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Le microbiote associé aux racines de riz dans différents contextes d'infection par des nématodes phytoparasites : une approche écologique d'un système plante-pathogène

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Les sciences nous racontent notre histoire : l'astronomie notre passé
et l'écologie notre avenir.

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Je dédie cette thèse à quiconque sera intéressé(e) par les prochaines pages...

Bonne lecture ✭



Abbreviations

- aka = also known as
- CA = conservation agriculture
- CEC = cation exchange capacity
- *cf.* = *confer* (see)
- CIRAD = centre de coopération internationale en recherche agronomique pour le développement
- CT = conventional tillage
- DALARM = department of agricultural land resources management
- DNA = deoxyribonucleic acid
- *e.g.* = *exempli gratia* (for example)
- EMP = earth microbiome project
- (E)SV = (exact) sequence variant
- *et al.* = *et alia* (and others)
- *etc.* = *et cetera* (and the rest)
- ETI = effector-triggered immunity
- GLM = generalized linear model
- HR = hypersensitive response
- *i.e.* = *id est* (that is)
- IAA = indole-3-acetic acid
- IRD = institut de recherche pour le développement
- ISR = induced systemic resistance
- ITS = internal transcribed spacer
- JA = jasmonic acid
- KO = KEGG (database) ortholog
- LC-MS = liquid chromatography - mass spectrometry
- NGS = next-generation sequencing
- NLR = nucleotide-binding leucine-rich repeat
- NMDS = non-metric multidimensional scaling
- OD = optical density
- OTUs = operational taxonomic units
- PAMP = pathogen-associated molecular pattern
- PCR = polymerase chain reaction
- PGP = plant-growth promotion
- pH = potential hydrogen
- PPN = plant-parasitic nematode
- PRR = pattern recognition receptor
- PTI = PAMP-triggered immunity
- QTL = quantitative trait loci
- RNA = ribonucleic acid
- RNAi = RNA interference
- rRNA = ribosomal RNA
- ROS = reactive oxygen species
- RKN = root-knot nematode
- SA = salicylic acid
- SAR = systemic acquired resistance
- SCAR = sequence characterized amplified region
- SOC = soil organic carbon
- sp. = species
- spp. = species *pluralis*
- subsp. = subspecies
- TKN = total Kjeldahl nitrogen
- TSA = tryptic soy agar
- TSB = tryptic soy broth
- VOC = volatile organic compound

<u>Units</u>:

- bp = base pair
- m = meter
- cm = centimeter
- mm = millimeter
- l = liter
- µl = microliter
- M = molar (mol/l)
- ppm = part per million
- meq = milliequivalent
- ha = hectare
- kg = kilogram
- mg = milligram
- ng = nanogram
- min = minute
- rcf = relative centrifugal force
- S (16S, 5.8S, 18S, *etc.*) = Svedberg
- °C = Celsius degree
- % = percent

<u>Chemical formula</u>:

- CaO = calcium oxyde
- CO_2 = carbon dioxide
- $C_6H_{12}O_6$ = glucose
- $HClO_4$ = perchloric acid
- HCN = hydrogen cyanide
- HNO_3 = nitric acid
- H_2O = water
- $K^{(+)}$ = potassium (ion)
- KCl = potassium chloride
- K_2O = potassium oxide
- MgO = magnesium oxide
- N = nitrogen
- NH_4^+ = ammonium
- NH_3 = ammonia
- NO_3^- = nitrate
- NPK = nitrogen, phosphorus, potassium
- N_2 = diazote
- O_2 = dioxygen
- P = phosphate
- Pi = inorganic phosphate
- P_2O_5 = phosphorus pentoxide
- SO_4^{2-} = sulfate anion (available sulfate)
- Zn = zinc

Glossary

- ★ autotrophic (adjective): able to self-nourish by using inorganic substances as a source of nutrients and using photosynthesis or chemosynthesis as a source of energy for carbon fixation (like most plants and certain bacteria and protists)
- ★ biocontrol (noun): biological control of a pest or pathogen population *via* the action or by-products of living organisms
- ★ biome (noun): distinct spatial unit consisting of a biological community (*e.g.* including the fauna and flora at geographical scale, or microorganisms at microscopic scale) that has formed in response to shared environmental conditions
- ★ disease suppressive soil (noun): soil having a low level of disease development and incidence even though a virulent pathogen and a susceptible host are present (Mazzola *et al.*, 2002). By opposition, conducive soils harbor plants with a higher level of disease expression.
- ★ dominant allele (adjective): variant of a gene on a chromosome masking or overriding the effect of a different variant (recessive allele) of the same gene on the other copy of the chromosome
- ★ dysbiosis (noun): altered composition of microbes, in contrast to a normal or "balanced" composition of microbes named eubiosis. It is also often associated with a lower diversity, a higher variability, and a diseased phenotype in humans.
- ★ ecosystem (noun): ecological system consisting of all the interacting biological entities and their physical environment
- ★ endophyte (noun): organism which lives inside a plant
- ★ entity (noun): a distinct biological object which contains in itself all the conditions essential to individuality. It can be an organism, a species, a gene, a sequence, *etc.*
- ★ eukaryote (noun): single-celled or multicellular organism whose cell(s) contain(s) a distinct, membrane-bound nucleus and other organelles enclosed by a plasma membrane
- ★ gall (noun): also called cecidia, a tumor-like organ on the external tissues of an organism caused by the infection of a parasite
- ★ heterotrophic (adjective): unable to produce its own food and therefore derives its intake of nutrition from other sources of organic carbon, mainly plant or animal matter
- ★ holobiont (noun): the host and its associated microbiota as one unit of selection that coevolves as one entity
- ★ interaction (noun): reciprocal reaction, *i.e.* action that occurs as two entities have an effect upon one another
- ★ microbial ecology (noun): scientific study of interactions between microbial communities and their environments
- ★ microbiome (noun): the microbiota and its "theater of activity" (Berg *et al.*, 2020). In this manuscript, the term microbiome is generally used in the broader meaning of microbiota, especially if its ecological niche is explicitly mentioned.
- ★ microbiota (noun): community of microorganisms living in a specific environment
- ★ niche (noun): match of an entity to a specific environmental condition. It describes how an entity responds to the distribution of resources and competitors and how it, in turn, alters those same factors.

★ nitrification (noun): process by which organisms (mainly bacteria) synthesize ammonia from nitrogen fixation following the equation $N_2 + 6\,H_2O \rightarrow 2\,NH_3 + 3\,O_2$

★ pathogen (noun): biotic causal agent of an infectious disease

★ pathogenicity (noun): ability of a pathogen to induce a disease

★ pathobiome (noun): pathogen integrated within its biotic environment. In the case of disease emergence, it is the host-associated pathogenic microbiome, in contrast to a "healthy" microbiome.

★ parasite (noun): organism in close interaction with another organism (host), that lives in or on it and gets its nutrients from or at the expense of it

★ parasitism (noun): ability of an organism to develop in or on a host while consuming its nutrients for its own growth and reproduction

★ photosynthesis (noun): process by which chloroplast containing organisms (mainly plants) and some other organisms use sunlight to synthesize organic matter from carbon fixation following the equation $6\,CO_2 + 6\,H_2O + \text{light} \rightarrow C_6H_{12}O_6 + 6\,O_2$

★ phytobiome (noun): plants and their environment including all microorganisms and macroorganisms living in, on or around the plants, and the abiotic components (soil, water, atmosphere, *etc*.)

★ prokaryote (noun): unicellular organisms that lack membrane-bound structures, the most noteworthy of which is the nucleus

★ protist (noun): any eukaryotic organism that is not an animal, plant, or fungus

★ protozoa (noun): informal term for a group of single-celled protists that feed by heterotrophy

★ quorum-sensing (noun): the ability to detect and respond to cell population density by gene regulation

★ reservoir (noun): population of organisms (*e.g.* plants or animals) or specific environment (*e.g.* air or water) in which an infectious pathogen naturally lives and reproduces without causing disease, or upon which the pathogen primarily depends for its survival

★ resilience (noun): measure of the speed at which a system returns to its original state after a perturbation (definition in Ecology)

★ resistance (noun): ability of a system to maintain its original state in the face of an external disturbance or perturbation (definition in Ecology), or the ability of a host to limit colonization by a pathogen and therefore its multiplication leading to its exclusion (definition in Pathology)

★ rhizosphere (noun): soil zone that surrounds and is influenced by the plant roots

★ saprotroph, saprophyte or saprobe (noun): organism that feeds on non-living organic matter (detritus)

★ symbiont (noun): organism living in a symbiosis with another

★ symbiosis (noun): interaction between two (or more) biological entities living in close physical association (Bulgarelli *et al*., 2013) to the advantage of both (or all entities, *i.e.* mutualistic relationship) or, to a larger extent, to the advantage of the symbiont(s) only (*i.e.* non-mutualistic relationship)

★ taxa (noun): taxonomic group of any rank, *i.e.* kingdom, phylum, class, order, family, genus or species

★ tolerance (noun): ability of a system to survive a given stress and to limit the damages it causes (definition in Ecology), or the ability of a host to reduce the effect of infection on its fitness regardless of the level of pathogen multiplication (definition in Pathology)

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General introduction

(English version)

Background on the subject

Plant diseases have played a major role in our civilization, just like the late blight disease on potatoes, caused by the pathogen* *Phytophthora infestans*, which forced 4.5 million starving Irishmen to flee the Great Famine in the mid 1800s. This oomycete was responsible for a crop loss of nearly 75% and a population decline of nearly a quarter in ten years, which represented about 1 million people (Brzustowski *et al.*, 2002). This example illustrates that the most important plant diseases for humans are certainly related to their impact on our agricultural systems. They are not the only threat to plant health, but they constantly put pressure on our food security, especially in maladapted systems where causes of yield reduction are poorly known and therefore poorly managed. Moreover, in the case of underground signs of the infection and non-specific symptoms like for the late blight disease or root-parasitic nematodes, the pathogen has less chance to be identified and more chances to become unmanageable. Besides, infection by phytopathogens does not always lead to a disease emergence or yield reduction (Weller *et al.*, 2002) but still remains a potential threat for our modern agriculture. Indeed, the socio-economic (*e.g.* growing world population) and environmental (*e.g.* climate changes) conditions stimulate rapid changes of our agrosystems (*e.g.* crop intensification and homogenization) which can be favorable for plant pathogens (Stukenbrock and McDonald, 2008; Kreye *et al.*, 2009). This encourages us to reconsider plant pathogens with an ecological view, as parasites* interacting with their plant hosts, themself interacting with a cohort of soil organisms submitted to environmental factors which can strengthen or weaken both entities, possibly giving advantage to one of them, in an apparently slow and silent process.

Plants are indeed evolving with plenty of microorganisms in their surrounding environment, called the microbiome*, and these interactions* can result in detrimental or beneficial effects for the plant health. How do plant-pathogen interactions fit into an ecological context? It is one of the ten big unanswered questions in plant-microorganism interactions (Harris *et al.*, 2020). Can the microbiome contribute to plant immunity? How does environmental variation shape the interaction between plant immunity and the microbiome? These are also some of the key questions in the emerging field of community ecology* (aka synecology) that have gained increasing attention recently (Teixeira *et al.*, 2019) and that arose in my thesis. Prosser and Martiny (2020) listed the four major themes emerging in microbial ecology* as following:

1) a theme about **eco-evolutionary processes** in the context of microbial community diversity. Microbial community ecology can not ignore evolution and, further, must simultaneously consider ecological and evolutionary processes that drive microbial composition and diversity in any community.
2) a theme about the **interactions within and between microbial communities**. After having described who (what microorganism) is in the communities and what functions they can bring, what matters most is how they interact between each other to make these potential functions actually functioning and how they can contribute to resilience* to biotic and abiotic stresses.
3) a theme about the importance of **space and compartimentation**. Investigations on the microscale are especially challenging and our ignorance has hindered conceptual advances.
4) a theme about the **responses of microbial community composition to environmental changes** and how compositional variation is related to functioning.

In the plant environment, nematodes are the most abundant soil animals on Earth (van den Hoogen *et al.*, 2019) and are of special interest because of the parasitic pressure they put on our cropping systems. They are very diversified and belong to every trophic group of the soil food web (Bongers and Ferris, 1999). Among the 24 genera of rice phytophagous nematodes, *Meloidogyne* spp., aka root-knot nematodes, profoundly affect rice yield (Jones *et al.*, 2013). In particular, *Meloidogyne graminicola* is ubiquist and particularly detrimental in South-East Asia, where the vast majority of this cereal is produced and consumed (Mantelin *et al.*, 2017). The prohibition of synthetic nematicides for public health concerns, combined with changes in cropping practices (*e.g.* aerobic rice and direct seeding) have led to an increase in disease emergence in the fields in the last decades (Ravindra *et al.*, 2017). In this context, the limits of conventional agriculture based on petrochemistry and a plant-centered vision for pest and pathogen management have been reached. Therefore, alternative approaches appear crucial to sustain world food security while preserving global health.

Native microorganisms (bacteria, fungi, protists*, *etc.*) offer promising potential in reducing the impact of soil-borne pathogens such as root-knot nematodes (Schlatter *et al.*, 2017). Many biological control agents of plant-parasitic nematodes have indeed been described (Stirling, 2015). They inhabit soil and might become plant residents (Edwards *et al.*, 2015). Within an assemblage of other microorganisms, they can have direct or indirect phytobeneficial effects, bringing additional plant traits of disease tolerance* or growth improvement (Trivedi *et al.*, 2020). However, soil disturbances can disrupt the biological processes on which soil health, and therefore plant health rely (Kibblewhite *et al.*, 2007; Saleem *et al.*, 2019). Obviously, many components implicate in the fragile balance to maintain soil disease suppressiveness against plant-parasitic nematodes, such as microbial activities, organic matter content, chemical composition and physical constitution (Silva *et al.*, 2018). Therefore, studying the impact of each component, alone or collectively, is required in order to build soil disease suppressiveness to plant-parasitic nematodes.

On one hand, the genetic basis and the histological, morphological and physiological impacts of rice response in a context of compatible or incompatible interaction with root-knot nematodes have been well described in several studies (Fuller *et al.*, 2008; Kyndt *et al.*, 2014; Petitot *et al.*, 2017; Phan *et al.*, 2018). On the other hand, although studies have shown the genetic basis of microbial adaptation to plant colonization and the plant response to phytobeneficial microorganisms (King, 2019; Wallner, 2020), we are just beginning to understand microbial diversity and assemblage in a natural context. Many factors shape the root assemblage such as soil type (edaphic factors), resident composition, plant genotype and age, to mention just a few (Edwards *et al.*, 2015; Hacquard *et al.*, 2016). Although we reckon that microorganisms have an important role in plant health and tolerance to root-knot nematodes (Pieterse *et al.*, 2016; Topalović *et al.*, 2020), we have little knowledge about the plant-associated microbiome in rice infected by root-parasitic nematodes. In this context, the factors that can modulate the disease output of the plant-parasite interaction remain complex to combine in agrosystems.

On this background, emerging themes in microbial ecology were studied during my thesis with the model system *Oryza sativa* and its root-associated microbiomes (with a focus on the communities of bacteria, fungi and nematodes) in order to understand ecological processes that could help farmers to limit the impact of phytoparasitic nematodes, especially the root-knot nematode *Meloidogyne graminicola* in Asian agrosystems.

Thesis outline

The approach and research questions

The work undertaken for this thesis applied an integrative approach on several **biotic** or **abiotic** components of agricultural systems in order to understand the host-parasite model system *Oryza sativa* - *Meloidogyne graminicola* (**figure 1**; **table 1**). This allowed us to address the following general question regarding the phytobiome* functioning in a broader view: **what are the effects of biotic and abiotic factors on rice and its associated microbiodiversity in different contexts of plant-parasitic infection?** All factors were not comparable within studies, but we have information on many of them to characterize the model system and we focused on a few factors to answer specific questions in each study. More specifically, in a first study (chapter 2), the impact of **the nematode infection by *M. graminicola* (microbiodiversity) and the edaphic properties (soil)** were analyzed on the bacterial community (microbiodiversity) associated with rice (plants) in the roots in three fields in Vietnam (scale) managed under conventional tillage (practices) and a humid subtropical climate (weather). In a second study (chapter 3), the impact of **the rice genotype (plants) and a conservation agriculture (practices)** were studied on the edaphic properties (soil), and on the communities of bacteria, fungi and nematodes (microbiodiversity) in the rhizosphere* (small scale) in an experimental field in Cambodia (large scale and associated weather) seven years after the transition to conservation agriculture (time). Ultimately, the impact of **the nematode infection by *M. graminicola* and the addition of bacterial rice endophytes* (microbiodiversity)** were studied on the rice phenotype (plants) in greenhouse assay (controlled conditions for the soil, the experimental time and the physical parameters on the plant growth) in a third study (chapter 4).

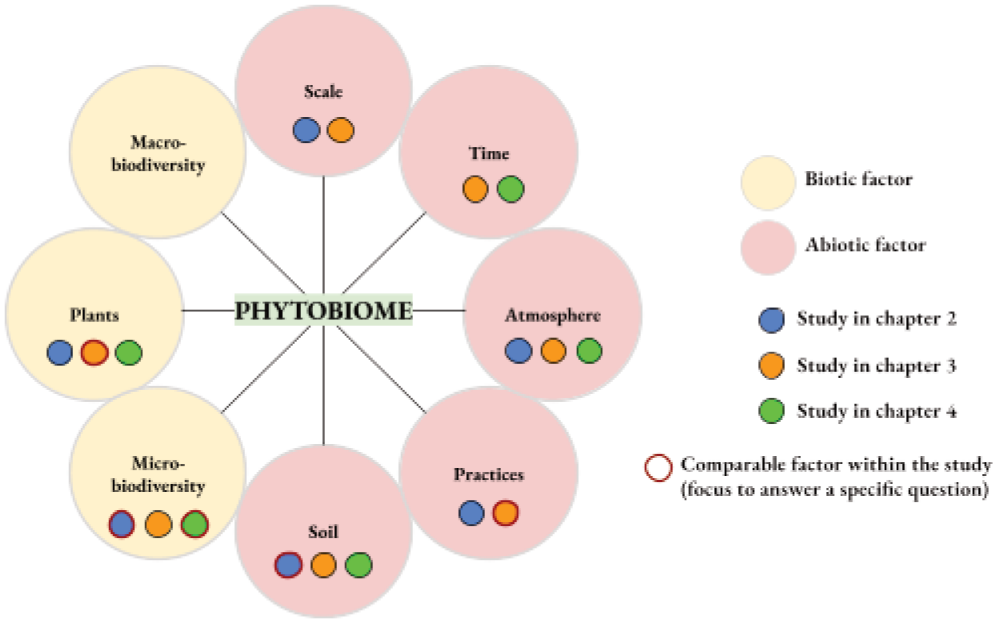

Figure 1. A global vision on some components of agricultural systems to integrate in order to fully understand the phytobiome complexity. The factors for which we have accessible information have been indicated for the studies of each chapter in this thesis, and the comparable ones within studies have been highlighted in red.

Table 1. Biotic and abiotic factors of the phytobiome studied in different contexts of infection by phytoparasitic nematodes in this thesis.

Factors (biotic or abiotic)	Chapter 2 (context 1)	Chapter 3 (context 2)	Chapter 4 (context 2)
Plants	• young rice plants • genotype *Oryza sativa* (*indica* subsp., Bac Thom n°7) • highly infected by *Meloidogyne graminicola*	• mature rice plants • **four genotypes of *Oryza sativa* (two *indica* subsp., IR504 and IR64, two *japonica* subsp., Azucena and Zhonghua)** • abundance of plant-parasitic nematodes in roots	• young and mature plants • genotype *Oryza sativa* (*indica* subsp., IR64) • sign and symptoms of the infection by *Meloidogyne graminicola*
Microbiodiversity	• **nematode infection (*Meloidogyne graminicola*)** • **bacterial community**	bacterial, fungal and nematode communities	• **nematode infection (*Meloidogyne graminicola*)** • **bacterial pretreatments (35 endophytes individually)**
Soil	**loamy soil in three fields (*cf.* physico-chemical properties in chapter 2)**	loamy soil (*cf.* physico-chemical properties in chapter 3)	mixture of silica sand and compost (7:3 in volume)
Practices	conventional tillage without cover crop and with a crop rotation in winter (onions)	**conservation agriculture (no-tillage, with *Stylosanthes guianensis* as a cover crop) and conventional tillage without cover crop**	not applicable in greenhouse
Scale	• large scale: Vietnam, Hải Dương (21°00'51.1" N - 106°19'33.0" E) • intermediate scale: three fields • small scale: roots (infected *versus* non-infected)	• large scale: Cambodia, Stung Chinit (12°32'55.6" N - 105°08'48.6" E) • small scale: rhizosphere	• large scale: not applicable in greenhouse • small scale: roots
Time	no data	seven years after the transition to conservation agriculture	sowing → bacterial pretreatment 6 days later → nematode infection 5 days later
Atmosphere	humid subtropical climate (*cf.* average measurements over the year in chapter 2)	humid subtropical climate associated with the geographical location	artificial: • 26°C by day and 24°C by night • 80% relative humidity • 12h of white light
Macrobiodiversity	no data	no data	no data

The thesis objectives

Specific questions were asked in the three studies (chapter 2, 3 and 4) of this thesis having two main objectives (**figure 2**):

Objective 1 : characterize the rice-associated microbiome...

In order to improve our knowledge on the model system *Oryza sativa* - phytoparasitic nematodes, we underwent the description of the associated communities in different contexts of infection.

(A) ...in the gall* of *Meloidogyne graminicola*

Since rice infection by the root-knot nematode *Meloidogyne graminicola* causes histological, morphological and physiological modifications, we hypothesized that the roots of rice infected by the nematode were also associated with a different bacterial community, potentially less diversified. The aim was thus to measure the effects of *M. graminicola* infection on the rice root microbiome at the plant level (infected roots *versus* non-infected roots), in terms of diversity, taxonomic structure, composition and interactions. The data used was collected in three highly infested fields in Vietnam in March 2017 and was the subject of a paper published three years later during this thesis (Masson *et al.*, 2020). Briefly, results showed that the rice infection by the plant-parasitic nematode *M. graminicola* was associated with profound changes in the microbiome and, surprisingly, higher diversity, richness and equitability. A predictive analysis suggested a shift of the bacterial metabolism in the gall to allow the community to adapt in a more nutrient-rich ecological niche*. The effects of soil properties on the bacterial community of rice roots were also assessed at the field level. This study of plant galls (aka cecidology) corresponds to chapter 2.

(B) ...under conservation agriculture

Previously, the occurrence of plant-parasitic nematodes was monitored under conservation agriculture in Cambodia in an experimental lowland rice field which was known to be conducive to the disease. At that time, no decrease was recorded compared to a type of conventional tillage (Suong *et al.*, 2018). Seven years after the transition, we observed that the plants were less infected. We hypothesized that the reduction of rice infection by phytoparasitic nematodes under this type of conservation agriculture was linked to modifications of soil properties and/or food web, which potentially harbored more antagonistic taxa* to plant-parasitic nematodes. The aim was thus to compare the effects of the two contrasted agricultural practices (conservation agriculture without tillage and with a cover crop *versus* conventional tillage without cover crop) on the rhizosphere communities of bacteria, fungi and nematodes, in terms of diversity, taxonomic structure and composition, and in terms of functioning according to their assignment to trophic guilds. Their combinations with four rice varieties of *Oryza sativa* (two *indica* subsp. and two *japonica* subsp., including one resistant to *M. graminicola*) were also assessed. The data used were collected in May 2018 and was the subject of a second paper that has been recently submitted. Briefly, results showed that the agricultural practices had more impact than the rice variety on the rhizosphere communities and that reduction of phytoparasitic nematodes (*Meloidogyne graminicola* in roots and *Hirschmanniella* spp. in

the rhizosphere) under conservation agriculture was associated with a maturation of the soil food web: under conservation agriculture, there was an accumulation of soil organic matter and nutrients available for the plants and the basal microorganisms in the soil food web, a higher richness and diversity, an increased relative abundance of saprophytic fungi and predatory-persistent nematodes, all of these potentially antagonizing phytoparasitic nematodes and improving plant development and defense. This study corresponds to chapter 3.

Objective 2: assess candidate bacteria for the biocontrol* of *M. graminicola*

Based on the field observations described through the first objective (*i.e.* there was a shift in the bacterial community of the non-infected roots compared to the galls, and conservation agriculture reduced the abundance of plant-parasitic nematodes in roots), we hypothesized that rice bacterial endophytes in the field under conservation agriculture could suppress plant-parasitic nematodes. The aim was then to assess the biocontrol effects of a set of bacteria collected in rice roots in the experimental field in Cambodia. We performed *in planta* tests to measure indirect beneficial effects on *Oryza sativa*, and *in vitro* tests to measure direct effects against *Meloidogyne graminicola*. Data was generated in controlled conditions (greenhouse at the IRD, Montpellier) during a screening test with 35 bacterial endophytes inoculated on rice plantlets. Signs (*e.g.* gall number) and symptoms (*e.g.* reduced biomass) of the infection were measured and allowed the selection of eight candidate bacteria for further investigation, during which we discovered biocontrol activities of some candidates. A complementary method was used to link the cultivable bacteria to the root microbiota and to the infection by *M. graminicola* in the field. This study corresponds to chapter 4.

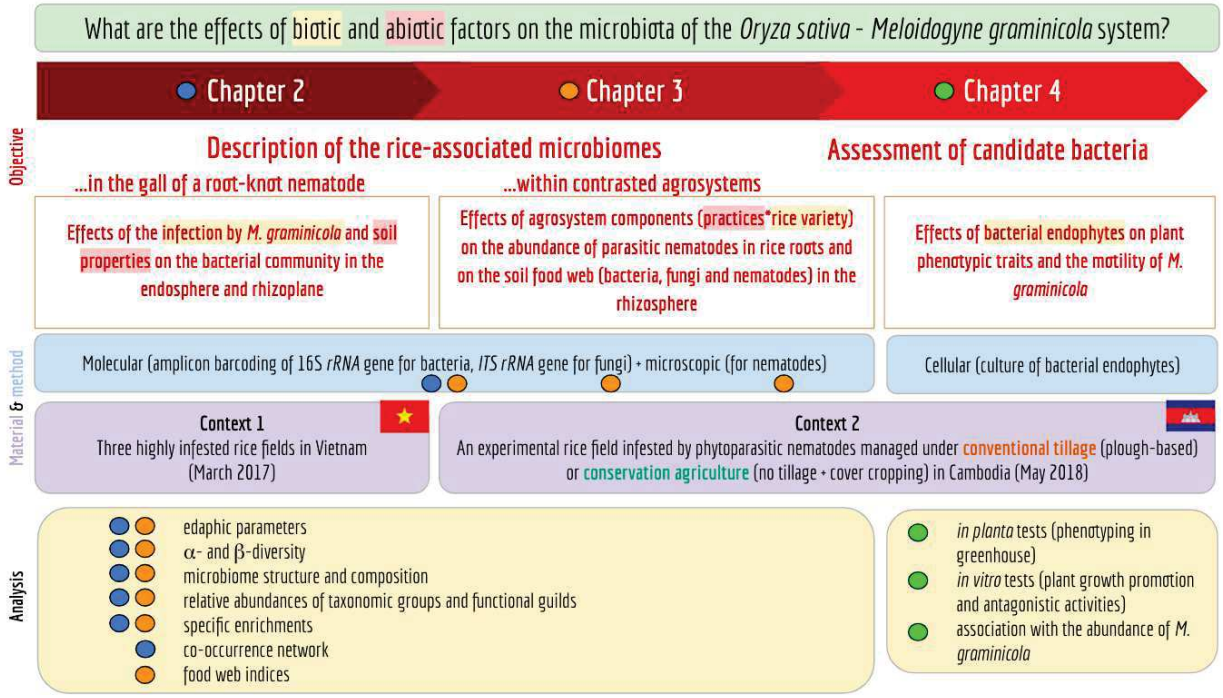

Figure 2. The general question, objectives, materials (two contexts), methods and analyzes for each study reported in the chapters of this thesis.

The materials and methods

Two **datasets** were generated in two different contexts and exploited with two **complementary methods** (*cf.* **chapter 1**):

1. one dataset collected in Vietnam in March 2017 in three fields naturally infested with *M. graminicola* (***context of infection***). Because all rice plantlets were heavily infected, each root system was divided into two sample types: infected (galled root tips), and uninfected root tips (with no visible galls). These samples isolated from the same plants were compared using an amplicon barcoding method targeting the *16S rRNA* marker gene, followed by *in silico* analyzes to search for the bacterial microbiome signature related to the *M. graminicola* infection. This type of data was exploited using the *Qiime2* pipeline available on the IRD *iTrop* server and other tools available on the *R* software for most of them.
2. a second dataset collected in Cambodia in May 2018 in an experimental field. This field was known to have high levels of infestation by phytoparasitic nematodes but turned less conducive to the infection according to our data (***context of disease suppression***). We used a molecular-based method by amplicon barcoding once again, but this time in both roots and the rhizosphere, and in the bacterial and fungal communities with the *16S* and *ITS rRNA* marker genes, respectively (performed by *Macrogen,* Seoul, South Korea). In addition, we used a microscopic-based method by morphological identification on the nematode community in the rhizosphere (performed by *ELISOL environnement*, Congénie, France), in the absence of a valid high-throughput molecular-based method for nematodes. These samples were used to study the soil food web associated with a combination of agricultural practices and rice variety. In parallel, the rice bacterial endophytes were isolated and used by cultivation techniques to test them *in planta* and *in vitro* in order to search for improvement of plant phenotypic traits (plant development and nematode tolerance) and nematode antagonism.

The manuscript flow

This manuscript is divided into four chapters. After the present general introduction (English and alternative shorter French versions), the first chapter gives a bibliographic synthesis on the subject of this thesis: the microbiota* associated with rice roots in the context of plant-parasitic nematode infection. It brings an ecological view to the plant-pathogen system *Oryza sativa - Meloidogyne graminicola*. The second chapter describes the root bacterial microbiome associated with rice infection, the so-called rice "gallobiome" of *M. graminicola*. The third chapter assesses the capacity of combinaisons of cropping systems components (agricultural practices*rice variety) to reduce the parasitic pressure in a rice field, and to modify the function and structure of the soil food web. The fourth chapter assesses candidate endophytic bacteria for the biocontrol of *M. graminicola* in laboratory (*in vitro* and *in planta*) tests. Finally, we present the main results and a general view on the *O. sativa - M. graminicola*-associated microbiome, with the limitations of this approach and some perspectives in the general discussion of this thesis (English and alternative shorter French versions).

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Introduction générale

(version courte française)

Contexte général sur le sujet

Les plantes, comme tout organisme vivant, sont soumises à des contraintes biotiques et abiotiques. Par exemple, le principal stress biotique qui réduit le rendement et la qualité de la production rizicole est la présence de “mauvaises” herbes (adventices), suivie par l'infection par l’agent pathogène fongique responsable de la pyriculariose du riz, *Pyricularia oryzae*. Les autres principaux stress biotiques sont les nématodes, les virus et les bactéries (Seck *et al.*, 2012). Selon une étude rassemblant plusieurs estimations de phytopathologistes (Savary *et al.*, 2019), les pertes de récolte de pommes de terre sont les plus élevées en raison du mildiou (3,24%) qui a causé la Grande Famine en Irlande au milieu du XIXème siècle, mais aussi du nématode à kyste (3,13%) dans le nord-ouest de l'Europe, et les pertes de récolte de soja sont les plus élevées en raison du nématode à kyste encore une fois (9,31%), en Amérique du Sud cette fois. Les nématodes phytoparasites sont en effet des agents pathogènes qui causent de sérieux dégâts sur diverses cultures dans le monde. En particulier, les nématodes à kyste et à galles sont les nématodes phytoparasites qui affectent le plus les cultures de riz (Jones *et al.*, 2013). En outre, le diagnostic est biaisé par leurs signes souterrains d'infection, au niveau des racines, ce qui entraîne une mauvaise gestion dans les systèmes de culture du riz, principal aliment de base pour des milliards de personnes dans le monde, et qui nécessiterait une augmentation de la production pour répondre à la demande mondiale d'environ 9 milliards de personnes en 2050 (Seck *et al.*, 2012). En particulier, le nématode à galles *Meloidogyne graminicola* est un agent pathogène émergent sur le riz, son principal hôte (Mantelin *et al.*, 2017). Ce nématode est signalé en Asie du Sud et du Sud-Est, en Chine, en Afrique du Sud, aux États-Unis, en Colombie et au Brésil, attaquant une autre culture majeure en plus du riz : le blé. Récemment, *M. graminicola* a été découvert en Italie (Fanelli *et al.*, 2017), ce qui met les pays européens en danger et pourrait inciter les autorités à organiser une quarantaine, et les scientifiques à évaluer des stratégies de contrôle, afin de limiter l'émergence de la maladie et son incidence, pour au final, supprimer la maladie.

Heureusement, l'infection par les nématodes phytoparasites ne conduit pas toujours au développement de symptômes et à la réduction du rendement au champ (Weller *et al.*, 2007). Les épidémies sont souvent liées aux conditions météorologiques, la pluie et l'humidité stimulant les conditions de nombreuses maladies dévastatrices, comme le mildiou par exemple (Johnson *et al.*, 2009). Dès 1974, dans leur ouvrage sur la lutte biologique contre les agents pathogènes des plantes, Baker et Cook soulignaient que l'environnement contrôle le résultat de toutes les interactions entre l’hôte, l’agent pathogène et l’agent antagoniste (du pathogène). Les différences dans l'expression de la maladie s'expliquent par des différences dans la composante environnementale du triangle de la maladie (hôte-agent pathogène-environnement). Cette composante environnementale est d’ailleurs interdépendante de la plante et de l’agent pathogène, puisqu'elle comprend tous les facteurs biotiques interagissant avec ce pathosystème (ainsi que les facteurs abiotiques agissant sur ces entités) et faisant émerger des effets sur la santé du système (Berg *et al.*, 2017). Ainsi, nous considérons aujourd'hui que la microbiodiversité peut jouer un grand rôle dans la suppression de la maladie en contribuant à des interactions bénéfiques avec le riz (Vacheron *et al.*, 2013) et/ou à des interactions antagonistes contre les nématodes phytoparasites (Stirling *et al.*, 2015), ce qui pourrait entraîner des cas asymptomatiques de l'infection et une régulation de la population de l’agent pathogène.

Au début de cette thèse, des connaissances étaient disponibles sur l'interaction riz - nématodes phytoparasite et sur l'interaction riz - endophyte, mais moins sur les trois entités en interaction. D'une part, la base génétique et les impacts histologiques, morphologiques et physiologiques de la réponse du riz dans un contexte d'interaction compatible ou incompatible avec les nématodes à galles ont été bien décrits dans plusieurs études (Phan *et al.*, 2021). D'autre part, bien que des études aient montré la base génétique de l'adaptation microbienne à la colonisation des plantes et la réponse des plantes aux microorganismes phytobénéfiques (King, 2019 ; Wallner, 2020), nous commençons tout juste à appréhender la diversité et à comprendre l'assemblage des communautés de microorganismes dans un contexte naturel. De nombreux facteurs façonnent le microbiote racinaire, comme le type de sol (facteurs édaphiques), la composition des résidents, le génotype et l'âge de la plante, pour n'en citer que quelques-uns (Hacquard *et al.*, 2016). Bien que nous reconnaissons que les microorganismes jouent un rôle important dans la santé des plantes et la tolérance aux nématodes à galles (Pieterse *et al.*, 2016 ; Topalović *et al.*, 2020), nous avons peu de connaissances sur le microbiote associé à la plante dans le riz infecté par des nématodes parasites des racines. Dans ce contexte, les facteurs pouvant moduler l'expression de la maladie restent complexes à combiner dans les agrosystèmes. Des thèmes émergents en écologie microbienne ont donc été étudiés au cours de cette thèse sur le modèle *Oryza sativa* et ses microorganismes associés aux racines (avec un focus sur les communautés de bactéries, champignons et nématodes), afin de comprendre les processus écologiques qui pourraient aider les agriculteurs à limiter l'impact des nématodes phytoparasites, en particulier du nématode à galles *Meloidogyne graminicola* dans les agrosystèmes rizicoles asiatiques.

Problématique et objectifs de thèse

Les travaux entrepris pour cette thèse entrent dans le cadre d'une approche intégrative de plusieurs composantes biotiques ou abiotiques des systèmes agricoles, afin de comprendre le fonctionnement du pathosystème *Oryza sativa - Meloidogyne graminicola* avec une vision écologique (**chapitre 1**). Cela nous a permis de répondre à la question générique suivante : quels sont les effets de facteurs biotiques ou abiotiques sur la microbiodiversité associée au riz dans différents contextes d'infection par des nématodes phytoparasites ? Dans une première étude (**chapitre 2**), l'impact de l'infection par le nématode *M. graminicola* et des propriétés édaphiques a été analysé sur la communauté bactérienne associée aux racines de riz dans trois champs au Vietnam. Dans une seconde étude (**chapitre 3**), l'impact du génotype de riz et d'une agriculture de conservation du sol a été étudié sur les propriétés édaphiques, et sur les communautés de bactéries, champignons et nématodes dans la rhizosphère de riz dans un champ expérimental au Cambodge, sept ans après la transition vers l'agriculture de conservation. Enfin, l'impact de l'infection par le nématode *M. graminicola* et de l'inoculation de bactéries endophytes de racines de riz a été étudié sur le phénotype du riz en serre dans une troisième étude (**chapitre 4**).

Les questions spécifiques de ces trois études avaient pour objectifs principaux de :

1) caractériser le microbiote associé au riz dans différents contextes d'infection par des nématodes phytoparasites

(a) dans la galle de *Meloidogyne graminicola*

L'infection du riz par le nématode à galles *M. graminicola* entraînant des modifications histologiques, morphologiques et physiologiques, avec création d'un organe nourricier appelé galle au niveau des racines, nous avons émis l'hypothèse que les racines de riz infectées par le nématode, et en particulier les galles, étaient associées à une communauté bactérienne différente, potentiellement moins diversifiée. L'objectif était donc de mesurer les effets de l'infection par *M. graminicola* sur le microbiote des racines de riz au niveau de la plante (racines infectées *versus* racines non infectées) en termes de diversité, structure taxonomique, composition et interactions. Les données utilisées ont été collectées dans trois champs fortement infestés au Vietnam en mars 2017 et ont fait l'objet d'un article publié trois ans plus tard au cours de cette thèse (Masson *et al.*, 2020). Brièvement, les résultats ont montré que l'infection du riz par *M. graminicola* était associée à de profonds changements dans le microbiote et, de manière surprenante, à une plus grande diversité, richesse et équitabilité du microbiote. Une analyse prédictive a suggéré des fonctions supplémentaires dans le métabolisme bactérien de la galle, potentiellement pour permettre à la communauté de s'adapter à une niche écologique plus riche en nutriments. Cette étude correspond au **chapitre 2**.

(b) en agriculture de conservation

Auparavant, l'occurrence des nématodes phytoparasites a été mesurée sur une rizière de bas-fond connue pour être infestée par les nématodes phytoparasites, et cultivée en agriculture de conservation dans le

cadre d'une expérience. À l'époque (trois et quatre ans après la transition), aucune diminution n'avait été enregistrée par rapport à un type de travail du sol conventionnel (Suong *et al.*, 2018). Sept ans après la transition, nous avons observé que le sol contenait moins de nématodes phytoparasites et que les plantes étaient moins infectées. Nous avons émis l'hypothèse que la réduction de l'infection du riz par les nématodes phytoparasites sous ce type d'agriculture de conservation était liée à des modifications des propriétés du sol et/ou du réseau trophique, qui abritait potentiellement plus d'espèces antagonistes aux nématodes phytoparasites. L'objectif était donc de comparer les effets des deux ensembles de pratiques agricoles (agriculture de conservation sans travail du sol et avec une plante de couverture *versus* travail du sol par labour conventionnel, sans plante de couverture) sur les communautés rhizosphériques de bactéries, champignons et nématodes, en termes de diversité, de structure taxonomique et de composition, ainsi qu'en termes de fonctionnement selon leur affectation à des guildes trophiques. Ces pratiques ont été combinées avec quatre variétés de riz *Oryza sativa* (deux subsp. *indica* et deux subsp. *japonica*, dont une résistante à *M. graminicola*) pour évaluer l'impact de ces génotypes. Les données utilisées ont été collectées en mai 2018 et ont fait l'objet d'un second article qui a été récemment soumis. En bref, les résultats ont montré que les pratiques agricoles avaient plus d'impact que la variété de riz sur les communautés de la rhizosphère et que la réduction du nombre de nématodes phytoparasites (*M. graminicola* dans les racines et *Hirschmanniella* spp. dans la rhizosphère) dans le cadre de l'agriculture de conservation était associée à une maturation du réseau trophique dans la rhizosphère. Nous avons observé une accumulation de matière organique du sol et de nutriments disponibles pour les plantes et les microorganismes basaux du réseau trophique, des modifications de la richesse et de la diversité des microorganismes (bactéries, champignons et nématodes), et une augmentation de l'abondance relative des champignons saprophytes et des nématodes prédateurs-persistants. Ces agents biologiques pourraient participer à la réduction du nombre de nématodes phytoparasites observée. Cette étude correspond au **chapitre 3**.

2) évaluer des bactéries candidates pour le biocontrôle de *M. graminicola*

Sur la base des observations de terrain décrites dans le premier objectif (*i.e.* il y a un microbiote spécifique dans la galle différent de celui des racines non infectées, et l'agriculture de conservation permet de réduire l'abondance des nématodes phytoparasites dans les racines), nous avons émis l'hypothèse que les bactéries endophytes de racines de riz dans le champs sous agriculture de conservation du sol pourraient participer à la réduction du nombre de nématodes phytoparasites. L'objectif était donc d'évaluer les effets de biocontrôle d'un ensemble de bactéries collectées dans les racines de riz dans le champ expérimental au Cambodge. Nous avons réalisé des tests *in planta* pour mesurer les effets bénéfiques indirects sur *Oryza sativa*, et des tests *in vitro* pour mesurer les effets directs contre *M. graminicola*. Les données ont été générées en conditions contrôlées (serres de l'IRD, Montpellier) lors d'un test de criblage avec 35 bactéries endophytes inoculées sur des plantules de riz. Les signes (par exemple, le nombre de galles) et les symptômes (par exemple, la réduction de la biomasse) de l'infection ont été mesurés pour évaluer la capacité des bactéries à augmenter la tolérance du riz à l'infection par *M. graminicola*. Cela a permis de sélectionner huit bactéries candidates pour une étude plus approfondie, au cours de laquelle nous avons découvert des activités de biocontrôle de certaines candidates. Nous avons aussi relié les bactéries cultivables au microbiote racinaire sur le terrain, et à l'abondance de *M. graminicola* dans les racines, afin de déceler des types d'association. Cette étude correspond au **chapitre 4**.

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Chapter 1

Bibliographic synthesis

Preamble

Parasitic: a word to describe organisms whose life depends on others. They can be on top of the food webs while plants are at the base (**figure 3**). They seem only to take advantage of all and to bring no benefit to their hosts, however they are in such close relationships with their hosts that it would be hard to separate them without affecting their hosts. So, rather than removing what we consider to be disadvantageous, let's build a better niche to strengthen the hosts in interaction with other organisms too from whom they can, in turn, take advantage. I am spoiling the take-home message that is proposed in this thesis, but it has already been sent elsewhere anyway: "Do not live against but with nature" (H. Reeves, 2009). Since the outcome of host-parasite interaction varies according to the adaptive capacity of each entity* and to environmental factors, we should bring evolutionary and ecological concepts back into plant pathology rather than thinking in the short term for each individual, be it plants or, implicitly, humans.

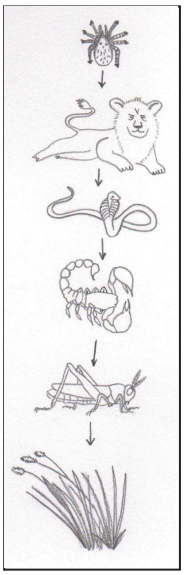

Figure 3. An example of a food chain where a parasite (a tick) is on top and a plant (a grass) is at the base (adapted from Hallé, 1999).

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Préambule

Parasites: un mot pour décrire des organismes dont la vie dépend d'autres. Ils peuvent être au sommet des réseaux trophiques alors que les plantes en sont à la base (**figure 3** - un exemple de chaîne alimentaire où un parasite (une tique) est au sommet et une plante (une herbe) à la base). Ils semblent seulement profiter de tous et n'apporter aucun bénéfice à leurs hôtes, cependant ils sont en relation si étroite avec eux qu'il serait difficile de les séparer sans affecter les hôtes. Alors, plutôt que de supprimer ce que nous considérons comme désavantageux, construisons une meilleure niche pour renforcer les hôtes en interaction également avec d'autres organismes dont ils peuvent, à leur tour, tirer profit. Je dévoile dès maintenant le message de la thèse qui est proposée dans ce manuscrit, mais qui n'est de toute façon pas inédit : "Ne pas vivre contre mais avec la nature" (H. Reeves, 2009). Puisque l'issue de l'interaction hôte-parasite varie en fonction de la capacité d'adaptation de chaque entité et des facteurs environnementaux, nous devrions ramener des concepts d'évolution et d'écologie dans la phytopathologie plutôt que de penser à court terme pour chaque individu, que ce soit les plantes ou, implicitement, les humains.

The disease triangle...

According to the **ontological** model, a disease is a foreign entity, or an agent lodged in the host. Ultimately, curing disease and restoring health amounts to expelling the intruder (Grmek *et al.*, 1998). Health and disease can also be seen as natural facts. In the **physiological** model, a disease can be defined as any disturbance or deviation from the normal physiological process that modifies its vital functions, morphology or biochemical processes and exists over time. What is "healthy" is then what contributes to survival or reproduction, is statistically normal and conforms to the design of reference within the species (Boorse *et al.*, 1977). These models are still reflected, at least partly, in current scientific concepts. In plant pathology, diagnosis of the health status (healthy *versus* diseased) is largely based on characteristic signs and symptoms expressed by diseased plants. **Signs** are either micro- or macroscopic structures directly formed by the causal agent of disease or by the result of interactions* between the causal agent and its host. **Symptoms** are the internal and external expressions of disease, not a product of the causal agent itself, but a product of its pathogenicity*. Together with the accompanying signs, symptoms make up the syndrome of disease. Diagnosis thus includes careful observation, classification and evaluation of facts (presence or absence, and syndrome severity), and a logical decision as to the cause. Identification of the causal agent is also essential to diagnosis (Britannica, 2021). To study disease occurrence, one central dogma of plant pathology is constituted by "the disease triangle" (**figure 4**). It was first published by Stevens in 1960 to illustrate the paradigm that "the existence of a disease caused by a biotic agent absolutely requires the interaction of a *susceptible* host, a *virulent* pathogen and an environment *favorable* for disease emergence" (Francl, 2001). It thus comprises three essential and distinct elements that contribute to disease emergence and that will be presented below.

The host

Plant pathologists focus on plants as hosts in their pathosystems. Plants are autotrophic* organisms at the first trophic level of the soil food web (**figure 5**). They are primary producers that fuel the food web *via* photosynthesis*, *i.e.* they are able to use solar energy to fix carbon dioxide from atmosphere and they add organic matter to soil (biomass such as dead cell, plant litter and root exudates) that constitute a basal ressource for the food web. Fungal and bacterial decomposers of the second trophic level break down plant residues, proteins and sugars released by roots into nutrients for themself and other organisms. Protozoa* and nematodes that graze on decomposers' by-products are thus concentrated near roots and much of the disease emergence or suppression occurs in this area (NRCS Soils, 2021). Some of the soil-borne organisms in the food web are detrimental to plants and are classified as plant pathogens. One main principle in plant pathology is that all plants have susceptibility to plant pathogens, unless they carry a genetic background for resistance* (Schulze *et al.*, 2005). In other words, most plants are resistant to most pathogens because of their immune system.

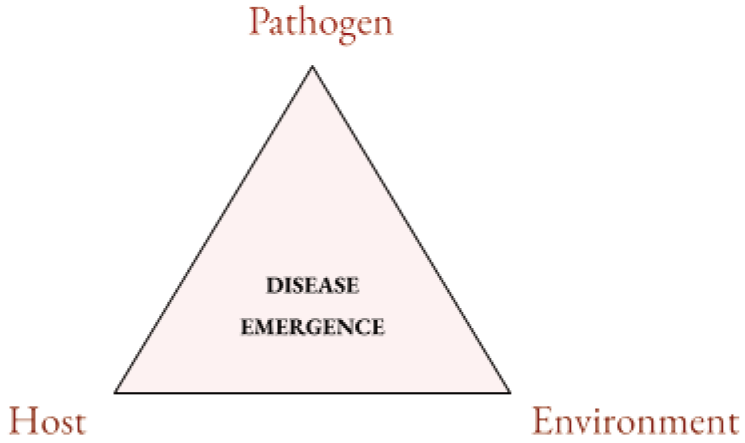

Figure 4. The equilateral plant disease triangle in which the three necessary causal elements of disease are positioned at the vertices (Stevens, 1960).

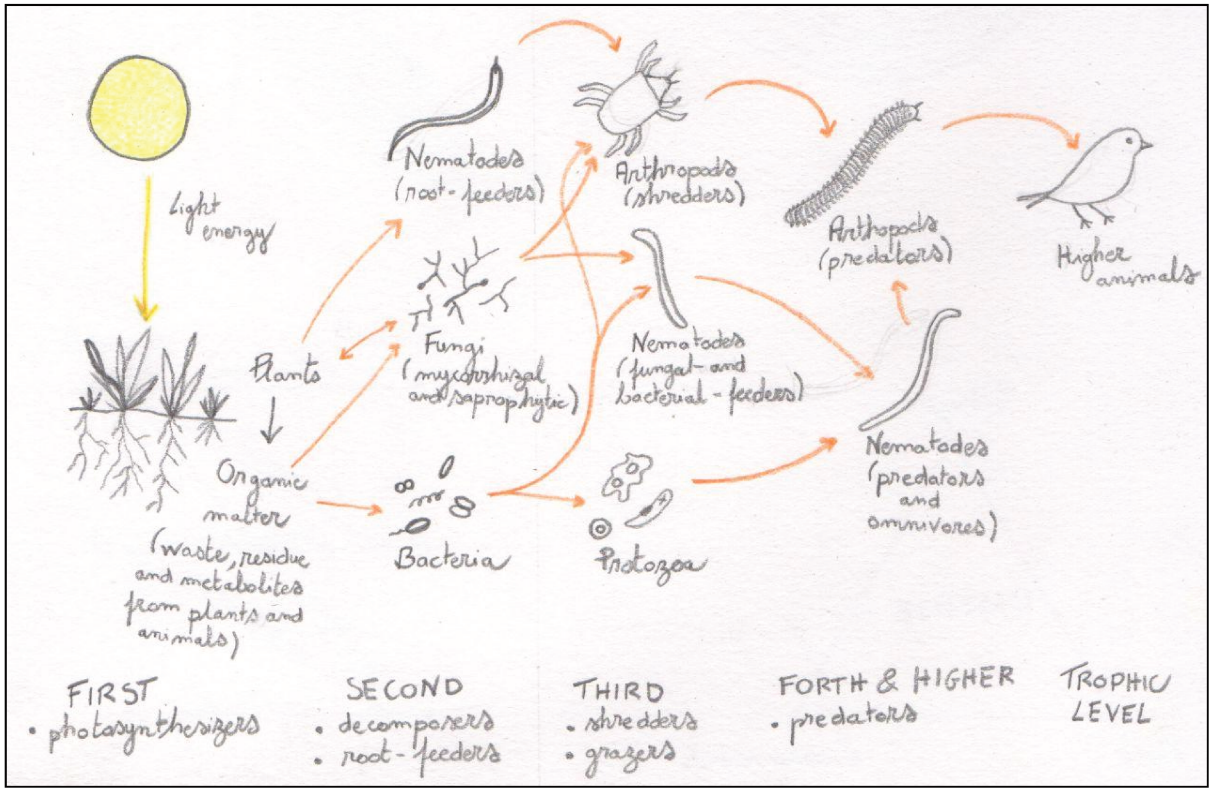

Figure 5. The soil food web (adapted from Orgiazzi *et al.*, 2016). Every soil organism occupies a trophic level in the food web according to its feeding habits (orange arrows). Basal species, such as plants, form the first trophic level and feed on no other living creature in the food web. Species in this level are also known as primary producers, as they are able to convert solar energy or chemical energy into organic matter. The intermediate levels are filled with organisms that feed on more than one trophic level (predator-prey relationships) and transfer energy to the upper trophic levels through a number of food pathways, starting from a basal species. The uppermost trophic level includes top predators that have no other species predating on them.

Two layers of plant defense

To fight against pathogens, two main amplitudes of defense based on two different recognition strategies can be differentiated.

Basal defenses: the PAMP-triggered immunity (PTI)

The first layer of defenses is referred to as innate immunity. It is inducible by the recognition of pathogens, more precisely, by the perception of molecules characteristic of pathogens (*e.g.* highly conserved structures) or characteristic of the pathogens' activities (*e.g.* cell wall fragments) that are all referred to as pathogen-associated molecular patterns (**PAMPs**). Plant cells express more than 100 different pattern recognition receptors (PRRs) mediating this perception, nucleotide-binding leucine-rich repeats (NLRs) being the most familiar class of innate immune receptors. Together, they constitute an effective surveillance system that enables plant cells to sense the extracellular presence of many different latent foes. This recognition triggers signal transduction cascades involving phosphorylation, Ca_2^+ signals and the rapid production of reactive oxygen species (ROS) in what is called the oxidative burst. Physical constitutive barriers are locally reinforced (*e.g.* cuticle, trichomes) with the synthesis of chemical compounds (*e.g.* lignin and callose that compose cell wall). Among these secondary metabolites, various defense proteins are generally toxic such as phytoalexins that are broad spectrum inhibitors belonging to the class of terpenoids, flavonoids, indole, alkaloids, *etc.* (Schulze *et al.*, 2005). Growth and defense-related hormones such as salicylic acid (SA) and jasmonic acid (JA) that specify the expression of various genes are also involved.

Specific defenses: the effector-triggered immunity (ETI)

Additionally, a second layer of inducible defenses is activated when *virulence* factors (also called **effectors**) are detected by plants. During a plant-pathogen interaction, some sources of plant resistance are indeed conferred by a single dominant* *resistance* gene (*R* gene) in the host whose product may specifically interact with the product of a corresponding *avirulence* gene (*Avr*) from the pathogen. This "gene-for-gene" hypothesis requires that both *R* and the matching *Avr* genes be present in the host and the pathogen, respectively (Flor, 1971). Such "incompatible" interaction between the plant, which is characterized as *resistant*, and the pathogen, which is characterized as *avirulent*, results in the initiation of a cascade of plant defense responses: the infected cells undergo a cell death programme, called the hypersensitive response (HR), which limits the spread of the pathogen at the infection site. Although some *R* genes can recognize several effectors, effector recognition requires large sets of *R* genes because effectors are not all conserved and pathogens produce a wide variety of effector molecules. In any other case, when either the *R* or *Avr* gene is absent, the interaction becomes "compatible" between the pathogen characterized as *virulent* and the plant characterized as *susceptible* that express a diseased phenotype (Bent and Mackey, 2007).

Models of coevolution for plant immunity strengthened by the environment

The evolutionary arms race between the molecular ammunition of the host and the pathogen puts a selective pressure that occurs constantly and forces species to adapt and coevolve. This antagonistic relationship leads to the necessity for the pathogen to have the best *virulence* factors to infect the host and for the host to have the best *resistance* factors to survive parasitism*, which changes the genetic constitution of both partners according to the Red Queen hypothesis (van Valen, 1973). In 2006, Jones and Dangl proposed a simple coevolutionary model of plant-pathogen interactions, called the "zigzag" model, that encompasses the first (PTI) and second (ETI) layers of the plant immune system. As every model, it is wrong because of its limitations to represent reality: *e.g.* it orders steps that might be simultaneous, it is restricted to the interaction between one *pathogen* and one plant at the molecular level, the distinction between the PTI and ETI might actually be blurry and, not least, it lacks an environmental context (Pritchard and Birch, 2014). Other dynamic, quantifiable and predictive models of coevolution between hosts and pathogens were proposed (van der Burgh and Joosten, 2019). However, further research is essential to explore the causes of amplitude differences in plant immune responses, in order to clearly determine what factors can make a host tolerant rather than *resistant* to a pathogen, enhance its immunity and reduce the disease emergence. The "strength" of plant immunity that allows resistance or tolerance to pathogens might be given by surrounding organisms in the plant environment, at a community level, where beneficial interactions might emerge and lead to coevolution, especially at the root interface.

Environmental role for the durability of the resistance

The "gene-for-gene" hypothesis is a monogenic resistance where only a single gene is involved with a major trait of total susceptibility or resistance (**qualitative** resistance). In addition, several *R* genes can be involved in a qualitative resistance (de Wit, 2002). Moreover, some sources of resistance are complex traits with more than one gene involved in both minor and/or major effects. In this polygenic and **quantitative** resistance, effects are partial and do not block the pathogen at the infection site but decrease symptom severity, pathogen colonization and multiplication, and can sometimes confer complete resistance to certain pathogens with a combination of quantitative trait loci (QTL) involved in the production of secondary metabolites, cell wall thickening, *etc.* (Niks *et al.,* 2015). Quantitative resistance is more complex but is durably more effective against a broad spectrum of pathogens even under disease *favorable* environments. Indeed, in an environment composed of a community of *susceptible* hosts and *virulent* pathogens, resistance can appear by the selection of a host genetic composition (*resistant*). In response, the becoming avirulent pathogen genotypes will differ and a new pathogen genetic composition will appear (*virulent*). This is called a G*G*E (genotype-genotype-environment) interaction (Schulze *et al.*, 2005) and it leads to less selection pressure on the pathogen than the "gene-for-gene" interaction (Lannou *et al.*, 2021). Looking at the dynamics of the interactions within the communities in the plant-pathogen environment can allow a more durable crop protection by limiting the pathogen proliferation and by not encouraging the emergence of new aggression strategies.

The "cry-for-help" strategy and plant growth-promotion (PGP) effects

One of the first responses of plants after a herbivore attack is the immediate air emission of volatile organic compounds (VOCs). VOCs are aldehydes, alcohols or acetates that can exert repulsive effects on phytophages, participate in intraplant communication and induce defense of neighboring plants, but also attract parasitoids and phytobeneficial organisms (Liu and Brettel, 2019). This strategy can also occur underground at the root-soil interface. Plant roots exude an enormous range of potentially valuable compounds into the rhizosphere. Root exudation includes the secretion of low-molecular weight compounds (ions such as free oxygen, molecules such as water, amino acids, organic acids, sugars, phenolics and other carbon-containing primary and secondary metabolites) that account for much of the diversity of root exudates, and high-molecular weight exudates (mucilage such as polysaccharides and proteins) that are less diverse but often compose a larger proportion of the root exudates by mass (Bais *et al.*, 2006). Although the functions of most root exudates have not been determined, several compounds play important roles in biological processes. Plants could attract or inhibit the growth of specific organisms through deposition of exudates into the rhizosphere (rhizodeposition) for its own benefit (Huang *et al.*, 2019; Worsley *et al.*, 2019). Recent evidence even suggests that plants appear to have evolved a "cry-for-help" response upon exposure to stress (*e.g.* attack of a parasite or a herbivore) by changing their root exudation chemistry leading to the recruitment of beneficial microorganisms to help minimize damages caused by the stress (Berendsen *et al.*, 2018; Rolfe *et al.*, 2019; Rizaludin *et al.*, 2021).

Phytobeneficial microorganisms can have many direct and indirects effects on plant growth promotion (PGP effects) which include fertilization (*e.g.* nitrogen fixation and phosphorus solubilization), stimulation (*e.g.* production of phytohormones) and protection (*e.g.* production of antagonistic molecules) (Bhattacharyya and Jha, 2012; Trivedi *et al.*, 2020). Plant hormones are also involved and linked to already described systemic resistances induced by microbes (ISR) or acquired upon a pathogen attack (SAR). Maithani *et al.* (2021) expose in more detail the molecules involved in the signalisation of stress alleviation. Root exudation and rhizodeposition would thus be major drivers of the positive and negative interactions with plants and could provide protection to subsequent generation in the same soil (Bakker *et al.*, 2018). As a result of these changes in soil organisms, plants modify not only its own physiology, but also the biological and physico-chemical properties of the soil that supports its growth (Passioura, 1991; Angers and Caron, 1998). Hence, plant immunity is not only determined by the genetic constitution (Violle *et al.*, 2007; Alizon, 2020) but also by the global composition of the surrounding microorganisms, and by the feedback effects of the interactions and properties that can emerge between the populations of organisms (plants, bacteria, fungi, *etc.*) in one specific environment.

The pathogen

Heterotrophic* plant-associated organisms have three main ways of utilizing plant biomass as a substrate. The majority are restricted to a saprophytic lifestyle, *i.e.* the degradation of dead plant material, because the defense system of living plants effectively prevents colonization. Only a limited number of organisms have evolved the ability to overcome the plant immune system and thus gained access to the resources of living plants. They establish either a **symbiotic** (plants also take advantage of the interaction)

or a **parasitic** (the interaction is detrimental to plants) relationship with their host. During completion of the parasite's life cycle, in some cases, hosts express a diseased phenotype. The foreign parasitic organisms are then called pathogens (Schulze *et al.*, 2005).

The causal agent of an infectious disease

To identify pathogens, one plant pathologist can refer to the classification of causal agents first postulated by Koch and resumed by "a certain pathogen will cause a certain infectious disease" (Henle, 1840). Four methodological steps were established: (1) the presumed causal agent must be observed in every occurrence of the disease; (2) it must be successfully isolated from the host; (3) once inoculated to another host, it should yield the same syndrome; (4) it must be recovered from the experimental host that was inoculated. Although it is often inapplicable (in the cases of unculturable organisms, healthy carriers or reservoir* hosts, unreproducible conditions of the diseases, *etc.*), if an agent fulfills these four gold standards, then, it will be classified as the pathogen responsible for the infectious disease it causes.

Infectious plant diseases are caused by pathogens such as fungi, oomycetes, bacteria, mycoplasma, viruses, viroids, nematodes or other plants. Some (including all viruses) are **biotrophic**, *i.e.* they keep their hosts alive while exploiting their resources for reproduction, some are **necrotrophic**, *i.e.* they kill their hosts and feed on its dead cells, and some are **hemi-biotrophic**, literally half-biotrophic, *i.e.* they kill their hosts at later stages of the colonization, becoming **saprotrophic** (Morris, 1992; Schulze *et al.*, 2005). Obligate saprotrophs* feed on dead plant cells or soil humus, are unable to live on living plants and thus don't cause disease. In contrast, obligate parasites can only take nutrients from the cells of a living plant, are thus biotrophs and can cause diseases (Dyakov, 2007). Besides, non-infectious plant diseases are caused by abiotic conditions such as extreme temperatures, toxic substances in the soil or the atmosphere, and an excess or a deficiency of an essential mineral. These are not transmissible.

A parasite characterized by its pathogenicity...

The motive force in the evolution of pathogens is an attempt to overcome the competition for resources with saprotrophs. By penetrating inside plants, pathogens can inhabit a refuge niche. However, it is not possible for most microorganisms due to the immune properties of the living cells. The easiest way to overcome host immunity is to kill plant cells. But the death of the host means a return to competition with other saprotrophs. Thus, "the evolution of parasitism is a way to biotrophic nutrition, which means replacement of the rough ways of breaking the host immunity (necrotrophic nutrition) by gentler ways ensuring live conditions of the host cells for a longer time" (Dyakov, 2007). Finally, parasitic pathogens can be seen as parasites able to induce an accidental disease, but not intending to kill the host. Frequently, parasitic processes are accompanied by proliferation of the affected tissue in the host, formation of tumors, galls, and other neoplasms where parasites find refuge.

To explicit the difference between a pathogen and a parasite, Shaner (1992) divided pathogenicity into two components: first, the **aggressiveness** (parasitic capacity) as a quantitative component that is evaluated by both the rates of penetration (infectious capacity) and multiplication in the host

(reproductive capacity), and second, the **virulence** (pathogenic capacity), as a qualitative component that reflects the intrinsic (genetic) capacity of a pathogen to cause symptoms and the success of the pathogen. Pathogens have to enter plant tissues, effectively suppress the plant immune system, gain access to plant resources and be able to grow and reproduce rapidly within plant tissues. Pathogenicity relies on virulence factors.

Virulence factors are brought by virulence genes carried by pathogens. Among them, there are toxins preventing cell functions, enzymes destroying cell walls, extracellular polysaccharides blocking the passage of fluid through the plant system, *etc.* All together, they interfere with the plant immune system and disturb physiological or developmental processes which cause the disease. However, not all virulence factors are operative with a particular host in a certain environment. It depends on various combinations of environmental factors. It can vary both experimentally (*in vitro* and *in vivo*) and spontaneously, and it can be enhanced, lost, and restored (Chamberlain *et al.*, 2014). Therefore, virulence is not a constant property of causal agents. Recently, it has even been suggested that pathogens have no structure or function unique to them, and that classification into "pathogenic" *versus* "non-pathogenic" or "virulent" *versus* "non-virulent" attributes a property to the organism that is instead a function of the host, the parasite, and their interaction (Méthot and Alizon, 2014). Biotrophic parasites, at least in the first phase, can even be forms of symbionts*, as parasites stay in living host cells or tissues, and even stimulate their metabolism (Dyakov, 2007).

...and by its host range and specialization

Parasites can be monophagous if they can parasitize plants within the same genus or several close genera, oligophagous if they broadly parasitize plants within the same host plant family, or polyphagous if they parasitize plants from a variety of families, orders, and even classes. The host range can thus be very small (**specialist** parasites), even limited to a single host species, or very large with the possibility of infecting many host species (**generalist** parasites). For a generalist pathogen, the **main host** is the most susceptible whereas **alternate hosts** are less susceptible. This qualitative vision where pathogens can be classified as *virulent* or *avirulent* for a host (and therefore plants classified as *host* or *non-host*) can be weighted by the quantitative notion of aggressiveness for the parasite (or susceptibility for plants). Indeed, some hosts are very favorable to the multiplication of a parasite, others less. We then obtain a measure of the degree of ecological specialization of a parasite (Schulze *et al.*, 2005). Some hosts, called **reservoirs** because they serve as a source of infection, harbor pathogens but suffer no disease. However, they contribute to the disease transmissibility and can cause important concerns if the number of individual hosts a pathogen affects increases dramatically in an area. In this case, the disease is said to have become epidemic, or precisely epiphytotic for plants (Britannica, 2021).

