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## Co-occurrence network inference analysis allows identification of keystone microbial species associated to soil compartments and environments in cultivated olive

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Large-scale microbiome studies are currently facing challenges to overcome the lack of knowledge in relation to the interactions occurring among microbial communities and their surrounding environment. As a result, the study of associations through co-occurrence network analysis may lead to a better understanding for the aggregation or exclusion interactions in microbial studies and it offers a mapping of how information flows among the members of the microbiome system. By using 16S and ITS high-throughput sequencing, we studied the associations of bacterial and fungal communities in different olive compartments (fruit, phyllosphere, stem, xylem sap and roots) and the surrounding soil (bulk and rhizosphere) from three olive genotypes ('Picual', 'Arbequina' and 'Frantoio') growing at three olive orchards (Úbeda, Baena and Antequera) which differ in physicochemical soil characteristics and climate, in Andalusia, Southern Spain. Results based on the analysis of amplicon sequence variant (ASVs) displayed distinct microbial association network behaviors according to plant or soil compartments. Thus, plant compartment showed a positive association between Actinobacteria and Proteobacteria whereas some negative associations were exhibited by fungal communities, mainly from phyla Ascomycota and Basidiomycota. On the other hand, the negative associations of fungi were more noticeable in the soil compartments and the bacterial phylum Firmicutes played a different role in the soil than in the plant compartments. Furthermore, members of the bacterial phyla Deinococcota and Armatimonadota were unique in plant compartments while the phylum Verrucomicrobiota was only detected in the soil compartment. Overall, 14 keystone species with positive and negative associations in aboveground and belowground compartments were predicted based on the network parameters of high closeness and degree, and a low betweenness centrality. Interestingly, Bradyrhizobium and Pseudonocardia were positioned as two common keystone species among the positive associations in both compartments. This powerful analysis can reveal new knowledge regarding specific microbial associations on soil and plant microbiomes and it can propose a possible road map to investigate potential microbial source migration from soil to olive compartments.

Study supported by Projects XF-ACTORS 727987 (EU-H2020) and AGL2016-75606-R (MICINN Spain and FEDER-EU). MA-M acknowledged the predoctoral contract for the Training of Personal Investigator (FPI- MICINN) with reference BES-2017-082361 and COST Action CA16107 EuroXanth.