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Agroprairie, a new intercropping system: what is the impact on the regulation of *Zymoseptoria tritici* and wheat microbiome ?

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Introduction

Increasing biodiversity in agroecosystems is desirable for many services, including disease regulation^{1,2}. The diversification offered by current approaches such as intercropping (e.g. often two crops in association) falls short of what is generally advocated by ecological studies. In this context, a new cropping system called "Agroprairie" has been designed³ (Figure 1) resulting from the association of alternating strips of wheat and diverse prairies (>10 plant species). We studied the effect of this new cropping system on the regulation of *Zymoseptoria tritici*, one of the most important wheat diseases in European countries.

We hypothesized that the well-known mechanism of the dilution effect⁴ influences the system, as well as the inhibition of pathogens⁵ linked to the modification of the plant microbiome in such diversified agroprairies (Figure 2).

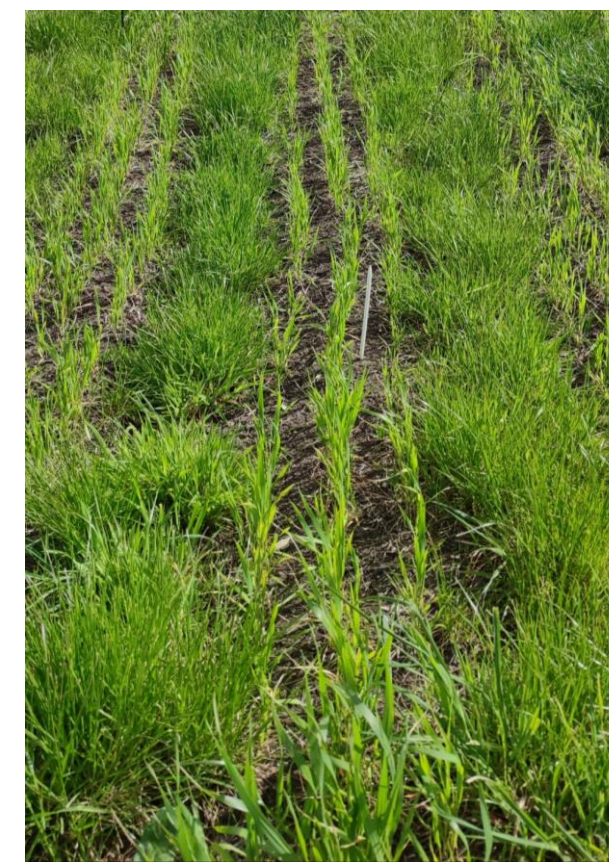


Figure 1: Agroprairies (INRAE Crouel)

