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**Study of the function and diversity of the microbial
community in plant phyllosphere and rhizosphere
and interactions with the environment**

A thesis submitted by
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Abstract

Phyllosphere and rhizosphere are plant-associated micro-habitats that are known to support diverse microbial communities whose structure is mediated by plants. We aimed to disentangle the mechanisms shaping the microbial communities in the phyllosphere and the soil root zone and identify their response to agricultural practices like soil organic amendment and pesticide application. The focus was on plants indigenous to Mediterranean ecosystems, some of them producing essential oils which are known to exert antimicrobial activities, and also cultivated plants.

We initially explored the factors shaping the microbial community of the phyllosphere in plants native to semi-arid Mediterranean ecosystems using q-PCR and amplicon sequencing approaches. We collected leaves at two largely contrasting seasons (summer and winter) from 8 perennial plants with varying attributes, that belong to different functional groups: (i) woody sclerophyllous evergreen, semi-deciduous and non woody shrubs (ii) aromatic and non-aromatic. We determined the abundance of bacteria, Crenarchaea, fungi, *Alternaria* and *Cladosporium* (main airborne fungi) via q-PCR, and the structure of the epiphytic bacterial, archaeal and fungal community *via* amplicon sequencing. We observed strong seasonal effects but no clear plant-host effects on microbial abundance: bacteria showing higher abundance in the winter, and all others in the summer. Plant-host and season were equal determinants of the composition of the bacterial and fungal communities, whereas the archaeal community showed plant-host driven patterns. Plant habit exhibited a stronger filtering effect on the epiphytic microbial communities compared to the aromatic plant nature which affected only the fungal community. The bacterial community was dominated by *Chloroflexi* and α -proteobacteria in the summer and winter respectively, with OTUs of *Sphingomonas*, *Rhizobia* and *Methylobacterium* favored in the winter. The archaeal community was dominated by the Soil Crenarchaeotic Group (SCG) and Aenigmarchaeota. The fungal community is mostly comprised of Ascomycota with *Capnodiales*, *Pleosporaceae* and *Dothioraceae* being key members whose abundance varied by plant host and season.

We extended our study on aromatic plants by exploring their use, as soil amendments. We employed a pot study to examine the impact of peppermint (*Menta piperita*), spearmint (*Menta spicata*) and rosemary (*Rosemarinus officinalis*), in comparison with an organic fertilizer, on the dynamics of key bacterial taxa,

Crenarchaea, fungi and functional microbial groups like ammonia-oxidizing bacteria (AOB) and archaea (AOA), sulfur-oxidizing bacteria (SOB) and *catA*-, *pcaH*-carrying bacteria involved in C cycling. We further explored possible interactions between soil amendments and the presence of tomato plant. Soil amendment with peppermint, spearmint and the organic fertilizer increased the abundance of proteobacteria and fungi, in contrast to rosemary, characterized by essential oils with a different chemical profile compared to mints, which benefited these copiotrophic microbial groups only in the presence of tomato plants. We further explored this complex interaction via amplicon sequencing analysis of bacteria, archaea and fungi. This verified the key role of rosemary soil amendment in shaping the bacterial, archaeal and fungal community and its beneficial role in the abundance of proteobacteria. Multivariate analysis identified OTUs belonging to Actinobacteria, mostly associated with undisturbed soil systems (i.e. *Blastococcus*, *Rubrobacter*, *Solirubrobacter*, *Agromyces*) that were negatively affected by rosemary amendment. On the contrary we observed a striking dominance of the cellulose-decomposing basidiomycetes *Minimedusa* in soils amended with rosemary. The known antibiotic properties of this fungus might explain the negative effects of rosemary soil amendment on *Nectriaceae* also observed.

We finally explored the potential impact of pesticides, as external perturbation factor, on the abundance and diversity of the microbial communities on plant leaves and the soil root zone. We tested the hypothesis that these two habitats support distinct microbial communities but exhibit a similar response (accelerated biodegradation or toxicity) to their repeated exposure to the biodegradable fungicide iprodione. Pepper plants received four repeated foliage or soil applications of iprodione which accelerated its degradation in soil ($DT_{50_1st}=1.23$ and $DT_{50_4th} = 0.48$ days) and on plant leaves ($DT_{50_1st} >365$ and $DT_{50_4th} = 5.95$ days). The composition of the epiphytic and soil bacterial and fungal communities, determined by amplicon sequencing, were significantly altered by iprodione. The archaeal epiphytic and soil communities responded differently; the former showed no response to iprodione. Three closely related iprodione-degrading *Paenarthrobacter* strains were isolated from soil and phyllosphere. They hydrolyzed iprodione to 3,5-dichloraniline (3,5-DCA) via the formation of 3,5-dichlorophenyl-carboxiamide and 3,5-

dichlorophenylurea-acetate, a pathway shared by other soil-derived arthrobaeters implying a phylogenetic specialization in iprodione biotransformation.

Overall, we showed that phyllosphere is a habitat colonized by diverse bacteria and fungi, while archaea are less abundant and diverse. The epiphytic microbial community in Mediterranean plants, is shaped by plant-host and seasonality. The use of aromatic plants as soil amendment was found to stimulate copiotrophic microorganisms and microorganisms allelopathic against soil-borne plant pathogens. Finally, we showed that the epiphytic microbiome, responds to pesticide applications, with some microbes became acclimated to degrade pesticides. This thesis has reported the first epiphytic bacterium, a *Paenarthrobacter* strain, that could degrade iprodione and also suggested an uncommon specialization of Arthrobaeter in the degradation of this fungicide.