The environment

A given host-parasite interaction is not only dependent on the immune state of the host or on the pathogenicity of the parasite but also on the wider environment. The environmental part of disease emergence has been implicitly linked to the abiotic conditions in which plants and pathogens evolve, but

all the biotic factors that gravitate around them also contribute directly to their needs and constitute their environment. Consequently, the expression of plant disease is considered to be the product of genetic factors related to plants, genetic factors related to pathogens, and environmental factors including genetics of surrounding micro- and macro-organisms (biotic factors) and abiotic factors (Lannou *et al.*, 2021). This section exposes the different types of environmental factors that can participate in disease emergence or suppression.

Abiotic factors

Each parasite has optima temperature, relative humidity, *etc.* for growth. Therefore, physical factors modulate its pathogenicity. **Soil characteristics** (or edaphic parameters) such as moisture, pH, type (clay, sand, silt) and fertility can be factors limiting or favoring disease emergence. For instance, certain pathogens are favored by high moisture levels (*e.g.* the fungi *Pythium* spp. and *Phytophthora* spp. causing water molds) or low moisture level (*e.g. Sclerotium cepivorum* causing the onion white rot and *Streptomyces scabies* causing the common scab of potato). However, scab is not normally a problem when the natural soil pH is about 5.2. Certain pathogens are favored by loam soils and others by clay soils. Raising or lowering the levels of nutrient elements required by plants through fertilization can also influence disease emergence: certain infectious diseases are frequently more destructive after application of excessive amounts of nitrogen fertilizer (Britannica, 2021). **Climatic characteristics** (frequency of rainfalls, wind, sunlight intensity, *etc.*) also have non-negligible effect on pathogenicity. Anthropogenic activities leading to climate changes (increase frequency of extreme temperatures resulting in more heat waves and lasting drought, *etc.*) and biodiversity erosion might favor disease emergence and severity, especially of soil-borne diseases (Delgado-Baquerizo *et al.*, 2020).

Abiotic factors also modulate plant immunity. It was shown for instance that the resistance conferred against *Meloidogyne incognita* in tomato plants by the temperature sensitive *Mi-1.2 R* gene breaks down above 28°C (Dropkin, 1969). There is no general tendency for the impact of physical factors on host-parasite interactions, but certain conditions might increase the pathogenicity of the parasite and/or decrease the resistance of the host, leading to disease emergence. Counterbalanced effects can also happen and lead to disease suppression.

Biotic factors

In addition to physical factors, the environment is composed of very diverse biological factors, including host and parasite entities (**figure 6**). There are more than 1.6 million species on Earth that can be divided into two empires or superkingdoms (*Prokaryota* and *Eukayota*) and seven kingdoms following the classification by Ruggiero *et al.* (2015). In the same environment, we can find *Bacteria* or *Eubacteria* and *Archaea* (prokaryotes*), and *Protozoa* or *Protista*, *Fungi*, *Plantae* and *Animalia* (eukaryotes*). In the soil, there are microscopic communities of diverse taxonomic levels of living organisms such as bacteria (Delgado-Baquerizo *et al.*, 2018), fungi (Větrovský *et al.*, 2020), nematodes (van den Hoogen *et al.*, 2019) and protists (Geisen *et al.*, 2020) that are at the base of biological processes, as well as macroscopic communities. Each of them can interact with hosts and parasites, and therefore modulate the disease

emergence or suppression. In disease complexes, where hosts are infected by more than one pathogen, intraplant multiplication of pathogens and symptoms are often affected (positively or negatively) by coinfection (Tollenaere *et al.*, 2017). This emphasizes the importance of plant interactions with multiple biotic factors that contribute to disease emergence or suppression. Viruses and bacteriophages, after infection of plant cells, can also be part of biotic factors (Scholthof *et al.*, 2011).

What makes an environment favorable to disease emergence is very complex to determine. It depends on the impact of abiotic factors such as soil and climatic parameters directly on diverse biotic factors, not only on hosts and parasites but also on every surrounding organism interacting with them or, to a greater extent, sharing the same ecosystem* in which they evolve. Indeed, abiotic factors modulate the capacity of biotic factors to grow and survive in the ecosystem. In turn, biotic factors modify the environmental characteristics (pH, nutrient accessibility, toxin concentration, space availability, *etc.*) that favor or disfavor the growth of other biotic factors. Therefore, every biotic and abiotic factor dynamically contributes to building a specific niche, allowing interactions to occur. Finally, the interaction network in soil could influence plant health status (Pauvert *et al.*, 2020). A branch of community ecology, synecology, has come to remind us that the environmental part of plant disease expression is also linked to the biotic context and modulates the outcome of host-parasite interaction (Lannou *et al.*, 2021). But due to the complexity to take into account all factors and to predict their direct or indirect effects on each other, the environmental part contributing to disease emergence is restricted to one distinct corner of the disease triangle and its importance is often underestimated (Barnejee *et al.*, 2019). More emphasis on the *favorable* network interactions with hosts to determine what contributes to disease suppression and plant health is needed.

...with a holistic view

As early as 1866, "ecology" was defined as the science addressing the interactions between organisms and their organic (biotic factors) or inorganic (abiotic factors) environment. "Though the organisms may claim our primary interest, when we are trying to think fundamentally, we cannot separate them from their special environment, with which they form one physical system", the ecosystem (Schulze *et al.*, 2005).

Understanding the fluidity of the interactions

Different biological entities make up an ecosystem and interact with each other, in strong or weak, direct or indirect, and positive or negative interactions (Schulze *et al.*, 2005). Ecological interactions (**figure 7**), according to their effect on both entities, can be classified into:

- mutualism (+|+): a mutually beneficial relationship, especially if it's an obligate mutualism (symbiosis*) in which neither species can survive without the other, *e.g.* a nitrogen-fixing bacterium, an endophytic mutualist (Hardoim *et al.*, 2015) or a mycorrhizal fungus (van der Heijden *et al.*, 2008) can all be symbionts of plant hosts.
- neutralism (0|0): a relationship without a beneficial or detrimental effect for both species, *e.g.* a plant (first trophic level) and a predatory (high trophic level). They are likely to live in the same environment but their direct interaction will (obviously) not modify their growth and reproduction.
- competition (-|-): a relationship that leads to mutual disadvantage for both species. They compete for the same resources, whether this is nutrients, water or space. It can happen between two species of the same niche and leads to the exclusion of one of them (Gause and Witt, 1935), *e.g.* the emission of an antibiotic or a repulsive compound (antibiosis) by a competitor microorganism in the soil in order to exclude other microorganisms and gain access to root exudates (Karimi *et al.*, 2017).
- commensalism (+|0): a relationship in which one species (A) benefits and the other (B) is not affected either negatively or positively, *e.g.* the biodegradation of cellulose that is produced by plants and consumed by commensal bacteria (Karimi *et al.*, 2017).
- amensalism (-|0): a relationship in which one species (A) is disadvantaged and the other (B) is not affected either negatively or positively, *e.g.* a physical or chemical modification of the environment leading to the release of toxic compounds from one bacteria, altering the environment to the detriment of other microorganisms (Karimi *et al.*, 2017).
- benefice|antagonism (+|-): a relationship in which one species (A) benefits to the detriment of the other (B). The most widespread antagonistic interaction is the predatorism relationship in which the predator (A), that is a free-living species, feeds on the prey (B), *e.g.* some predatory nematodes feed on bacteria and fungi. Another antagonistic interaction is parasitism in which the parasite (A) is physically associated with its host (B) for at least part of its biological cycle, *e.g.* plant-parasitic nematodes. Another variant of this interaction is herbivorism in which the insect feeds from a plant. Some parasites and herbivores are known to harbor pathogenicity toward their hosts.

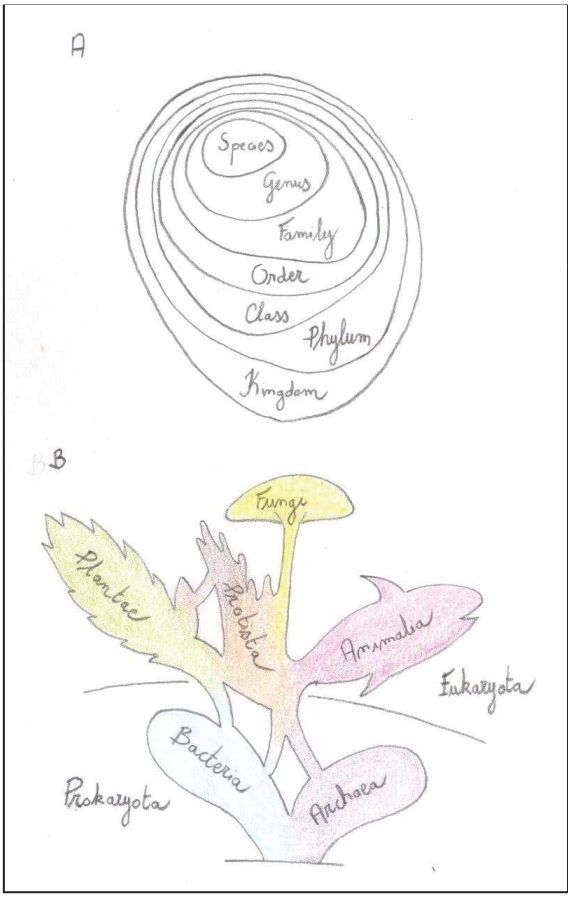

Figure 6. Classification of life. All living organisms are classified into nested groups or taxa: kingdom ⊃ phylum ⊃ class ⊃ order ⊃ family ⊃ genus ⊃ species (**A**) and belong to this schematic tree of life (**B**).

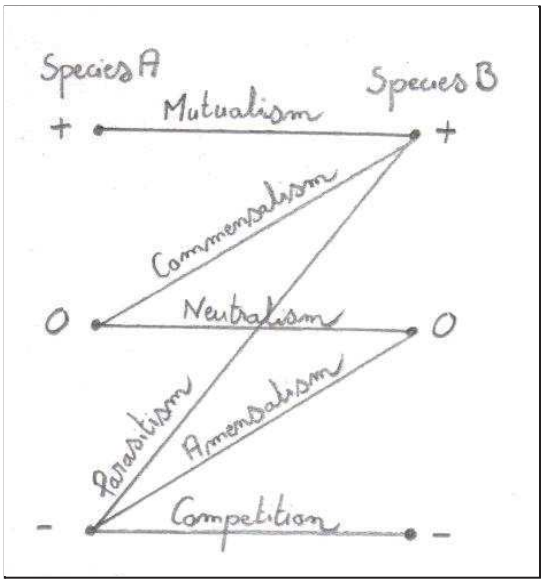

Figure 7. Classification of ecological interactions according to their effect (+ for beneficial, **0** for neutral and - for detrimental) on both biological entities (species A and species B). The benefice|antagonism interaction is illustrated by parasitism.

In practice, few examples exactly fit these descriptions. Since the net effects of species interactions vary in both sign (-, 0, +) and magnitude (from strong to weak) as a function of the biotic and abiotic context, we can say that ecological interactions are context-dependent (Chamberlain *et al.*, 2014). Mutualism, in particular, is more variable than predatorism, probably because mutualism has on average weaker interaction strengths, which may lead to greater variation. Surprisingly, despite the expectation that variation would be lowest in most controlled studies where non-target effects can be best eliminated, laboratory studies have the greatest proportion of sign changes, and the highest variation in magnitude of outcomes, probably because laboratory studies allow to isolate more effectively the effects of different contexts on interaction outcomes. Studying the interactions with plants in more or less controlled environments provides a context to determine the factors involved in disease emergence.

In fact, for an organism seeking to establish a beneficial interaction, the boundaries between mutualism, commensalism and parasitism with its host are fluid, and these interactions may best be viewed as a continuum rather than as fixed categories in nature (Méthot and Alizon, 2014): organisms can transition along a gradient from mutualism to parasitism. Additionally, with a phytopathologist's point of view, microorganisms can also transition along a gradient from symbionts to pathogens (Newton *et al.*, 2010). At the extremities of the two gradients, parasites colonize their hosts but cause only what might be described as collateral damage by their physical presence (the sign of the infection) and by taking resources from their hosts without being detrimental. By contrast, pathogens can actively damage hosts for their only own trophic benefit, frequently causing necrosis and therefore being detrimental. In other words, microorganisms can be parasites and behave as pathogens only at certain stages of their life cycle and under specific circumstances, *e.g.* the hemi-biotroph *Phytophthora infestans* (causal agent of the potato late blight) that can have a symptomless biotrophic growth phases in its life cycles before necrotic lesions are formed. Conversely, neutral plant endophytes* which complete their life cycle in plants, showing only internal signs of the infection, fit this definition of "parasites". Additionally, they can have PGP effects, and therefore behave as mutualists. Thus, the dynamic nature of the interactions can vary during the life cycle of the two associates.

In an agricultural context, it is usually the farmer's aim to favor plants and to eliminate other organisms if they are known only as pathogens. An interactionist approach can help in clarifying the intrinsic and extrinsic origins of pathogenicity and refining the principles employed by practitioners to classify organisms (Méthot and Alizon, 2014) and treat them accordingly. In ecology, pathogenicity is rather viewed as a dynamical feature of an interaction between a host and a parasite, rather than an intrinsic characteristic of the parasite. After identification of a plant-associated pathogen, one must also consider all kinds of interaction that can emerge: the ones that can strengthen the plant immunity (mutualistic or commensalistic associations) and the ones that can weaker the parasite pathogenicity (competitive, amensalistic or other antagonistic associations) in order, *in fine*, to suppress the disease.

Being more inclusive & redesigning the ecological boundaries

An ecosystem is a **physical** unit where biogeochemical processes happen, such as the decomposition of organic matter, providing the necessary nutrients to ensure life, and where biological entities interact with each other, shaping their environment. It is a thermodynamically open system, where

energy and matter also get lost to the atmosphere or the hydrosphere, such as nitrogen losses during nitrification* and denitrification, or nutrient losses *via* leaching and run-off. An ecosystem is also a **functional** unit where ecosystem processes, such as primary productivity and evapotranspiration, translate into ecosystem services, such as food and timber production, when used by humankind (Schulze *et al.*, 2005). Among the ecosystem services, regulation of pests and pathogens is of special concern here. Thus, hosts, parasites and their surrounding environment are included in an ecosystem.

Each ecosystem is characterized by its biome that is the major type of organisms it welcomes and that potentially follows the same pattern of variability across ecosystems (macro-ecological pattern). In the field of landscape ecology, at the biggest scale of ecological units, we study the biomes of tropical rainforest, savanna or toundra for instance, characterized by major types of flora and fauna. In microbial ecology, we study the microbiomes characterized by an assemblage of microorganisms (the microbiota) which interact with each other, live in the same habitat, and form their ecological niche together such as the root, leaf or soil microbiome for instance. The term microbiome, as it was originally postulated, includes not only the communities of microorganisms (communities of bacteria, archaea, fungi, algae and protists), the so-called microbiota, but also their "theater of activity" (Berg *et al.*, 2020). The latter involves the whole spectrum of molecules produced by microorganisms, including their structural elements (nucleic acids, proteins, lipids, polysaccharides), and molecules produced by hosts (root exudates for example). The core microbiota is a suite of members shared among microbial consortia from similar habitats, which is important for understanding stability, plasticity, and functioning across complex microbial assemblages.

Microbes are ubiquitous, have a vast genetic, metabolic and physiological diversity, occupy the broadest range of environments, and are essential for all biogeochemical processes and for the existence of all animals and plants (Prosser, 2020). Ecology of microbiomes is essential for predicting an ecosystem's "health" and its resilience (Prosser and Martiny, 2020). Since plants form the critical base of food chains in nearly all ecosystems, a focus is made on the plant microbiome. In particular, the rhizosphere microbiome plays an important role in plant growth, nutrition and health (Pieterse *et al.*, 2016). Indeed, plants invest a significant proportion of their photosynthetically-fixed carbon sources in the maintenance of rhizosphere microbiota, for example, *via* root exudation, rhizodeposition and quorum-sensing* mimics. In return, beneficial microbes provide important services to the plant as they improve root architecture, enhance nutrient uptake, and provide protection against plant pathogens, especially soil-borne.

Mechanisms of beneficial effects of plant-associated microbiotas can be direct or indirect (Trivedi *et al.*, 2020). **Direct** effects are mediated through **1) nitrogen fixation** by diazotrophic bacteria that can fix atmospheric nitrogen (N_2) and might actively transport ammonium (NH_4^+) and nitrate (NO_3^-) to the plant, or through **2) enhancing the plant nutrient uptake** from the soil, and unlocking essential nutrients from minerals by bacteria producing organic acids and siderophores which solubilize or chelate minerals into plant-available ions such as phosphate (Pi), potassium (K^+) or iron (Fe^{3+}). Benefits can also be **indirect**, as the plant-associated microbiota protects the plant against pathogens or pests through antagonism, or through ISR in plants. Extending the boundaries of the plant system to the phytobiome, including the plant-associated microbiota, is needed to understand the processes and functions of the microbiota in plant health and disease suppression (*cf.* **The "cry-for-help" strategy and plant growth-promotion (PGP) effects**).

Letting it evolve

In a more holistic view, hosts and their associated microbiota are seen as inseparable entities whose ecology and evolution are inseparably entwined (Agler *et al.*, 2016). There has been a fundamental paradigm shift in our understanding of microorganisms and it is now accepted that all eukaryotic individuals can be analyzed as meta-organisms of coevolved, tightly integrated, prokaryotic communities (Guerrero *et al.*, 2013; Berg *et al.*, 2015). The hologenome theory of evolution (Zilbert-Rosenberg and Rosenberg, 2008) considers the host and its associated microbiota as one unit of selection (so-called meta-organism or holobiont*) that coevolves as one entity. The term holobiont recognizes hosts and their obligate symbionts but also emphasizes the diversity of facultative symbionts and their dynamic associations within a host. As host-microbe interactions shape the reciprocal physiology, host phenotypes are profoundly affected by their complex microbial communities, in both cooperative and competitive ways (Theis *et al.*, 2016). Following this view, the beneficial interplay of the host and its microbiome is responsible for maintaining the health of the holobiont characterized by a "balanced" microbiome (eubiosis, in contrast to dysbiosis*), and prevents diseases often associated with a pathobiome*.

This association responds to a complex entanglement of ecological and evolutionary phenomena. Health defined as a dynamic conceptualisation of harmony or equilibrium established between the entities of the system (physiological model of Boorse *et al.*, 1977) fits within this eco-evolutionary framework. Therefore, ecological and evolutionary factors come together in determining whether a biological association is pathogenic or not (Méthot and Alizon, 2014). This concept also considers the fact that pathogens represent only a tiny fraction of organisms and that ecological or environmental modifications can disturb this equilibrium. When disruption occurs, it would have a cascading impact on the immune system and would offer an advantage for the expression of the disease (Walker *et al.*, 2017; Liu *et al.*, 2020).

In agricultural systems, humans have created disease emergence factories that are particularly vulnerable to epidemics of plant pathogens. Modern agriculture indeed contributes to the disruption of ecosystem stability by promoting the simplification and homogenization of cropping systems (Stukenbrock and McDonald, 2008). Commensals can become pathogenic in such environments and this can increase the global disease incidence. But the reverse is also true: soil microorganisms can suppress diseases (**BOX 1 - Soil disease suppression**) and pathogens can even end up protecting their hosts against more virulent parasites (Méthot and Alizon, 2014). There are solutions to suppress plant diseases but only one possible outcome: the reconceptualization of our production systems with crop protection based on natural regulations and the plant capacity to defend themselves against aggression. This transition cannot be based on a simple substitution logic (*i.e.* pesticides simply replaced by biopesticides on huge plots of land in monoculture). It is a question of moving from the age of chemicals to the age of biology, of moving away from highly artificial systems in which plants are under excessive fertilization and synthetic protection, in order to make the most of soil functions, to take advantage of the stimulating and protective effects of the microbiota, and to fully express the potential of plant immunity (Lannou *et al.*, 2021).

BOX 1 - Soil disease suppression

Disease suppressive soil* have been originally defined as "soils in which the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but therefore, the disease is less important, although the pathogen may persist in the soils" (Baker and Cook, 1974). In these soils, a combination of factors would make the environment *unfavorable* to disease emergence and limit disease development through time, even in the presence of a *susceptible* host and a *virulent* pathogen. It is strongly suggested that the mechanisms rely on biotic factors which strengthen the plant immunity (PGP effects, "cry-for-help" strategy, ISR, *etc.*) or inhibit the growth of soil-borne pathogens (by competitive exclusion due to specific microbes and microbial consortia) (Mazzola, 2002; Schlatter *et al.*, 2017). Plants would be able to protect themselves by recruiting defensive microbes *via* active mechanisms and by constituting two structural layers of defense: the endophytes and the microbes in the rhizosphere (Dini-Andreote, 2020). Such microbes should be available for plant-association in the soil surrounding the roots so, again, biotic factors are intertwined with soil abiotic factors to be suppressive, and plant health is closely linked with soil health and ecological functions. Many species of fungi and bacteria involved in suppressiveness against phytoparasitic nematodes for example have been identified: the bacteria *Bacillus thuringiensis* which is an antagonist to *Meloidogyne* spp. and induces resistance in plants and *Pasteuria penetrans* which parasitizes juveniles of *Meloidogyne* spp., or the fungus *Monacrosporium lysipagum* which traps many species of phytoparasitic nematodes, *etc.* (Silva *et al.*, 2018). Moreover, avoiding disturbances such as tillage which disrupts the interactions and therefore the coevolution of these native suppressive organisms, and incorporating organic residues can contribute to build suppressive soils against phytoparasitic nematodes. Other physical and chemical aspects of the soil should be monitored since any of those factors is a single driver in disease suppressive soils (**figure 8**).

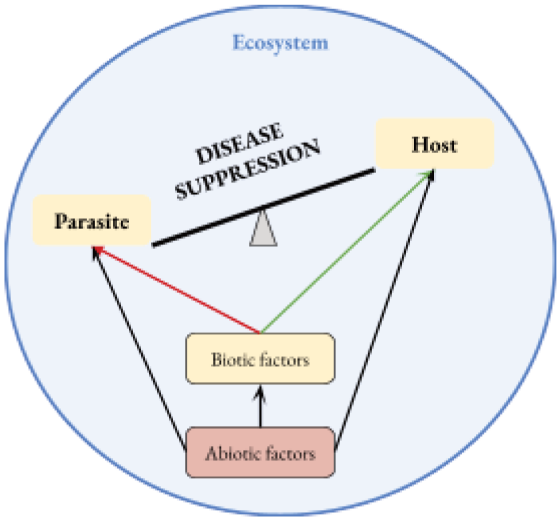

Figure 8. A simplified diagram of soil disease suppression. In such a system, a parasite interacting with its host would not be detrimental because biotic factors disadvantage the parasite (red arrow) and strengthen the host (green arrow), resulting in a healthy balance for the host. Meanwhile, abiotic factors also contribute to this balance by modulating each biological entity (black arrow). Disrupting the system can counterbalance the advantage toward the parasite and result in a disease emergence.

A plant-pathogen system...

Rice as the host plant

A model system for biologists

The rice plant *Oryza sativa* is an annual short-day plant that has the ability to produce many culms or stems from the germination of a single grain. The stem is a hollow stubble with nodes that ends in a branched panicle bearing determinate inflorescences that produce grains called "paddy" rice. The roots are fasciculated and are found at a relatively shallow depth in the soil (**figure 9**).

Rice can be considered a model plant for at least three aspects. Firstly, since it is a monocotyledonous plant distinct from the dicotyledonous model system *Arabidopsis thaliana*, it represents an interesting alternative model, although its generation time is much longer (from three to six months according to the rice variety compared to six weeks for *A. thaliana*). Moreover, since it is a self-pollinating plant, it was the first poaceae to be genetically transformed, which has facilitated the study of the function of many genes and the activity of promoters. Methods used for rice transformation are protoplast transformation, *Agrobacterium*-mediated transformation and the particle bombardment of embryos. Thus, rice transformation is accessible to all plant molecular biologists. Secondly and consequently, researchers established rice as a model system for the study of the organization of poaceae genomes. The presence of the subspecies *japonica* and *indica* provided suitable material for constructing molecular maps, because hybrids between them set enough seeds in the F2 generation and exhibited a high degree of DNA polymorphism (Izawa and Shimamoto, 1996). The genome of *japonica* variety Nipponbare was sequenced, assembled and annotated for the first time in 2005 (Matsumoto *et al.*, 2005). It has a relatively small genome size of 321 Mb (Kawahara *et al.*, 2013) and simple diploid structure (2n = 24 chromosomes). Later, 3,000 *O. sativa* rice accessions were sequenced as part of the "3K rice genomes project" (Li *et al.*, 2014) and recently compared to the Nipponbare reference genome (Wang *et al.*, 2018). Rice benefits from a large genetic diversity based on thousands of cultivated and wild varieties worldwide that are a major source of agronomically important genes, and many have been incorporated into cultivated rice. Thirdly, in phytopathology, rice allows the exploration of mechanisms that govern compatible or incompatible interactions with bacteria (*e.g. Xanthomonas* spp.), fungi (*e.g. Magnaporthe* spp.), viruses (*e.g.* RYMV) or plant-parasitic-nematodes (*e.g. Meloidogyne* spp.). In addition, many studies on rice intend to explore the dynamic interplay between plants and their associated microbiota, in the field of community ecology (Bacilio-Jiménez *et al.*, 2003; Hardoim *et al.*, 2015; Ding *et al.*, 2019).

A crop to sustain human food

The maize, rice and wheat cereals are the three most widely grown and produced crops in the world. Global production on average from 2009 to 2019 was approximately 1 billion tonnes for maize, 0.730 billion tonnes for rice and 0.718 billion tonnes for wheat (FAOSTAT, 2021). However, maize is

mainly used to feed livestock whereas rice ranks first as a source of energy for humans; it provides 20% of their energy needs while wheat provides 19% and corn 5% (Dawe *et al.*, 2010). Although rice farming is important to particular regions in some developed countries, especially in the inter-tropical area, it is of much greater importance to low- and lower-middle-income countries, where it accounts for 27 % of the calories in the poorest countries, mainly in Asia. Data showed that, although rice consumption is spread across income classes relatively equally in these countries, the poorest people actually consume relatively little wheat: most of the wheat consumption is done by people in the upper part of the income distribution (who are not below the poverty line). Thus, rice is clearly a very important food crop to sustain the poors.

We estimate that in the first half of the 2000s, there were approximately 144 million rice farm households in the world, the vast majority in developing countries. China and India are by far the greater producers of rice worldwide, followed by Indonesia, Bangladesh, Vietnam, Thailand and Myanmar (**figure 10 B**). From 2009 to 2019, Vietnam and Cambodia, for example, produced about 43 millions and 9.5 tonnes of rice, respectively, and it was the most produced commodity in these countries. Despite Asia's dominance in rice production (**figure 10 A**) and consumption, rice is also very important in other parts of the world such as in parts of western Africa. Rice is grown on both small and large farms that are generally smallest in Asia and Africa (< 1 ha).

Two rice species (*Oryza* spp.) are cultivated and believed to have evolved from one of the wild species through a long-term domestication (Sang and Ge, 2007; Khush, 1997):

- *O. sativa* was probably first domesticated in the Yangtze River Valley in China, after which it spread to other parts of Asia and has now a worldwide distribution due to its high yield (Vaughan *et al.*, 2008). Two subspecies termed *japonica* and *indica* are speciated in *O. sativa*.
- *O. glaberrima* is native from the basin of the Upper Niger River in western Africa and remains restricted to that region.

Rice production systems are diverse and can be characterized in many ways, but one of the most important is based on water source. **Irrigated** rice is grown using water supplies that supplement rainfall and natural runoff, such as water from large scale human-made surface irrigation systems or groundwater. Use of these additional supplies, coupled with good drainage, gives greater control over the level of water in the field and provides favorable growing conditions for rice. This lowland cultivation leads to higher yields with irrigation and therefore 62% of rice area is irrigated. **Rainfed** rice is grown using only rainfall and natural runoff, and these systems are more heterogeneous than irrigated rice systems. Within the category of rainfed rice, several distinct systems present different management challenges: rainfed lowland, upland/dryland, and deepwater (Dawe *et al.*, 2010). Yield growth of total rice has slowed in recent years to rates below the rate of population growth. Furthermore, growth in area harvested is much slower today than it was in the past, as the lands most suitable for rice are already under cultivation. Thus, it will be a major challenge to increase yield growth in the future so as to enable the world to feed a growing population at prices that are affordable to the poor. This goal has not yet been accomplished, as evidenced by the large numbers of people around the world who are still undernourished (FAO, 2010).

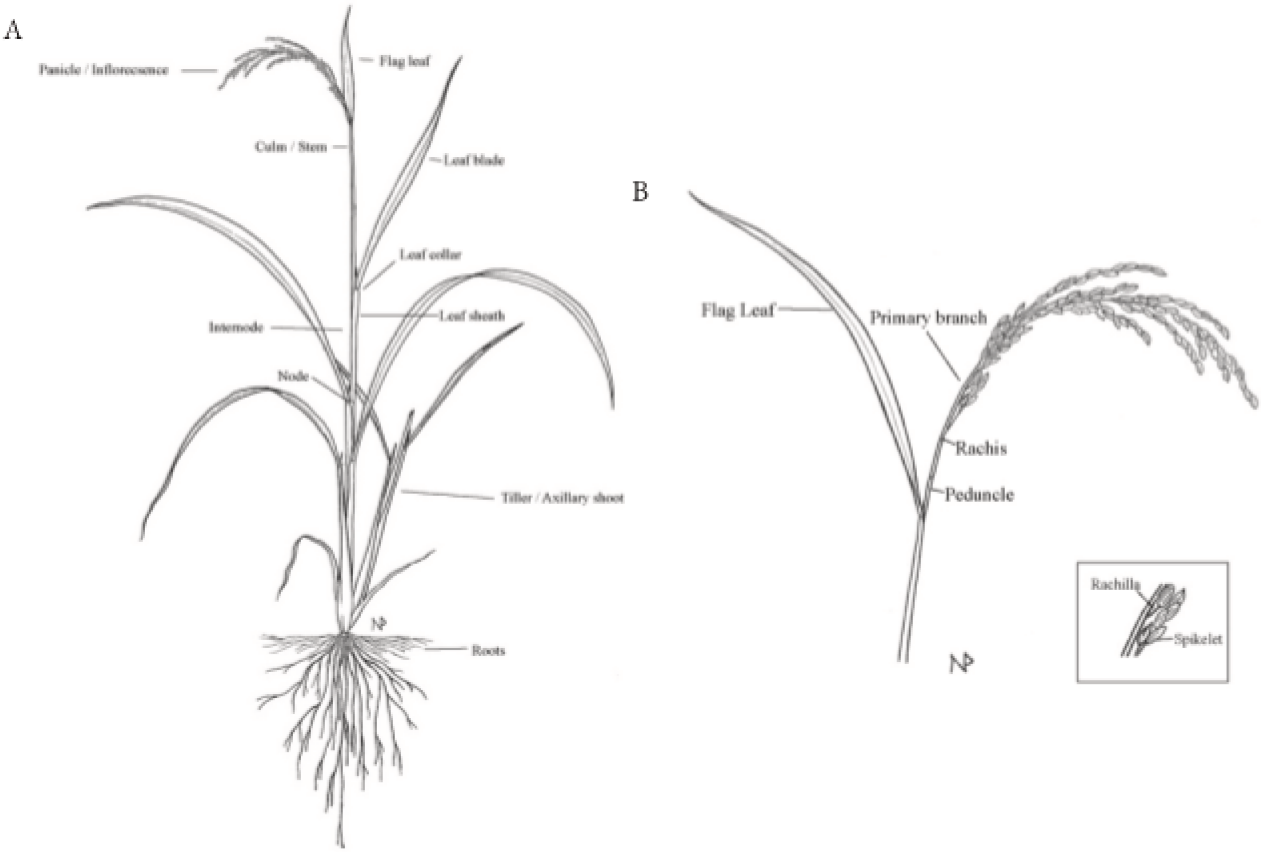

Figure 9. Anatomy of the rice plant *Oryza sativa* (variety Nipponbare), whole plant (**A**) and panicle (**B**). Drawings by Nicholas Polato from archive.gramene.org.

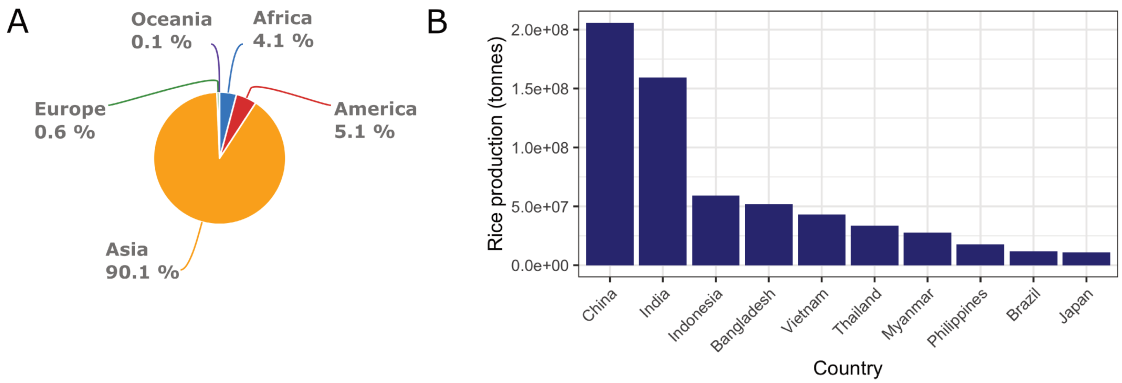

Figure 10. Production of rice (**A**) shared per continent and (**B**) in the top ten producer countries, from 2009 to 2019 (FAOSTAT, 2021).

Root-knot nematodes as the disease-causing pathogens

Soil-borne organisms...

Nematodes are non-segmented roundworms with a very simple but optimized structure (**figure 11**). The basic anatomy of nematodes is sometimes described as a tube (the endoderm with the **alimentary system** and **reproductive systems**) inside another tube (the ectoderme or body wall). They neither have a respiratory or a circulatory system, but they do have a so-called **excretory-secretory system** which is connected to small pores in the body wall (Smant, 2012). They also have a **nervous system** to coordinate movements and to sense their environment. They can be found in very diverse habitats, even hostile ones such as hot springs, deserts and Antarctica. Soil nematodes, like the model bacterial-feeder *Caenorhabditis elegans*, are translucent and microscopic (from 0.1 to several mm). Despite their little visibility, they are by far the most abundant animals in the soil; they account for an estimated four-fifths of all soil animals, filling all trophic levels in the soil food web. According to an estimation by van den Hoogen *et al.* (2019), there are about $4.4 \pm 0.64 \times 10^{20}$ nematodes with a total biomass of approximately 0.3 gigatonnes that inhabit surface soils across the world. They are highly abundant in sub-Arctic regions (38% of total), more than in temperate (24%) or tropical (21%) regions. This distribution is mainly driven by soil enrichment and structure: it is the content of organic matter, rather than climatic conditions, that ultimately determines the abundance of nematodes in soil.

... with a parasitic way of life

Nematodes have a way of life from free-living, like most species, to parasitic. The evolution of plant parasitism in nematodes has occurred independently on several occasions (van Megen *et al.*, 2009) giving rise to at least four different groups of plant-feeding nematodes, characterized by an oral stylet to perforate plant cell wall. Over 4,100 plant-parasitic nematodes (PPNs) are responsible for considerable economic losses in worldwide agriculture (more than 80 billion $US losses annually according to Nicol *et al.*, 2011). They mainly attack the roots of plants, but some species invade the aerial parts including seeds (*e.g. Aphelenchoides* spp.). We can differentiate **migratory** (move between feeding periods), such as *Hirschmanniella* spp., from **sedentary** (modify plant cells into a permanent feeding structure), and **ectoparasites** (reproduce outside host plants), such as *Criconema* spp., from **endoparasites** (reproduce whilst being in their hosts). Most of the considerable nematode damage to crop plants is due to infection by the sedentary endoparasitic PPNs, including the cyst nematodes and the root-knot nematodes of the genera *Heterodera* and *Globodera*, and *Meloidogyne*, respectively (Jones *et al.*, 2013).

Meloidogyne genus

Meloidogyne spp. are obligate biotrophs, meaning that they are absolutely dependent on plant hosts for existence; access to the vascular system of the host is essential for their success in parasitism. After hatching, the juvenile nematodes at stage 2 (J2) penetrate plants just above the root tip (elongation zone) and migrate intercellularly (apoplastic pathway) through the cortex. Here, they enter the vascular cylinder and move up to the differentiation zone to settle down. One single infective nematode can induce a feeding

structure made of several swelling vascular cells on which the nematodes feed on. These giant cells and the surrounding plant cells undergo **hypertrophy** (*i.e.* abnormal increase in cell volume) and **hyperplasia** (*i.e.* excessive division of cells that become multinucleated) leading to the formation of a tumor-like structure, the so-called **gall**. After the initiation of the giant cells, the juveniles rapidly develop into a dimorphic adult stage after two molts (to J3 and J4). Males remain vermiform and migrate in the plant or leave the root while female nematodes develop and remain inside the infection site. They reproduce by an array of possible strategies: parthenogenesis (asexual reproduction) and/or amphimixis (sexual reproduction), depending on the species (Phan, 2021). Females lay eggs in a gelatinous mass (**figure 12**). Abundant juvenile nematodes at stage 1 (J1) can be observed within the eggs where they undergo their first molt to become pre-parasitic (J2). After hatching, if the infective nematodes at stage 2 are released in soil, they locate roots by chemotaxis (Reynolds *et al.*, 2011) and enter a new development cycle. Amphimictic *Meloidogyne* spp. are very polyphagous: they affect all crops worldwide, from vegetable crops to cereal crops.

Meloidogyne graminicola

Meloidogyne graminicola, commonly named as the rice root-knot nematode, is one of the most prevalent PPNs in rice agrosystems. It is considered to be a major threat to rice agriculture (Mantelin *et al.*, 2017), particularly in Asia, where changes of agricultural practices in response to environmental and socioeconomic conditions have led to a dramatic increase in *M. graminicola* populations on rice, its main host (de Waele and Elsen, 2007; Phan, 2021). *M. graminicola* was first described from grasses and oats by Golden and Birchfield in 1965 in Louisiana (USA). It has a relatively fast life cycle compared to other *Meloidogyne* species, completed in 19–27 days on rice depending on the temperature range, which usually ranges from 22 to 29 °C in the areas where it is found. Factors such as soil structure, temperature, pH, redox state and moisture, as well as plant growth stage and crop cycle duration, can affect the capacity of the nematode to survive in the ecosystem. It is adapted to flooded conditions where the soil saturation corresponds to the optimal humidity for nematode growth. Therefore, *M. graminicola* can be a devastating plant pathogen in irrigated rice agrosystems and is classified as a quarantine pest in many countries (EPPO, 2021).

A potential plant pathogen

The characteristic hook-shaped galls (**figure 12**) mainly at the root tips are a sign of the infection by *M. graminicola*. They strongly impair root development and physiology. Symptoms caused by *M. graminicola* are the disruption of water and nutrient transport, stunting, chlorosis and loss of vigor. It results in poor growth and reproduction of the plants with substantial yield losses in crops that can represent up to 87% of the rice production (Netscher and Erlan, 1993). Infection by *M. graminicola* also predisposes rice to other diseases (Kyndt *et al.*, 2014). Since the signs are below ground and the above ground symptoms are not specific, the diagnosis is compromised. The apoplastic movement of root-knot nematodes inside roots does not cause extensive cell damage. However, cells do respond to the nematode infection by initiating basal plant defenses. For instance, secondary compounds such as chlorogenic acid in *Solanaceae* exert a weak nematicidal activity that can be enhanced if metabolized into a more toxic

compound such as caffeic acid (Lannou *et al.*, 2021). In order to sustain the intimate relationship with their host, *M. graminicola* suppresses the plant immunity with effectors. In particular, the genes related to the JA pathway, to the PR13/thionin gene family and to the phenylpropanoid pathway are repressed in giant cells and/or in gall tissues (Mantelin *et al.*, 2017). The JA pathway plays a determinant role in rice basal immunity against *M. graminicola* (Nahar *et al.*, 2011).

Some sources of plant resistance to *M. graminicola* have been identified in African rice species (*O. glaberrima* and *O. longistaminata*) as well as in a few Asian rice varieties. However, knowledge on the molecular basis of plant defenses is extremely limited; ascarosides have just recently been identified as PAMPs (Manohar *et al.*, 2020). Moreover, only one PRR (*NILR1*) has been characterized whereas the PAMP recognized hasn't been identified yet, whilst many effectors have been found (*MIF*, *MiCRT*, *MiMIF-2*, *MiISE5*, *etc.*) (Lannou *et al.*, 2021). Moreover, *R* genes are generally effective against a very limited range of species (*e.g. Mi-1.2* in a wild relative of cultivated tomato *S. lycopersicum* is effective against *M. incognita*, *M. arenaria* and *M. javanica*) and their introgression by hybridation in distant species may confer yield penalties or undesirable agronomic traits (Fuller *et al.*, 2008). Genetic modification of plants, CRISPR-Cas9-based targeted genome editing and RNAi for gene silencing in plant-parasitic nematodes may be considered to protect crops against *M. graminicola*. However, although durability of *R* genes to sedentary plant nematodes has been generally high (Fuller *et al.*, 2008), another concern is that a virulent race of the cognate pathogen will evolve and break the resistance. Therefore, we need more sustainable and integrative strategies.

Sustainable means control

"Once a soil has been converted to intensive agricultural production and has lost its natural suppressiveness, pest species become more abundant. One convenient and effective solution is to apply a pesticide. However, many of the pesticides used to control nematodes, and those that have been used in the past, are broad-spectrum biocides that further reduce levels of organisms which might otherwise contribute to natural soil suppressiveness." Ferris (Stirling, 2015)

Chemical-based pesticides are not considered as a viable solution any more. On the contrary, they lead to intense directional selection pressure that is not evolutionarily sustainable because they force the pathogens to become more virulent and aggressive, and interfere with the potentially beneficial organisms, ultimately weakening the plant immunity. In another way to control diseases induced by PPNs, the notion of sustainable management is only just emerging, with different perceptions among researchers and farmers (Lannou *et al.*, 2021). Three main roads are open:

➔ **Inducing plant defenses**

Some chemical and biological compounds are able to trigger the plant defense machinery, leading to induced resistance. Induced resistance to *M. graminicola* in rice has been shown to be feasible (Nahar *et al.*, 2011; Pottie, 2021). Nevertheless, defense activators also tend to have negative effects on plant growth, because of the trade-off between growth and defense. Priming is a more efficient type of induced resistance, where defense responses are not directly activated, but only induced when plants are subsequently

challenged by a pathogen or pest. Consequently, the metabolic investment of the plant is reduced compared with constitutive defense activation. Another option that may induce resistance in plants is the addition of organic compounds to the substrate such as biochar, a solid coproduct of biomass pyrolysis (Mantelin *et al.*, 2017).

➔ **Controlling the pathogen attack**

Biological control, or *biocontrol*, is a relatively broad concept that encompasses a range of strategies that ultimately results in a reduction in pest population, or in the capacity of the pest to cause damage, through the action of parasites, predators and other antagonistic organisms (Stirling, 2015). It is essentially the exploitation of living agents (including viruses) to combat pestilential organisms for diverse purposes providing human benefits (Stenberg *et al.*, 2021). Biocontrol strategies can employ biocontrol agents (micro- or macro-organisms such as nematophagous fungi, parasitic bacteria, symbiotic mycorrhizae, *etc.*) or nature-based substances (*e.g.* antimicrobial or repulsive molecules such as flavonoids and neem oil in which azadirachtin is the main active compound that has a nematostatical activity) directly on the field or indirectly by stimulating their development. Soil-native biocontrol agents have been identified in fields and can offer a promising strategy in order to suppress the root-knot nematode disease by using non-destructive agricultural practices (Silva et al., 2018; Topalović *et al*., 2020).

"One thing I have learnt during my career is that plant-parasitic nematodes are rarely the only cause of suboptimal crop performance. If a poor-growth problem is soil-related, it will generally have multiple causes, and so it is important to provide holistic solutions rather than a temporary fix that just focuses on the nematode component. Biological control has been a continuing interest, but from my perspective, it is only one of many tools that can be used by farmers to improve soil health and limit losses from plant-parasitic nematodes." Stirling (2015)

➔ **Preventing diseases**

Many prophylaxis and sanitary practices can be applied to reduce the emergence of diseases and their incidence: use of nematode-free tools, constant immersion of rice in irrigated fields, crop rotation with resistant, non-host plants such as mustard, sesame, millet (Rahman, 1990) or trap plants, *etc*. However, each of these strategies has their own drawbacks. Continuous flooding conditions are not always possible due to water scarcity, and are not recommended in order to limit the emission of methane, a greenhouse gas effect produced by *Archaea* in anoxic paddy fields which are contributing from 10 to 25% of global emissions (Sakai *et al.*, 2007). The efficiency of crop rotation could be compromised by the relatively wide host range of *M. graminicola*, which is able to propagate in reservoir weeds commonly found in tropical fields, such as several *Cypereae* and *Echinochloa* species. Moreover, although the *M. graminicola* population declines rapidly after four months of crop rotation, some eggs can remain viable for up to 14 months in water-logged soils (Bridge and Page, 1982), indicating that crop rotations must include a long sequence without rice for greater efficiency. This may be unacceptable for growers who rely on rice (Mantelin *et al.*, 2017). Efficient control of a pest such as *M. graminicola* requires a combination of means feasible according to each crop system and the farmers' acceptance, but there is a chance that preventing the specific disease induced by *M. graminicola* will result in improving general plant and soil health in a longer term.

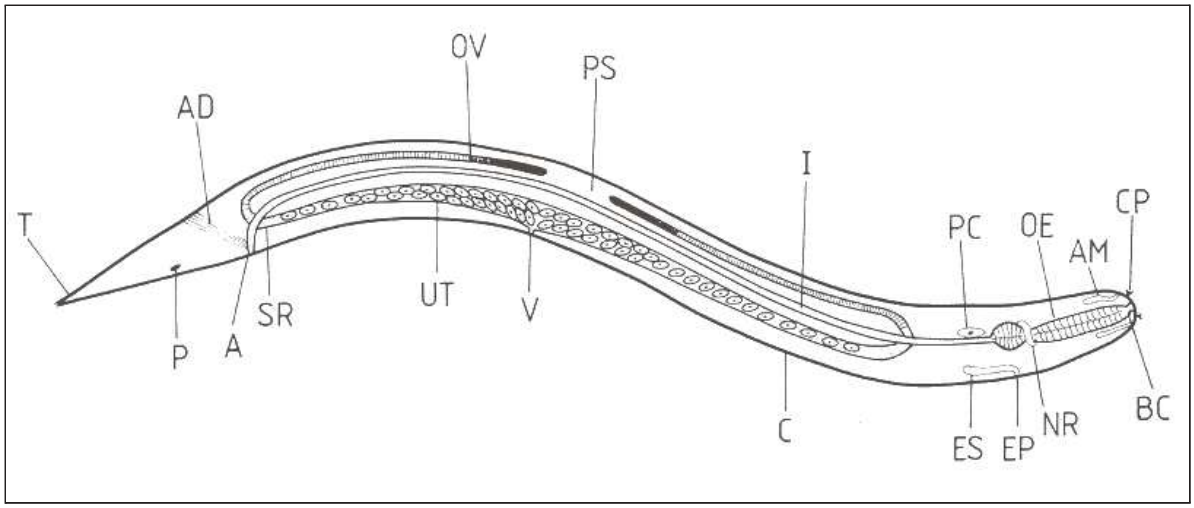

Figure 11. Generalized picture of a female nematode showing the typical morphological features (A, anus; AD, anal dilator muscles; AM, amphids; BC, buccal cavity; C, cuticle; CP, cephalic papillae; EP, excretory pore; ES, excretory system; I, intestine; NR, nerve ring; OE, oesophagus; OV, ovary; P, phasmids; PC, pseudocoelomocyte; PS, pseudocoelom; SR, seminal receptacle; T, tail; UT, uterus; V, vulva (Smant, 2012).

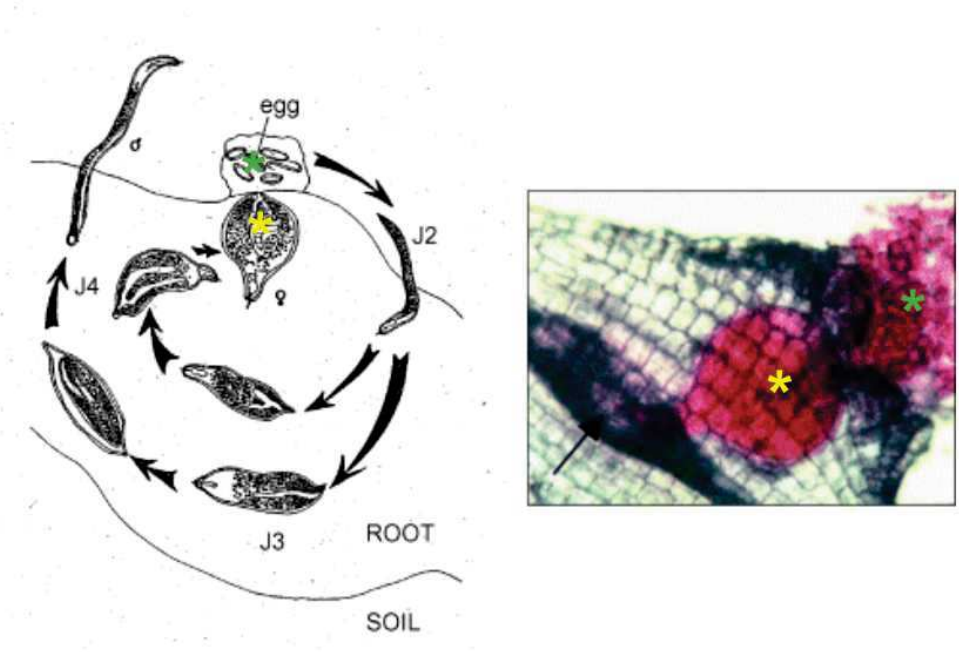

Figure 12. Development cycle of *Meloidogyne* sp. (left) and a root with a gall stained with fuchsin to track a female nematode (pear-shaped, indicated by a yellow star) within the root and an egg mass (indicated by a green star) expelled outside of the root (right). Juveniles at stage 2 (J2) measure between 350 and 510 mm (adapted from Abad *et al.*, 2003).

... to study the associated microbiomes

In microbial ecology research, two complementary methods are commonly used.

The cultivable method

The first method used to study microbes implies to isolate them from fresh samples (*e.g.* roots, rhizosphere, bulk soil) and to grow, isolate, purify and conserve them on culture media in order to maintain the microbial material alive and to use it for *in vitro* or *in vivo* experiments (El-Sayed *et al.*, 2014). *In vitro* tests include characterization of PGP traits (*e.g.* N_2 fixation, NH_3 production, P solubilization, Zn solubilization, siderophore production, IAA production), antagonistic traits (*e.g.* chitinase activity for antifungal trait, cellulase and protease activity, siderophore production, HCN production, SA production) and antagonistic activity against other organisms (*e.g.* PPNs, bacteria of the same microbiome). *In vivo* tests include confirmation of PGP traits *in planta* (improvement of plant growth and reproduction traits), reduction of symptoms after pathogen inoculation, characterization of colonization patterns, *etc*. This method is advantageous because it requires only basic expertise and material in microbiology. However, its main limitation is the very little percentage of cultivable microbes. In plants, only less than 10% of plant-associated bacteria are recovered by a basic cultivable method because they require specific growth conditions. Nonetheless, the culturability can be improved by using plant-based culture media (Sarhan *et al.*, 2019) and a high throughput cultivable technique, termed "culturomics", which require more material and time resources (Zhang *et al.*, 2021).

The amplicon barcoding method

To overcome some of the limitations of cultivation-based techniques, another method that is molecular-based and "omics" imply high throughput NGS and *in silico* analyzes. With the amplicon barcoding technique, one marker gene is amplified simultaneously in all the DNA sequences of different samples that have been previously labeled by a unique barcode. The steps involved are: 1) DNA extraction of environmental samples (using commercialized kits for extraction and, facultatively, purification for better quality data), 2) marker gene amplification and barcoding (by genomic services providers) and, 3) *in silico* analysis of sequence abundance at different taxonomic levels (**figure 6 A**), richness and diversity (**BOX 2 - How to describe diversity?**), predicted ecological functions and interaction networks, *etc.* This technique is powerful to rapidly generate a big amount of data which allows us to deeply describe microbiomes. It has revealed a previously unimaginable amount of microbial diversity, including newly discovered phyla whose existence was not suspected (Berg *et al.*, 2015). It not only allows us to know who (what organisms) there are in the samples, how many and how diverse, but also allows us to predict what they are doing, how they interact, *etc.* Another advantage is that lots of open source pipelines are available for the analyzes, encouraging the sharing of knowledge and expertise, the reproductivity of the analyzes and the cross-comparison of studies. However, it still requires bioinformatic expertise and experimental validation of data interpretation.

Several primer pairs can be used according to the targeted marker gene and community (**table 2**), with more or less accuracy and efficiency (Lucaciu *et al.*, 2019). A better exploration of the microbiomes can be acquired by untargeted techniques, with shotgun metagenomic, that is the sequencing of the whole genomes of microbiomes in environmental samples (Sessitsch *et al.*, 2012). But it requires even deeper sequencing, bioinformatic expertise and infrastructure.

Table 2. Primer pairs commonly used with the amplicon barcoding method to target different microbial communities and marker genes.

Community	Marker gene	Primer pair	Amplicon size	Reference
Bacteria and *Archaea*	V4 region (*16S rRNA*)	515FB/806RB	~291 bp	Earth Microbiome Project (Bates *et al.*, 2010)
	V3 and V4 regions (*16S rRNA*)	341F/805R	~464 bp	Herleman *et al.*, 2011; Wasimuddin *et al.*, 2020; **chapter 3**; **chapter 4**
	V3 and V4 regions (*16S rRNA*)	337F/806R	~469 bp	**chapter 2**
	subunit β of DNA gyrase (*gyrB*)	gyrB_aF64/gyrB_aR353	~290 bp	Watanabe *et al.*, 2001; Barret *et al.*, 2015
Fungi	ITS1 region (between *18S* and *5.8S rRNA*)	ITS1/ITS2	~290 bp	White *et al.*, 1990
	ITS2 region (between *5.8S* and *28S rRNA*)	ITS3/ITS4	~330 bp	White *et al.*, 1990; **chapter 3**

List of primer sequences:

- 337F: 5'-CCTACGGGAGGCWGCAG-3'
- 341F: 5'-CCTACGGGNGGCWGCAG-3'
- 515FB: 5'-GTGYCAGCMGCCGCGGTAA-3'
- 805R: 5'-GACTACHVGGGTATCTAATCC-3'
- 806R: 5'-GACTACHVGGGTMTCTAAT-3'
- 806RB: 5'-GGACTACNVGGGTWTCTAAT-3'
- gyrB_aF64: 5'-MGNCCNGSNATGTAYATHGG-3'
- gyrB_aR353: 5'-ACNCCRTGNARDCCDCCNGA-3'
- ITS1: 5'-TCCGTAGGTGAACCTGCGG-3'
- ITS2: 5'-GCTGCGTTCTTCATCGATGC-3'
- ITS3: 5'-GCATCGATGAAGAACGCAGC-3'
- ITS4: 5'-TCCTCCGCTTATTGATATGC-3'

BOX 2 - How to describe diversity?

To describe entities (species, families, ESVs, *etc.*) found in samples collected in habitats, diversity can be estimated at several nested types, named by Whittaker (1960):

- **α-diversity**: within-sample diversity, that is, the local diversity within a community of organisms and per area in an habitat
- **β-diversity**: between-sample diversity differentiation, that is, a dimensionless comparative diversity between several habitats
- **γ-diversity**: landscape diversity, that is, the diversity at a larger scale that includes more than one community in a habitat. (Schulze *et al.*, 2005)

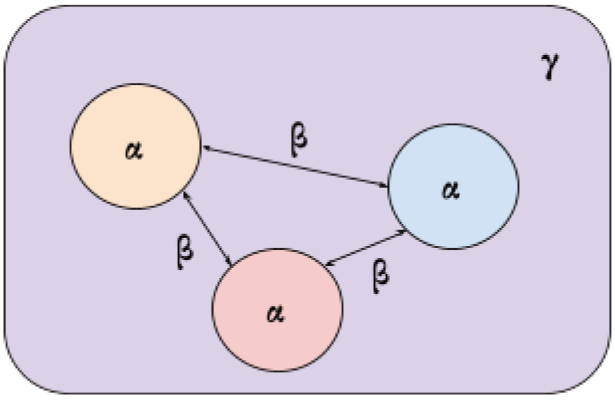

Figure 13. The nested types of diversity measures.

Classical measures of diversity include the followings:

- **Count** = number of individuals of each entity
- **Richness** = total number of entities (regardless of their counts)
- **Evenness** = equitability or uniformity of the distribution of entities (relative abundance of their counts). An entity represented abundantly or by a single individual might not bring the same contribution to the ecosystem. The presence of very dominant entities mathematically leads to the rarity of the scarcity of certain others: it is therefore quite intuitive that the maximum diversity will be reached when the entities have a very uniform distribution.
- **Dissimilarity** = disparity or divergence between entities, with or without count weighting.

Indices were developed to estimate these measures of diversity. The most commonly used are:

- **Shannon index H** (Shannon and Weaver, 1949) = assessment of both richness and evenness based on Shannon formula for entropy to quantify the uncertainty in predicting the entity identity with:

$$H = -\Sigma[(pi)*\log(pi)]$$

where:

pi = proportion of individuals of i-th entity in a sample = n/N

where:

n = individuals of a given entity

N = total number of individuals in the sample.

Values typically range from 1.5 (low diversity) to 3.5 (high diversity). It does not tell us whether a high value is due to a high richness or to a more even distribution of individuals.

- **Pielou's index E** (Pielou, 1966) = assessment based on Shannon index to quantify mainly the evenness with:

$$E = H/\ln(S)$$

where:

S = total number of entities in the sample.

Values range from near 0 (one dominant entity) to 1 (equitability between entities).

- **Simpson's index D** (Simpson, 1949) = assessment of both richness and evenness.

$$D = 1-\Sigma[(pi)^2]$$

Values range from 0 (high diversity) to 1 (low diversity). It can be interpreted as the probability that two individuals drawn at random are of different entities.

- **Jaccard's similarity coefficient J** (Jaccard, 1901) = assessment using information on entity absence or presence in several samples (A and B).

$$J(A,B) = |A \cap B|/|A \cup B|$$

Values range from 0 (low diversity) to 1 (high diversity). It can also be expressed as a percentage, or as dissimilarity ($1-J$), the latter representing the **Bray-Curtis dissimilarity** (Bray and Curtis, 1957).

There are other ways of estimating diversity while taking into account unobserved species (*e.g.* **Chao's index**, Chao and Chiu, 2006). **Hill's numbers** (Jost, 2006), which are all expressed in the same units, and ratios derived from these numbers, can also be used to compute diversity indices. Other ways to describe diversity include aspects of genetics, morphology, biochemistry, biogeography or functional roles within ecosystems.

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Chapter 2

The "gallobiome" of *Meloidogyne graminicola* in a highly infested rice field

Preamble

In order to extend the knowledge on the interaction between the rice *Oryza sativa* and the root-knot nematode *Meloidogyne graminicola*, which are both part of an interaction network with many microorganisms in the field, we undertook the description of the microbial communities at the infection site, and more specifically, of bacteria in the gall. The samples were collected in Vietnam in 2017. Three rice fields located in the Red River Delta, an intensive agricultural region in the East of Hanoi (**figure 14**), have been attacked by a pest. The farmers, deploring the disaster (100% losses), allowed the researchers to investigate. The causal agent was identified as *Meloidogyne graminicola*, and the rice variety as well as onions grown in the off-season on these fields were found to be highly susceptible to the infection (Nguyen *et al.*, 2020). The emergence of the disease did not seem to be due to the physicochemical properties of the soil because they were similar to other uninfested fields, but rather to the agricultural practices (monoculture, direct-sowing and poor water management) that had favored the nematode outbreak. We used this case study as a fundamental study to investigate whether a specific microbiome was associated with the infection and, if so, with which characteristics in terms of bacterial diversity, structure, taxa enrichment and co-occurrence network. The aim was not to determine whether the modifications we indeed observed (*e.g.* shift in the composition, relative abundances and connectivity of taxa) were the causes or the consequences of the infection, but to describe the bacterial microbiome associated with infected roots. We also identified some taxa that could have a role, for example, in helping the nematode during the invasion of the plant, in helping the plant to defend itself against the nematode, or that were simply able to survive in the supposedly nutrient-rich and highly competitive environment of the gall. The gall microbiome, which we called the "gallobiome", could thus be explored and was the subject of my first publication (**figure 15**) entitled: "Deep modifications of the microbiome of rice roots infected by the parasitic nematode *Meloidogyne graminicola* in highly infested fields in Vietnam" (Masson *et al.*, 2020). Some brief modifications have been made here to fit the format of the thesis manuscript and to clarify the results. Supplemental analyzes have also been made in the last section. "Funding" section can be read directly in the journal FEMS Microbiology Ecology (doi: 10.1093/femsec/fiaa099). This work opens a field of study on the implications of the root microbiome for plant immunity against root-knot nematode diseases.

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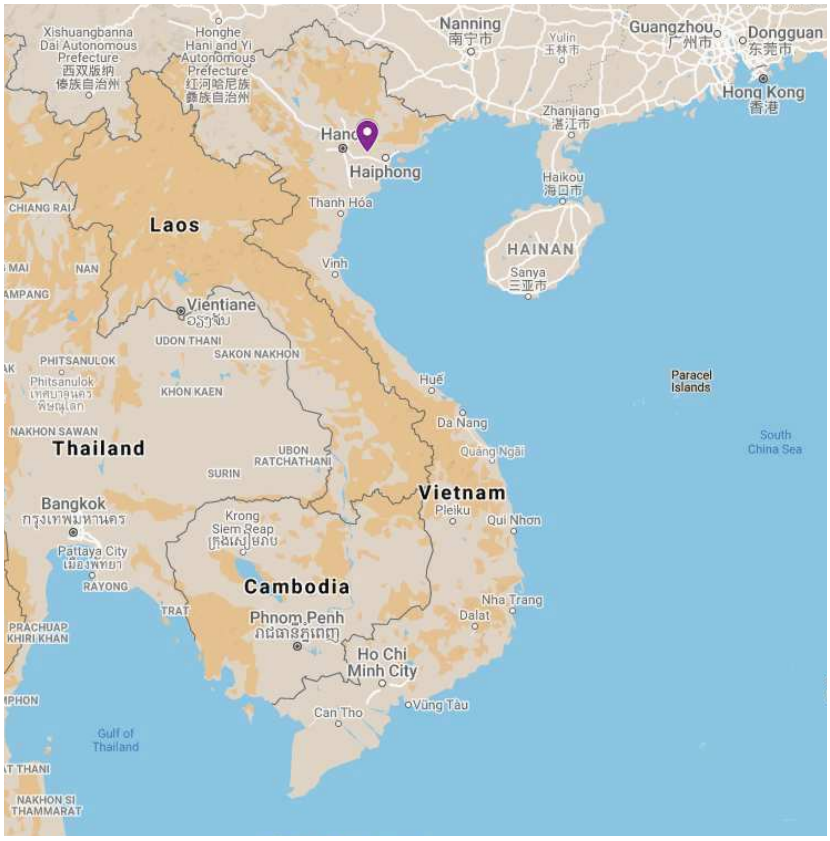
Préambule

Afin d'étendre les connaissances sur l'interaction entre le riz *Oryza sativa* et le nématode à galles *Meloidogyne graminicola*, qui font tous deux partie d'un réseau d'interaction avec les nombreux micro-organismes du champ, nous avons entrepris la description des communautés microbiennes au niveau du site d'infection, et plus spécifiquement des bactéries dans la galle. Les échantillons ont été collectés au Vietnam en 2017. Trois rizières situées dans le delta du Fleuve Rouge, une région agricole intensive à l'est de Hanoi (**figure 14**), avaient été attaquées par un ravageur. Les agriculteurs, déplorant la catastrophe (100% de pertes), ont permis aux chercheurs d'enquêter. L'agent causal a été identifié comme étant *Meloidogyne graminicola*, et la variété de riz ainsi que les oignons cultivés en contre-saison sur ces champs se sont avérés très sensibles à l'infection (Nguyen *et al.*, 2020). L'émergence de la maladie ne semblait pas être due aux propriétés physicochimiques du sol, car elles étaient similaires à celles d'autres champs non infestés, mais plutôt aux pratiques agricoles (monoculture, semis direct et mauvaise gestion de l'eau) qui avaient favorisé l'apparition du nématode. Nous avons utilisé cette étude de cas comme étude fondamentale pour déterminer si un microbiome spécifique était associé à l'infection et, si oui, avec quelles caractéristiques en termes de diversité bactérienne, de structure, d'enrichissement taxonomique et de réseau de co-occurrence. L'objectif n'était pas de déterminer si les modifications que nous avons effectivement observées (*e.g.* changement dans la composition, les abondances relatives et la connectivité des taxons) étaient les causes ou les conséquences de l'infection, mais de décrire le microbiome bactérien associé aux racines infectées. Nous avons également identifié certains taxons qui pourraient avoir un rôle, par exemple, en aidant le nématode pendant l'invasion de la plante, en aidant la plante à se défendre contre le nématode, ou simplement en étant capable de survivre dans l'environnement de la galle supposé riche en nutriments et hautement compétitif. Le microbiome de la galle, que nous avons appelé le “gallobiome”, a ainsi pu être exploré et a fait l'objet de ma première publication (**figure 15** - résumé graphique de l'article publié associé au chapitre 2 - L'infection du riz par le nématode phytoparasite *Meloidogyne graminicola* est associée à de profondes modifications du microbiome racinaire en termes de composition de la communauté bactérienne, de diversité et de structure de réseau avec des taxons bactériens spécifiques, enrichis et hautement connectés) intitulée : “Modifications profondes du microbiome des racines de riz infectées par le nématode parasite *Meloidogyne graminicola* dans des champs fortement infestés au Vietnam” (Masson *et al.*, 2020). Quelques brèves modifications ont été apportées à l’article pour l’adapter au format du manuscrit de thèse et pour clarifier les résultats. Des analyses supplémentaires ont également été effectuées dans la dernière section. La section “Financement” peut être lue directement dans le journal FEMS Microbiology Ecology (doi : 10.1093/femsec/fiaa099). Ce travail ouvre un champ d'étude sur les implications du microbiome racinaire dans l'immunité des plantes face à la maladie des nématodes à galles.



21°00’51.1’’ N - 106°19’33.0’’ E



Figure 14.
Localization of the investigated fields in chapter 2 and picture of the farmer showing infected seedlings.

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Localisation des champs examinés dans le chapitre 2 et photo de l’agricultrice montrant les semis infectés.

