

PLANET project

Plant and soil health indicators from an agroecological perspective for sustainable rice production in Cambodia

PPNs cause serious losses in rice production

- rice yield losses due to PPN range from 10% to 25% worldwide
- Southeast Asia produce 90% rice production
- Pesticide is the most common method to control PPN in Cambodia



PPN infected rice field



=> shift of Cambodian rice farming systems towards a sustainable and safe production

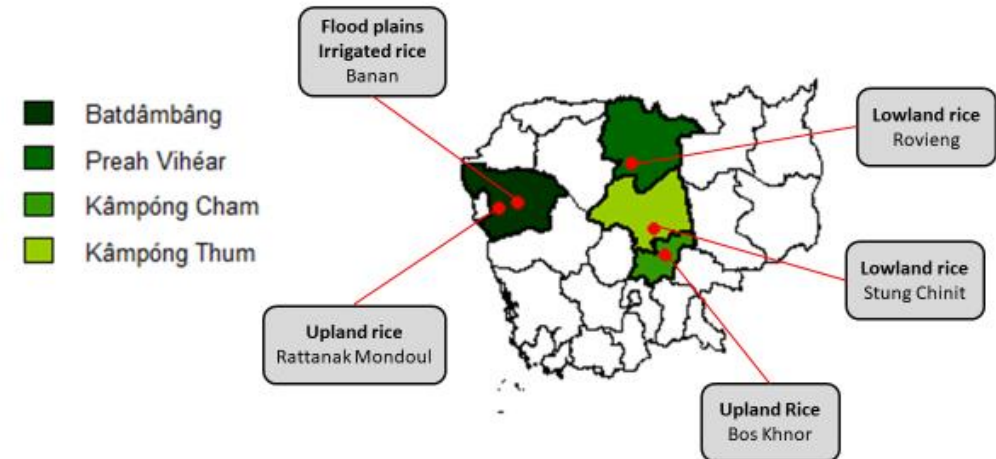
Conservation agriculture in Cambodia

- Minimum soil perturbation
- Permanent soil cover
- Crop rotation

=> maintain biodiversity of soil organisms

=> relies on natural processes

=> increase yield



- **CA** (no tillage + cover crops)
- **CT** (conventional tillage + no cover crop)

Which PPNs are present in Stung chinit?

Method: - ITS/18S/28S metabarcoding
- Shot-gun sequencing

Database: SILVA, ntNCBI, rice PPN references

Sample: Nematode population from rice root Phka Rumduol

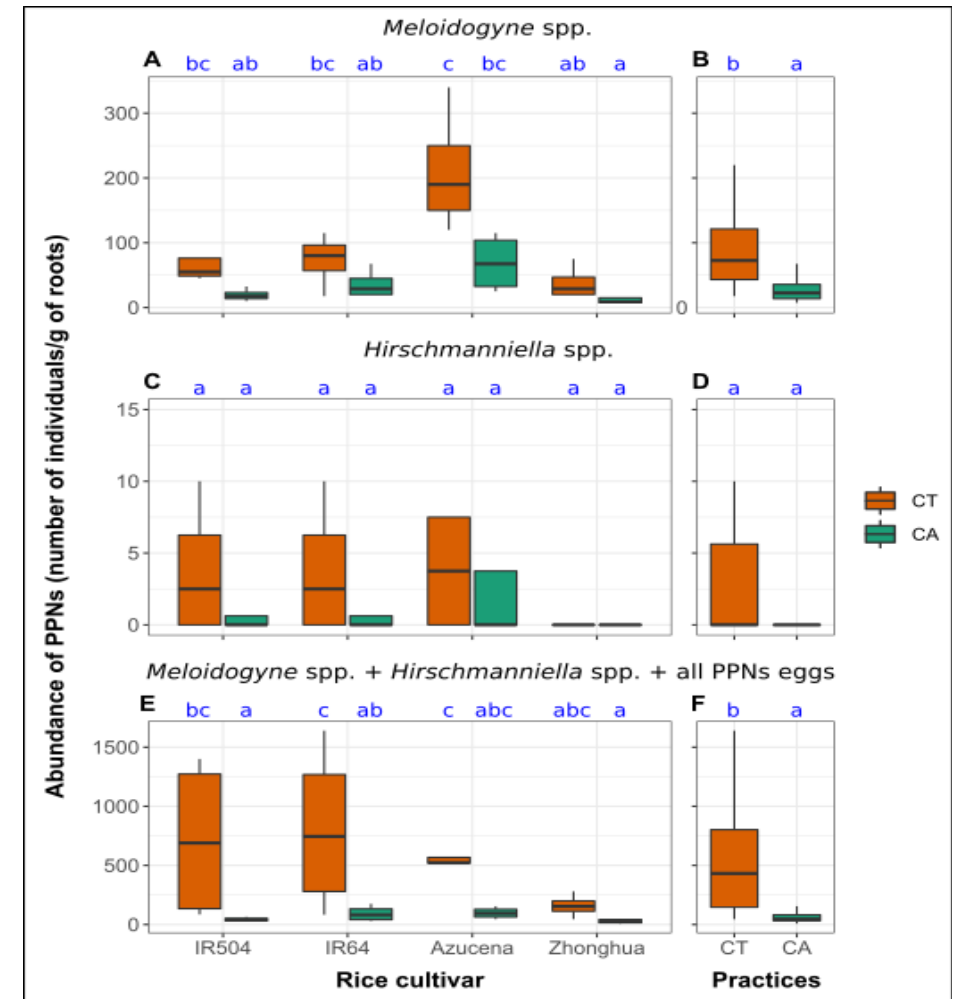
3 genus among 24 parasitic nematode in rice were found:

Genus	Amplicon read coverage	Shot-gun reads coverage
<i>Meloidogyne</i>	48252	6405
<i>Hirschmanniella</i>	1321	16
<i>Tylenchorhynchus</i>	1074	11

- Does CA allow to reduce PPN or make plant tollerant to PPN?
- CA has specific microbiome that participate in the biocontrol of this pest?

