

Resistant and susceptible pea lines harbour different root-rot pathogens and antagonistic fungi

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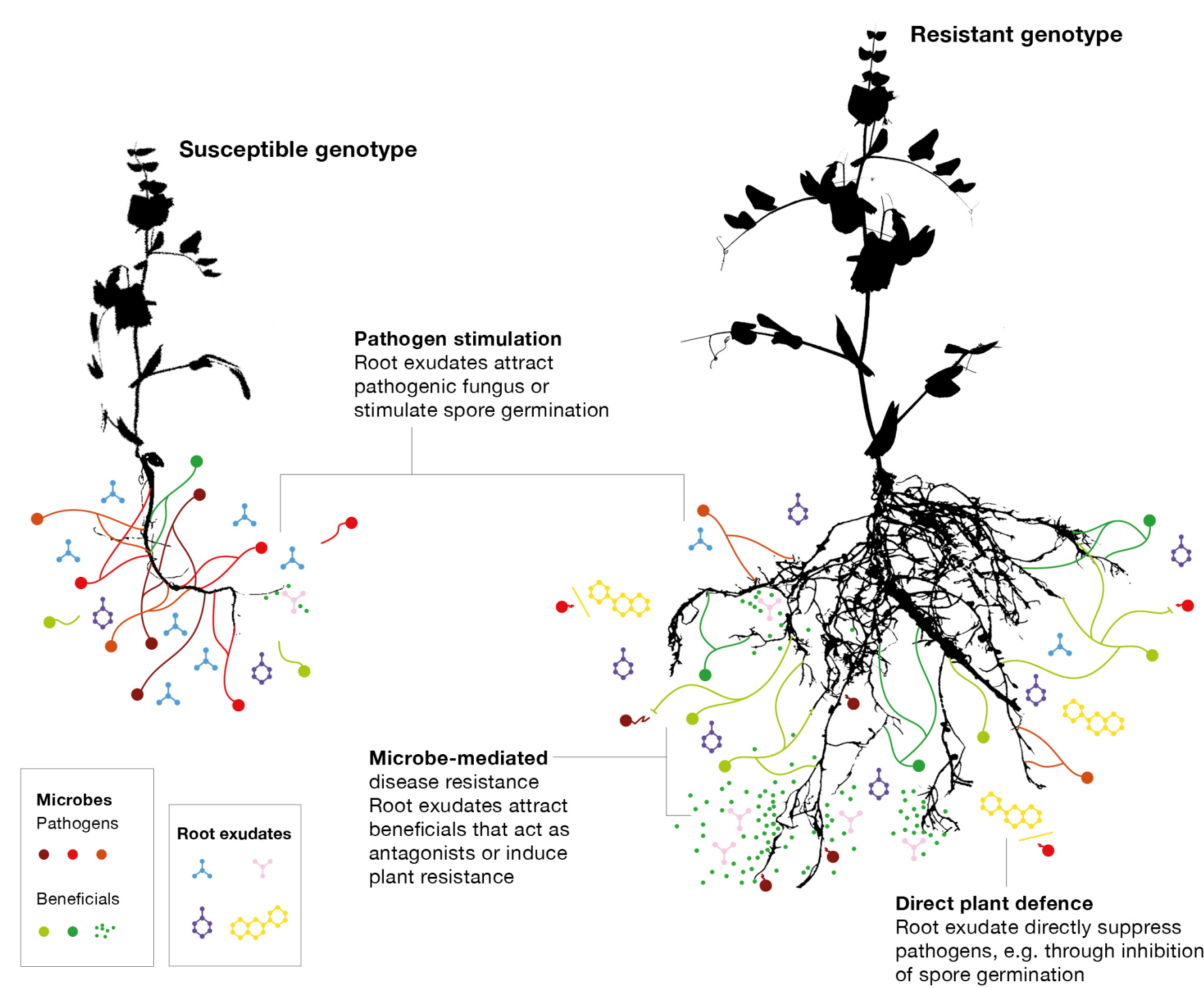
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Abstract

Pea (*Pisum sativum* L.) is a valuable protein source and important crop in low-input farming systems. Fungal root diseases threaten cultivation, and resistance in current pea varieties against multiple pathogens is lacking. To fully acknowledge the rhizosphere microbiome as a part of the plant environment, eight pea genotypes with contrasting root rot resistance levels were selected and tested on four soils with different disease pressure. Our controlled conditions pot experiment showed a significant genotype x soil type interaction for root disease index. Furthermore, the quantification of pathogens and potential antagonists suggests different roles of these fungi in pea root disease.