Abstract

Meloidogyne graminicola, also known as the rice root-knot nematode, is one of the most damaging plant-parasitic nematode, especially on rice. This obligate soil-borne parasite induces the formation of galls that disturb the root morphology and physiology. Its impact on the root microbiome is still not well described. Here, we conducted a survey in Northern Vietnam where we collected infected (with galls) and non-infected root tips from the same plants in three naturally infested fields. Using a metabarcoding approach, we discovered that *M. graminicola* infection caused modifications of the root bacterial community composition and network structure. Interestingly, in infected roots, we observed a higher diversity and richness (+24% observed ESVs) as well as a denser and more complex co-occurrence network (+44% nodes and +136% links). We identified enriched taxa that include several hubs, which could serve as potential indicators of the nematode infection or biocontrol agents. Moreover, the community of infected roots was more specific suggesting changes in the functional capabilities to survive in the gall environment. We thus described the signature of the gall microbiome (the "gallobiome") with shifting abundances and enrichments that lead to a strong restructuration of the bacterial community.

Keywords: lowland rice; root-knot nematode; gall microbiome; bacterial community; co-occurrence network; metabarcoding

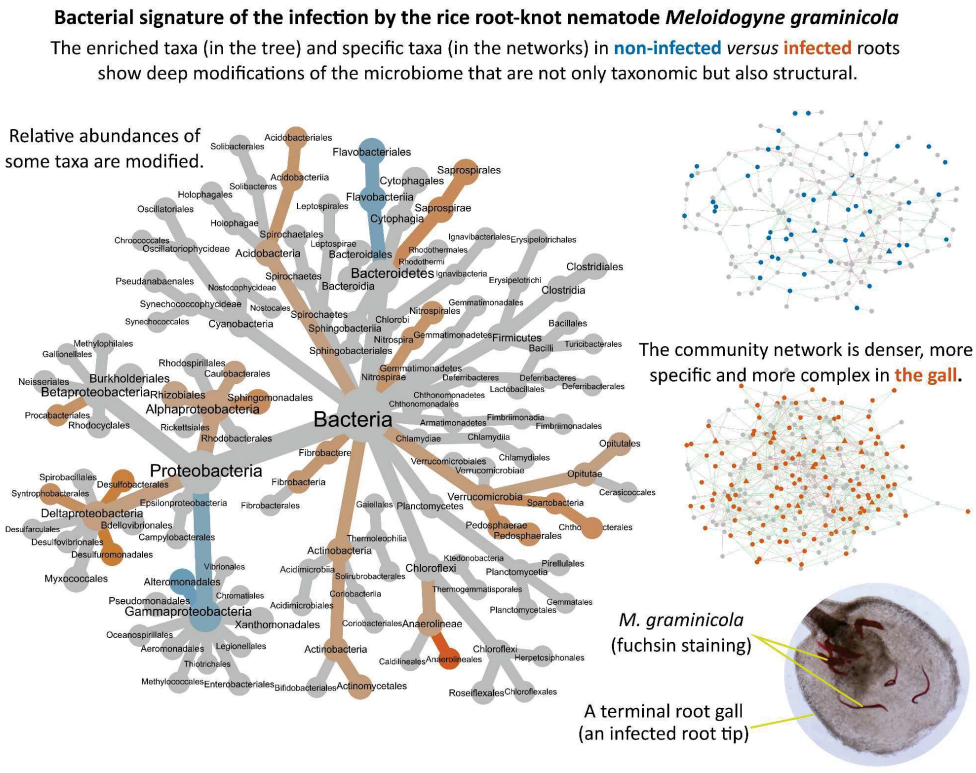

Figure 15. Graphical abstract of the published article associated with chapter 2. Rice infection by the plant-parasitic nematode *Meloidogyne graminicola* is associated with deep modifications of the root microbiome in terms of bacterial community composition, diversity and network structure with specific, enriched and highly connected bacterial taxa.

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Introduction

Plant-parasitic nematodes are known to cause significant crop losses (Nicol *et al.*, 2011) and of these, *Meloidogyne* spp. are considered one of the most severe in terms of economic importance (Jones *et al.*, 2013). *Meloidogyne* spp. are telluric obligate parasites that accomplish their life cycle in roots and have a short free-living stage in soil. They are also known as root-knot nematodes (RKNs) because they distort the root vascular system by creating large deformations at the root tips, called galls, which are essential for their growth and reproduction. Indeed, the infectious juveniles settle in the root, where they form a feeding site by inducing giant plant cells near the endodermis, and accomplish several molts over their life cycle of 20 to 30 days (Cabasan *et al.*, 2012; Cabasan *et al.*, 2014). Hyperplasia and hypertrophy of the surrounding cells in the feeding site lead to the formation of characteristic hook-shaped galls that appear from two to four days after infection and that will limit root development. These giant feeding cells act as specialized sinks providing the nematodes with their nutrient requirements for reproduction (Jammes *et al.*, 2005). As a result, the root system is atrophied, disrupting the transport of water and nutrients into the plant and compromising rice yield (Bridge and Page, 1982). *Meloidogyne graminicola* has a particularly detrimental impact in Asia where a large part of the world's rice is produced and consumed. In flooded conditions, yield losses associated with *M. graminicola* infections of up to 80% have been reported (Plowright and Bridge, 1990). Therefore, it is considered as a major threat to rice agriculture (Mantelin, *et al.* 2017).

Plants and their associated microorganisms (microbiota) form a holobiont that can be considered as coevolved species assemblages consisting of bacterial, archaeal and diverse eukaryotic species (Zilber-Rosenberg and Rosenberg, 2018). Microbial communities indeed inhabit different plant compartments like the root endosphere (root interior), the rhizoplane (root surface) and the rhizosphere (soil influenced by the root) (Edwards *et al.*, 2015; Ding *et al.*, 2019). Key insights reveal close parasitic relationships between these microorganisms and the plant host along a mutualism and a pathogenesis gradient (Newton *et al.*, 2010). Through metabolic interplay and signaling, microorganisms can stimulate germination and plant growth, prevent diseases, and promote stress resistance and general fitness (Berg *et al.*, 2017). Due to the advance of -omic tools, microbe-based agronomic approaches such as the exploitation of the plant microbiota as a solution against RKNs are promising (Sánchez-Cañizares *et al.*, 2017) .

The physiological impact of *M. graminicola* on rice has been widely described (Jain *et al.*, 2012; Cabasan *et al.*, 2014; Patil and Gaur 2014). Contrastingly, its impact on the root microbiota is less known, although the impact of plant pathogens on the plant-associated microbiotas is suspected to have an importance on plant health and yield (Vannier *et al.*, 2019). At the plant level and particularly at the root site, Back *et al.* (2002) have identified synergistic interactions between plant-parasitic nematodes and soil-borne pathogens. In particular, the release of plant root exudates into the rhizosphere, known as "rhizosphere effect", is considered as an important factor in the shaping of the assemblages of microorganisms (Zhalnina *et al.*, 2018). Due to the physiological impact of *M. graminicola* on the rice roots, the plant exudation pattern can be modified and so the nematode can indirectly affect the microbiome, *i.e.* the microbiota and its its "theater of activity" (Berg *et al.*, 2020). Indeed, *M. graminicola* could affect the root-associated bacteria by modifying the plant hormonal balances (*e.g.* ethylene, jasmonic and salicylic acids), inducing the production of secondary metabolites (terpenoids and flavonoids) or defense proteins (Pathogenesis-Related proteins, thaumatin and thionin) as described in the transcriptomic analysis of Petitot *et al.* (2017). *M. graminicola* could also have an impact on root-associated microorganisms by carrying its own microbiota as it was shown for *M. incognita*, another RKN (Elhady *et al.*, 2017). Finally, these direct and indirect effects could lead to modifications of the root microbiome that play an important role in plant health (Pieterse *et al.*, 2016).

The relationship between plant microbiotas and RKNs has been described in few studies. For instance, communities and functions of endophytes in tomato plants were compared before and after infection by *M. incognita* in a greenhouse assay (Tian *et al.*, 2015). Some bacterial groups have been found specifically enriched in the root galls and carry genes that may be associated with the nematode pathogenesis. Another study focused on the characterization of rhizosphere microbiotas of eggplant and cucumber infected by *M. incognita* transplanted on tomato plants, in a greenhouse assay as well (Zhou *et al.*, 2019). The authors highlighted some nematicidal effects and plant benefits that can be associated to taxa such as *Pseudomonas* sp. and *Bacillus* sp. with biocontrol activity. *Bacillus* strains were also antagonist toward one fungal pathogen of the *Meloidogyne*-based disease complex studied by Wolfgang *et al.* (2019). However, to our knowledge, no study has investigated the relationship between *M. graminicola* infection, rice and its root-associated microbiota in a natural environment.

In the present study, we characterized root-associated bacterial communities (comprising both endosphere and rhizoplane colonizing bacteria) of rice roots infected by *M. graminicola* (with apparent galls) and of non-infected roots (no apparent galls) from the same plants in three highly infested fields in Vietnam. We hypothesized that the root-associated microbiota was originally the same and that there was a restructuration of the microbiota because of or leading to the infection. Using a metabarcoding approach, we aimed to assess the effect of the infection by *M. graminicola* on the microbiome by investigating the differences in the following features: bacterial diversity and composition, community structure, enriched taxa, and potential hub taxa in co-occurrence networks.

Material & methods

Field description

The survey was conducted in Vietnam on the 11th of March 2017 in Nam Sách district, Hải Dương province (21°00'51.1" N and 106°19'33.0" E). Prospected lowland fields were located within the Red River delta, on loamy soil under a humid subtropical climate (**figure 16**). The three rice fields surveyed were inside a ten hectares area with three crop rotations per year: two rice cultures and one onion culture. Farmers have grown onions for a decade in winter before cultivating two cycles of rice in spring and summer. Chemical fertilizers consisted of 800 to 850 kg of NPK per ha for the rice crop, and 1,000 to 1,500 kg of P_2O_5 + 300 kg of urea + 200 kg of KCl per ha for the onion crop. Some pesticides were applied whenever pests appeared in the field but the names of the substances could not be recovered. For the first rice cropping cycle in 2017, 15 days after tillering, rice variety Bac Thom n°7 (*Oryza sativa* subsp. *indica*) was broadcasted. In spring of 2017, due to unusual water scarcity, the fields suffered from drought stress for up to 20 days. Nearly four weeks after seeding, almost all seedlings died, presenting leaf chlorosis and small root systems with formed swelling galls. The three fields were highly infested and devastated (**figure 16 E**).

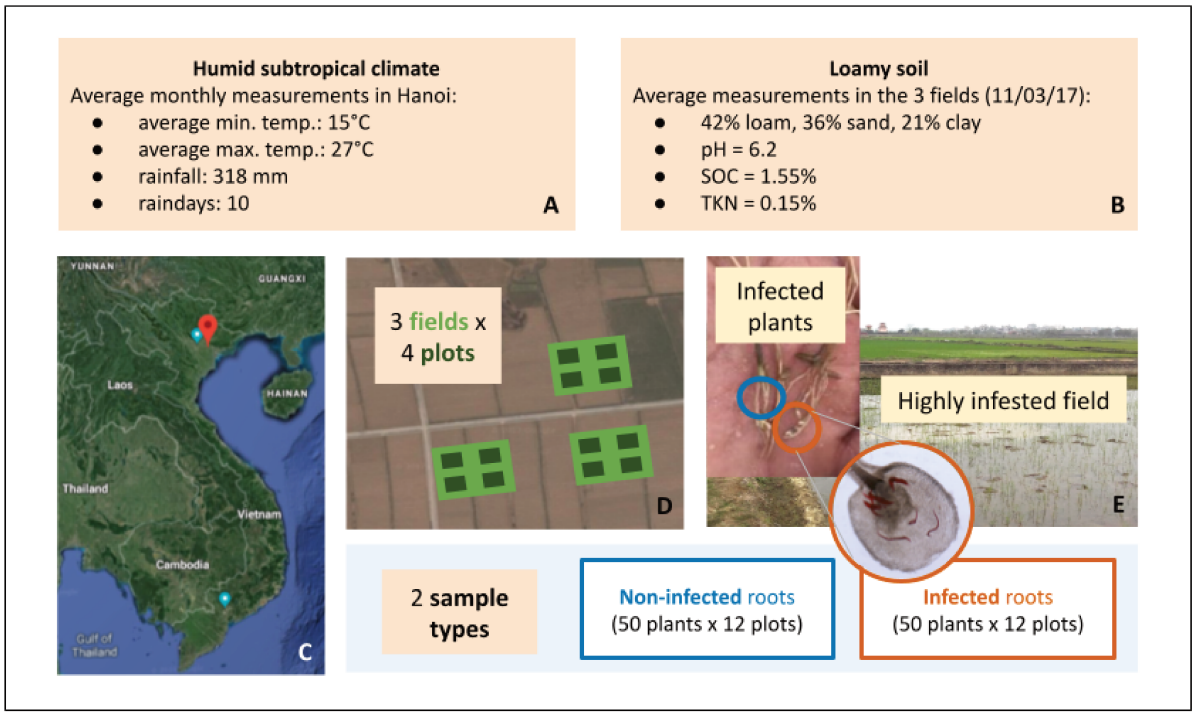

Figure 16. Sampling site and design. Climatic features (**A**, data from worldweatheronline) and soil features (**B**, data from the VNUA) of the site localized in Hải Dương (**C**, red point) near Hanoï (upper blue point) in Northern Vietnam. Map of the infested fields and plots (**D**). Picture of one of the three infested fields used to constitute the two sample types (**E**).

Plant sampling and nematode identification

Each of the three rice fields of 3,000 m^2 was subdivided in four plots of 100 m^2, resulting in 12 plots in total (**figure 16 D**). Four weeks after seeding, 50 plants at the vegetative phase in each plot were randomly picked up and carefully scanned for the presence of hook-shaped galls characteristic of *M. graminicola* infection. As all rice plantlets were infected, each root system has been divided into two sample types: the infected roots with galls and the non-infected roots without any visible gall. Only non-necrotic roots were collected. An average of three root tips (about 2 cm) with or without galls according to the sample type were collected from the same plant. This part of the root corresponds to the growing zone including both the proliferation zone and the elongation zone, with galls (if any) since the nematodes usually settle in the root tip. We pooled the root tips of 50 plants per sample type and per plot. In total, we collected 600 plants to constitute n = 24 samples. The samples were kept in separate labeled plastic bags at 4°C until laboratory analysis within 24 h. The presence of *M. graminicola* was confirmed in galls collected at random by acid fuchsin staining and by molecular identification of plant-parasitic nematodes extracted from roots (Nguyen *et al.*, 2020). SCAR markers were used and a fragment of the *rRNA* gene including the ITS-1 and a part of the 5.8S and 28S was sequenced (Bellafiore *et al.*, 2015).

Soil sampling and physico-chemical analysis

Five soil samples were collected from each of the 12 plots at 0-5 cm depth and were mixed to create one composite sample per plot. Soil properties were analyzed at the Soil Science Department Faculty of Land Management at the Vietnam National University of Agriculture (VNUA in Hanoi, Vietnam) with methods described in Motsara and Roy (2008). Briefly, soil pH was determined using a 1:5 ratio of soil/distilled water-KCl 1 M mixture and measured with a pH meter D-51 (Horiba Ltd., Kyoto, Japan). Cation exchange capacity (CEC) was determined by the ammonium acetate method. Soil organic carbon (SOC) was determined by the Walkley and Black method and the quantification of total Kjeldahl nitrogen (TKN) was determined by the method of Kjeldahl. Total phosphorus (P_2O_5) was determined by digestion with $HClO_4$ and colorimetric method. Total sulfur (S) was determined after di-acid (HNO_3-$HClO_4$) digestion and turbidimetric method, soluble or available sulfate (SO_4^{2-}) by barium sulfate precipitation and turbidimetric method. Soil texture was determined by the pipette method (Robinson), and aggregate stability was determined using the wet sieving apparatus (Eijkelkamp instrument, stroke = 1.3 cm, at about 34 times/min, 0.053 mm and 0.25 mm mesh sieve).

PCR amplification and metabarcoding sequencing

The root samples were washed with sterile water to remove the rhizospheric soil attached to the roots. The 50 root tips for each plot were pooled according to their sample type (with gall/without galls) and grinded in liquid nitrogen in a sterile mortar. DNA was extracted from 15 mg of powder of root tissues using the PowerSoil® DNA Isolation Kit (Qiagen, Netherland) following the manufacturer's instructions. Samples were pooled contributing exactly the same amount (50 ng/µl) of DNA in the final library. PCR amplification, library and MiSeq Illumina sequencing were performed by *Macrogen* (Seoul,

South Korea) using primers 337F (16S_V3F, 5'-CCTACGGGNGGCWGCAG-3') and 806R (16S_V4R, 5'-GACTACHVGGGTATCTAATCC-3') to amplify the V3 and V4 hypervariable regions of the *16S rRNA* gene.

Sequences processing

QIIME 2 bioinformatic platform (Bolyen *et al.*, 2019) was used to obtain exact sequence variants (ESVs) feature table and its taxonomy. More concretely, paired-end reads were primer and adapter removed by *cutadapt* (Martin, 2011). To extract the ESV feature table, forward and reverse read truncation at 277 and 242 bp respectively based on quality plot inspection, default chimera removal, and denoising were conducted by *DADA2* (Callahan *et al.*, 2017). We initially had 1,878,244 reads and we filtered out low frequency (less than ten) and singleton/doubleton features which represented 9.65% of the reads. Taxa were assigned by a Naive Bayes classifier, which was trained for the V3+V4 region from the GREENGENES 16S rRNA database (version 13.8). Lastly, ESVs with no assignment at phylum level (10.78% of the initial reads) or assigned to mitochondria or chloroplast (3.15%) were removed. These different filtering steps resulted in removing in total 23.59% of the reads in the dataset. After the removal of these reads, the sequencing depth was still very good, ranging from 39,679 reads (sample 2.2I) to 74,622 reads (sample 2.3N) with homogeneous variances (standard deviation = 8,484). We finally ended up with 2,202 ESVs. The script written on *R* software (version 3.5.2, *R* Development Core Team, 2018) to make the analysis and generate the figures is available on GitLab under the project ID 17993041 (gallobiome_haiduong_2017). The data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under the accession number PRJEB37618.

Microbiota structure analysis

The rarefaction curves of the samples were checked (**sup. figure 1**) and there was no need to rarefy the data (McMurdie and Holmes, 2014). Indeed, the sequencing depth was sufficient for all samples to reach a plateau around 20,000 reads. The packages *dplyr* (Wickham *et al.*, 2021), *phyloseq* (McMurdie and Holmes, 2013), *microbiome* (Lahti and Sudarshan, 2020) were used to handle data with the *R* software. To visualize the infection and field effects on the microbiota structure, a non-metric multidimensional scaling (NMDS) with Bray-Curtis distance was drawn using the package *Vegan* (Oksanen, 2009). We performed a permutation test to check the multivariate homogeneity of variance with the function *betadisper*. After that, we performed an Adonis test (permutational multivariate analysis of variance using distance matrices) to look for an effect of the root infection by the nematode (*infection* effect) or the sampling localization (*field* effect) on the microbiota structure. An *envfit* test was used to look for correlations of the community structure with environmental variables. The package *ggplot2* (Wickham *et al.*, 2009) was used to build the figures.

Diversity analysis

To assess the diversity of the bacterial communities within and between samples, we measured the observed ESVs richness and calculated the Shannon and Pielou's indices using the package *Vegan*. After that, we performed statistical tests with a generalized linear model (GLM). The best fit for the three measurements was found with a gamma distribution and a log scale. Only significant effects (*infection* effect, *field* effect and dependency between the two effects) are indicated on the figures by asterisks. The tests were performed using the *R* packages *MASS* (Venables *et al.*, 2002) and *car* (Fox *et al.*, 2020).

Phylogenetic and differential abundance testings

To better characterize the rice root microbiota composition at a phylogenetic level, we drew an unrooted phylogenetic tree with specifically enriched taxa in non-infected or in infected roots, using the package *Metacoder* (Foster *et al.*, 2017). *Metacoder* is a set of tools for parsing, manipulating, and graphing data classified by a hierarchy such as taxonomic data. In a nutshell, it sums the reads counts per taxon (*i.e.* calculates the total ESV reads count), converts them to proportions for every taxonomic level and represents them on a tree. We drew a detailed (with all enriched taxa) and a simplified (with total ESV reads count > 50) version of the same tree. The *Metacoder* trees allowed us to visualize the overall enrichment of bacterial clades along the phylogenetic tree, highlighting some signatures of enrichment, in order to focus our further analyzes. In parallel, we performed *DESeq2* (Love *et al.*, 2014) with an adjusted *p*-value lower than 0.05. *DESeq2* is used to calculate differential abundances of entities between two conditions, allowing us to compare the abundance of taxa in non-infected *versus* infected roots. One limit of *DESeq2* in our analysis is that it ignores the compositionality of the community because it calculates the abundance for each individual ESV. Consequently, it can inform us about the differential abundance for one ESV, but it doesn't inform us about the enrichment at taxonomic levels that aggregate several ESVs. However, we could calculate the proportion of reads count of an enriched ESV in non-infected *versus* infected roots by the sum of ESV reads count, respectively in non-infected or in infected samples, divided by the total ESV reads count of this taxon. We focused on the enriched taxa at order and phylum levels according to the *Metacoder* trees. For the *DESeq2* analysis, we focused on the enriched ESVs with full assignment (until species level) or assigned at genus level (if unassigned or uncultured at species level) to represent them on a graph. We also calculated their relative abundance in all samples (both sample types) by the sum of reads count of an enriched ESV, divided by the total reads count of all ESVs.

Co-occurrence networks construction

Co-occurrence networks were generated with the packages *SPIEC-EASI* (Kurtz *et al.*, 2015) and *ggnet* (Briatte, 2020). ESVs present in non-infected or in infected roots were separated in two files in order to construct the two bacterial community networks with a threshold of 80% for taxa prevalence. We used the following parameters in *SPIEC-EASI* to compute the networks: model inference procedures by neighborhood selection, lambda.min.ratio = 5e-4 and nlambda = 80 for sparsity path. Network properties and taxa specificities were analyzed with the package *igraph* (Amestoy *et al.*, 2020) and detailed on the figures. To identify highly connected taxa in those networks, we considered the 5% most connected ESVs in terms of betweenness centrality, closeness centrality and node degree as described and used by Agler *et al.* (2016). We identified these highly connected ESVs as "hubs".

Results

The infection was associated with a shift in the microbiota structure

First of all, the total number of reads was similar in both sample types: 715,488 in infected roots and 719,666 in non-infected roots, with a sequencing depth by sample allowing a full exploration of the bacterial communities as observed with rarefaction curves (**sup. figure 1**). The NMDS ordination drawn with the table of ESVs showed that the bacterial community structure of the infected roots was distinct from the one of the non-infected roots (**figure 17 A**). Indeed, 17% of the variance was explained by the nematode infection ($p < .001$, *infection* effect). The field localization of the samples also had a significant effect (20%) on the community structure ($p < .001$, *field* effect). This effect on the community structure can be seen on the ordination along the NMDS2 axis (**figure 18**). We also looked into correlations between the bacterial community structure and environmental variables. We found that the pH ($p < .05$, $R^2 = 0.37$), the available sulfate ($p < .05$, $R^2 = 0.35$), the soil organic carbon ($p < .05$, $R^2 = 0.32$), the clay texture ($p < .05$, $R^2 = 0.27$) and the total phosphorus ($p < .05$, $R^2 = 0.25$) were the variables significantly correlated to the community structure. Field 3 for example had a higher total sulfur and clay texture, and a lower pH, total carbonates and the total phosphorus (**sup. table 1**). There was no significant interaction between this field effect and the infection effect (*infection* x *field* effect: $p = 0.25$) meaning that the infection effect on the bacterial community structure was not dependent on the sampling location and *vice versa*.

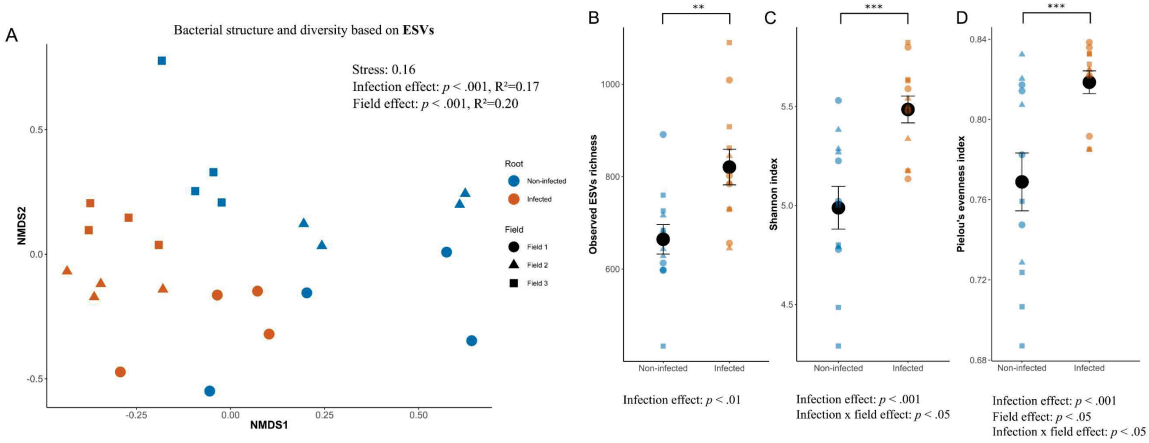

Figure 17. NMDS ordination (**A**) and diversity indices (**B**, **C** and **D**) of the bacterial community structure of infected rice roots by *Meloidogyne graminicola* (with galls) and non-infected roots (without galls). Observed ESVs richness (**B**), Shannon index (**C**) and Pielou's index (**D**).

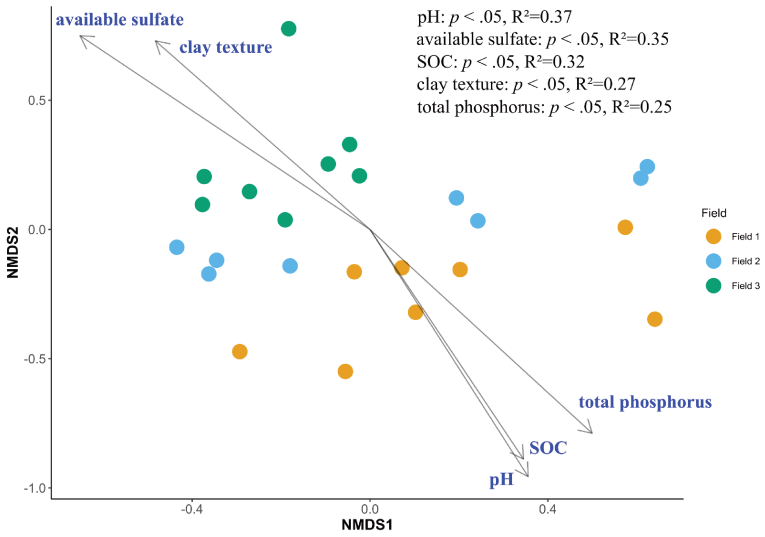

Figure 18. NMDS ordination of the bacterial community structure of infected rice roots by *Meloidogyne graminicola* (with galls) and non-infected roots (without galls) on which environmental vectors significantly responsible for the field effect were added.

Richness and diversity increased with the infection

We represented the observed exact sequence variants (ESVs) richness, Shannon and Pielou's indices in **figure 17** from **B** to **D**. The number of observed ESVs was significantly impacted by the RKN infection ($p < .01$). Indeed, the ESVs richness was 24% higher in infected roots than in non-infected roots (**sup. table 2**). The Shannon and Pielou's indices also significantly increased with the infection ($p < .001$, +9.96% and +6.46%, respectively). The infection by *M. graminicola* was thus associated with an increase in species richness, diversity and evenness of the bacterial community in the roots. We also observed a dependency between the infection and the field effect for both Shannon and Pielou's indices (*infection* x *field* effect: $p < .05$) meaning that the soil properties shaped the bacterial diversity differently in response to the nematode infection. However, the infection effect always had a significantly higher impact on the bacterial diversity than the field effect: $p < .001$ for the infection effect on Pielou's index *versus* $p < .05$ for the field effect and $p < .001$ for the infection effect on Shannon index *versus* no significant impact for the field effect.

Abundances of several bacterial taxa were modified with the infection

To explore the composition of the bacterial communities and identify enriched taxa in non-infected or in infected roots, we performed two independent analyzes: 1) one analysis with the package *Metacoder* that measured the enrichment of taxonomic groups at any taxonomic level (grouped ESVs from phylum to species level) and 2) the other analysis with the package *DESeq2* that measured the potential enrichment for every single ESV.

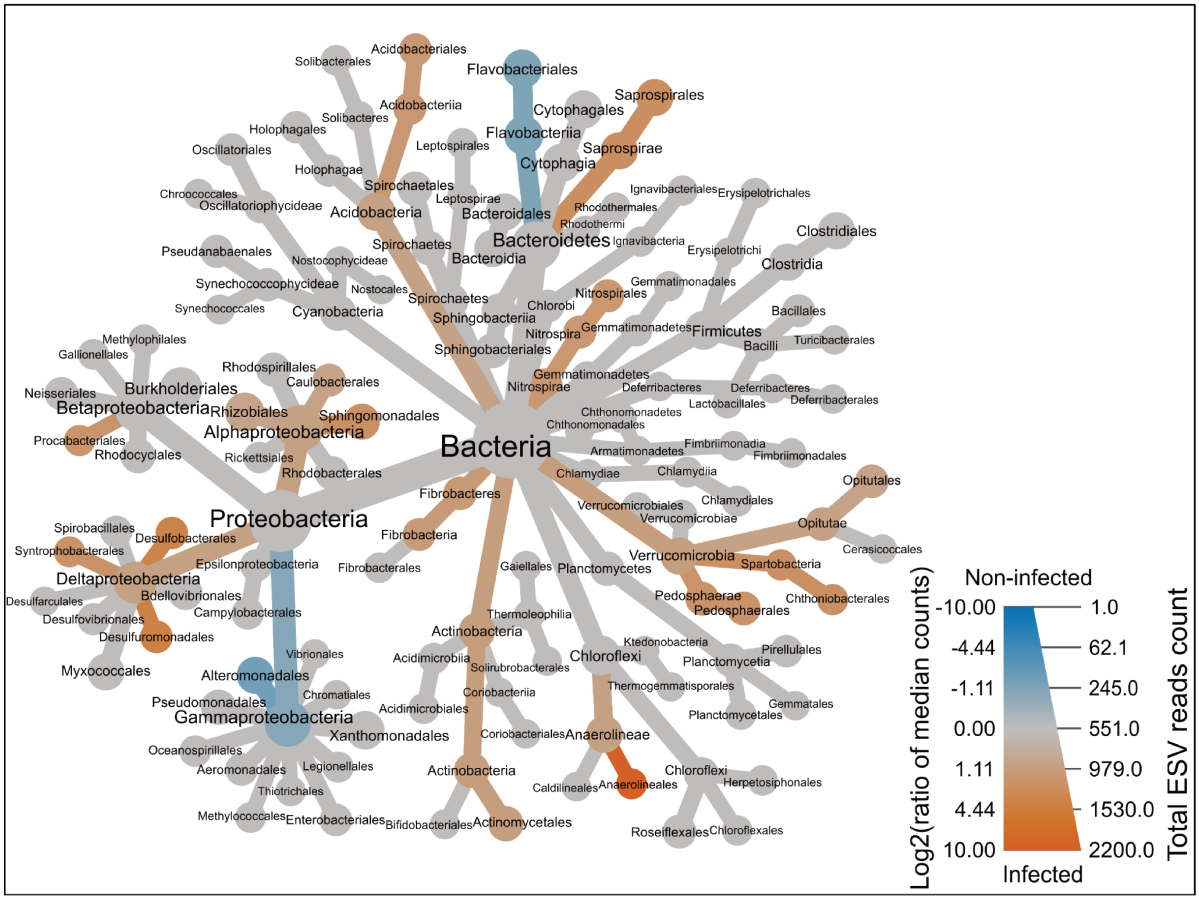

Figure 19. Phylogenetic tree of enriched taxa. Each node represents a taxon. The color indicates the differential abundance (in binary logarithm scale of the ratio of the median reads counts) between the median counts of taxa in infected (orange) and non-infected (blue) rice roots. The size node indicates the total ESV reads count for the taxon. Only taxa at order or higher levels with total ESV reads count > 50 are shown in this simplified tree version (until order level).

The analysis with *Metacoder* and additional calculations allowed us to represent the overall distribution and the relative abundances of taxa on a phylogenetic tree and to highlight specific taxonomic signatures of bacterial enrichments in non-infected or infected roots (**figure 19**). Firstly, more taxa were enriched in infected rice roots, as shown by the higher prevalence of orange nodes and branches on the representation, which was consistent with a higher observed richness and diversity in infected roots. Secondly, some branches were fully or partially colored meaning that the enrichment could be more or less restricted to some clades. Thirdly, the enrichments were not only restricted to close taxa, but they were spread among the branches, especially in infected roots. At the phylum level (**figure 20 A**), *Actinobacteria* for example had a higher prevalence in infected roots: about 70% of these taxa in our samples were found in infected roots (**sup. table 3**). In contrast, no phylum was found to be specifically enriched in non-infected roots. At order level (**figure 20 B**), more orders were enriched in infected roots (16 orders) than in non-infected roots (2 orders). By rank of decreasing prevalence in infected roots, some enriched orders with high total abundance included [*Pedosphareales*], [*Saprospirales*], *Actinomycetales*, *Sphingomonadales*, *Rhizobiales*, and *Opitutales*. For example, about 65% of all ESVs assigned to *Rhizobiales* (107 ESVs) and *Actinomycetales* (44 ESVs) were found in the infected roots (**sup. table 3**). At species level, eight species

were enriched in infected roots whereas four species were enriched in non-infected roots according to the *Metacoder* detailed trees (**sup. figure 2**). For instance, these species were assigned to *Rhizobiales* (*Agrobacterium sullae*, *Ensifer adhaerens* and *Pleomorphomonas oryzae*) for the ones enriched in infected roots and *Flavobacteriales* (*Flavobacterium succinicans*), or *Xanthomonadales* (*Silanimonas mangrovi* and *Stenotrophomonas maltophilia*) for the ones enriched in non-infected roots. The *Metacoder* tree showed a high restructuration of the microbiota associated with the infection as shown by the enrichments of diverse clades at different taxonomic levels.

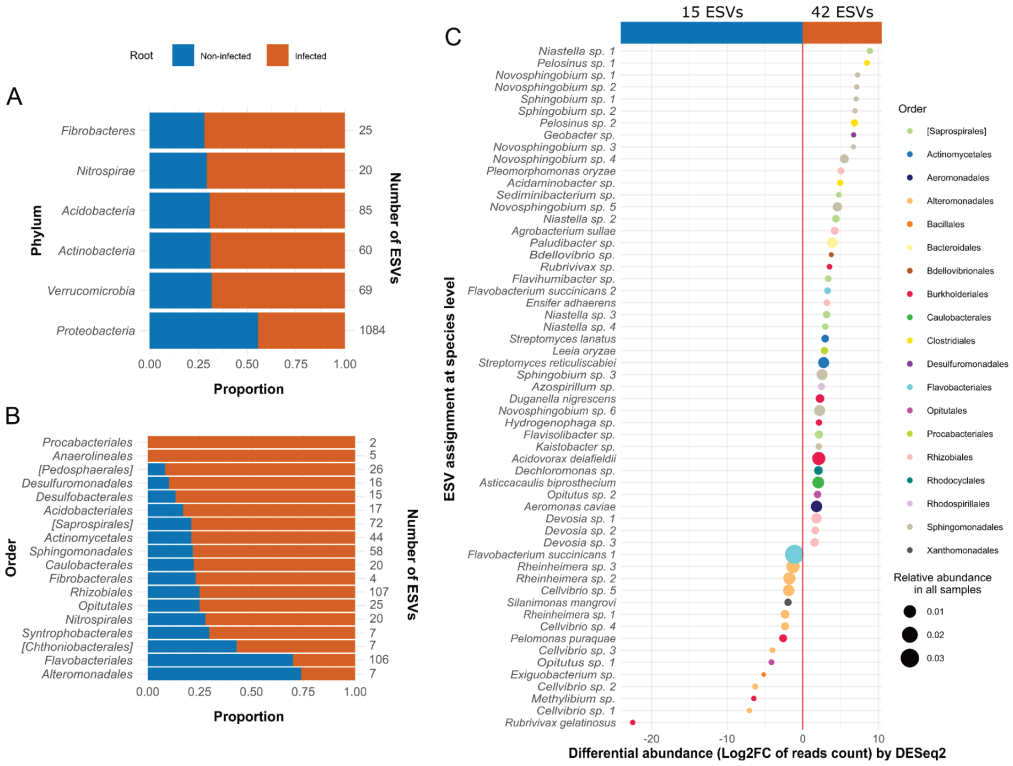

Figure 20. Enriched taxa according to the *Metacoder* (**A** and **B**) and the *DESeq2* (**C**) analyzes. Proportion of reads count in infected *versus* non-infected root and number of ESV per taxa at phylum (**A**) and order (**B**) levels. The *Proteobacteria* phylum, which was not significantly different between the two sample types, is presented to allow comparison with the other significantly enriched phyla and because it is the most dominant bacterial phylum associated with rice roots. Differential abundance of the enriched ESVs with full assignment (until species level) or assigned at genus level (if unassigned or uncultured at species level) (**C**).

Table 3. Summary on numbers of the enriched ESVs according to the *DESeq2* analysis.

Number of ESVs enriched in...	**...infected roots**	**...non-infected roots**
Total	81	17
With **assignment at genus level** (unassigned or uncultured at species level)	42	15
With **full assignment** (until species level)	11	4

The analysis with *DESeq2* enabled the identification of single ESVs enriched or depleted between non-infected and infected roots with assignment at different taxonomic levels. The results (**table 3**) gave 81 ESVs enriched in infected roots including 11 with a full assignment (*Pleomorphomonas oryzae*, *Agrobacterium sullae*, *Ensifer adhaerens*, *Streptomyces lanatus*, *Leeia oryzae*, *Streptomyces reticuliscabiei*, *Asticcacaulis biprosthecium*, *Duganella nigrescens*, *Acidovorax delafieldii*, *Aeromonas caviae* and *Flavobacterium succinicans*), and 17 ESVs enriched in non-infected roots including four with a full assignment (*Rubrivivax gelatinosus*, *Pelomonas puraquae*, *Silanimonas mangrovia*, *Flavobacterium succinicans*). Enrichment of ESVs with assignment at species or genus levels according to the *DESeq2* analysis were represented in **figure 20 C** and given in the corresponding **sup. table 4**. In non-infected roots, 15 ESVs (over the 17 enriched) were assigned at extended species level whereas there were 42 ESVs (over the 81 enriched) in infected roots. For instance, the most enriched in non-infected roots was *Rubrivivax gelatinosus* which was highly enriched ($2^{22.5}$ times, $p < .001$) but very rare in terms of relative abundance (0.02% with 226 reads count in non-infected samples that was not highly enriched with the *Metacoder* analysis). The least enriched but most abundant in non-infected roots was *Flavobacterium succinicans* ($2^{1.11}$ times enriched, $p < .01$, 3.11% of relative abundance and 31,345 reads count in non-infected samples). Moreover, there was another ESV assigned to *Flavobacterium succinicans* enriched in infected roots as well ($2^{3.28}$ times enriched, $p < .01$, 0.07% of relative abundance with 875 reads count in infected samples). This species was actually the most abundant in both non-infected and infected roots but had a higher proportion in infected roots (**sup. figure 2**), hence the need for a complementary aggregating tool such as *Metacoder* to visualize its type of enrichment. The ESVs with a full assignment that were found enriched in the *Metacoder* trees and/or with *DESeq2* were detailed in **table 4**.

Table 4. Compilation of informations for taxa with full assignment (until species level) that are enriched according to the *DESeq2* (single ESV) and/or *Metacoder* (aggregated ESVs) analyzes, and identified as specific taxa and/or hub taxa in networks according to the *SPIEC-EASI* analysis, by order of decreasing relative abundance. Enrichments are indicated in the binary logarithm scale of the ratio of the median reads counts.

ESV	**Enrichment of single ESV (*DESeq2*)**	**Proportion** (in the enriched sample type)	**Relative abundance** (in all samples)	**Assignment**	**Enrichment of aggregated ESVs (*Metacoder*)**	**Specificity (*SPIEC-EASI*)**	**Connectivity (*SPIEC-EASI*)**
6b5fc6d4fb430a2cd66300aef054058a	-1.1	70%	3.11%	*Flavobacterium succinicans*	-1.6	non-specific	non-hub
477b56546f0b35d5df855c45fa3ceeae	3.3	88%	0.07%			infected-specific	hub in infected
303f65442c3fdfa0cfeac97ed0b29a09	2.1	76%	1.12%	*Acidovorax delafieldii*	1.7	non-specific	non-hub
35a93467ec381d7da6449fa326cf5552	2.1	78%	0.79%	*Asticcacaulis biprosthecium*	1.9	non-specific	hub in both networks

740ba29c6c30bce2c57d055dde9938ca	1.8	79%	0.71%	*Aeromonas caviae*	1.0	non-specific	non-hub
ab6a2446d56f9061879d8896e64f1a45	2.8	83%	0.64%	*Streptomyces reticuliscabiei*	2.6	infected-specific	non-hub
0dcb89d978e3c73b67fc1d1ff53423e7	2.3	55%	0.25%	*Duganella nigrescens*	1.4	non-specific	non-hub
aggregated ESVs	not significant	80%	0.20%	*Chitinimonas taiwanensis*	-1.6	non-specific	non-hub
a982a0f1091cb2e2cf172c626a0ae01a	4.2	93%	0.18%	*Agrobacterium sullae*	not significant	infected-specific	non-hub
8bd19edbb5a3d33673f3639814d0f01e	-2.6	87%	0.17%	*Pelomonas puraquae*	-3.3	non-specific	non-hub
99667ea0549e388ede7ed5404ae5136b	3.0	86%	0.16%	*Streptomyces lanatus*	2.6	infected-specific	non-hub
c3596b8775ccb4e66a30abea48c4cfc3	-2	79%	0.13%	*Silanimonas mangrovi*	-2.3	non-specific	hub in infected
440d431a0f0e1c2550f0e22a04355a7a	2.9	87%	0.12%	*Leeia oryzae*	1.8	infected-specific	non-hub
e4bb06ed5943ababeb94ffc8e25435fb72	5.0	96%	0.09%	*Pleomorphomonas oryzae*	not significant	infected-specific	non-hub
1a880bdea6cfd625c5042b36fcbe038b	3.2	88%	0.08%	*Ensifer adhaerens*	not significant	infected-specific	non-hub
aggregated ESVs	not significant	73%	0.03%	*Stenotrophomonas maltophilia*	-4.3	one non-infected-specific ESV	non-hub
6512d48085087fe43c596efafea45322	-22.5	88%	0.02%	*Rubrivivax gelatinosus*	0.1	one infected-specific ESV	one ESV hub in non-infected + one ESV hub in infected

The co-occurrence network was more complex and specific in the gall

Co-occurrence networks were constructed in order to visualize and analyze the impact of the infection on the microbiota network and to identify hub taxa. The networks computed with the package *SPIEC-EASI* were different between non-infected and infected roots, in terms of node number, connectivity and specificity (**figure 21**). The community network of non-infected roots (**figure 21 A**) was composed of 180 ESVs with 260 links in total (174 positive and 86 negative) whereas the network of

infected roots (**figure 21 B**) was composed of 260 taxa with 616 links (424 positive and 192 negative). The bacterial network of infected roots was denser (260 taxa *versus* 180 taxa), which was consistent with the higher species richness described earlier. It was also more connected (degree distribution of 3.14 in non-infected *versus* 5.02 in infected network). This higher complexity suggested potentially more interactions and a higher stability in the infected community. In both networks, positive links ratio was similar (174/260 = 0.67 in non-infected network whereas 424/616 = 0.69 in infected network) meaning that the types of predicted interactions were unaffected in the overall community. Furthermore, in the non-infected network, the majority of ESVs were shared with the infected network, with only 27% specific taxa (48/180), while 49% of the taxa (128/260) were specific to the infected network. This showed a higher specificity of the bacterial network in roots infected by *M. graminicola*.

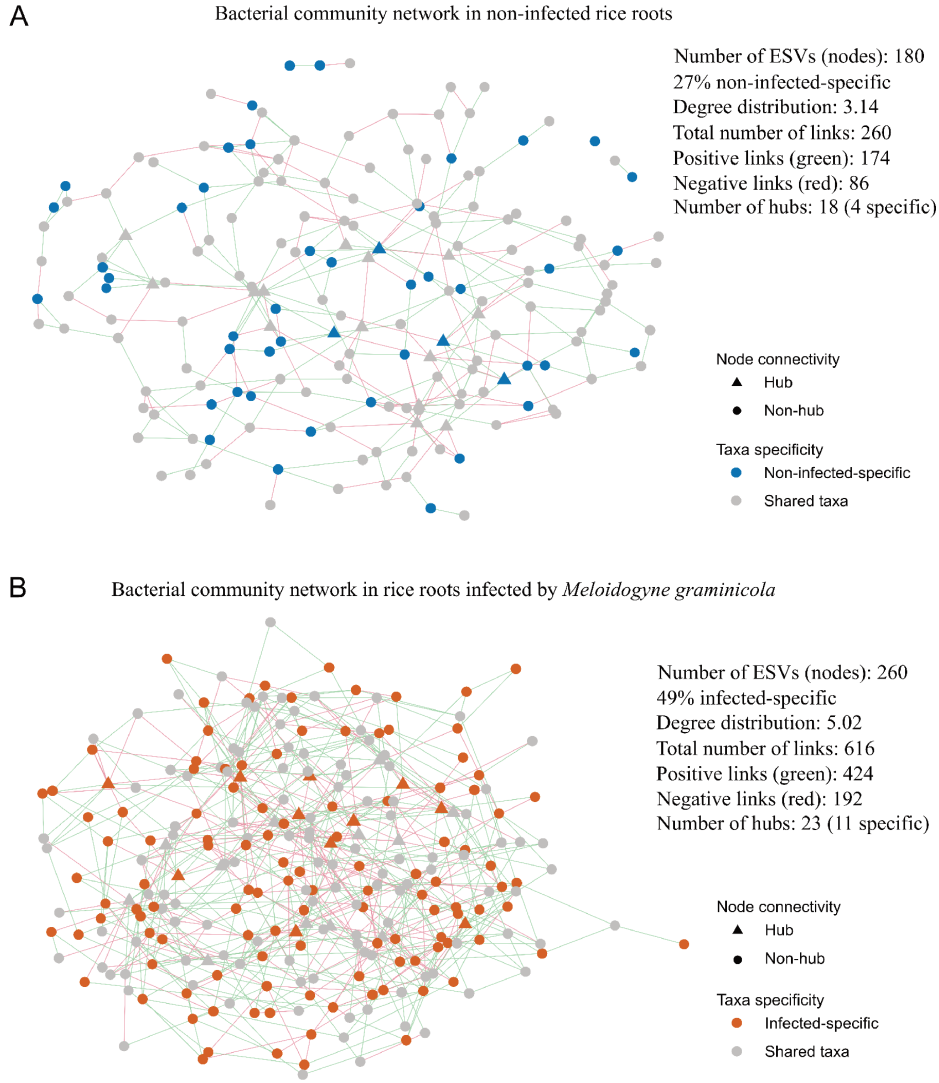

Figure 21. Co-occurrence networks of taxa in non-infected (**A**) and in infected roots (**B**). Each taxon is represented by a node. The green lines between nodes are positive links (positive co-occurrence) and the red lines are negative links (negative co-occurrence). Identified "hub" taxa are represented by triangles. Non-infected-specific taxa are in blue in **A** and infected-specific taxa are in orange in **B**. The other shared taxa are in gray. Only the ESVs present in 80% of each sample type are included in these networks.

We identified hub taxa according to the betweenness centrality, closeness centrality and node degree values of the ESVs present in the networks (**sup. figure 3**). We assumed that hubs were highly connected taxa based on these three features, so we selected the 5% most connected to have a few numbers of taxa. Hence, we identified 18 hub taxa in the non-infected network, and 23 hub taxa in the infected network (**sup. table 5**). In the non-infected network (**figure 21 A**), four hub taxa (22%) were specific to the non-infected condition whereas in the infected network (**figure 21 B**), 11 hubs (48%) were specific to the infected condition. Moreover, 12 hubs (66%) in the non-infected network were shared taxa between both networks and 10 hubs (43%) in the infected network were shared taxa. Once again, all these characteristics about potentially important taxa for the structure and the composition of the network suggested a higher specificity in the overall structure of the infected bacterial community.

Discussion

Our characterization of the bacterial community (combined endosphere and rhizoplane) of non-infected and infected rice root tips in three highly infested paddy fields in Northern Vietnam showed that the infection by the RKN *M. graminicola* led to a large restructuration of the microbiota. There were indeed two distinct structures of the bacterial community based on ESVs taxonomy and abundances, with differences in diversity including a higher richness and evenness in infected roots, and different phylogenetic composition with a strong signature of some taxa enriched or depleted in either non-infected or infected roots. This shift in the gall microbiome, for which we propose the name "gallobiome", suggested a different network of interactions in the infected root tissues. Computational analysis performed in this study indeed showed a denser, more connected and more specific community network in the case of the infection.

The signature of the bacterial gallobiome of *M. graminicola*

We observed an increase in bacterial diversity in rice roots in the context of the infection by the RKN *M. graminicola*. Not only the species richness increased but also the evenness, meaning that the taxa abundances were more equal and that there were fewer rare taxa in infected roots. It is indeed clearly visible on the *Metacoder* tree (**figure 19**) where there were diverse and numerous enriched taxa in infected roots, whereas in non-infected roots only two branches were enriched. Differences in the structure and diversity of the bacterial communities were explained by both the infection effect and the field effect independently.

Concerning the infection effect, such a shift in the microbiome leading to a microbiome restructuration is called a dysbiosis and this phenomenon is under current broad investigations in human and other animals (DeGruttola *et al.*, 2016). Imbalanced human microbiomes, compared to healthy microbiomes, can be associated with diseases according to many studies (Xuan *et al.*, 2014; Casén *et al.*, 2015). In plants, few studies described a shift in the microbiome as a function of plant health or disease. Koskella *et al.* (2017) examined interactions between the bacterial pathogen *Pseudomonas syringae* pv. *aesculi* (Pae), the leaf miner moth pest *Cameraria ohridella*, and the bark-associated bacterial microbiota of the horse chestnut tree. They found a clear loss of diversity and associated shift in the microbiota composition of trees as a signature of the disease. In our study, we found on the contrary an increase of species richness in infected roots. It could mean that the infection was not only associated with a change of the microbiome as a dysbiosis, but it was also associated with a change of the ecological niche because of root morphological and physiological modifications induced by the nematode. As sedentary biotrophic parasites, RKNs modify the plant's metabolism in order to complete their life cycle (Trudgill and Blok, 2001). Therefore, the gall is a nutrient rich environment from which a large diversity of bacteria seems also to benefit. If so, the microbial restructuration would be an indirect consequence of the infection. However, the microbial restructuration that we observed could be a temporal state of the plant health status. To know if the shift in the microbiome happened before (if so it would be a cause of the infection) or after the infection (if so it would be a consequence of the infection), a dynamic sampling would be required. This is what has been done by Lebreton *et al.* (2019) with the parasite

Plasmodiophora brassicae on the cabbage *Brassica rapa*. They analyzed the bacterial and fungal communities during the infection in both roots and rhizosphere. They observed a drastic shift of the fungal community from healthy plants between the last two sampling dates, especially in plant roots. The shift of the bacterial community in our study would fit with these observations, knowing that the plantlets were highly infected in the fields in Vietnam. Thus, the microbiome state described here would characterize a relatively late stage of the infection, a snapshot of well-formed galls in highly infected plants.

Concerning the field effect, as the physicochemical properties of the fields were slightly different, we expected different ecological niches and consequently different microbiomes in the three fields. We indeed observed different microbiome structures and diversities based on ESVs and KOs (orthologous genes, see **Additional analyzes** part) between the fields. According to a multifactorial analysis (Shakya *et al.*, 2013), soil properties can be responsible for 9.1% of the variances in β-diversity (pairwise UniFrac distances). Other authors previously found that among all soil factors, pH has the largest effect on the bacterial rhizosphere communities (Lauber *et al.*, 2009). We also found in our study that pH was the measured environmental factor that affected the structure of the bacterial community the most.

As just mentioned above, we found that the α-diversity increased in the presence of *M. graminicola*. That was consistent with the simple explanation that the nematode carried its own microbiota inside the gall and that the taxa enriched in infected roots were part of the nematode's microbiota. *M. graminicola*'s microbiota has not been published yet, but other nematode's microbiota have been studied. The closest to date is the one of the other RKN *M. hapla* collected from different soils in Germany (Topalović *et al.*, 2019). Although the microbiota depends on host as well as many other factors (Shakya *et al.*, 2013; Hacquard *et al.*, 2015), this study confirmed that only a few microorganisms (14 strains) were able to attach to the nematode's cuticle. Little is known about the internal microbiota of RKNs, but these obligatory plant parasites ingest plant solutes through a stylet whose diameter of 340-510 nm limits the entry of microorganisms (Hussey and Mims, 1991). Other plant parasitic nematodes may nevertheless carry endosymbionts (Haegeman *et al.*, 2009) but, to date, none have been isolated from the RKNs (Brown, 2018). In other words, even if our data included *M. graminicola*'s microbiota in the infected roots, it could explain only a small part of the total increased richness (+24% *i.e.* +157 ESVs). Besides, specific taxa were also revealed in non-infected roots suggesting indirect reasons for the modifications of the root microbiota. We propose that the shift in the bacterial composition was mainly due to changes in the plant physiology and morphology caused by the infection that benefited opportunistic bacteria from the surrounding soil, and slightly due to the colonization of the nematode's microbiota. The rhizosphere effect, or so-called gall effect in our case, could be responsible for the shift in the gallobiome *via* root exudation (Sasse *et al.*, 2018) especially at the root tip where flux of primary metabolites are mostly located (Canarini *et al.*, 2019).

Potential roles for enriched bacterial taxa related to the infection

Back and colleagues (2002) described the mechanisms by which plant-parasitic nematodes and soil-borne pathogens can work together to infect a plant. These mechanisms imply nematode-induced wounds, nematodes-induced physiological changes to the host plant (giant cell for example), reduction of

host resistance and modifications within the rhizosphere (microbial substrate preference or rhizosphere effect). Interactions including competition (*e.g.* antagonism) and mutualism (*e.g.* syntrophy) could play an important role in the infection. For example, a study conducted by Berendsen *et al.*, (2018) showed that the infection of *Arabidopsis* plants with a biotrophic pathogen can promote growth of a specific microbiota in the rhizosphere to aid in their defense. In our study, the complex structure and the higher number of bacterial taxa (+44%) and co-occurrence evidence (+136%) in the infected network suggests more interactions than in the non-infected network. Another study conducted by Carrión *et al.* (2019) also showed an increased complexity in the co-occurrence network when facing a pathogen invasion of *Rhizoctonia solani* inoculated on sugar beet plants. Many enriched species in the gallobiome of *M. graminicola* are known to evolve mechanisms that allow them to grow and survive in highly competitive environments like soil and rhizosphere. For instance, among the enriched species in infected roots (**table 4**), *Ensifer adhaerens* is a predator of Gram negative bacteria (Casida, 1982). It is able to attach to other bacteria and to cause their lysis. It has already been described as an endophyte of rice roots (Xiaoxia *et al.*, 2010) and, interestingly, as an occupant of *Fabaceae* nodules (Rogel *et al.*, 2001) which is also a nutrient-rich environment. About *Duganella nigrescens*, another enriched species in infected roots, little is known but it is closely related to *Duganella violacienigra*, a rice endophyte (Sun *et al.*, 2008). This later is known to produce violacein, a blue-purple secondary metabolite that has numerous biological activities involved in competitive interactions, including antibacterial, antiviral, antiprotozoan and antitumor effects (Ballestriero *et al.*, 2014). Thus, some bacterial taxa were enriched at the infected root tips potentially for different reasons that may be involved in plant defense.

Flavobacterium succinicans was the most abundant species in all roots and was enriched in non-infected roots according to the analysis with *Metacoder*. It has been described as a freshwater commensal and may possess opportunistic pathogenic responses according to Bernardet and Bowman (2006) that is consistent with the state of the plants exposed to water in the field during the sampling and to the nematode infection. In Tian and colleagues (2015), *Flavobacteriales* were found enriched in tomato roots that are infected by the RKN *M. incognita*. To know about their functional role in the community, they identified a vast range of CAZymes mainly involved in oligosaccharide degradation or simple sugar utilization, suggesting that these bacteria might be involved in carbohydrate metabolism. In our study, metabolic pathways related to carbohydrate degradation were found enriched in non-infected roots (*e.g.* sucrose degradation, see **Additional analyzes** part). During the infestation process and the gall formation, the nematode *M. graminicola* uses cellulases (Phan *et al.*, 2020) and hyperplasia and hypertrophy of the plant cells involve plant cellulases that can potentially release carbohydrates in the environment and feed opportunistic bacteria such as *F. succinicans*. Moreover, the ESVs assigned to *F. succinicans* and enriched in the infected network were identified as a hub in the infected networks, meaning that this bacterium may play a key role in the community structure during the infection. It would be interesting to check experimentally in a synthetic microbial community (SynCom) experiment if it is indeed a keystone taxon, *i.e.* a highly connected taxa that individually or in a guild exerts a considerable influence on microbiome structure and functioning irrespective of its abundance across space and time (Banerjee *et al.*, 2018).

Stenotrophomonas maltophilia, the last enriched species in non-infected roots according to the *Metacoder* analysis, has been identified as a rice root endophyte in a field in China (Zhu *et al.*, 2012). Many

strains of this species can produce antibiotics that protect plants and compounds that can promote plant growth. The strain *S. maltophilia* R3089 for example, can produce an antifungal compound named maltophilin. Although it has been found inactive against bacteria (Jakobi *et al.*, 1996), it can play an important role within the overall microbiome. More importantly for this study, another strain, *S. maltophilia* G2, isolated from soil in China was found to have a high nematotoxic activity against a free-living nematode (*Panagrellus redivivus*) and a plant-parasitic nematode (*Bursaphelenchus xylophilus*) (Huang *et al.*, 2009). In our study, *S. maltophilia* was present in non-infected roots and it would be interesting to test under controlled conditions its potential role in preventing the establishment of *M. graminicola* in rice roots. Such bacteria would be potential candidates for biocontrol strategies and could be tested *in vitro* against RKNs by using a complementary cultivable approach.

Conclusion

In this study, we aimed at improving our understanding on the impact of RKNs infection on the root microbiome by describing the two bacterial communities of non-infected roots and infected roots (with galls of *M. graminicola*). We clearly observed a specific signature of the gallobiome with shifting abundances and enrichments found in the two community networks that was also associated with a restructuring of the microbiota. It is interesting to notice that these deep taxonomic and structural modifications of the microbiota were also associated with a shift in the predicted functional capabilities of the microbiome (see **Additional analyzes** part). Efforts to link taxa to putative functions or roles can be pointless without experimental validations, and such validations can be difficult to obtain from a complex community influenced by many environmental factors. However, in the perspective of limiting the infection by *M. graminicola*, our study could help to select candidate bacteria for biocontrol strategies in the field. An ideal candidate would carry nematicidal activity and/or direct beneficial effects to the plant (that can be tested *in vitro* and *in planta*) and would be able to survive in the same environment as the nematode. Thus, the presence of predicted functions specialized for bacterial survival in the gall and the connectivity in the bacterial community could be important criteria for selection of several candidates. For example, *Streptomyces lanatus* and *S. reticuliscabiei* belonging to *Actinobacteria* were all enriched in infected roots. This phylum is known for antibiotic production and degradation of complex polysaccharides, and some strains have nematicidal activity (Xu *et al.*, 2011). Because of their ability to colonize and survive in the gall environment and their potential biocontrol activity, testing the potential of this consortium bacteria to limit the infection by *M. graminicola* in a rice field could be an interesting perspective.

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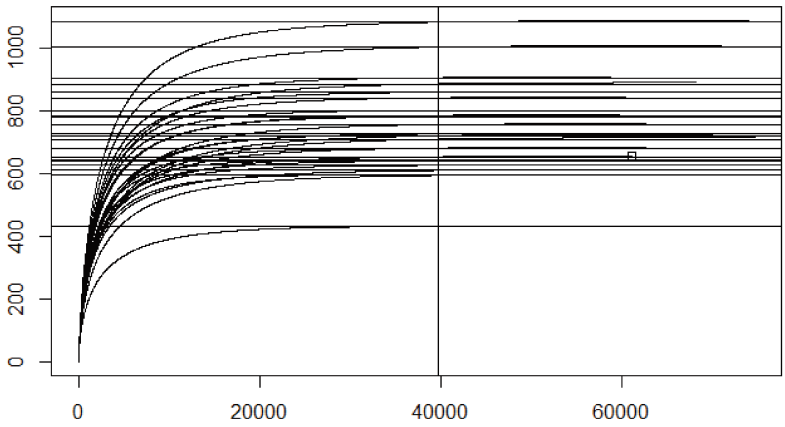
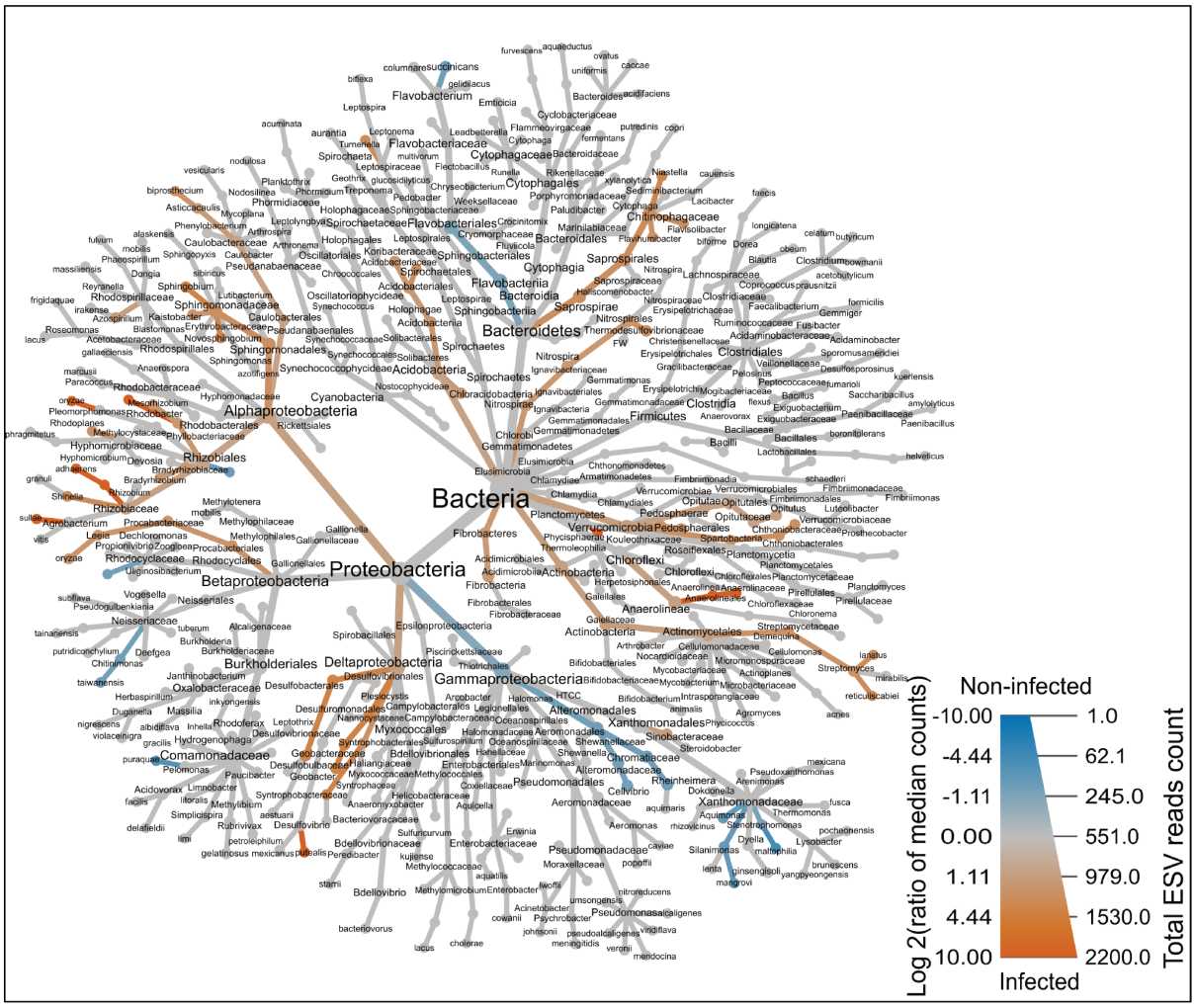
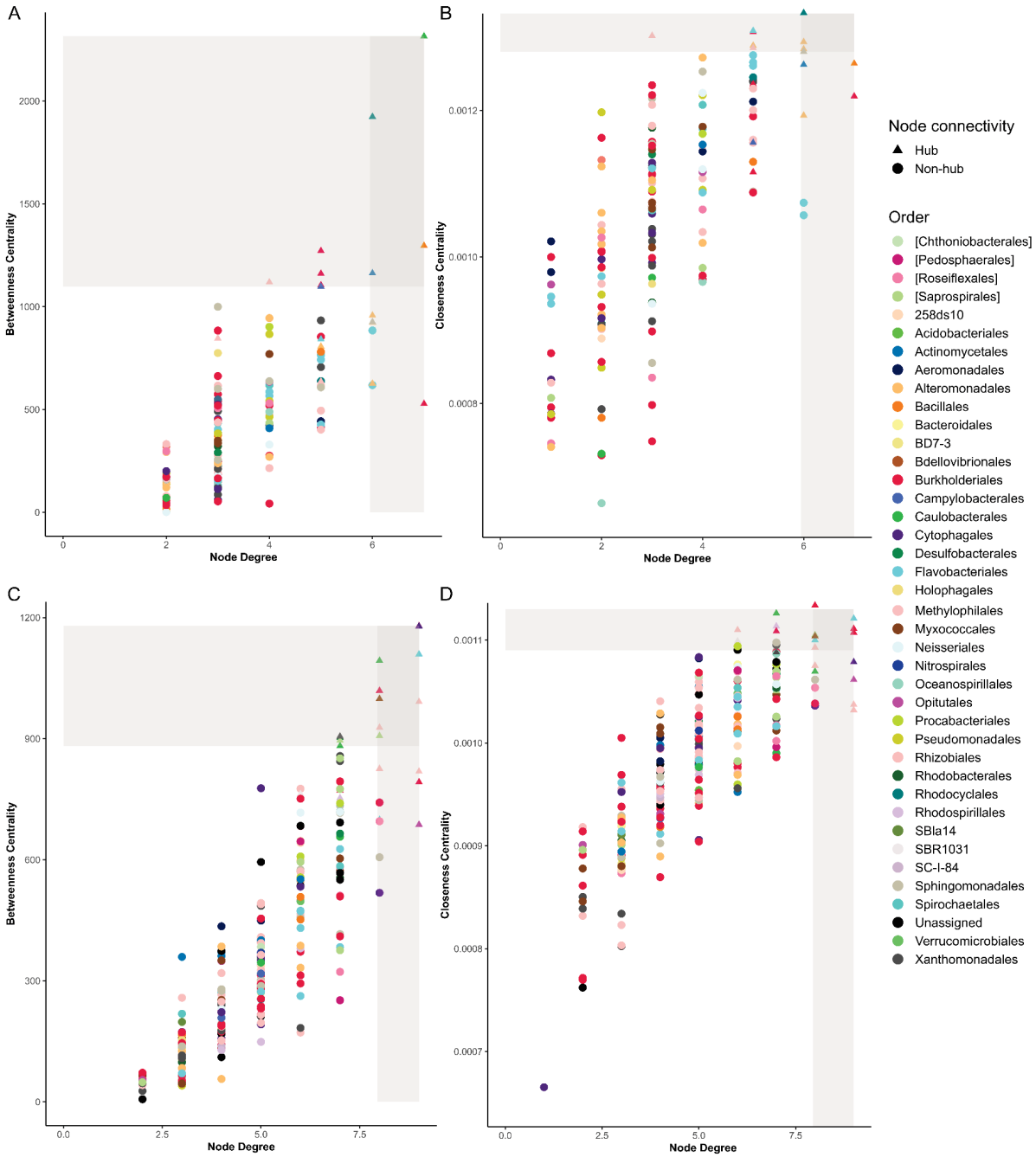
Supplemental figures and tables



Sup. figure 1. Rarefaction curves of the samples: number of ESVs = f(total reads count).