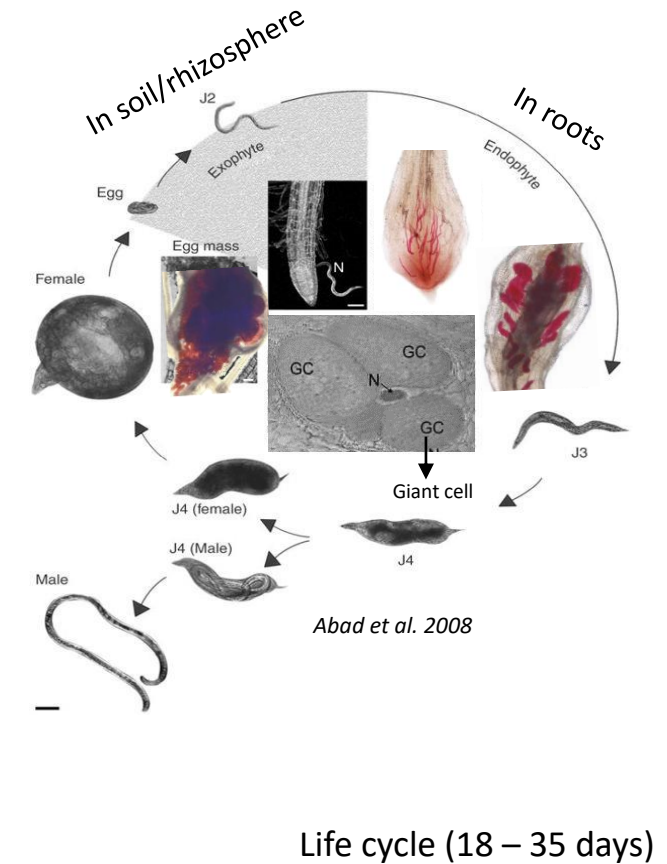
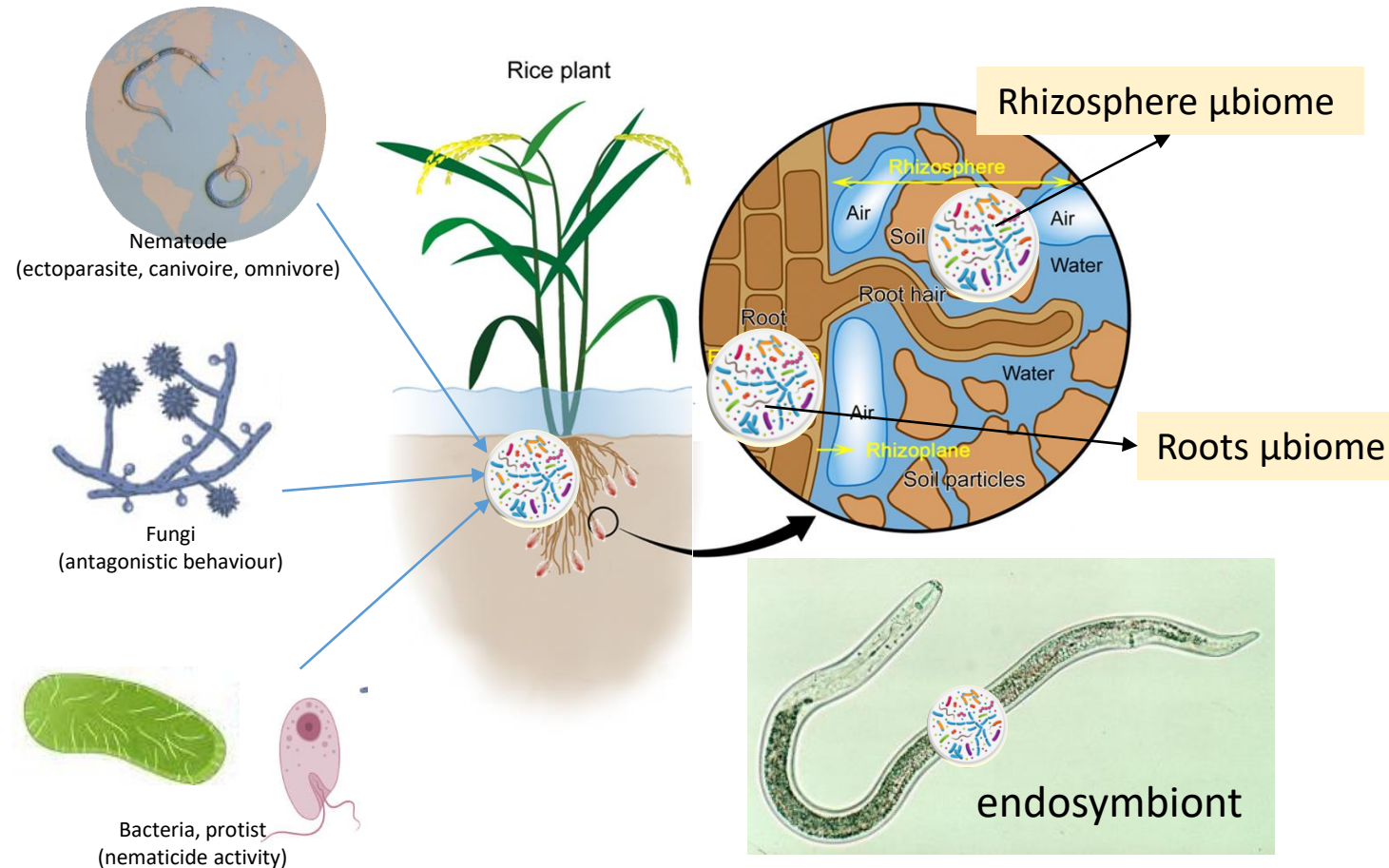
Sample: Nematode population colected from 4 varieties in CA and CT practice

Method: microscopic observation



Masson et al. unpublished data

Which microorganisms can control PPN?



- Nematode, bacteria, fungi and other protists can control PPN
- Microorganisms in roots, rhizosphere, and endosymbiont can have impact on PPN living and infection
- Root-knot nematode spends most of its lifecycle inside the roots => Roots microbiome and endosymbiont should have more impact on PPN ?

Amplicon barcoding in Stung chinit samples under CT and CA



1 field managed under 2 types of **practices**:

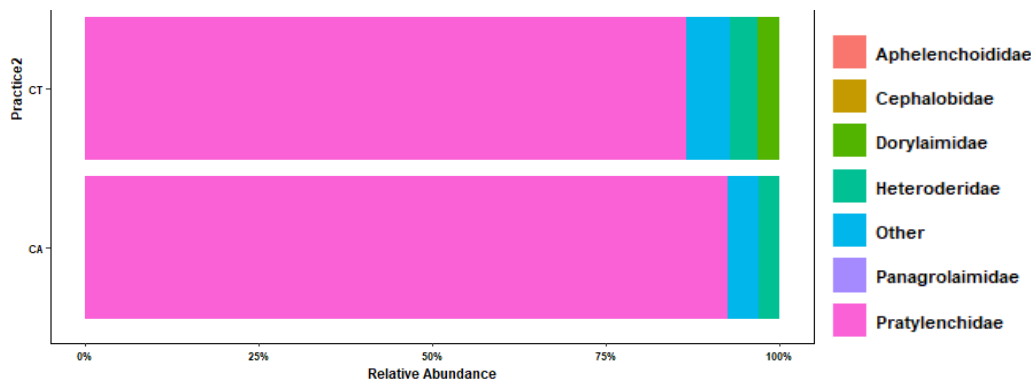
- **CA** (no tillage + cover crops)
- **CT** (conventional tillage + no cover crop)

Rice varieties	Species	Host of <i>Mg</i> ?	Replications	Compartments
IR64	<i>O.s. indica</i>	Susceptible	4 reps per practices	Root and Rhizosphere
IR504	<i>O.s. indica</i>	Susceptible		
Azucena	<i>O.s. japonica</i>	Susceptible		
Zhonghua	<i>O.s. japonica</i>	Resistance		

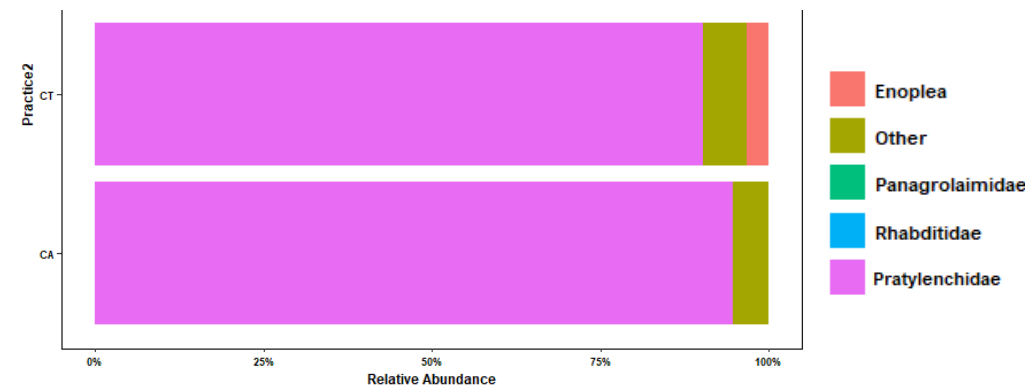
Compartment	#Samples	DNA extraction	μ biome	Amplicon barcode	Database
Root	32	PowerSoil kit	Nematode	18S	SILVA/ntNCBI/ rice PPN refs
Rhizosphere	32		Fungi	ITS	UNITE/ntNCBI
			Bacteria	16S	SILVA/ntNCBI