Microbes are important for plant health

Recent studies on plant resistance have shown that the plant associated microbiome plays a crucial role for plant health. There is evidence that the plant genotype determines the composition of the plant associated microbial community. Plants release a variety of chemical compounds from their roots; these root exudates encompass defence molecules and compounds that attract certain microbes. Whereas many microbes are pathogenic to plants and cause economically important diseases, many can

be beneficial promoting plant growth or resistance. Understanding the interactions between plants and microbes poses a leverage point for resistance breeding. Incorporating the plant microbiome in plant breeding could be a sustainable solution for improved, resistant crop varieties.



Further reading: Wille et al. (2019) Insights to plant-microbe interactions provide opportunities to improve resistance breeding against root diseases in grain legumes. *Plant, Cell & Environment*.

1 - Levels of pea root rot on four different soils and occurrence of pea pathogens in diseased roots

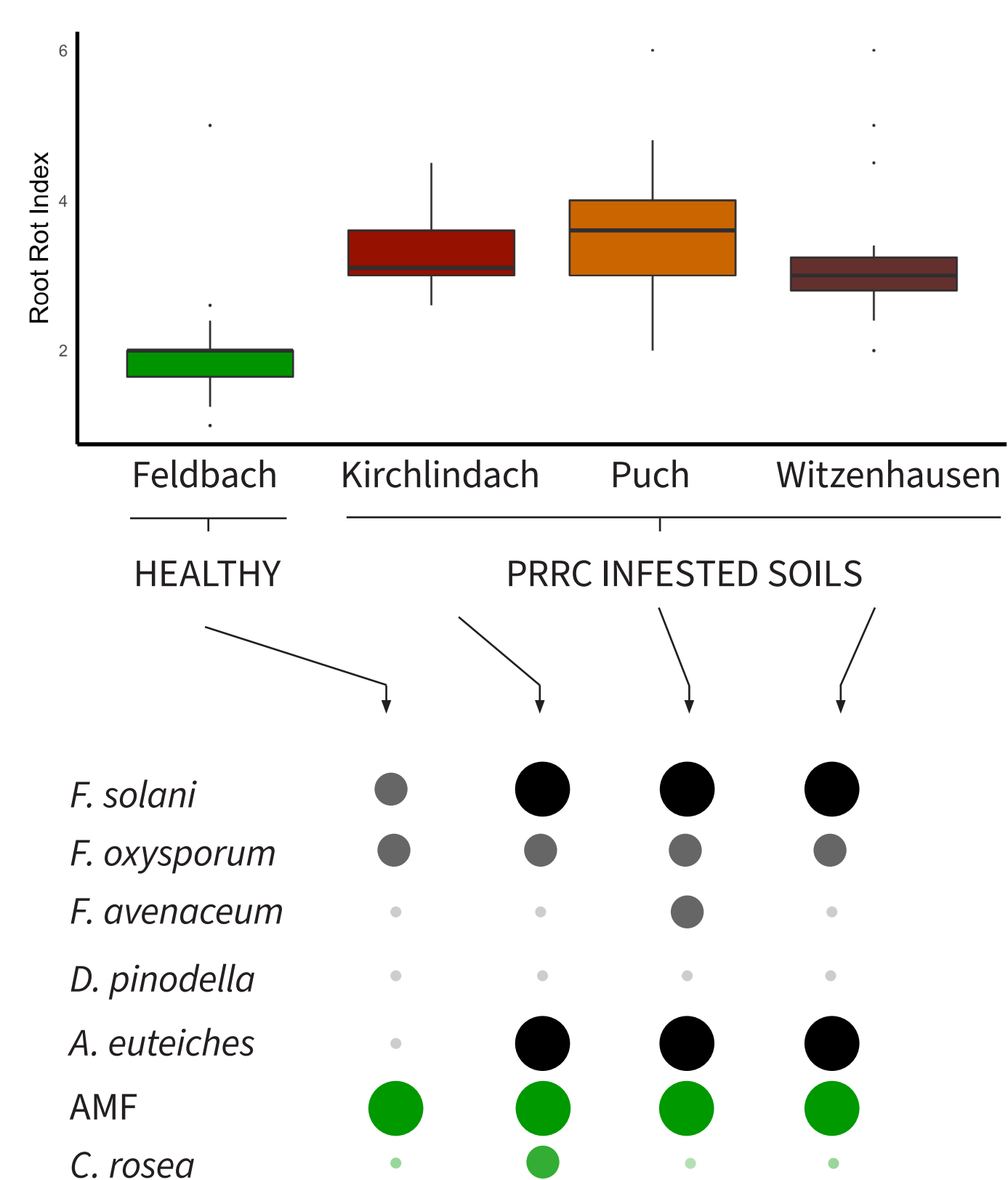


Figure 1. Pea root rot and qPCR data assessed on eight pea lines. TOP: Root rot index (RRI) evaluated for eight pea genotypes on four different soils (controlled conditions; four replications). BOTTOM: Occurrence (high, moderate and low) of five fungal pathogens and two beneficials in pea roots grown in the four soils.

- 'Feldbach' soil shows overall low level of root rot
- 'Kirchlindach', 'Puch' and 'Witzenhausen' soils show higher root rot
- The composition of major fungal pathogens in pea roots varies among the four soils

