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Examination of biotic and abiotic controls of soil bacterial diversity under perennial shrubs in xeric soils

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ABSTRACT

In xeric environments, organic carbon is provided by above and belowground plant-litter components. The plants also act as an aboveground physical barrier. Both these biotic and abiotic features contribute to 'fertile-island' formation. Fifty *Hammada scoparia* shrubs and 50 artificial plants were randomly marked at a study site. The latter allowed simulation of the physical (abiotic) impact of perennial-plant cover and thus allowed distinguishing between biotic and strictly abiotic impacts on under-canopy soil bacterial diversity in a desert ecosystem. Soils were collected monthly over one year from under canopies of *H. scoparia* and artificial plants, and from the control area between the shrubs. The presence or absence of real plants and seasonality was the main drivers of bacterial diversity in soils. Simple canopy cover, as offered by the artificial plants, induced non-significant shifts in the diversity of the dominant bacteria.

1. Introduction

Water availability and organic matter inputs are the main limiting factors that largely determine microbial community composition and functions in desert ecosystems (Barness et al., 2009; Whitford, 2002; Yu et al., 2012). In the Negev Desert, rain events are usually relatively short and scattered over long periods during the winter season (November–April) (Evenari et al., 1982). Dew is a more reliable source of moisture that triggers and prolongs biological activity during the long, dry season (Garner and Steinberger, 1989; West and Skujins, 1978).

The spatial structure of arid ecosystems has been described as a plant-centered mosaic, creating 'islands of fertility' (Schlesinger et al., 1996) around shrubs that supply a wide spectrum of carbon sources to the decomposer population while providing an aboveground physical 'umbrella' (Berg and Steinberger, 2010; Wichern et al., 2007). In deserts, primary production is based mainly on perennial plants that have access to vertical moisture and nutrient pools that are essentially governing their ecophysiological adaptations (Evenari et al., 1982; Whitford, 2002). One of the main ecophysiological adaptations of perennial plants in desert ecosystems is based on surface reduction through a wide range of shedding mechanisms (e.g., leaves, stems, branches, roots) in order to maximize water utilization (Evenari et al., 1982).

The above and belowground biotic components of terrestrial ecosystems essentially depend on each other since plants provide carbon sources for the soil fauna. Perennial shrubs allow soil microbial communities to enhance and prolong their above and belowground activities (Kaffe-Abramovich et al., 2010; Santos and Whitford, 1981; Sarig et al., 1994; Steinberger and Whitford, 1983; Whitford, 2002), and enhance their dispersion and composition (Berg and Steinberger, 2008; Larson and Pierce, 1994; Steinberger and Loboda, 1991;). Berg and Steinberger (2010) have shown the importance of perennial-shrub contribution as two separate elements: the organic part (e.g., fruits, seeds, and belowground root parts) (Hartley et al., 2007; Kidron, 2009; Loranger-Merciris et al., 2006; Smolander and Kitunen, 2002) and the aboveground physical part (e.g., as a barrier to protect the under canopy area against high radiation and wind velocity). Plant and microbial-community structure and diversity are closely integrated through the utilization of dead residue, root exudates, and litter (Dornbush, 2007; Shmueli et al., 2007; Wichern et al., 2007). Smalla et al. (2001) and Kowalchuk et al. (2002) reported that the diversity of soil microorganisms differed under different plant species. Field observations have shown canopies to physically influence their surroundings, e.g. they decrease radiation and narrow temperature range. This plays a key role in determining soil microbial-community composition and activity but has been little studied.

We aimed to examine the impact of biotic and abiotic factors due to perennial-shrub stands and plant inputs to soil on the soil microbial community in a desert ecosystem. For this *Hammada scoparia*, the most widespread perennial shrub at the study area was used as a model.

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Microbial diversity was monitored in the vicinity of *H. scoparia* shrubs, which integrated both the abiotic and biotic effects of perennial plants, while the samples collected in the vicinity of the artificial shrubs simulated the physical role of the plant presence, simulating only the abiotic parameters.

Based on the above, we hypothesized that soil microbial diversity would be regulated by the dual role of the perennial shrubs and that both biotic and abiotic impacts associated with these shrubs would affect the soil microbial community and its diversity.

2. Material and methods

2.1. Study site

The field study was conducted at the Avdat Farm Long-Term Ecological Research Station in the Negev Desert highlands of Israel at 600 m above sea level. The area has a temperate desert climate, with hot summers (June mean maximum temperature 32 °C and mean minimum temperature 17.7 °C) and cool winters (January mean maximum temperature 14.8 °C and mean minimum temperature 5.4 °C). The multi-annual mean rainfall at Avdat Station is 90 mm, and occurs in scattered showers only during the winter season (November to April). An additional source of moisture is dew, which falls heavily during the autumn months (September–November) (Evenari et al., 1982). The soils are brown, shallow, rocky desert soils (brown lithosols), loessial, and gray desert soils (loessian sierozems) (Dan et al., 1972), with a water-holding capacity (WHC) of 41% and mean soil total organic-carbon (TOC) content of 0.37%. The soil, formed over calcareous bedrock, had a $CaCO_3$ content of 58.4% and pH of 7.9 (Oren and Steinberger, 2008).

