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Medicago truncatula genotype drives the plant nutritional strategy and its associated rhizosphere bacterial communities

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Context and objective

Harnessing plant microbiome through plant genetics is gaining of interest to improve plant nutrition and health. While genome-wide association studies (GWAS) allowed identifying genes involved in microbiome recruitment to improve plant growth, only few recent studies have assessed the effect of plant genetics on its associated microbiome in relation to plant performance.

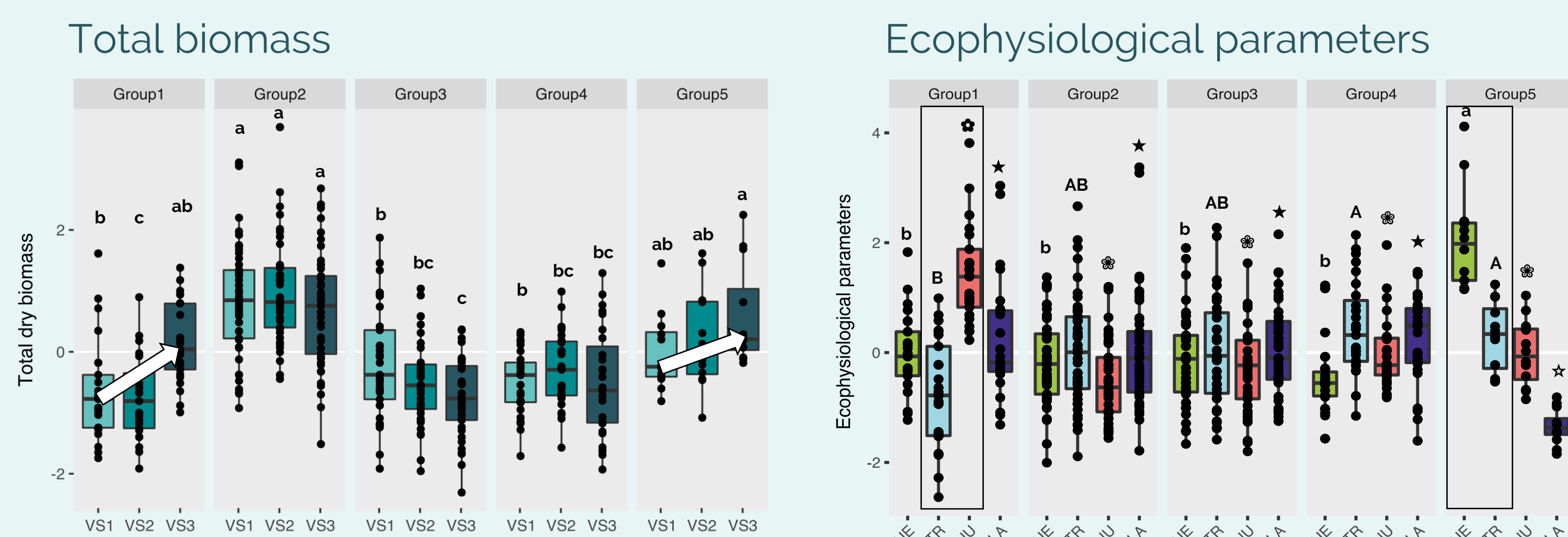
In this study, we conducted the first GWAS analysis on the microbiome associated to the model legume, *Medicago truncatula*, and considering also both plant growth and plant nutritional strategy. The aim of this study was to identify plant genes potentially involved in the recruitment of bacteria whose relative abundance predicts plant traits of interest.

Materials and methods

- A core collection of 155 accessions representing *Medicago truncatula* diversity was studied.
- Several plant traits were measured at three sampling dates during the vegetative stage to describe both establishment of plant structures (e.g. leaves, roots) and estimate their efficiency to uptake carbon and nitrogen.
- Rhizosphere bacterial community were assessed using amplicon sequencing.
- Random forest analyses were used to identify bacterial OTU predicting plant traits of interest.
- Genome-wide association studies were performed to identify plant loci potentially involved in the plant-microbe interaction.