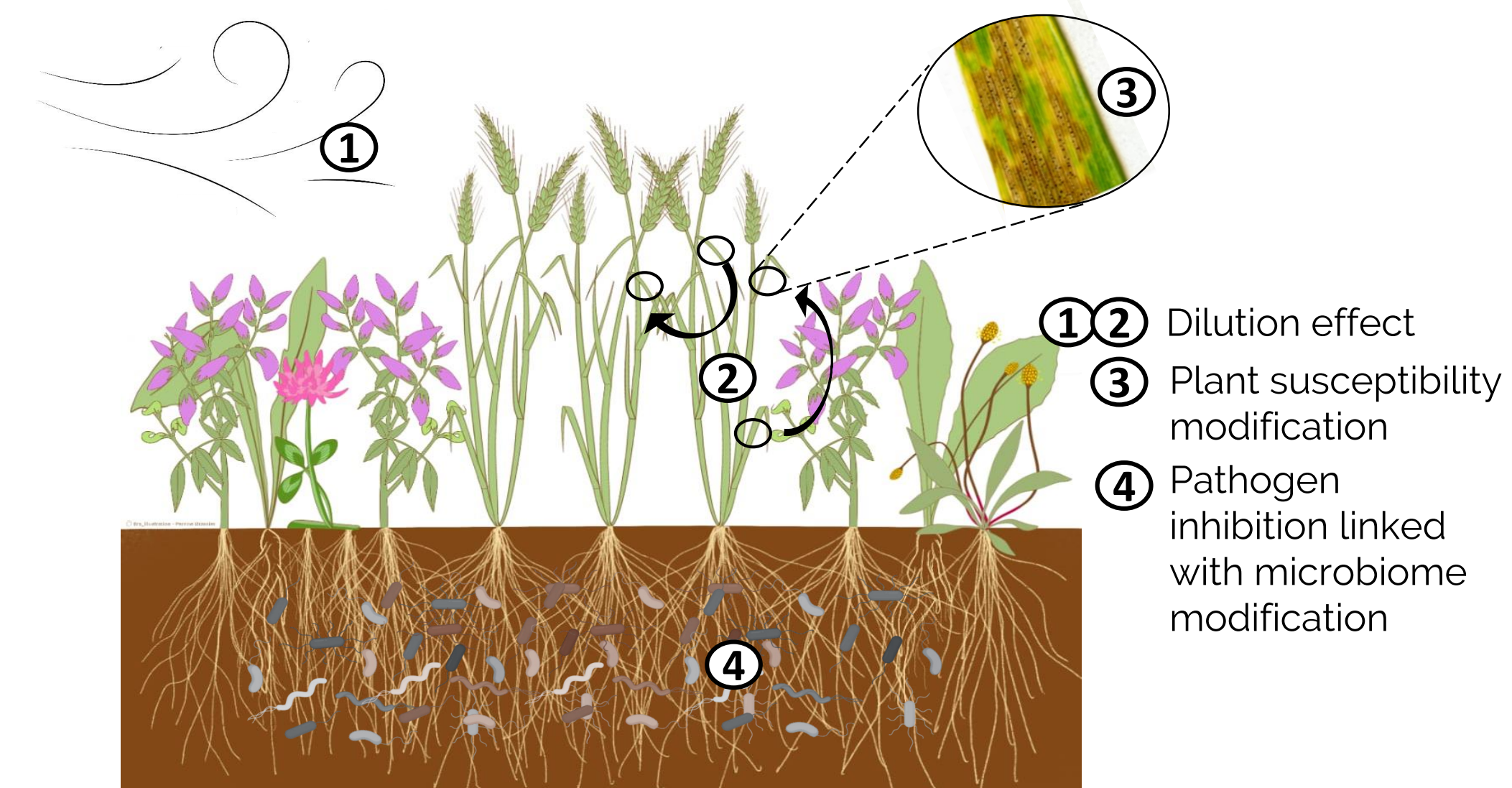


Figure 2: *Zymoseptoria tritici* regulation mechanisms which may be involved in « agroprairies »

Experimental Design

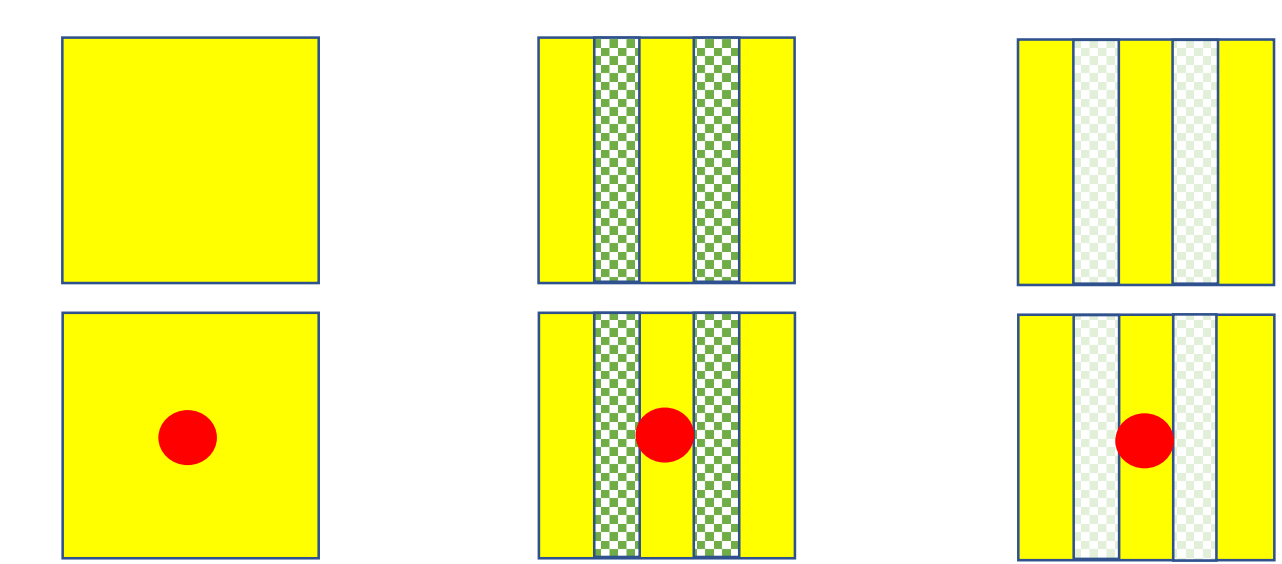
24 micro-plots (3m x 3.5m)

