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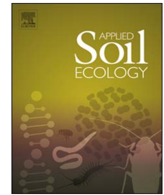

Short communication

# Bulk soil bacterial community mediated by plant community in Mediterranean ecosystem, Israel

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ABSTRACT

The Israeli Mediterranean ecosystem has a distinctive flora with high levels of plant diversity. Previous studies of soil microbial ecology from the region have paid little attention to the possibility of distinctive soil bacterial communities associated with particular habitats. This study looked at bacterial communities present in the bulk soil from the closed canopy of four indigenous plants – *Quercus ithaburensis, Calicotome villosa, Sarcopoterium spinosum*, and *Rhamnus puncata*. Soil DNA was amplified for the 16S rRNA gene and sequenced using MiSeq Illumina platform. Results indicated that the bacterial communities differed between open and closed canopy with no significant difference in community structure within closed canopy, with soil moisture and organic matter content significantly influenced the community. Only the relative abundance of *Gemmatimonadetes* varied significantly with the open canopy having the highest abundance, and the relative abundance of *Spartobacteri*a and *Saprospirae* was unusually high compared to other Mediterranean soils. There was no difference in OTU richness between plant species. Additionally, this study revealed that open canopy community between plants was nested within closed canopy bacterial community implying that open canopy community potentially serves as a source for the closed canopy. It is then possible that the rhizospheric influence extends beyond the root-soil interface and to the surrounding bulk soil. Overall, this study indicates that in Israeli Mediterranean ecosystems native plants extend a selection beyond the rhizosphere, and like other studies from Mediterranean ecosystems pH does not seem to play an integral role in structuring bacterial communities.

## 1. Introduction

Plants have long been known to have associations with microbes that live in the soil surrounding the roots, known as the rhizosphere (Egamberdieva et al., 2008; Kowalchuk et al., 2010; Quiza et al., 2015; Schlaeppi and Bulgarelli, 2015). The secretion of photosynthesis-derived metabolites into the rhizosphere (rhizodepostion) is proposed to be the mechanism the plant uses to assemble and maintain this microbial community (Berendsen et al., 2012; Dakora and Phillips, 2002; Jones et al., 2009). This rhizosphere effect has been widely documented in agricultural crops and mesocosm studies (Bouffaud et al., 2014; Buee et al., 2009). However, these processes are not yet well understood in natural environments such as forests and other highly diverse ecosystems.

Recent studies have indicated that the rhizosphere effect leads to divergent bacterial community dynamics between rhizosphere and surrounding bulk soil (Bakker et al., 2015), even under heavy metal contamination (He et al., 2017), and even community networks differ considerably (Fan et al., 2017). Majority of these studies have only been reported in agricultural systems and little is known whether these differences exist in bulk soil communities in natural ecosystem. There is a need for more studies that focus on the rhizosphere and bulk soil communities of native plant communities.

The Israeli Mediterranean biome is predicted to experience increased rainfall and variations in rainfall distribution over the region (Ben-Gai et al., 1998). Undoubtedly, this change in precipitation will lead to changes in vegetation cover (Mouillot et al., 2002; Pereira et al., 2007), and in Mediterranean biomes pedogenesis process occur during the winter season- the rainy season (Verheyen and Rosa, 2005). This increased nutrient mobilisation will lead to soil changes in carbon

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**Table 1**
Bulk soil physical and chemical characteristic ± standard deviations.

| Plant species | Soil Moisture (%) | Organic Matter (%) | pH |
|---|---|---|---|
| Control | 32.1 ± 3.52 | 0.12 ± 0.02 | 7.84 ± 0.33 |
| *Rhamnus puncata* | 31.32 ± 0.90 | 0.11 ± 0.008 | 7.72 ± 0.38 |
| *Calicotome villosa* | 32.05 ± 0.78 | 0.13 ± 0.01 | 7.95 ± 0.30 |
| *Sarcopoterium spinosum* | 30.91 ± 2.70 | 0.10 ± 0.03 | 8.29 ± 0.04 |
| *Quercus ithaburensis* | 41.70 ± 3.499 | 0.23 ± 0.11 | 8.31 ± 0.30 |

storage, primary production, and litter decomposition. Consequently, the majority of microbial studies from the region focused on these key processes (Bachar et al., 2010; Li and Sarah, 2003; Sherman and Steinberger, 2012). These studies and more have shed some light on what could be delimiting bacterial activity and abundance; however, none have looked at the influence plants have on the bacterial community directly beneath the canopy.

The aim of this study is to discern whether there would a distinction in bulk soil bacterial community between different native plants of the Israeli Mediterranean ecosystem. To this end, 16S rRNA gene dataset was used to examine the bacterial community structure, taxonomic composition, and diversity pattern.