Nematode population on roots

Using ntNCBI database



Using SILVA database



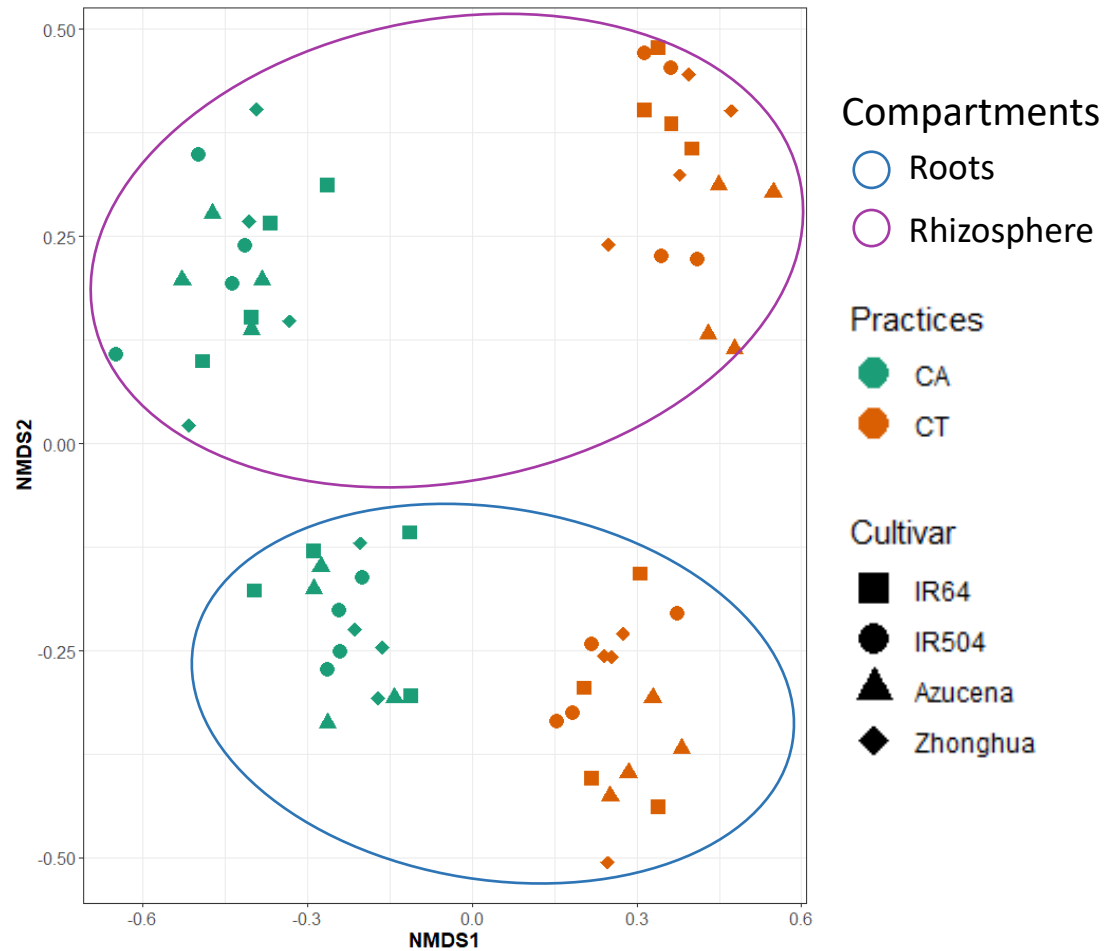
Using homemade rice PPNs references

	CA		CT	
	Genus	#reads	Genus	#reads
Azucena	Hirschmanniella	59031	Hirschmanniella	92153
	Meloidogyne	28	Meloidogyne	321
IR504	Hirschmanniella	58548	Hirschmanniella	46828
			Meloidogyne	753
IR64	Hirschmanniella	49021	Hirschmanniella	57023
	Meloidogyne	492	Meloidogyne	516
Zonghua	Hirschmanniella	54764	Hirschmanniella	58516
			Meloidogyne	119

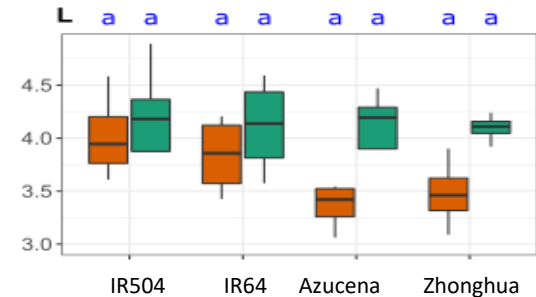
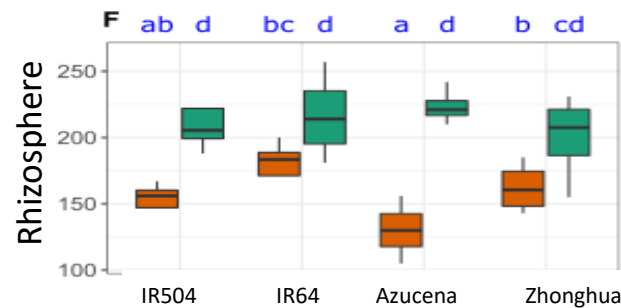
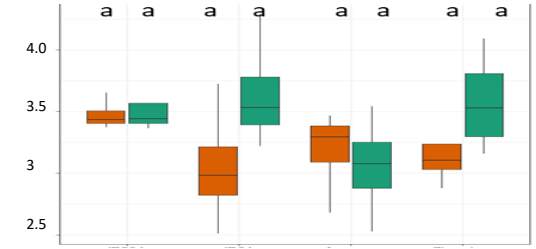
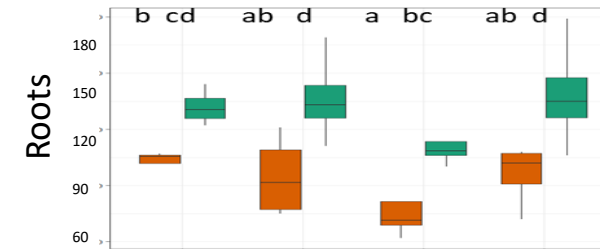
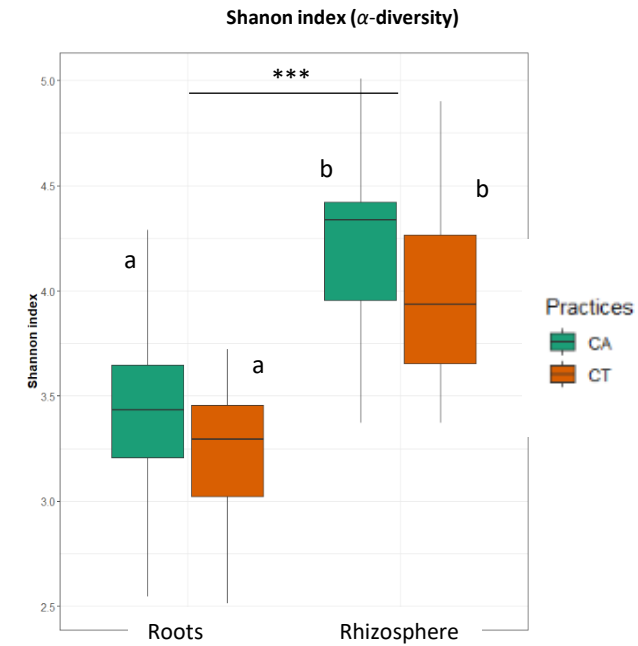
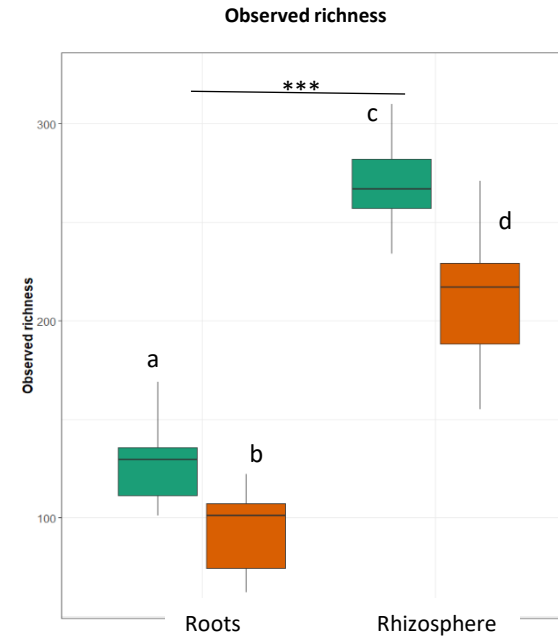
Pratylenchidae (*Hirschmanniella*) is the most abundance which is not the case