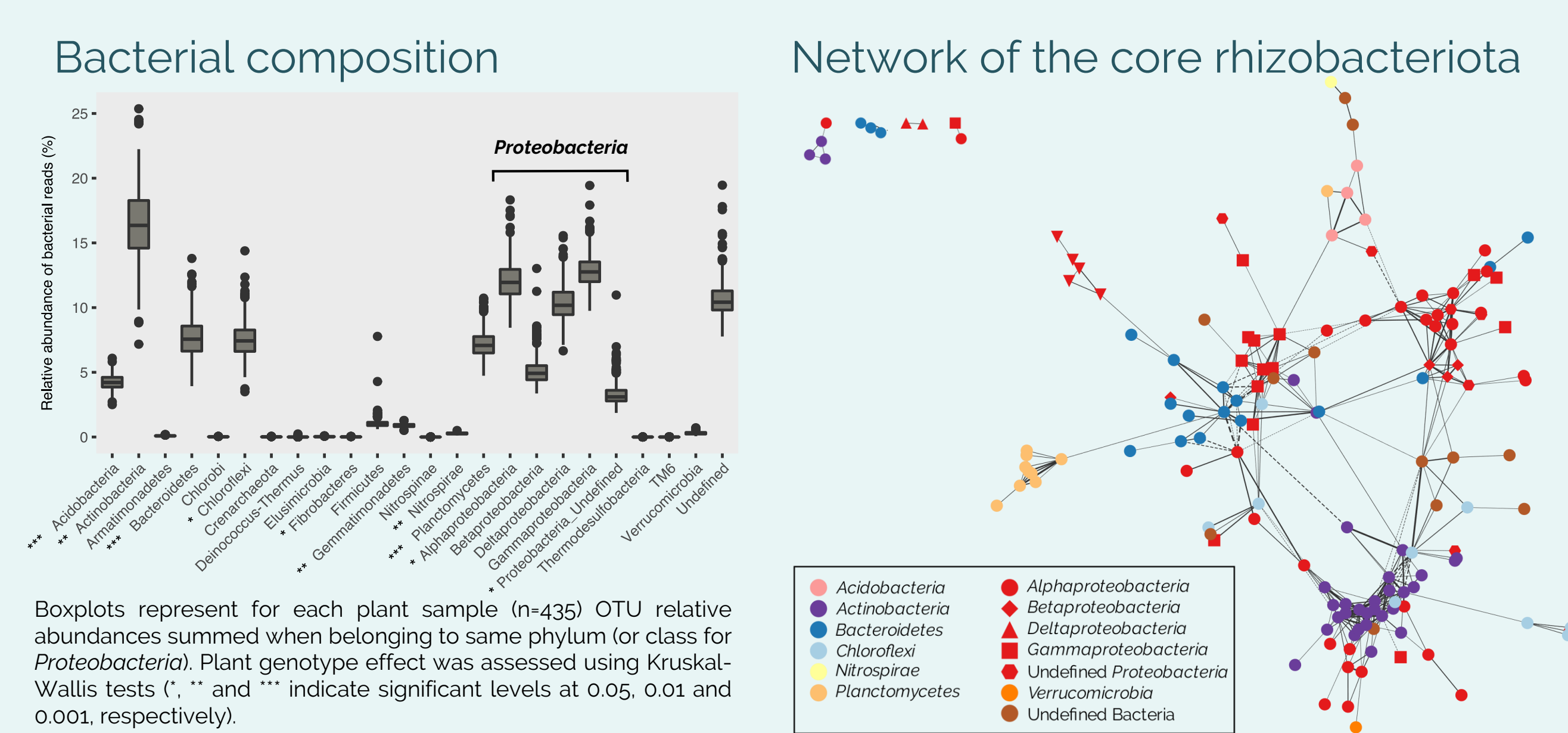
Results

Plant growth and nutritional strategies are contrasted within the *M. truncatula* core collection



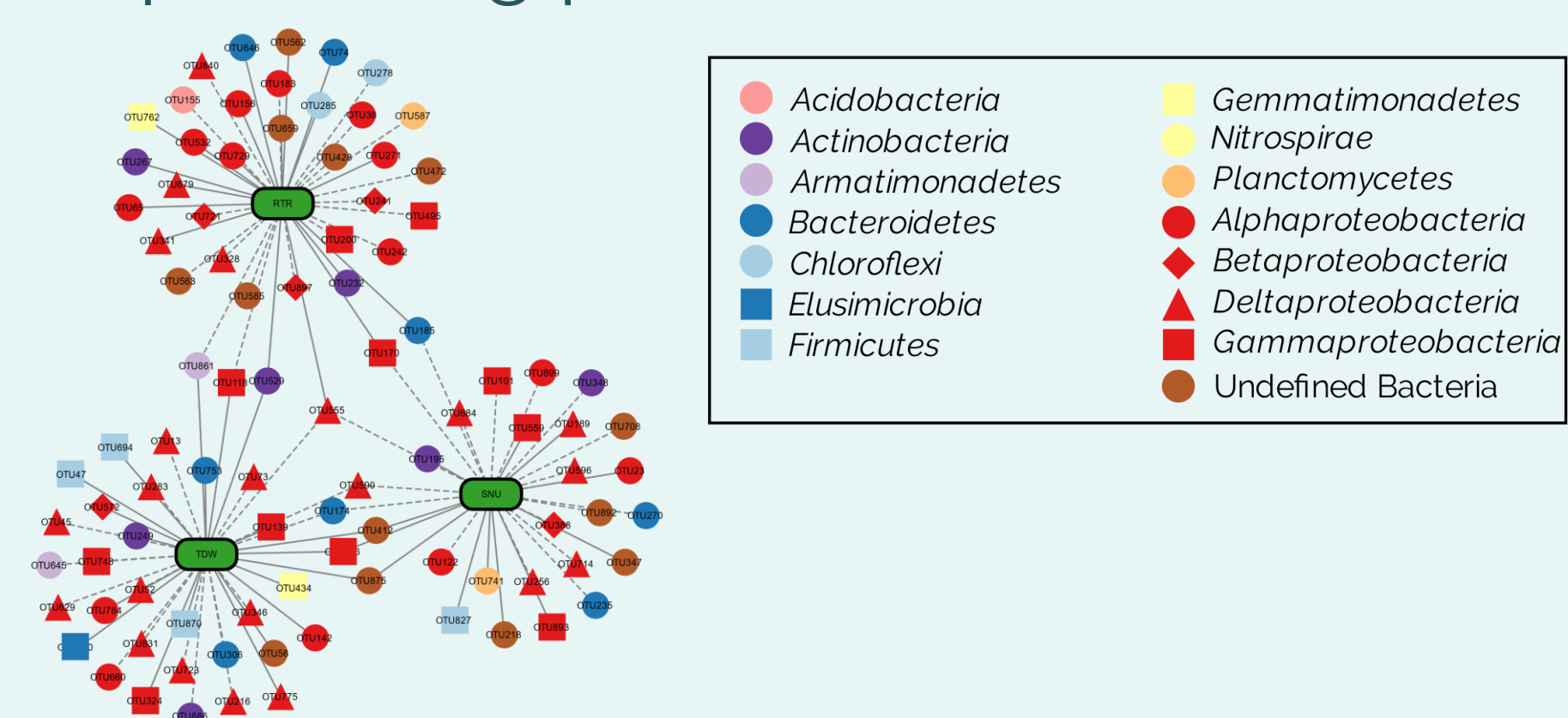
The five groups of plant genotypes were defined using the hclust "ward.D2" linkage method in R software based on 13 non-correlated ecophysiological variables. Boxplots were plotted using the average data per plant genotype (n=145) that have been centered and scaled for each variable. Abbreviations: VS1, VS2 and VS3: vegetative stage 1, 2 and 3 (488, 734 and 848 degree-days after sowing, respectively); RUE: radiation use efficiency; RTR: root/total biomass ratio; SNU: specific nitrogen uptake; NLA: conversion factor of nitrogen to leaf area