## 2. Methods

### 2.1. Study site and sampling

The study site was located in a humid Mediterranean ecosystem (N 33° 0′, E35° 15′) in northern Galilee mountains of Israel as previously described in (Levi et al., 2012; Sherman and Steinberger, 2012) (Table 1). Briefly, the soil is classified as montmorillonitric terra rossa which is produced by the weathering of the sandstone bedrock. Bulk soil samples were collected randomly along a transect beneath the canopy of separate shrubs of four dominant plant species representing Mediterranean-type ecosystems: *Rhamnus puncata* (RP)*, Calicotome villosa* (CV), *Sarcopoterium spinosum* (SS), *Quercus ithaburensis* (QI), and control samples were collected from bare patches between shrubs. The top 0–10 cm of the soil was collected using a small garden trowel to scoop about 100 g. Soil DNA was quickly extracted after sampling using the MOBIO Power Soil DNA extraction kit (MOBIO laboratories, Carslbad, Ca, USA) as per manufacturer instructions. The isolated DNA was stored at −80° prior to PCR amplification.

### 2.2. PCR amplification and sequencing

Amplificaiton of the 16S rRNA gene was performed using primer pair Bakt341F (5′- CCTACGGGNGGCWGCAG-3′) and Bakt805R (5′-GACTACHVGGGTATCTAATCC-3′), and PCR conditions as previously reported (Herlemann et al., 2011). All samples were pooled in equimolar concentration and were pair-end sequenced using the Illumina MiSeq (Illumina Inc., CA, USA) at Macrogen (Macrogen Inc., Seoul, South Korea). The sequences have been deposited to MG-RAST under access number 47663253.3–47632979.3 (http://metagenomics.anl.gov/linkin.cgi?project=mgp82226) (Meyer et al., 2008).

### 2.3. Sequence analyses

In PANDAseq (Masella et al., 2012) a 0.9 quality score (which is the most stringent criteria to remove errors) was used to pair-assemble the sequence data. The assembled sequence data obtained was processed following the MiSeq SOP in Mothur (Schloss et al., 2009). The sequences were then analysed as previously described (Tripathi et al., 2012).

### 2.4. Statistical analyses

To compare the diversity indices across samples and sites, samples were standardised by subsampling at randomly to 98000 reads. OTUs and rarefaction values were calculated using the Mothur platform (Schloss et al., 2009). To discern whether the bulk soil communities varied between treatment (open canopy vs. closed canopy) and within treatments (between plant-associated bulk soil). The generated OTU table was used to calculate the Bray-Curtis community dissimilarity matrix and diversity indices. Principal Coordinate Analyses (PCoA) was used for ordinations with vectors indicting edaphic variables influencing the community structure. To assess the significance each of plant type in affecting community structure, Analysis of Similarity (ANOSIM) and Permutational Multivariate Analysis of Variance (PerMANOVA) both with 999 permutations. Lastly, the multivariate dispersion in the samples was measured and confirmed using Analysis of Variance (ANOVA) with 999 permutations.

The Shannon and Simpson diversity indices were calculated from the resultant OTU. OTU accumulation curve was constructed to determine total number of recovered OTUs as a function of the sampling effort (number of samples). In addition, a Preston log-normal curve was constructed to evaluate the estimated richness and occurrence of rare taxa (a more visible normal distribution indicating improved recovery of rare taxa in samples).

Furthermore, to determine the level of nested across community, a nested analysis was performed using BINMATNEST with default input parameters (Rodríguez-Gironés and Santamaría, 2010). The significance of the nested was further tested using null models as previously described in (Dong et al., 2016; Geel et al., 2015). Finally, the relative abundance of the dominant bacterial phyla and classes was assessed across all sampled site. All statistical analyses were performed using the Vegan package (Oksanen et al., 2013) in R Studio (Team, 2014).

## 3. Results

The PCoA plot showed a clear distinction in bacterial community between the closed and open canopy (Fig. 1A), but no significant difference in community structure between plant types (Fig. 1B). The PCoA could only explain 36% of the observed variation, but the goodness of fit i.e. how well the point fit into the ordination was significant (PerMANOVA = 0.588, $p < 0.05$). And of all measured environmental variables, soil organic matter $R^2 = 0.50$, ($p < 0.01$) and soil moisture ($R^2 = 0.48$, $p = 0.051$) both played a significant role in delimiting the bacterial community structure (Fig. 1).

Interestingly, multivariate dispersion analysis was significant (F value = 8.583, $p < 0.05$), this implies that the bacterial community structure is influenced not only by difference in between bacterial composition (between samples), but also by differences within a sample plant types (replicates of a sample). Highlighting the influence of bacterial diversity on community structure. Similarly, the presence/absence distance (Jaccard) showed similar results (Fig. S1). Mantel test indicated that the edaphic variable significantly influenced the community structure (Mantel R = 0.48, $p < 0.05$).

There were no significant differences in the alpha diversity indices (Fig. 2) and OTU richness (Fig. S2). The OTU accumulation curve indicates that though sampling in terms of numbers of reads was intensive, there were still more OTUs that could be sampled (Fig. S3A). Interestingly, the Preston log-normal model indicated that there were few rare OTUs (as indicated by the truncation point being far below the mode) and that the sampling effort had recovered most of the expected OTUs (area under the curve) (Fig. S3B). The nestedness matrix temperature was significantly lower than the null models indicating high levels of nestedness. The packed matrix generated from the analysis indicated that the open canopy community was indeed nested within the other communities (Table S1, $p < 0{,}0001$).

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