assemblage of microorganisms and not because of a single taxa.

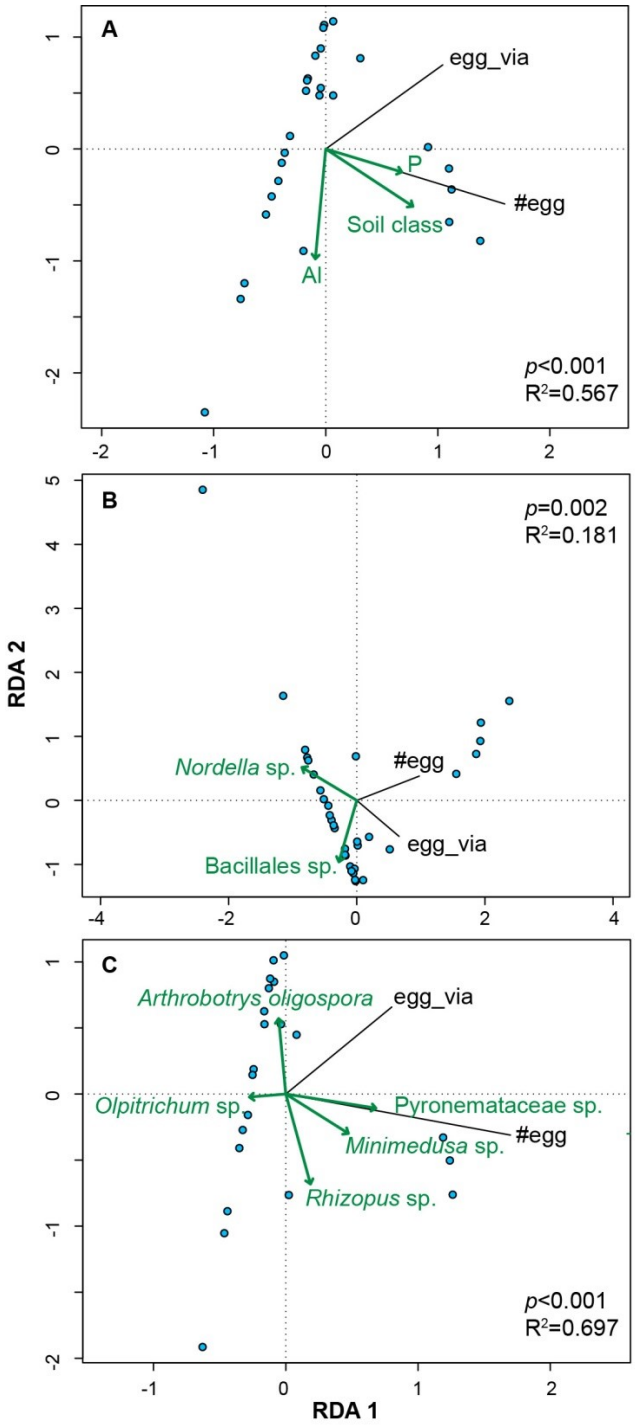

Fig. 9: Distance-based redundancy analysis (db-RDA) ordination of soybean cyst nematode egg viability (egg_via) and the number of eggs per 100g of soil (#egg) with edaphic factors (A), bacterial taxa present in soil (B) and fungal taxa present in soil (C).

4.5 Discussion

Soil microbial communities fulfill many ecological services from nutrient cycling to pesticide degradation (Barrios, 2007). They are an important driver of soil health as they influence plant disease development as well as yield potential of each field (Barrios, 2007). The composition of these communities is however greatly influenced by plant type, soil properties and cultivation methods (Garbeva *et al.*, 2004). Changes in land use are thus predicted to have major effects on soil biodiversity that need to be evaluated for the development of more sustainable systems (Foley *et al.*, 2005). In this study, we characterized the soil microbiome associated with soybean in Canada and its interaction with SCN, the most devastating disease of soybean in North America (Koenning and Wrather, 2010).

The comparison of the alpha diversity revealed that fungal communities were only slightly more diverse in Ontario than in Quebec and bacterial communities were equally diverse between the two areas. The most dominant phyla (Proteobacteria, Actinobacteria, Acidobacteria and Bacteroidetes) and their proportion were also comparable between provinces and similar to those of other soil microbiome studies in various field crops (Peiffer *et al.*, 2013; Sugiyama *et al.*, 2014; Li *et al.*, 2016). The phylum Chloroflexi was surprisingly well represented in all samples. This phylum is generally not dominating in soil microbiome and, although it was reported in soybean fields (Mendes *et al.*, 2014; Sugiyama *et al.*, 2014), the abundance observed here is relatively high. These filamentous bacteria, mostly aerobic and thermophilic, were shown to have an affinity with sandy soil that provides better oxygen supply and with manure application (Zhang *et al.*, 2013; Ding *et al.*, 2014). Since this type of fertilizer is commonly used in field crops and several fields had sandy soils, it could explain the unusual abundance of Chloroflexi. On the fungal side, Ascomycota and Basidiomycota were dominating as expected (Liu *et al.*, 2011; Dong *et al.*, 2017; Zhou *et al.*, 2017). Bacterial core microbiomes of both provinces were also very similar and dominated by taxa involved in nitrogen fixation and cycling. The well-known nitrogen-fixing genera *Bradyrhizobium* forms symbiotic relationship with plants of the Fabaceae family (Hennecke, 1990). Soybean fields are often inoculated with *Bradyrhizobium* so its wide distribution in our dataset is not surprising. It was shown that *Rhizobium* inoculation also influenced the composition of the other members of the community and increase the connections in rhizobacterial networks (Zhong *et al.*, 2019). This would therefore contribute to the

homogenization of bacterial communities between new and established soybean-growing area. The presence of an OTU assigned to *Variibacter*, only in the core microbiome from Quebec, is of particular interest. Indigenous Bradyrhizobia are well adapted to specific bioclimatic conditions and could surpass the efficacy of commercial strains for nodulation and productivity (Abou-Shanab *et al.*, 2017). A previous characterization of indigenous strains Bradyrhizobia from eastern Canada have shown a great diversity and the superiority of some isolates in terms of nodulation and nitrogen fixation when compared to commercial inoculant (Bromfield *et al.*, 2017). Therefore, this diversity represents a valuable potential, which could be lost with the intensification of soybean cultivation and systematic inoculation with commercial strains. Because *Variibacter* represent a putative indigenous strain of N-fixing rhizobia in soils from Quebec, its properties should be investigated further.