=> DNA extraction methods does not allow to retrieve all nematode DNA!

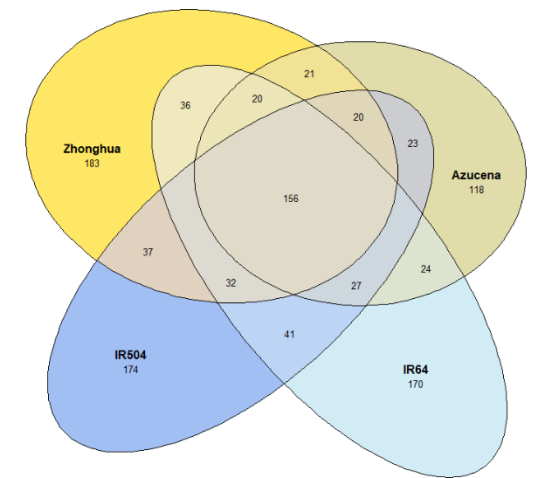
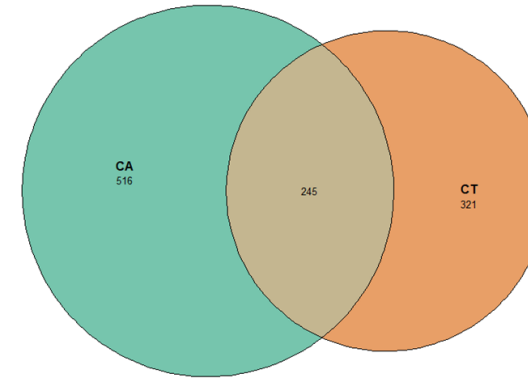
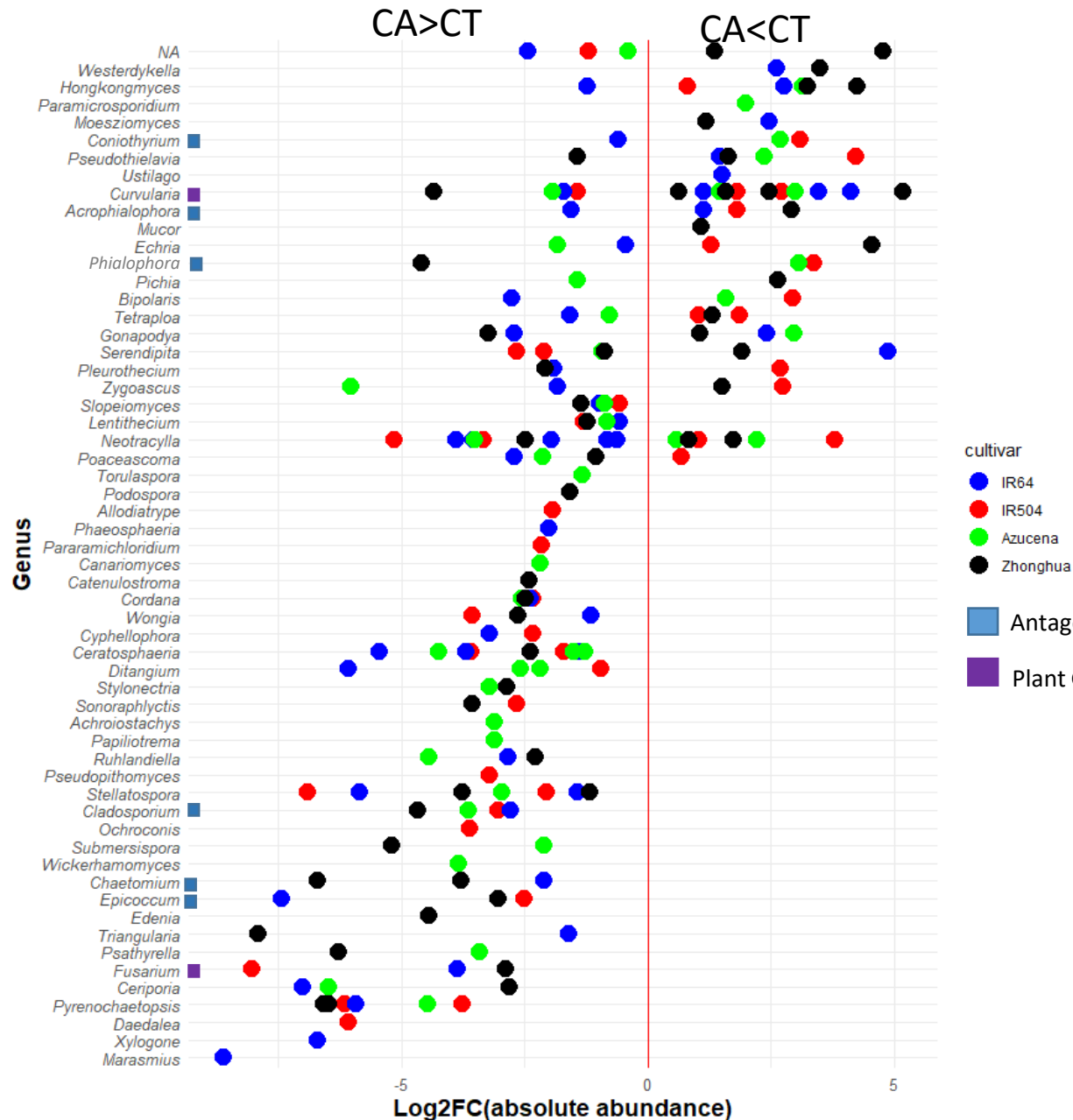
Diversity of the fungi communities



- **Compartment** level: Richness and diversity higher in Rhizosphere
- **Practice** level: Richness higher in CA
- **Cultivar** level: Higher richness in CA in all cultivar