Sup. figure 2. Phylogenetic tree of enriched taxa. Each node represents a taxon. The color indicates the differential abundance (in binary logarithm scale of the ratio of the median reads counts) between the median counts of taxa in infected (orange) and non-infected (blue) rice roots. The size node indicates the total ESV reads count for each taxa. All taxa are shown in this detailed tree version (until species level).



Sup. figure 3. Identification of hub ESVs. The betweenness centrality, closeness centrality and node degree have been used for the identification of hubs (triangles) in non-infected (**A** and **B**) and infected (**C** and **D**) rice roots. The 5% most connected taxa have been highlighted by the gray area for each feature. This allowed us to select 23 taxa hubs in infected roots and 18 taxa hubs in non-infected roots.

Sup. table 1. Physicochemical properties of the soil samples.

Field n°	Plot n°	pH KCl	SOC (%)	TKN (%)	P_2O_5 (ppm)	S (ppm)	SO_4^{2-} (ppm)	CEC (meq/ 100g)	Texture			Aggregate		
									< 0.002 (clay)	0.002-0.02 (loam)	0.02-2 (sand)	< 0.053	0.053-0.25	0.25-2
1	1	5.7	1.61	0.15	0.27	0.036	34.3	13.1	20.5	45.5	34	67.8	20.9	11.3
	2	5.7	1.63	0.15	0.29	0.026	25.7	13.1	20.4	43.3	36.3	68.6	20.7	10.7
	3	5.8	1.67	0.17	0.32	0.019	25.7	13.2	20.9	41.4	37.7	63.3	21	15.6
	4	5.5	1.61	0.14	0.29	0.017	35.7	13.2	21.9	42.4	35.7	51.9	36.7	11.5
Mean		**5.68**	**1.63**	**0.15**	**0.29**	**0.02**	**30.36**	**13.15**	**20.9**	**43.17**	**35.94**	**62.9**	**24.82**	**12.28**
2	1	5.7	1.48	0.14	0.25	0.026	36.4	13	19.7	42.5	37.8	61.1	26.2	12.6
	2	5.8	1.46	0.14	0.28	0.019	33.6	12.5	19.2	43	37.8	61.2	25.2	13.6
	3	5.8	1.48	0.15	0.27	0.024	47.1	12.3	20.1	43.5	36.4	61.1	24.4	14.5
	4	5.7	1.45	0.15	0.24	0.014	41.4	12.8	22.6	41.4	36	66.2	23.8	10
Mean		**5.75**	**1.47**	**0.15**	**0.26**	**0.02**	**39.64**	**12.65**	**20.39**	**42.61**	**36.99**	**62.39**	**24.93**	**12.69**
3	1	5.8	1.39	0.13	0.24	0.021	41.4	13.2	22.7	40.2	37.1	59.2	23	17.7
	2	5.6	1.57	0.16	0.27	0.033	42.9	13.2	21.6	41.5	36.9	58.5	21	20.4
	3	5.7	1.59	0.16	0.29	0.025	51.4	13.3	23.1	44.4	32.5	64.5	19	16.5
	4	5.7	1.61	0.16	0.28	0.014	37.1	13.4	23.3	43.4	33.3	65.3	21.7	12.9
Mean		**5.7**	**1.54**	**0.15**	**0.27**	**0.02**	**43.21**	**13.28**	**22.66**	**42.4**	**34.96**	**61.89**	**21.21**	**16.9**

Sup. table 2. Diversity indices of the bacterial community of infected roots by *Meloidogyne graminicola* (with galls) and non-infected roots (without galls) with mean ± standard deviation.

Field n°	Plot n°	Sample type	Observed ESVs richness	Shannon index	Pielou's index
1	1	infected	656	5.13	0.79
		non-infected	597	4.78	0.75
	2	infected	802	5.59	0.84
		non-infected	613	5.02	0.78
	3	infected	784	5.48	0.82
		non-infected	598	5.23	0.82
	4	infected	1,009	5.80	0.84
		non-infected	891	5.53	0.81
2	1	infected	788	5.50	0.82
		non-infected	643	5.38	0.83

	2	infected	645	5.34	0.82
		non-infected	628	5.29	0.82
	3	infected	845	5.54	0.82
		non-infected	716	4.79	0.73
	4	infected	730	5.18	0.79
		non-infected	683	5.269	0.81
3	1	infected	729	5.18	0.79
		non-infected	433	4.29	0.71
	2	infected	862	5.63	0.83
		non-infected	726	5.00	0.76
	3	infected	1,090	5.82	0.83
		non-infected	760	4.80	0.72
	4	infected	908	5.64	0.83
		non-infected	684	4.49	0.69
Mean		**infected**	**821 +/- 133**	**5.49 +/- 0.24**	**0.82 +/- 0.02**
Mean		**non-infected**	**664 +/- 111**	**4.99 +/- 0.37**	**0.77 +/- 0.05**

Sup. table 3. Total number and proportion of ESV reads count, and number of ESVs of enriched taxa. Taxa are either enriched in non-infected or infected roots if proportion is significantly > 0.50 in one sample type according to the *Metacoder* analysis.

Taxa name	Taxa level	Total ESV reads count		Proportion		Number of ESVs
		non-infected	infected	non-infected	infected	
*Proteobacteria**	phylum	659,947	526,877	0.56	0.44	1,084
Verrucomicrobia	phylum	4,572	9,801	0.32	0.68	69
Actinobacteria	phylum	9,417	20,644	0.31	0.69	60
Acidobacteria	phylum	2,417	5,442	0.31	0.69	85
Nitrospirae	phylum	567	1,373	0.29	0.71	20
Fibrobacteres	phylum	3013	7731	0.28	0.72	25
Alteromonadales	order	143,603	50,468	0.74	0.26	70
Flavobacteriales	order	81,115	44,451	0.65	0.35	106

[Chthoniobacterales]	order	236	741	0.24	0.76	7
Syntrophobacterales	order	78	232	0.25	0.75	7
Nitrospirales	order	159	412	0.28	0.72	20
Opitutales	order	2,378	4,891	0.33	0.67	25
Rhizobiales	order	60,279	96,542	0.38	0.62	107
Fibrobacterales	order	12	40	0.23	0.77	4
Caulobacterales	order	8,100	15,466	0.34	0.66	20
Sphingomonadales	order	13,037	35,497	0.27	0.73	58
Actinomycetales	order	8,925	19,737	0.31	0.69	44
[Saprospirales]	order	3,825	16,362	0.19	0.81	72
Acidobacterales	order	89	383	0.17	0.83	17
Desulfobacterales	order	589	5,148	0.10	0.90	15
Desulfuromonadales	order	266	1,403	0.16	0.84	16
[Pedosphaerales]	order	619	2,871	0.18	0.82	26
Anaerolineales	order	30	263	0.10	0.90	5
Procabacteriales	order	0	86	0	1	2

* The *Proteobacteria* phylum, which is not significantly different between the two sample types, is indicated in this table to allow comparison with significant phyla and because it is the most dominant bacterial phylum associated with rice roots.

Sup. table 4. Table of enriched ESVs with full (until species) or at genus level (if unassigned or uncultured at species level) assignment and their differential abundance (log2FC) calculated with *DESeq2*.

ESV	Phylum	Order	Species	*DESeq2* (log2FC)
6512d48085087fe43c596efafea45322	*Proteobacteria*	*Burkholderiales*	*Rubrivivax gelatinosus*	-22,49
f33f66d383dbb880418494cf610046a0	*Proteobacteria*	*Alteromonadales*	*Cellvibrio sp. 1*	-7,06
4e2176d0a164028cc7d5f96f06797239	*Proteobacteria*	*Burkholderiales*	*Methylibium sp.*	-6,47
13a85ec596f58bca5fd4a5c612d41091	*Proteobacteria*	*Alteromonadales*	*Cellvibrio sp. 2*	-6,30
bceda017b8762829850d74ca447eaf39	*Firmicutes*	*Bacillales*	*Exiguobacterium sp.*	-5,18
7f3fc3de0291f08b16f0ca102d93c2aa	*Verrucomicrobia*	*Opitutales*	*Opitutus sp. 1*	-4,15
9fb82f32eb82975222f78b04260eaa3a	*Proteobacteria*	*Alteromonadales*	*Cellvibrio sp. 3*	-4,02
8bd19edbb5a3d33673f3639814d0f01e	*Proteobacteria*	*Burkholderiales*	*Pelomonas puraquae*	-2,61

da51cbe5c5b594c4105af4245a2ae4b3	*Proteobacteria*	*Alteromonadales*	*Cellvibrio sp. 4*	-2,35
9c7881f72f1d31b8c29869014453a40a	*Proteobacteria*	*Alteromonadales*	*Rheinheimera sp. 1*	-2,35
c3596b8775ccb4e66a30abea48c4cfc3	*Proteobacteria*	*Xanthomonadales*	*Silanimonas mangrovi*	-1,97
efbf6b9e354b736c8cd722030a46082c	*Proteobacteria*	*Alteromonadales*	*Cellvibrio sp. 5*	-1,87
3c12dccd725c6cde499f3e93dec8f40f	*Proteobacteria*	*Alteromonadales*	*Rheinheimera sp. 2*	-1,79
a542db1043409f96f7c76b5d7cda4cd9	*Proteobacteria*	*Alteromonadales*	*Rheinheimera sp. 3*	-1,31
6b5fc6d4fb430a2cd66300aef054058a	*Bacteroidetes*	*Flavobacteriales*	*Flavobacterium succinicans 1*	-1,11
4321e723ae5dacb46cfc28dd212cd5a2	*Proteobacteria*	*Rhizobiales*	*Devosia sp. 3*	1,58
e656f69b552c09a5cc975676a527995e	*Proteobacteria*	*Rhizobiales*	*Devosia sp. 2*	1,66
3646b140b6d089f9b690fc2dc20650b9	*Proteobacteria*	*Rhizobiales*	*Devosia sp. 1*	1,82
740ba29c6c30bce2c57d055dde9938ca	*Proteobacteria*	*Aeromonadales*	*Aeromonas caviae*	1,83
7f1b86f616352b76dec9021d1970a12a	*Verrucomicrobia*	*Opitutales*	*Opitutus sp. 2*	1,97
35a93467ec381d7da6449fa326cf5552	*Proteobacteria*	*Caulobacterales*	*Asticcacaulis biprosthecium*	2,06
9ca3963a3a8135e6f0c0e4908f5e8be0	*Proteobacteria*	*Rhodocyclales*	*Dechloromonas sp.*	2,08
303f65442c3fdfa0cfeac97ed0b29a09	*Proteobacteria*	*Burkholderiales*	*Acidovorax delafieldii*	2,12
cdd68ff4900c660f92c27420592d501d	*Proteobacteria*	*Sphingomonadales*	*Kaistobacter sp.*	2,13
f655e7f7c73991fe696ea3f4c9671ce2	*Bacteroidetes*	*[Saprospirales]*	*Flavisolibacter sp.*	2,14
f478892b80ba4959a6e905cb167f8806	*Proteobacteria*	*Burkholderiales*	*Hydrogenophaga sp.*	2,15
faf6ed7acd39bca6ba22aaef30e9e9e3	*Proteobacteria*	*Sphingomonadales*	*Novosphingobium sp. 6*	2,24
0dcb89d978e3c73b67fc1d1ff53423e7	*Proteobacteria*	*Burkholderiales*	*Duganella nigrescens*	2,29
9e55bd0c112c7a000168549e84084033	*Proteobacteria*	*Rhodospirillales*	*Azospirillum sp.*	2,48
9f66e83f0909b6a8fbe18e0c65cce3c9	*Proteobacteria*	*Sphingomonadales*	*Sphingobium sp. 3*	2,56
ab6a2446d56f9061879d8896e64f1a45	*Actinobacteria*	*Actinomycetales*	*Streptomyces reticuliscabiei*	2,77
440d431a0f0e1c2550f0e22a04355a7a	*Proteobacteria*	*Procabacteriales*	*Leeia oryzae*	2,88
99667ea0549e388ede7ed5404ae5136b	*Actinobacteria*	*Actinomycetales*	*Streptomyces lanatus*	2,97
f56ad4f98e704aa65899dbded23e844e	*Bacteroidetes*	*[Saprospirales]*	*Niastella sp. 4*	2,97
0806ce4613d94cd48156fb21aafa46fc	*Bacteroidetes*	*[Saprospirales]*	*Niastella sp. 3*	3,16
1a880bdea6cfd625c5042b36fcbe038b	*Proteobacteria*	*Rhizobiales*	*Ensifer adhaerens*	3,21
477b56546f0b35d5df855c45fa3ceeae	*Bacteroidetes*	*Flavobacteriales*	*Flavobacterium succinicans 2*	3,28

225df02ca193f11c3ec4ab066f68c3bb	*Bacteroidetes*	*[Saprospirales]*	*Flavihumibacter sp.*	3,38
22bf88a41f63317518e44a3a8c79f002	*Proteobacteria*	*Burkholderiales*	*Rubrivivax sp.*	3,54
4e759f23222d348d64cf8b049d992bc7	*Proteobacteria*	*Bdellovibrionales*	*Bdellovibrio sp.*	3,77
a0db6c9d574f9e89016ba0702cf6e837	*Bacteroidetes*	*Bacteroidales*	*Paludibacter sp.*	3,91
a982a0f1091cb2e2cf172c626a0ae01a	*Proteobacteria*	*Rhizobiales*	*Agrobacterium sullae*	4,23
74e5439bd31d45d8af425a7f40b1fd0a	*Bacteroidetes*	*[Saprospirales]*	*Niastella sp. 2*	4,37
a7f7b848ed4fcbddf67080d6cd9683b4	*Proteobacteria*	*Sphingomonadales*	*Novosphingobium sp. 5*	4,59
ba32adabf60cc79dfcaa8a29b0a3d55e	*Bacteroidetes*	*[Saprospirales]*	*Sediminibacterium sp.*	4,77
cd5752285fa3753c4e5b1d8bced6d0e9	*Firmicutes*	*Clostridiales*	*Acidaminobacter sp.*	4,96
e4bb06ed5943abeb94ffc8e25435fb72	*Proteobacteria*	*Rhizobiales*	*Pleomorphomonas oryzae*	5,04
40a43181ba38fb7d5df3fddc108d4a6d	*Proteobacteria*	*Sphingomonadales*	*Novosphingobium sp. 4*	5,50
d25321b9cd0a63de0de3389567b04ddd	*Proteobacteria*	*Sphingomonadales*	*Novosphingobium sp. 3*	6,69
80518120a2116ffe75ffde413475a8a9	*Proteobacteria*	*Desulfuromonadales*	*Geobacter sp.*	6,72
d2ef3262e6b5c6cebf64baa1ffd9f366	*Firmicutes*	*Clostridiales*	*Pelosinus sp. 2*	6,84
60ef976d04fab209bf207a49356e1d5c	*Proteobacteria*	*Sphingomonadales*	*Sphingobium sp. 2*	6,90
bba8e37b4ae867a45f1f09a983f7e722	*Proteobacteria*	*Sphingomonadales*	*Sphingobium sp. 1*	7,06
cee53717f70a8a8da7ff22f8dfb16bda	*Proteobacteria*	*Sphingomonadales*	*Novosphingobium sp. 2*	7,13
a79def26e468aa1e32bf97b94b8af7a2	*Proteobacteria*	*Sphingomonadales*	*Novosphingobium sp. 1*	7,24
c900116778842c99a8780d483ecdc383	*Firmicutes*	*Clostridiales*	*Pelosinus sp. 1*	8,48
c1e245dd68c3726213efbae5315a98e4	*Bacteroidetes*	*[Saprospirales]*	*Niastella sp. 1*	8,86

Sup. table 5. Table of hub ESVs identified by their betweenness centrality, closeness centrality and node degree calculated with *SPIEC-EASI*, with their specificity in the network and their assignment at order and species levels.

ESV	Hub in network	Betweenness centrality	Closeness centrality	Node degree	Specificity	Order	Species
1476ab9832bdce151d5ecf11963cef7d	non-infected	806.946739	0.00128866	5	non-infected-specific	*Alteromonadales*	*Rheinheimera* sp.
18aaad2ffc7edc3b076eaa1b54a8a115	non-infected	957.422226	0.001293661	6	non-infected-specific	*Alteromonadales*	unassigned
1b3a529ff03bd783348c84795a2fb75b	non-infected	923.583926	0.001283697	6	non-specific	*Alteromonadales*	*Shewanella* sp.
3c12dccd725c6cde499f3e93dec8f40f	non-infected	626.211689	0.001193317	6	non-specific	*Alteromonadales*	*Rheinheimera* sp.
4ab20c8d8ba438fdc1de73fcf3611d4d	non-infected	636.995669	0.001285347	5	non-specific	*Rhizobiales*	*Rhizobium* sp.

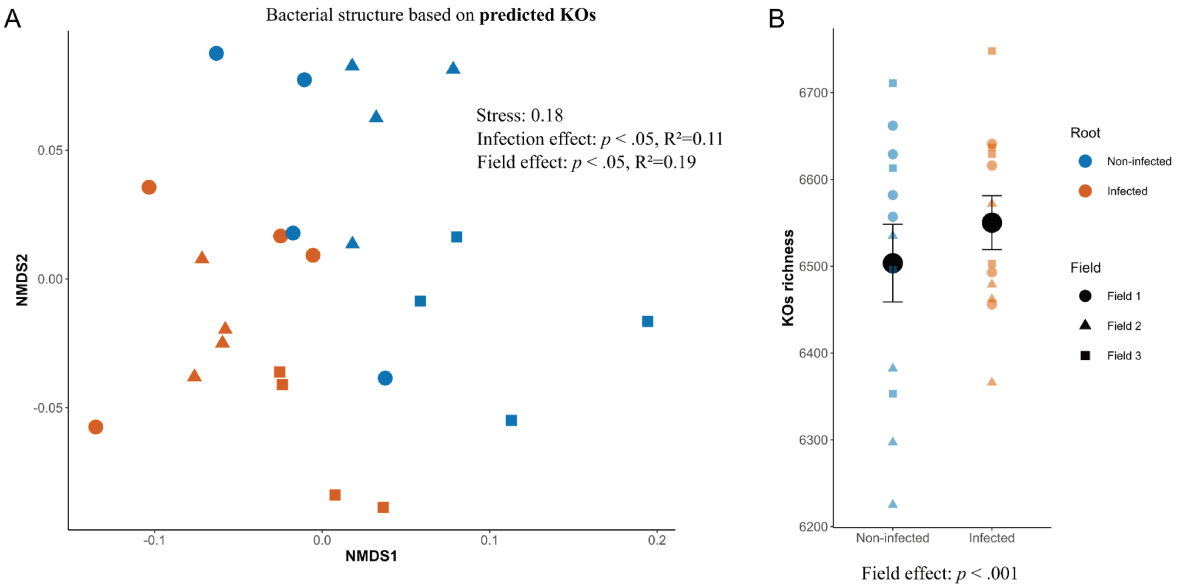
50a0836c5fd765a777 83ad22b377a8a2	non-infected	1,296.609876	0.001264223	7	non-specific	*Bacillales*	*Exiguobacterium* sp.
511b96076fc9f3ae42 f162ad2676e929	non-infected	1,104.85971	0.001236094	5	non-specific	*Burkholderiales*	*Methylibium* sp.
52dd603c8be246d5d 270bbb24cf6cd8d	non-infected	1,162.78522	0.001262626	6	non-infected-specific	*Actinomycetales*	*Cellulomonas* sp.
6128b612bdbabdf54 fe10ffceba69899	non-infected	527.95522	0.001219512	7	non-specific	*Burkholderiales*	unassigned
6b92e1fa0f1901e6f7 db767cbe5bca49	non-infected	1,271.710866	0.00130719	5	non-specific	*Burkholderiales*	*Rubrivivax* sp.
7990182f24d7b1715 eb85594ee085dce	non-infected	845.877587	0.001302083	3	non-specific	*Rhizobiales*	unassigned
9ca3963a3a8135e6f0 c0e4908f5e8be0	non-infected	1,923.826937	0.001333333	6	non-specific	*Rhodocyclales*	*Dechloromonas* sp.
bfc0d52b05d8ccf82c e11ad73eeeca67	non-infected	925.434766	0.00128041	6	non-specific	*Sphingomonadales*	*Blastomonas* sp.
c5e2f144bfc58e8458 a5d61c629f5fde	non-infected	1,161.201517	0.001116071	5	non-specific	*Burkholderiales*	*Rhodoferax* sp.
e87d142b2e55876d5 03355f2eb09cd95	non-infected	841.512641	0.001308901	5	non-infected-specific	*Flavobacteriales*	*Flavobacterium succinicans*
fa9f4f0810c07ba423 4f810b967f8503	non-infected	1,098.001487	0.001156069	5	non-specific	*Campylobacterales*	*Arcobacter* sp.
35a93467ec381d7da6 449fa326cf5552	non-infected / infected	2,315.303016 / 1,093.33298	0.001371742 / 0.0010695187	7 / 8	non-specific	*Caulobacterales*	*Asticcacaulis biprosthecium*
3ffa20123528359e92 ca7428ec712fb0	non-infected / infected	1,119.116046 / 569.1204	0.001150748 / 0.0011098779	4 / 6	non-specific	*Rhizobiales*	unassigned
0ce9280406fd028b18 b1f8f72d9b85a8	infected	927.41895	0.0010928962	8	non-specific	*Methylophilales*	*Methylotenera mobilis*
0ff364032f73daa5bca 9f4eb45642c29	infected	902.14381	0.0010799136	7	infected-specific	*Rhodobacterales*	*Rhodobacter* sp.
225df02ca193f11c3e c4ab066f68c3bb	infected	907.07498	0.0011049724	8	infected-specific	*[Saprospirales]*	*Flavihumibacter* sp.
2500d8fe4acdc852b8 d5a44495806d85	infected	737.19694	0.0010976948	7	infected-specific	*Flavobacteriales*	*Flavobacterium* sp.
32b03eacbfa8a85721 01cca9186bad78	infected	1,109.27727	0.0011210762	9	non-specific	*Flavobacteriales*	*Flavobacterium* sp.
410063411f2adb883 a281281603dc2ea	infected	762.98519	0.0010989011	6	infected-specific	*SBR1031*	unassigned
4187fed434317f3fd8 3e8a5169fdd14b	infected	882.15781	0.0011261261	7	non-specific	*Caulobacterales*	*Caulobacter* sp.
56546f0b35d5df855c 45fa3ceeae	infected	699.19281	0.00110011	8	infected-specific	*Flavobacteriales*	*Flavobacterium succinicans*
56e200b71e5898bf1 0a17b731d3b73bd	infected	825.55662	0.0010752688	8	infected-specific	*Rhizobiales*	*Bradyrhizobium* sp.
6669ecf3866bf4fc1f3 eca6c4f4ae26c	infected	792.61053	0.0011111111	9	non-specific	*Burkholderiales*	*Hylemonella gracilis*
6e7604ec6f61c2fee0d 97a8d8b3d057d	infected	818.75426	0.0010373444	9	infected-specific	*Rhizobiales*	*Bradyrhizobium* sp.
80a689cad1c7313920 942ea5c0e0b343	infected	1,179.29035	0.0011074197	9	non-specific	*Burkholderiales*	*Rhodoferax* sp.
95f18da015b9dfaf19 944ae8c31227bb	infected	895.47193	0.0010504202	7	infected-specific	*[Saprospirales]*	unassigned
9c5e92b178b419493 b47d71cafdb8480	infected	753.15089	0.0011135857	7	infected-specific	*Rhodospirillales*	unassigned

b13d1e34ca6d4a5fad58f2f69b33d571	infected	771.91259	0.0011086475	7	non-specific	*Burkholderiales*	*Rubrivivax gelatinosus*
b1483adc61b768846903642428c852f9	infected	1,018.88829	0.0011337868	8	non-specific	*Burkholderiales*	*Hydrogenophaga* sp.
bb493e536998e79f77073b916108c7cf	infected	991.4302	0.0010319917	9	infected-specific	*Rhizobiales*	unassigned
c3596b8775ccb4e66a30abea48c4cfc3	infected	905.64404	0.0010881393	7	non-specific	*Xanthomonadales*	*Silanimonas mangrovi*
d00b35e4fc09e7080aec0ab00aa0eb5e	infected	687.12645	0.0010615711	9	infected-specific	*Opitutales*	*Opitutus* sp.
de0950f8c27cfd535295f9fe9fdb00e1	infected	998.92287	0.0011037528	8	non-specific	*Myxococcales*	unassigned
e3fdaa38d04fba15dc1726b8eaca28e1	infected	1,178.65991	0.0010787487	9	non-specific	*Cytophagales*	unassigned

Additional analyzes and perspectives

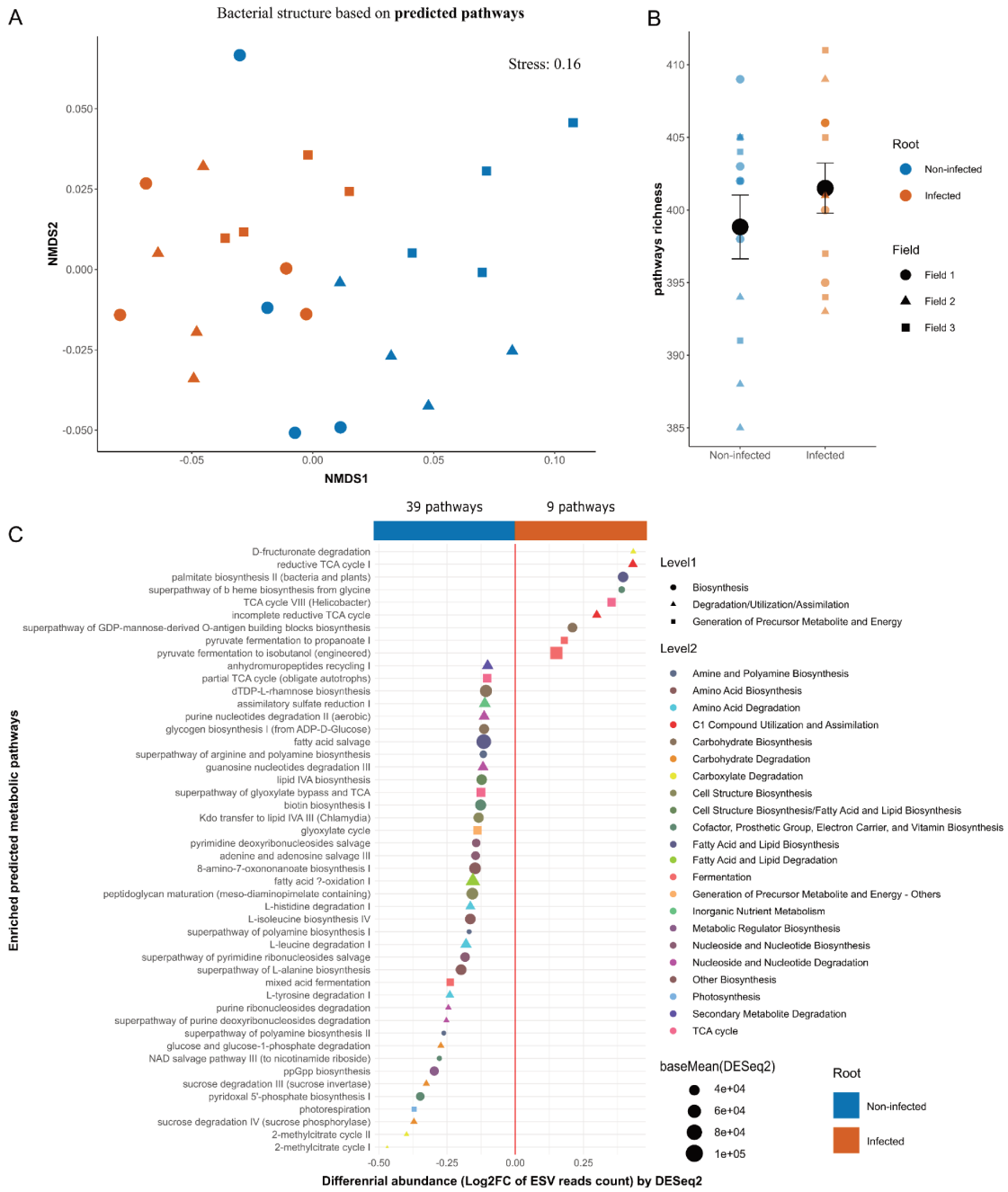
Toward a specialization of the bacteria in the gallobiome?

To know if the shift in the taxonomic composition of the infected roots was also associated with a shift in the functional capacities of the microbiome, we looked into the genetic pool of the bacteria using a computational approach. In order to do that, we performed a predictive analysis of the functions carried by the bacterial communities present in each sample type with the software *PICRUSt2* (Douglas *et al.*, 2019). *PICRUSt2* is a prediction tool for functional abundances and metagenome content based only on marker gene sequences such as the *16S rRNA* gene used in this study. The results were based on orthologous genes (KOs) from the KEGG database and on metabolic pathways from the MetaCyc database.



Sup. figure 4. Effect of the nematode infection on the predicted KOs of the bacterial communities based on predictions with *PICRUSt2*. Composition and structure of predicted KOs represented by an NMDS ordination (**A**) and number of predicted KOs (**B**) in infected roots by *Meloidogyne graminicola* (with galls) or non-infected roots (without galls) collected from different rice fields in Northern Vietnam.

The analysis of the structure based on predicted KOs showed an infection effect (**sup. figure 4 A**, $p < .05$, $R^2=0.11$) with a clustering according to the sample type, suggesting a shift in the overall predicted functional capabilities of the communities based on the infection status. Besides, although there were more numerous and diverse bacteria in infected roots, the KOs richness was interestingly similar in both sample types (**sup. figure 4 B**), suggesting more redundancy and/or convergence of the functions required to survive in infected roots. The analysis based on predicted metabolic pathways followed the same tendencies, *i.e.* a distinct structure with no significant difference in richness (**sup. figure 5 A** and **B**).



Sup. figure 5. Effect of the nematode infection on the predicted metabolic pathways of the bacterial communities based on predictions with *PICRUSt2*. Composition and structure of predicted pathways represented by an NMDS ordination (**A**), number of predicted pathways (**B**) and most enriched and abundant MetaCyc pathways (**C**) in infected roots by *Meloidogyne graminicola* (with galls) or non-infected roots (without galls) collected from different rice fields in Northern Vietnam.

A further analysis of the predicted metabolic profiles highlighted a putative specialization of the overall bacterial metabolism toward anaerobic or microaerophilic pathways that might indeed occur in the gall environment. Initially, 420 pathways were predicted, and after a differential analysis with *DESeq2*, a total of 158 pathways were found to be enriched, with 77 in non-infected roots and 81 in infected roots. Some pathways were highly enriched but had a very low number of ESVs involved. To have a general

overview on the functional capabilities of the microbiomes, we focus on the most enriched (based on a log2FC(reads count) > |0.1|) and abundant (based on a base mean of reads count > 20,000) pathways in **sup. figure 5 C**. We finally obtained 48 pathways including 39 enriched in non-infected roots and nine in infected roots.

Most of the enriched pathways in infected roots (D-fructuronate degradation, reductive TCA cycle I, TCA cycle VIII, incomplete reductive TCA cycle, pyruvate fermentation to propanoate I and pyruvate fermentation to isobutanol) were easily linkable as described below. It has been first described in the strict anaerobic bacterium *Thermotoga maritima* that the degradation of pectin, a plant cell wall component, can result in the formation of D-galacturonate and D-fructuronate (Blamey and Adams, 1994). This degradation could occur in the gall by synergistic action of bacteria and nematodes during the infestation process (Goto *et al.*, 2013) after alteration of the root cell wall. Indeed, *M. graminicola* move by apoplastic pathway and therefore necessitate an arsenal of enzymes able to degrade pectin that might have been acquired by bacterial horizontal gene transfer (Phan *et al.*, 2020), and the idea that a part of the microbiome can assist the nematode parasitism has been developed by Topalović and Vestergård (2021). After pectin degradation, GDP, a product of D-fructuronate (or D-galacturonate that can be converted to D-fructuronate after several reactions) degradation, could enter the glycolysis to produce more pyruvate in aerobic conditions. Pyruvate could then be decarboxylated in acetyl-CoA following the tricarboxylic acid (TCA) cycle, aka Krebs cycle. When substrates are limited, the cycle can become "incomplete" or "reductive". For example, in response to anaerobic or microaerophilic growth conditions, pyruvate can still be converted to essential intermediate molecules *via* fermentation or incomplete reductive TCA cycle (Wood *et al.*, 2004). These linkable predicted metabolic pathways, if indeed occurring in the gallobiome, suggested a specialized activity in response to a particular environment in the gall with apparently a low oxygen availability for the bacteria.

Hence, a whole biochemical superpathway from a plant cell wall compound (pectin) toward energy production for bacteria through fermentation seemed plausible, potentially involving competition and synthrophy within the community. As the development of RKNs depends on oxygen availability (van Gundy and Stolzy, 1961), the gall would consequently not be a strictly anaerobic environment but likely with transient hypoxic conditions depending on the nematode infection stage. Our results suggested that bacteria adapted to survive in the gall environment and within the infected community were predicted to carry more genes involved in alternative pathways in order to survive in low availability of oxygen or other nutrients in the gall. In addition to this, by using induced-giant cells as nutrient source, the RKN can induce extensive changes in vascularization to transport nutrients toward the giant and surrounding cells at the feeding site (Bartlem *et al.*, 2014) and this could change the nutrient availability and allow opportunistic bacteria to feed directly from the plant (cell degradation, rhizodeposition or root exudation) or from other bacteria at the infection site. Altogether, these results suggested a shift in the functional capabilities of the microbiome in the infected roots by *M. graminicola* in the rice fields in Hải Dương that could serve to describe a core gallobiome that would rely on functions rather than on taxonomy (Lemanceau *et al.*, 2017). Finally, this analysis with *PICRUSt2* is potentially conclusive regarding the environmental context of the gall but remains speculative because, to date, our knowledge of environmental genomes is fragmentary, limits prediction and requires fundamental validations (Sun *et al.*, 2020).

Potential indicator taxa of the rice infection by *M. graminicola*

Agler *et al.* (2016) clarified important terms related to network analyzes whose topology originated from Estrada (2007). First, a node is a taxa representing operational taxonomic units (OTUs) grouped at a specific level (*e.g.* genus level or ESV level as in our previous network analysis of the gallobiome). An edge is a line connecting nodes and represents correlations between the nodes (*e.g.* positive or negative co-occurrence in our network). Connectivity is an important concept to describe how central a node is in the network, *i.e.* how well connected it is to the rest of the network. It is measured by node parameters degree (*i.e.* the number of direct correlations to a node), betweenness centrality (*i.e.* the fraction of cases in which a node lies on the shortest path between all pairs of other nodes), and closeness centrality (*i.e.* the reciprocal of the sum of distances to all other nodes). Then, different kinds of nodes can be characterized: a "hub" node is significantly more connected within the network than other nodes according to all three node parameters, and an "edge" node is poorly connected within the network and likely has little influence on microbial community structure. Finally, a "keystone" node is a hub node that fundamentally underlies the observed network structure. Ecologically important species are responsible for the microbial community structure and are therefore keystone species. Without them, the dynamics of the community changes and the observed network would look significantly different. However, hubs are not necessarily keystones. Indeed, some hubs are "only" important in their "neighborhood" within the network and the overall taxa would not depend on them, so they would not be keystones.

In our study, we characterized hubs as the 5% most connected taxa in terms of higher betweenness centrality, closeness centrality and node degree following the study by Agler *et al.* (2016). This resulted in a total of 41 hubs with 56% in the infected network including 48% specific, whereas 22% were specific in the non-infected network. In another study, Karimi *et al.* (2019) characterized hubs as the 20 most connected taxa in terms of node degree only. This method now results in 60% in the infected network including 25% specific, whereas 38% are specific in the non-infected network, and showed an inverted pattern of hub specificity. In addition to the method used, the cutoff could also affect the outcome (Agler *et al.*, 2016) and should be checked. Above all, an adequate computational method can confirm the characterization of taxa as a keystone (Berry and Widder, 2014) and a simple and highly standardized qPCR-based approach can also be used for keystone analysis (Berg *et al.*, 2020).

Keystones, either pathogenic or beneficial, can manipulate host immunity to establish a successful relationship with the host and disturb microbiota composition (Brader *et al.*, 2017). They can thus be structuring factors of the microbiota (Banerjee *et al.*, 2018). Keystone species can also (but not necessarily) be characterized as "indicator" taxa. They are highly indicative of an ecological context such as a habitat type, a specific community, an environmental change, etc. In our study of the gallobiome, specific hubs could be indicator taxa of the plant health status, that is infected or non-infected roots by *M. graminicola*. A list is provided in **sup. table 5**. A better method using a correlation index for example could have been used (Cáceres *et al.*, 2010) however, we identified specific hub in both networks, such as ESVs assigned to *Rheinheimera* sp. and *Cellulomonas* sp. in the non-infected network, and to *Bradyrhizobium* sp., *Rhodobacter* sp. and *Flavihumibacter* sp. in the infected network that could serve as indicator taxa in regard to the infection by *M. graminicola*.

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Chapter 3

The soil food web and microbiodiversity under conservation agriculture in a suppressive field

Preamble

After having characterized the bacterial community associated with the infection by the root-knot nematode *M. graminicola* in rice roots (*cf.* **Chapter 2**), we underwent a broader characterization of the microbial community including bacteria and fungi, as well as the nematode community, associated with a combination of agricultural practices and rice varieties in the rhizosphere. The aim was to assess the potential of conservation agriculture to reduce the impact of plant-parasitic nematodes and, *in fine*, to identify taxa potentially involved in soil disease suppressiveness of plant-parasitic nematodes. Data was collected in Cambodia in 2018. Previously, an experimental field in the Eastern region of the Tonlé Sap lake (**figure 22**) was set up in 2011 by collaborators from CIRAD and DALARM originally to monitor the carbon sequestration, the soil health and the plant yield under conservation agriculture. Since 2014, the parasitic pressure was monitored because the soil is conducive to plant-parasitic nematodes in this intensive agricultural region and concerns emerged with the disease. *Meloidogyne graminicola* and *Hirschmanniella mucronata* were indeed highly abundant in rice roots and in the soil, three and four years after the transition to conservation agriculture (Suong *et al.*, 2018). At that time, they were more abundant within conservation agriculture compared to the plot with conventional tillage. Yet, the yield was maintained, suggesting an establishment of soil disease suppressiveness. Seven years after the transition, in 2018, we found that the parasitic pressure was this time lower in roots under conservation agriculture. We hypothesized that it was linked to the biological activity and modifications of the soil food web. Since soil nematodes were known as excellent indicators of the soil food web (Bongers and Ferris, 1999) and soil-borne microbes were known to have potential biocontrol activity against plant-parasitic nematodes (Silva *et al.*, 2018), we explored the rhizosphere communities associated with different rice varieties within conservation agriculture in contrast to a type of conventional tillage without cover crops. We showed that the reassembly of the bacterial, fungal and nematode communities observed seven years after the transition to conservation agriculture compared to conventional tillage were associated with a reduction of the parasitic pressure. This study was the subject of my second article (**figure 23**) entitled: "Maturation of the soil food web under conservation agriculture is associated with suppression of rice-parasitic nematodes" (Masson *et al.*, submitted). I gathered in this chapter the submitted article and additional analyzes. This work gives rise to new hypotheses regarding the implementation and the mechanisms of soil disease suppressiveness discussed in the last section of this chapter.

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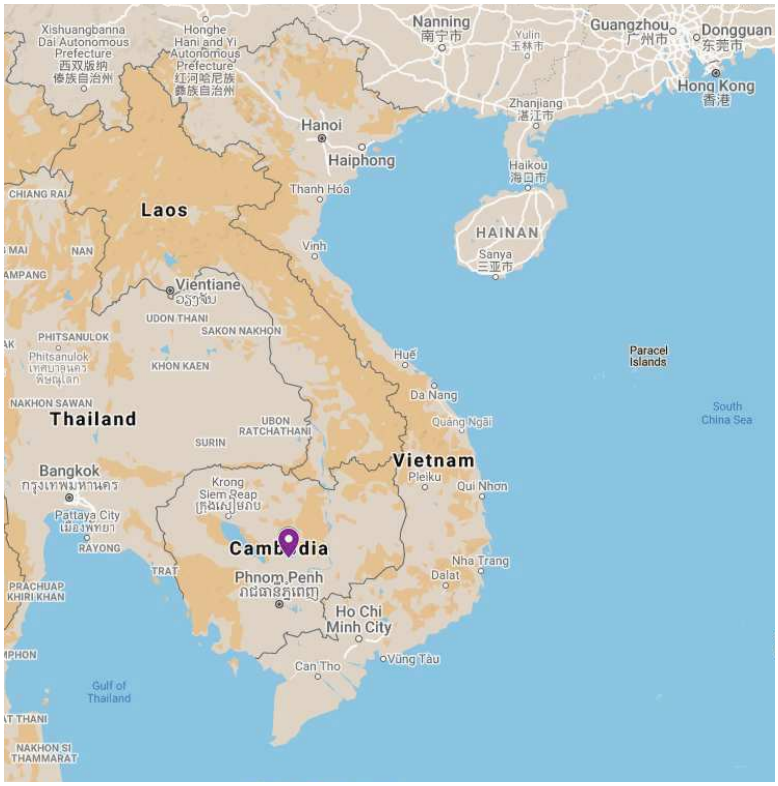
Préambule

Après avoir caractérisé la communauté bactérienne associée à l'infection par le nématode à galles *M. graminicola* dans les racines du riz (*cf.* **Chapitre 2**), nous avons procédé à une caractérisation plus large de la communauté microbienne comprenant les bactéries et les champignons, ainsi que la communauté des nématodes, associée à une combinaison de pratiques agricoles et de variétés de riz dans la rhizosphère. L'objectif était d'évaluer le potentiel de l'agriculture de conservation pour diminuer l'impact des nématodes phytoparasites et, *in fine*, d'identifier des taxons potentiellement impliqués dans la suppression des maladies causées par les nématodes phytoparasites. Les données ont été collectées au Cambodge en 2018. Auparavant, un champ expérimental dans la région à l'est du lac Tonlé Sap (**figure 22**) avait été mis en place en 2011 par des collaborateurs du CIRAD et du DALARM à l'origine pour suivre la séquestration du carbone, la santé du sol et le rendement des plantes sous agriculture de conservation. Depuis 2014, la pression parasitaire est suivie car le sol est propice aux nématodes phytoparasites dans cette région d'agriculture intensive et des inquiétudes ont émergé avec la maladie. *Meloidogyne graminicola* et *Hirschmanniella mucronata* étaient en effet très abondants dans les racines de riz et dans le sol, trois et quatre ans après la transition vers l'agriculture de conservation (Suong *et al.*, 2018). À ce moment-là, ils étaient plus abondants sous agriculture de conservation par rapport à la parcelle sous un labour conventionnel. Pourtant, le rendement était maintenu, ce qui suggérait l'établissement d'un sol suppresseur de maladies. Sept ans après la transition, en 2018, nous avons constaté que la pression parasitaire était, cette fois, plus faible dans les racines sous agriculture de conservation, et nous avons émis l'hypothèse que cela était lié à l'activité biologique et aux modifications du réseau trophique du sol. Puisque les nématodes du sol sont connus comme d'excellents indicateurs du réseau trophique du sol (Bongers et Ferris, 1999) et que les microbes du sol sont connus pour avoir une potentielle activité de biocontrôle contre les nématodes phytoparasites (Silva *et al.*, 2018), nous avons exploré les communautés rhizosphériques associées à différents variétés de riz sous deux types de pratiques agricoles contrastées. Nous avons montré que le réassemblage des communautés bactériennes, fongiques et de nématodes observé sept ans après la transition vers l'agriculture de conservation étaient associées à une réduction de la pression parasitaire en comparaison à l'agriculture conventionnelle. Cette étude est l'objet de mon deuxième article (**figure 23** - résumé graphique de l'article soumis associé à ce chapitre) intitulé : "La maturation du réseau trophique du sol dans le cadre de l'agriculture de conservation est associée à la suppression des nématodes parasites du riz" (Masson *et al.*, soumis). J'ai rassemblé dans ce chapitre l'article soumis et des analyses supplémentaires. Ce travail donne lieu à de nouvelles hypothèses concernant la mise en place et les mécanismes des sols suppresseurs de maladies qui sont discutés dans la dernière section de ce chapitre.



12°32'55.6" N - 105°08'48.6" E



Figure 22.

Localization of the investigated field in this chapter and picture of the field managed within conventional tillage (on the left) or conservation agriculture (on the right).

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Localisation du champ examiné dans ce chapitre et photo du champ géré sous agriculture conventionnelle (à gauche) ou agriculture de conservation (à droite).

Abstract

Meloidogyne spp. and *Hirschmanniella* spp. are among the most damaging plant-parasitic nematodes (PPNs). They threaten the production of rice, the main staple food in Asia. Cropping systems that promote biocontrol and plant tolerance to diseases are put forward as sustainable solutions to protect rice from these pests. In particular, cropping systems managed under conservation agriculture (CA) are promising because they improve soil health and functioning. We investigated the effect of two cropping system components, (i) conservation agriculture practices, *i.e.* no-tillage with a cover crop *Stylosanthes guianensis* (variety Nina), or belonging to conventional plow-based tillage with no cover crop, and (ii) the rice variety using IR504, IR64, Azucena and Zhonghua 11, on PPNs in roots and on communities (bacteria, fungi and nematodes) in the rhizosphere, in a field in Stung Chinit, Cambodia. We used a molecular technique by amplicon barcoding to target microbial marker genes (*16S* and *ITS rRNA* gene) and a microscopic technique to identify and quantify nematodes in the rhizosphere compartment. Globally, the variety had fewer effects than the agricultural practices on the plant infection by nematodes and on the assembly of the three rhizosphere communities. Under CA, the abundance of PPNs extracted from the roots was reduced by 88%. Soil quality was substantially improved (+83% of TKN, +34% of available P, +10% of exchangeable K, +110% of SOC, +30% for the CEC), thus providing more basal resources for microbial decomposers, especially fungi (+164% putative saprotrophs). Characterization of the three rhizosphere communities (bacteria, fungi and nematodes) revealed a shift in the structures associated with the soil enrichment. Both microbial richness (+3% for bacteria and +38% for fungi) and diversity (Shannon index, +11% for fungi and +5% for nematodes) increased. The relative abundances of taxa and was modified by CA with notably more mycorrhizal fungi (+329% *Glomeromycota* spp.) and fewer *Pratylenchidae* nematodes (-92% *Hirschmanniella* spp.) in the rhizosphere. The reassembly of the communities using CA was associated with a regulation of the PPN populations. The reduction in *Meloidogyne* spp. abundance in roots (-64%) was correlated with the maturation of the food web (maturity index, +10% under CA) and with the increase in the relative abundance of omnivorous nematodes in the rhizosphere (+68% under CA). Seven years of CA in this field enabled the whole soil food web to mature, thus creating a favorable niche for potentially predatory nematodes and microbes antagonistic against PPNs. This study confirms that CA is an alternative to nematicides to limit infection by PPNs in rice cropping systems.

Keywords: rice-based cropping systems; soil microbiota; nematode community; pest management practices; soil suppressiveness; trophic groups

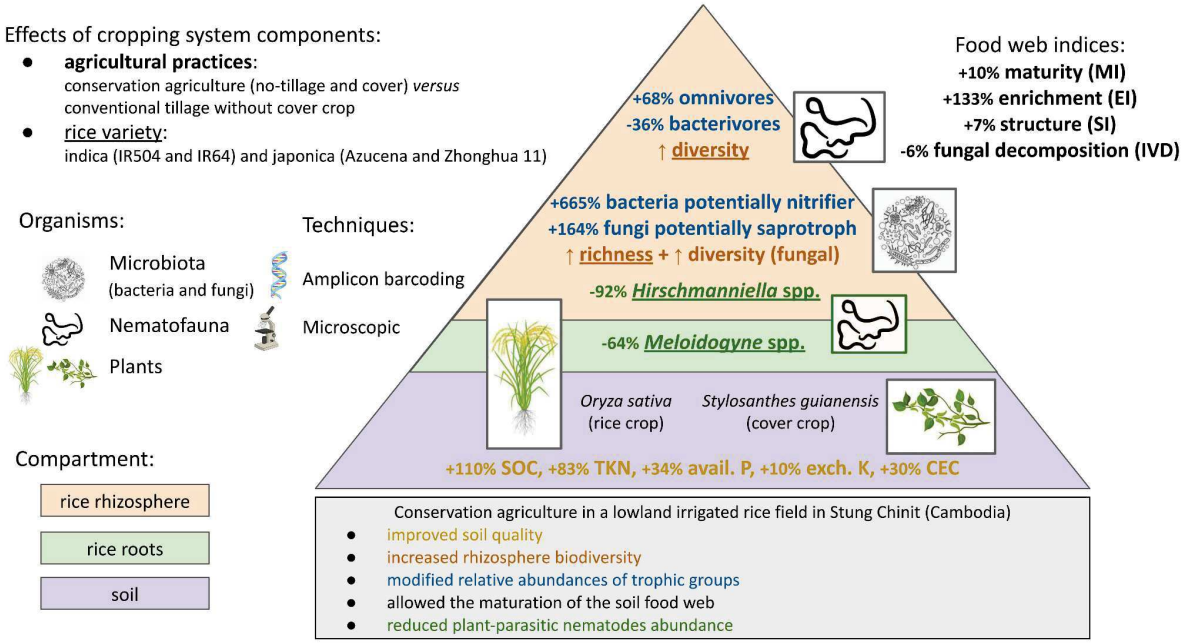

Figure 23. Graphical abstract of the submitted article associated with this chapter. The highlights were the following:

- Conservation agriculture reduced the abundance of rice-parasitic nematodes in roots.
- The communities of bacteria, fungi and nematodes in the rhizosphere were explored.
- No-tillage and cover crops enriched the soil in basal resources.
- Microbial diversity increased with potentially more decomposers such as saprotrophs.
- The food web was more mature and harbored more persistent and predatory nematodes.

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Les points saillants étaient les suivants :

- L'agriculture de conservation a réduit l'abondance des nématodes parasites du riz dans les racines.
- Les communautés de bactéries, champignons et nématodes dans la rhizosphère ont été explorées.
- Les communautés ont été plus impactées par les pratiques agricoles que par la variété de riz.
- Le non-travail du sol et les plantes de couverture ont enrichi le sol en ressources basales.
- La diversité microbienne a augmenté avec potentiellement plus de décomposeurs tels que les saprotrophes.
- Le réseau trophique était plus mature et abritait davantage de nématodes persistants et prédateurs.

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Introduction

Rice is the world's main staple crop and is mainly produced in South-East Asia. In Cambodia, it accounts for more than 80% of cultivated land and is the largest export commodity (Yu and Fan, 2011). From 2017 to 2019, Cambodia was one of the world's top 10 rice-exporting countries, with an annual income of 360 million $US (FAOSTAT, 2021). Plant-parasitic nematodes (PPNs) are a serious threat to rice production and can reduce yields by between 16% to 80% (Netscher and Erland, 1993; Soriano *et al.*, 2000), *i.e.* cause an estimated loss of 80 billion $US per year (Nicol *et al.*, 2011, Jones *et al.*, 2013). *Meloidogyne* (Göeldi, 1892) and *Hirschmanniella* (Sher, 1968) are the two main genera of PPNs that affect rice production in South-East Asia (de Waele and Elsen, 2007). *Meloidogyne*, also known as root-knot nematodes, are sedentary endoparasitic nematodes and cause the formation of galls on the roots, whereas *Hirschmanniella* are migratory endoparasitic nematodes. These parasites damage the root architecture, disrupt water and nutrient transport through the roots and increase crop susceptibility to other diseases (Kyndt *et al.*, 2017).

Methods to reduce PPNs infection are available, but all have known limitations. For example, although next-generation nematicides are now available on the market, they still have an environmental cost and are toxic to non-target organisms (Ebone *et al.*, 2019; Oka, 2020). Another method of control is using rice genotypes that are resistant to PPNs. Some resistance genes have been identified, but they are rare and occur mainly in sparsely cultivated rice species (*e.g. O. glaberrima*), making it difficult to transfer useful traits to widely grown rice varieties. A few rare resistant *O. sativa* varieties have been identified, but their introgression may have yield penalties or confer undesirable agronomic traits (Fuller *et al.*, 2008; Mantelin *et al.*, 2017). In addition, an increasing number of resistance-breaking nematode pathotypes is being reported, thus requiring continuous effort by rice breeders to select varieties that are resistant to new nematode

pathotypes (Davies and Elling, 2015; Phan *et al.*, 2018). Finally, traditional cultivation systems mainly based on water management (continuous flooding and crop rotation) had been used for centuries to control rice PPNs and reduce yield losses, but tillage followed by seed broadcasting on non-flooded rice fields has become the most common cultivation system in recent decades, notably due to the Green Revolution (Pingali, 2012) and the increasing scarcity of water and labor force (Thrall *et al.*, 2010).

Agricultural approaches emerged a few decades ago, based on the substitution of external inputs by an improved management of ecological processes (Altieri, 1989). In these systems, farmers seek to optimize biotic and abiotic interactions within agroecosystems, to limit the prevalence of pests and diseases. These "ecologized agricultures" (sensu Ollivier and Bellon, 2013) emphasize the importance of soil biodiversity and rely on agroecosystem self-regulation. Soil organisms indeed provide a wide range of ecosystem services, including pests and diseases regulation (Kibblewhite *et al.*, 2008). Nematodes (also called the nematofauna) are excellent indicators of soil functions (Bongers and Ferris, 1999; Yeates, 2003; Villenave *et al.*, 2009). The abundance and diversity of nematodes provide insight into the soil biological functioning as they occupy different levels of the soil food web (Ekschmitt *et al.*, 2001). While some nematodes are parasitic (*i.e.* PPNs and entomopathogenic nematodes), others regulate bacterial and fungal populations (bacterivorous and fungivorous nematodes) or feed on other organisms including nematodes (predatory nematodes). Studying the structure and assembly of these communities provides insights into the effects of biological activities on plant health.

Plants and their associated microbes grouped under the term "microbiota" (Berg *et al.*, 2020) form an assemblage of co-evolved species that is often referred to as a "holobiont" (Zilber-Rosenberg and Rosenberg, 2008). The assembly of the rice-associated microbiota has been shown to be driven by a variety of factors (Edwards *et al.*, 2015) including the host genotype (Hardoim *et al.*, 2011; Tabrett and Horton, 2020) and cultivation practices. Many studies have shown that plants can modulate their associated above- or below-ground microbiota to dynamically adjust to their environments (Vandenkoornhuyse *et al.*, 2015) *via* signaling (Venturi *et al.*, 2016) and root exudation (Vives-Peris *et al.*, 2020). Plants can recruit beneficial microbes to defend soil-borne pathogens (Liu *et al.*, 2020; Berendsen *et al.*, 2012). Phytobeneficial microbes can prevent plant diseases either by promoting plant growth and development (Bhattacharyya and Jha, 2012; Vejan *et al.*, 2016) or through antagonistic effects on pathogens (Mhatre *et al.*, 2018; Stirling, 2015). Suppressive soils are a natural source of microbiota with a high potential to suppress PPNs, including root-knot nematodes (Topalović *et al.*, 2020a) and cyst nematodes (Hussain *et al.*, 2018) using different mechanisms (Silva *et al.*, 2018; Gamalero *et al.*, 2020). However, soil suppressiveness is complex because it involves both biotic (Mazzola *et al.*, 2002; Schlatter *et al.*, 2017) and abiotic factors (Agler *et al.*, 2016; Islam *et al.*, 2020). In rice cropping systems, there is an insufficient understanding of the effects of different agricultural practices and variety on the assembly of rhizosphere communities, in particular bacteria, fungi and nematodes.

Conservation agriculture (CA) can be considered as an "ecologized" cropping system that improves soil health and functioning (FAO, 2021). It relies on minimum soil disturbance (reduced or no-tillage), permanent soil cover (living cover crops or dead organic cover) and crop rotation (as long and diversified as

possible). These practices have significant impacts on soil communities. A previous study showed that the use of no-tillage and cover crops has improved soil physicochemical properties (SOC and nutrient availability) and increased microbial biomass (bacteria and fungi) during a three-year rotation of rice, corn and soybean in Laos (Lienhard *et al.*, 2012). Microbial functional diversity was also increased under CA (Tang *et al.*, 2020), suggesting that CA can improve crop tolerance to pathogens (van Elsas *et al.*, 2002; Doni *et al.*, 2019; Wang *et al.*, 2020). For instance, a study showed that the use of no-tillage and crop rotation helped control the rice cyst nematode *Heterodera elachista* (Ito *et al.*, 2015a). However, the potential of CA in PPN control in rice under irrigated conditions and its effects on the microbiota and the nematofauna at the plant-soil interface have not yet been fully understood.

To assess the potential of CA to improve plant health, an experiment was set up in 2011 in a lowland and sandy rice field in Stung Chinit, Kampong Thom province, Cambodia. The field was managed under either conventional plow-based tillage (hereafter CT), or a type of CA with direct sowing of rice on cover crops crushed with a roller to form a layer of mulch before sowing, and with no tillage. In 2018, seven years after the transition to CA, we observed a reduction in the abundance of PPNs in roots under CA compared to CT, and investigated which soil parameters were linked with this reduction. In this study, we hypothesized that the reduction in the abundance of PPNs was associated with modifications in the soil food web caused by the cropping system. Thus, we characterized the communities of bacteria, fungi and nematodes in the rice rhizosphere in response to two components of the cropping system: agricultural practices and the rice variety. More specifically, parasitism, soil properties and community assembly of these three rhizosphere communities were investigated in four varieties (two *O. sativa* subsp. *indica* named IR504 and IR64, and two *O. sativa* subsp. *japonica* named Azucena and Zhonghua 11, the latter being resistant to *Meloidogyne graminicola*) grown using CA or CT. We analyzed the α- (richness and Shannon index) and β- (structure and dispersion) diversity, the relative abundances of taxa and guilds and their specific enrichments in each community. Finally, we discussed correlations observed between the reduction in PPN abundance and soil parameters, biodiversity or soil food web indices.

Material & methods

Field characterization, historical management and experimental design

The field experiment was established in April 2011 on a 2.6 ha tropical lowland rice parcel in Stung Chinit, Santuk district, Kampong Thom province, close to the Tonlé Sap lake in Cambodia (12°32′55″ N - 105°08′47″ E). Most rainfall in this region occurs in the early wet season (April to July) and the main wet season (July to October). The soil is a sandy loam (~ 69% sand, 18% silt and 13% clay) belonging to the "Prey Khmer group" in the Cambodian agronomic soil classification system (White *et al.*, 1997), equivalent to red-yellow podzols according to the FAO soil taxonomy (Suong *et al.*, 2019). A field plot experiment comparing a type of conventional tillage (CT) and a type of conservation agriculture (CA) no-till mulch-based cropping system using four different *Oryza sativa* varieties has been implemented. The experiment thus comprised eight treatments in a randomized complete block design with four replications, and each of the eight blocks had a total area of 55 m^2 (13.75 x 4 m). Four blocks were managed under CA and the other four under CT.

Before the experiment, in 2017, two rice cycles of *O. sativa indica* were cultivated: IR504 sown as an early wet season rice in March and Phka Rumduol sown in July. After harvesting in 2017, under CT, the soil remained bare until it was plowed and rice was sown for the 2018 season. Under CA, before the harvest of the second rice cycle (Phka Rumduol) in mid-November 2017, seeds of *Stylosanthes guianensis* (variety Nina), a legume cover crop, were broadcast (8 kg/ha). On March 15, 2018, two weeks before rice was sown, the cover crop was terminated by rolling twice with a roller-crimper followed by the application of a mix of 3 l/ha of glyphosate (N-(phosphonomethyl)glycine) and 1 l/ha of 2,4-D (2,4-dichlorophenoxyacetic acid) immediately after rolling.

On March 28, 2018, four rice varieties were sown per block: two varieties of *O. sativa* indica (IR504 and IR64) and two varieties of *O. sativa japonica* (Azucena and Zhonghua 11). These varieties are not photosensitive and have a relatively short cycle (less than four months). We chose a diversity of varieties based on their use in Cambodia and their different responses to the PPN infection. The IR64 variety was developed by IRRI in 1985 with a combination of many valuable traits including high yield, quality and disease resistance (Mackill and Khush, 2018), although it is sensitive to PPNs such as *M. graminicola* (Phan *et al.*, 2018). Azucena is the most sensitive to PPNs (data not shown); Zhonghua 11 was the only resistant variety in our set (Phan *et al.*, 2018). Prior to sowing, a base dressing with 200 kg/ha of thermophosphate (16% P_2O_5, 28% CaO, 18% MgO) was applied. The varieties were sown by hand manually by inserting four to five seeds in three-centimeters at ten-centimeter intervals in a straight row. Three four-meter long rows of each variety spaced 30 cm apart were planted in each block. In all rows, 120 holes were filled with a total of 3,840 to 4,800 seeds. Following sowing, the whole field (CA and CT plots) was treated with 1 l/ha of 2,4-D and 0.15 l/ha of organic vegetable oil to control weed development, and a top dressing was applied with 100 kg/ha of DAP (diammonium phosphate, 16 N - 20 P_2O_5 - 0 K_2O/ha) and 50 kg/ha of KCl (potassium chloride, 30 kg K_2O/ha). Thirty days later, 75 kg/ha of urea (34.5 kg N/ha) was also applied on the whole field.

Plant and soil sampling

Sampling was done one month after sowing (May 1, 2018) when the lowland field was not under water. Sampling was done in the block corresponding to each variety and each type of agricultural practices, giving a total of 32 samples. To characterize the nematofauna in the rhizosphere and the abundance of PPNs in the roots, ten plants per condition were carefully collected for each analysis. To characterize the microbial communities (bacteria and fungi), five plants per condition were collected. The soil surrounding the roots was also collected and pooled to create a composite sample per condition for the soil analysis. All the samples for analysis of the rhizosphere compartment were taken in the middle rows in order to avoid the edge effect. Samples were immediately placed in plastic bags, transported to the laboratory, then stored at 4°C until analysis.

Soil analysis

Soil properties were analyzed with the methods described in detail in Motsara and Roy (2008). Briefly, soil samples were air-dried at room temperature and pH was determined using a 1:2:5 ratio of soil:distilled water:KCl 1 M mixture and measured with a pH meter D-51 (Horiba Ltd., Kyoto, Japan). Available phosphorus (avail. P) was determined with the Bray II method, exchangeable potassium (exch. K) with a flame photometer, soil organic carbon (SOC) using the Walkley and Black method, total Kjeldahl nitrogen (TKN) using the method of Kjeldahl and cation exchange capacity (CEC) by the ammonium acetate method.

PPN abundance in roots

Plant-parasitic nematodes (PPNs) were extracted from fresh root samples following the method of Bellafiore *et al.* (2015). Briefly, samples were put in a 0.6% hypochlorite solution for three minutes and crushed in a blender to extract nematode eggs and juveniles. The mixture was then filtered through successive sieves of 250, 75 and 25 µm to collect them. Juveniles belonging to the genera *Meloidogyne* and *Hirschmanniella* were counted, in addition to all the PPN eggs, and reported as abundance of PPNs/g of root.

Nematofauna processing

The nematofauna in the soil surrounding the roots (the rhizosphere) of the fresh plant samples following was analyzed by ELISOL Environnement (Congénies, France) using the standardized ISO 23611-4 procedure (ISO, 2007). The nematodes in each sample were extracted from 150 g of soil composite fresh soil samples using a modified elutriation system (Seinhorst, 1962; Villenave *et al.*, 2009). After fixing in a formalin glycerol mixture and transferring to slides, the composition of soil nematofauna was determined at family level (and genus level if possible) through microscopic observation at 400x magnification. A total of 44,019 nematodes were counted (min = 202, median = 1,369, max = 2,789 per sample). Nematode

density was recorded as the total number of individuals/100g of dry soil. Food web indices as defined by Ferris and Bongers (2006 and 2009) in the rhizosphere were also calculated: EI; enrichment index (a measure of resource availability, especially nitrogen, and activity of primary decomposers), SI; structural index (a measure of the degree of trophic links, stability and capacity to recover from stress calculated with the slow-growing and reproducing predatory and omnivorous nematodes with c-p values of 3, 4 and 5), IVD; index of organic matter decomposition pathway (a measure of primary organic matter decomposition, also known as nematode channel ratio of the fungal-feeders over the bacterial-feeders) and MI; maturity index (a measure of environmental disturbance and stability based on free-living nematodes).