Vegetation is a mixture of perennial shrub communities with a large variety of annuals. Predominant perennials at the research site are *H. scoparia*, *Zygophyllum dumosum* (*Z. dumosum*), *Artemisia sieberi* (*A. sieberi*), and a variety of annual plants and geophytes.

2.2. Experimental set-up

In November of 2005, 50 *H. scoparia* shrubs were randomly marked (by using randomized statistical table- to determine shrub location) at the study site within a 10,000 m^2 fenced area. Additionally, 50 artificial plastic plant-like shrubs (about 45 cm high and 40 cm in diameter), chosen to mimic *H. scoparia's* canopy cover, were randomly placed (see above) in the study area (with about 2 m between any two shrubs).

Sampling started one year after the set-up of the experiment, and continued for a period of one year (December 2006 to November 2007). Samples were collected early morning randomly under the shrubs. Soil cores of 7-cm in diameter were collected from the depth of 0–10 cm. The samples were collected monthly ($n = 4$) from 3 locations: (1) under the canopy of *H. scoparia*, (2) under the canopy of the artificial plants, and (3) from the space between the shrubs (control samples). Each soil sample (12 samples per month), was labeled, placed in an individual plastic bag, and transported in a cooler to the laboratory. All soil samples were sieved (mesh size 2 mm) to remove root particles and other organic debris, and kept at 4 °C. Before any analyses, soil samples from consecutive months belonging to the same season were pooled as follows: December, January, and February — winter, March, April, and May — spring, June, July, and August — summer and September, October, and November –—autumn. All biological and chemical analyses were performed within 72 h after collection. Subsamples were stored at −80 °C until DNA extraction.

2.3. Methods

Soil moisture was determined gravimetrically by drying samples at 105 °C for 48 h, and expressed as a percentage of dry weight.

Organic-matter content, as described by the loss on ignition method, was determined by igniting samples at 490 °C for 8 h (loss on ignition carbon, LOI-C).

Total soluble nitrogen (TSN) was determined in soil extracts [0.01 M $CaCl_2$, 1:2.5 soil solution ratio (Houba et al., 1987)] using a Skalar autoanalyzer (S.F.A.S., 1995) that measures NH_4 and NO_3 content.

DNA extraction — soil DNA was extracted using the MB DNA extraction kit (Minerva Biolabs, GmbH). DNA quality and concentrations were confirmed via electrophoresis in a 1% agarose gel.

2.4. PCR-DGGE analysis of total bacterial community

The PCR-DGGE method, based on 16S ribosomal DNA and denaturing gradient gel electrophoreses fingerprinting technology, is a common screening method for bacterial diversity and monitoring spatial and temporal changes in the dominant members of the community in response to changes in environmental parameters (Mette and Neils, 2002). Here we used it to investigate changes in microbial-community structure during four seasons for three test conditions: under the *H. scoparia* shrubs, in the vicinity of the artificial plants, and in the control areas between the shrubs.

The universal eubacterial primer pair 341F, with a GC clamp at its 5′ end (Sheffield et al., 1989), and 907R (Muyzer et al., 1993) was used to obtain the 16S rDNA amplicons used in the DGGE. Each 50-μl reaction mixture contained the following: 1.5 U *Taq* DNA polymerase (Red *Taq*; Sigma, St. Louis, MO), *Taq* buffer containing a final magnesium concentration of 2.5 mM, dNTPs (20 nmol each), 12.5 μg bovine serum albumin, 25 pmol of each primer, and 1.2 μl DNA template. Thermocycling was carried out with an initial denaturation step of 95 °C for 4 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and elongation at 72 °C for 30 s. The program was completed with a final elongation step at 72 °C for 10 min. The PCR products were verified by agarose gel electrophoresis (1%). DGGE was performed on 6% (w/v) acrylamide gels containing a linear urea-formamide gradient ranging from 30 to 60% denaturant (with 100% defined as 7 M urea and 40% (v/v) formamide). Gels were run for 17 h at 100 V in the Dcode Universal Mutation System (Bio-Rad Laboratories, Hercules, CA). DNA was visualized after staining with Gelstar (Invitrogen Corp., Carlsbad, CA) by UV transillumination (302 nm) and photographed with a Kodak KDS digital camera (Kodak Co., New Haven, CT).

2.5. Cluster analysis of DGGE community fingerprints

DNA fingerprints obtained from the 16S rDNA DGGE amplicon-banding patterns ($n = 4$) were digitized by using Fingerprinting II Informatix software (Bio-Rad Laboratories). Lanes were normalized to equal amounts of total signal after background subtraction. Cross-comparison was done using a marker combining PCR products of selected fragments with broad migration range. A neighbor-joining tree based on the unweighted pair-group method using arithmetic average (UPGMA) dendrogram using the Dice coefficients, was produced.

2.6. Statistical analysis

All data obtained in this study were subjected to statistical analysis of variance using the SAS model (ANOVA, Duncan's multiple range tests, and Pearson correlation coefficient), and were used to evaluate differences between separate means. Significant differences ($P < 0.05$) between parameters are represented in the figures by small letters.

3. Results

3.1. Soil moisture

The total amount of rainfall at the study site during the rainy season (December 2006–May 2007) was 170 mm, which was double the