1 wheat variety: Rubisko
4 replicates

6 treatments:

- 1 monocrop
- 2 types of « agroprairies »: wheat intercropped with fast-growing (FL) or slow-growing (SL) perennial species and a high proportion of legume species
- Inoculated ● or not with *Z. tritici* on the 16th May 2024 (flowering stage)

Monocrop Agroprairie_FL Agroprairie_SL



Material and methods

-25 plants per plot, and 2 leaf layers (Leaf 1 and 2) were observed

-Severity (level 1-9) and incidence (% of diseased plants) were noted at 4 five different dates (between flowering and ripening stages)

-Disease index and the disease index AUDPC (Area Under Disease Progress Curve) were calculated to illustrate the dynamic of the disease.

$$\text{Disease index} = \frac{\sum(\text{number of diseased leaves in each level} \times \text{value of the corresponding level})}{\text{total leaf number investigated} \times \text{value of the highest level}}$$

-24th April 2024 : Rhizosphere and root sampling of wheat and perennial species

-DNA extraction

-16S rRNA gene long read sequencing

Results 1

- Agroprairies affected the receptivity of wheat to *Z. tritici*, with a lower AUDPC for the wheat intercropped with fast growing perennial species (agroprairie_FL) in inoculated plots. No significant difference was noticed between the monocrop and the wheat intercropped with slow growing perennial species (agroprairie_SL) (Figure 3B).
- In the case of a natural epidemic, agroprairies did not significantly affect the AUDPC (Figure 3A).
- However, slow growing perennial species in agroprairie_SL significantly reduced the wheat disease incidence in control plots (Figure 4A).

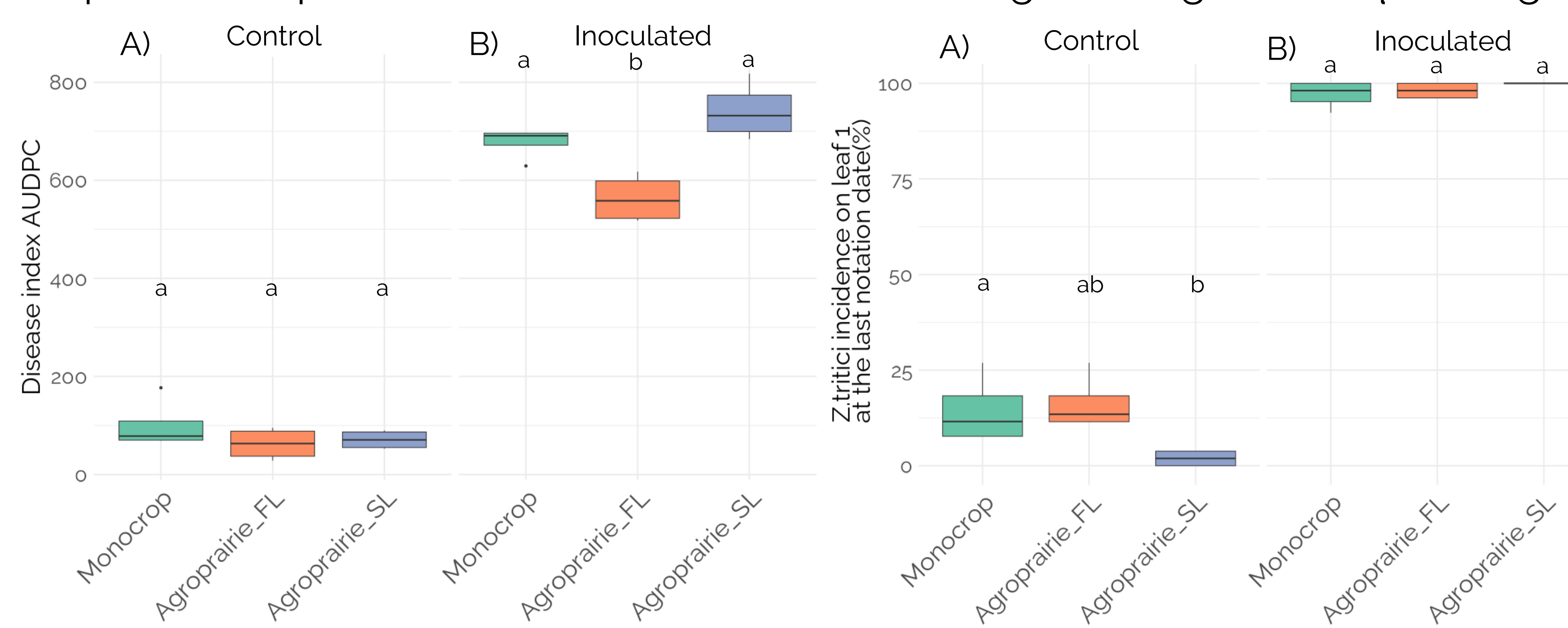


Figure 3: AUDPC for not inoculated (A) and inoculated plots (B). Different letters represent significant differences among treatments by Tukey's HSD test at P < 0.05.