Microbiome processing

Bacterial and fungal communities in the rhizosphere of the fresh plant samples were analyzed using molecular techniques. DNA was extracted from a 0.25-g composite sample of the rhizosphere using the PowerSoil® DNA Isolation Kit (Qiagen, Netherland) following the manufacturer's instructions. Samples were pooled and each contributed exactly the same amount (50 ng/µl) of DNA in the final library. PCR amplification, library and MiSeq Illumina sequencing were performed by Macrogen (Seoul, South Korea) using bacterial primers 341F (16S_V3F, 5'-CCTACGGGNGGCWGCAG-3') and 805R (16S_V4R, 5'-GACTACHVGGGTATCTAATCC-3') to amplify the V3-V4 region of the *16S rRNA* gene (Sinclair *et al.*, 2015), and using fungal primers ITS3F (5'-GCATCGATGAAGAACGCAGC-3) and ITS4R (5'-TCCTCCGCTTATTGATATGC-3) to amplify the *rRNA-ITSII* region (White *et al.*, 1990; Mitchell and Zuccaro, 2006). The sequencing data for this study are accessible in the ENA database under the accession number PRJEB47939.

The data was analyzed using the *QIIME 2* (v2020.2) pipeline (Bolyen *et al.*, 2019) on the IRD *i-Trop* cluster. The function *DADA2 denoise-paired* (Callahan *et al.*, 2016) with default parameters was used to correct sequencing errors, to infer exact amplicon sequence variants (ESVs) and to remove chimeric sequences. For bacteria, forward and reverse reads were trimmed at 17 and 21 bp, respectively, to remove primers and adapters, quality-truncated at 274 and 210 bp respectively, and merged with a minimum overlap of 20 bp. For fungi, only forward reads have been processed according to the method of Pauvert *et al.* (2019) and 20 bp were trimmed to remove primers. Taxonomic affiliations were assigned by a naive Bayes classifier which was trained for the V3-V4 region using the database SILVA 138 for bacteria and the database UNITE 04.02.2020 (all eukaryotes) for fungi.

Approximately 33% and 74% of input reads passed the denoising and chimera filters for the 16S and the ITS marker, respectively. We subsequently filtered out plasts (chloroplasts and mitochondria) and other unwanted ESVs (unassigned at domain level or assigned to *Eukaryota*) to keep only ESVs assigned to the *Bacteria* or *Archaea* kingdoms for the 16S marker. These removed reads accounted for 0.5% of the total preprocessed reads. Only 42 ESVs were assigned to *Archaea* and were consequently filtered out in the *phyloseq* object before the analysis. For the ITS marker, we filtered out the unassigned ESVs at domain level. These removed reads accounted for 25.5% of the total preprocessed reads. Then we kept only ESVs assigned to the *Fungi* kingdom. These removed reads accounted for 36.3% of the total preprocessed reads. Finally, we

ended up with 99.5% and 38.2% of the total preprocessed reads for respectively the 16S marker and the ITS marker. We used a microscopy-based technique to identify and quantify nematodes because of the difficulty involved obtaining DNA from a community of nematodes, the lack of appropriate primers and public databases (Geisen *et al.*, 2018; Schenk *et al.*, 2020). According to the rarefaction curves (**sup. figure 6**), the samples reached a plateau, meaning the sequencing depth was sufficient so there was no need to rarefy the datasets (McMurdie and Holmes, 2014). Only one sample of nematofauna (CA, Zhonghua 11, repetition 3) did not reach the plateau and was consequently discarded from the analysis. The scripts for the hereinabove *QIIME 2* pipeline and the following *R* analyzes written for this study are available on GitLab under the project ID 27138799 (soilfoodwebwithinCA_stungchinit_2018).

Analyzes were performed using *R* software, version 4.0.3 (*R* Development Core Team, 2020). The packages *dplyr* (Wickham *et al.*, 2021a), *magrittr* (Milton Bache *et al.*, 2020), *tidyverse* (Wickham *et al.*, 2019), *tidymodels* (Kuhn and Wickham, 2021) and *stringr* (Wickham, 2019) were used to handle data. The packages *phyloseq* (McMurdie and Holmes, 2013), *microbiome* (Lahti and Sudarshan, 2021), *vegan* (Oksanen *et al.*, 2020) and *eulerr* (Larsson, 2020) for the Venn diagrams were used to analyze the community metrics. Non-metric multidimensional scaling representations (NMDSs) based on Bray-Curtis distances were drawn using the function *metaMDS*, the homogeneity of the multivariate dispersions was tested using the function *vegdist*, the dispersion was tested using the function *betadisper*, the effects of the treatments on community structure were tested with a permutational multivariate analysis using the functions *permutest* and *adonis* with "practices" (agricultural practices) and "variety" (rice variety) as fixed effect and "block" as random factor, and correlations between the structure of the communities and environmental variables were explored using the function *envfit*.

The packages *nlme* (Pinheiro *et al.*, 2021), *lme4* (Bates *et al.*, 2015), *MASS* (Venables *et al.*, 2002), *car* (Fox *et al.*, 2020), *multcomp* (Hothorn *et al.*, 2008) and *emmeans* (Russel *et al.*, 2021) were used for statistical analyzes. A linear mixed model (function *glm*) with "practices" and "variety" as fixed effect and "block" as random factor was fitted. In case of non-normality, data were transformed by f(x) = log10(x+1) for PPN abundance in the roots and f(x) = log10(x) for the soil variables (function *lme*, package *nlme*). A generalized linear mixed model (function *glmer*, package *lme4*) was used for the analysis of the diversity (family = "poisson" for the richness and family = gaussian(link = "identity") for the Shannon index. The effects "practices" and "variety" (with interaction term) were assessed using analysis of variance (ANOVA) followed by a Tukey's honest significant difference (HSD) *post hoc* test, and were considered significant at $p < .05$. Estimated marginal means (least-squares means) were given with the functions *cld* (package *multcomp*) and *emmeans* (adjust = "tukey").

We used the package *DAtest* (Russel *et al.*, 2018) for differential abundance testing of features (bacterial and fungal ESVs or nematode families). Enrichments were analyzed on each variety and type of practices after trimming low abundant features (min.samples = 3, min.reads = 10). The best statistical tests (LIMMA for the microbiota and negative binomial for the nematofauna) were used. Features were then filtered based on significance ($p < .05$). Bacteria, fungi and nematodes were assigned to guilds using respectively the FAPROTAX (Louca *et al.*, 2017), FUNGuild (Nguyen *et al.*, 2016) and NEMAPLEX

(Ferris, 1999) databases. Functional guilds were divided into two non-overlapping groups: group 1 included reactions with chemical elements and the use of small molecules (manganese oxidation, methanol oxidation, methanotrophy, nitrate reduction, nitrification and respiration of sulfur compounds) and group 2 included degradation of larger molecules or polymers and the fermentation processes (xylanolysis + fermentation, ureolysis + fermentation, ureolysis, hydrocarbon degradation, fermentation + aromatic compound degradation, fermentation, chlorate reducers, chitinolysis, cellulolysis and aromatic compound degradation). Among the 11,919 bacterial ESVs, a total of 788 (6.6%) were assigned, 572 to group 1 and 416 to group 2. For the putative fungal trophic guilds, among the 2,062 ESVs, 756 (36.7%) were found in the database (140 highly probable, 346 probable and 270 possible) that could be attributed to one or several of the three trophic modes (symbiotrophy, saprotrophy and pathotrophy). All nematodes were assigned to one of the following trophic group (Yeates *et al.*, 1993): plant-feeding (including facultative or obligatory plant-feeding nematodes), fungal-feeding, bacterial-feeding, unicellular eukaryote-feeding (including nematodes feeding on protists, fungal spores and whole yeast cells), predatory (including predators of nematodes that are mainly specialist) and omnivorous (including nematodes feeding on a combination of fungi and unicellular eukaryote, and including predators of nematodes that are mainly generalists). In addition to their trophic group, nematode families were assigned to a structural guild that characterize their life strategy (from copiotroph to persistor, Bongers and Bongers, 1998) defined as: cp1 for enrichment opportunists, cp2 for basal fauna, cp3 for early successional opportunists, cp4 for intermediate succession and disturbance sensitivity and cp5 for long-lived intolerant species.

Finally, the packages *Hmisc* (Harrell, 2021) and *corrplot* (Wei et al., 2017) were used for the correlation analysis (type = “spearman”, adjust = “fdr”). Drawings were done with the packages *ggplot2* (Wickham *et al.*, 2009), *cowplot* (Wilke, 2020) and *svglite* (Wickham *et al.*, 2021b). *Inkscape* software was used to finalize the figures.

Results

Reduction in PPN abundance in roots under CA

The abundance of PPNs extracted from the rice roots (**figure 24** and **sup. table 6**) revealed significant effects of both cropping system components (agricultural practices and the rice variety). The abundance of *Meloidogyne* spp. depended on both the variety tested (**figure 24 A**, $p < .001$) and the type of practices (**figure 24 B**, $p < .001$). We observed a reduction of 64% in *Meloidogyne* spp. under CA (35 ±32 PPNs/g of roots) compared to CT (98 ±85 PPNs/g of roots) with variability depending on the variety. The fewest *Meloidogyne* spp. were found in the roots of the resistant Zhonghua 11 variety (26 ±23 PPNs/g of roots) and the most in the roots of the Azucena variety (139 ±103 PPNs/g of roots). Abundance in IR504 and IR64 were intermediate: respectively 45 ±37 and 55 ±37 PPNs/g of roots. For *Hirschmanniella* spp., we observed a tendency to a reduction under CA (1 ±4 PPNs/g of roots) compared to under CT (3 ±4 PPNs/g of roots), although the reduction was not significant (**figure 24 D**, $p = .216$). A similar trend was observed for *Meloidogyne* spp. with the variety effect (**figure 24 C**), Zhonghua 11 having the lowest abundance of *Hirschmanniella* spp. (0 ±0 PPNs/g of roots) and Azucena the highest (4 ±6 PPNs/g of roots). The effects of the cultivation practices ($p < .001$, **figure 24 F**) and of the rice variety ($p < .01$, **figure 24 E**) were significant when the total abundance of these two genera of PPNs included the eggs of all PPNs: fewer PPNs were present under CA (65 ±50 PPNs/g of roots) than under CT (560 ±518 PPNs/g of roots) and again the Zhonghua 11 variety harbored fewest PPNs than the other varieties (93 ±95, 331 ±257, 379 ±581, 447 ±606 PPNs/g of roots for Zhonghua 11, Azucena, IR504 and IR64, respectively).

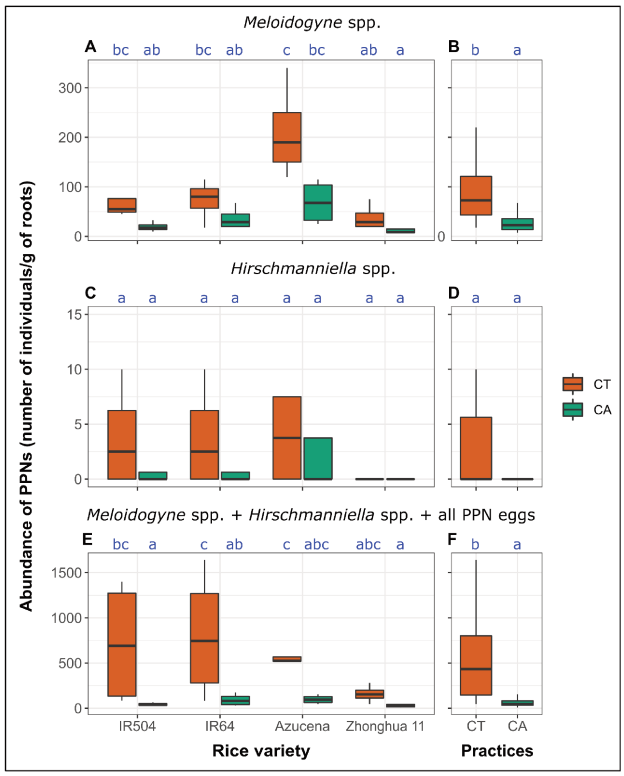

Figure 24. Abundance of plant-parasitic nematodes (PPNs) in roots of four rice varieties (IR504, IR64, Azucena and Zhonghua 11) managed under a type of conventional tillage (CT) or conservation agriculture (CA). Effect of the rice variety on the left panel (**A**, **C** and **E**) and effect of the practices (all varieties combined) on the right panel (**B**, **D** and **F**). Abundance of *Meloidogyne* spp. (**A** and **B**), *Hirschmanniella* spp. (**C** and **D**) or the sum of both genera in addition to all PPNs eggs (**E** and **F**) were measured by the number of individuals/g of roots and assessed by an estimated marginal means (groups are indicated on top of each bar) on a mixed linear model of the number of individuals +1 with a log scale (including a random effect for the block).

Enrichment in soil organic matter and nutrients under CA

Agricultural practices impacted six out of the seven soil variables: with the exception of pH, all the variables were significantly higher under CA than under CT (**table 5**, **sup. table 7**). There was an increase of 110% in SOC ($p < .001$), 83% increase in TKN ($p < .001$), 34% in available P ($p < .001$), 30% in CEC ($p < .001$) and 10% in exchangeable K ($p < .05$).

Table 5. Effect of practices (CA: conservation agriculture *versus* CT: conventional tillage) on soil properties as assessed by an anova on a mixed linear model of the soil properties with a log scale (including a random effect for the block). Means ±standard deviations for the pH, available phosphorus (avail. P), exchangeable potassium (exch. K), total Kjeldahl nitrogen (TKN), soil organic carbon (SOC) and cation exchange capacity (CEC). Statistically different soil properties are in bold and *F*-values for the effect of the practices are in **sup. table 7** with minor effects of the rice variety (IR504, IR64, Azucena and Zhonghua 11).

Soil properties	CT	CA
pH	5.32 ±0.09	5.23 ±0.16
avail. P (ppm)	**13.85 ±3.34**	**18.57 ±4.02**
exch. K (meq/100 g)	**0.29 ±0.05**	**0.32 ±0.04**
TKN (%)	**0.030 ±0.008**	**0.061 ±0.011**
SOC (%)	**0.95 ±0.28**	**1.99 ±0.27**
CEC (meq/100 g)	**8.78 ±2.01**	**11.41 ±2.19**

Effects of the cropping system on the diversity of the rhizosphere communities

Amplicon sequencing yielded a total of 1,095,186 reads (min = 28,341, median = 33,892, max = 45,755) for the 16S marker and 1,153,809 reads (min = 25,131, median = 37,635, max = 42,549) for the ITS marker with all samples having more than 1,000 read counts. Hereafter, the term "features" refers to bacterial and fungi exact sequence variants (ESVs) obtained by the amplicon barcoding and bioinformatic taxonomic assignments, or to the nematode families counted and identified by the microscopy-based technique. For the microbiota, we obtained 361,889 high quality reads with a median of 10,832 reads per sample (min = 7,510 and max = 17,834) assigned to a total of 11,919 ESVs for *Bacteria*, and 326,487 high quality reads with a median of 10,234 reads per sample (min = 4,471 and max = 16,476) assigned to 2,062 ESVs in total for *Fungi*. These microbial features were shared or specific to the cropping system components within the bacterial (**figure 25 A** and **D**) or fungal (**figure 25 B** and **E**) communities. The fraction shared by both types of practices was larger for fungal ESVs (17%) than for bacterial ESVs (12%). The remaining ESVs were specific to either CA or CT. The fraction of fungal ESVs specific to CA was relatively larger (50% under CA compared to 33% under CT) than the fraction of bacterial ESVs (46% under CA compared to 42% under CT). The fraction of fungal ESVs shared by all varieties was larger (13%) than the fraction of

bacterial ESVs (8%). The fraction of bacterial ESVs specific to each variety was 19% and the fraction of fungal ESVs was 16%. For nematodes, we obtained 32 families in total. All nematode families found under CT were also found under CA (**figure 25 C**). A few more were specific to CA (22%). Most of the nematode features were shared by all four varieties (69%), very few were specific to a particular variety (9% to Azucena, 3% to IR504) and none to Zhonghua 11 or to IR64 (**figure 25 F**).

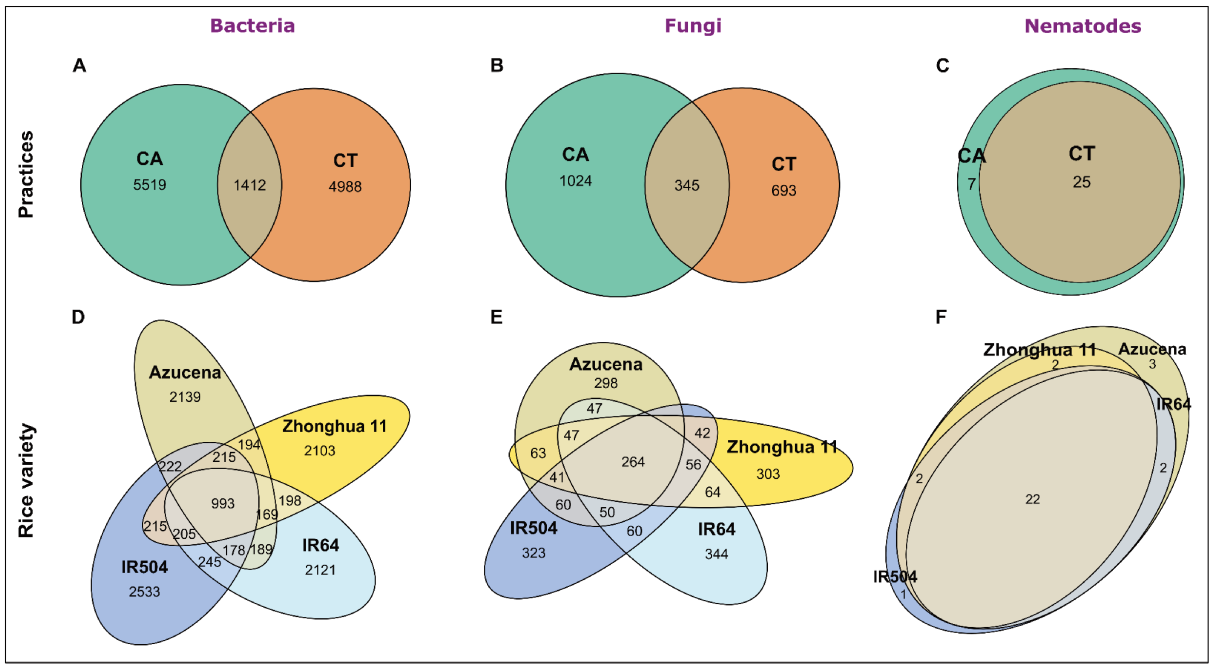

Figure 25. Venn diagrams of the rhizosphere communities of bacteria (**A** and **D**), fungi (**B** and **E**) and nematodes (**C** and **F**). The numbers indicate the feature counts (ESVs for bacteria and fungi, or microscopically identified families for nematodes) shared between the types of agricultural practices (CT: conventional tillage and CA: conservation agriculture) (**A**, **B** and **C**) and the rice varieties (IR504, IR64, Azucena or Zhonghua 11) (**D**, **E** and **F**).

The cropping system components had an effect on the diversity of both the microbiota (bacteria and fungi) and the nematofauna (**figure 26** and **sup. table 8**). First, there was a shift in β-diversity induced by practices (**figure 26 A**, **B** and **C**) that explained around 25% of the variance in the structure of all three rhizosphere communities (**sup. table 8**), bacteria being the least impacted (R^2 = 0.21, p < .001). The dispersion of the nematofauna was higher under CA than under CT (F = 12.67, p < .01). The variety had no significant effect on the β-diversity. Soil properties were correlated with the structure of the three communities (**sup. figure 7**): the increases in SOC, TKN, available P and CEC were correlated with the shift of the structure of the three rhizosphere communities toward CA. In addition, pH was positively correlated with the shift of the fungal community, again, toward CA.

Secondly, the effects of the communities on the α-diversity were more contrasted. The microbial richness was higher under CA (**figure 26 E**, chisq = 7.25 with p < .01 for bacteria, **figure 26 G**, chisq = 146.83 with p < .001 for fungi). There were respectively about 3% and 38% more ESVs in the bacterial and fungal communities under CA compared to under CT. A similar trend was observed in the nematofauna

(**figure 26 I**) with 7% more families under CA. The microbial richness was also influenced by the variety (**figure 26 D**, chisq = 64.79 with $p < .001$ for bacteria, and **figure 26 F**, chisq = 9.06 with $p < .05$ for fungi) with an interaction between the two effects (chisq = 137.50 with $p < .001$ for bacteria due to IR504 that increased richness, whereas IR64 reduced it under CA, chisq = 26.70 with $p < .001$ for fungi with Azucena showing the highest difference between CA and CT, while IR64 and Zhonghua 11 the smallest). The Shannon index for fungi was higher under CA (**figure 26 M**, chisq = 5.81 with $p < .05$, +11%) and for nematodes (**figure 26 O**, chisq = 3.86 with $p < .05$, +5%). The Shannon index for nematodes was also impacted by the variety (**figure 26 N**, chisq = 13.26 with $p < .01$).

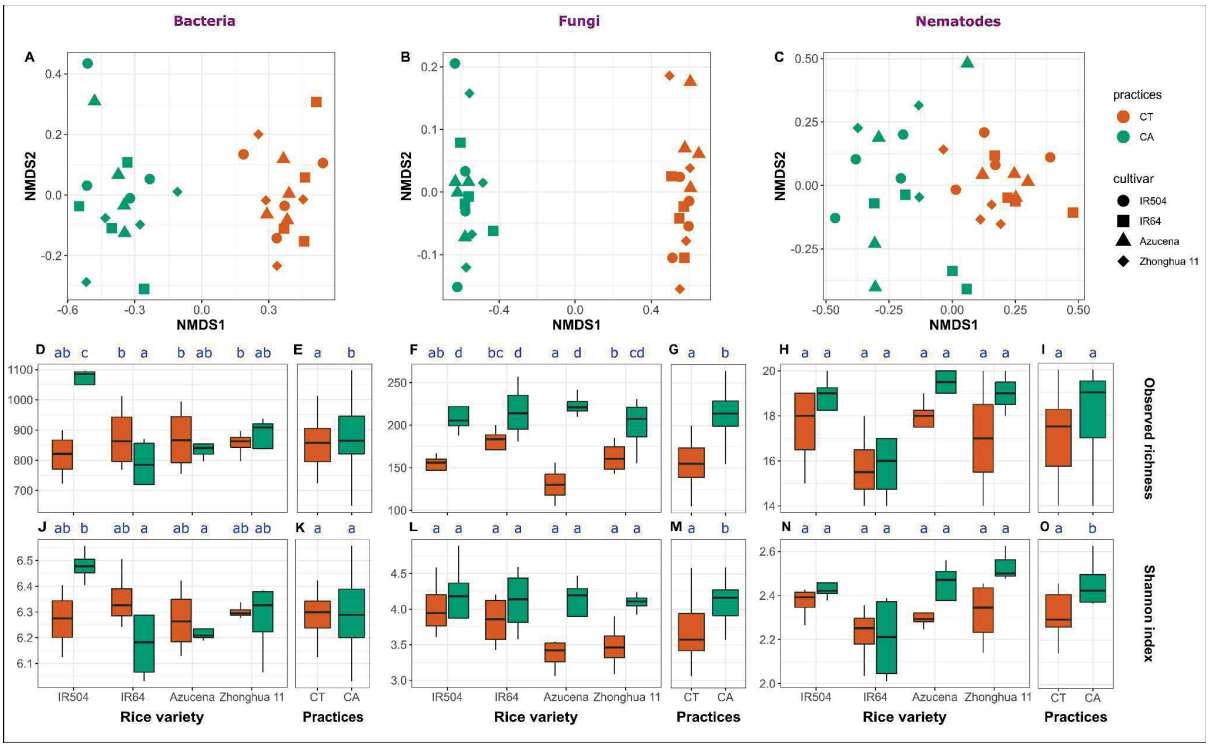

Figure 26. Diversity of the communities of bacteria (**A**, **D**, **E**, **J** and **K**), fungi (**B**, **F**, **G**, **L** and **M**) and nematodes (**C**, **H**, **I**, **N** and **O**) in the rhizosphere of the four rice varieties (IR504, IR64, Azucena or Zhonghua 11) managed under a type of conventional tillage (CT) or conservation agriculture (CA) as represented by non-metric multidimensional scalings (NMDSs) based on Bray-Curtis distances (**A**, **B** and **C**), observed richness (from **D** to **I**) and Shannon index (from **J** to **O**) assessed by an estimated marginal means (groups are indicated on top of each bar) on a generalized linear mixed model of the diversity index with a Poisson distribution for the observed richness or a gaussian distribution for the Shannon index (including a random effect for the block). Detailed effects of the practices and the rice variety on these diversity indices in **sup. table 8**. Soil variables projected on top of the NMDSs in **sup. figure 7**. Stressplot = 0.10 (**A**), 0.080 (**B**) and 0.20 (**C**).

Modified differential abundances of taxa and trophic groups under CA

The effects of the cropping system on the relative abundances of the taxa are presented in **figure 27** (effect of the practices) and **sup. figure 8** (effect of the variety). In the bacterial community, 14/42 phyla

were impacted by the practices: the relative abundance of *Armatimonadota* (+28%, $p < .05$), *FCPU426* (+37%, $p < .05$) and *Verrucomicrobiota* (+30%, $p < .001$) was higher under CA while the relative abundance of *Chloroflexi* (-43%, $p < .001$), *Cyanobacteria* (-61%, $p < .01$), *Fibrobacterota* (-75%, $p < .001$), *GAL15* (-82%, $p < .05$), *Hydrogenedentes* (-75%, $p < .05$), *Latescibacterota* (-77%, $p < .001$), *MBNT15* (-61%, $p < .001$), *Myxococcota* (-23%, $p < .05$), *Nitrospirota* (-75%, $p < .001$), *RCP2-54* (-75%, $p < .01$) and *Spirochaetota* (-31%, $p < .01$) was lower under CA. We found an effect of the variety on *Chloroflexi* (Azucena < IR504 < Zhonghua 11 < IR64, $p < .001$), *Fibrobacterota* (Azucena < IR64 < Zhonghua 11 < IR504, $p < .05$) and *MBNT15* (IR64 < Zhonghua 11 < IR504 < Azucena, $p < .05$). In the fungal community, 6/13 phyla were impacted by the practices: the relative abundance of *Ascomycota* (+109%, $p < .001$), *Blastocladiomycota* (+392%, $p < .05$), *Glomeromycota* (+329%, $p < .01$), *Monoblepharomycota* (+540, $p < .01$) was higher under CA while the relative abundances of *Mucoromycota* (-41%, $p < .01$) and *Rozellomycota* (-65%, $p < .001$) was lower under CA. We observed an effect of the variety on *Kickxellomycota* (Zhonghua 11 = IR504 < Azucena < IR64, $p < .05$). In the nematofauna, 12/31 families were impacted by the practices: the relative abundance of *Achromadoridae* (+582%, $p < .01$), *Anatonchidae* (absent under CT, $p < .05$), *Aphelenchoididae* (+176%, $p < .05$), *Belondiridae* (absent using CT, $p < .05$), *Cephalobidae* (+93%, $p < .01$), *Qudsianematidae* (+77%, $p < .001$), *Qudsianematidae* unsure (+340%, $p < .001$) and *Rhabdolaimidae* (+364%, $p < .001$) was higher under CA while the relative abundances of *Ironidae* (-60%, $p < .001$), *Leptolaimidae* (-75%, $p < .001$), *Pratylenchidae* (-92%, $p < .01$) and *Prismatolaimidae* (-69%, $p < .001$) was lower under CA. We observed an effect of the variety on *Anatonchidae* (absent in IR504 and IR64, Azucena < Zhonghua 11, $p < .05$) and *Ironidae* (IR64 < Zhonghua 11 < Azucena < IR504, $p < .05$).

Differential abundance testing (**figure 28**) revealed contrasted taxonomic enrichment profiles depending on the rhizosphere communities. In the communities of bacteria and nematodes, respectively 53% and 64% of the enriched features were enriched under CA whereas in the community of fungi, 65% of the enriched features were enriched under CT (**table 6**). Some bacterial ESVs (**figure 28 A**), *e.g. Methylocystis* sp., *Bacillus* sp., *Opitutus* sp. and *Geotalea* sp., were enriched in only one variety under one type of practices. Other bacterial ESVs, *e.g. Candidatus Koribacter* and *Bryobacter* sp., were enriched in several varieties under both types of practices. The remaining ESVs had stronger signatures of the effect of practices because they were enriched in several varieties under only one type of practices, *e.g. Aquicella* sp. under CT, *Citrifermans* sp. and *Acidibacter* sp. under CA. All fungal ESVs (**figure 28 B**) were also enriched under one type of practices or the other, *e.g. Moeszimyces* sp. under CT or *Gibberella* sp. under CA, except for unclassified *Rozellomycota*, which displayed a particular pattern: fungal ESVs were highly enriched under CT in all varieties except in Zhonghua 11, in which two ESVs were enriched under CA. In the community of nematodes (**figure 28 C**), the signatures of all enriched taxa were even stronger: 18 families were exclusively enriched under CA and 10 were exclusively enriched under CT. Overall, slightly more enriched features were enriched under CA compared to CT (**table 6**, 140:126). Different taxonomic enrichment profiles were also influenced by the variety. Zhonghua 11 was the only variety that constantly had more enriched features under CA than under CT (57% in total). Conversely, Azucena had more enriched features under CT than under CA (59% in total).

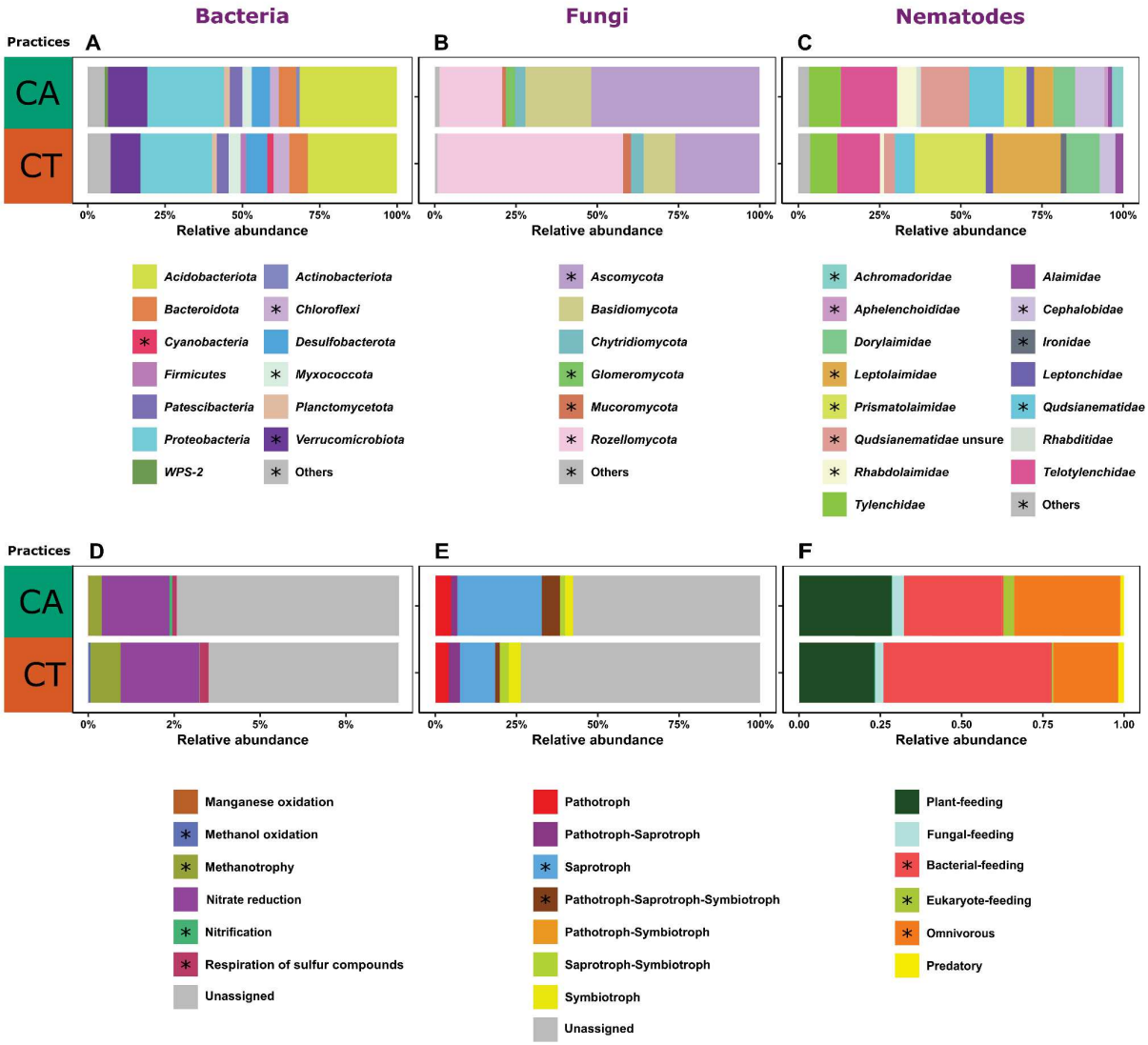

Figure 27. Relative abundances of taxa (**A**, **B** and **C**) and functional guilds (**D**, **E** and **F**) in the communities of bacteria (**A** and **D**), fungi (**B** and **E**) and nematodes (**C** and **F**) in the rhizosphere of the four rice varieties (IR504, IR64, Azucena or Zhonghua 11) managed under a type of conventional tillage (CT) or conservation agriculture (CA). Taxa at phylum level (**A** and **B**) or family level (**C**). "Others" had a relative abundance < 1% each. Features were assigned to either ecological functions from the FAPROTAX database (**D**), trophic modes from the FunGuild database (**E**) or trophic groups from the Nemaplex database (**F**). Asterisks indicate effects of the practices on taxonomic or functional guilds with a $p < .05$. Effects of the variety in **sup. figure 8**. Alternative guilds for bacteria in **sup. figure 9**.



Figure 28. Enrichments of bacterial (**A**), fungal (**B**) and nematode (**C**) features grouped at genus (**A** and **B**) or family (**C**) levels in the rhizosphere of the four rice varieties (IR504, IR64, Azucena or Zhonghua 11) managed under a type of conventional tillage (CT) or conservation agriculture (CA). Colored squares indicate the functional guilds if assigned. The enrichments ($p < .05$) were assessed on features present in at least 25% of the samples for each variety with the package *DAtest*. Features without affiliation at genus (**A** and **B**) level were named "Unclassified" followed by the highest assigned taxonomic group.

Table 6. Summary of the differential abundance testing on bacterial, fungal and nematode features in the rhizosphere of four rice varieties (IR504, IR64, Azucena or Zhonghua 11) managed under a type of conventional tillage (CT) or conservation agriculture (CA). Number of enriched features under CA *versus* under CT (CA:CT) and total numbers of features (in parenthesis). The enrichments were assessed on features present in at least 25% of the samples for each variety with the package *DAtest*. For each condition, more enriched features were found under CA or under CT.

	Bacteria	*Fungi*	*Nematoda*	Total
IR504	33:15 (48)	1:7 (8)	4:5 (9)	38:27 (65)
IR64	37:33 (70)	3:4 (7)	5:0 (5)	45:37 (82)
Azucena	21:31 (52)	2:4 (6)	4:4 (8)	27:39 (66)
Zhonghua 11	23:22 (45)	2:0 (2)	5:1 (6)	30:23 (53)
Total	114:101 (215)	8:15 (23)	18:10 (28)	140:126 (266)

Some bacterial functions related to the decomposition of relatively small (**figure 27 D**) or large molecules (**figure 27 A**) were sensitive to the type of practices: taxa putatively associated with nitrification (+665%, $p < .01$), chitinolysis (+443%, $p < .05$) and ureolysis (+101%, $p < .05$) were more abundant under CA, while those associated with hydrocarbon degradation (-56%, $p < .001$), methanol oxidation (absent under CA, $p < .001$), methanotrophy (-57%, $p < .001$) and respiration of sulfur compounds (-53%, $p < .01$) were less abundant under CA. Only three of the enriched bacterial ESVs were assigned to a functional guild (**figure 28 A**): one to methanotrophy enriched using CT (*Methylocystis* sp.) and two to nitrate reduction enriched using CA (*Azospira* sp. and *Opitutus* sp.). Some fungi putatively associated with trophic modes were relatively more abundant under CA (**figure 27 E**): pathotrophs-saprotrophs-symbiotrophs (+251%, $p < .001$) and saprotrophs (+164%, $p < .01$). Five of the enriched fungal ESVs were assigned to a guild (**figure 28 B**): one to pathotrophy enriched under CT (*Moesziomyces* sp.), two to saprotrophy enriched under CA (*Rhizophlyctis rosea* and *Xenomyrothecium tongaense*) and two to pathotrophy-saprotrophy-symbiotrophy enriched under CA (*Saitozyma flava* and *Gibberella intricans*). In the nematofauna (**figure 27 F**), the relative abundances of unicellular eukaryote-feeders (+582%, $p < .01$) and omnivorous nematodes (+68%, $p < .05$) were higher under CA at the expense of bacterial-feeders (-36%, $p < .05$). The enriched families (**figure 28 C**) were assigned to one plant-feeders enriched under CT (*Psilenchidae*), seven bacterial-feeders enriched under either CT (*Leptolaimidae*, *Prismatolaimidae*, *Alaimidae* and *Panagrolaimidae*) or CA (*Cephalobidae*, *Rhabditidae* and *Rhabdolaimidae*), two fungal-feeders enriched under CA (*Aphelenchoididae* and *Leptochidae*), one unicellular eukaryote-feeder enriched under CA (*Achromadoridae*), four omnivorous enriched under CT (*Ironidae* and *Dorylaimidae*) or CA (*Qudsianematidae* and unsure *Qudsianematidae*).

Shift in the soil food web indices and structural guilds under CA

Nematofaunal indices revealed higher enrichment index (EI) (24.2 ±18.5 > 10.4 ±6.8, $p < .05$), structural index (SI) (91.4 ±4.0 > 85.8 ±3.7, $p < .001$) and maturity index (MI) (3.3 ±0.2 > 3.0 ±0.1, $p < .001$), and a lower index of organic matter decomposition (IVD) (89.6 ±8.0 < 95.1 ±2.9, $p < .01$) under CA compared to under CT. The higher enrichment and structure indices of the food web under CA are visible in **sup. figure 10 A**. The structural guilds of the nematode families (**sup. figure 10 B**) revealed a lower relative abundance of early successional opportunists (cp3, -32%, $p < .05$), and a higher relative abundances of species with intermediate succession and sensitivity to disturbance (cp4, +45%, $p < .05$) and long-lived species with high sensitivity to disturbance (cp5, +409%, $p < .01$) under CA.

Correlations between the reduction in PPN abundance and soil abiotic and biotic variables

Correlations were found between the reduction in PPN abundance and the CA edaphic and biotic signature (**figure 29**). The abundance of *Meloidogyne* spp. in rice roots was correlated with soil chemical properties (r = -0.49, $p < .01$ with the TKN, and r = -0.39, $p < .05$ with the CEC), with diversity measurements (0.4 < r < 0.5 , $p < .01$ with the NMDS1 coordinates of the three rhizosphere communities and r = -0.48, $p < .01$ with the fungal richness), and with food web indices (r = 0.36, $p < .05$ with the IVD and r = -0.37, $p < .05$ with the MI). The abundance of both phytoparasitic genera including all PPN eggs was also correlated with the same variables, in addition to the NMDS2 coordinates of the bacterial community (r = 0.37, $p < .05$), but without the fungal richness and the food web indices (although r = -0.34, $p = .055$ with the MI). The abundance of *Hirschmanniella* spp. was correlated with other variables that were only linked to the nematofauna: the total abundance of PPNs in the rhizosphere (r = 0.39, $p < .05$), the Shannon index (r = -0.39, $p < .05$) and the EI (r = 0.39, $p < .05$). Correlations were also found between the reduction in PPN abundance and the relative abundances of functional guilds (**sup. figure 11**). The abundance of *Meloidogyne* spp. was correlated with the abundance of omnivorous nematodes (r = -0.36, $p < .05$). The abundance of both phytoparasitic genera including all PPN eggs was also correlated with the abundance of omnivorous (r = -0.40, $p < .05$), in addition to with the abundance of saprotrophic fungi (r = -0.44, $p < .05$) and predatory nematodes (r = 0.36, $p < .05$).

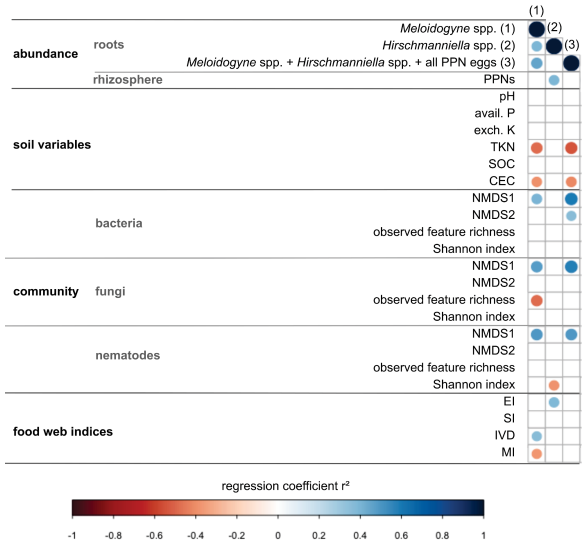

Figure 29. Heatmap of correlations ($p < .05$) linking the abundance of PPNs with soil variables, diversity measurements of the rhizosphere communities and food web indices (EI; enrichment index, SI; structural index, IVD; index of organic matter decomposition pathway and MI; maturity index) associated with the four rice varieties (IR504, IR64, Azucena or Zhonghua 11) managed within a type of conventional tillage or conservation agriculture. A complementary heatmap of correlations between the abundance of PPNs and the abundance of trophic guilds in **sup. figure 11**.

Discussion

In this study conducted on an irrigated lowland rice field, we observed that CA improved the soil quality (+110% of SOC, +83% of TKN, +34% of available P, +10% of exchangeable K, +30% for the CEC), increased the biodiversity (richness: +3% for bacteria and +38% for fungi; Shannon index: +11% for fungi and +5% for nematodes), modified the relative abundances of functional guilds (notably +164% potentially saprotroph fungi and +665% of potentially nitrifiers bacteria, -37% of bacterial-feeding nematodes and +68% of omnivorous nematodes), allowed the maturation of the soil food web (+9% for the maturity index, +132% for the enrichment index and +7% for the structure index) and reduced the abundance of PPNs (-64% of *Meloidogyne* spp. in roots and -92% *Hirschmanniella* spp. in the rhizosphere). Some taxa were enriched under either CA (*e.g.* one pathotrophic fungus) or CT (*e.g.* two saprotrophic fungi) and the varieties also displayed different enrichment patterns. The analysis of the structural guilds revealed that there were fewer early successional opportunists nematodes (-32% cp3) and more persistent nematodes (+45% cp4 and +409% cp5) under CA. We also found correlations associated with the abundance of PPNs, notably between the reduction in *Meloidogyne* spp. abundance in roots and improved soil variables (TKN with r = 0.49 and CEC with r = 0.39), the increases of fungal richness (r = 0.48), and decomposition and maturation indices (r = -0.36 and 0.37, respectively) of the soil food web.

The reduction in PPNs was observed seven years after the transition to CA

Suong *et al.* (2019) identified two PPNs species in rice roots in this field located in Stung Chinit: *Meloidogyne graminicola* (present at all developmental stages) and *Hirschmanniella mucronata* (present at the tillering and milky stages). These authors found that in 2014 and 2015, a few years after the conversion from CT to CA, the abundance of *Meloidogyne graminicola* and *Hirschmanniella mucronata* was about seven times higher under CA than under CT. In the present work, we collected the samples at the tillering stage and extended our investigation to the genus level of these species. For a broader view of the dominant PPNs in this field, we counted the PPNs belonging to the *Meloidogyne* and *Hirschmanniella* genera in addition to the eggs of all PPNs. Our results showed the opposite trend in 2018: the total number of PPNs (*Meloidogyne* spp. + *Hirschmanniella* spp. + the eggs of all PPNs) was about nine times lower under CA than under CT. Moreover, the abundance of PPNs studied was lower under CA in 2018 (65 PPNs/g of roots) than it was in 2014 or 2015 (364 PPNs/g of roots on average). Despite the higher pressure due to the PPN infection in 2014 and 2015, the rice yield was maintained in both years (Suong *et al.*, 2019). In the present study, we focused on the effects of the cropping system components on the rhizosphere communities that might benefit plant health and showed that, after seven years, the pressure from PPNs was lower due to practices that improved crop health *via* enhanced soil fertility and biodiversity.

It has been suggested that practices affect the nematode community much more than the crop (Neher *et al.*, 1999; Berkelmans *et al.*, 2003). However, the chosen variety impacted the PPN population in the roots. This was particularly clear for *Meloidogyne* spp. possibly because they are sedentary nematodes and this have a closer relationship with the plant, and also because our varieties differed in their susceptibility to *Meloidogyne graminicola*. The Zhonghua 11 variety, which is resistant to the infection by *Meloidogyne*

spp. (Phan *et al.*, 2018), showed the lowest abundance of PPNs, whereas Azucena variety was the most susceptible to *Meloidogyne* spp. in our study. Meanwhile, the abundance of *Hirschmanniella* spp. in roots was only slightly impacted by the tested practices, potentially because the biological cycle of these migratory nematodes makes them less affected by tillage and cover crops. Yet, under CA, *Hirschmanniella* spp. were less abundant in the rhizosphere (-92% *Pratylenchidae* that were only represented by *Hirschmanniella* spp.) as already observed by Berkelmans *et al.* (2003) in a 12-year experiment under low-input and organic management systems, and by Natthidech *et al.* (2021) in another seven-year experiment under a similar type of CA in Cambodia.

CA practices substantially modified rhizosphere nematofauna by generating a distinct community structure associated with a higher diversity. Another study also showed that reduced tillage (but not organic matter input) increased nematode diversity and the stability of the food web in long-term field experiments in Europe (Bongiorno *et al.*, 2019). In particular, the study by Berkelmans *et al.* (2003) showed that agricultural practices modified the nematofauna by modulating their trophic levels. In our study, the relative abundance of total plant-feeders was not significantly impacted under CA but other trophic groups and the structural guilds were modified (notably more omnivorous and more cp4 and 5). Berkelmans *et al.* (2003) reported that although the differences observed could disappear after a short disruptive management (*i.e.* tillage), the nematofauna then stabilized over time and regained its original structure at the 12-year long experiment. Since nematodes have key positions in the food web, shifts in their community are generally also associated with restructurations of other soil communities.

An enrichment of soil resources triggered a bottom-up effect in the food web

Here, we validated our hypothesis that CA benefited the soil food web in the rice field in Stung Chinit. Indeed, the mulch of cover crops under CA (first trophic level) was a source of organic matter (SOC) and nutrients (NPK) for the microbial decomposers (second trophic level). Improved soil quality associated with increased richness and diversity (especially fungal) restructured the microbial communities in the rhizosphere. Previous studies also showed that a shift to CA has a major effect on soil biodiversity and functions (Chabert and Sarthou, 2017). Long-term no-tillage associated with organic input (Wang *et al.*, 2017) or even cover crops alone (Wang *et al.*, 2020) enhanced the diversity and stability of the soil microbiota, although this may depend on the cropping system (Kim *et al.*, 2020). Consequently, farming systems such as CA can improve soil quality by increasing the diversity and abundance of functional guilds (Kibblewhite *et al.*, 2008). In the soil communities under CA, there was possibly more nitrification due to an enrichment of bacteria such as *Azospira* spp. (Park *et al.*, 2020) and *Opitutus* spp. (Chin *et al.*, 2001) and more saprotrophy due to an enrichment of fungi such as *Rhizophlyctis rosea* (James *et al.*, 2006) and *Xenomyrothecium tongaense* (Sterkenburg *et al.*, 2018). The latter species belongs to *Ascomycota* and can play an active role in breaking down plant biomass (Ma *et al.*, 2013; Challacombe *et al.*, 2019).

The changes observed in the bacterial and fungal communities under CA in turn structured populations of fungal- and bacterial-feeding nematodes (third trophic level). Fungal-feeders are generally less abundant than bacterial-feeders in highly disturbed soil systems such as conventional agricultural soils

(Villenave *et al.*, 2009). Soil disturbances such as tillage favor a nematode community dominated by less sensitive, opportunistic and fast-growing bacterial feeders (Ferris *et al.*, 1996; Yeates, 2003). In this study, we observed an increase of the fungal- to bacterial-feeder ratio under CA, as revealed by the modified relative abundances and the lower IVD. This measure of primary organic matter decomposition implies that under CA, decomposition was mainly driven by fungal activity rather than by bacterial activity, as already reported under low-input and organic management systems (Berkelmans *et al.*, 2003). In our study, the structure and diversity of the fungal community were the most affected by the practices, which could be due to their particular sensitivity to tillage, especially for mycorrhizal fungi (Gupta *et al.*, 2019) such as *Glomeromycota* spp.

Next, at the fourth trophic level of the soil food web, we observed relatively more omnivorous nematodes under CA. We also observed more eukaryote-feeding nematodes, but in our study, this trophic group was only represented by one family (*Achromadoridae* spp.) and could have been grouped with omnivorous and predatory nematodes (Villenave *et al.*, 2009). Nonetheless, the abundance of such rare nematodes could be linked to the higher diversity of nematofauna under CA and possibly represent additional soil functions. Interestingly, another study showed that increased organic resources may cascade up the food chain and affect higher trophic levels up to macro-invertebrates, after 14 years of CA in a field with wheat as the main crop (Henneron *et al.*, 2014). Similarly, a study revealed that omnivorous nematodes were more abundant after six years of no-tillage in a soybean field, and that the structure and maturity indices were higher than in the plot under conventional tillage (Okada and Harada, 2007).

Finally, we found a more advanced maturity in the whole soil food web under CA. Changes in the structural guilds indeed resulted in a more enriched and more stable food web. This observation is based on the lower abundance of early successional opportunists (cp3), and the higher abundances of species with intermediate succession and disturbance sensitivity (cp4) and long-lived intolerant species (cp5). In Berkelmans *et al.* (2003), the SI and EI were also lower under one type of CT than under low-input and organic management systems. The ban on tillage and the use of cover crops have already been shown to increase enrichment and structure indices and reduce IVD, with variable effects depending on the type of cover crop used (Ito *et al.*, 2015b). Two families of cp3 bacterial-feeders (*i.e. Leptolaimidae* and *Prismatolaimidae*), one family of cp4 predators (*i.e. Anatonchidae*, absent under CT) and one family of cp5 omnivores (*i.e. Qudsianematidae*) significantly contributed to these changes in our study. Finally, due to the enrichment of soil basal resources and avoidance of soil disturbance, CA enabled some species to inhabit the soil and enabled the food web to mature. Villenave *et al.* (2009) also found that systems with direct seeding harbor fewer opportunists and a more complex nematofauna, including taxa that are sensitive to perturbations, than systems that include tillage. Such mature soil can be “suppressive”, meaning that there are sufficient antagonists of various kinds in the food web to reduce populations of pathogenic species (Ferris *et al.*, 2001).

Mechanisms of PPN suppression potentially occured in the field

Enrichment of soil resources (*e.g.* SOC and NPK) was correlated with a reduction in PPN abundance in plant roots suggesting that the improvement in soil quality due to agricultural practices reached the PPN population. Indeed, the reduced abundance of *Meloidogyne* spp. in roots was correlated

with the increase in MI and relative abundance of omnivorous nematodes. Similarly, Berkelmans *et al.* (2003) reported that the percentage of suppression of *M. javanica* was correlated with increases in EI and SI. The reduced abundance of *Hirschmanniella* spp. in the rhizosphere also suggests that the field under CA was suppressive for these PPNs.

Based on these correlations and on the literature, we propose that suppression of PPNs observed under CA is due to both direct or indirect antagonism. Direct antagonism can involve antagonistic microbes and omnivorous or predatory (generalists or specialists) nematodes. Indeed, organisms at high trophic levels in soil food webs can play a role in suppressing plant parasites (Devi and George, 2017). For example, a study showed that the top-down soil suppressiveness of a parasitic nematode, *Meloidogyne incognita*, was related to the ratio predators/prey and to the prevalence of predatory nematodes (Sánchez-Moreno and Ferris, 2006). Another study of the transition from CT to CA in an upland rice field showed that, following an increase in SOC, six years were required for predatory nematodes to appear and to play an active role in biocontrol (Ito *et al.*, 2015a). This delay is comparable to the time needed in the Stung Chinit field to show a reduction in PPN infection. In the rhizosphere under CA, we indeed observed more omnivorous nematodes such as *Qudsianematidae* spp. which are generalist predators able to feed on the microbiota and microfauna, and specialist predators such as *Anatonchidae* spp. (absent under CT) and *Mononchidae* spp. that feed only on the microfauna (Khan and Kim, 2007). Interestingly, species of *Qudsianematidae* have been described to prey on *Hirschmanniella oryzae* (Bilgrami and Gaugler, 2005). Omnivorous and predatory nematodes could be responsible for a top-down regulation of *Hirschmanniella* spp. in the rhizosphere in our study. In contrast, Henneron *et al.* (2014) found no increase in predators perhaps because the conventional field was not tilled in the sampling year. All these results underline the importance of avoiding tillage and of providing a continuous supply of organic inputs through the use of cover crops to allow the soil food web to mature and to create a favorable niche for persistors-predators.

Microbes may also play a direct or indirect role as biological control agents of PPNs, as suggested by the negative correlation between *Meloidogyne* spp. abundance in roots and the higher fungal richness under CA. Some fungi are indeed known to be direct antagonists of PPNs including the nematode-trapping fungus *Arthrobotrys* spp., *Dactylellina* spp. or *Mortierella* spp., the endoparasitic fungus *Catenaria* spp. and the egg and female parasitic fungi *Purpureocillium* spp., *Dactylella* spp. or *Trichoderma* spp. (Topalović *et al*., 2020b) that were all found in our samples. Such fungi can impact PPN populations (Jaffee *et al.*, 1997; Jaffee and Strong, 2005; Stirling, 2015). Indirect antagonistic mechanisms can involve microbes able to induce systemic resistance. For example, *Glomeromycota* spp., which were enriched under CA, are obligate associates of plants and may be able to protect tomato and pepper against *M. incognita* (Rodriguez-Heredia *et al.*, 2020). Other arbuscular mycorrhizal fungi such as *Glomus mosseae* have also been shown to reduce penetration by -and life development rate of- *M. incognita* in tomato as well (Vos *et al.*, 2011). Although soil suppressiveness seems to involve both abiotic and biotic factors, Topalović *et al*. (2020a) and Watson *et al.* (2020) have demonstrated that microbes from specific soil may trigger high reduction of root-knot nematode populations. In the rice field in Stung Chinit, CA could have created a favorable environment for the development and plant recruitment of biocontrol agents to suppress PPNs. Further investigations are now required to fully understand the mechanisms of soil suppressiveness and their contribution to crop health and productivity (Trivedi *et al.*, 2020) in this field.

Conclusion

An experiment was conducted in a rice field in Cambodia to monitor the PPN infection under contrasted cropping systems: conservation agriculture (CA: no-tillage and cover crops) *versus* conventional agriculture (CT: including tillage) in combination with four rice varieties (IR504, IR64, Azucena, Zhonghua 11). We found that after seven years, rice roots were less infected by PPNs under CA. Our data reinforces results of previous studies showing that CA favors soil ecosystem services: no-tillage cropping systems combined with the use of cover crops increased organic matter inputs above and belowground, and consequently triggered a structuring and enrichment of the whole soil food web. We suggested that the food web maturity is associated with the development of a soil biota that prey on (*e.g.* predatory nematodes) or antagonize (*e.g.* trapping fungus) nematodes, and promote the plant growth and defense (*e.g.* mycorrhizal fungi). CA resulted in a disease suppression of PPNs. This could have led to the reduction in PPN abundance, especially *Meloidogyne graminicola* in roots and *Hirschmanniella mucronata* in the rhizosphere. CA relieves parasitic pressure on rice and possibly counterbalanced disease outbreaks. Further research is needed to unravel the mechanisms involved in the reduction in PPN abundance. Even though the rice variety is an important component of the cropping system because it provides resistance at the plant level, *i.e.* resistance to *Meloidogyne graminicola* with Zhonghua 11, the four tested varieties had very little effect on the rhizosphere communities. However, this requires further investigation into the ability of the variety to recruit specific microorganisms and to interact with them. Finally, by improving soil quality and crop health, CA is a very promising alternative cropping system to support the transition to more sustainable rice production in South-East Asia. The description of the soil food web in this study provides a snapshot of an agroecosystem that requires more monitoring to evaluate the full potential of CA for the regulation of pest and pathogen population, and for other services including the support of nitrogen and carbon cycles.

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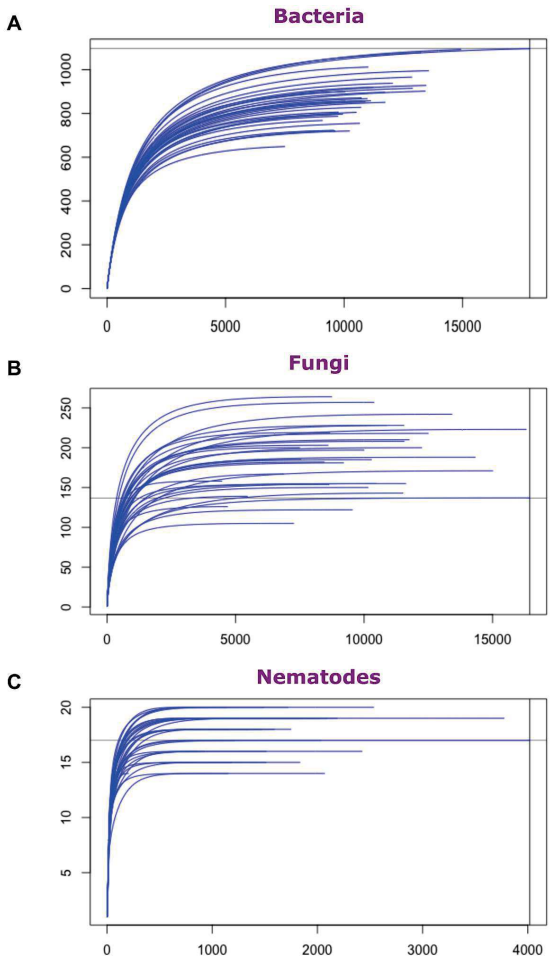
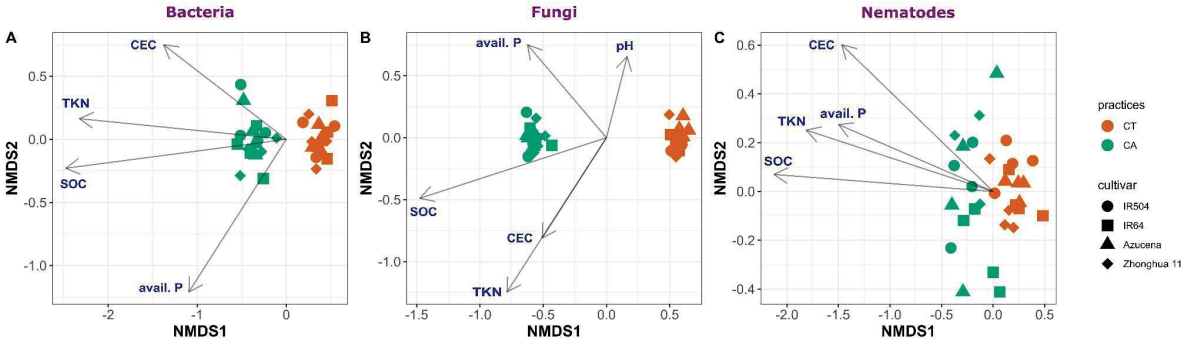
Supplemental figures and tables



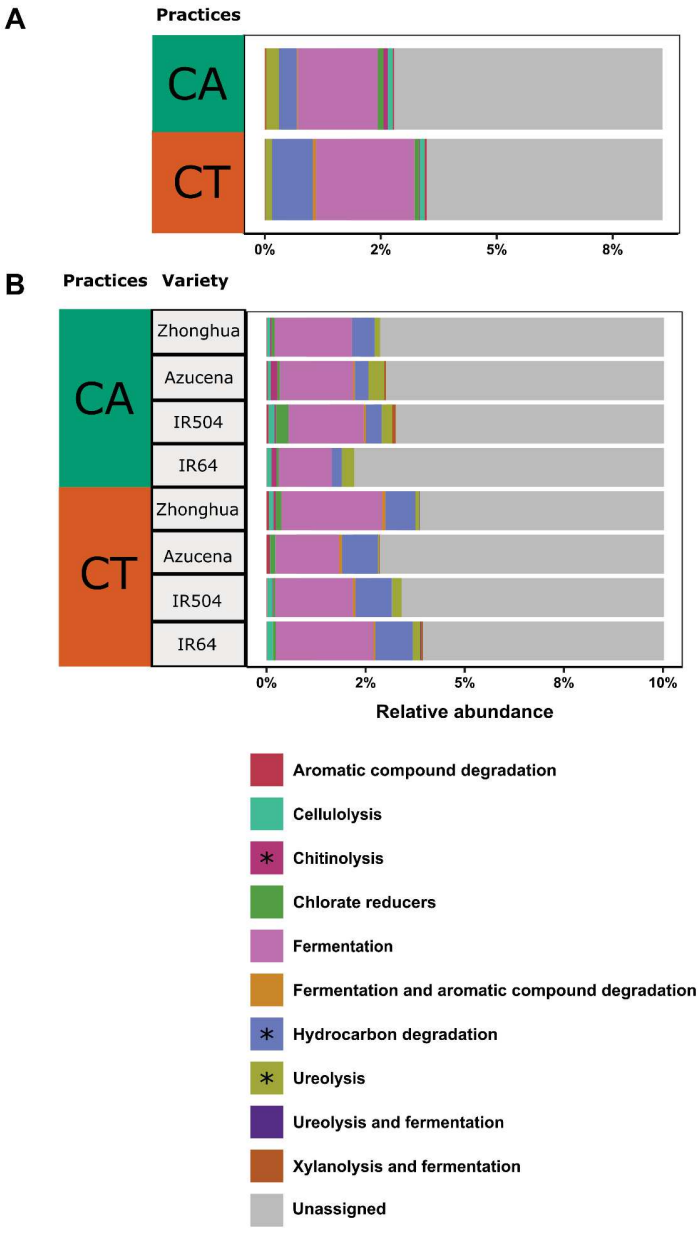
Sup. figure 6. Rarefaction curves for the communities of bacteria (**A**) and fungi (**B**): number of ESVs = f(total reads count), or for the community of nematodes (**C**): number of families = f(total individuals count).



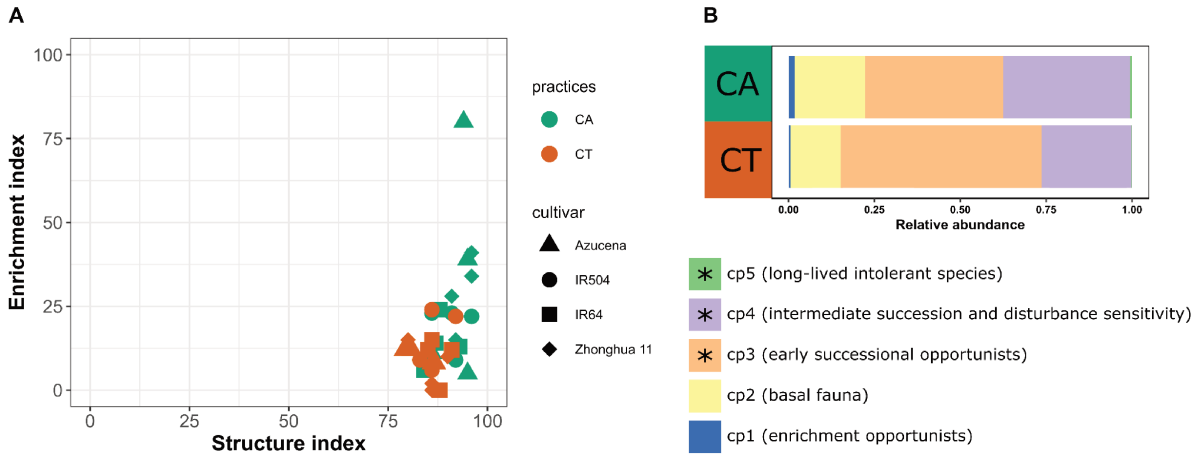
Sup. figure 7. Soil variables projected on the NMDSs of the communities of bacteria (**A**), fungi (**B**) and nematodes (**C**) with the function *envfit* of the package *vegan*. Only significant variables: pH, available phosphorus (avail. P), total Kjeldahl nitrogen (TKN), soil organic carbon (SOC) and cation exchange capacity (CEC). Stressplot = 0.10 (**A**), 0.080 (**B**) and 0.20 (**C**).



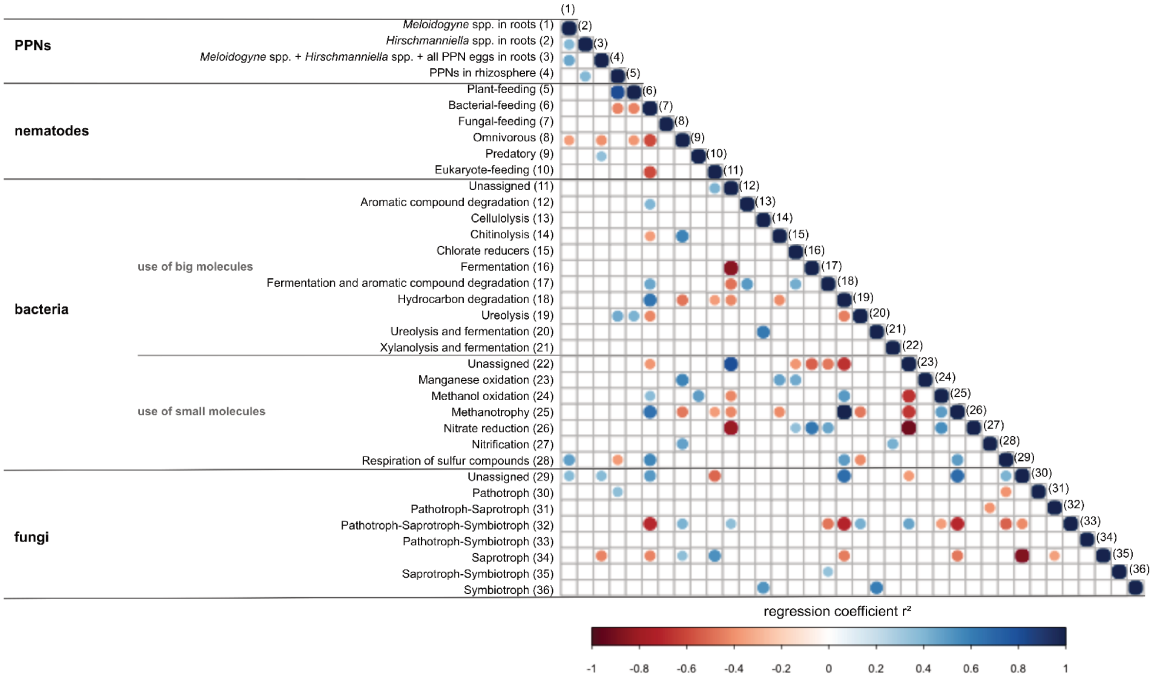
Sup. figure 8. Relative abundances of taxa (**A**, **B** and **C**) and functional guilds (**D**, **E** and **F**) in the communities of bacteria (**A** and **D**), fungi (**B** and **E**) and nematodes (**C** and **F**) in the rhizosphere of four rice varieties (IR504, IR64, Azucena or Zhonghua 11) managed within a type of conventional tillage (CT) or conservation agriculture (CA). Taxa at phylum level (**A** and **B**) or family level (**C**). "Others" had a relative abundance < 0.01. Features were assigned to either ecological functions from the FAPROTAX database (**D**), trophic modes from the FunGuild database (**E**) or trophic groups from the Nemaplex database (**F**). Asterisks indicate effects of the variety with $p < .05$. Effects merged by practices are in **figure 27**. Alternative guilds for bacteria are in **sup. figure 9**.