Differential taxonomic enrichments of fungi in roots

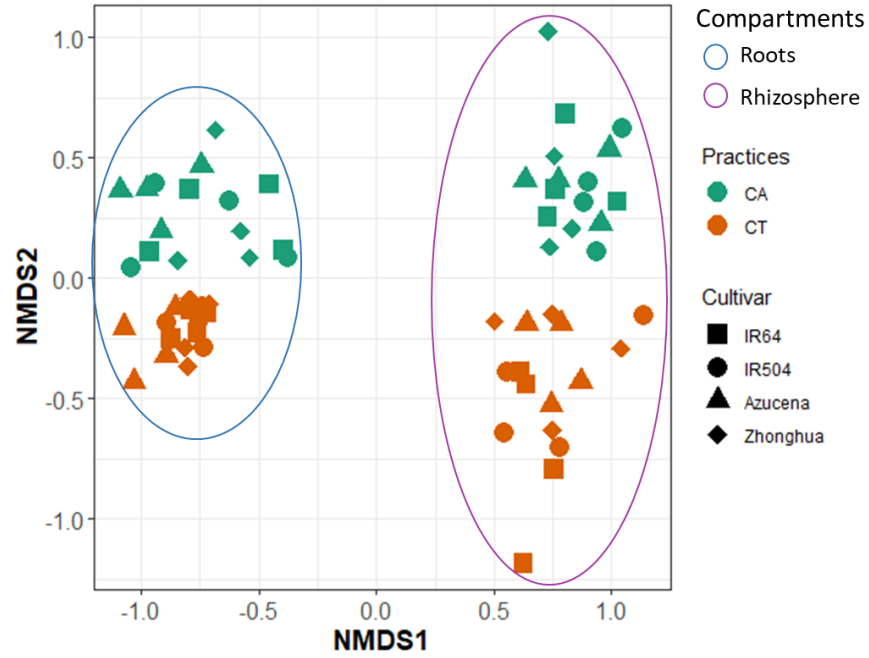


Venn diagrams of feature counts (SVs) in the roots communities of fungi

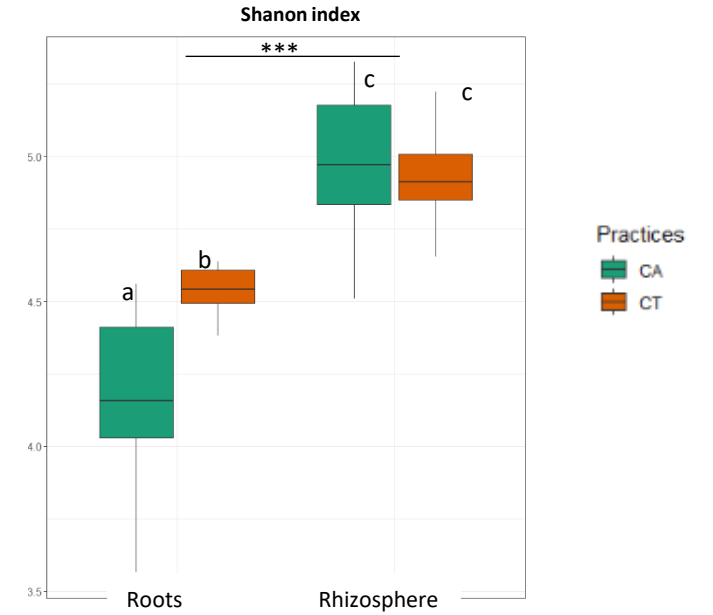
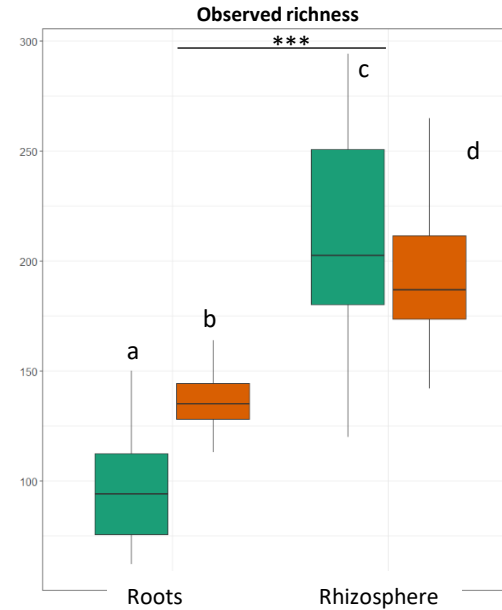
- Higher #SVs in CA
- Specific SVs and genus associated to cultivar
- Specific fungi genus associated to CA

=> Do these fungi has antagonistic behavior against PPN?
=> Are they PGPF ?

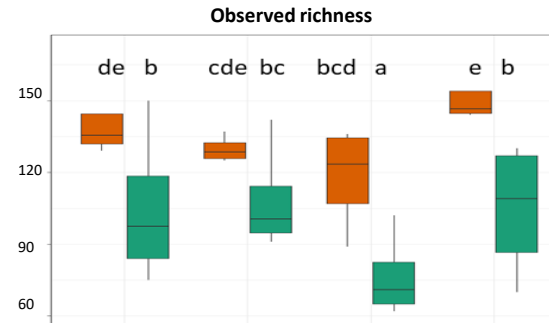
Diversity of the bacteria communities



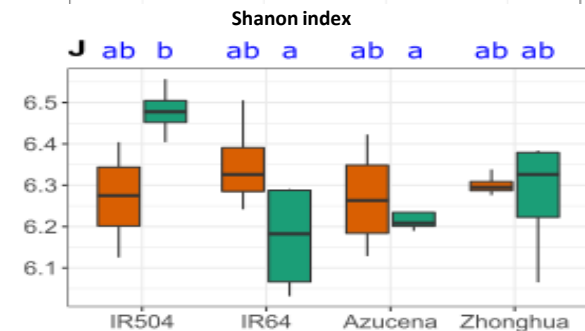
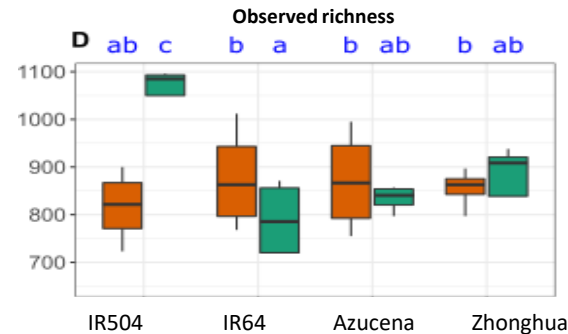
- **Compartment** level: Richness and diversity
- Rhizosphere >> Roots
- **Practice** level:
- In Rhizosphere: Richness CA >> CT
- In roots: Richness and diversity CT >> CA
- **Cultivar** level: Richness and diversity
is not always higher in CT in all cultivar