Although the diversity indexes and core microbiomes were similar between provinces, analysis of the beta-diversity revealed a clear difference between provinces in the assemblage of soil communities, especially for fungal taxa. A distance-based redundancy analysis (db-RDA) was carried out to identify the relationship between different factors and the composition of the communities. Surprisingly, crop history (number of previous years with soybean cultivation), cultivation practices (till or no-till) or soil class did not correlate with bacterial assemblage. This may indicate that the soybean crop is the main driver of the bacterial community assemblage and will dictate most of its composition, even after a single growing season. This would be consistent with many other studies (Kourtev *et al.*, 2002; Berg and Smalla, 2009). Edaphic factors had a minor correlation and, among the tested parameters, only the concentration of aluminium and nitrate were shown to be significantly linked to the composition of bacterial communities. High concentrations of Al are known to influence some bacteria (Piña and Cervantes, 1996) but it is not clear here if the effect was direct or if soluble Al and bacterial communities were co-influenced by an external parameter (e.g. pH). However, Pearson correlations highlighted individual OTUs that had been experimentally tested with these edaphic factors in the past. For example, the abundance of Chloroflexi JG30-KF-CM66 was strongly correlated with aluminium concentration (Wegner and Liesack, 2017). Our results also revealed that the abundance of some OTUs were correlated with nitrate concentrations in soil. Nitrogen in all its forms is known to influence microbial communities (Peacock *et al.*, 2001; Ramirez *et al.*, 2010). Inputs of this element come from various sources including the atmospheric deposition and dinitrogen fixation via biological fixation (Jarvis

et al., 1996; Randall and Mulla, 2001). However, the majority of the inorganic N pool in soil comes from fertilizer application (Almasri and Kaluarachchi, 2004). Elevated concentrations of nitrate promote the development of many bacteria involved in N cycling, but also influence the whole community (Zhong *et al.*, 2019). Therefore, the specific OTUs highlighted in this study could potentially serve as indicator species for a more precised management of nitrate concentration in soil. Although considered essential for plant growth and to increase crop yields, fertilizers have often been associated with groundwater pollution, potentially affecting human health and causing eutrophisation of fresh water (Randall and Mulla, 2001; Almasri and Kaluarachchi, 2004).

Soil fungal communities were clearly distinct between provinces and no significant db-RDA model was found when investigating the effect of edaphic factors. These communities therefore appeared to be influenced by other factors and at a much slower pace than bacterial ones since they are not as homogenous after a single year of soybean.

Since its first detection in North America in 1954, SCN has spread to almost every soybean-producing areas in the United States (Davis and Tylka, 2000), and to Canada (Anderson *et al.*, 1988). It was rapidly detected in new soybean fields in Quebec (Mimee *et al.*, 2014) even if reproduction success was initially reduced in those conditions (Mimee *et al.*, 2016). It is predicted that SCN presence could still expand and be greatly favored by climate change (Gendron-St-Marseille *et al.*, 2019). Worst, it was shown that the species exhibits a very high genetic diversity allowing it to adapt easily to new environment and that continuous selective pressure with resistant cultivars contributes to the spread of more virulent populations (Gendron-St-Marseille *et al.*, 2018). The pest is therefore a significant threat to the Canadian soybean industry and new tools will have to be developed for an effective management. In this study, we confirmed the lower SCN densities in soil of infested fields from Quebec (considered as having a recent history of soybean production) compared to southwestern Ontario (known to have a long history of soybean production) while egg viability was not significantly different between provinces.

Interestingly, when individual field characteristics were assessed, some edaphic factors and microbial community assemblage were significantly associated with egg number or viability. Al concentration was negatively correlated with SCN egg viability while P levels positively influenced the number of eggs. The nematicidal effect of Al was confirmed *in vivo* on the model nematode *Caenorhabditis elegans* at high concentration while lower levels decreased its

mobility (Dhawan *et al.*, 2000). It is also known that acidic soils increase Al toxicity and reduce P availability (Liao *et al.*, 2006) and that high SCN population densities are associated with high soil pH (Pedersen *et al.*, 2010). Soil class was also significantly influencing SCN with the lighter sandy soils exhibiting higher numbers of SCN eggs, consistent with many previous studies (Heatherly and Young, 1991; Koenning and Barker, 1995; Avendano *et al.*, 2004).

Of particular interest in this study is the significant associations of specific microbial assemblages with SCN egg number or viability in db-RDA models. Members of the Bacillales order were found to be negatively correlated to the number of eggs in soil. This is consistent with many previous examples of SCN-antagonistic species from this order proposed as potential biocontrol agents, such as *Bacillus firmus* and *Pasteuria penetrans* (Chen and Dickson, 1998; Zhang *et al.*, 2014). A fungal taxa annotated as *Arthrobotrys oligospora* was also significantly correlated with reduced numbers of SCN eggs. This is a model organism for interractions between fungi and nematodes and one of the most common nematode capturing fungus (Niu and Zhang, 2011). Interestingly, when computing Pearson correlations for individual significant OTUs on egg number and viability, very few remained significant, indicating that they probably act synergistically and that the effect of a single antagonistic species is not sufficient to obtain a measurable effect. These results are in contradiction with the current commercial strategy of adding a single non-native biocontrol species to the system and suggest that the promotion of an assemblage of indigenous species could lead to a better biocontrol of SCN.

It was previously shown that cysts were not a sterile environment and could be colonized by different microorganisms. Our results indicated that the diversity of the bacterial and fungal communities are much less in cysts than in soil. Also, even if the same phylum were found in the cysts, their proportions and composition were clearly different than for soil communities. Actually, the cystobiome appeared to be relatively similar in all the fields, no matter the province or soil conditions and one specific taxa, *Shewanella* sp., was present in all cyst samples even if it was not in the core soil microbiome. This is consistent with Nour *et al.* (Nour *et al.*, 2003) who showed that some bacterial taxa were ubiquitous in cysts samples. Hussain *et al.* (Hussain *et al.*, 2018) also observed that the nematode antagonist *Pasteuria* sp. was more abundant in cysts than in bulk soil. The same study concluded that the bacterial community in cysts was initially established by the consecutive selection of bacterial taxa from the root endosphere (Hussain *et al.*, 2018). However,

it is also possible that the cystobiome is vertically transmited by the female to its progeny inside the cyst. Although the mechanisms of this surprising difference between soil microbiome and cystobiome are not well understood, these results open interesting research avenues for the management of SCN. Manipulating the cystobiome, either directly or through a better selection of cultural practices, could help to reduce SCN viability, limit its establishment in new areas and increase soybean yield in affected fields.