Sup. figure 9. Relative abundances of alternative guilds in the communities of bacteria in the rhizosphere of four rice varieties (IR504, IR64, Azucena or Zhonghua 11) managed within a type of conventional tillage (CT) or conservation agriculture (CA). Features were assigned to ecological functions from the FAPROTAX database that were not overlapping the guilds in **figure 27**. Practices (**A**) and variety (**B**) effects. Asterisks indicate effects of the practices with a $p < .05$. No variety effect was found.



Sup. figure 10. Food web structure and function based on the nematofaunal indices of four rice varieties (IR504, IR64, Azucena and Zhonghua 11) managed within a type of conventional tillage (CT) or conservation agriculture (CA): (**A**) Enrichment index (y axis) ~ Structure index (x axis) diagram and (**B**) Relative abundances of nematodes associated to cp (coloniser-persistor) values. Asterisks indicate effects of the practices with a $p < .05$.



Sup. figure 11. Heatmap of correlations ($p < .05$) between the abundance of PPNs and functional guilds of the rhizosphere communities (bacteria, fungi and nematodes) associated with four rice varieties (IR504, IR64, Azucena or Zhonghua 11) managed under a type of conventional tillage or conservation agriculture.

Sup. table 6. Effects of the practices (CA: conservation agriculture *versus* CT: conventional tillage) and the rice variety (IR504, IR64, Azucena and Zhonghua 11) on the abundance of plant-parasitic nematodes (PPNs) as assessed by an anova on a mixed linear model of the number of individuals +1 with a log scale (including a random effect for the block). Abundances of *Meloidogyne* spp., *Hirschmanniella* spp. and the sum of both genera in addition to the eggs of all PPNs were measured by number of individuals/g of roots and *F*-values are reported in this table. Significativity codes for *p*: *** if < .001, ** if < .01, * if < .05, NS if non-significant.

	Meloidogyne spp.	*Hirschmanniella* spp.	Sum of both genera (including all PPNs eggs)
practices	**22.76 *** **	1.63 (NS)	**49.27 *** **
variety	**10.21 *** **	1.30 (NS)	**5.14 ** **
practices × variety	0.46 (NS)	0.20 (NS)	0.16 (NS)

Sup. table 7. Effects of the practices (CA: conservation agriculture *versus* CT: conventional tillage) and the rice variety (IR504, IR64, Azucena and Zhonghua 11) on the soil properties as assessed by an anova on a mixed linear model of the soil properties with a log scale (including a random effect for the block). *F*-values for the effects on pH, available phosphorus (avail. P), exchangeable potassium (exch. K), total Kjeldahl nitrogen (TKN), soil organic carbon (SOC) and cation exchange capacity (CEC). Significativity codes for the *p*: *** if < .001, ** if < .01, * if < .05, NS if non-significant.

	pH	avail. P	exch. K	TKN	SOC	CEC
practices	3.47 (NS)	**14.86 *** **	**4.85 * **	**86.28 *** **	**74.66 *** **	**17.30 *** **
variety	0.68 (NS)	1.64 (NS)	1.56 (NS)	2.46 (NS)	0.57 (NS)	1.41 (NS)
practices × variety	0.04 (NS)	1.19 (NS)	2.10 (NS)	0.24 (NS)	0.60 (NS)	**4.05 * **

Sup. table 8. Effects of the practices (CA: conservation agriculture *versus* CT: conventional tillage) and the four rice variety (IR504, IR64, Azucena and Zhonghua 11) on the *β*- and *α*-diversity of the rhizosphere communities of bacteria, fungi and nematodes as assessed by an adonis test for the structure (including a random effect for the block), the *betadisper* function from the package *vegan* for the dispersion, and an anova on a generalized linear mixed model of the abundances with a Poisson distribution for the richness or a gaussian distribution for the Shannon index (including a random effect for the block). Significativity codes for p: *** if < .001, ** if < .01, * if < .05, "NS" if non-significant.

	Bacteria				*Fungi*				*Nematoda*			
	β-diversity		α-diversity		β-diversity		α-diversity		β-diversity		α-diversity	
	Structure	Dispersion	Richness	Shannon	Structure	Dispersion	Richness	Shannon	Structure	Dispersion	Richness	Shannon
	R^2	F	Chisq	chisq	R^2	F	chisq	chisq	R^2	F	chisq	chisq
practices	**0.21** ***	0.01 (NS)	**7.25** **	0.06 (NS)	**0.28** ***	2.97 (NS)	**146.83** ***	**5.81** *	**0.28** ***	**12.67** **	0.43 (NS)	**7.40** **
variety	0.08 (NS)	0.95 (NS)	**64.79** ***	6.96 (NS)	0.09 (NS)	0.73 (NS)	**9.06** *	3.21 (NS)	0.07 (NS)	0.31 (NS)	0.52 (NS)	**13.26** **
practices × variety	0.08 (NS)		**137.50** ***	**13.89** **	0.08 (NS)		**26.70** ***	2.35 (NS)	0.07 (NS)		0.97 (NS)	2.81 (NS)

Additional analyzes and perspectives

On the way for a better description of the communities

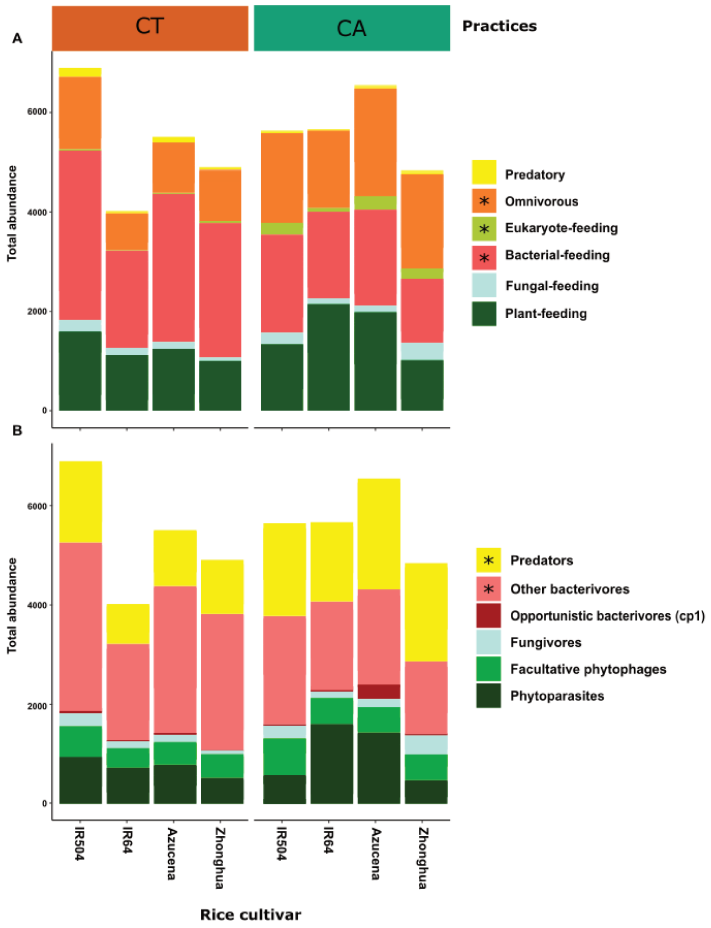
Using total abundances

To better characterize the community, in addition to the relative abundances, the total abundances are also meaningful because they show the individual changes of the groups (Alteio *et al.*, 2021). In the nematode community for example, the relative abundances (**figure 27 F**) and the total abundances (**sup. figure 12 A**) show complementary information, especially if separate groups are shown (by sample, or by variety and practices here). Despite apparent variable total abundance for each condition (*e.g.* total abundance in IR504 > total abundance in IR64 under CT), the relative abundances were conserved across varieties (relative abundances in IR504 = relative abundances in IR64 under CT). We can thus observe that the relative enrichments (*i.e.* eukaryote-feeding and omnivorous) or depletion (*i.e.* bacterial-feeding) were confirmed in absolute abundances: only these guilds gave the same significant results (*i.e.* -33% bacterial-feeding, $p < .05$, +715% eukaryote-feeding, $p < .001$ and +86% omnivory, $p < .05$) and were not overpassed by the abundance of other guilds. Moreover, although absolute abundances of plant-feeding nematodes were higher in most varieties under CA than under CT (-15% for IR504, +35% for IR64, +59% for Azucena, +35% for Zhonghua 11), it was simply weighted by the total abundances of nematodes that were also higher in most varieties (-18% for IR504, +40% for IR64, +19% for Azucena, +32% for Zhonghua 11). Consequently, it resulted in similar relative abundances of plant-feeding nematodes across practices, but specific depletions were observed with the taxonomic assignment (*e.g.* -92% *Pratylenchidae* spp., $p < .001$).

Using structural guilds

In an ecological context, functions are more meaningful than phylogenetic taxa, so we focused our study on the soil functions through the soil food web, especially nematodes because they are known as excellent bioindicators of the soil functions (Bongers et Ferris, 1999). Since no marker gene was available for an amplicon barcoding method on the nematode community, we used morphological traits to taxonomically and functionally assign nematodes. During analysis, we compared the functional assignment of nematodes with NEMAPLEX (**sup. figure 12 A**) to another functional assignment performed by the ELISOL company that includes both the trophic and the structural guilds (**sup. figure 12 B**). In this classification, predators include both specialists and generalists and therefore were relatively more abundant under CA (+76%, $p < .01$), in accordance with the NEMAPLEX classification showing that it was mainly generalist predators (*i.e.* omnivorous) that were enriched (+68%, $p < .05$). Moreover, bacterivores other than cp1 were relatively less abundant under CA (-29%, $p < .05$), in accordance with the NEMAPLEX classification again, showing that the total bacterivores were depleted (-36%, $p < .05$). It might be due to the shift in the bacterial composition. Bacteria can also be classified according to their life strategy along a gradient from copiotrophs to oligotrophs and within the competitor–stress tolerator–ruderals framework. A study showed that cover crops and no-till shifted soil microbial community life strategies (Schmidt *et al.*, 2018). Cover crops shifted the communities toward ruderals-organisms and no-till shifted them toward slow

growing stress tolerators. It would be interesting to analyze the life strategy of bacteria to see if we would also find less abundant bacterial copiotrophs under CA that could explain less cp1 bacterial-feeding nematodes in the soil food web.



Sup. figure 12. Total abundance of nematodes in the rhizosphere of four rice varieties (IR504, IR64, Azucena or Zhonghua 11) managed within a type of conventional tillage (CT) or conservation agriculture (CA) using the NEMAPLEX (**A**, trophic guilds), ELISOL (**B**, combined trophic and structural guilds) assignments. Asterisks indicate effects of the practices with a $p < .05$.

Optimizing the barcoding method

Because they are microscopic animals but almost visible to the eye, and because they are highly abundant in soils and essential for its functions, nematodes are morphologically and functionally well described. However, the microscopic method to study a whole nematode community is time-consuming and has poor resolution: only 32 families were counted in this study and did not allow to deeply describe the diversity within and between sample types compared to the molecular method with thousands unique ESVs for bacteria and fungi (**figure 25**). An amplicon barcoding approach would give high-throughput results and help to rapidly determine for example what combination of practices*variety would be more susceptible to PPNs. Different primers, targeting either 18S (NF1 and 18Sr2b) or ITS (ITS3F and ITS4R) regions of

rRNA gene, are being tested in the team but the limitation for their development remains in the efficiency of DNA extraction and on the genome complexity of nematodes. Indeed, because of genome duplications or heterozygosity, the abundances of some species are either overestimated or underestimated, and overly biased. This bias due to variation in gene copy number between species also exists in other communities but it can be avoided for example in the bacterial community by using a different gene marker such as *rpoB* (Ogier *et al.*, 2019) or by normalizing data with the *CopyRighter* tool (Angly *et al.*, 2014). This requires a sufficient database of reference genomes that is not accessible nowadays for nematodes and above all, no conservative region has been identified in nematodes that could easily discriminate against all species. Another approach would be to combine amplicon barcoding and metagenomic methods. Nonetheless, their functional characterization was powerful in our study: all nematode families were assigned to a trophic (**figure 28 C**) and a structural guild (**sup. figure 10 B**) that allowed the calculation of nematofaunal indices and the description of the whole food web (*i.e.* at all trophic levels).

Toward a specialization of the rhizosphere microbiota?

Studies showed that rice genotype is a key factor of the assemblage of the microbial community in rice (Hardoim *et al.*, 2011; Alonso *et al.*, 2020). In order to limit the disease in a field, the variety can indeed be seen as a key component in the agrosystem, because of the genotypic background it carries (for example *R* genes against *Meloidogyne* spp. in the Zhonghua 11 variety), and because of phenotypic traits they can express within certain abiotic conditions (enrichments of bacteria from the rhizosphere, the so-called "cry-for-help" strategy). With only four varieties were tested, our data showed that the rice variety had no significant effect on the β-diversity (structure and dispersion) of the rhizosphere communities, and little effect on the ***α***-diversity: the Shannon index of IR64 tended to be lower in the nematode community, and the richness was significantly higher for IR504 under CA in the bacterial community and globally for all varieties under CA, especially for Azucena, in the fungal community (**figure 26**). These variety effects on the microbial communities were dependent on the practices (**sup. table 8**) and that was also obvious on the enrichments (**table 6**). IR504 and IR64 had slightly more total enriched features under CA *versus* CT (58% and 55%, respectively). Azucena had a higher number of enriched features under CT *versus* CA (59% in total, 60% in the bacterial community, 67% in the fungal community and 50% in the nematode community). Conversely, Zhonghua 11 was the only variety that had a higher number of enriched features under CA *versus* CT in all communities (57% in total, 51% in the bacterial community, 100% in the fungal community and 83% in the nematode community). Specifically, two bacterial ESVs assigned to nitrate reduction (*Azospira* sp. and *Opitutus* sp.) were enriched with Zhonghua 11 under CA, whereas one ESVs assigned to a pathotrophic fungi (*Moeszimyces* sp.) and one family of plant-feeding nematodes (*Psilenchidae* sp.) were enriched with Azucena under CT (**figure 28**). Zhonghua 11 and Azucena are two *O. sativa* subsp. *japonica* that have contrasted phenotypic traits (roots of Azucena are more developed, as observed in the field) and bacteria could have different host preference (Wippel *et al.*, 2021). If we assume that plants can recruit microorganisms in the rhizosphere for their survival (*cf.* the **"cry-for-help" strategy** in **chapter 1**), Zhonghua 11 seemed more adapted for such a strategy under CA than under CT. However, these potential enrichments observed may not be an active process by the plants. It would be interesting to study their

capacity to attract microorganisms under CA or CT (compared to a bulk soil) and to measure phenotypes of plant growth and tolerance to PPNs with more varieties of diverse genetic backgrounds.

Potential antagonistic taxa of plant-parasitic nematodes

The biological basis of suppressiveness to soil-borne plant pathogens has been described in studies (Weller *et al.*, 2002). Classically, suppressiveness is classified into general suppression, which owes its activity to the total microbial biomass in soil and is not transferable between soils, and specific suppression, which owes its activity to the effects of individual or select groups of microorganisms and is transferable. Specific suppressiveness to plant-parasitic nematodes have been identified for example with the bacteria *Pasteuria penetrans* against *Meloidogyne incognita* and *M. javanica* in a seven-year monoculture of tobacco in a field naturally infested (Weibelzahl-Fulton *et al.*, 1996). Other antagonistic taxa against PPNs have been identified in fields and their mechanisms of action unraveled (**sup. table 9**). In our data, three of these microbial taxa were relatively enriched within either CA (*Bradyrhizobium*, $p < .05$, +36% and *Trichoderma*, $p < .05$, +183%) or CT (*Bacillus*, $p < .01$, +74%). *Bradyrhizobium* and *Trichoderma* could be potentially involved in soil disease suppressiveness of *Meloidogyne* spp. and *Hirschmanniella* spp. in this field by producing toxins and promoting ISR more efficiently under CA.

Sup. table 9. Non-exhaustive list of bacterial and fungal taxa native from the soil that are involved in antagonistic interaction against plant-parasitic nematodes with known mechanisms of action.

	Genus (Topalović *et al.*, 2020b)	**Species** (Silva *et al.*, 2018)
Bacteria	*Pseudomonas*	*Pseudomonas fluorescens*
	Bacillus	*Bacillus thuringiensis*
	Pasteuria	*Pasteuria penetrans*
		Pasteuria nishizawae
		Candidatus Pasteuria usgae
	Rhizobium	
	Streptomyces	
	Lysobacter	
	Arthrobacter	
	Variovorax	
Fungi	*Purpureocillium*	*Purpureocillium lilacinum (Paecilomyces lilacinus)*
	Pochonia	*Pochonia chlamydosporia*
	Trichoderma	*Trichoderma harzianum*
		Monacrosporium lysipagum

	Arthrobotrys	
	Dactylellina	
	Drechslerella	
	Mortierella	
	Haptocillium	
	Hirsutella	
	Catenaria	
	Dactylella	
	Nematophtora	

Within the nematode community, families of omnivorous nematodes were enriched under CA such as *Anatonchidae*, *Mononchidae* and *Qudsianematidae*. In the litterature, species of *Anatonchidae* have been described as predators of plant-parasitic nematodes such as *A. ginglymodontus* and *A. tridentatus* preying on *Meloidogyne hapla* and *A. tridentatus* preying on *Pratylenchus* spp. (Khan and Kim, 2007). Several of commonly occurring species of *Mononchidae* feed extensively, though not exclusively, on plant-parasitic and other nematodes. The prey preference for *Mononchoides gaugleri*, for example, is very high for *Meloidogyne incognita*, and relatively high for *Hirschmanniella oryzae* (Bilgrami, 2008). Concerning species of *Qudsianematidae*, they belong to the order *Dorylaimida* that is probably the most diverse of all nematode taxa and the most poorly studied (Ferris, 1999). However species such as *Laimydorus baldus* and *Discolaimus major* have been described to prey on *Hirschmanniella oryzae* (Bilgrami and Gaugler, 2005). Moreover, plant-parasitic nematodes in the order *Dorylaimida* are ectoparasites that could be competitors of other endoparasites. This hypothesis has been tested in the work by Garcia *et al.* (2008). They showed that high population densities of the original native communities of plant-parasitic nematodes have a limiting effect on the installation/invasion phase of *Meloidogyne chitwoodi*. Therefore, although the resolution of the microscopic method did not allow us to identify the nematodes at species level, several nematodes could have antagonistic effects against *Meloidogyne* spp. and *Hirschmanniella* spp. in the field under CA.

Further investigations should be done between the abundance of PPNs and potentially antagonistic taxa, and this requires validation of their mechanisms of action since correlations can be spurious. Generally, antagonism by competitive exclusion suggests a negative correlation (more antagonists is linked to less PPNs). However, predatorism seems more complex since there is a dependency of the predator for its prey. It suggests a positive correlation (more predatory is linked to more prey) but theoretically, its overgrowth will cause the prey population to collapse. Therefore, its own population will collapse in turn. It will give prey the opportunity to settle again in the niche and less predatory will be linked to more prey. Predatorism, similarly to parasitism, is then expected to trigger various types of association. In resource-consumer interactions indeed, population evolution can alter the dynamics of the interactions (Derocles *et al.*, 2017).

Moreover, in a disturbed soil, the presence of preys doesn't necessarily imply the presence of nematode predators since they are more susceptible to perturbations which might break the population dynamics (Abrams, 2003). A time series sampling would allow to see a potential population regulation or stabilization through time and identify which taxa could be involved, and subsequent direct antagonistic tests would unravel the specific mechanisms that could suppress the plant-parasitic nematodes.

Another approach is to create a cross-kingdom network of interaction. Because bacteria, fungi and nematodes compete within a similar niche in the soil food web of the rhizosphere, interactions between members of these phyla are likely to occur. Using next-generation sequencing is a promising way to link all organisms, from above and belowground, prokaryotes and eukaryotes, in the same network (Vacher *et al.*, 2016). Direct and indirect interactions can be represented with co-occurrence networks that provide a quantitative framework to unify the study of biodiversity and ecosystem function. However, the taxonomic observations of the nematode community have been acquired with a lower resolution than the fungal and bacterial communities and the trophic guilds for bacteria have been poorly assigned, which makes the network challenging to build. Moreover, as explained earlier, interpreting co-abundance patterns is not straightforward as complex oscillatory dynamics, indirect interactions or trophic cascades may alter the structure of co-occurrence networks (Derocles *et al.*, 2017). More focused work is needed to choose the right method of network learning and construction in order to understand the ecosystem stability and maturation of the soil food web with our data.

Beyond the disease regulation and other ecosystem services of CA

Soil is the essential component of food, energy and water security (Hatfield *et al.*, 2017). Soil properties (SOC, pH, available water capacity, *etc.*) are linked to soil functions (support for plants, source of water and nutrients, niche for organisms, *etc.*) that provide ecosystem services (food provisioning, disease regulation, *etc.*). However, soil degradation is driven by tillage (Bouthier *et al.*, 2014) and residue removal in modern agriculture. The benefits of conservation agriculture extend beyond reducing erosion to overall improvement in the soil resource capable of ensuring greater production and reduced degradation (Lal, 2015a and 2015b). In this chapter, we focused on the pathogen regulation through the use of no-till, rice variety and cover crops, but a more systemic approach is essential to assess all the benefits of CA. The reduction in PPN abundance depends also on the cover crop susceptibility. *Crotalaria* spp. and *Tagetes* spp. for example produce nematicidal compounds (Silva *et al.*, 2018) whereas others are susceptible. In addition to the disease regulation, CA also has a role in promoting biological activity that cascade up the soil food web and can have an impact up to the macrofauna (Henneron *et al.*, 2014) such as earthworms that feed on plant residue and bacteria, and directly serve as food resources for higher animal predators. Moreover, earthworms can create a soil porosity with a strong pore continuity important for water flow and saving. CA can bring many valuable services to humankind that would need more awareness and more policy advocacy for transition toward a less destructive agricultural system.

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Chapter 4

The effects of bacterial endophytes on rice infected by *Meloidogyne graminicola*

Preamble

After having characterized the reassembly of the rhizosphere communities of rice in a field potentially suppressive to the disease caused by plant-parasitic nematodes (*cf.* **Chapter 3**), we focus in this chapter on the potential phytobeneficial effects of bacterial endophytes native from this field, directly or indirectly against the root-knot nematode *Meloidogyne graminicola* (**figure 31**). We used a cultivable method in order to recover a diversity of strains and to characterize their interaction *in planta* with the rice *Oryza sativa* subsp. *indica* variety IR64 (**figure 30**), and *in vitro* against *M. graminicola*. The aim was not to assess the whole diversity of bacteria in the samples, but to assess the activities of selected bacterial endophytes 1) on the plant-parasitic nematode infection, and the plant development and tolerance in a controlled environment, 2) on plant-growth promotion traits, and 3) on the nematode motility by direct confrontation. First, we defined *in planta* variables that were affected by the nematode infection before to treat the plants with bacterial addition and to look for a compensation of the deleterious effects caused by the nematode, regardless of the bacteria inoculated. The biomass ratio (root mass/shoot mass) was interestingly anticorrelated with the gall density, bringing additional information on the aerial plant development. Then, we screened the effects of the single inoculations of selected candidate bacteria. Although we lacked statistical power on most variables with individual bacterial pretreatments and therefore we could not find significant effects of specific bacteria during this screening, we observed different patterns of plant response to the bacterial pretreatments, suggesting different strategies resulting in an increase of the plant tolerance to the disease caused by *M. graminicola*. We selected eight candidate strains for further investigations and confirmed plant-growth promotion traits *in vitro*. Antagonistic tests by direct confrontation with the nematode were promising on the nematostatic or nematicidal effect of two candidate strains. A complementary amplicon barcoding method was used in order to match the cultivable strains with taxa found in the root microbiota in the field and to obtain more information about their relative abundance and their correlation with the abundance of *M. graminicola* in roots. We found three strains positively correlated with a higher abundance of nematode juveniles in roots, requiring more research in order to explore their effect on the plant tolerance. This last chapter, which gathers experimental results from field, greenhouse and laboratory experiments collected since the beginning of my thesis, relates to the other chapters of this thesis and opens new perspectives to unravel the mechanisms of soil disease suppressiveness in the field under conservation agriculture in Stung Chinit.

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Préambule

Après avoir caractérisé le réassemblage des communautés rhizosphériques du riz dans un champ potentiellement suppresseur de la maladie causée par les nématodes phytoparasites (*cf.* **Chapitre 3**), nous nous intéressons dans ce chapitre aux potentiels effets phytobénéfiques des bactéries endophytes originaires de ce champ, directement ou indirectement contre le nématode à galles *Meloidogyne graminicola* (**figure 31** - résumé graphique pour le chapitre 4). Nous avons utilisé une méthode cultivable afin de récupérer une diversité de souches et de caractériser leur interaction *in planta* avec la variété de riz *Oryza sativa* subsp. indica IR64 (**figure 30**), et *in vitro* contre *M. graminicola*. L'objectif n'était pas d'évaluer la diversité totale des bactéries dans les échantillons, mais d'évaluer les activités des bactéries endophytes sélectionnées 1) sur l'infection par les nématodes phytoparasites, et le développement et la tolérance des plantes dans un environnement contrôlé, 2) sur les traits de promotion de la croissance des plantes, et 3) sur la motilité des nématodes par confrontation directe. Dans un premier temps, nous avons défini *in planta* les variables qui étaient affectées par l'infection du nématode avant de traiter les plantes avec un ajout de bactéries et de rechercher une compensation des effets délétères causés par le nématode, quelque soit les bactéries inoculées. Le ratio de biomasse (masse du système racinaire/masse du système aérien) s'est révélé être anticorrélé avec la densité des galles, apportant une information supplémentaire sur le développement du système aérien. Ensuite, nous avons examiné les effets des inoculations uniques des bactéries candidates. Bien que nous ayons manqué de puissance statistique sur la plupart des variables avec les traitements bactériens individuels et que nous n'ayons donc pas pu trouver d'effet significatif de bactéries spécifiques au cours de ce criblage, nous avons observé différents types de réponse des plantes aux traitements bactériens, suggérant différentes stratégies résultant en une augmentation de la tolérance des plantes à la maladie causée par *M. graminicola*. Nous avons sélectionné huit souches candidates pour des études plus approfondies et avons confirmé des caractéristiques de promotion de la croissance des plantes *in vitro*. Des tests antagonistes par confrontation directe avec le nématode ont été prometteurs sur l'effet nématostatique ou nématicide de deux souches candidates. Une méthode complémentaires par barcodage d'amplicons a été utilisée afin de faire correspondre les souches cultivables avec les taxons trouvés dans le microbiome racinaire sur le terrain et d'obtenir plus d'informations sur leur abondance relative et leur corrélation avec l'abondance de *M. graminicola* dans les racines. Nous avons trouvé trois souches positivement corrélées avec une plus grande abondance de juvéniles de nématodes dans les racines, nécessitant plus de recherche afin d'explorer leurs effets sur la tolérance de la plante. Ce dernier chapitre, qui rassemble les résultats expérimentaux obtenus sur le terrain, en serre et en laboratoire depuis le début de ma thèse, est lié aux autres chapitres de cette thèse et ouvre de nouvelles perspectives pour élucider les mécanismes de suppressivité des maladies sur le terrain cultivé en agriculture de conservation à Stung Chinit.

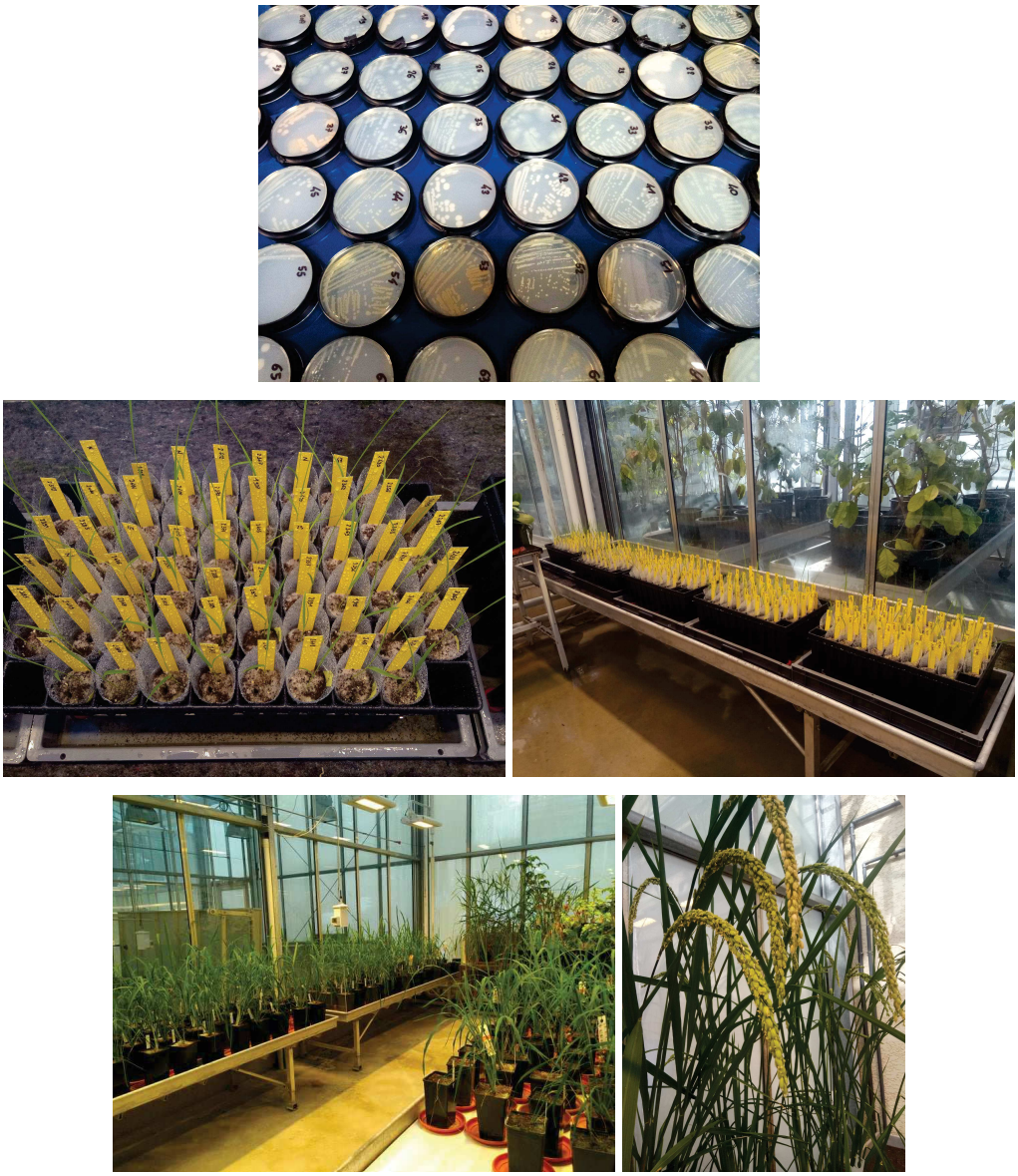

Figure 30.
Pictures of the bacterial isolates cultivated in Petri dishes (upper picture), of the screening test on rice plantlets (test n°1, middle pictures) and of the additional test on mature plants with the selected candidate bacteria (test n°2, lower pictures).

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Photos des isolats bactériens cultivés dans des boîtes de Pétri (photo du haut), du test de criblage sur les plantules de riz (test n°1, photos du milieu) et du test supplémentaire sur les plantes matures avec les bactéries candidates sélectionnées (test n°2, photos du bas).

Abstract

Plant-parasitic nematodes are a major threat for agriculture, especially *Meloidogyne graminicola* on rice, its main host and a staple food for the human population. *M. graminicola* colonizes rice by the roots and uses its resources, causing a reduction in the plant development and grain yield. Rice is also colonized by endophytes that can interfere by improving plant growth and by limiting the symptoms of the infection. Therefore, endophytes represent a sustainable solution to suppress the disease caused by *M. graminicola*, but little is known about the nature of bacterial endophytes in rice fields infested by *M. graminicola* and their strategies to suppress the disease. In this study, 68 rice endophytes native from a lowland field in Cambodia were collected and the biocontrol potential of 35 of them was assessed in greenhouse tests with the variety *Oryza sativa* IR64 susceptible to *M. graminicola*. We first measured the effect of the nematode infection and bacterial addition on the plant growth and infection in order to identify plant phenotypic traits related to the disease caused by *M. graminicola* and compensated by the bacterial inoculation such as the biomass ratio (root mass/shoot mass) which indicates an increased tolerance against the infection by *M. graminicola*. Then, results revealed that the bacterial pretreatments displaying similar plant phenotypic responses were grouped into four clusters, suggesting different phytobeneficial strategies. Eight candidate bacteria were selected for further *in vitro* tests. We found some plant-growth promotion activities (auxin production, siderophore production, tricalcium phosphate solubilization, *etc.*) and strong antagonistic activities against *M. graminicola*, notably with two strains (*Stenotrophomonas maltophilia* and *Pseudomonas kilonensis*). Also, by using an amplicon barcoding method, we found that three strains (*Burkholderia cepacia*, *B. contaminans* and *Novosphingobium humi*) were increasers associated with the abundance of *M. graminicola* in roots, suggesting several scenarios on the nature of their relationship with rice. Supplementary tests on plant yield and seed vigor, and further research on the mechanisms of interaction with the plant and within the root microbiota are required, but this study showed that bacterial endophytes have valuable potential in disease suppression of *M. graminicola* in rice fields.

Keywords: bacterial endophytes; *Meloidogyne graminicola*; rice; plant tolerance; nematicidal activity; disease suppression

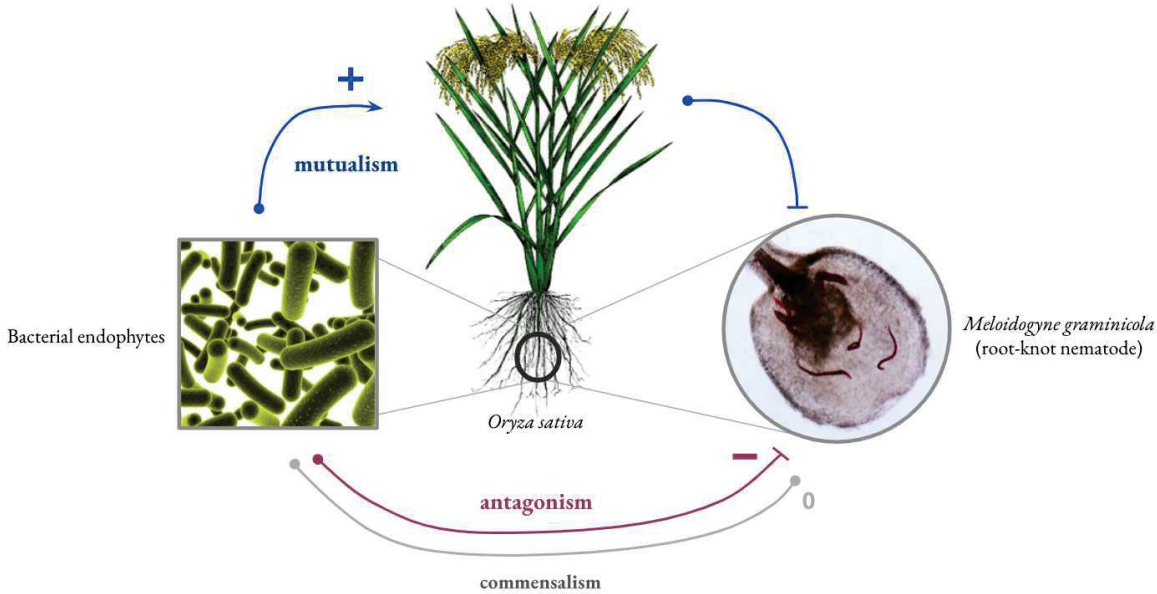

Figure 31. Graphical abstract for chapter 4. Direct (antagonism) and indirect (mutualism) phytobeneficial effects against the infection by *Meloidogyne graminicola* were measured by single inoculations of bacterial endophytes isolated from rice roots on plants later infected by the phytoparasitic nematode. Observed (or unobserved) effects may also result from other types of interactions with naturally associated microorganisms with rice or the nematode (*e.g.* commensalism).

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Les effets phytobénéfiques directs (antagonisme) et indirects (mutualisme) contre l'infection par *Meloidogyne graminicola* ont été mesurés par des inoculations uniques de bactéries endophytes isolées des racines de riz sur des plantes infectées par le nématode phytoparasite. Les effets observés (ou non observés) peuvent également résulter d'autres types d'interactions avec des micro-organismes naturellement associés au riz ou au nématode (*e.g.* commensalism).

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Introduction

Pests and phytoparasites are major threats to agriculture. Among them, we encounter more than 4,100 species of phytoparasitic nematodes (Decraemer and Hunt, 2006) and 24 genus have been reported to be associated with rice (Prot, 1994). Studies estimate that plant-parasitic nematodes are responsible for more than 80 billion $US losses in worldwide agriculture annually (Nicol *et al.*, 2011) and that at least 20% of these losses are related to rice production (Sasser and Freckman, 1987), notably due to the emerging root-knot nematode *Meloidogyne graminicola* (Mantelin *et al.*, 2017). The nematode infection by *M. graminicola* in rice is manifested by the presence of terminal hook-like galls on roots. It adversely affects uptake of nutrients and water and translocation of photosynthates and minerals (Williamson and Hussey, 1996). It leads to stunting and chlorosis of the rice plants that appear in uneven yellowing and dwarfing patches of infected plants within nurseries or main fields. The leaf size is reduced, newly emerged leaves appear distorted and crinkled along the margins, tillering is poor and earhead emergence is delayed. The plants show depletion in vigor, and higher susceptibility to biotic and abiotic stresses such as drought and other diseases (Kyndt *et al.*, 2017). Moreover, heavily infected plants flower and mature early and the earheads have poorly filled or no grains (Dutta *et al.*, 2012; Ravindra *et al.*, 2017). Consequently, it has a negative domino effect on crop yield (Jain *et al.*, 2012).

To limit their infection, fumigant nematicides were the first chemical agents to be used and were widely applied to high-value crops, like tobacco (Ebone *et al.*, 2019). These substances had the objective of sterilizing the soil, thereby removing any pests and phytoparasites, including nematodes. Therefore, a large portion of the fumigant nematicides is non-selective and can affect and reduce a broad range of non-target organisms such as antagonists or free-living nematodes that are essential for plant health and soil functions. The fumigant nematicides were banned or are currently being withdrawn in many countries due to their adverse effects on health (Oka *et al.*, 2020). New-generation nematicides were developed and made available on the market. However, these synthetic substances can still be toxic to non-target organisms, especially to aquatic organisms (like fluensulfone for which the mode of action is unclear) and even to plants themselves (Oka *et al.*, 2020). Extensive use of synthetic pesticides contaminates soil, remains in

crops, leaches to the groundwater and pollutes the whole food chain (Rasmussen *et al.*, 2015). It has major adverse impacts on ecosystems, especially in developing countries (Sarkar, 2021). In South-East Asia, farmers heavily depend on synthetic pesticides as their main method of pest control and tend to overuse them (Schreinemachers *et al.*, 2017; Schreinemachers *et al.*, 2020). Besides, maximum residue limits, *i.e.* the maximum concentrations of pesticide residues to be legally permitted in food commodities and that are required for importation, force farmers, especially rice producers, to turn toward other approaches of pest and phytoparasitic management (Lopes *et al.*, 2019).

Alternative solutions such as the use of biopesticides (biological pesticides) are promoted. They are pesticides based on living organisms such as plants, animals, and microorganisms including fungi, bacteria, viruses or protozoa (Essiedu *et al.*, 2020). Recently, the use of endophytes as biopesticides is drawing special attention as an attractive option for management of some plant diseases, resulting in minimal impact to the environment (Liu *et al.*, 2017; de Silva *et al.*, 2019; Gao *et al.*, 2020). While most, if not all, synthetic pesticides are neurotoxic, endophytes have other modes of action including direct or indirect effects that are phytobeneficial: pest antagonism such as parasitism, predation or competition, and promotion of host growth, defenses and tolerance to stresses. Biopesticides can be industrially produced and made available in chemical products (Wilson and Jackson, 2013; Ebone *et al.*, 2019; Seong *et al.*, 2021). Although evidence shows their effectiveness against a vast range of crop pests, misapplication of biopesticides may result in no significant or adverse effects on the environment. Moreover, producing microbial pesticides at an industrial scale is not always reachable and their use is not always affordable by the farmers. Therefore, the first limitation remains the farmer's dependency on commercially available products.

A more sustainable strategy is to build soil disease suppressiveness to phytoparasitic nematodes (Silva *et al.*, 2018). Promoting microbial diversity in the field by regenerative agriculture is suggested as the key to preserve plant and soil health (Berg *et al.*, 2017; Giller *et al.*, 2021). A diversity of phytobeneficial and/or endophytic organisms to limit the disease by phytoparasitic nematodes have indeed been naturally found (Siddiqui and Shaukat, 2003a; Stirling *et al.*, 2015; Topalović *et al.*, 2020). In rice, fungal endophytes have been identified and their mechanisms against plant-parasitic nematodes are mostly associated with the endophyte-plant mutualism (Le *et al.*, 2009; Sikora *et al.*, 2008). However, how bacterial endophytes can interfere with the infection by plant-parasitic nematodes is not well described. In this study, we hypothesized that rice bacterial endophytes from the same root microbiome can simultaneously improve plant tolerance to the infection by the root-knot nematode *M. graminicola* and directly antagonize it. We used a cultivable method to isolate bacterial endophytes collected in a field in Cambodia under conservation agriculture that is suppressive to plant-parasitic nematodes compared to under conventional tillage (Masson *et al.*, submitted). We assessed their effects on the rice *Oryza sativa* variety IR64 susceptible to *M. graminicola* in greenhouse assays by measuring the *in planta* effects upon 35 bacterial pretreatments on the signs (number of galls and infective juveniles) and symptoms (growth and reproductive traits) of the infection to look for an increased plant tolerance and a reduced infection. Eight bacterial endophytes representing the diversity of the isolates were tested *in vitro* to look for plant-growth promotion activity and direct antagonism against *M. graminicola*. We also used an amplicon barcoding method to link bacterial isolates with the suppression of *M. graminicola* in the field.

Material & methods

Plant material sampling and bacterial endophyte cultivation

Sampling was done one month after sowing (May 1, 2018) in a lowland rice field in Stung Chinit, Cambodia (12°32′55″ N - 105°08′47″ E) that is known to have been conducive to the infection by plant-parasitic nematodes (Suong *et al.*, 2019) and that potentially turned suppressive against the disease caused by *Meloidogyne graminicola* under a type of conservation agriculture (Masson *et al.*, submitted). Roots belonged to different varieties of *O. sativa* (IR504, IR64, Azucena and Zhonghua 11). To obtain a high diversity of cultivable bacteria while restricting the number of samples to analyze, we focused on bacteria associated with conservation agriculture under which we assumed that the higher labile carbon (Suong *et al.*, 2019) should have promoted microbial activity and biodiversity. We also focused on IR64 under conventional tillage because it is an agronomically improved variety but susceptible to *M. graminicola* (Nguyen *et al.*, 2021). For the cultivable recovery of bacterial endophyte, one three-centimeter root tip of five plants were pooled together to constitute composite samples, done in four replicates for each of the five conditions (all four varieties under conservation agriculture + IR64 under conventional tillage). Samples were immediately placed in plastic bags, transported to the laboratory then stored at 4°C. For the bacterial endophyte isolation, a surface disinfection of the roots was done in a solution of sodium hypochlorite (3.2%) for 1 min followed by five successive washes in sterilized water for 20 min the first, then 5 min the others. The roots were grinded in sterile water with a sterile ceramic bead using a FastPrep-24™ for 40 sec at frequency 6 m/sec. Solutions of crushed surface-disinfected roots were centrifuged at 80 rcf for 1 min to remove plant debris, diluted to a dilution factor of 10^{-3} and plated on Petri dishes containing TSA culture medium (Merck KGaA, Darmstadt, Germany) at low concentration (10%) and cycloheximide (200 mg/l) to avoid bacterial bloom. After incubation at 28°C for 48h, colonies as diverse as possible based on visual shape, opacity and color were manually picked up, isolated on TSA 50% and reincubated.

Bacterial endophyte sequencing and identification

The *16S rRNA* genes of the purified bacterial isolates were amplified by colony PCR with the universal couple of forward FGPS6 (5'-GGAGAGTTAGATCTTGGCTCAG-3') and reverse FGPS1509 (5'-AAGGAGGGGATCCAGCCGCA-3') primers (Sy *et al.*, 2001). The PCR amplifications were performed in 25 µl final reaction mixture in sterile water containing DreamTaq Buffer (5X), 2.5 mM of each dNTP, 0.4 µM of each primer and 0.125 µl of DreamTaq DNA Polymerase (Thermo Fisher Scientific). Bacteria were added by touching a colony with a sterile toothpick directly soaked into the PCR reaction mixture. The PCR thermocycler performed an initial denaturation at 94°C for 2 min, 35 cycles of denaturation, annealing, and elongation at 94 °C for 30 sec, 56°C for 30 sec, and 72°C for 30 sec, respectively, and a final elongation step at 72°C for 10 min. To check the amplicon size (1,480 bp), they were visualized under UV light after gel electrophoresis (1% agarose, TAE buffer, 100 V for 25 min) and staining in a bath containing 0.5 µg/ml of ethidium bromide for 5 min followed by a bath in clear water for

10 min. The PCR products were sent to *Macrogen* (Seoul, South Korea) for sequencing. A total of 478 bacteria were sequenced. The chromatograms were manually corrected if needed and the first best match by BLAST (NCBI database "rRNA_typestrains/16S_ribosomal_RNA") was assigned. Based on these assignments, we kept at least one isolate representing the diversity of all the species by cultivating the original colonies in TSA 50% for 16 h under agitation (200 rpm) at 28°C and mixing them with 60% of sterile glycerol in collection tubes. Thus, a collection of 68 bacterial endophyte strains was stored at -80°C for following assays (**sup. table 10**)

In planta tests of individual bacterial pretreatments with *M. graminicola*

Based on a broad literature review looking for evidence of plant association and/or beneficial effects, we selected 35 bacteria from the collection (excluding known or suspected opportunistic human pathogens) in order to screen the effects of individual bacterial pretreatments on rice infected by *M. graminicola*. We intended to select bacteria as diverse as possible based on their assignment and their phylogenetic distribution on a tree built with *MEGA X* software (Kumar *et al.*, 2016). Sequences were first aligned and trimmed to a total of 350 positions. The evolutionary history was inferred by using the maximum likelihood method (1,000 bootstrap replications) based on the Kimura 2-parameter model (Kimura *et al.*, 1980). The tree with the highest log likelihood (-2,163.37) was saved.

Test n°1: screening test of 35 candidate bacteria on rice plantlets

Seeds of *O. sativa* subsp. *indica* variety IR64 were sowed at 0.5 cm depth in 286 conical pots (two seeds per pot) containing 100 ml of a mixture (7:3 in volume) of silica (Sibelco™) and compost (Jiffy™ M2). The compost was previously sieved through a mesh (diameter = 0.5 cm) to facilitate its removal from the roots after dumping. The strongest plantlets showing homogenized germination were kept. Bacterial strains from the collection were grown on TSA 50% then in TSB 50% at 28°C for 48h then over the night under agitation at 200 rpm, respectively. Broth cultures were centrifuged at 185 rcf for 10 min and washed with sterile osmosis water. Six days after sowing (plants measured 5-10 cm high), 1 ml of bacterial culture at OD (600 nm) = 0.8 was randomly inoculated at the base of the plantlets in three or four replicates. Five days later, 2 ml containing 170 infective juveniles of *M. graminicola* (VN18, isolated by Bellafiore and colleagues, 2015) were inoculated at the base of the plantlets. Nematodes had been extracted 10 days before from roots of IR64 following the protocol of Bellafiore *et al.* (2015). To drain the nematodes in the soil, eight ml of water were added in each pot. Sterile osmosis water replaced the cultures of bacteria or nematodes for the mock inoculations ("mock bacterial pretreatment" and "mock nematode treatment"). Plants were grown at 26°C by day and 24°C by night, with 80% of relative humidity and 12 h of white light per day, and regularly watered with similar volumes. To avoid border effects, pots were moved every two or three days. Measurements were done on the 25 days-old plants (plants measured about 25 cm): root and shoot systems were dried in an oven at 42°C for 1 week and weighted, photosynthetic activity (performance index; Pi) was measured with a fluorometer meter (PEA, Hansatech™), the number of galls in the entire root system and at the base (galls ≥ 1 mm in the area ≤ 2 cm around the stem emergence) were counted after plant removal and cleaning of the root system in water.

Test n°2: additional test of selected candidate bacteria on rice yield and quality

To measure variables linked to the rice yield and quality of seed produced by rice infected by *M. graminicola*, the same protocol was applied with selected candidate bacteria in pots containing 2 l of the silica:compost mixture, in four to five replicates. The photosynthetic activity was measured at three dates during the vegetative stage (55, 63 and 69 days after the infection nematodes) and averaged. Four months after sowing, the number and total mass of the panicles were measured. The seeds were harvested and weighted. Root and shoot systems were also dried and weighted. The nematodes were extracted from the roots and counted under a microscope.

In vitro test of eight endophyte strains

We performed *in vitro* tests to look for PGP effects by growing candidate bacteria (**table 7**) on culture media. Siderophore production was measured on a TSA medium containing chrome azurol S (Schwyn and Neilands, 1987). Note that this medium is toxic to Gram positive bacteria. The development of a yellow, orange or violet halo indicated that the bacteria was a siderophore producer. Phosphate solubilization was measured on a medium containing tricalcium phosphate (Gupta et al., 1994) and PVK (Pikovskaya, 1948) media. Bacteria was considered solubilisant if there was a halo of solubilization on both media. Catalase activity was measured by emerging a colony in a drop of peroxide hydrogen. An effervescence, visible by the formation of bubbles, indicated a positive result. Auxin (indole-3-acetic acid) production from L-tryptophan was estimated by colorimetric method (Gordon and Weber, 1950).

We also performed *in vitro* tests to look for antagonistic activity of eight bacterial strains against *M. graminicola* by direct confrontation. The test was performed in five technical replicates and three biological replicates. In 12-well microplates, on the top of each well, a sieve of 10 µm was inserted, allowing only motile juveniles to pass through it and to drop at the bottom of the well. Above each sieve, 1 ml of the solution of nematodes and 2.5 ml of the solution of bacteria were added to obtain 3.5 ml of final solution at 100 J2/ml and OD (600 nm) = 0.8 for bacteria. The plates have been incubated at 28°C for 48 h. Nematodes in the filtrate that passed through the sieve were counted. In order to do so, the sieve was carefully removed with a wrench, the filtrate was homogenized with a pipette and 1 ml was poured in a counting cell. All nematodes were counted on ⅛ of the counting cell under the microscope. To identify what fraction of the bacteria was active, we tested bacterial cells in water, in the initial and diluted supernatant, and with the addition of proteinase K (final concentration at 0.1 and 1 mg/l). To identify if the activity was nematostatic (reversible) or nematicidal, after direct confrontation with the bacteria, the remaining nematodes above the sieve were placed in new wells with 3.5 ml of water. Recovered motile nematodes were counted 24 h later in the filtrate.

Data analysis

Analyzes were performed using *R* software, version 4.0.3 (*R* Development Core Team, 2020). The collection of packages *tydiverse* (Wickham *et al.*, 2019) was used to handle and represent data. The packages *car* (Fox *et al.*, 2020), *lsr* (Navarro, 2015), *rstatix* (Kassambara, 2021) and *agricolae* (de Mendiburu, 2021) were used for the statistical analysis. Shapiro and Levene tests were used to check data normality and homoscedasticity, respectively. To analyze the nematode infection (mock bacteria pretreatment + nematode treatment *versus* mock bacterial pretreatment + mock nematode treatment) and the bacterial addition (bacterial pretreatment + nematode treatment *versus* mock bacterial pretreatment + nematode treatment), a t-test (with a Welch correction for heteroscedastic data) or a Wilcoxon test were used for parametric or non-parametric data, respectively. To analyze the bacterial pretreatments (individually or by cluster), a one-way anova or Kruskal test were used for parametric or non-parametric data, respectively. Additionally, post-hoc tests were done using a pairwise t-test or a Dunn test, respectively, with a method for adjustment of the *p*-value (false discovery rate). A Tukey test was used to obtain groups of significance shown on the graphs. The *d* of cohens was used to calculate size effect. The packages *FactoMineR* (Husson *et al.*, 2020) and *factoextra* (Kassambara and Mundt, 2020) were used to draw the PCA. The package *Hmisc* (Harrel, 2021) was used to calculate and draw the matrix of correlations. The package *pheatmap* (Kolde, 2019) was used to cluster bacterial pretreatments. The script written on *R* software (version 4.0.3, *R* Development Core Team, 2020) to make the analysis and generate the figures is available on GitLab under the project ID 29546592 (cultivable_montpellier_2021).

Amplicon barcoding and sequences processing

In parallel to the cultivable method, the root sampling was similarly done in the field in all conditions (conservation agriculture and conventional tillage for all four varieties) to analyze the bacterial community by amplicon barcoding. The composite root samples of three-centimeter root tips of five plants were washed with sterile water to remove the rhizospheric soil attached to the roots. They were grinded in liquid nitrogen in a sterile mortar and DNA was extracted from 15 mg of powder of root tissues using the PowerSoil® DNA Isolation Kit (Qiagen, Netherland) following the manufacturer's instructions. Samples were pooled contributing exactly the same amount (50 ng/µl) of DNA in the final library. PCR amplification, library and MiSeq Illumina sequencing were performed by *Macrogen* (Seoul, South Korea) using primers 341F (16S_V3F, 5'-CCTACGGGNGGCWGCAG-3') and 805R (16S_V4R, 5'-GACTACHVGGGTATCTAATCC-3') to amplify the V3 and V4 hypervariable regions of the *16S rRNA* gene. *QIIME 2* bioinformatic platform (Bolyen *et al.*, 2019) was used to obtain exact sequence variants (ESVs) abundance table and taxonomy according to the processing in Masson *et al.* (submitted). We performed a BLAST of the endophyte sequences against the obtained ESVs in order to analyze the prevalence and relative abundance of the cultivable bacteria in roots. We also identified the ESVs that exhibited significant increases (*i.e.* increasers) or decreases (*i.e.* decreasers) associated with the abundance of *M. graminicola* in roots extracted for the field experiment (Masson *et al.*, submitted), by using the package *TITAN2* (Bakker *et al.*, 2020) with the arguments "minSplt = 5, numPerm = 250, nBoot = 500".

Results

Effects of the nematode infection

The effects of the infection by the plant-parasitic nematode *Meloidogyne graminicola* on the rice *Oryza sativa* were first measured during test n°1 on plantlets 25 days after sowing. The infected plantlets (mock bacterial pretreatment + nematode treatment) clustered at the opposite side of the non-infected plantlets (mock bacterial pretreatment + mock nematode treatment) on a PCA (**figure 32 A**) and were characterized by modifications of the growth and development (**figure 32** from **B** to **H** and **sup. table 11**): the infected plantlets presented galls, both systemically ($p < .01$, +100%) and locally at the base of the root system ($p < .001$, +100%) and, consequently, had an increased gall density ($p < .01$, +100%) compared to the non-infected plantlets with no sign of the infection. *M. graminicola* infection also reduced the biomass ratio (root/shoot) ($p < .05$, -25%) and the photosynthetic activity (Pi) ($p < .05$, -45%).

Correlations were found between these variables (**figure 33**). The total number of galls was positively correlated with the shoot mass ($p < .001$, $r^2 = 0.31$), with the root mass ($p < .01$, $r^2 = 0.26$) and therefore with the total biomass ($p < .001$, $r^2 = 0.32$). The total number of galls was not significantly and directly correlated with the photosynthetic activity ($p = 0.5$ and $r^2 = 0.07$) but the photosynthetic activity was positively correlated with the biomass ($p < .001$, $r^2 = 0.34$ with the root mass and $p < .001$, $r^2 = 0.49$ with the shoot mass). The gall density was negatively correlated with the total biomass ($p < .001$, $r^2 = -0.55$), especially with the root mass ($p < .001$, $r^2 = -0.66$) but also with the shoot mass ($p < .001$, $r^2 = -0.39$), therefore with the biomass ratio ($p < .001$, $r^2 = -0.42$), and finally with the photosynthetic activity ($p < .01$, $r^2 = -0.27$).

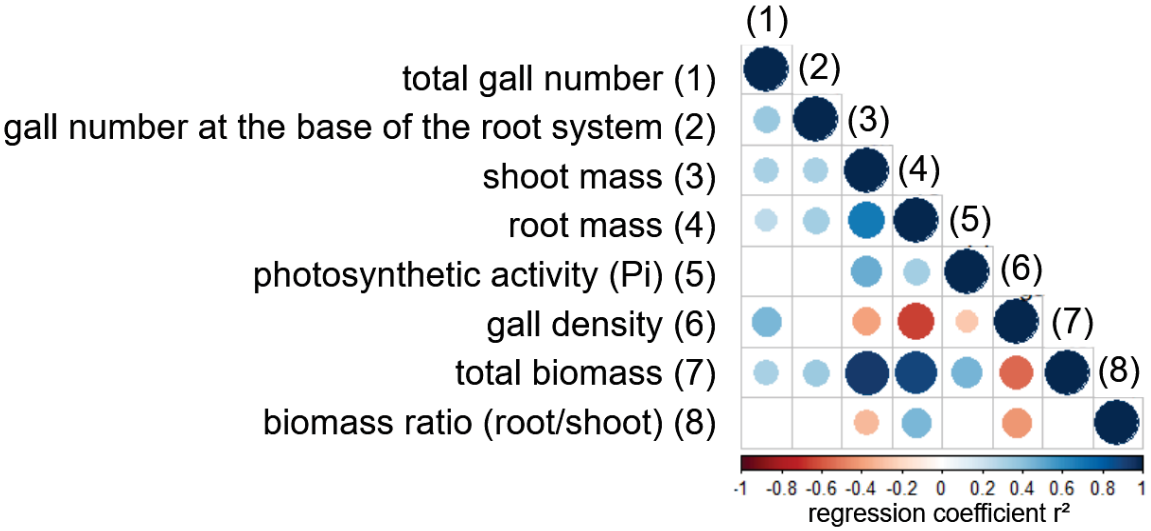

Figure 33. Matrix of correlations based on variables measured on plantlets infected by the nematode *M. graminicola* and inoculated by bacterial endophytes. The point size indicates the significance: for all $p < .05$, the size point increases with the significance. The color indicates the sign of the correlation (red for negative, blue for positive).

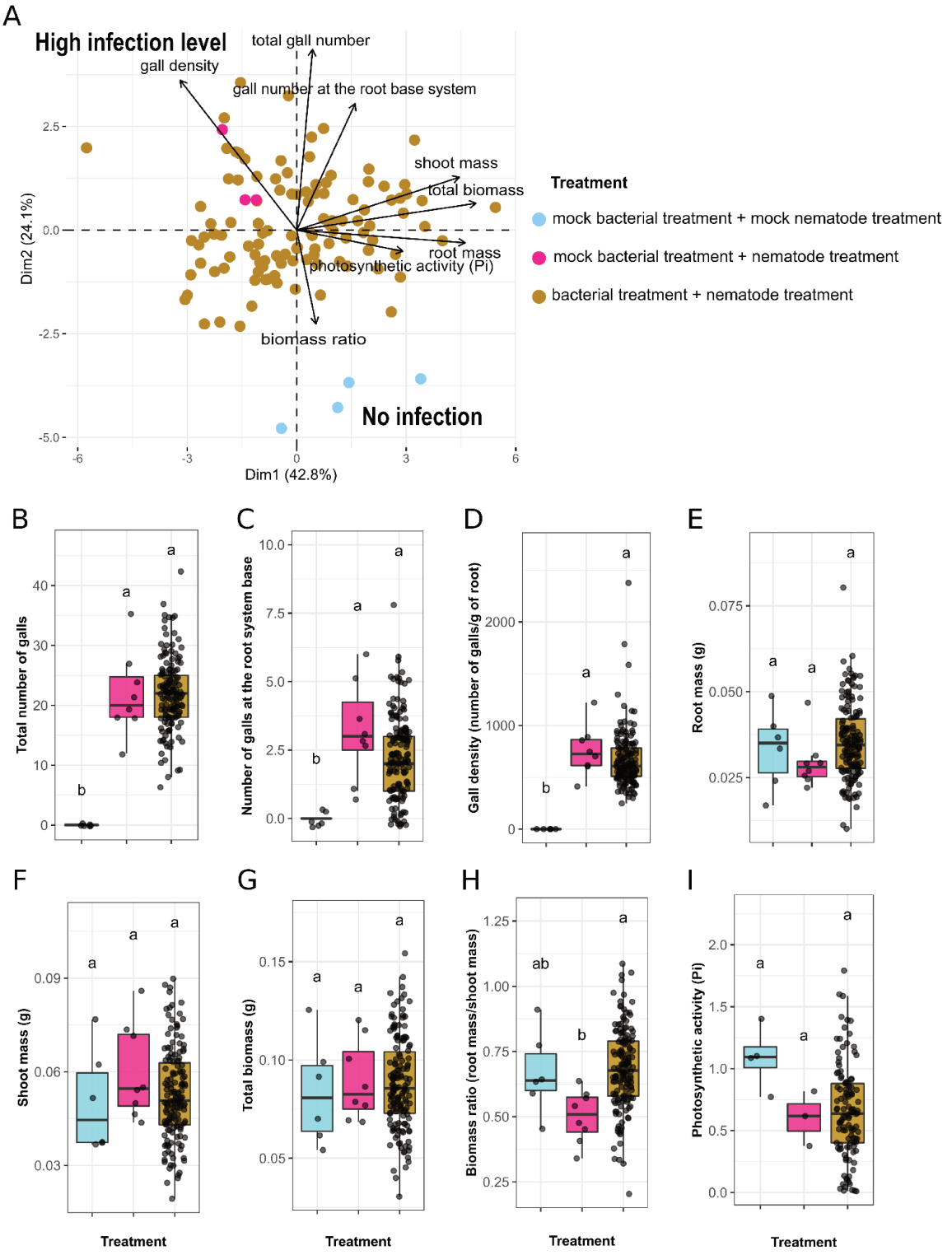

Figure 32. PCA and boxplots of rice plantlets to look for effects of the infection by *M. graminicola* (mock bacterial pretreatment + nematode treatment *versus* mock bacterial pretreatment + mock nematode treatment) and of the addition of bacterial endophytes (bacterial pretreatment + nematode treatment *versus* mock bacterial pretreatment + nematode treatment) in a screening test (test n°1). Mock (pre)treatments were done using sterile water.

The effects of the nematode infection were also measured during the test n°2 (**sup. table 11** and **sup. figure 14**) on mature plants until seed harvesting. The infected plants (mock bacterial pretreatment + nematode treatment) had an increased number of nematodes per root mass ($p < .001$, +100%), and lower root ($p < .05$, -81%) and shoot ($p < .001$, -66%) masses compared to the non-infected plants (mock bacterial pretreatment + mock nematode treatment). Their photosynthetic activity tended to decrease ($p = .90$, -53%). They produced fewer ($p < .05$, -61%) and lighter ($p < .01$, -51%) panicles that harbored less seeds ($p < .01$, -93%) which had a lighter total mass ($p < .01$, -93%).

Effects of the bacterial endophyte inoculation on infected rice

The effects of the bacterial addition was assessed in the test n°1 with 35 bacterial strains on plantlets (**figure 32** and **sup. table 11**). The bacterial addition (bacterial pretreatment + nematode treatment *versus* mock bacterial pretreatment + nematode treatment) had inverted effects on several variables compared to the nematode infection. In particular, the bacterial addition was characterized by an increased biomass ratio ($p < .001$, +37%) that reached the level of non-infected plantlets. Although not significant, it was associated with an increased root mass ($p = .073$, +20%) and a decreased shoot mass ($p = .217$, -12%). We also observed tendencies to minimize the effects of the nematode infection with the bacterial addition: a reduction of the gall number at the root base system ($p = .136$, -28%) and of the gall density ($p = .177$, -12%). In the test n°2 with fewer candidates (**sup. figure 14** and **sup. table 11**), the bacterial addition also tended to minimize effects of the nematode infection, although it was not significant: the number of juvenile nematodes related to the root mass ($p = .417$, -37%), the shoot mass ($p = .809$, +42%), the photosynthetic activity ($p = .394$, +52%), the number of panicles ($p = .364$, +36%) and the total mass of seeds ($p = .145$, +137%).

In planta assessments of bacterial candidates on the nematode infection

Variables that mostly differentiate the bacterial pretreatments were linked to the biomass and to the gall number (**figure 33 A**). The total biomass and gall density each contributed to more than 15% of the dimensions 1 et 2 combined, especially more than 35% for the total biomass in dimension 1 and almost 30% for the gall density in dimension 2. Some plantlets had relatively high contributions to the dimensions, especially ones inoculated by the bacteria n°2423, n°2419, n°2388, n°2366 and n°2392 (contribution > 2.5%). However, plantlets were not clearly clustered by bacterial endophytes. Due to the low number of replicates, there was a high variance within the bacterial pretreatments and therefore, no significant effect could be found individually compared to the mock bacterial pretreatment, except for the biomass ratio ($p < .001$, effect size > 0.20, **sup. table 11** and **sup. figure 13**). Nonetheless, some strong tendencies were observed for several bacteria.