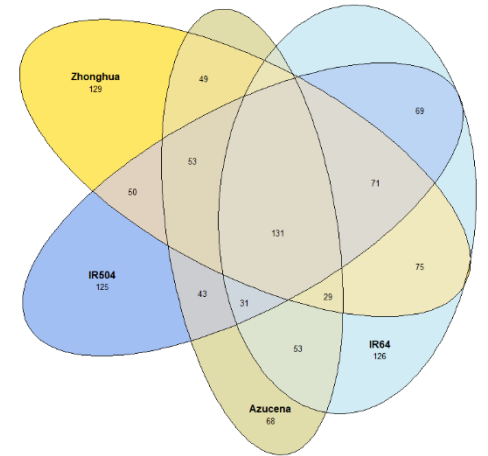
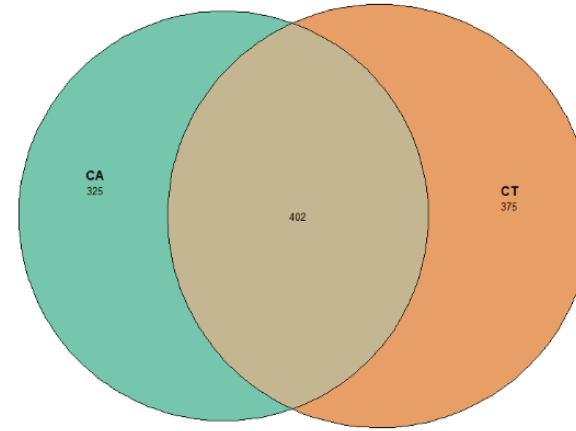
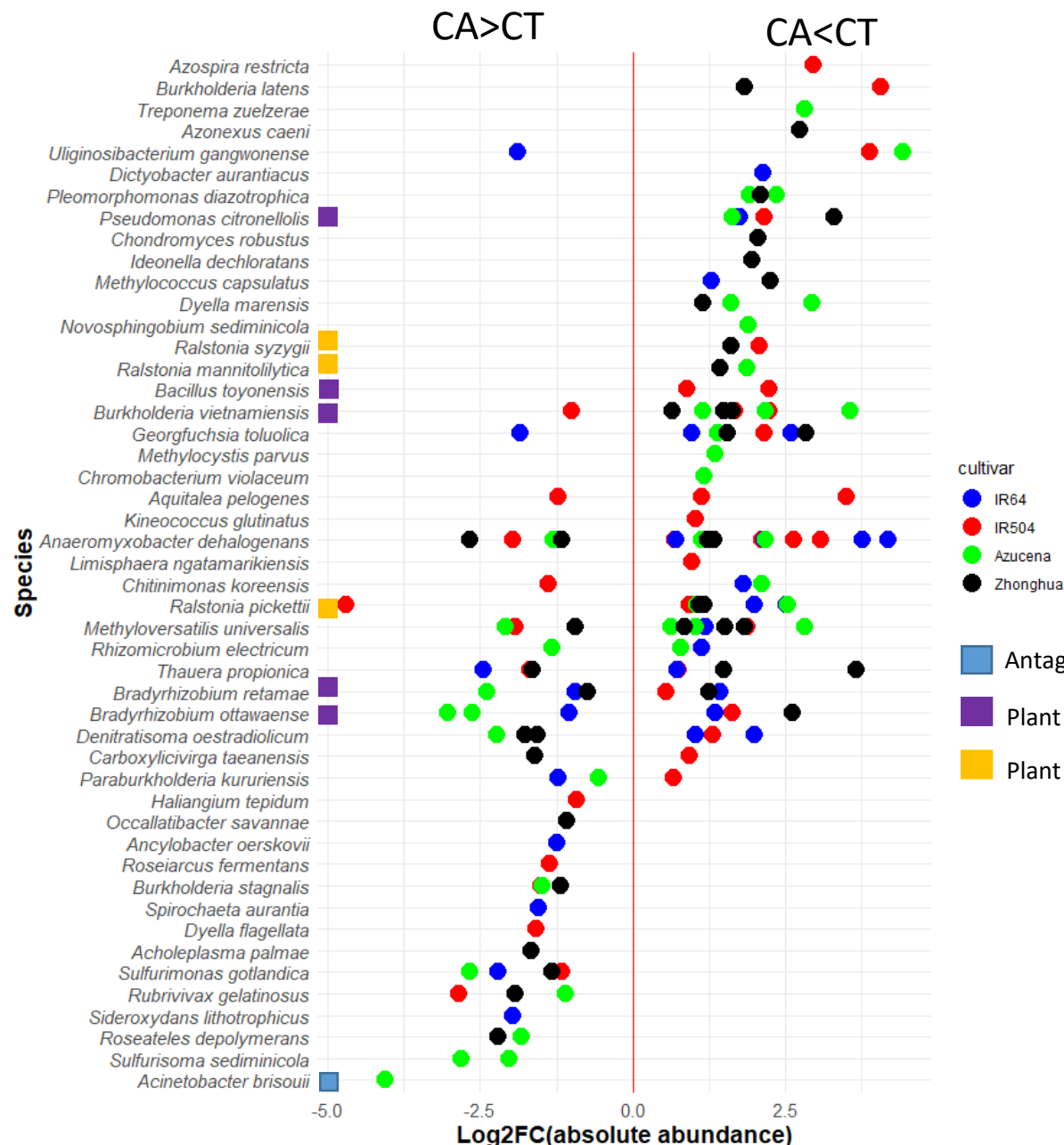
Roots



Rhizosphere



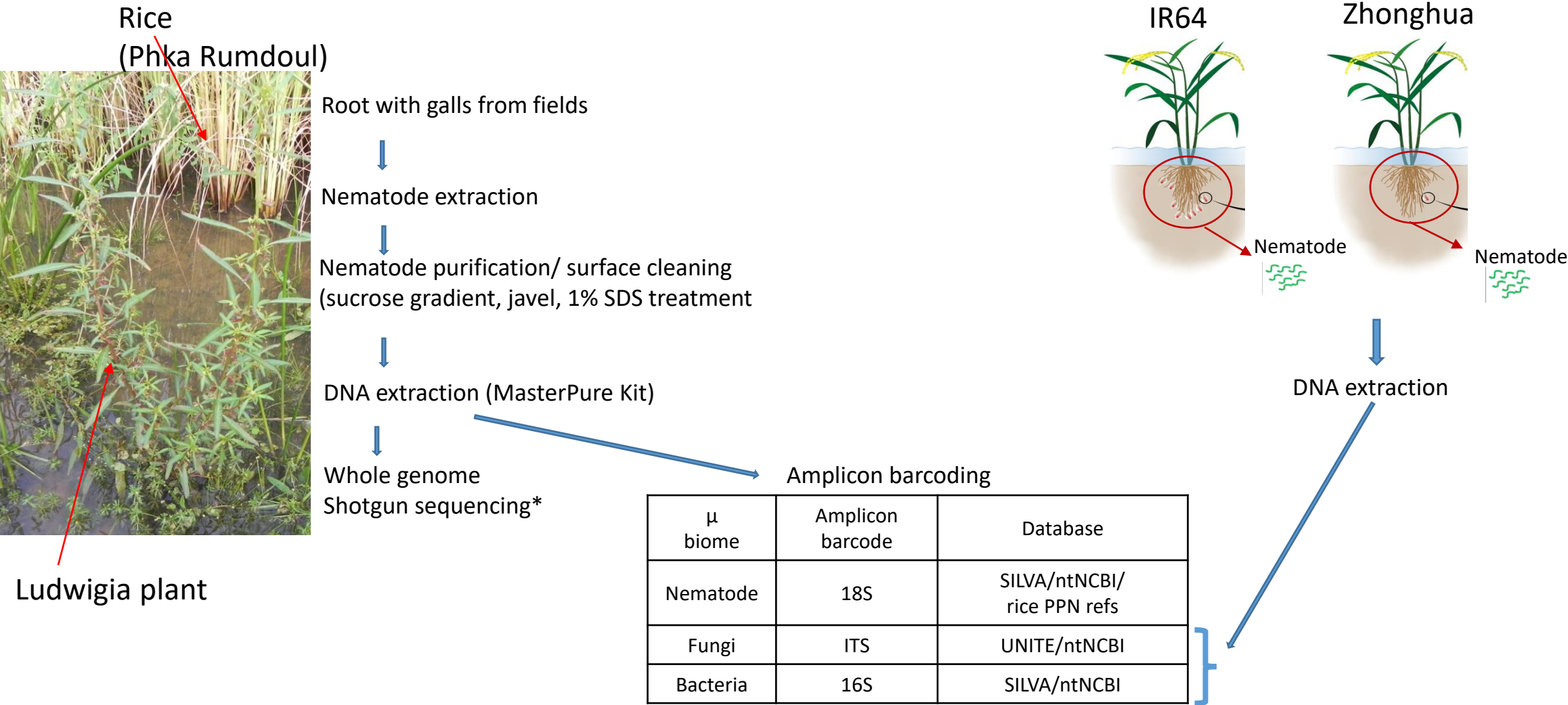
Differential taxonomic enrichments of **bacteria** in roots