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5. Discussion

Considering the high and still growing interest of Canadian farmers for soybean and good perspectives for expansion resulting from both climate change and breeding programs, it is safe to assume that soybean will remain a top crop in Canada for years to come (Egli and Bruening, 2000; Statistics Canada, 2018; Gendron-St-Marseille *et al.*, 2019). However, because of the introduction and spread of SCN, known to be virtually impossible to eliminate from a field once infected, undertaking actions to better manage and limit the spread of this devastating pest as soon as possible is essential (Niblack and Tylka, 2012). The only current viable options are the use of resistant varieties and rotation with non-host crops. However, reports of SCN populations overcoming soybean resistance are numerous (Young, 1992; Niblack, 2005; Howland *et al.*, 2018). New ways to fight this pest are thus desperately needed and one of the possible solutions could be taking advantage of the presence of SCN antagonistic microorganisms naturally present in infected fields by valorising them.

With that in mind, this thesis was divided into two distinct but intertwined objectives, acquiring new knowledge on the microbiome associated with soybean in Eastern Canada and the impact of soil and cyst microbiota on SCN development.

The first part focused on gathering information about the soil microbiome present in Canadian soybean fields. Our results showed that the microbiome was quite similar to what have been found in other field crops studies. The core bacterial microbiomes were also very similar between both provinces and the prevalence of nitrogen associated bacterial taxa was high, no matter how long soybean has been grown in the fields. This suggests that this crop drastically influences the soil bacterial microbiome during a single year of growth. However, those similarities were limited to the core microbiome and the assemblage of soil communities were shown to be different between provinces, for both bacteria and fungi. While none have been highlighted in RDA models for the fungi, two edaphic factors were revealed as statistically influencing the bacterial communities' assemblage, aluminium and nitrate concentrations. Thus, the hypothesis proposing that the soil microbiome in Quebec would be different from Ontario because of the shorter period of time soybean has been cultivated was only partially right. The

amount of time soybean has been cultivated seemed to be of little impact and only for fungal communities. Communities, especially for bacteria, were probably affected by other confounding factors not investigated as part of the current study (e.g. bioclimatic variables).

Then, we studied the composition of the cystobiome. It became clear that this micro-environment was a lot poorer in terms of biodiversity than the soil. Quebec and Ontario cystobiomes shared a lot of similarities and more importantly, the composition of the communities and the proportion of the various taxa present for both fungi and bacteria were drastically different from the soil microbiome. These results completely denied the initial hypothesis that cystobiome would be very similar to soil microbiome. Furthermore, the core cystobiomes comprised only one OTU in both provinces, *Shewanella* sp., while it was absent from the soil core microbiome. This highlighted the fact that SCN cysts are a distinct ecological niche. It also suggested that females could vertically transmit an important part of their cystobiomes to their offspring in the cyst (Ewald, 1987). Infecting the developing cysts with antagonistic microorganisms assemblages could thus be a potential avenue for future SCN management. This could be done by treating soybean seed with antagonistic endophytes - if this mode of dissemination is confirmed - which would infect the feeding females. This would greatly reduce the reproductive success of SCN with each new generation and the associated yield losses. This would also reduce the spread of the pest to new areas.

Finally, db-RDA models revealed that some bacterial and fungal assemblages in soils were associated to a hindered SCN reproduction. It is important to note that the results of subsequent individual correlations are to be considered very carefully. In the follow-up investigation, Pearson correlations were prefered to Spearman ones beacause db-RDA models are linear and Spearman correlations do not reflect well this kind of data. However, it is imperative to note that the most of the individual correlations in this study were affected by either one or both of the previously mentioned issues linked to Pearson correlations and that they were used as explorative tools. Amongst the microorganisms associated with lowered SCN reproduction, OTUs of the Bacillales bacterial family and the fungus *Arthrobotrys oligospora* had the most significant correlation, hence being potential SCN biocontrol agents. However, further analysis using individual Pearson correlations suggested that the previously observed correlations were of very little significance when analysed alone. It was particularly true when *A. oligospora* was alone and it might indicate

that a synergy with other microorganisms is necessary to have a significant effect. That goes against the commercial strategy to add a single non-native species known to have an antagonistic effect in infected fields and valorising the assemblages of indigenous species could be a more efficient way to manage SCN. Some soil edaphic factors also had an effect on SCN development. Aluminium concentration was negatively correlated to reduced egg viability. Higher phosphorus concentrations and sandier soils were both linked to higher number of eggs in soil. Therefore, valorising cultural practices that influence these parameters could be a first step to make recommendations that are in line with a good management of SCN.

6. Conclusion

The combination of the recent soybean popularity in Canada and the establishment of SCN in the country confront producers to a difficult situation. This crop brings numerous advantages to them and growing it as much as possible can be very tempting. However, once a field is infected by SCN, there are very few viable options to manage the infestation and mitigate the damages caused by this pest. Adopting a crop rotation system including SCN resistant lines and non-host crops is currently the best method available but this strategy is already showing its limits and its efficiency was demonstrated to drop drastically after some time. Finding new ways to fight SCN are mandatory and a potential avenue would be to use SCN antagonistic microorganisms. Some of these microorganisms are already available commercially but their efficiency is limited and their usage implies the inputs of organisms in a field without knowing if these are naturally present in it, therefore likely impacting the communities' composition. Since in Eastern Canada a characterization of the microbiome associated with soybean and SCN was never made before, it was impossible to predict the impacts of the usage of such organisms.

The results of this study bring a whole new knowledge component about the microbiome associated with soybean and SCN in Eastern Canada, brushing a portrait of the actors present in these ecological niches. This information could be precious in a context of SCN biocontrol. Results have also highlighted various parameters that are correlated to changes in SCN viability. These newly acquired data could thus be used in the development of alternative and more sustainable ways to manage SCN rather than the crop rotation system involving resistant lines of soybean currently used, while limiting the disturbances to the ecosystems of infected fields.