2 - Relationship between rhizosphere microbes and root rot resistance

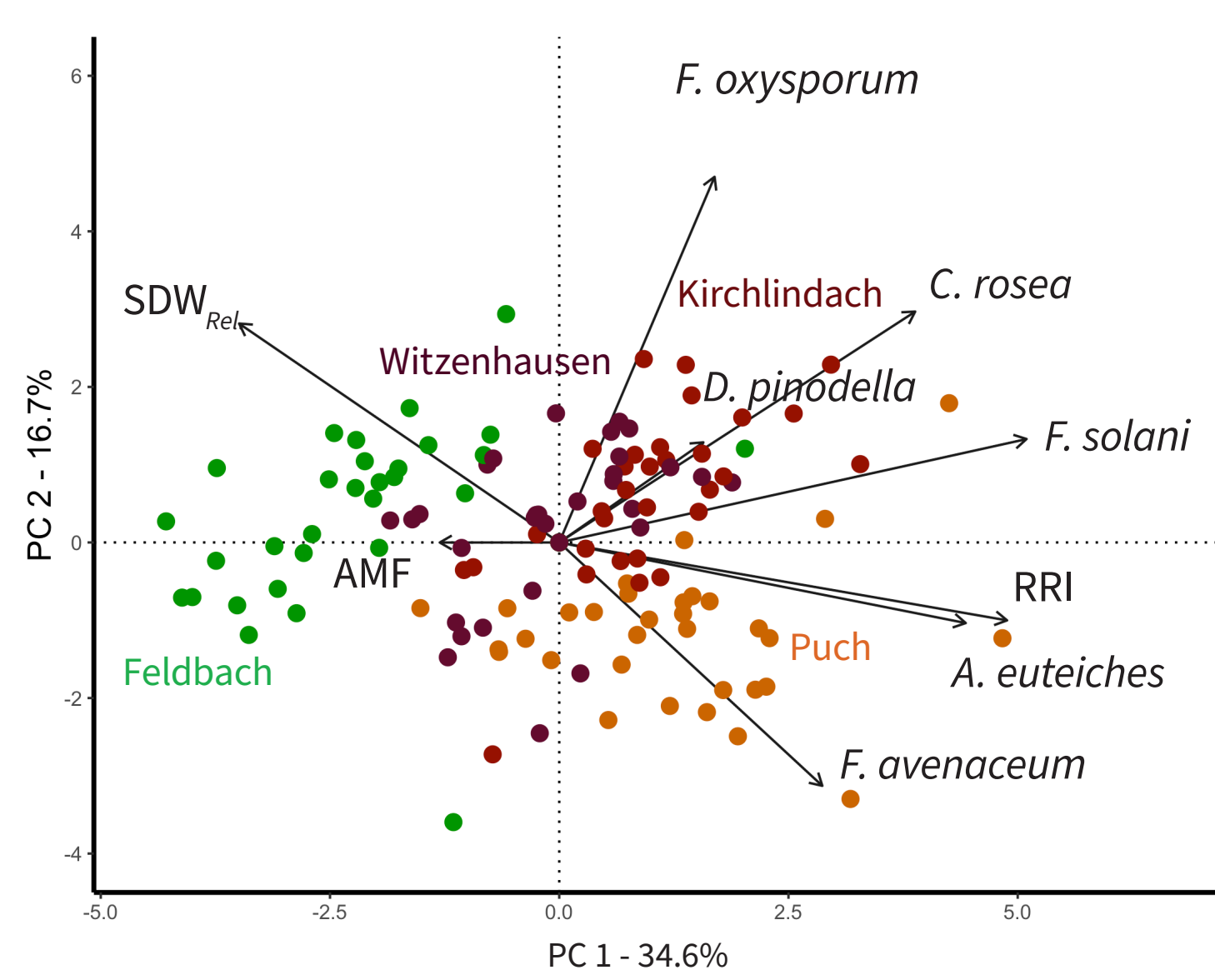


Figure 2. Principal component analysis of qPCR (six fungal taxa and *A. euteiches*) and phenotypic data (Root rot index, RRI and relative shoot dry weight, $SDW_{Rel.}$) of eight pea genotypes evaluated for root rot resistance on four different soils.

- qPCR quantification and root rot resistance data separate the soils in a healthy (i.e. 'Feldbach') and a sick group
- 'Puch' soil, showing a higher abundance of *F. avenaceum* in diseased pea roots, groups away from the two other sick soils
- The pathogen *A. euteiches* shows a close relation with root rot index (RRI)
- AMF quantities show negative relationships with *A. euteiches* and *F. solani* quantities

3 - Significant correlations between root rot index and microbial quantities in diseased roots