Figure 4: *Z. tritici* incidence (%) on the first leaf layer at the last notation date.

Results 2

- Agroprairies affected the roots microbiome but not the rhizosphere alpha diversity (data not shown).
- Agroprairies affected the roots microbiome beta diversity (Figure 5A).
- The disease index AUDPC might be linked with roots Beta diversity (Figure 5B; p=0.048). However, further analyses are required.

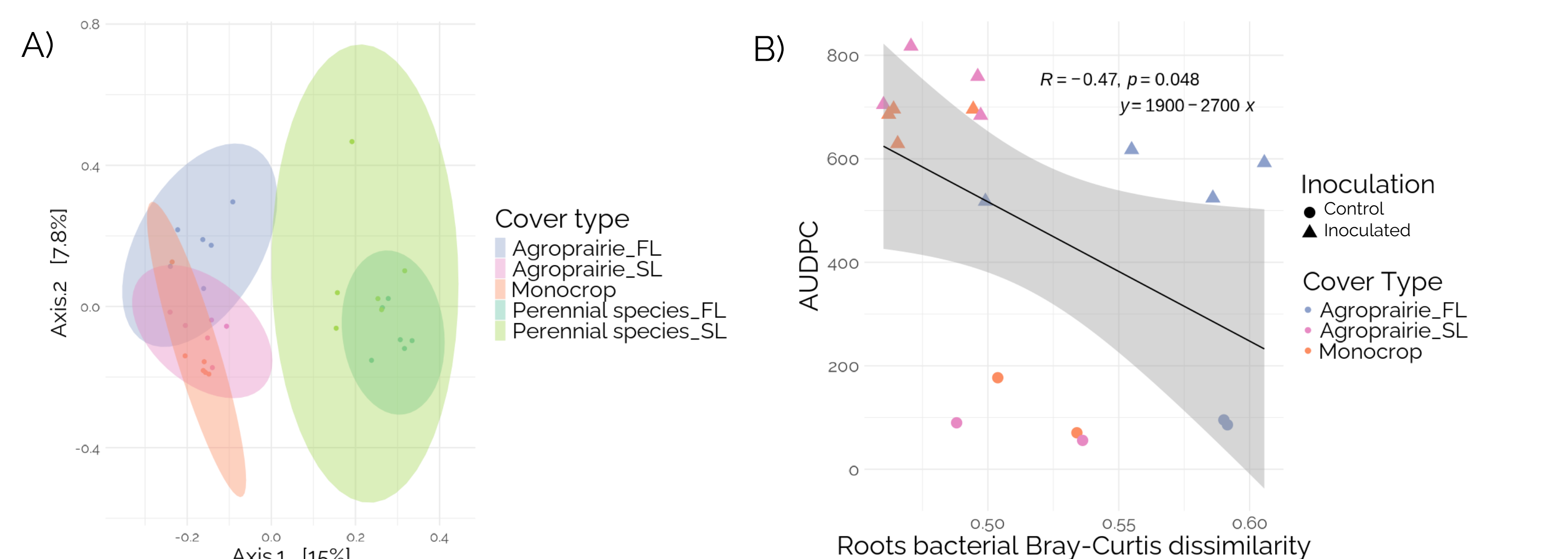


Figure 5: Effects of cover type on the roots bacterial community beta diversity. The PCoA plot was based on the bacterial Bray-Curtis distance at the OTU level (A). Pearson correlation between Bray Curtis dissimilarity of roots bacterial communities and AUDPC (B).

Conclusion : Our findings provide insights into the role of "agroprairies" in *Zymoseptoria tritici* regulation. It appears that the susceptibility of wheat to *Zymoseptoria tritici* and the incidence of the disease were reduced in agroprairies. We found a distinct effect of the different types of agroprairies on the wheat root microbiome. Complementary analyses are carried out to investigate the fungal microbiome and its role in the disease development.

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