Since the SCN infestation is still new in Quebec and that populations apparently have difficulties to build up in the fields, it is mandatory to take full advantage of it. If using a crop rotation system with resistant lines of soybean and non-host crops is a good beginning to limit the SCN spreading, using indigenous nematicidal microorganisms could be the next thing when it comes to SCN management. However, to do so, more research is needed on these specific parameters to confirm their potential as SCN biocontrol agent. The soil aluminium concentrations and the presence of specific OTUs of the Bacillales bacterial family and the fungus *Arthrobotrys*

oligospora need to be specifically studied to pinpoint the mechanisms of their potential effects on SCN or at least confirm their efficiency. Assays in greenhouses would be ideal to control every variable in the system and more importantly to rule on their effectiveness to lower SCN viability and reproduction.

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Appendices

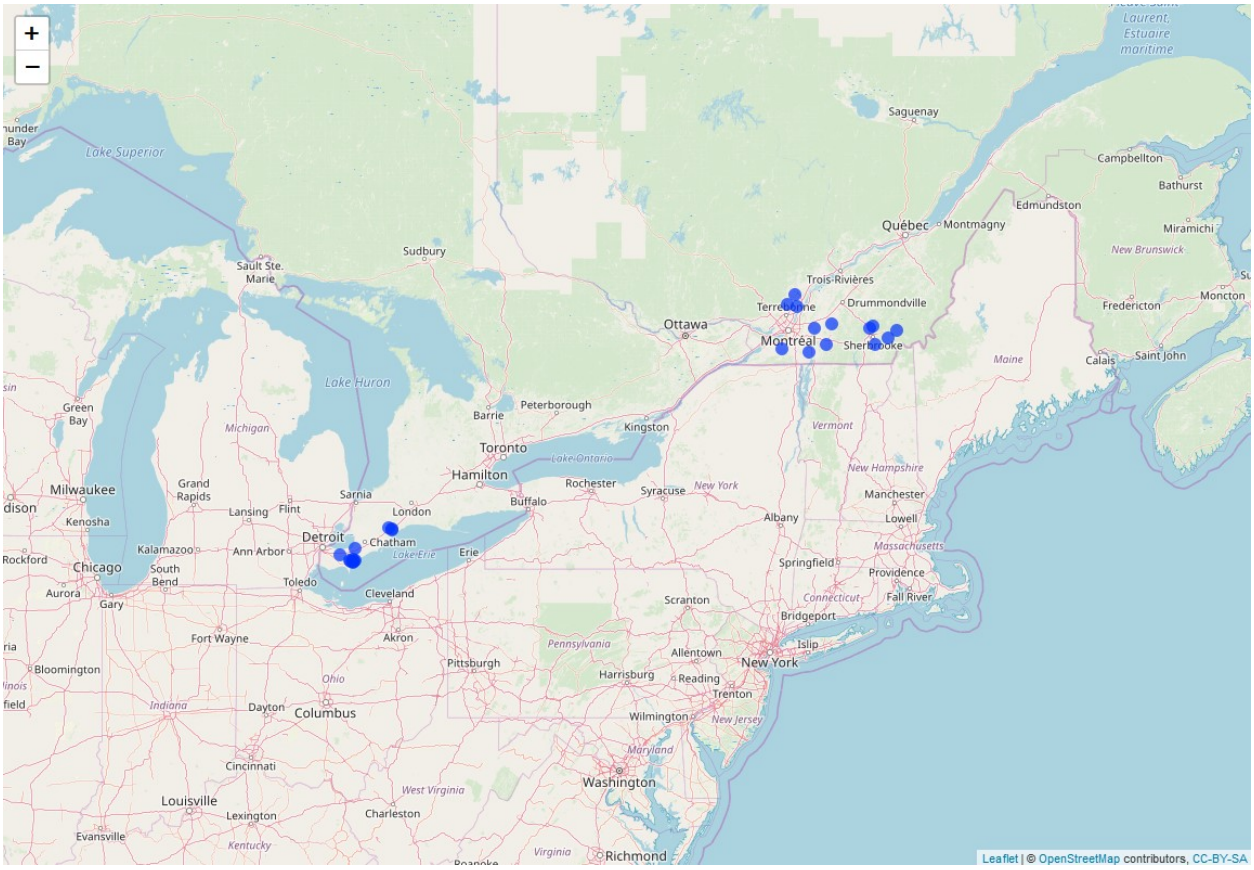

Fig. S1: Locations of sampling sites in Eastern Canada.

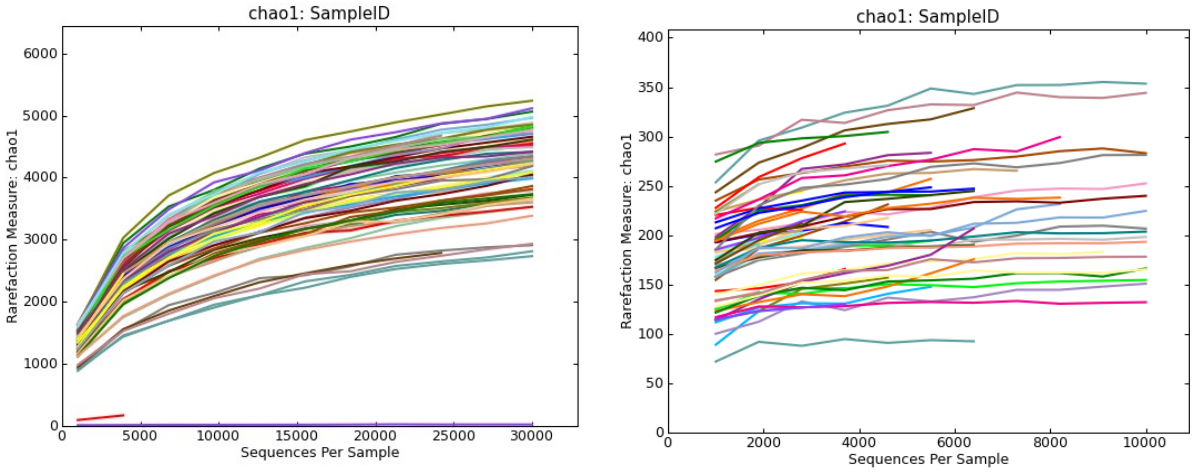

Fig. S2: Rarefaction curves based on Chao1 indexes for bacterial (A) and fungal (B) sequences obtained by amplicon sequencing of the 16S and ITS rRNA regions.