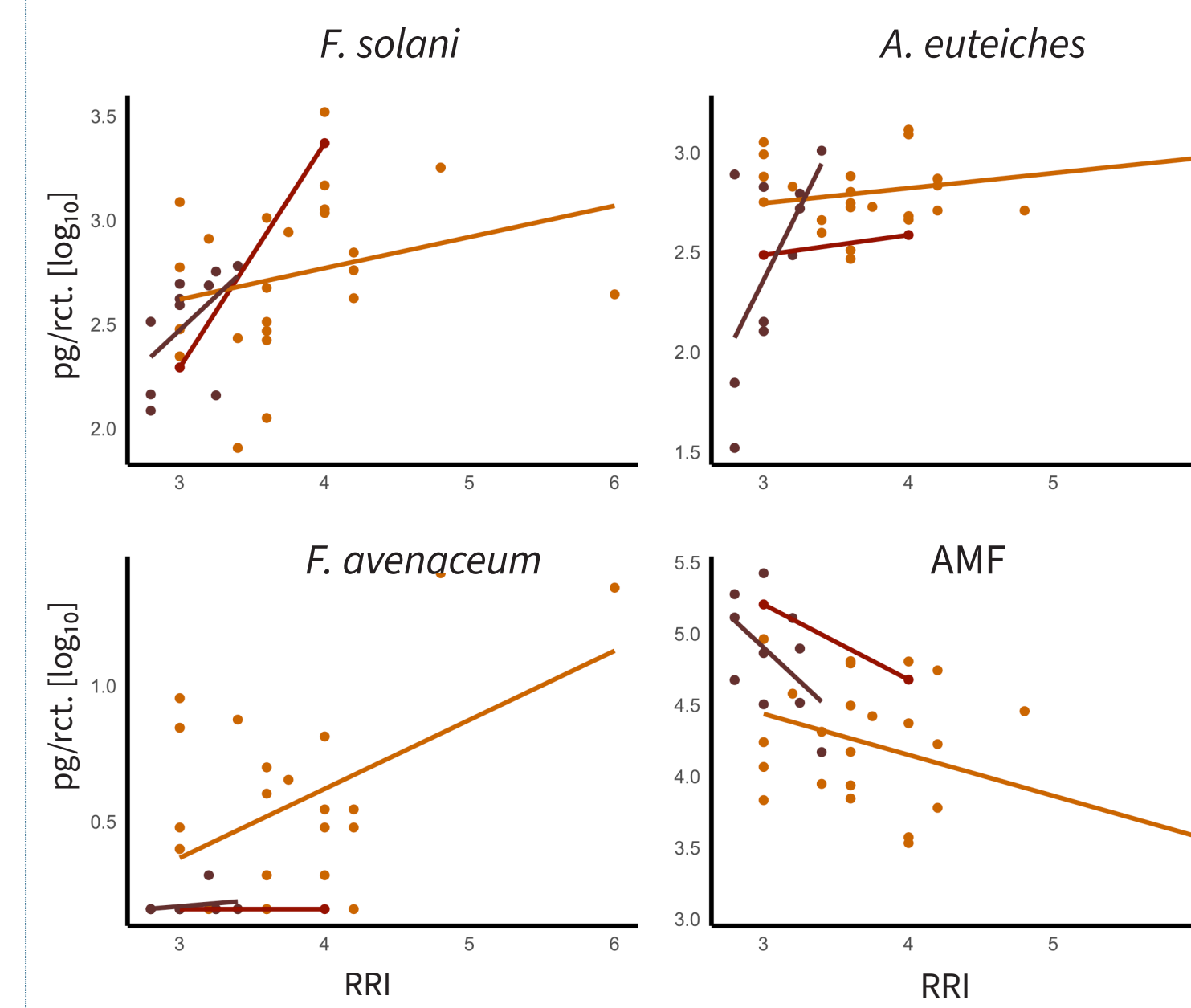


Figure 3. qPCR quantities of three pea pathogens (*F. solani*, *F. avenaceum* and *A. euteiches*) and arbuscular mycorrhizal fungi (AMF) in pea roots plotted against root rot (RRI).

- The two pathogens *F. solani* and *A. euteiches* show positive correlations with RRI (over all three sick soils: $p < 0.01$)
- *F. avenaceum*, detected only in pea roots grown on 'Puch' soil, shows a positive correlation with RRI ($p < 0.05$)
- AMF quantities show a negative correlation with RRI (over all three sick soils: $p < 0.01$)

4 - Significant differences in microbial quantities between resistant and susceptible pea genotypes