In particular, the bacteria n°2369 ($p < 0.001$, +73%) and n°2399 ($p < 0.001$, +65%) had higher biomass ratios compared to the mock bacterial pretreatment (**sup. figure 13**). It was mainly due to the root mass that tended to increase ($p = 0.367$, +51% for the bacteria n°2369, $p = 0.472$, +47% for the bacteria n°2399) whereas the shoot mass tended to decrease ($p = 0.790$, -12% for the bacteria n°2369, $p =$

0.770, -13% for the bacteria n°2399). Some other bacteria also tended to increase the root mass like the bacteria n°2405 (p = .368, +67%), n°2421 (p = .368, +63%) and n°2391 (p = .476, +62%), whereas the total number of galls was maintained (p = .700). As a consequence, the gall density tended to decrease (p = .508, -39% for the bacteria n°2405, p = .753, -34% for the bacteria n°2421, p = .752, -28% for the bacteria n°2391). Conversely, the bacteria n°2418 tended to have the highest gall density (p = .900, +99%) and tended to decrease root (p = .954, -24%) and shoot (p = .745, -28%) masses, maintaining a biomass ratio similar than the mock bacterial pretreatment (p = .990, -5%).

Using a matrix of the variables measured on plantlets (**figure 34 C**), we obtained a clustering of the bacterial pretreatments. On the PCAs (**figure 34 A** and **B**), we could observe a first cluster which showed a similar pattern than the infected plantlets (nematode treatment in the upper left corner as in **figure 33 A**), a second cluster mainly characterized by a decrease of all variables, a third cluster mainly characterized by an increased biomass, especially roots, and which showed a similar pattern than the non-infected plantlets (mock nematode treatment in the bottom right corner as in **figure 34 A**), and a fourth cluster showing no strong or specific pattern. Effects of clustered bacterial pretreatments were found (**figure 34 D** to **K** and **sup. table 11**): the cluster n°1 (including the mock bacterial pretreatment) showed a lower biomass ratio compared to all the other clusters (p < .001), a higher gall density than clusters n°2 (p = .070, +35%) and n°3 (p < .01, +59%), and a lower root mass than clusters n°3 (p < .001, -37%) and 4 (p < .001, -20%); the cluster n°2 (including the bacteria n°2357 and n°2372) showed fewer galls at the root base system compared to all other clusters (p < .001) and lower root (p < .001, -29%) and shoot (p < .01, -22%) masses compared to the cluster n°3; the cluster n°3 (including the bacteria n°2399 and n°2405) showed a lower gall density compared to the cluster n°1 (p < .01, -37%) and a higher root mass compared to all the other clusters (p < .01); the cluster n°4 (including the bacteria n°2370, n°2388, n°2409 and n°2413) showed any extreme mean value for these variables, but a higher root mass than the cluster n°1 (p < .05, -26%).

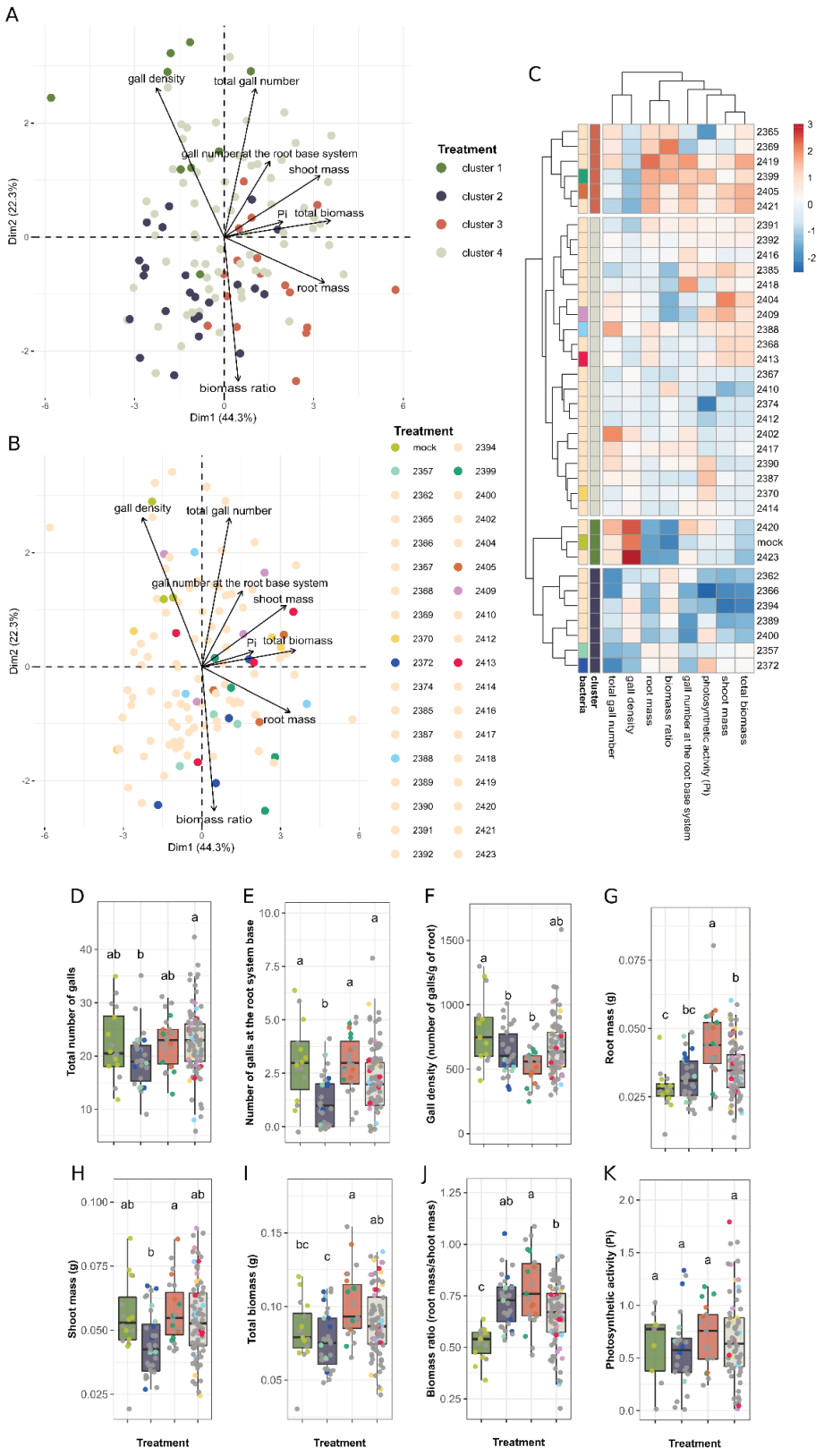

Figure 34. PCA (**A** and **B**), heatmap (**C**) and boxplots (from **D** to **K**) of plantlets to look for effects of the pretreatment of individual bacterial endophytes grouped by cluster during test n°1. Plantlets were all infected by the nematode *M. graminicola*. Clustering of bacteria sharing similar plant phenotypic traits was done based on the dendrogram of the mean values for each variable.

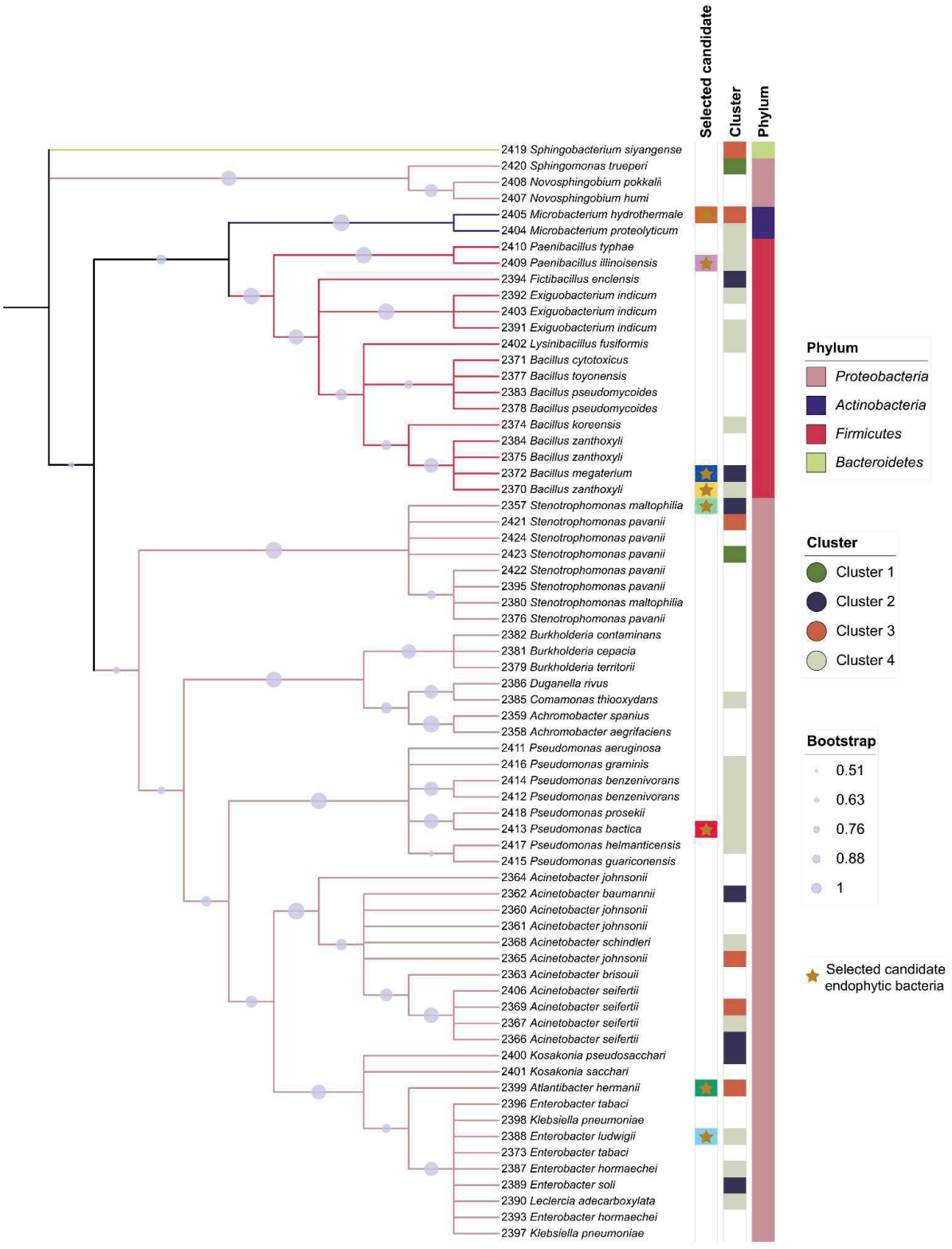

Figure 35. Phylogenetic tree based on the sequencing of the *16S rRNA* gene of the endophyte bacteria strains in collection. The ones marked by a star were the candidates selected for further tests.

In vitro tests of bacterial candidates

We focused on eight selected candidate bacteria displaying diverse and strong effects on the plant (**sup. table 12**) for further tests. The candidates were strains of *Stenotrophomonas maltophilia* (n°2357, *Proteobacteria*, cluster n°2), *Bacillus zanthoxyli* (n°2370, *Proteobacteria*, cluster n°4), *Bacillus megaterium* (n°2372, *Proteobacteria*, cluster n°2), *Enterobacter ludwigii* (n°2388, *Proteobacteria*, cluster n°4), *Atlantibacter hermannii* (n°2399, *Proteobacteria*, cluster n°3), *Microbacterium hydrothermale* (n°2405, *Actinobacteria*, cluster n°3), *Paenibacillus illinoisensis* (n°2409, *Firmicutes*, cluster n°4) and *Pseudomonas baetica* (n°2413, *Proteobacteria*, cluster n°4). They were distributed in three out of four phyla present in the collection of bacterial endophytes, and all clusters except for the cluster n°1 that include the mock bacterial pretreatment (**figure 35** and **sup. table 10**).

To disentangle the mechanisms involved during the interaction between the plant, the bacteria and the nematode, we performed *in vitro* tests to look for plant-growth promotion traits (**table 7**). All bacteria harbored a catalase activity and were able to produce auxin from L-tryptophan. The strains of *Enterobacter ludwigii* (n°2388) and *Pseudomonas baetica* (n°2413) were able to produce siderophores and to solubilize tricalcium phosphate. The strain of *Atlantibacter hermannii* (n°2399) was also able to solubilize tricalcium phosphate. By direct confrontation with the nematode (**figure 36 A**), we found that the strains of *Stenotrophomonas maltophilia* (n°2357) and *Pseudomonas baetica* (n°2413) decreased the nematode motility ($p < .001$, -84% and -66%, respectively). Focusing on this latter antagonistic effect (**figure 36 B**), we found that the supernatant isolated from the cells still exhibited a strong activity (-97%). Successional dilutions of the supernatant gradually decreased the antagonistic activity (by 32% with a dilution factor equal to five and by 71% with a dilution factor equal to ten). Treatments with proteinase K did not totally inactivate the antagonistic activity, even at high concentration: the nematode motility was still reduced by 28% with 1 g/l of proteinase K. Finally, treatments with water did not allow the recovery of the juvenile motility, even in the diluted supernatants that exhibited lower antagonistic activity (**figure 36 C**).

Table 7. Characterization of the candidate bacteria for some *in vitro* PGP effects.

Bacteria	Cluster	Gram	Siderophore production	Tricalcium phosphate solubilization	Catalase	Auxin production (µg / ml)
n°2357	2	-	+	-	+	40.13
n°2370	4	+	NA	-	+	10.38
n°2372	2	+	NA	-	+	21.80
n°2388	4	-	+	+	+	74.17
n°2399	3	-	-	+	+	53.80
n°2405		+	NA	-	+	63.08
n°2409	4	+	NA	-	+	22.95
n°2413		-	+	+	+	22.71

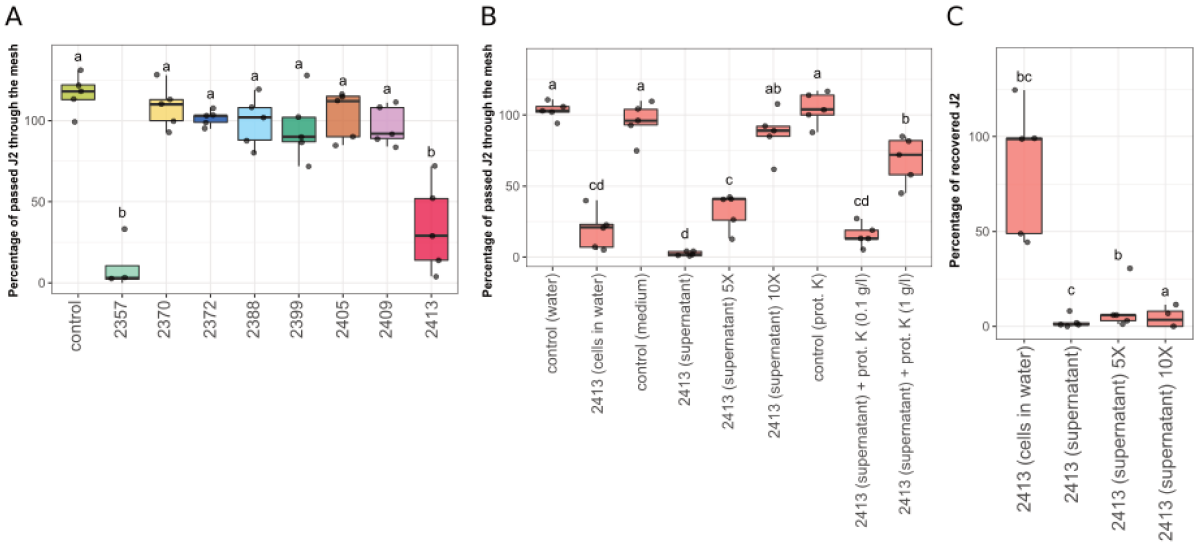

Figure 36. Boxplots of the percentage of nematodes that passed through a mesh 48 h after direct confrontation with the selected candidate bacteria (**A**), with different treatments of the bacteria n°2413 (**B**) and after recovering in water for 24 h (**C**).

Link between the cultivable bacteria and the abundance of PPNs in roots

For each cultivable bacteria, we matched their sequence with an amplicon barcoding dataset from roots samples (**sup. table 10**). We found a positive correlation between the abundance of *M. graminicola* in the roots in the field and three endophytic strains of the collection: *Burkholderia cepacia* and *B. contaminans* (R = 0.46, 100% identity), and *Novosphingobium humi* (R = 0.21, 100% identity) (**figure 37**). The ESVs had a relative abundance in roots of 0.12% and 0.17%, respectively, were present in half of the 32 samples, and were present under conservation agriculture with a relative abundance of 0.10 % and 0.05 %, respectively (**sup. table 13**).

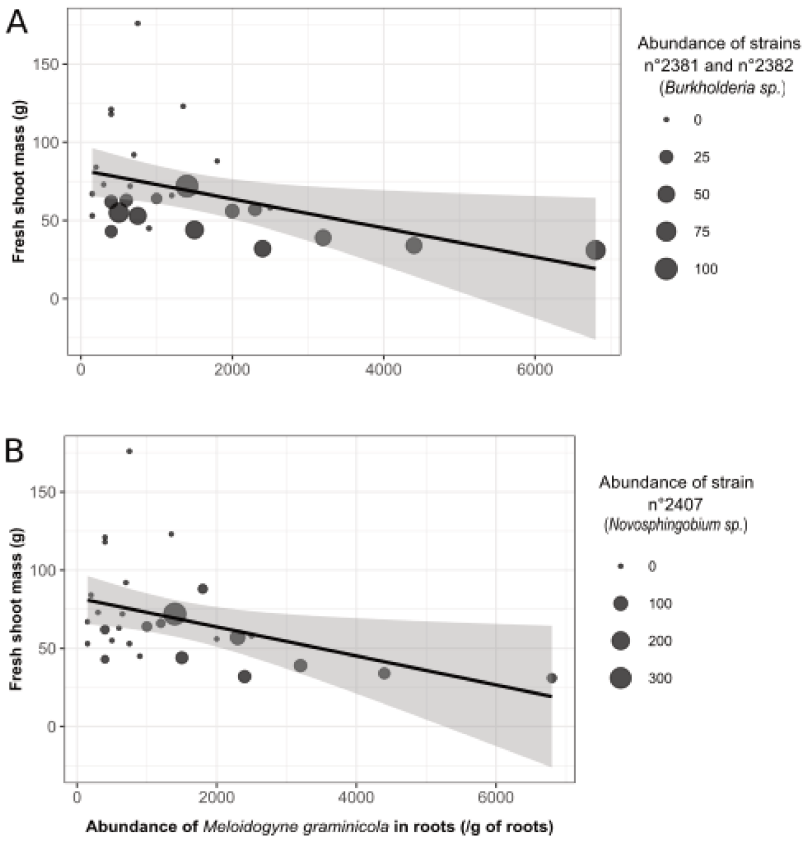

Figure 37. Association with the abundance of ESVs matched with the sequences of cultivable rice endophytes (*Burkholderia sp.* in **A** and *Novosphingobium sp.* in **B**) along the gradient of abundance of *M. graminicola* extracted from rice roots in the field.

Discussion

In this study, we showed that the infection by *Meloidogyne graminicola* was characterized by a decreased biomass ratio as soon as 14 days after the infection of plantlets (test n°1). The gall density negatively correlated with the root and shoot masses, and with the biomass ratio. On mature plants (test n°2), the infection additionally showed impacts on the next plant generation, notably fewer and lighter panicles and seeds. From the 68 bacterial endophytes collected on a lowland rice field in Cambodia, we screened 35 of them (test n°1) and found reversed effects, in particular the biomass ratio was increased by the bacterial addition (regardless of the bacterial genotype) compared to the nematode infection. However, we found different effect sizes, notably on the gall number and shoot mass. This suggests different levels of tolerance to the infection by *M. graminicola*, and different mechanisms of compensation by the bacterial pretreatments, depending on the cluster the candidate bacteria belongs to. Eight candidates displaying strong and diverse plant-phenotypic traits were selected for further assays. *In vitro* tests indicated that the selected strains exhibited some plant-growth promotion activities and that two strains exhibited antagonistic activities against *M. graminicola*. Two additional strains from the collection were positively correlated with the abundance of *M. graminicola* in roots in the field.

Most bacterial endophytes were beneficial to rice infected by *M. graminicola*

To look for an increase of tolerance to the disease caused by the nematode *M. graminicola* in rice plants, we first observed the signs of the infection by the number of hook-shaped galls characteristic of *M. graminicola* and juveniles extracted from roots to confirm the success of the infection (**figure 32** for test n°1, **figure 34** for test n°2 and **sup. table 11**). Symptoms of the nematode infection were manifested by the weaker plant reproductive traits: infected plants produced less and lighter seeds, but statistical power was too weak with individual bacterial pretreatments to conclude on the effects in test n°1 and n°2. However, the gall density in rice plantlets (test n°1) upon bacterial pretreatments decreased with endophytes of the clusters n°2 and n°3. Galeng-Lawilao and colleagues (2018) showed by a correlation coefficient analysis that the severity of root galling was negatively correlated with the number of panicles, the percentage of filled grains and the yield. They also found a negative correlation between the root galling and the root and shoot weight, similarly to our results. However, the minimal gall density (number of galls/root mass) that causes a measurable reduction in plant growth or yield varies with nematode species, host plant and environment (Barker and Olthof, 1976). The rice *Oryza sativa* variety IR64 used in this study is characterized as susceptible to *M. graminicola* (Soriano *et al.*, 1999; Nguyen *et al.*, 2021) because it allows the nematode development and reproduction, by contrast with resistant varieties that limit it. Additionally, both types of varieties may suffer either little injury (they will thus be characterized as tolerant), even when heavily infected with nematodes, or much injury (they will thus be characterized as sensitive), even when relatively lightly infected with nematodes. Resistance/susceptibility can be determined by measuring nematode reproduction, whereas tolerance/sensitivity can be determined by measuring the effect of nematode population on plant growth and yield (Galeng-Lawilao *et al.*, 2018).

We found an increased biomass ratio (root/shoot) with the bacterial addition in infected plantlets (test n°1), suggesting that bacteria can induce tolerance in the variety IR64, especially in the young plants ($p < .001$, +37%). In a study by Anwar and Van Gundy (1989), the biomass ratio (root length/leaf area) was also used to differentiate tolerant from sensitive grape plants. Both types of varieties had a reduced ratio due to the infection, but the tolerant ones exhibited a higher ratio than the susceptible ones because the shoot area also decreased in the susceptible varieties whereas it was maintained in the tolerant ones. In our study, the root mass and biomass ratio for most bacterial pretreatments were increased in cluster n°2, n°3 and n°4, compared to cluster n°1 that include the mock bacterial pretreatment (**figure 34**). It is known that bacteria are able to produce phytohormones and to influence plant growth (Arkhipova *et al.*, 2005; Costacurta *et al.*, 2008). Auxin production, for example, is widespread among plant-associated bacteria and plays a critical role in directly increasing plant growth and development (Ali *et al.*, 2009). All tested candidate bacteria were indeed able to produce auxin (**table 7**) and some were additionally able to solubilize phosphate, which can improve plant nutrition and indirectly benefit plant growth and tolerance (Trivedi and Sa, 2008). Besides, we found no increase of biomass ratio with the bacterial addition in mature plants (test n°2). The degree of symptom manifestation indeed differs with the age of the plants, mature plants being less susceptible (Rahman and Evans, 1987)

The effects of the nematode infection also differ with the environment, including both abiotic factors such as practices (Vinod *et al.*, 2015) and biotic factors such as the rhizosphere and endosphere microbiota (Tian *et al.*, 2015; Zhou *et al.*, 2019). Endophytes can colonize internal host tissues, including gall tissue, and have biocontrol activity of plant-parasitic nematodes (Siddiqui and Shaukat, 2003a). It was likely that the endophytes of the cluster n°1, since it included the mock pretreatment, had no observed impact on the plant development because of unsuccessful invasion or inhibition of the bacterial activity by other residents of the plant community (Mallon *et al.*, 2018). The plantlets treated with endophytes of the cluster n°4 showed a higher root mass, but the shoot mass was not increased, and the gall density was similar to the plantlets treated with endophytes of the cluster n°1, suggesting a beneficial effect of the bacteria on root growth but few or no increase in plant tolerance to *M. graminicola*. The plantlets treated with endophytes of the cluster n°3 had an exacerbated positive effect on the root growth and negative effect on the gall density, and had the highest biomass ratio (the effect being significant for bacteria n°2369 and n°2399), suggesting a high tolerance to the nematode infection. In contrast, the plantlets treated with endophytes of the cluster n°2 that also had a gall density lower than the plantlets treated with endophytes of cluster n°1, had the lowest shoot biomass, suggesting a developmental cost on plants interacting with bacteria for which we have no control to measure in our tests. Such ecological cost may result from trade-offs between induced resistance and the plant interaction with beneficial organisms (Walters and Heil, 2007).

Antagonism and mutualism were potentially involved

Antagonism against *M. graminicola*

We focused further investigations on selected candidate endophytes representing the diversity of the isolates from the collection and the diversity of *in planta* effects. Direct confrontation with the nematode *M. graminicola* showed that the strain of *Pseudomonas baetica* (n°2413, phylum *Proteobacteria*) had a high capacity to immobilize the infective juveniles at stage 2. As they could not recover their mobility after 24 h in water, we supposed they were dead and that the bacteria had a nematicidal effect. Since the proteinase K at maximal concentration (advised by the manufacturer) in the supernatant reduced the immobility, we suppose that the activity was due to a protein secreted by the bacteria. Nematicidal activity of *Pseudomonas* strains has already been observed *in vitro* and confirmed *in planta* (Siddiqui and Shaukat, 2003b; Lee *et al.*, 2011). Notably, the 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* CHA0 strain was able to cause a high mortality and to inhibit the egg hatching of the root-knot nematode *M. javanica*. It was also able to induce systemic resistance, and might be the first active secondary metabolite produced by a bacteria found in a disease suppressive soil (Weller *et al.*, 2002). Further tests are needed to identify the compound or mechanism responsible for the nematicidal effect of the strain of *Pseudomonas baetica* which is an interesting phytobeneficial endophyte because it was also able to produce siderophores, solubilize phosphate and detoxify ROS. Another strain, *Stenotrophomonas maltophilia* (n°2357, phylum *Proteobacteria*) also had a high capacity to immobilize the infective juveniles at stage 2, was able to produce siderophores, and to detoxify ROS. Moreover, *Stenotrophomonas maltophilia* has been found enriched in non-infected rice roots (Masson *et al.*, 2020) and a strain has already exhibited a nematotoxic activity against a plant-parasitic nematode, *Bursaphelenchus xylophilus* (Huang *et al.*, 2009).

Mutualism with the host plant

A strain of *Enterobacter hormaechei* (n°2388, phylum *Proteobacteria*) was also able to produce siderophores. These high-affinity iron (III) ion transport agents could efficiently complex iron, inhibiting the growth of certain soil-borne organisms, including plant-parasitic nematodes (Schroth and Hancock, 1982). With the strain of *Atlantibacter hermannii* (n°2399, phylum *Proteobacteria*), which belonged to the cluster n°3, it was also able to solubilize tricalcium phosphate. They were thus able to convert insoluble phosphates compounds in a form accessible to the plant that is important to increase the plant yield (Hayat *et al.*, 2010) and whose plant uptake might be compromised by the root infection. Moreover, all eight selected candidates exhibited a catalase activity. This microbial detoxification enzyme can represent an advantage to compete with other microorganisms, to adapt to chemical stress (Zamocky *et al.*, 2008) and to facilitate endophytic colonization (Trivedi *et al.*, 2020). We found no phylogenetic signature with the measured effects on our eight selected candidates, but it could be interesting to look for a functional pattern broadly. A similar approach could be used to select more candidates in order to study the whole diversity of the root-associated bacteria in rice infected by root-knot nematodes.

Other types of interactions within the microbiome

We observed that the plantlets inoculated by endophytes of the cluster n°2 had less galls at the root base system, where inoculations were done. A strain in this cluster, *Bacillus megaterium* (n°2372, phylum *Firmicutes*) had no effect on the nematode motility, suggesting that it didn't exhibit direct antagonistic

activity (aka antibiosis) on the infective juveniles, but it has already been described as a competitor *in planta* (Flor-Peregrin *et al.*, 2014) and is used in biopesticides (Radwan *et al.*, 2012). It has indeed been shown that *B. megaterium* reduces the penetration, migration and gall formation of *M. graminicola* in rice, and the egg hatching by over 60% (Padgham and Sikora, 2007).

The two strains of *Burkholderia* spp. and the strain of *Novosphingobium* sp. that have been positively associated (*i.e.* they are increasers) with the abundance of nematode juveniles in roots in the field (**figure 37** and **sup. table 13**) suggest several scenarios. Depending on the effect on the plant, they could induce either a tolerance (phytobeneficial effect) or a sensitivity (deleterious effect) to the infection, or have no effect (commensalism). In our study, the strains of *Burkholderia* spp. and *Novosphingobium* sp. were associated with the nematode infection but not with a higher dried shoot mass. Therefore, we supposed they were not phytobeneficial. Strains of *Burkholderia* sp. have already been found associated with the infection of another species of root-knot nematode, *M. incognita*, in roots of cotton (Hallman *et al.*, 1998) and were also associated with an increased root-galling index of *M. graminicola* on rice (Padgham and Sikora, 2007). However, strains of *Burkholderia* sp. are able to fix free nitrogen (Estrada *et al.*, 2002), suggesting an improvement of rice growth and tolerance, and to antagonize phytopathogens, notably root-knot nematodes (Meyer *et al.*, 2000; Guyon *et al.*, 2003). In our study, the strains of *Burkholderia* sp. were present all along the gradient of abundance of *M. graminicola*, therefore they could be opportunistic bacteria. Due to the dual effects of *Burkholderia* spp. in interaction with the plants, to the limitations in the resolution of the strain assignment, and to the genetic variability within species, we cannot compare between the literature and our field study and conclude on the nature of this opportunistic behavior (commensalism or others). Endophytic strains of *Novosphingobium* sp. have also been found in rice (Zhang *et al.*, 2016; Rangjaroen *et al.*, 2017) with potentially plant-growth promotion effects, and with *M. incognita* on tomato (Cao *et al.*, 2015). The authors suggest that these strains could develop a commensalistic or symbiotic relationship with the nematodes because of their ability to degrade lignin and cellulose compounds. Moreover, several ESVs assigned to *Novosphingobium sp.* have been found specifically enriched in the rice gallobiome of *M. graminicola* (Masson *et al.*, 2020). In this study, the strain of *Novosphingobium* sp. was less abundant in the sample with few juveniles of *M. graminicola*, suggesting that this strain was indeed associated with the nematode establishment process in roots, being potentially beneficial to the nematode (Topalović and Vestergård, 2021).

Conclusion

We collected 68 endophytic bacteria in a soil potentially suppressive to the phytoparasitic nematode *M. graminicola* and tested 35 of them. Since microbial interactions with plants can range from mutualistic to pathogenic depending on the context, we assessed the effects of these bacterial isolates on the rice *Oryza sativa* challenged with *M. graminicola* in order to determine the nature of their interaction. *In planta* tests revealed that most treatments of endophytes in greenhouse conditions increased rice tolerance to the nematode stressor and/or reduced the infection. Additionally, *in vitro* plant-growth promotion traits and nematicidal activity of some strains make them valuable native rice-associated microorganisms to suppress the nematode disease. Finally, the global phytobeneficial effects of endophytes might depend on the bacterial strategies, on their abundance, and on their interactions with other residents of the microbiota associated with rice or the nematode. One perspective of this work would be to test the effect on plant yield of a consortium of microorganisms designed to cooperatively suppress the disease caused by *M. graminicola*.

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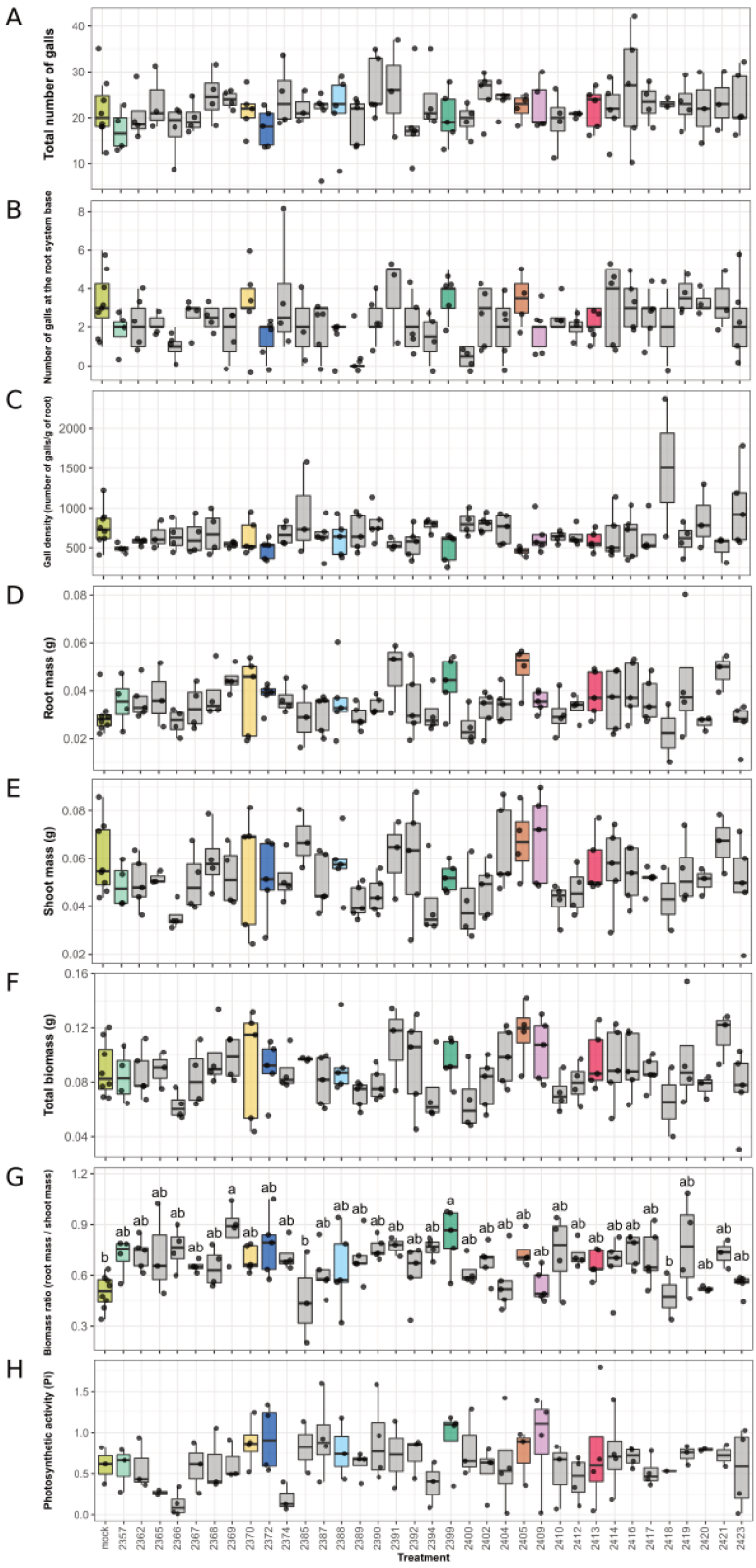
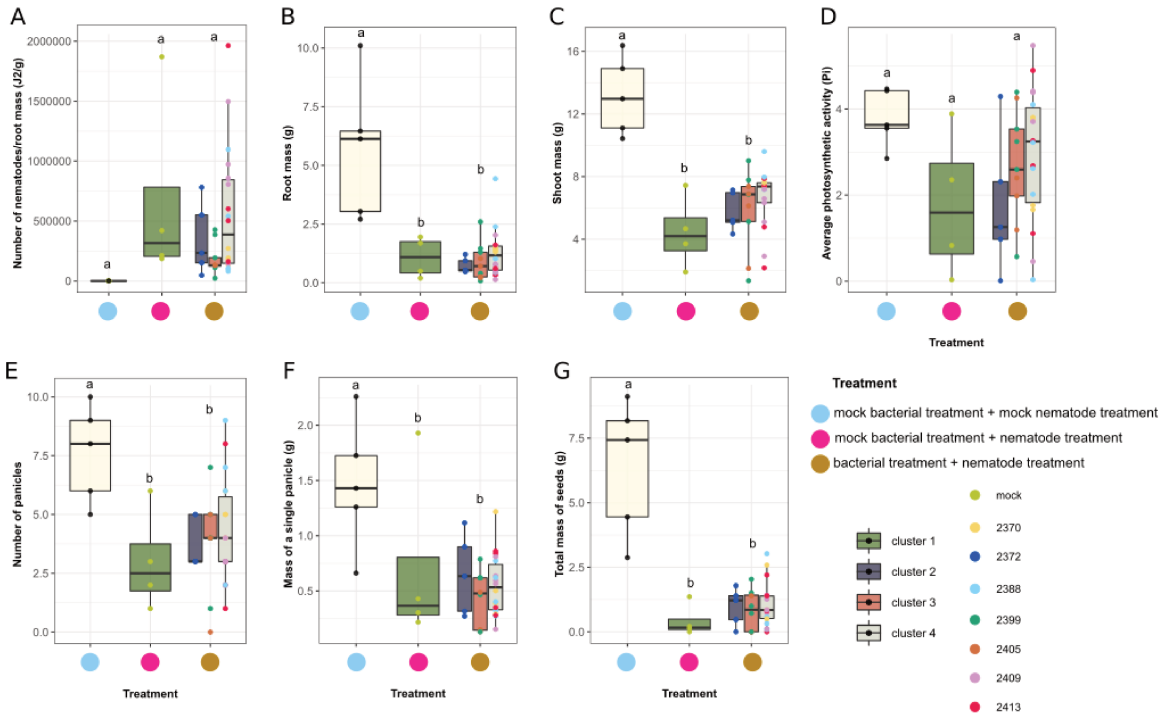
Supplemental figures and tables



Sup. figure 13. Boxplots of plantlets to look for effects of the bacterial pretreatments individually. Plantlets were all infected by the nematode *M. graminicola* (test n°1)



Sup. figure 14. Boxplots of mature plants to look for effects of the pretreatment of individual selected bacterial endophytes grouped by cluster (test n°2).

Sup. table 10. Bacterial endophytes native from the experimental field in Stung Chinit (Cambodia) that have been stored in collection. The background color indicates the cluster whose bacteria belong to (cluster 1, cluster 2, cluster 3 and cluster 4) according to a screening on rice plantlets to look for tolerance traits of the disease caused by *M. graminicola*. The highlighting color of the collection number indicates candidates that were selected for further experiments. Sequences of the *16S rRNA* gene were BLASTed against the NCBI database to assign them a name, and on the dataset of the bacterial community in roots from the same field. We found correlations between the abundance of ESVs in bold and *M. graminicola* extracted from rice roots in the field.

Collection number	NCBI name	Percentage of identity	ESV identity	Percentage of identity
2357	*Stenotrophomonas maltophilia*	98.856	387349bbf301de818017a263010277fa	98.361
2358	*Achromobacter aegrifaciens*	99.605	73e942a482dd2e331939a3e7e1a3fa42	91.375
2359	*Achromobacter spanius*	98.833	73e942a482dd2e331939a3e7e1a3fa42	89.664
2360	*Acinetobacter johnsonii*	99.714	ce65fce9fe4844bcc30786828d45f21a	97.669
2361	*Acinetobacter johnsonii*	99.667	ce65fce9fe4844bcc30786828d45f21a	97.500
2362	*Acinetobacter baumannii*	99.857	445254fd8f28417a46239f5d3a97fe58	97.664
2363	*Acinetobacter brisouii*	98.404	692ef1c8da6de95493822a33c9554bab	100.000
2364	*Acinetobacter johnsonii*	92.222	ce65fce9fe4844bcc30786828d45f21a	92.683
2365	*Acinetobacter johnsonii*	99.286	ce65fce9fe4844bcc30786828d45f21a	96.977
2366	*Acinetobacter seifertii*	100	445254fd8f28417a46239f5d3a97fe58	100.000
2367	*Acinetobacter seifertii*	100	445254fd8f28417a46239f5d3a97fe58	100.000
2368	*Acinetobacter schindleri*	99.714	9cce6aabbfd925399bf1111383f9d0a5	99.766
2369	*Acinetobacter seifertii*	100	445254fd8f28417a46239f5d3a97fe58	100.000
2370	*Bacillus zanthoxyli*	99.714	253a7230f2465f29a4f710bbbf490c22	99.532
2371	*Bacillus cytotoxicus*	98.288	1e9e730ad6482a72b481b66e8fe9c382	97.424
2372	*Bacillus megaterium*	99.857	714b0378efe0b8744e24aa04b48008d3	99.766
2373	*Enterobacter tabaci*	99.825	32ab1812bd3770f4b7f6aad7273783da	99.730
2374	*Bacillus koreensis*	99.857	1e9e730ad6482a72b481b66e8fe9c382	98.595
2375	*Bacillus zanthoxyli*	99.714	253a7230f2465f29a4f710bbbf490c22	99.532
2376	*Stenotrophomonas pavanii*	99.508	387349bbf301de818017a263010277fa	99.169

2377	*Bacillus toyonensis*	100	8cb24777cb48dde0aac60dfeca125d10	100.000
2378	*Bacillus pseudomycoides*	99.459	8cb24777cb48dde0aac60dfeca125d10	99.065
2379	*Burkholderia teritorii*	100	e7ae7d7ef5b6152a0837b6cfed70bd7a	100.000
2380	*Stenotrophomonas maltophilia*	99.600	387349bbf301de818017a263010277fa	99.038
2381	*Burkholderia cepacia*	99.524	**ae67b413f957a845132c368fd32d1fce**	100.000
2382	*Burkholderia contaminans*	99.524	**ae67b413f957a845132c368fd32d1fce**	100.000
2383	*Bacillus pseudomycoides*	99.857	8cb24777cb48dde0aac60dfeca125d10	100.000
2384	*Bacillus zanthoxyli*	99.733	253a7230f2465f29a4f710bbbf490c22	99.532
2385	*Comamonas testosteroni*	99.867	7af6d7fa861a2616576d401731c07a4f	96.729
2386	*Duganella rivus*	98.857	3fb20a72f9c73dd635762b94a45d9a6a	98.829
2387	*Enterobacter hormaechei*	99.590	33864bb560755ddca68811bd7f19a072	99.766
2388	*Enterobacter ludwigii*	96.279	32ab1812bd3770f4b7f6aad7273783da	97.101
2389	*Enterobacter soli*	98.889	32ab1812bd3770f4b7f6aad7273783da	98.168
2390	*Leclercia adecarboxylata*	99.057	32ab1812bd3770f4b7f6aad7273783da	99.379
2391	*Exiguobacterium indicum*	99.857	5b07228d0efad0e55e6dc884fd1d7a6a	99.766
2392	*Exiguobacterium indicum*	100	d5fe955ed527a47f75b3147dcb5c988f	100.000
2393	*Enterobacter hormaechei*	99.600	32ab1812bd3770f4b7f6aad7273783da	99.317
2394	*Fictibacillus enclensis*	99.143	102bef129e1efb6d53f60521c8df0920	95.785
2395	*Stenotrophomonas pavanii*	99.714	387349bbf301de818017a263010277fa	99.532
2396	*Enterobacter tabaci*	98.571	32ab1812bd3770f4b7f6aad7273783da	98.810
2397	*Klebsiella pneumonia*	99.342	24d821dddfbe031b81a44817e6d6a4c1	100.000
2398	*Klebsiella pneumonia*	99.865	24d821dddfbe031b81a44817e6d6a4c1	99.532
2399	*Atlantibacter hermannii*	99.184	9b747cc81030e98da7def4519aec9d05	98.175
2400	*Kosakonia pseudosacchari*	99.394	9b747cc81030e98da7def4519aec9d05	97.892
2401	*Kosakonia sacchari*	99.143	9b747cc81030e98da7def4519aec9d05	99.532
2402	*Lysinibacillus fusiformis*	99.857	8cb24777cb48dde0aac60dfeca125d10	94.200
2403	*Exiguobacterium indicum*	99.857	5b07228d0efad0e55e6dc884fd1d7a6a	100.000

2404	*Microbacterium proteolyticum*	99.714	cfa64d02b5eeb7ba0aa94f848cf49acf	97.789
2405	*Microbacterium hydrothermale*	99.714	cfa64d02b5eeb7ba0aa94f848cf49acf	98.034
2406	*Acinetobacter seifertii*	100	445254fd8f28417a46239f5d3a97fe58	100.000
2407	*Novosphingobium humi*	99.870	**485602dcc0f041e5a1f507f2708ae81f**	99.751
2408	*Novosphingobium pukkalii*	99.863	c3c934de8a53419458c67f2a7de0024f	99.502
2409	*Paenibacillus illinoisensis*	99.857	8999521e413899c0ba2a3417842097c5	92.254
2410	*Paenibacillus typhae*	99.865	8999521e413899c0ba2a3417842097c5	91.315
2411	*Pseudomonas aeruginosa*	100	f65e122df0f197f65964147a79034c02	99.532
2412	*Pseudomonas benzenivorans*	98.927	8338de26814ceac363faf0656a5b4058	98.603
2413	*Pseudomonas baetica*	99.867	8338de26814ceac363faf0656a5b4058	97.424
2414	*Pseudomonas benzenivorans*	93.929	8338de26814ceac363faf0656a5b4058	91.086
2415	*Pseudomonas guariconensis*	100	d1e7c970dac89bea848fc295abeb8624	100.000
2416	*Pseudomonas graminis*	98.973	8338de26814ceac363faf0656a5b4058	98.829
2417	*Pseudomonas helmanticensis*	99.714	d1e7c970dac89bea848fc295abeb8624	98.829
2418	*Pseudomonas prosekii*	99.429	8338de26814ceac363faf0656a5b4058	97.892
2419	*Sphingobacterium siyangense*	99.571	ca9d9340a0100e32e5412019b1824ea2	91.726
2420	*Sphingomonas trueperi*	100	65de1eaa5c80fac9e5a2c5b52441ee29	100.000
2421	*Stenotrophomonas pavanii*	99.091	387349bbf301de818017a263010277fa	97.892
2422	*Stenotrophomonas pavanii*	99.714	387349bbf301de818017a263010277fa	99.297
2423	*Stenotrophomonas pavanii*	98.857	387349bbf301de818017a263010277fa	97.892
2424	*Stenotrophomonas pavanii*	99.000	387349bbf301de818017a263010277fa	97.892

Sup. table 11. Statistical results of variables measured on rice in the two *in planta* tests. As a factor, "nematode infection" compared mock bacterial pretreatment + nematode treatment *versus* mock bacterial pretreatment + mock nematode treatment (two groups), "bacterial addition" compared bacterial pretreatment + nematode treatment *versus* mock bacterial pretreatment + nematode treatment (two groups) and "bacterial pretreatments" compared each bacterial pretreatment individually *versus* mock bacterial pretreatment + nematode treatment (36 groups) or by cluster (4 groups). *p*-values can be a little (< .05), moderately (< .01) or highly (< .001) significant and effect size (*d* of Cohen) can be small (> 0.20), medium (> 0.50) or large (> 0.80). NA: not enough replicates to perform statistical tests.

		Nematode infection		Bacterial addition		Bacterial pretreatments (individually)		Bacterial pretreatments (by cluster)	
Assay	**Variable**	***p*-value**	**Effect size**	***p*-value**	**Effect size**	***p*-value**	**Effect size**	***p*-value**	**Effect size**
Test n°1: 35 candidates	total number of galls	< .01	> 0.80 (+)	.921	0.04 (+)	.700	0.16	< .05	0.04
	number of galls at the root base system	< .001	> 0.80 (+)	.136	> 0.50 (-)	.098	> 0.80	< .001	0.14
	gall density	< .01	> 0.80 (+)	.177	> 0.20 (-)	.193	0.06	< .01	0.06
	root mass	.500	> 0.20 (-)	.073	> 0.50 (+)	.098	0.10	< .01	0.08
	shoot mass	.300	> 0.50 (+)	.217	> 0.50 (-)	.100	> 0.20	< .001	0.14
	total biomass	.700	> 0.20 (+)	.850	0.06 (-)	NA	> 0.20	< .001	0.11
	biomass ratio (root/shoot)	< .05	> 0.80 (-)	< .001	> 0.80 (+)	< .001	> 0.20	< .001	0.18
	photosynthetic activity (Pi)	< .05	> 0.80 (-)	.856	0.15 (+)	.474	0.01	0.583	0.06
Test n°2: 7 selected candidates	number of J2/g of root mass	< .01	> 0.80 (+)	.417	> 0.50 (-)	.054	> 0.20	0.134	0.08
	root mass	< .05	> 0.80 (-)	NA	0.10 (+)	.181	0.10	0.560	0.03
	shoot mass	< .001	> 0.80 (-)	0.809	> 0.80 (+)	.087	0.18	0.162	0.07
	photosynthetic activity (Pi)	.090	> 0.80 (-)	.394	> 0.50 (+)	.900	0.13	0.456	0.11
	number of panicles	< .05	> 0.80 (-)	.364	> 0.50 (+)	.100	> 0.20	0.731	0.05
	mass of a single panicle	< .01	> 0.80 (-)	.676	> 0.20 (-)	.710	0.08	0.594	0.03
	total mass of seeds	< .01	> 0.80 (-)	.145	> 0.50 (+)	.629	0.06	0.524	0.02

Sup. table 12. Effect sizes (*d* of Cohen in contrast with the mock bacterial pretreatment) of the variables measured on plantlets infected by the nematode *M. graminicola* and inoculated by candidate endophytic bacteria (test n°1). These candidates were selected for further tests against *M. graminicola*.

Bacteria	Gall density	Number of galls at the root base system	Root mass	Shoot mass	Biomass ratio (root/shoot)	Photosynthetic activity (Pi)
n°2357	> 0.80 (-)	> 0.80 (-)	> 0.50 (+)	> 0.50 (-)	> 0.80 (+)	0.11 (+)
n°2370	> 0.20 (-)	0.03 (-)	> 0.80 (+)	> 0.20 (-)	> 0.80 (+)	> 0.50 (+)
n°2372	> 0.80 (-)	> 0.80 (-)	> 0.80 (+)	> 0.50 (-)	> 0.80 (+)	> 0.80 (+)
n°2388	> 0.50 (-)	> 0.80 (-)	> 0.50 (+)	0.16 (-)	> 0.80 (+)	> 0.20 (+)
n°2399	> 0.80 (-)	> 0.20 (+)	> 0.80 (+)	> 0.50 (-)	> 0.80 (+)	> 0.80 (+)
n°2405	> 0.80 (-)	0.17 (+)	> 0.80 (+)	> 0.50 (+)	> 0.80 (+)	> 0.20 (+)
n°2409	> 0.20 (-)	> 0.80 (-)	> 0.50 (+)	> 0.50 (-)	> 0.20 (+)	> 0.80 (+)
n°2413	> 0.50 (-)	> 0.50 (-)	> 0.80 (+)	0.17 (-)	> 0.80 (+)	> 0.20 (+)

Sup. table 13. Prevalence and relative abundances of ESVs matched with the cultivable bacterial endophytes. Prevalence is the number of samples where the ESV is present (32 samples in total). Relative abundance is calculated with the reads count of each ESV divided by the total reads count in the dataset of interest. We found correlations between the abundance of ESVs in bold and *M. graminicola* extracted from rice roots in the field.

Collection number	ESV identity	Prevalence	Relative abundance in the all dataset	Relative abundance under CA
2415, 2417	d1e7c970dac89bea848fc295abeb8624	1	0,006	0,000
2412, 2413, 2414, 2416, 2418	8338de26814ceac363faf0656a5b4058	3	0,016	0,006
2404, 2405	cfa64d02b5eeb7ba0aa94f848cf49acf	3	0,013	0,015
2387	33864bb560755ddca68811bd7f19a072	2	0,006	0,013
2386	3fb20a72f9c73dd635762b94a45d9a6a	5	0,015	0,018
2357, 2376, 2380, 2395, 2421, 2422, 2423, 2424	387349bbf301de818017a263010277fa	2	0,004	0,003
2362, 2366, 2367, 2369, 2406	445254fd8f28417a46239f5d3a97fe58	3	0,006	0,008
2373, 2388, 2389, 2390, 2393, 2396	32ab1812bd3770f4b7f6aad7273783da	5	0,022	0,021
2397, 2398	24d821dddfbe031b81a44817e6d6a4c1	2	0,003	0,006
2368, 2399, 2400, 2401	9cce6aabbfd925399bf1111383f9d0a5	2	0,006	0,000
2360, 2361, 2364, 2365	ce65fce9fe4844bcc30786828d45f21a	9	0,048	0,043
2411	f65e122df0f197f65964147a79034c02	4	0,008	0,008
2358, 2359	73e942a482dd2e331939a3e7e1a3fa42	14	0,027	0,022
2420	65de1eaa5c80fac9e5a2c5b52441ee29	23	0,131	0,150
2377, 2378, 2383, 2402	8cb24777cb48dde0aac60dfeca125d10	20	0,260	0,359
2363	692ef1c8da6de95493822a33c9554bab	17	0,096	0,094
2408	c3c934de8a53419458c67f2a7de0024f	4	0,006	0,008
2394	102bef129e1efb6d53f60521c8df0920	14	0,050	0,027
2385	7af6d7fa861a2616576d401731c07a4f	11	0,010	0,008
2379	e7ae7d7ef5b6152a0837b6cfed70bd7a	29	0,432	0,344
2371, 2374	1e9e730ad6482a72b481b66e8fe9c382	12	0,012	0,014
2381, 2382	**ae67b413f957a845132c368fd32d1fce**	14	0,120	0,104
2370, 2375, 2384	253a7230f2465f29a4f710bbbf490c22	14	0,137	0,144
2419	ca9d9340a0100e32e5412019b1824ea2	5	0,010	0,006
2372	714b0378efe0b8744e24aa04b48008d3	10	0,087	0,102

2391, 2403	5b07228d0efad0e55e6dc884fd1d7a6a	14	0,243	0,335
2409, 2410	8999521e413899c0ba2a3417842097c5	3	0,004	0,005
2392	d5fe955ed527a47f75b3147dcb5c988f	6	0,078	0,115
2407	**485602dcc0f041e5a1f507f2708ae81f**	13	0,165	0,055

Additional analyzes and perspectives

The response of rice interacting with *M. graminicola* and bacterial endophytes

Plant response depends on plant perception and bacterial colonization pattern, as shown by King *et al.* (2019). In this study, two related bacterial endophytes with contrasted colonization pattern engaged different plant responses: while *Burkholderia vietnamiensis* colonized root cells intracellularly and enhanced a systemic JA regulation at early stages of the interaction then repressed it, *Paraburkholderia kururiensis* colonized root cells intercellularly and induced a transient delayed of the systemic JA up-regulation. In our system, to study the response of rice infected by *M. graminicola* upon pretreatments with potentially phytobeneficial bacteria, we could first check whether endophytes can colonize rice in our greenhouse conditions and what are their colonization pattern, by using bacterial strains tagged with a reporter gene such as the DsRed and microscopic observations.

It is known that the inoculation of beneficial rhizobacteria (Vacheron *et al.*, 2013; Liu *et al.*, 2017) and of root-knot nematodes (Kyndt *et al.*, 2014; Petitot *et al.*, 2018) independently induces important changes in the hormonal balance and through hormone-related transcriptional regulation in rice roots. Research has also shown that plant defense mechanisms can be stimulated in an immediate plant response induced after infection through modulation of genes, called ISR or SAR (Maithani *et al.*, 2021). ISR is activated by beneficial rhizobacteria, while SAR is tolerance initiated by pathogens, but both provide more resistance towards certain biotic stressors upon exposure to an exogenous chemical or biotic stimulus (Khanna *et al.*, 2021; Pottie *et al.*, 2021). In general, studies that assess mean controls of plant-parasitic nematodes are associated with microbial-mediated ISR or priming. Priming refers to a specific subset of the ISR response, in which a defense response is not triggered directly by the ISR stimulus, but is expressed more rapidly or more intensely in plants treated with the ISR stimulus upon later exposure to a biotic stressor (Pottie *et al.*, 2021).

In order to assess the potential of endophytic bacteria to activate or repress, locally and/or systemically, the plant defenses and to face a nematode attack, priming and ISR could be studied with transcriptomics and metabolomics (Mhlongo *et al.*, 2018). We can also target genes and metabolites that are involved in the plant defense. For a long time, SA was associated with defense against biotrophic pathogens while JA and ethylene contributed to defense against necrotrophic and herbivorous pathogens. However, studies showed that it is much more complex and that these three hormonal signaling pathways are interacting. The consensus is that ethylene would inhibit root-knot nematode infection *via* the JA pathway and that SA would activate basal plant defenses against nematodes (Gheysen et Mitchum, 2019). Therefore, we could target genes involved in these pathways to study the transcriptional response of rice infected by *M. graminicola* and inoculated with phytobeneficial bacteria identified in this study. To look for a potentialisation of the response, several bacteria could be co-inoculated, and to compare with the basal plant response, commensalistic bacteria could be used as a control.

Finally, one aspect that should also be taken into account is the dose effect. We set an inoculum of bacteria based on the same OD, but due to differences in the bacterial shapes, consistencies and exudations, it can result in different concentrations of bacterial cells. For example, a liquid culture of the strain n°2405 (*Microbacterium sp.*) was 10^5 times more concentrated in bacterial cells than a liquid culture of the strain n°2370 (*Bacillus sp.*) at OD = 0.5 (600 nm). Since processes and signals such as quorum-sensing, secreted effectors, antibiotics, or competition for scarce nutrients, drive competition or cooperation between microbes and therefore influence both the nature and intensity of plant-microbe interactions (Harris *et al.*, 2020), the dose effect should be normalized (using the same cell concentration or the same relative abundances as the ones found in the root microbiota) in subsequent analyzes of the rice response to both endophytes and *M. graminicola*.

Designing a microbial consortium to cooperatively suppress *M. graminicola*

Although the plant response with one bacteria might be complex, rice actually interacts with many more and diverse organisms. Therefore, including other organisms in the system is more realistic and can be more efficient since synergism is usually a better strategy for reaching greater effects. Synthetic microbial communities (SynComs) are small consortia of microorganisms designed to mimic the observed function and structure of the microbiota in natural conditions (de Souza *et al.*, 2020). Generally, to design SynComs involves applying concepts from both microbial ecology and genetics by: 1) identifying and incorporating robust and prevalent plant colonizers, such as those belonging to core microbiotas, in order to increase SynCom stability and robustness to natural invasion, and 2) selecting microbial candidates by screening approaches based on the microbial genome in search of traits related to functions beneficial to plants. This selection can be done computationally to select bacteria without *a priori*.

Its use can be expanded to include a desired set of microbial traits for enhancing crop resiliency against stressful conditions such as the biotic stressor *M. graminicola*. In our case, such a consortium of microorganisms to study can include:

- bacteria, fungi, protists or any organisms that are naturally associated with rice and are preferentially found in its cropping systems. This requires the capacity to cultivate them (Liu *et al.*, 2019). Therefore, it implies modifying cultivation methods by using specific media (*e.g.* with plant extracts) and testing their potential interactions, as original studies recently highlighted the importance of cooperation to protect a phytobeneficial soil fungus (Büttner *et al.*, 2021) and the importance of cross-feeding in specific conditions to stimulate a phytobeneficial bacteria (Sun *et al.*, 2021).
- organisms that exhibit various types of interaction with the model system *Oryza sativa* - *Meloidogyne graminicola* to gain efficiency in the reduction of the nematode infection (mutualism with rice, antagonism against the root-knot nematode, commensalism, *etc.*). For example, many fungi are known to antagonize plant-parasitic nematodes (Stirling *et al.*, 2015) and commensal bacteria have been shown to modulate the root immune system of *Arabidopsis* (Teixeira *et al.*, 2021).

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General discussion

(English version)

Main results of this thesis

At the beginning of this thesis, knowledge was available about the interactions between rice and plant-parasitic nematodes or microorganisms (bacteria and fungi) but few studies addressed questions about the factors modulating the interaction of the three entities together in rice cropping systems, especially with the bacterial community. The first chapter of this thesis questioned what characterizes the disease caused by *Meloidogyne graminicola* on rice and exposed the reasons why an ecological view is required on the *Oryza sativa - Meloidogyne graminicola* pathosystem. The plant phenotype is indeed shaped by both the environment and the genetics (Singh *et al.*, 2019). In this ecoevolutionary framework, we studied the rice-associated microbiomes in different contexts of infection by *M. graminicola* (**figure 38**, objectif 1 A and B). In the second chapter, we described for the first time the gall microbiome of *M. graminicola* in infested rice fields, that we proposed to call the "gallobiome". More specifically, we characterized the assembly of the bacterial community in the roots morphologically modified by the infection. We showed that this new ecological niche in roots was a refuge for the survival of the parasite and its associated and specific microbiota, characterized by higher richness, diversity and evenness (**figure 15**). *M. graminicola* is mainly an endoparasite but it also has a short exophytic phase in its life cycle during which it can interact with soil and rhizosphere organisms. In the third chapter, we then described the rhizosphere communities of rice cultivated in contrasted cropping systems. We basically studied the impact of abiotic factors (agricultural practices) on simultaneous biotic factors (bacteria, fungi and nematodes interacting together within the soil food web). An important result lies in the fact that conservation agriculture improved microbiodiversity and limited the infection by *M. graminicola*. Since it was associated with the maturation of the soil food web, a population regulation through predatorism by nematodes and antagonism by microbes could have directly occured (**figure 23**). Bacterial endophytes from roots collected in this field were tested for their biocontrol potential (antagonism against *M. graminicola* and benefits to *O. sativa*) in a greenhouse experiment and *in vitro* assays. We measured plant phenotypic traits upon treatments of bacteria representing the microbiodiversity of rice roots in order to identify biotic factors suppressive to the disease or the damages caused by *M. graminicola* in the field (**figure 31**). Interesting strains that exhibited phytobeneficial effects and/or nematicidal or nematostatic activity were reported in the last chapter (**figure 38**, objectif 2).

In this general discussion, we will first give a global picture of the rice-associated microbiota in the different contexts of infection by *M. graminicola* or disease suppression studied in this thesis, by showing phylogenetic trees built with the two generated NGS datasets, and by making connections between the different factors of the phytobiome. Then, we will present the limitations of this approach, before proposing complementary analyzes and perspectives of special interest to elevate and refine the knowledge on the *Oryza sativa - Meloidogyne graminicola* pathosystem. Finally, we will conclude on remaining open questions on soil disease suppressiveness.

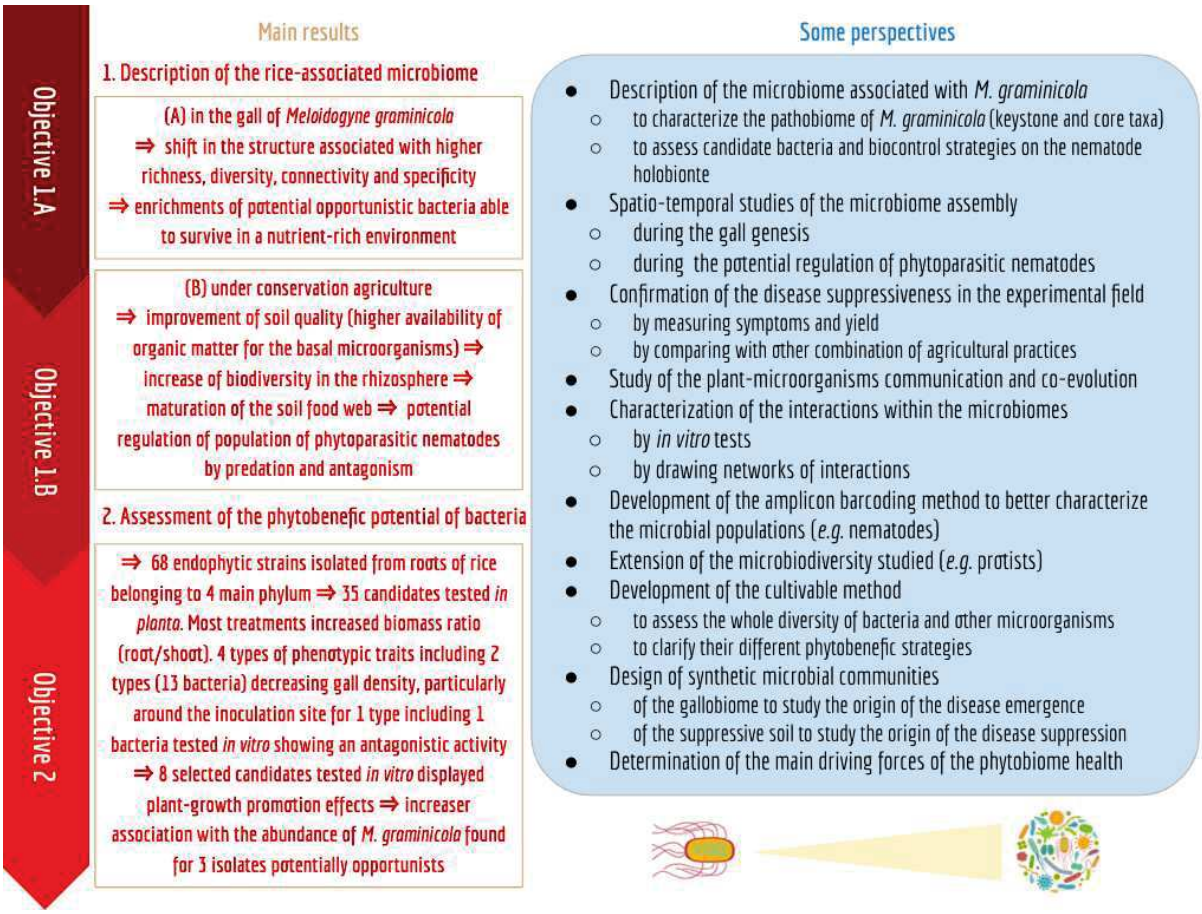


Figure 38. The main results obtained in the studies of this thesis and some perspectives of this work.

A picture on the rice pathobiome of *M. graminicola*

Global phylogenetic trees were drawn to explore the phylogenetic distribution and the taxa specificity with the two datasets generated for this thesis (**figure 39**). They represent the presence/absence of each ESV in different environmental conditions (what plant compartment, what infection status, what practices and for which *O. sativa* subspecies). The branch lengths were ignored to facilitate the visualization (one ESV = one branch, whatever the taxonomic level) and the ESV abundances were not considered. These trees provide a general description of the rice-associated microbiota in a context of infection by *M. graminicola*, that is also, in fact, the rice pathobiome of *M. graminicola*.