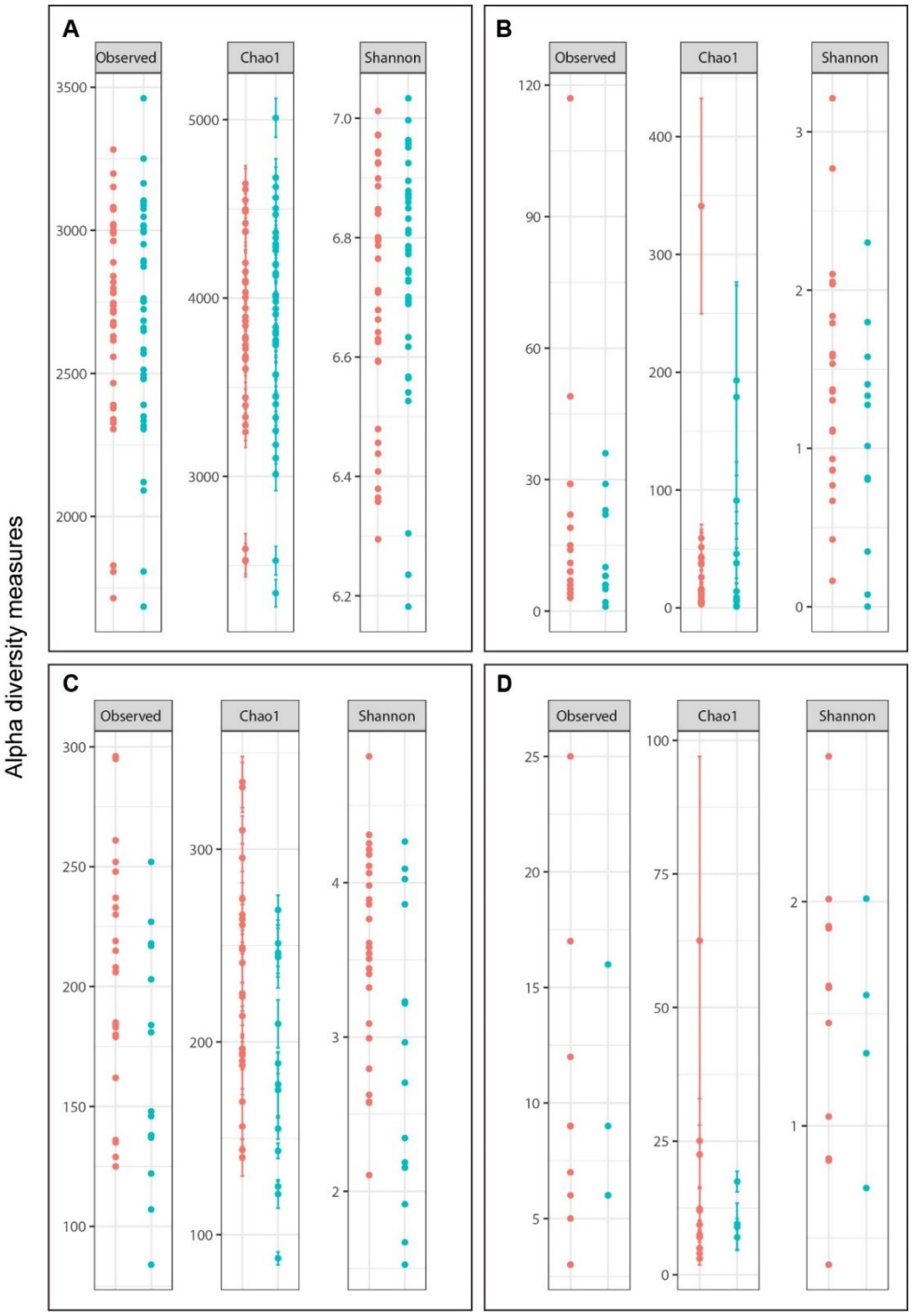

Fig. S3: Observed richness, Chao1 and Shannon indexes of bacterial soil samples (A), bacterial cysts samples (B), fungal soil samples (C) and fungal cysts samples (D) from the Canadian provinces of Ontario (red) and Quebec (blue).

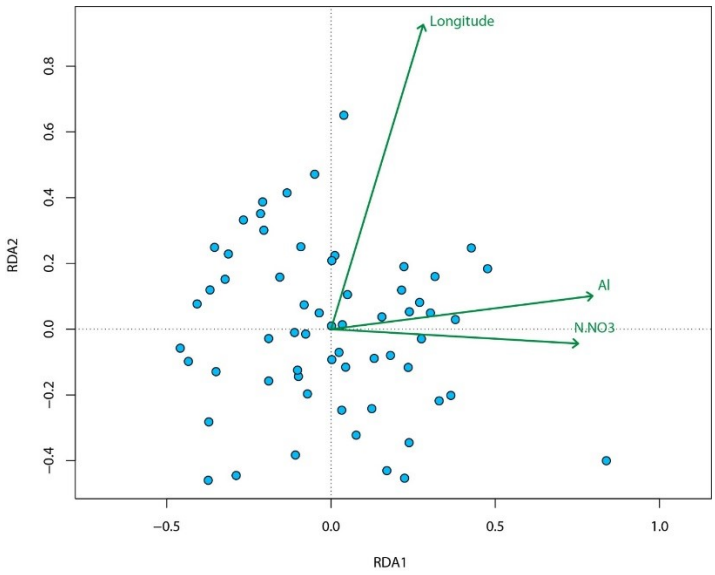

Fig. S4: Distance-based redundancy analysis (db-RDA) ordination of edaphic factors (aluminum and nitrate concentration) and longitude with the bacterial soil communities.

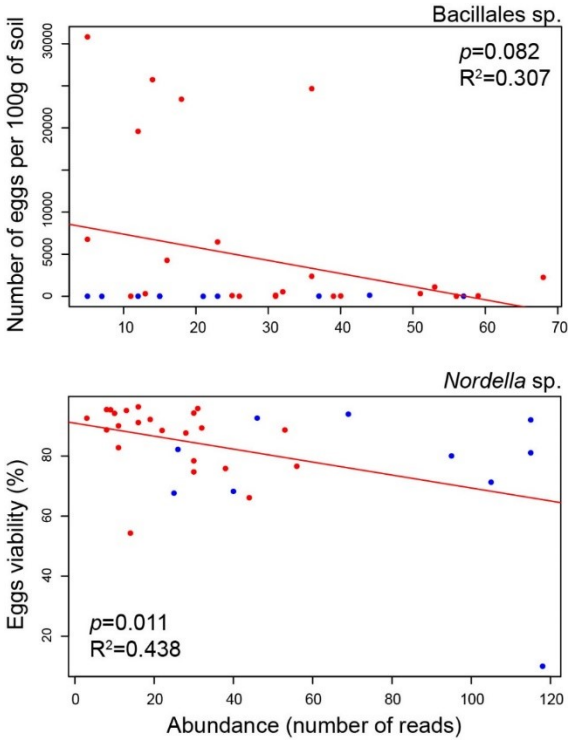

Fig. S5: Pearson correlations of the most significant bacterial OTUs linked with egg viability and the number of eggs in samples from the Canadian provinces of Ontario (red) and Quebec (blue).