Venn diagrams of feature counts (SVs) in the roots communities of bacteria

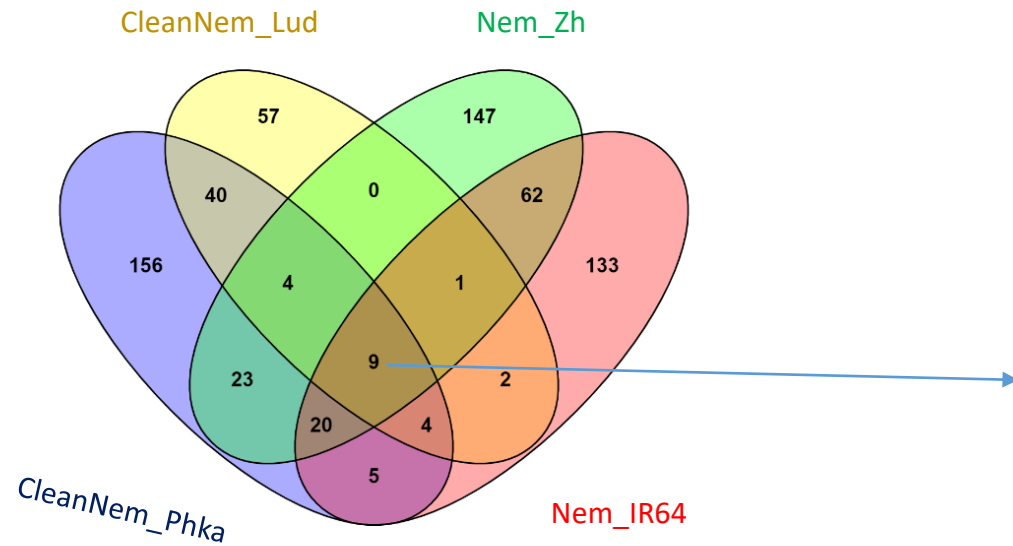
- Slightly higher #SVs in CT
- Specific SVs and species associated to cultivar
- Specific species genus associated to CA
 - => Do they has antagonistic behavior against PPN?
 - => Are they PGPB?

Amplicon barcoding for endosymbionts of *Mg*



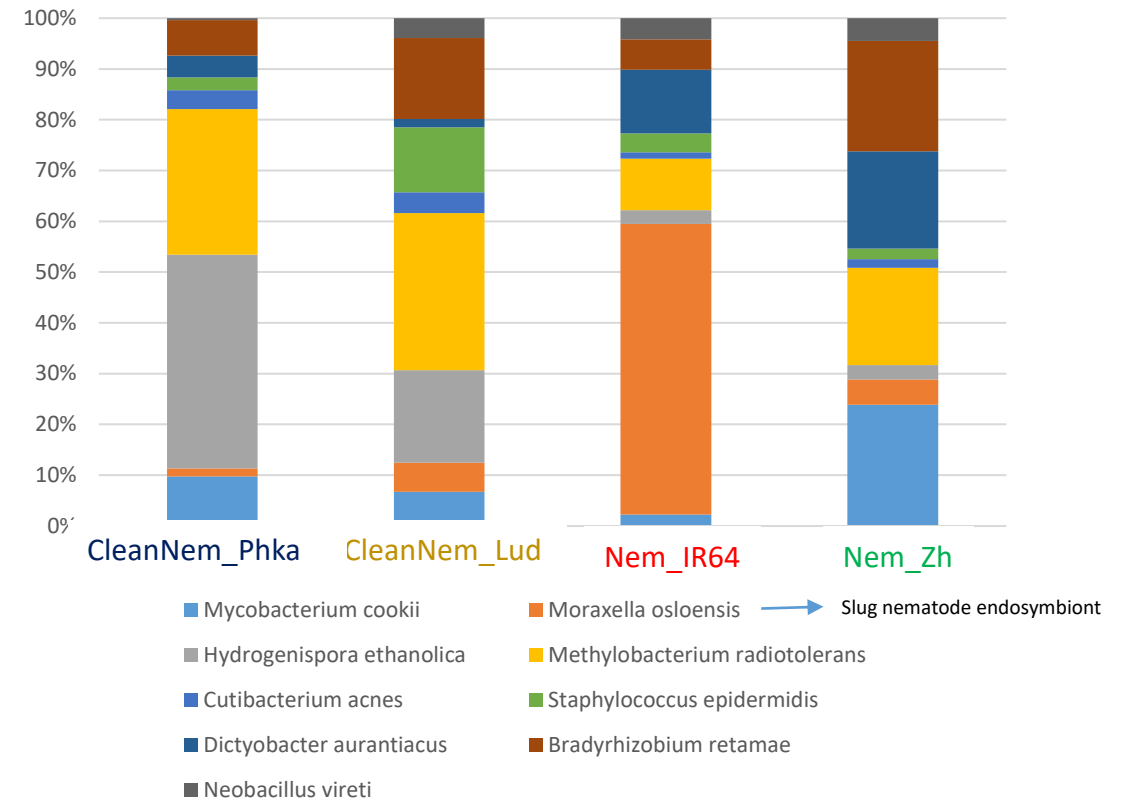
**Meloidogyne graminicola* genome coverage (600x)

Potential set of endosymbionts of *Mg*



Venn diagrams of bacteria species found in 4 samples

*Note: in Ludwigia root: only *Mg* was found



Relative abundance of 9 bacteria species found in four Nematode sample

Main messages and remaining questions

1. Both **CA** and **CT** practices showed **higher** richness and diversity of microbiome (fungi and bacteria) in **rhizosphere** than root
2. on roots : **CA** practices associated to **higher** richness and diversity of **fungi** community compare to **CT**
3. on roots : **CA** practices associated to **lower** richness and diversity of **bacteria** community on roots compare to **CT**
4. **Specific microbiome** (fungi and bacteria) associated to rice roots under **CA** and **CT** were found
 - ? Do they have nematocide/ antagonist activity
 - ? Do they promote plant development?
5. A set of potential bacterial endosymbiont of *Mg* was detected
 - ? How they impact *Mg* infection to plant? Do they participate to promote plant development ?

Future works

- Do CA practice resulted in promoting plant developments and/or reduction of PPN?
 1. study CA between different fields/ rice agro-ecosystems
 - (i) Kampong Thom (Stung-chinit, irrigated rice on sandy soil),
 - (ii) Preah Vihear (rain fed upland and irrigated rice on upper terraces)
 - (iii) Battambang (irrigated rice on clayed soils)
 2. cultivable method to test biocontrol activity and soil suppressiveness (reduction of the ability of PPNs to infect rice plants) of specific microbiome (fungi and bacteria) associated to rice roots under CA
 3. cultivable method to test PGPB and PGPF activities of fungi and bacteria in rice
- Do *Mg* microbiome impact to plant microbiom that resulting in promoting plant development?
 1. More replications of *Mg* microbiome samples
 2. Detection of bacteria/fungi in *Mg* (using FISH/microscopic flourescence)
 3. Cultivable method and infect in Rice => PGPR effect?