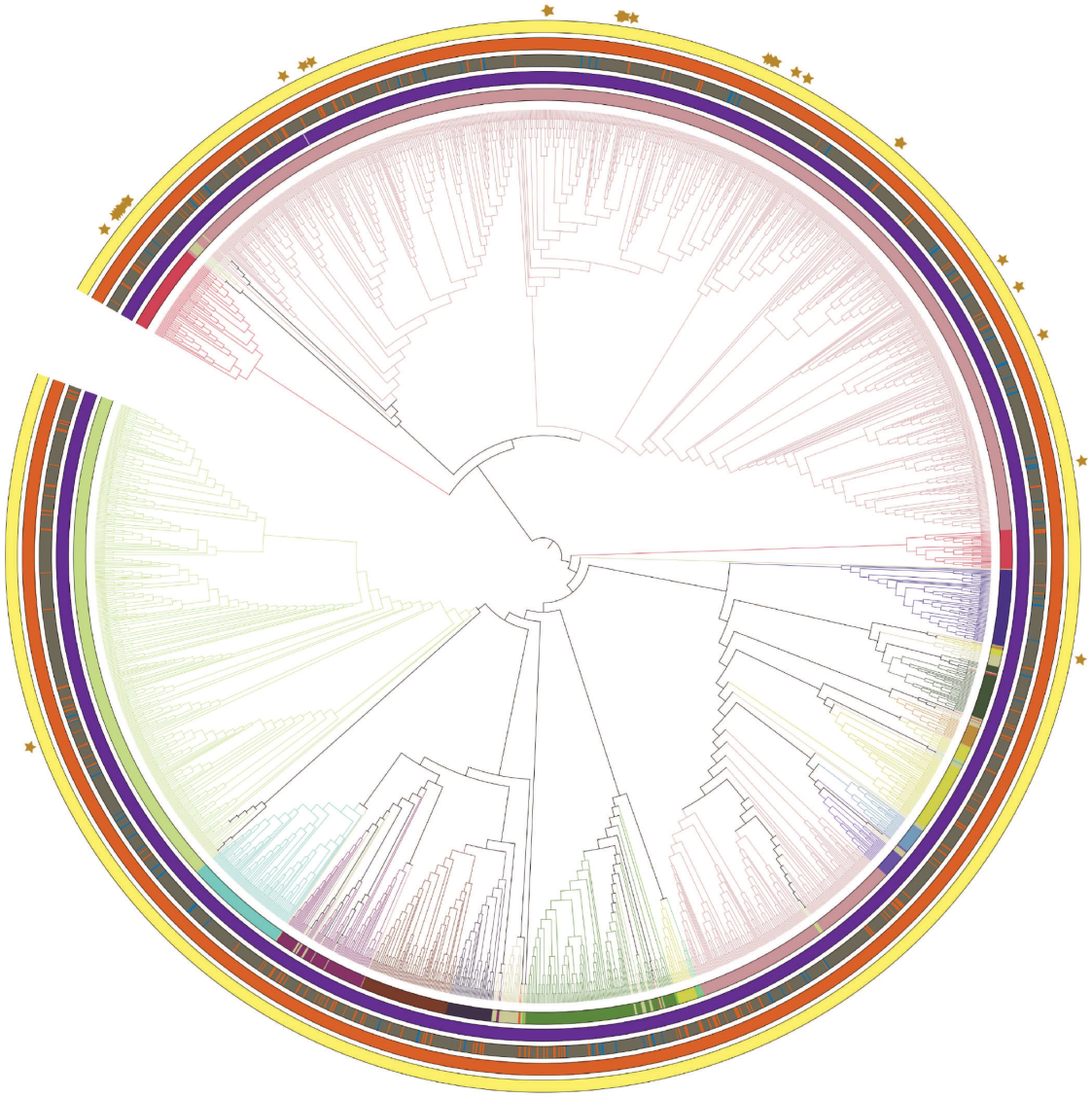
Looking at the distribution along the phylogeny in **dataset 1** (**A**, *Bacteria*, rice root), *Proteobacteria* is the main phylum: almost half of the branches belong to this phylum, which is consistent with the literature (Trivedi *et al*., 2020). The second most represented phylum is *Actinobacteria*, followed by less represented phyla: *Firmicutes*, *Cyanobacteria*, *Verrucomicrobia*, *Chloroflexi*, *Acidobacteria*, *etc*. In **dataset 2** (**B**, *Bacteria*, rice root and rhizosphere), we expected more diversity since it includes both compartments and since the rhizosphere compartment has a higher diversity than the root compartment. Surprisingly, there is a similar number of phyla (48 in **A** and 49 in **B**), but a higher richness (13 phyla with > 30 reads in **A** and 22 in **B**). This might be due to the facts that the preprocessing for these trees are different, and that the gallobiome in the fields in Vietnam is very diversified (*cf.* **chapter 1**). In the eukaryotic tree (**C**, *Fungi* and *Nematoda*, rhizosphere), *Ascomycota* is the most represented (> ⅓ of total number of branches), followed by *Basidiomycota*, *Rozellomycota* and *Glomeromycota*. The fungal assignment is less complete (more unassigned ESVs) and less diversified than the bacterial one (11 fungal phyla in **C** and 31 bacterial phyla in the rhizosphere in **C**), but still more than the nematode community (5 families). Nematodes might have indeed been fewly represented, because of the method (lack of efficiency of the DNA extraction, biais of amplification and insufficient database for assignment) that is not appropriate for the nematodes, as already discussed in **chapter 3**. However, we observe that nematodes, gathered between *Rozellomycota* and *Ascomycota* on the tree, are mainly assigned to the *Pratylenchidae* family, *Hirschmanniella* genus including some to *H. mucronata* species, predominant in the field, which are also found in rice roots by microscopic observations. Besides, most of the nematodes (⅘ families) were PPNs (*Criconematidae*, *Meloidogynidae*, *Pratylenchidae* and *Telotylenchidae*), which could confirm their predominance in the field, while admitting the database is poorly provided in sequences due to the lack of interest for other nematode guilds and due to their high genome complexity. Moreover, this method by amplicon barcoding overestimate the number of *Hirschmanniella* spp. in roots compared to the microscopic observation (*cf.* **chapter 3**) and shotgun sequencing or metabarcoding methods (Ngan Thi Phan, personal communication). This bias is mainly due to the DNA extraction kit (Stéphane Bellafiore, personal communication).

Overally, taxa in the bacterial community (**B**) are much more specific to the rhizosphere than to the roots (root:rhizosphere:both = 386:1,885:1,387, 62% specific). It is also slightly more specific to conservation agriculture than to conventional tillage (CA:CT:both = 998:870:1,790, 51% specific) and to *indica* subsp. than to *japonica* subsp. (*japonica*:*indica*:both = 281:384:2,993, 18% specific). In detail, more bacteria in the rhizosphere are associated with conservation agriculture, as seen by the overlapping layers of

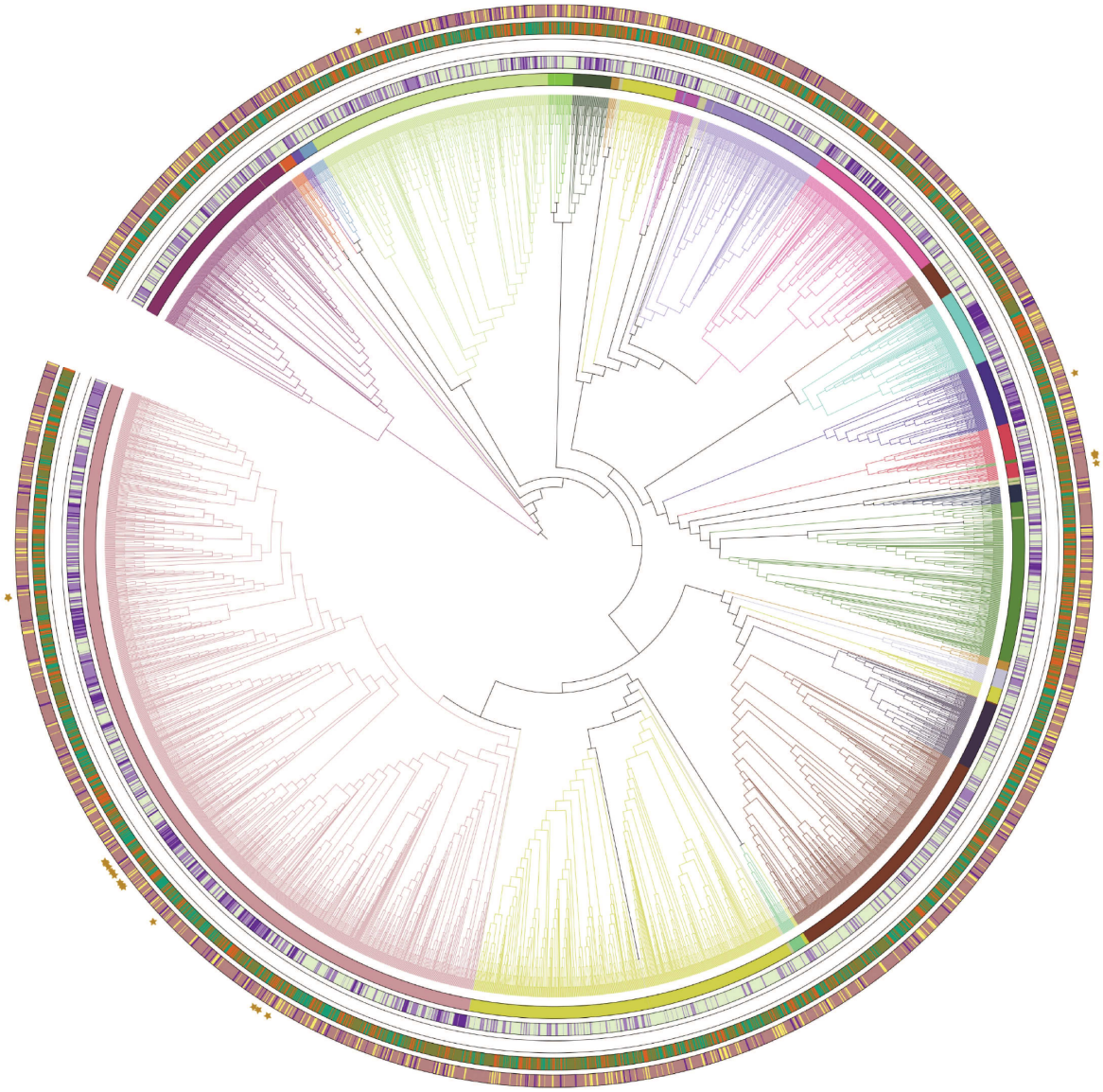
information but more obviously by the Venn diagram in **chapter 3** (**figure 25**). Nonetheless, we observe on the tree that *Cyanobacteria* spp. are more specific to conventional tillage, as seen by the relative abundance in **chapter 3** and as expected in a waterlogged soil (Sinha *et al.*, 1996) due to soil compaction after repeated tillage. *Rozellomycota*, a fungal phylum abundantly found in aquatic environments (Grossart *et al.*, 2016), is also more specific to conventional tillage. The pattern of specificity for the fungal community and the nematofauna in the rhizosphere (**C**) is exacerbated for the practices, with more taxa specific to CA (CA:CT:both = 351:193:459, 54% specific) which is not surprising since tillage is highly disruptive for the mycorrhizal fungi for example (*e.g. Glomeromycota* spp.) and the cover crops under conservation agriculture offers more ressource for saprotrophic fungi notably (*e.g. Ascomycota* spp.) as described in **chapter 3**. There is a similar ratio for both rice subspecies and slightly less total specificity than the bacterial community (*japonica*:*indica*:both = 78:85:840, 16% specific). The capacity of different rice genotypes to shape the root-associate microbiota and, in response to that, the capacity of the soil microbiota to bring adaptive traits to rice is worth more investigations with the bacterial community (*cf.* **chapter 3**).

Concerning the microbiota associated with the infection by *M. graminicola*, the *Metacoder* tree (**chapter 2**, **figure 19**) is a better representation since it includes quantitative information (relative abundance of ESVs on the branches). Nonetheless, on the tree of the dataset 1, we can observe that *Verrucomicrobia* (already observed in **chapter 2**) and the closely related phylum *Patescibacteria* are more specific to the gall than to the non-infected roots. On the tree of the dataset 2, the qualitative type of information about the abundance of *M. graminicola* in roots makes it difficult to represente. One last piece of information is given by the stars that indicate the strains from the endospheric microbiota in the field in Cambodia that have been recovered by a cultivable method and stored in collection. They are spread on the main phylum but obviously represent a tiny part of the total diversity in the roots and in the rhizosphere (**A** and **B**). This method can be highly optimized.

(**A**) **Dataset 1** - rice root - *16S rRNA* gene - *Bacteria* - GREENGENES database - 2,237 branches



(**B**) **Dataset 2** - rice root and rhizosphere - *16S rRNA* - *Bacteria* - SILVA database - 3,658 branches



(**C**) **Dataset 2** - rice rhizosphere - *ITS rRNA* - *Fungi* and *Nematoda* - UNITE database - 1,003 branches



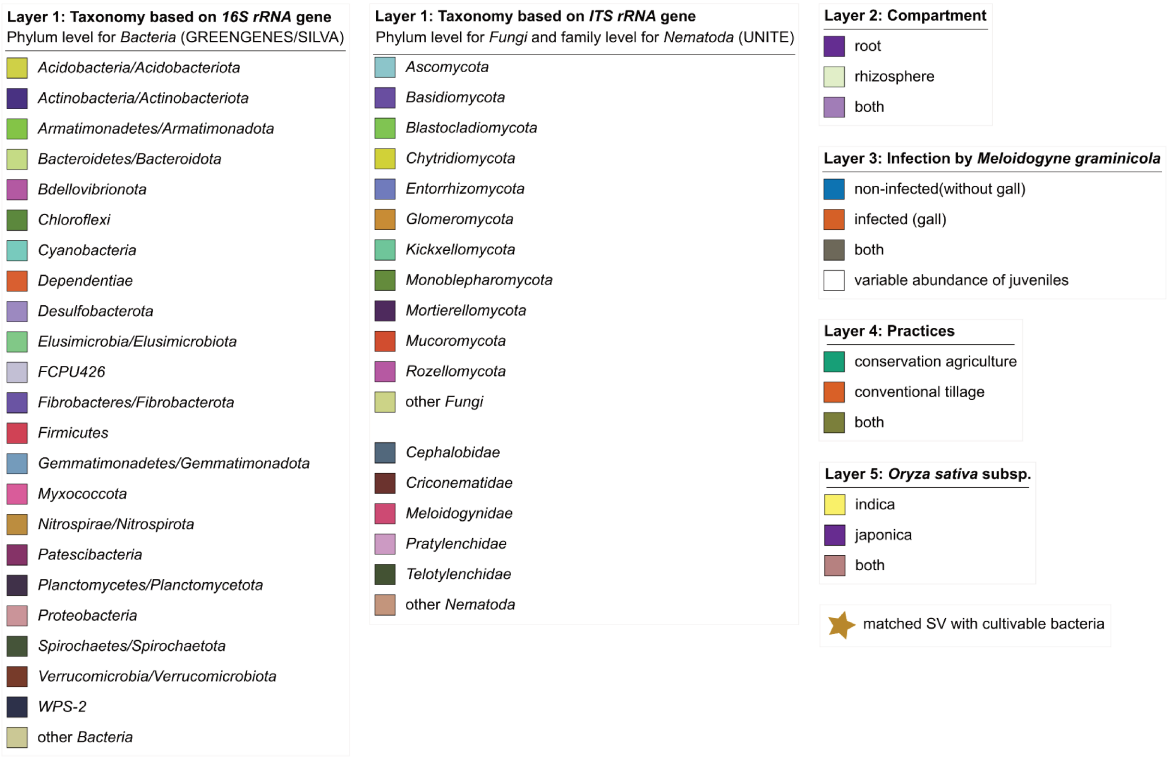


Figure 39. Phylogenetic trees of bacteria, fungi and nematodes made with the two NGS datasets used in this thesis. ESVs of the *16S rRNA* marker gene were assigned with the GREENGENES database in the dataset 1, or the SILVA database in the dataset 2. ESVs of the *ITS rRNA* marker gene were assigned with the UNITE database (version 04.02.2020) in the dataset 2. *Qiime2* platform was used to build rooted trees including ESVs which were only assigned to *Bacteria*, *Fungi* or *Nematoda* phyla with a total abundance > 10 reads and a prevalence > 1 sample. Unassigned ESVs at phylum or family levels were grouped as others (in addition to ESVs with < 30 reads for *Bacteria*). The representations were done with *iTOL*. Four concentric layers of presence/absence information were annotated, from the center to the outside: taxonomy (with colored branches), compartment (root and/or rhizosphere), infection (infected and/or non-infected), practices (conservation agriculture and/or conventional tillage without cover crop), *Oryza sativa* subspecies (*indica* and/or *japonica*) and recovered cultivable strains (indicated by a star). The scripts to prepare the data with *Qiime2* and the files to annotate the trees with *iTOL* are available on GitLab under the project ID 30046176 (fusiondatasets_montpellier_2021).

To fully describe the pathobiome of *M. graminicola*, we need to integrate as much information about the biotic and abiotic context as possible (**table 1** and **8**). In this ambitious approach, informative elements on the environmental factors will always be missing. With the dataset 2, plant traits and symptoms are lacking. Moreover, the biodiversity of only a few communities are included, and macrobiodiversity, time and other factors are totally missing. Therefore, it is a unique snapshot of the rice-associated microbiota in different contexts with *M. graminicola* that could never be observed a second time, although a part of the microbiota could be consistently found (the so-called core microbiota). However, specific taxa could have an impact on the plant health and particular observations on the assembly can be very informative about the impact of a specific environment. For example, spatio-temporal studies give dynamic pictures so, one interesting perspective would be to make a time series in the two contexts. 1) By making a kinetic sampling

of the gall microbiome during its genesis in a greenhouse assay, we could be able to determine at which stage the deep modifications appear in the gall microbiome, whether, how and when it is becoming dysbiotic: during the nematode invasion of plant roots, during the induction when target cells have been selected by the nematode and become giant or when the nematode progresses through its life cycle (Goto *et al.*, 2021). 2) By continuing the monitoring of the experimental field in Cambodia, at different developmental stages of rice and through years, we could follow the maturation of the soil food web and determine how and when plant-parasitic nematode are regulated: vegetative stage, reproductive stage or maturation stage of rice (Moldenhauer *et al.*, 2001) more than seven years after the transition to conservation agriculture. In addition, sampling more than one compartment (Gao *et al.*, 2021) would inform us if modifications are restricted to the gall or occur in other parts of the infected plants, and sampling at large scale in the field would allow us to explore the progress and heterogeneity of the maturation of the soil food web, since the infection by PPNs in the field is usually visible by heterogeneous patches of plant growth delay. Exceptionally, the fields in Vietnam were homogeneously conducive to the disease caused by *M. graminicola*, showing a high level of infection in the whole fields.

Table 8. Biotic and abiotic factors in the phytobiome studied through the two NGS datasets generated for this thesis. Note that singletons, doubletons and low frequent ESVs (< 10 reads) were filtered in **dataset 1** (for both **chapter 2** and tree **B**), singletons and low frequent ESVs (< 10 reads) were filtered in **dataset 2** (for trees **C** and **D** but not for **chapter 3**).

Factors (biotic or abiotic)		**Dataset 1 (chapter 2)**	**Dataset 2 (chapter 3)**
Plants	Infection by *Meloidogyne graminicola*	**infected** or **non-infected** roots	abundance of juveniles in roots
	Genotype of *Oryza sativa*	Bac Thom n°7 (subsp. ***indica***)	IR504 and IR64 (subsp. ***indica***), Azucena and Zhonghua 11 (subsp. ***japonica***)
Practices		conventional tillage without cover crop (**CT**)	conservation agriculture (**CA**) and conventional tillage without cover crop (**CT**)
Scale	Plant compartment	root	rhizosphere (root in **chapter 4**)
	Geographical localization	Vietnam, Hải Dương	Cambodia, Stung Chinit
Soil		42% loam, 36% sand, 21% clay pH = 6.2 SOC = 1.55% TKN = 0.15%	69% loam, 18% sand, 13% clay pH = 5.3 SOC = 1.47% TKN = 0.05%
Micro-biodiversity	*Bacteria*	Count: 1,435,166 reads Total richness: 2,202 ESVs Average Shannon index: 5.24	Count: 361,889 reads Total richness: 11,919 ESVs Average Shannon index: 6.29
	Fungi	not studied	Count: 326,487 reads Total richness: 2.062 ESVs Average Shannon index: 3.87
	Nematoda	not studied	Count: 44,019 observations Total richness: 32 families Average Shannon index: 2.27

Compare microbiomes avoiding the pitfalls of the integrative approach

Crossing datasets is very useful in order to find specific and general patterns of association between the microbiota and environmental factors and to determine the core microbiota, *i.e.* commonly associated microorganisms, and the structuring factors of the rice-associated microbiota in different contexts with *M. graminicola*. However, the brief overall description of the microbiome datasets of this thesis (**figure 39**) highlights the pitfalls when integrating different levels of information from different datasets. The most obvious yet the most tempting is to compare datasets that are not generated with the same method and need transformation or internal reference. For example, the bacterial communities processed in **datasets 1** (tree **A**) and **2** (tree **B**) could not be represented together on the same tree because we used different pipelines of analysis, with different databases for taxonomic assignment (GREENGENES for **dataset 1** and SILVA for **dataset 2**). Testing different pipelines of analysis can be fastidious but, since the processing strongly influences the accuracy of the data (Pauvert *et al.*, 2019), optimizing and standardizing the analysis is highly recommended to perform meaningful comparisons, especially during meta-analysis of microbiome datasets. Multidisciplinary research programs such as the Earth Microbiome Project (EMP) characterized the global microbial taxonomic and functional diversity by creating huge databases of *16S rRNA* gene amplicon sequences (Thompson *et al.*, 2017). The EMP recommended protocols for different steps of the analysis that we followed: DNA extraction, amplicon sequencing with recommended primers (Gilbert *et al.*, 2014), and *Qiime2* processing which has to be adapted and developed for each community (*e.g.* nematode). In addition to the *in silico* processing, the method used to collect and store samples is important to generate sequences of good quality. Upstream, the experimental design is important too and necessitates an adequate amount of replicates to have conclusive results. Some plant-associated microbiota analysts recommend taking at least five replicate samples per plant organ or sample type to compensate for this inherent variability, and including bulk soil as a reference from which the root-associated microbiota has been most likely acquired (Lucaciu *et al.*, 2019). Though, the facility to generate data gained through NGS technology can result in a deluge of unmanageable data which must be countered by following these simple recommendations and clearly asking the scientific question in the first place.

In the past, our understanding of microbial ecology was limited by our ability to grow microorganisms in the laboratory. Prosser and some colleagues regret the fact that the majority of studies in microbial ecology address technical, rather than scientific challenges, and that microbial ecology is nowadays technique-based (Berg *et al.*, 2017). “Most [studies] are descriptive, do not address scientific aims or questions and are not designed to increase understanding or test hypotheses.” (Prosser 2020) They advocate for a renewed focus on hypothesis-driven approaches in microbial ecology. The study in **chapter 2** was motivated by the simple hypothesis that infected roots had a different microbiome than non-infected roots, which was confirmed in terms of structure, diversity enrichment, connectivity, *etc*. This descriptive study suggested that the microbiota was indeed specialized to survive in the gall environment and allowed us to know in which aspects. Although a shotgun metagenomic method would have been more appropriate to defend this hypothesis by functional predictions, this study constitutes the required basis of knowledge about the interaction between *Oryza sativa*, *Meloidogyne graminicola* and their associated bacteria. The study in **chapter 3** investigated the potential of conservation agriculture to reduce the phytoparasitic pressure through changes in the soil food web, based on the hypothesis that a regulation at several trophic

levels of the soil food web and of different natures of interactions was involved. In this complex network of interaction, we observed processes that occurred in the field (structuration, enrichment and therefore maturation of the soil food web, decomposition of organic matter through the fungal channel) and that could have reduced the nematode infection. Since the microbiodiversity is at the base of these processes, we intended to identify the biological actors in the last study in **chapter 4**. We focused our investigation on the bacterial endophytes from rice roots that are in close relationship with both the plant and the nematode, and therefore might play an active role in the reduction of the infection.

Focusing on these original questions for each study resulted in the integration of a minimal number of clearly defined factors within the phytobiome (**table 8**). In other words, to avoid the pitfalls of this approach using NGS technology, we compared contrasted conditions with specific measurements (**table 9**). For example, to test the link between the infection by *Meloidogyne* spp. and the rice-associated microbiota, we used qualitative information (infected *versus* non-infected roots) from plants with a high level of symptoms in **dataset 1**, and quantitative information (abundance of juveniles in roots) from a field infested with PPNs in **dataset 2**. However, we lack phenotypic characterization such as the symptoms of the disease caused by PPNs in **dataset 2** in field since infected plants are not necessarily diseased in suppressive soils (but were symptomatic in the greenhouse, *cf.* **chapter 4**). Besides, contrasted agricultural practices revealed differences in the rhizosphere communities, whereas the effect of the rice varieties was less obvious, possibly because the genotype has a limited effect on the rhizosphere microbiota as seen in other studies (Simonin *et al.*, 2020) and also because the environment brings high heterogeneity in the assembly of the plant microbiota and makes the host genotype hardly comparable (Wagner *et al.*, 2016; Wagner, 2021). Moreover, to observe a pattern, we need observations with contrasted conditions and/or a higher number of conditions. We compared only four individual varieties while there are more than 3,000 rice varieties. Combining the varieties by subspecies to test the genotype effect gave more contrasted results on the γ-diversity (**table 9**) therefore, by redefining the factors studied, we can confirm the little impact of the plant genotype on the richness of the rhizospheric communities (bacteria, fungi and nematodes together) and highlight the importance of this factor on the diversity, if confirmed.

Table 9. Brief summary of the diversity results of the two NGS datasets for comparison within each study on rice. Significativity codes for *p*: *** if < .001, ** if < .01, * if < .05, NS if non-significant.

Chapter 2 (dataset 1) - Comparison of infected *versus* non-infected roots			
Bacteria	Richness (**) Shannon index (***) Pielou's evenness (***)	821 5.49 0.82	664 4.99 0.77
Chapter 3 (dataset 2) - Comparison of rhizosphere within conservation agriculture *versus* conventional tillage without cover crop			
Bacteria	Richness (**) Shannon index (NS)	883 6.29	855 6.30
Fungi	Richness (***) Shannon index (*)	214 4.07	155 3.68
Nematoda	Richness (NS) Shannon index (**)	17 2.33	16 2.21

Chapter 3 (dataset 2) - Comparison of rhizosphere of *Oryza sativa* subsp. *indica versus japonica*			
Bacteria	Richness Shannon index	886 6.32	853 6.27
Fungi	Richness Shannon index	191 4.01	179 3.73
Nematoda	Richness Shannon index	16 2.24	18 2.31
Bacteria, Fungi and *Nematoda*	Richness Shannon index	1093 12.57	1050 14.01

To explore more globally the rice-associated microbiota in different contexts with *M. graminicola*, these two datasets could be implemented in a meta-analysis to be cross-study compared with other data applying the same methods. Combining data from multiple individual studies to address macroecological patterns at a larger scale remains methodically challenging and plagued with biases (Ramirez *et al.*, 2017). The EMP was confronted to the limits of the method using OTUs and already reached its maximal capacity of data processing but, in order to have better understanding of the effects of environmental stressors such as the nematode infection or the agricultural practices on the rice-associated microbiota, some databases meet the challenge such as the Microbiome Stress Project (Rocca *et al.*, 2019). These standardized computational methods could allow for example to find general responses to conservation agriculture and rice genotype, to find indicator taxa of the plant infected by or tolerant to *M. graminicola*, and more generally to predict the alterations on the microbiome in responses to anthropogenic perturbations such as tillage, pollution and climate change. In particular, although we found a field effect (*cf.* **chapter 2**), the three infested agricultural fields prospected in Vietnam (**dataset 1**) were geographically close therefore, the described gallobiome might be specific to their geographical localization in Vietnam, with their typical soil type and climate that have an impact on the microbiome. Similarly, the experimental field monitored in Cambodia (**chapter 3**, **dataset 2**) might give a unique pattern and this limits the conclusion of the study about conservation agriculture. To find common robust pattern about conservation agriculture, we could use for example other experimental fields set up by Florent Tivet from CIRAD and Vira Leng from DALARM comparing different combinations of practices in conservation agriculture (other leguminous crops, reduced tillage, *etc.*) in different rice production systems (lowland, upland, flood plains rice, *etc.*) and soil types (sandy, clayed, *etc.*). Cross-study comparison with the microbiomes in this thesis could participate in the research for the main environmental factors shaping the plant-associated microbiota in different contexts with PPNs. Moreover, there is an increasing recognition of the potential for parasite-associated microbiotas to influence and shape host-parasite interactions (Yurchenko and Lukes, 2018), even for *M. graminicola* (Topalovic *et al.*, 2021). However, no microbiota of *M. graminicola* has been published yet. Filling this gap could redefine the outlines of the microbiotas we have described, especially of the gallobiome, and give us new angles of attack to limit the incidence of the disease caused by *M. graminicola* on rice. Also, compare the microbiota of *M. graminicola* with other parasite-associated microbiotas, within the Parasite Microbiome Project for example (Dheilly *et al.*, 2019), is an option to fundamentally understand host-parasite-microbiota interactions and ultimately improve the health of plants or any host.

Other approaches to study the mechanisms of interactions

Within an evolutionary frame

As a golden rule in Science, correlation doesn't mean causality. The presence of microorganisms surrounding plants or nematodes can be a stochastic event. So, we wondered what are the mechanisms involved in the association between the rice, the phytoparasitic nematodes and the microbiota. Mechanisms of plant-pathogen coevolution have been intensively described and modelized at molecular level (Dodds and Rethjen, 2010). Researchers have demonstrated a crosstalk between both interacting entities: plants have the capacity to recognize pathogens through strategies involving conserved and variable pathogen elicitors, meanwhile pathogens manipulate the defense response through secretion of virulence effector molecules (*cf.* **chapter 1**). But environmental processes can change the outcome of the interaction by increasing the plant tolerance to pathogens (Roden *et al.*, 2009). Recently, more focus was put on the mechanisms of plant-pathogen interaction at the ecosystem level (Peyraud *et al.*, 2016). Plants and their microbiotas are increasingly defined as a unit, aka the holobiont, where the microbiotas provide genomic and functional extension, *i.e.* a manifestation of the effects of plant genes on their environment inside and/or outside of the organism. Commonly, the rhizosphere is seen as a plant-extended root phenotype (de la Fuente Canto *et al.*, 2020). The authors of this review discussed physical, chemical and biological processes and traits such as nutrient and water acquisition, tolerance to abiotic stresses, *etc.* confirming the extended phenotype which ultimately benefits plants. To do so, plants have the capacity to shape the physicochemical properties in the rhizosphere through investment of carbon in root exudation in the rhizosphere (Canarini *et al.*, 2019), meanwhile microorganisms possess substance preferences for consumption of compounds found in the soil or deposited in the rhizosphere by plants (Zhalnina *et al.*, 2018).

Such mechanisms of coevolution between plants and their associated microbiotas can be studied using analytical instruments, and targeted or untargeted high throughput techniques. For example, in the gallobiome (*cf.* **chapter 2**), additional analysis predicted a potential functional specialization of microorganisms. An analysis of the metabolites could have supported the enrichment of fermentative pathways by targeting alcoholic compounds and complex carbohydrates by gas chromatography and mass spectroscopy, respectively. For another example, in our study of the response of the rhizosphere microbiota to the four rice genotypes (*cf.* **chapter 3**), based on the observation that the variety Azucena had the lowest richness and diversity (especially fungal) and the highest abundance of PPNs in roots under CT, we can hypothesize that its exudation was less effective to attract phytobeneficial microorganisms and therefore less advantageous than the other genotypes. This could be verified by first comparing the exudation pattern of this variety in a synthetic soil microbial community (whose composition is known), by analyzing the primary and secondary metabolites in the rhizosphere with a shotgun LC-MS method. Plant-phenotypic traits (linked to the growth and the infection) could be measured to link the plant susceptibility with the microbiota structures. Following these results, enriched microorganisms in the rhizosphere, potentially attracted by plants, could be depleted in the synthetic soil microbial community, and the measurements could be repeated. If the exudation pattern is unchanged and the plant-phenotypic traits are maintained, then it would mean that the association is rather stochastic than due to a response of the plant and confer no

advantage. Otherwise, enriched microorganisms could be attracted by plants through specific exudates because of their phytobeneficial effects. Secondly, genetically modified plants in their metabolic pathways of, if possible, specific exudates could be created to observe if there is a response of the microbiota to altered exudation. If so, it would mean that the association is unspecific or not established through exudation. But if we observe that both entities are responding to modifications of their partner (modified soil microbiota and modified plant exudation), it could be interesting to check if the specific plant exudation and the bacterial attractiveness can be maintained under CA and through time, with the hypothesis that a strong association is conserved because it is advantageous for both parties that are, in fact, coevolving. Interestingly, plants can also produce exudates that repel phytoparasitic nematodes (Wuyts *et al.*, 2006; Escudero *et al.*, 2014). Studying the plant-microbiota-phytoparasitic nematode interaction using high-throughput techniques (transcriptomics, *cf.* **perspectives** in **chapter 4**, metagenomics, metabolomics) at the rhizosphere interface will enable us to describe if and how the plant and its associated microbiota are communicating and evolving together, in response to biotic stress such as phytoparasitic nematodes.

Designing synthetic microbial communities...

The molecular-based technique by amplicon barcoding and the cultivable techniques are complementary (*cf.* **chapter 1**). On one hand, the amplicon barcoding technique allowed us to know the presence and abundance of bacteria in roots infected by *M. graminicola* (*cf.* **chapter 2**) and in the roots under conservation agriculture (*cf.* **chapter 3**). On the other hand, the cultivable technique allowed us to isolate endophytic bacteria and to test their potential beneficial effects on rice (*cf.* **chapter 4**). We can match the ESVs in the bacterial microbiotas to the sequences of cultivable bacteria and obtain their abundance in the studied conditions as in **table 10** for candidates of special interest. Based on the different patterns of potential enrichments, we could choose a combination of candidate bacteria to test in a synthetic microbial community using different approaches (Bernstein, 2019). Synthetic microbial communities can serve as model systems to ask questions about the performance and stability of microbial communities (top-down approach). They can also serve to study which conditions are necessary to generate interaction patterns like symbiosis or competition and how they emerge (bottom-up). An intermediate approach would be to create a combination based on the conditions in which they could accommodate (the environment that favors their growth) and the phenotypic traits they could give to plants, designed to induce an emergence of phytobeneficial effects on plants. Such a combination could include:

- "**bioindicators**" of the condition we want to create. For example, *Stenotrophomonas maltophilia/Bacillus megaterium*, *Duganella rivus* and *Microbacterium hydrothermale* have been found more abundant in non-infected roots. They could be active microbes to suppress the disease caused by *M. graminicola* as already discussed for each of them in regards to our results. However, they are absent under CT, potentially because they are sensitive to tillage or inhibited by other microorganisms. *Paenibacillus illinoisensis*, another example, is known in the litterature to have a strong chitinolytic activity which can reduce egg hatching (Jung *et al.*, 2002), acting as a parasite. In our studies, it is indeed more abundant in infected roots, but seems to have few impacts, and it is little abundant in the field in Cambodia, even totally absent under CA. So, characterizing their mechanism of interaction

with plants (to obtain beneficial effects) and with other bacteria (to avoid inhibition), and assessing their persistence in the soil is required for an efficient use of bioindicators in syncoms.

- a **diversity** of microbes with different phytobeneficial strategies in order to limit the nematode infection by direct or indirect effects:
  - mutualism with plants. Some microbes, particularly fungi, are known to increase plant tolerance to the infection by PPNs such as *Paecilomyces lilacinus*, *Fusarium oxysporum*, *Trichoderma* spp. (Sikora *et al.*, 2008). This thesis has confirmed that CA favors growth of the mutualistic fungi *Glomeromycota* spp. (*cf.* **chapter 3**). It has also contributed to the characterization of potentially bacterial mutualists such as *Pseudomonas baetica* (*cf.* **chapter 4**) which is more abundant under CA and exhibits PGP effects *in vitro*.
  - antagonism against the nematode. We know a lot of microbial taxa able to antagonize PPNs with more or less specificity. Natural enemies include nematophagous fungi such as nematode-trapping fungi of the order *Orbiliales* and fungal parasites of the genera *Stylopage*, *Catenaria* and *Hirsutella*, nematophagous oomycetes of the genera *Myzocytiopsis*, *Haptoglossa*, *Nematophtora* and *Lagenidiaceae*, cyst and egg parasite of the genera *Pochonia* and *Purpureocillium*, and bacteria of the genus *Pasteuria* (Stirling *et al.*, 2015). There are many more that are not named here and others that remain to be discovered. This thesis has contributed to the identification of specific antagonistic consortia against *M. graminicola* (*e.g.* the bacteria *Stenotrophomonas maltophilia* and *Pseudomonas baetica*). Generalist predators and free-living nematodes, mites and collembola and obligate parasites such as viruses can also participate in the general antagonism of PPNs.
  - antagonism against helpers of the nematode infection. For example, ESVs assigned to *Novosphingobium* sp. have been found specifically enriched in the gall (*cf.* **chapter 2**, **sup. table 4**) and characterized as increasers of the infection (*cf.* **chapter 4**). Due to the ability of *Novosphingobium* spp. to degrade carbohydrates such as cellulose, a cell-wall compound, they could be opportunist during the infection process and help parasitism of the nematode (Topalović and Vestergård, 2021). ESVs assigned to *Novosphingobium* (family *Sphingomonadaceae*) have been found in a preliminary core microbiota of *M. graminicola* (Thi Phan Ngan, personal communication). Cao and colleagues (2015) also detected *Sphingomonadaceae* in a core microbiota of *M. incognita* on tomato. Moreover, they suggested a symbiotic role for some other bacteria (*Sinorhizobium* spp. and *Devosia* spp.) in relation to the plant and the nematode, considering their importance in nitrogen fixation. ESVs of *Bradyrhizobium* were specifically found in infected roots (*cf.* **chapter 2**) and *Bradyrhizobium retamae* was found in the core microbiota of *M. graminicola* (Thi Phan Ngan, personal communication), suggesting the gall is also a niche for nitrogen-fixing bacteria, potentially benefiting the plant. A few other species were found in this preliminary core microbiota of *M. graminicola* such as *Moraxella osloensis*, an endosymbiont of the slug-parasitic nematode *Phasmarhabditis hermaphrodita* (An *et al.*, 2008). Although the pattern of abundance of cultivable *Novosphingobium* spp. is unclear in our last study (**table 10**) and these associations remain speculative, considering not only the plant-associated microbiota but also the pathobiome (microbiome of the pathogen) of *M. graminicola* is an underexplored biocontrol strategy to date and an opportunity to reconsider parasitism outcome.

- **keystone** microbes, not necessarily phytobeneficial, but required to stabilize the synthetic microbial community via syntrophy, competition with invasive microbes, *etc.* For example, *Flavobacterium succinicans* has been found enriched in non-infected roots and identified as hub in infected roots (*cf.* **chapter 2**). It can live in both environments possibly due to its appetite for a broad range of carbohydrates. It could also contribute to the structure of the microbiota by breaking down complex carbohydrates that other microbes can then use (syntrophy). However, the best way to maintain a phytobeneficial syncom stable seems to be by creating the same environment in which the are found and expected effects were observed, that means not only considering the biological agents but also the abiotic factors to engineer the *Meloidogyne graminicola - Oryza sativa* pathosystem.

Table 10. Ratios of ESV reads affiliated with candidate cultivable bacterial endophytes in the two NGS datasets. Percentage identity with GREENGENES database is indicated (dataset 1). *Cf.* percentages of identity with NCBI and SILVA databases in **chapter 4**, **sup. table 10**. Total reads count is 1,653,757 in **dataset 1** (766,870:886,887 in infected:non-infected) and 532,926 in **dataset 2** (261,612:271,315 in CA:CT, roots only). Highlighting colors indicate the conditions in which the ESV is more abundant (potential enrichment): infected or non-infected, and CA (conservation agriculture) or CT (conventional tillage).

n°BRIO	NCBI assignment	ESV (dataset 1)	ESV (dataset 2)	infected *versus* non-infected	CA *versus* CT
2357	*Stenotrophomonas maltophilia*	cefa04bd189097bac7e246d4505ebeca (96.97%)	387349bbf301de818017a263010277fa	43:128	21:0
2370	*Bacillus zanthoxyli*	c6f61b95201848ba396fcf8f9f393bd8 (99.53%)	253a7230f2465f29a4f710bbbf490c22	302:579	79:646
2372	*Bacillus megaterium*	fcad212ec9ca292f4e0f19dbad8caec4 (98.05%)	714b0378efe0b8744e24aa04b48008d3	142:243	40:421
2386	*Duganella rivus*	34ca32bda8e437ef0c51d7f1ecda65e5 (97.90%)	3fb20a72f9c73dd635762b94a45d9a6a	85:568	74:0
2388	*Enterobacter ludwigii*	741058eb21ec4b6e57432389d0b7fe1f (97.12%)	32ab1812bd3770f4b7f6aad7273783da	398:686	107:6
2399	*Atlantibacter hermanii*	bf8c9c2d2bf5f63b5201d63ba69923ae (98.91%)	9b747cc81030e98da7def4519aec9d05	51:61	74:29
2405	*Microbacterium hydrothermale*	4141229207a0929c629e48f9b137fb18 (98.04%)	cfa64d02b5eeb7ba0aa94f848cf49acf	29:41	67:0
2407	*Novosphingobium humi*	40a43181ba38fb7d5df3fddc108d4a6d (99.75%)	485602dcc0f041e5a1f507f2708ae81f	1431:2900	0:878
2408	*Novosphingobium pukkalii*	1cd29a12798b9ade4a4bc9a0f9e45bc9 (97.97%)	c3c934de8a53419458c67f2a7de0024f	393:119	13:14
2409	*Paenibacillus illinoisensis*	63fbf4767c8bd2e6a825e4764dd4cebc (97.68%)	8999521e413899c0ba2a3417842097c5	42:18	0:19
2413	*Pseudomonas baetica*	0087467b4fbb7dd9fdeb2328972de33d (99.77%)	8338de26814ceac363faf0656a5b4058	404:67	82:0

...toward building soil disease suppressiveness

Two types of soil disease suppression have been described: specific and general suppression, and theories have been developed around these two models (Schlatters *et al.*, 2017). General suppression is due to the collective action of a given soil/plant-associated microbiota that hinders the establishment of a pathogen, mostly through competitive exclusion. This type of suppression is untransferable across distinct soil types. Specific suppression is mediated by the concerted enrichment and activity of specific microbial taxa that effectively antagonize the pathogen. This occurs mostly through the ecological mechanisms of competition, parasitism, and/or antagonism. This type of suppression is transferable to conducive soils. However, as explained by Dini-Andreote (2017), this categorisation can be merged by simply realizing that biotic competition, parasitism, and/or antagonism are all types of negative interactions between organisms that lead, to a large extent, toward competitive exclusion. Nonetheless, we can differentiate general suppression due to indirect phytobeneficial effects which confer a relatively broad tolerance to plants against various biotic or abiotic stresses, and specific suppression due to negative interactions directly on the nematode. This illustrates that concepts in both phytopathology and ecology are needed to understand the basis of soil disease suppressiveness. However, to what extent soil disease suppressiveness can be generalized against a broad range of disease remains unknown. Syncom approaches can help to answer this question. To what extent soil disease suppressiveness can be transferable between soils depends on the source and recipient status (*i.e.* diversity, composition, and structure) of the two soil communities. Transferability, or the successful establishment of organisms within a novel niche, can be explained by concepts of invasion ecology and microbial community coalescence (Rillig *et al*., 2015). How long soil disease suppressiveness can be heritable could depend on the structural environmental conditions. The driving force of disease suppressiveness might lie on a balance that tilts for positive interactions with rice. But by disrupting the interactions (biodiversity loss, niche destruction by tillage, soil depletion, *etc.*), the plant could lose advantage for its nutrition and development, and therefore become more sensitive to diseases and less resilient to other stresses. Therefore, by reducing soil perturbations such as tillage and increasing microbial diversity and activity through the addition of green manure, the positive balance could persist. This thesis has contributed to determine some of the structural abiotic factors of soil disease suppression, *i.e.* reduced tillage combined with the use of cover crop in the experimental field in Cambodia (*cf.* **chapter 3**) that has phytobeneficial effects through the microbiodiversity activity (*cf.* **chapter 4**) and, on the contrary, the factors that can lead to a disease emergence (*cf.* **chapter 2**), *i.e.* a specific microbiota associated with the gall that could have been driven or induced by the nematode in Vietnam. More aspects of the phytobiome and pathobiome should be studied, in order to improve plant health in agricultural systems. Is soil disease suppressiveness applicable to current agricultural systems? There is an urge to integrate ecological concepts with principles of plant pathology, in order to provide innovative ways to engineer the phytobiome, and to release the parasitic pressure on farmers and human food security. But this is rather a question of priorities than feasibility.

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Conclusion générale

(version courte française)

Principaux résultats

Au début de cette thèse, des connaissances étaient disponibles sur les interactions entre le riz et les nématodes phytoparasites ou les micro-organismes (bactéries et champignons), mais peu d'études abordaient les questions relatives aux facteurs modulant l'interaction des trois entités ensemble dans les systèmes rizicoles. Le premier chapitre de cette thèse s'interroge sur ce qui caractérise la maladie causée par *Meloidogyne graminicola* sur le riz et expose les raisons pour lesquelles une vision écologique est nécessaire sur le pathosystème *Oryza sativa - Meloidogyne graminicola*. Le fait que *M. graminicola* ne soit pas seulement un agent pathogène mais aussi un parasite est mis en avant pour rappeler que l'interaction de *M. graminicola* avec le riz peut être modulée pour avoir un effet moins délétère, notamment *via* les microorganismes du sol connus pour être potentiellement phytobénéfiques. Dès lors, des approches d'écologie des communautés sont indispensables pour tendre vers une meilleure compréhension des interactions entre agents pathogènes au sein du microbiote associé à la plante, et de leurs conséquences sur la santé de la plante. Le phénotype de la plante est en effet façonné à la fois par l'environnement et la génétique (Singh *et al.*, 2019). Dans ce cadre éco-évolutif, nous avons étudié le microbiote associé au riz dans différents contextes d'infection par *M. graminicola* ou suppression de la maladie. Dans le deuxième chapitre, nous avons décrit pour la première fois le microbiote de la galle de *M. graminicola* dans des rizières infestées, que nous avons proposé d'appeler le "gallobiome". Plus spécifiquement, nous avons caractérisé l'assemblage de la communauté bactérienne dans les racines morphologiquement modifiées par l'infection. Nous avons montré que cette nouvelle niche écologique dans les racines était un refuge pour la survie du parasite et de son microbiote associé et spécifique, caractérisé par une plus grande richesse, diversité et équitabilité. Dans le troisième chapitre, nous avons décrit les communautés rhizosphériques du riz cultivé dans des systèmes de culture contrastés. Nous avons essentiellement étudié l'impact des facteurs abiotiques (pratiques agricoles) sur les facteurs biotiques (bactéries, champignons et nématodes interagissant ensemble au sein du réseau trophique du sol). Un résultat important réside dans le fait que l'agriculture de conservation a amélioré la microbiodiversité et limité l'infection par *M. graminicola*. Puisqu'elle était associée à la maturation du réseau trophique du sol, une régulation de la population par prédation par les nématodes et antagonisme par les microbes pourrait avoir eu lieu directement sur les nématodes phytoparasites. Les endophytes bactériens provenant des racines collectées dans ce champ ont été testés pour leur potentiel de biocontrôle (antagonisme contre *M. graminicola* et bénéfices pour *O. sativa*) dans une expérience en serre et des essais *in vitro*. Nous avons mesuré les traits phénotypiques des plantes lors de traitements avec des bactéries représentant la microbiodiversité des racines de riz afin d'identifier les facteurs biotiques supprimant la maladie causée par *M. graminicola* sur le terrain. Les souches intéressantes qui ont montré des effets phytobénéfiques et/ou une activité nématicide ou nématostatique ont été rapportées dans le dernier chapitre.

Quelques perspectives

Conception de communautés microbiennes synthétiques...

Les techniques moléculaires par barcodage d'amplicons et les techniques de microbiologie pour cultiver des micro-organismes sont complémentaires (*cf.* **chapitre 1**). D'une part, la technique du barcodage d'amplicons nous a permis de connaître la présence et l'abondance des bactéries dans les racines infectées par *M. graminicola* (*cf.* **chapitre 2**) et dans les racines en agriculture de conservation (*cf.* **chapitre 3**). D'autre part, les techniques de culture nous ont permis d'isoler des bactéries endophytes et de tester leurs effets bénéfiques potentiels sur le riz (*cf.* **chapitre 4**). Nous pouvons faire correspondre les séquences du microbiote bactérien avec les séquences des bactéries cultivables et obtenir leur abondance dans les conditions étudiées comme dans le **tableau 10** pour les candidats d'intérêt particulier. Sur la base des différents types d'enrichissements potentiels, nous pourrions choisir une combinaison de bactéries candidates à tester dans une communauté microbienne synthétique en utilisant différentes approches (Bernstein, 2019). Les communautés microbiennes synthétiques peuvent servir de systèmes modèles pour poser des questions sur la performance et la stabilité des communautés microbiennes. Elles peuvent également servir à étudier quelles conditions sont nécessaires pour générer des schémas d'interaction comme la symbiose ou la compétition et comment ils émergent. Une approche intermédiaire consiste à créer une combinaison basée sur les conditions dans lesquelles les candidates pourraient s'adapter (l'environnement qui favorise leur croissance) et les traits phénotypiques qu'elles pourraient donner aux plantes, conçue pour induire l'émergence d'effets phytobénéfiques sur les plantes. Une telle combinaison pourrait inclure :

- des microbes **bioindicateurs** de la condition que nous voulons créer. Par exemple, les bactéries *Stenotrophomonas maltophilia*/*Bacillus megaterium*, *Duganella rivus* et *Microbacterium hydrothermale* ont été trouvées plus abondantes dans les racines non infectées. Elles pourraient être actives pour supprimer la maladie causée par *M. graminicola* comme déjà discuté pour chacune d'entre elles dans le cadre de nos résultats. Cependant, elles sont absentes sous labour conventionnel, potentiellement parce qu'elles sont sensibles au travail du sol ou inhibées par d'autres microorganismes. *Paenibacillus illinoisensis*, un autre exemple, est connue dans la littérature pour avoir une forte activité chitinolytique qui peut réduire l'éclosion des œufs (Jung *et al.*, 2008), agissant comme un parasite. Dans nos études, elle est effectivement plus abondante dans les racines infectées, mais semble avoir peu d'impact, et elle est peu abondante au champ au Cambodge, voire totalement absente sous agriculture de conservation. Ainsi, la caractérisation de leur mécanisme d'interaction avec les plantes (pour obtenir des effets bénéfiques) et avec d'autres bactéries (pour éviter l'inhibition) et l'évaluation de leur persistance dans le sol sont nécessaires pour une utilisation efficace des bioindicateurs dans les communautés microbiennes synthétiques.
- une **diversité** de microbes avec différentes stratégies phytobénéfiques afin de limiter l'infection par les nématodes par des effets directs ou indirects :
    - mutualisme avec les plantes. Certains microbes, notamment les champignons, sont connus pour augmenter la tolérance des plantes à l'infection par les nématodes phytoparasites tels que *Paecilomyces lilacinus*, *Fusarium oxysporum*, *Trichoderma* spp. (Sikora *et al.*, 2008). Cette thèse a confirmé que l'agriculture de conservation favorise la croissance des champignons mutualistes *Glomeromycota* spp. (*cf.* **chapitre 3**). Elle a également

contribué à la caractérisation de bactéries potentiellement mutualistes comme *Pseudomonas baetica* (*cf.* **chapitre 4**) qui est plus abondante sous agriculture de conservation et possède des effets *in vitro* de promotion de la croissance du riz.

- antagonisme contre le nématode. Nous connaissons un grand nombre de taxons microbiens capables d'antagoniser les nématodes phytoparasites avec une spécificité plus ou moins large. Les ennemis naturels incluent des champignons nématophages tels que les champignons piégeurs de nématodes de l'ordre des *Orbiliales* et les parasites fongiques des genres *Stylopage*, *Catenaria* et *Hirsutella*, des oomycètes nématophages des genres *Myzocytiopsis*, *Haptoglossa*, *Nematophtora* et *Lagenidiaceae*, des parasites des kystes et des œufs des genres *Pochonia* et *Purpureocillium*, et des bactéries commes les parasites du genre *Pasteuria* (Stirling *et al.* , 2015). Il y en a beaucoup d'autres qui ne sont pas nommés ici et d'autres encore qui restent à découvrir. Cette thèse a contribué à l'identification de consortia antagonistes contre *M. graminicola* (*e.g.* les bactéries *Stenotrophomonas maltophilia* et *Pseudomonas baetica*). Les prédateurs généralistes et les nématodes, acariens et collemboles libres, ainsi que les parasites obligatoires tels que les virus, peuvent également participer à l'antagonisme général des nématodes phytoparasites.
- antagonisme contre les auxiliaires de l'infection par les nématodes. Par exemple, des séquences attribuées à des bactéries assignées à *Novosphingobium* sp. (famille des *Sphingomonadaceae*) ont été trouvées spécifiquement enrichies dans la galle (*cf.* **chapitre 2**) et caractérisées comme des "augmentatrices" de l'infection (*cf.* **chapitre 4**). En raison de la capacité des *Novosphingobium* spp. à dégrader les glucides tels que la cellulose, un composé de la paroi cellulaire, elles pourraient être opportunistes pendant le processus d'infection et favoriser le parasitisme du nématode (Topalović et Vestergård, 2021). Des séquences attribuées à *Novosphingobium* ont été trouvées dans un microbiote central préliminaire de *M. graminicola* (Thi Phan Ngan, communication personnelle). Cao et ses collègues (2015) ont également détecté des *Sphingomonadaceae* dans un microbiote central de *M. incognita* sur tomate. En outre, ils ont suggéré un rôle symbiotique pour certaines autres bactéries (*Sinorhizobium* spp. et *Devosia* spp.) en relation avec la plante et le nématode, compte tenu de leur importance dans la fixation de l'azote. Des séquences de *Bradyrhizobium* spp. ont été spécifiquement trouvées dans les racines infectées (*cf.* **chapitre 2**) et de *Bradyrhizobium retamae* dans le microbiote central de *M. graminicola* (Thi Phan Ngan, communication personnelle), ce qui suggère que la galle est également une niche pour les bactéries fixatrices d'azote, et ce qui pourrait bénéficier à la plante. Quelques autres espèces ont été trouvées dans ce microbiote central préliminaire de *M. graminicola* comme *Moraxella osloensis*, un endosymbionte du nématode parasite des limaces *Phasmarhabditis hermaphrodita* (An *et al.*, 2008). Bien que la signature de l'abondance des *Novosphingobium* spp. cultivables ne soit pas claire dans notre dernière étude (**tableau 10**) et que ces associations restent spéculatives, considérer non seulement le microbiote associé aux plantes mais aussi le pathobiote de *M. graminicola*, *i.e.* le microbiote associé à *M. graminicola*, est une stratégie de biocontrôle trop peu explorée à ce jour et une opportunité de reconsidérer les effets du parasitisme.

- des microbes "**clés de voûte**", pas nécessairement phytobénéfiques, mais nécessaires pour stabiliser la communauté microbienne synthétique par le biais de la syntrophie, de la compétition avec les microbes invasifs, *etc.* Par exemple, *Flavobacterium succinicans* a été trouvée enrichie dans les racines non infectées et identifiée comme hub dans les racines infectées (*cf.* **chapitre 2**). Elle peut vivre dans les deux environnements, probablement en raison de son appétit pour une large gamme de glucides. Elle pourrait également contribuer à la structure du microbiote en décomposant des glucides complexes que d'autres microbes peuvent ensuite utiliser (syntrophie). Cependant, la meilleure façon de maintenir stable un communauté microbienne synthétique phytobénéfique semble être de recréer le même environnement dans lequel les agents biologiques et les effets attendus ont été observés, ce qui signifie qu'il ne faut pas

seulement prendre en compte les agents biologiques mais aussi les facteurs abiotiques pour façonner le pathosystème *Meloidogyne graminicola - Oryza sativa*.

...pour la promotion de sols suppresseurs de maladies

Deux types de suppression des maladies par le sol ont été décrits : la suppression spécifique et la suppression générale, et des théories ont été développées autour de ces deux modèles (Schlatters *et al*., 2017). La suppression générale serait due à l'action collective d'un microbiote associé au sol ou à la plante qui entraverait l'établissement d'un agent pathogène, principalement par exclusion compétitive. La suppression spécifique serait médiée par l'enrichissement et l'activité concertés de taxons microbiens spécifiques qui s'opposeraient efficacement à l'agent pathogène. Cela se produirait principalement par le biais des mécanismes écologiques de compétition, de parasitisme et/ou d'antagonisme. Cependant, comme l'explique Dini-Andreote (2017), cette catégorisation peut être fusionnée en réalisant simplement que la compétition biotique, le parasitisme et/ou l'antagonisme sont tous des types d'interactions négatives entre organismes qui mènent, dans une large mesure, à l'exclusion par compétition. Néanmoins, nous pouvons différencier la suppression générale due aux effets phytobénéfiques indirects qui confèrent une tolérance relativement large aux plantes contre divers stress biotiques ou abiotiques, et la suppression spécifique due aux interactions négatives directement sur le nématode. Cela montre que des notions collectives de phytopathologie et d'écologie sont nécessaires pour comprendre la base de la suppression des maladies du sol. Cependant, on ne sait pas encore dans quelle mesure la suppression des maladies du sol peut être généralisée à un large éventail de maladies. Les approches de communautés synthétiques peuvent aider à répondre à cette question. La suppression des maladies pourrait être transférée d'un sol à l'autre en fonction de l'assemblage du microbiote source et de celui du récepteur et peut être expliquée par les concepts d'écologie des invasions et de coalescence des communautés microbiennes (Rillig *et al*., 2015). La durée de l'hérédité de la suppression des maladies pourrait dépendre des conditions environnementales structurelles. La force motrice de la suppression des maladies pourrait reposer sur une balance qui penche pour des interactions positives avec le riz. Mais en perturbant ces interactions (perte de biodiversité, destruction de la niche par le travail du sol, épuisement des ressources nutritives, *etc.*), la plante pourrait perdre des avantages pour son développement et devenir plus sensible aux maladies et moins tolérante aux autres stress. Par conséquent, en réduisant les perturbations du sol telles que le labour et en augmentant la diversité et l'activité microbienne par l'ajout d'engrais verts, l'équilibre positif pourrait persister. Cette thèse a contribué à déterminer certains des facteurs abiotiques structurels des sols suppresseurs de la maladie causé par *M. graminicola*, tel que la réduction du travail du sol combiné à l'utilisation de plantes de couverture dans le champ expérimental au Cambodge (*cf.* **chapitre 3**) qui a des effets phytobénéfiques à travers l'activité de la microbiodiversité (*cf.* **chapitre 4**) et, au contraire, les facteurs qui pourraient conduire à l'émergence de la maladie (*cf.* **chapitre 2**), *i.e.* un microbiote spécifique associé à la galle qui aurait pu être conduit ou induit par le nématode au Vietnam. D'autres aspects du phytobiome et du pathobiome devraient être étudiés, afin d'améliorer la santé des plantes dans les systèmes agricoles. La suppression des maladies est-elle applicable aux systèmes agricoles actuels ? Il est urgent d'intégrer les concepts écologiques aux principes de la pathologie végétale, afin de trouver des moyens novateurs de façonner le phytobiome et de réduire la pression parasitaire sur les agriculteurs et sur la sécurité alimentaire humaine. Mais il s'agit plutôt d'une question de priorités que de faisabilité.

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Abstract

Plants are interacting with plenty of surrounding organisms in their environment. The effects that emerge can result in detriment or benefit for plants. Nematodes, the most abundant soil animals, are playing a great role in soil fertility and are excellent bioindicators of the soil functioning through their diverse lifestyles. Among them, *Meloidogyne graminicola* is an obligate parasitic nematode of rice, its main host and a staple food for the human population. During its life cycle in rice, *M. graminicola* creates a specialized niche at the infection site in roots, called gall, that turns into a nutrient sink for the nematode feeding. It can disrupt the plant growth and ultimately cause a serious grain yield loss. Rapid socio-economic and environmental changes increased this parasitic pressure in rice cropping systems, urging farmers and researchers to consider the disease emergence with an ecological view. Pathogens could indeed cause little damage to plants thanks to a cohort of phytobeneficial microorganisms in disease suppressive soils. Therefore, agricultural systems promoting microbiodiversity such as soil conservation agriculture are brought forward. However, little is known about the complexity of simultaneous effects of biotic and abiotic factors on the plant-parasitic infection in rice cropping systems. In this thesis, we characterized the rice-associated gall microbiome of *M. graminicola* for the first time, in highly infested fields in Vietnam. Using an amplicon barcoding method on the *16S rRNA* coding gene, we observed deep modifications between galls and non-infected roots: a shift in the microbiota structure, a higher richness and diversity, taxa enrichments and a specific network of bacteria able to live in the "gallobiome" (*i.e.* biome of gall), potentially as opportunistic organisms. In a second step, since we noticed that rice was less infected by *M. graminicola* seven year after the transition from conventional tillage to conservation agriculture in an experimental field in Cambodia, we could explore the rhizosphere communities of bacteria (*16S rRNA*), fungi (*ITS2 rRNA*) and nematodes (microscopic observations) in this cropping system. Under conservation agriculture, we found that there was an accumulation of soil organic matter and nutrients available for plants and basal microorganisms which were more abundant and diversified, especially fungi. Through cascading effects, the soil food web became more mature and potentially harbored more mutualistic organisms for rice and antagonistic organisms to plant-parasitic nematodes. This hypothesis was tested in the last part, using a complementary cultivable method to recover bacterial endophytes from rice roots of the experimental field. We performed *in planta* tests to measure the potential of single bacterial inoculation to induce indirect beneficial effects on rice infected by *M. graminicola*, and *in vitro* tests to measure plant-growth promotion effects and direct effects against *M. graminicola*. We found phytobeneficial strains exhibiting plant-growth promotion traits, and maintaining the shoot mass while infected, therefore improving the plant tolerance to the nematode infection. Some strains were able to reduce the root galling and/or to antagonize the nematode, and some others were associated with the infection in the field and with the gallobiome, suggesting they could be opportunistic and/or assist the nematode. More research is required to assess the potential of a microbial consortium to cooperatively modulate the interactions in the pathosystem *Meloidogyne graminicola - Oryza sativa*. The approach used in this thesis revealed that promoting microbiodiversity through agricultural practices is a promising strategy to suppress the disease caused by *M. graminicola* and sustain rice health.

Keywords: *Oryza sativa*; amplicon barcoding; gall microbiome; *Meloidogyne graminicola*; conservation agriculture; soil disease suppressiveness

Résumé

Les plantes interagissent avec une multitude d'organismes dans leur environnement, dont les nématodes qui sont d'excellents bioindicateurs du fonctionnement du sol grâce à leurs divers modes de vie. Parmi eux, *Meloidogyne graminicola* (*Mg*) est un nématode parasite obligatoire du riz, son hôte principal et un aliment de base pour la population humaine. Au cours de son cycle, *Mg* crée une niche spécialisée au niveau du site d'infection dans les racines, appelée galle, qui se transforme en un puits de nutriments pour son alimentation. Il perturbe la croissance de la plante et, en fin de compte, peut provoquer une perte sévère du rendement en grains. Les changements socio-économiques et environnementaux rapides ont accru cette pression parasitaire dans les rizicultures, incitant les agriculteurs et les chercheurs à considérer l'émergence de la maladie avec une vision écologique. Les agents pathogènes pourraient en effet causer peu de dommages aux plantes grâce à une cohorte de micro-organismes phytobénéfiques dans les sols qui supprimerait les maladies. C'est pourquoi les agrosystèmes favorisant la microbiodiversité, comme l'agriculture de conservation du sol, sont mis en avant. Cependant, peu de choses sont connues sur la complexité des effets simultanés entre les facteurs biotiques et abiotiques sur l'infection parasitaire dans les rizicultures. Dans cette thèse, nous avons caractérisé pour la première fois le microbiote associé aux galles de *Mg*, dans des champs de riz fortement infestés au Vietnam. En utilisant une méthode par barcodage d'amplicon, nous avons observé de profondes modifications entre les galles et les racines non-infectées tels que de plus grandes richesse et diversité, ainsi qu'un réseau spécifique de bactéries capables de vivre dans le "gallobiome" (le biome de la galle), potentiellement comme organismes opportunistes. Dans un deuxième temps, puisque nous avons remarqué que le riz était moins infecté par *Mg* sept ans après la transition en agriculture de conservation dans un champ expérimental au Cambodge, nous avons pu explorer les communautés rhizosphériques de bactéries, de champignons et de nématodes dans ce système de culture. Nous avons constaté une accumulation de matière organique du sol et de nutriments disponibles pour des micro-organismes plus abondants et diversifiés en agriculture de conservation comparé à un labour traditionnel. Par des effets en cascade, le réseau alimentaire du sol est devenu plus mature et abriterait plus d'organismes mutualistes pour le riz et antagonistes des nématodes phytoparasites. Cette hypothèse a été testée dans une dernière partie, en utilisant une méthode complémentaire cultivable pour récupérer des bactéries endophytes des racines de riz du champ expérimental. Nous avons effectué des tests *in planta* pour mesurer la capacité des souches à induire des effets bénéfiques indirects sur le riz infecté par *Mg*, et des tests *in vitro* pour mesurer les effets de promotion de la croissance des plantes et les effets directs contre *Mg*. Nous avons trouvé des souches phytobénéfiques présentant des caractéristiques de promotion de la croissance des plantes, tout en maintenant la masse foliaire pendant l'infection, améliorant ainsi la tolérance de la plante au nématode. Certaines souches étaient capables de réduire le nombre de galles et/ou d'antagoniser le nématode, et d'autres étaient associées à l'infection sur le terrain et dans le gallobiome, suggérant qu'elles pourraient être opportunistes. Des recherches supplémentaires sont nécessaires pour évaluer le potentiel d'un consortium microbien à moduler de manière synergique les interactions dans le pathosystème *Meloidogyne graminicola - Oryza sativa*. L'approche utilisée dans cette thèse a révélé que la promotion de la microbiodiversité à travers les pratiques agricoles est une stratégie prometteuse pour supprimer la maladie causée par *Mg* et améliorer la santé du riz.

Mots-clés : *Oryza sativa*; amplicon barcoding; microbiote; *Meloidogyne graminicola*; conservation agriculture; sols suppresseurs de maladies