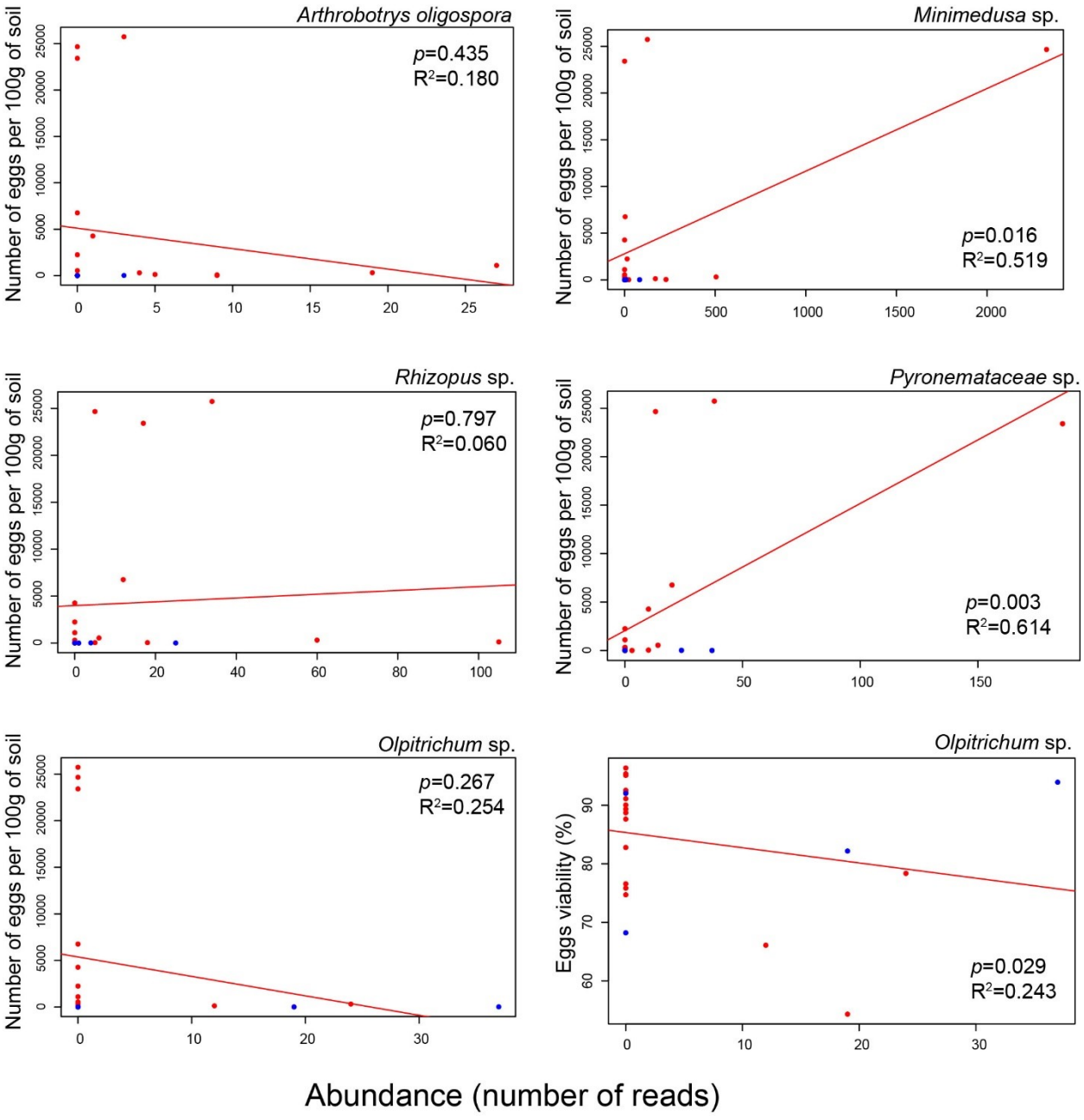

Fig. S6: Pearson correlations of the most significant fungal OTUs correlated with egg viability and the number of eggs in samples from the Canadian provinces of Ontario (red) and Quebec (blue).

Table S1: Sequences of primers used for amplicon sequencing of bacterial communities