Rhizosphere bacterial communities are contrasted within the *M. truncatula* core collection



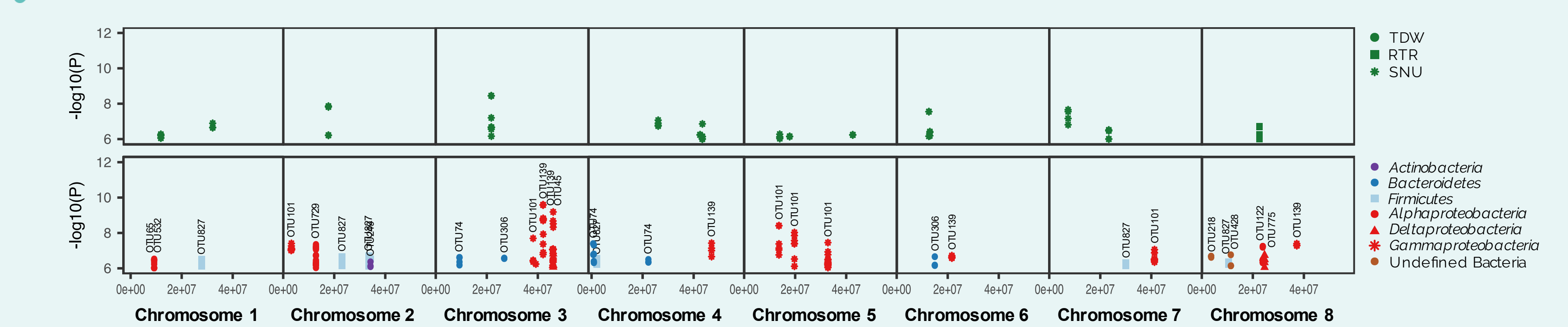
Boxplots represent for each plant sample (n=435) OTU relative abundances summed when belonging to same phylum (or class for Proteobacteria). Plant genotype effect was assessed using Kruskal-Wallis tests (*, ** and *** indicate significant levels at 0.05, 0.01 and 0.001, respectively).

Identification of bacterial candidates predicting plant traits



Regression random forest analyses were performed to identify bacterial OTU that are major predictors of plant phenotypic variables of interest that are represented in this network. Black dashed and solid lines represent negative and positive associations, respectively. Abbreviations: total dry plant weight (TDW), root/total biomass ratio (RTR), plant specific nitrogen uptake (SNU).

Identification of plant loci associated with these bacterial candidates



For SNU, 21 genes identified, including genes involved in root hair cell development, in nodulation, in transition from vegetative to reproductive phase, and in defense response.

For bacterial OTU, 112 genes identified, including genes involved in response to abscisic acid and salicylic acid, in terpenoid biosynthetic process, in lignin biosynthetic process, and in root development.

Conclusion

The different genotypes of the *M. truncatula* core collection showed contrasting plant growth, carbon and nitrogen nutritional strategies and rhizosphere bacterial communities. Moreover, plant loci were associated with the occurrence of bacterial OTUs, which are correlated to other OTUs in a well-connected network community, and can predict plant ecophysiological traits of interest. Together, these results suggest that plant genetics can affect the plant growth and nutritional strategies by harnessing keystone bacteria in a well-connected interaction network.