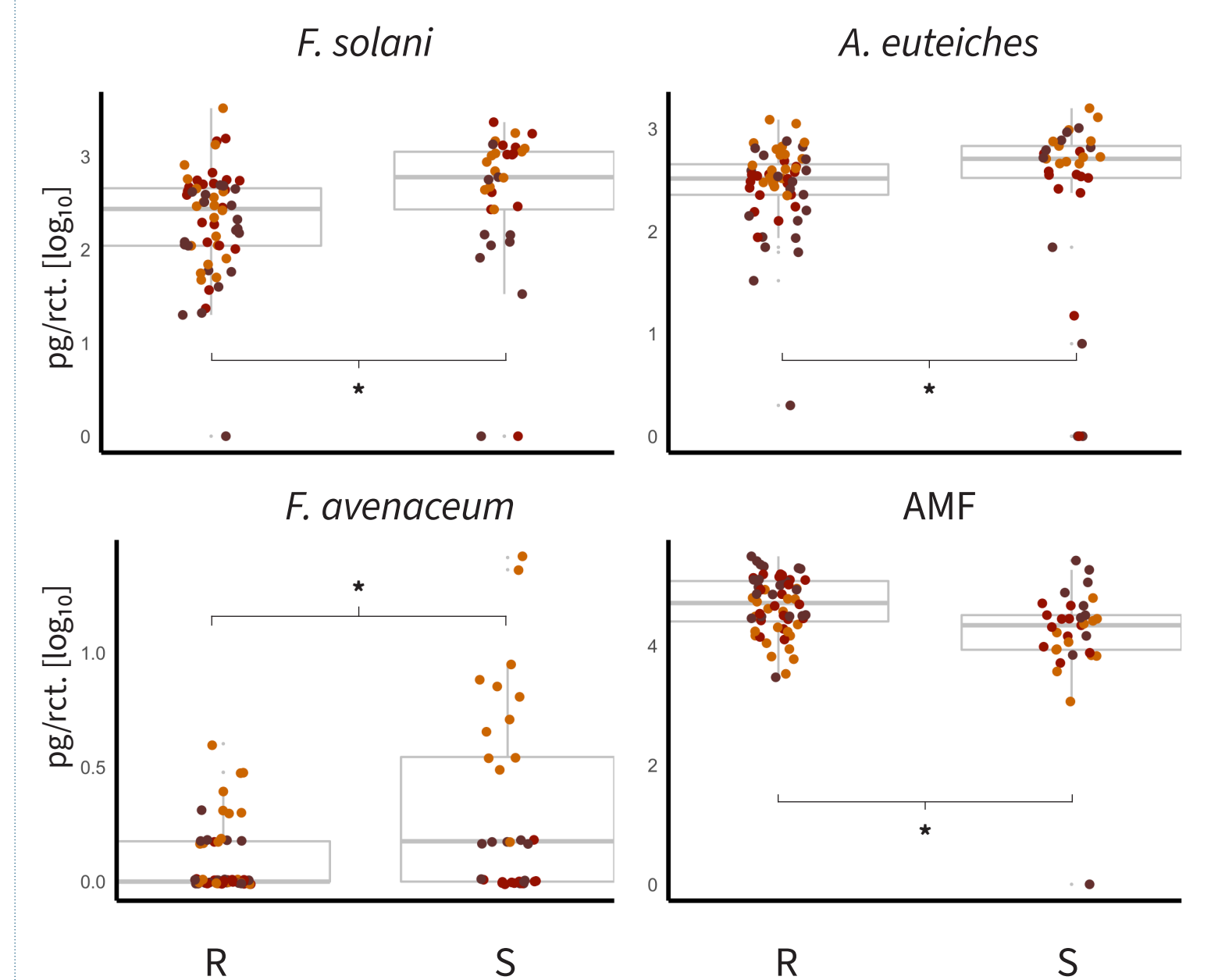


Figure 4. qPCR quantities of three pea pathogens and arbuscular mycorrhizal fungi (AMF) in infected roots of resistant (R) and susceptible (S) pea genotypes. Five and three pea genotypes ($n = 4$) were pooled for the two groups.

- Resistant pea genotypes harbour significantly lower quantities of the three pathogens than susceptible genotypes (Wilcoxon's rank sum test: $p < 0.01$)
- Resistant pea genotypes harbour significantly higher quantities of AMF than susceptible genotypes (Wilcoxon's rank sum test: $p < 0.01$)

Material & methods

- Initially, 261 pea cultivars, breeding lines and landraces were screened for resistance on a pea root rot complex (PRRC) infested soil under controlled conditions
- Eight genotypes with contrasting levels of root rot resistance were selected and tested on four soils (Healthy soil: 'Feldbach'; sick soils: 'Kirchlindach', 'Puch' and 'Witzenhausen'). Five weeks after sowing root rot (RRI: 1 = healthy - 6 = dead plant) was

scored for each genotype and growth performance was calculated as the ratio between biomass of plants grown on infested soil and biomass on a sterilised control soil ($SDW_{Rel.}$; 1 = no growth reduction). Additionally, total DNA was extracted from freeze-dried roots to quantify selected microbial taxa. The amount of fungal DNA (pg/reaction) was obtained by comparing the sample Ct to a standard curve (absolute quantification)

Summary & Conclusion

- Absolute quantification of pathogenic and beneficial fungi/oomycetes by quantitative PCR (qPCR) allows to quantify key microbial players in the pea root rot complex
- Pea plants grown in four different soils show abundant variation in the composition of root infecting microbes
- Known pea pathogens *F. solani* and *A. euteiches* are highly abundant in diseased roots from all three sick soils

- Other pea pathogens *D. pinodella* and *F. avenaceum* are present at intermediate levels in 'Witzenhausen' and 'Puch' soils, respectively
- Resistant pea genotypes show lower amounts of pathogens and higher amounts of AMF in the roots
- Higher arbuscular mycorrhizal fungi (AMF) colonisation in less diseased plants could indicate the bio-protection effect by these known plant symbionts
- Future analysis will determine genotypic (G), soil (E) and G x E effects steering the composition of pea pathogens and beneficial microbes