Name	Illumina linker	Barcode	Primer
799F_1	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GAGCAACATCCTT	AACMGGATTAGATACCCKG
799F_2	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TGTTGCGTTTCTGT	AACMGGATTAGATACCCKG
799F_3	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	ATGTCCGACCAAGT	AACMGGATTAGATACCCKG
799F_4	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	AGGTACGCAATTGT	AACMGGATTAGATACCCKG
799F_5	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	ACAGCCACCCATCGA	AACMGGATTAGATACCCKG
799F_6	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TGTCTCGCAAGCCGA	AACMGGATTAGATACCCKG
799F_7	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GAGGAGTAAAGCCGA	AACMGGATTAGATACCCKG
799F_8	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GTTACGTGGTTGATGA	AACMGGATTAGATACCCKG
799F_9	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GTTACGTGGTTGATAT	AACMGGATTAGATACCCKG
799F_10	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TACCGCCTCGGAATGA	AACMGGATTAGATACCCKG
799F_11	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	CGTAAGATGCCTATGA	AACMGGATTAGATACCCKG
799F_12	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TACCGGCTTGCATGCGA	AACMGGATTAGATACCCKG
799F_13	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	ATCTAGTGGCAATGCGA	AACMGGATTAGATACCCKG
799F_14	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	CCAGGGACTTCTTGCGT	AACMGGATTAGATACCCKG
799F_15	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	CACCTTACCTTAGAGTGG	AACMGGATTAGATACCCKG
799F_16	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	ATAGTTAGGGCTGAGTGG	AACMGGATTAGATACCCKG
799F_17	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GCACTTCATTTCGAGTGG	AACMGGATTAGATACCCKG
799F_18	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TTAACTGGAAGCCCTGTGG	AACMGGATTAGATACCCKG
799F_19	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	CGCGGTTACTAACCTGGAG	AACMGGATTAGATACCCKG
799F_20	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GAGACTATATGCCCTGGAG	AACMGGATTAGATACCCKG
1115R_1	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	GAGCAACATCCTA	AGGGTTGCGCTCGTTG
1115R_2	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TGTTGCGTTTCTTC	AGGGTTGCGCTCGTTG
1115R_3	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	ATGTCCGACCAATC	AGGGTTGCGCTCGTTG
1115R_4	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	AGGTACGCAATTTC	AGGGTTGCGCTCGTTG
1115R_5	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	ACAGCCACCCATCTA	AGGGTTGCGCTCGTTG
1115R_6	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TGTCTCGCAAGCCTA	AGGGTTGCGCTCGTTG
1115R_7	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	GAGGAGTAAAGCCTA	AGGGTTGCGCTCGTTG
1115R_8	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	GTTACGTGGTTGGATA	AGGGTTGCGCTCGTTG
1115R_9	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TACCGCCTCGGAGATA	AGGGTTGCGCTCGTTG
1115R_10	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	CGTAAGATGCCTGATA	AGGGTTGCGCTCGTTG
1115R_11	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TACCGGCTTGCAACTCA	AGGGTTGCGCTCGTTG
1115R_12	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	ATCTAGTGGCAAACTCA	AGGGTTGCGCTCGTTG
1115R_13	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	GTTACGTGGTTGATAT	AGGGTTGCGCTCGTTG
1115R_14	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TACCGCCTCGGAATGA	AGGGTTGCGCTCGTTG
1115R_15	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	CGTAAGATGCCTATGA	AGGGTTGCGCTCGTTG
1115R_16	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TACCGGCTTGCATGCGA	AGGGTTGCGCTCGTTG
1115R_17	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	ATCTAGTGGCAATGCGA	AGGGTTGCGCTCGTTG
1115R_18	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	CCAGGGACTTCTTGCGT	AGGGTTGCGCTCGTTG
1115R_19	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	CACCTTACCTTAGAGTGG	AGGGTTGCGCTCGTTG
1115R_20	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	ATAGTTAGGGCTGAGTGG	AGGGTTGCGCTCGTTG

Table S2: Sequences of primers used for amplicon sequencing of fungal communities

Name	Illumina linker	Barcode	Primer
ITS86F_1	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GAGCAACATCCTT	GTGAATCATCGAATCTTTGAA
ITS86F_2	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TGTTGCGTTTCTGT	GTGAATCATCGAATCTTTGAA
ITS86F_3	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	ATGTCCGACCAAGT	GTGAATCATCGAATCTTTGAA
ITS86F_4	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	AGGTACGCAATTGT	GTGAATCATCGAATCTTTGAA
ITS86F_5	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	ACAGCCACCCATCGA	GTGAATCATCGAATCTTTGAA
ITS86F_6	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TGTCTCGCAAGCCGA	GTGAATCATCGAATCTTTGAA
ITS86F_7	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GAGGAGTAAAGCCGA	GTGAATCATCGAATCTTTGAA
ITS86F_8	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GTTACGTGGTTGATGA	GTGAATCATCGAATCTTTGAA
ITS86F_9	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GTTACGTGGTTGATAT	GTGAATCATCGAATCTTTGAA
ITS86F_10	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TACCGCCTCGGAATGA	GTGAATCATCGAATCTTTGAA
ITS86F_11	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	CGTAAGATGCCTATGA	GTGAATCATCGAATCTTTGAA
ITS86F_12	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TACCGGCTTGCATGCGA	GTGAATCATCGAATCTTTGAA
ITS86F_13	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	ATCTAGTGGCAATGCGA	GTGAATCATCGAATCTTTGAA
ITS86F_14	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	CCAGGGACTTCTTGCGT	GTGAATCATCGAATCTTTGAA
ITS86F_15	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	CACCTTACCTTAGAGTGG	GTGAATCATCGAATCTTTGAA
ITS86F_16	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	ATAGTTAGGGCTGAGTGG	GTGAATCATCGAATCTTTGAA
ITS86F_17	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GCACTTCATTTCGAGTGG	GTGAATCATCGAATCTTTGAA
ITS86F_18	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TTAACTGGAAGCCCTGTGG	GTGAATCATCGAATCTTTGAA
ITS86F_19	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	CGCGGTTACTAACCTGGAG	GTGAATCATCGAATCTTTGAA
ITS86F_20	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GAGACTATATGCCCTGGAG	GTGAATCATCGAATCTTTGAA
ITS4_1	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	GAGCAACATCCTA	TCCTCCGCTTATTGATATGC
ITS4_2	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TGTTGCGTTTCTTC	TCCTCCGCTTATTGATATGC
ITS4_3	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	ATGTCCGACCAATC	TCCTCCGCTTATTGATATGC
ITS4_4	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	AGGTACGCAATTTC	TCCTCCGCTTATTGATATGC
ITS4_5	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	ACAGCCACCCATCTA	TCCTCCGCTTATTGATATGC
ITS4_6	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TGTCTCGCAAGCCTA	TCCTCCGCTTATTGATATGC
ITS4_7	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	GAGGAGTAAAGCCTA	TCCTCCGCTTATTGATATGC
ITS4_8	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	GTTACGTGGTTGGATA	TCCTCCGCTTATTGATATGC
ITS4_9	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TACCGCCTCGGAGATA	TCCTCCGCTTATTGATATGC
ITS4_10	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	CGTAAGATGCCTGATA	TCCTCCGCTTATTGATATGC
ITS4_11	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TACCGGCTTGCAACTCA	TCCTCCGCTTATTGATATGC
ITS4_12	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	ATCTAGTGGCAAACTCA	TCCTCCGCTTATTGATATGC
ITS4_13	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	GTTACGTGGTTGATAT	TCCTCCGCTTATTGATATGC
ITS4_14	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TACCGCCTCGGAATGA	TCCTCCGCTTATTGATATGC
ITS4_15	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	CGTAAGATGCCTATGA	TCCTCCGCTTATTGATATGC
ITS4_16	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TACCGGCTTGCATGCGA	TCCTCCGCTTATTGATATGC
ITS4_17	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	ATCTAGTGGCAATGCGA	TCCTCCGCTTATTGATATGC
ITS4_18	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	CCAGGGACTTCTTGCGT	TCCTCCGCTTATTGATATGC
ITS4_19	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	CACCTTACCTTAGAGTGG	TCCTCCGCTTATTGATATGC
ITS4_20	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	ATAGTTAGGGCTGAGTGG	TCCTCCGCTTATTGATATGC