THANK YOU



6 samples:



Nematode

Nem_IR64

Nem_Zh



Galls (infected)

Gall_IR64

Gall_Zh

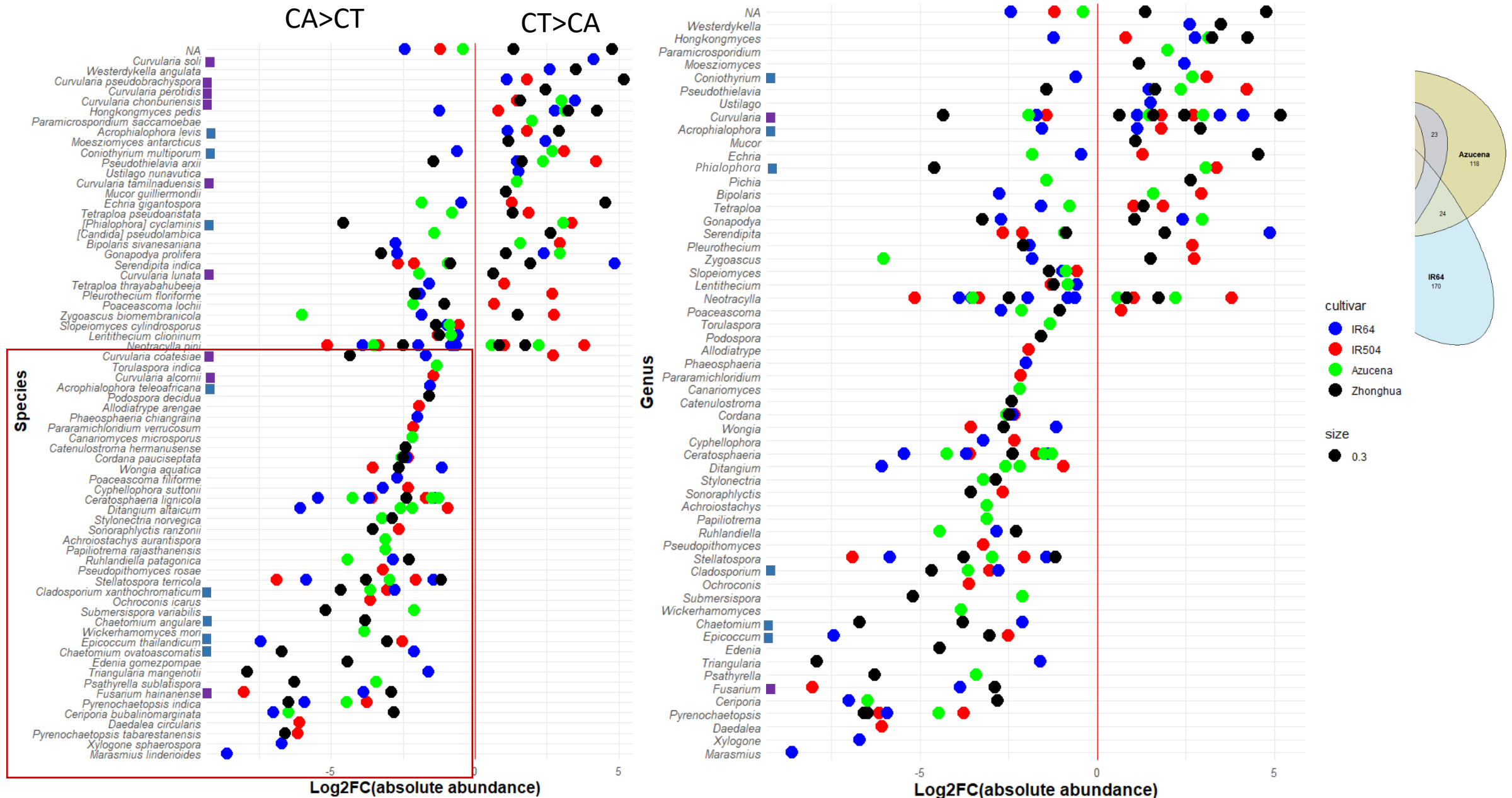


Roots (non-infected)

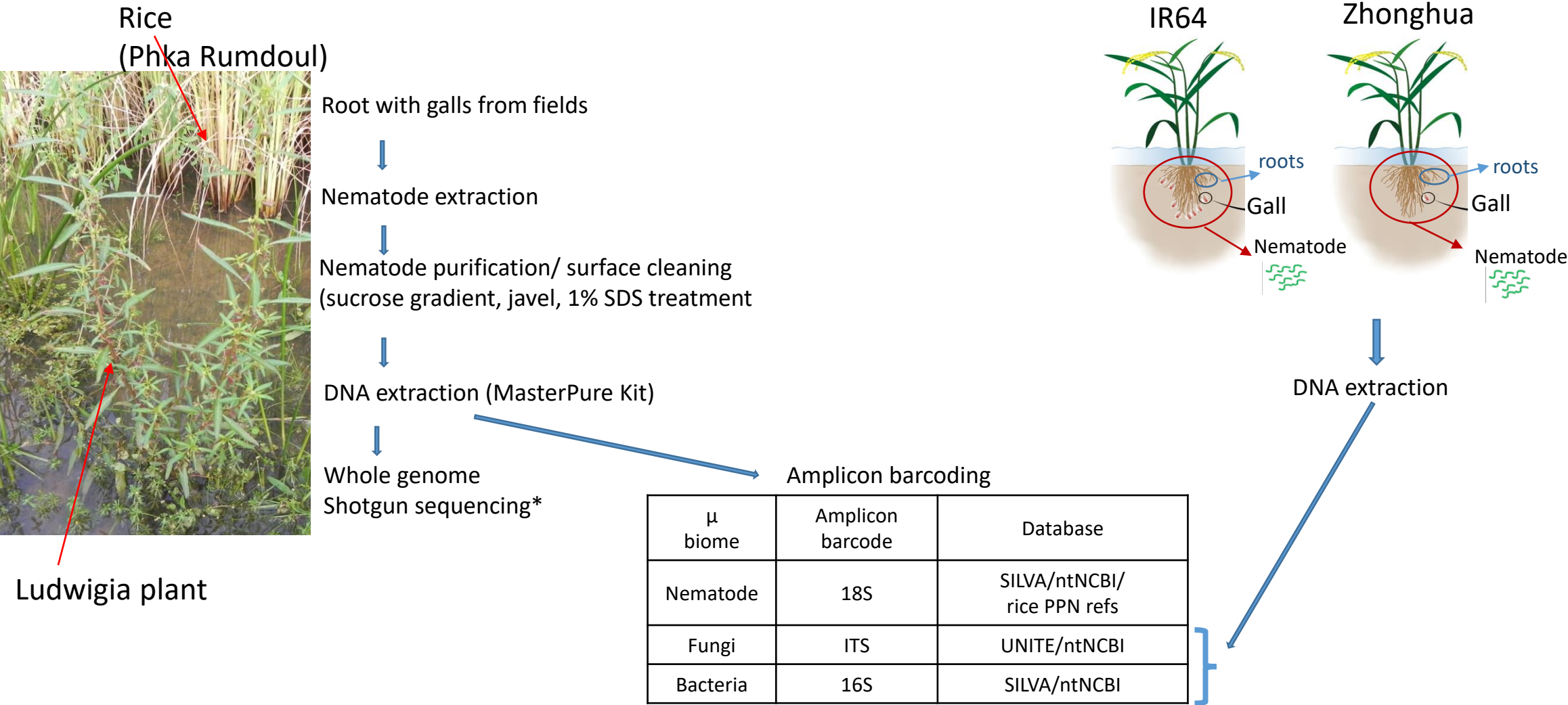
Root_IR64

Root_Zh

Differential taxonomic enrichments of fungi in roots under CA and CT



Amplicon barcoding for endosymbionts of *Mg*



**Meloidogyne graminicola* genome coverage (600x)

6 samples:



Nematode

Nem_IR64

Nem_Zh



Galls (infected)

Gall_IR64

Gall_Zh



Roots (non-infected)

Root_IR64

Root_Zh