Table S3: Number of sequencing reads obtained per samples for the analysis of bacterial communities (16S)

Sample	Number of sequences	Sample	Number of sequences	Sample	Number of sequences	Sample	Number of sequences	Sample	Number of sequences	Sample	Number of sequences
ON9.K.A	607	ON10.K.B	247	ON3.S.C	118399	ON8.S.A	87787	QC4.S.A	67344	QC12.S.B	23491
ON9.K.B	48	ON10.K.C	57	ON4.S.A	60947	ON8.S.B	60754	QC4.S.B	59597	QC12.S.C	42128
ON9.K.C	4289	ON11.K.A	170	ON4.S.B	67026	ON8.S.C	72336	QC4.S.C	93718	QC11.S.A	60388
ON1.K.A	800	ON11.K.B	560	ON4.S.C	69390	QC2.S.C	62247	QC7.S.A	61511	QC11.S.B	82069
ON1.K.B	1646	ON11.K.C	337	ON5.S.A	77367	QC4.K.A	136	QC7.S.B	113742	QC11.S.C	65209
ON1.K.C	118	ON12.S.A	8082	ON5.S.B	38055	QC4.K.B	71	QC7.S.C	68742	QC5.S.A	140088
ON2.K.A	232	ON12.S.B	70276	ON5.S.C	68050	QC4.K.C	202	QC13.S.A	70547	QC5.S.B	86559
ON2.K.B	2154	ON12.S.C	77567	ON6.S.A	56852	QC7.K.A	980	QC13.S.B	52622	QC5.S.C	109156
ON2.K.C	97	ON9.S.A	83352	ON6.S.B	67699	QC7.K.B	668	QC13.S.C	82788	QC6.S.A	114930
ON3.K.A	1163	ON9.S.B	29028	ON6.S.C	46747	QC7.K.C	1474	QC3.S.A	73310	QC6.S.B	85062
ON3.K.C	44	ON9.S.C	69186	ON7.S.A	116628	QC6.K.A	4	QC3.S.B	110188	QC6.S.C	49142
ON4.K.A	110	ON1.S.A	58363	ON7.S.B	99527	QC2.K.B	67	QC3.S.C	66844	QC2.S.A	60712
ON4.K.B	996	ON1.S.B	41722	ON7.S.C	132816	QC2.K.C	219	QC8.S.A	132325	QC2.S.B	62366
ON4.K.C	85	ON1.S.C	68351	ON10.S.A	68636	QC10.K.A	28	QC8.S.B	79817	QC10.S.A	76729
ON6.K.A	109	ON2.S.A	51720	ON10.S.B	74405	QC10.K.B	150	QC8.S.C	76018	QC10.S.B	80832
ON6.K.B	100	ON2.S.B	69979	ON10.S.C	59952	QC10.K.C	59	QC1.S.A	74429	QC10.S.C	88516
ON6.K.C	3364	ON2.S.C	79442	ON11.S.A	62158	QC9.S.A	83254	QC1.S.B	73941		
ON7.K.A	620	ON3.S.A	64267	ON11.S.B	44706	QC9.S.B	56697	QC1.S.C	59552		
ON10.K.A	65	ON3.S.B	60710	ON11.S.C	75133	QC9.S.C	49526	QC12.S.A	56898		

Table S4: Number of sequencing reads obtained per samples for the analysis of fungal communities (ITS)

Sample	Number of sequences	Sample	Number of sequences	Sample	Number of sequences	Sample	Number of sequences	Sample	Number of sequences
ON9.K.C	15	ON9.S.C	4740	ON7.S.A	67278	QC10.K.B	1	QC1.S.B	61564
ON2.K.A	76	ON1.S.A	48068	ON7.S.B	49	QC10.K.C	55	QC1.S.C	54069
ON2.K.B	1278	ON1.S.B	59435	ON7.S.C	36115	QC9.S.B	31312	QC12.S.A	5651
ON2.K.C	53	ON1.S.C	54244	ON10.S.A	114280	QC9.S.C	3	QC12.S.B	80
ON3.K.A	48	ON2.S.A	88309	ON10.S.B	49179	QC4.S.A	35619	QC11.S.A	48291
ON4.K.C	183	ON2.S.B	90852	ON10.S.C	70451	QC4.S.B	42818	QC11.S.B	16438
ON6.K.A	100	ON2.S.C	67064	ON11.S.A	44085	QC4.S.C	24944	QC11.S.C	151
ON6.K.B	47	ON3.S.A	91992	ON11.S.B	139234	QC7.S.A	8261	QC5.S.A	34643
ON6.K.C	152	ON3.S.B	31101	ON11.S.C	33597	QC7.S.B	23397	QC5.S.B	53139
ON10.K.B	12	ON3.S.C	19175	ON8.S.A	69331	QC7.S.C	53109	QC5.S.C	24711
ON10.K.C	15	ON4.S.A	58538	ON8.S.B	49628	QC13.S.A	75701	QC6.S.A	28842
ON11.K.A	34	ON4.S.B	47196	ON8.S.C	55088	QC13.S.B	1	QC6.S.B	1672
ON11.K.B	79	ON4.S.C	22859	QC4.K.A	46	QC3.S.A	69797	QC6.S.C	43148
ON11.K.C	59	ON5.S.A	19606	QC4.K.B	2	QC3.S.B	67890	QC2.S.A	45083
ON12.S.A	31	ON5.S.B	38159	QC4.K.C	8	QC3.S.C	51023	QC2.S.B	37365
ON12.S.B	35020	ON5.S.C	57567	QC7.K.A	392	QC8.S.A	35153	QC2.S.C	42737
ON12.S.C	84344	ON6.S.A	74234	QC2.K.B	39	QC8.S.B	23936	QC10.S.A	59038
ON9.S.A	29696	ON6.S.B	32849	QC2.K.C	38	QC8.S.C	40534	QC10.S.B	75439
ON9.S.B	49656	ON6.S.C	52581	QC10.K.A	12	QC1.S.A	54680	QC10.S.C	19209