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Experimentally testing the species-habitat size relationship on soil bacteria: A proof of concept

Manuel Delgado-Baquerizo[a,b,*], David J. Eldridge[c], Kelly Hamonts[b], Peter B. Reich[b,d], Brajesh K. Singh[b,e]

[a] Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO, 80309, USA
[b] Hawkesbury Institute for the Environment, Western Sydney University, Penrith, 2751, New South Wales, Australia
[c] Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, New South Wales, 2052, Australia
[d] Department of Forest Resources, University of Minnesota, St. Paul, MN, 55108, USA
[e] Global Centre for Land Based Innovation, University of Western Sydney, Building L9, Locked Bag 1797, Penrith South, NSW, 2751, Australia

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ABSTRACT

The species-area relationship is one of the most widely reported ecological theories accounting for biodiversity of plants and animals. However, we lack solid experimental data demonstrating whether this key ecological theorem also applies in the microbial world. Here, we conducted a microcosm study to evaluate the role of habitat area in driving the diversity, abundance, composition and functioning (i.e., four enzyme activities linked to organic matter decomposition) of soil bacterial communities. Thus, we aim to evaluate whether the principle of species-area relationship is potentially applicable to soil microbes. We established a fully factorial experimental design of three island sizes (~9, 50 and 150 cm^2) by two sterile soils (low, high resources). After six months of glasshouse incubation, habitat-area was positively related to bacterial richness, relative abundance of Chloroflexi, Verrucomicrobia and δ-proteobacteria, and soil functions in both soils. Soil with higher resources always had the greatest bacterial richness and functions. Our findings provide a proof of concept by demonstrating the potential importance of both habitat-area and resource availability in driving soil bacterial biodiversity and functioning.

1. Introduction

The relationship between habitat-area and number of plant and animal species is one of the most consistent ecological patterns in terrestrial ecosystems (MacArthur and Wilson, 1967; Hoyer and Canfield, 1994; Brunet and Medellín, 2001). Larger islands support a greater absolute number (i.e. not standardized to a common area) of plant and animal species than smaller islands (MacArthur and Wilson, 1967). As this popular theorum was developed without explicitly considering the microbial world, much less is known about the extent to which microbe diversity (i.e. number of species) conforms to predictions of Island Biogeography Theory (Green and Bohannan, 2006; Barberán et al., 2014). Bell et al. (2005) provided the first evidence that habitat size could drive diversity of bacteria using water-filled tree holes as its island model. However, the observational nature of this study and the multiple confounding factors surrounding the selected type of island led to serious criticism of this study (Fenchel and Finlay, 2005). In addition, Zinger et al. (2014) and Barreto et al. (2014) provided evidence that in aquatic environments, bacterial communities display a taxa-area relationship; however their results are also based on observational correlations.

Observational relationships have been questioned because of the inability to establish a cause-and-effect relationship between explanatory and responses variables. In other words, an experimental proof of concept for the microbial species-area relationship is needed to support future studies aiming to detect these types of relationships in real world ecosystems. Importantly, island vary in their availability of resources (e.g., soil fertility). Given the importance of resource availability in shaping the diversity and functioning of terrestrial ecosystems (Tilman, 1982; Waldrop et al., 2006; Maestre et al., 2015), any attend to evaluate the link between island size, and microbial diversity and function, needs to account for resource availability as a potentially important regulator of these relationships. Considering that soil microbes are major drivers of the rates and stability of key soil processes such as organic matter decomposition and nutrient cycling (Bodelier, 2011; Singh et al., 2009; Bardgett and van der Putten, 2014; Delgado-Baquerizo et al., 2017), improving our understanding of the ecological patterns driving soil microbial diversity is essential to predict changes

* Corresponding author. Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO, 80309, USA.
E-mail addresses: M.DelgadoBaquerizo@gmail.com, M.delgadobaquerizo@uws.edu.au (M. Delgado-Baquerizo).

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Table 1
Location, climate and main soil properties for Soils A and B.

	Soil A	Soil B
Location (°)	−34.00, 145.73	−33.73, 148.20
Mean annual temperature (°)	17	16
Annual precipitation (mm)	418	656
Altitude (m)	113	335
pH	6.36	7.35
Clay (%)	33	37
Bulk density (g cm^{-3})	1.43	1.17
Organic matter (%)	5.21	8.16
Dissolved organic N (mg N kg^{-1} soil)	0.00	40.48
NH_4^+ (mg N kg^{-1} soil)	2.99	6.40
Available P (mg P kg^{-1} soil)	2.18	11.23

in ecosystem functioning under changing environments.

Herein we posit that habitat-area drives the diversity (i.e. number of species – richness) and functioning of soil microbes. Specifically, we hypothesized that i) larger islands provide more space for microbial colonization resulting in greater microbial diversity and functioning; and ii) resource availability plays an essential role during island colonization (i.e. islands with higher amount of resources result in a higher soil microbial diversity and functioning).

2. Methods

2.1. Study design

To test our hypotheses, we conducted a microcosm study in which we evaluated the role of habitat-area in driving the diversity, abundance, composition and functioning (enzyme activities) of bacterial communities. We established a fully factorial experimental design with two factors: island size (three levels: ~9, 50 and 150 cm^2) and soil type, including relatively low (Soil A) vs. high (Soil B) nutrient availability (Table 1; Fig. 1a). Soils for this study were collected during March 2014 from two semiarid woodlands (*Eucalyptus* spp.) in eastern Australia. At each site, a composite soil sample (twenty soil cores) was collected (top 20 cm) under tree canopies. The full description of the site characteristics and soil properties are available in Table 1. We found significant differences ($P < 0.05$) in all soil variables between the two locations in this study (Table 1). Soil properties were measured using standardized protocols as described in Maestre et al. (2012).

Following field sampling, the soil was highly homogenized, sieved (< 2 mm mesh) and sterilised using gamma radiation (50kGy; see Delgado-Baquerizo et al., 2016a for a similar approach). Soils were re-sterilised seven days later (Gamma radiation, 50 kGy) to remove all microbial spores. We used gamma radiation because it causes minimal changes to the physical properties of soils compared to other methods such as autoclaving (Wolf et al., 1989; Lotrario et al., 1995). Sterilised soil diluted in nutrient medium (peptic digest of animal tissue 1.5 g L^{-1}, yeast extract 1.5 g L^{-1}, sodium chloride 5 g L^{-1}, beef extract 1.5 g L^{-1} each from DIFCO laboratories, USA) exhibited no growth 5 days after incubation at 28 °C.

2.2. Microcosm construction

Microcosms were constructed by carefully placing sterile soil in petri dishes of three sizes (1cm depth). Five replicates were established, resulting in 30 microcosms (two soil types x three island sizes x five replicates). Microcosms were placed close to each other (~5 cm) in a random spatial grid (6 × 5). The position of each microcosm in the grid was changed about every 5 days to avoid positional effects. Microcosms were placed indoors in a glasshouse, watered regularly with autoclaved sterile water, and incubated for six months to allow "natural" microbial colonization (i.e. by airborne microbial spores). Given that current empirical evidence suggests that microbial succession occurs from days

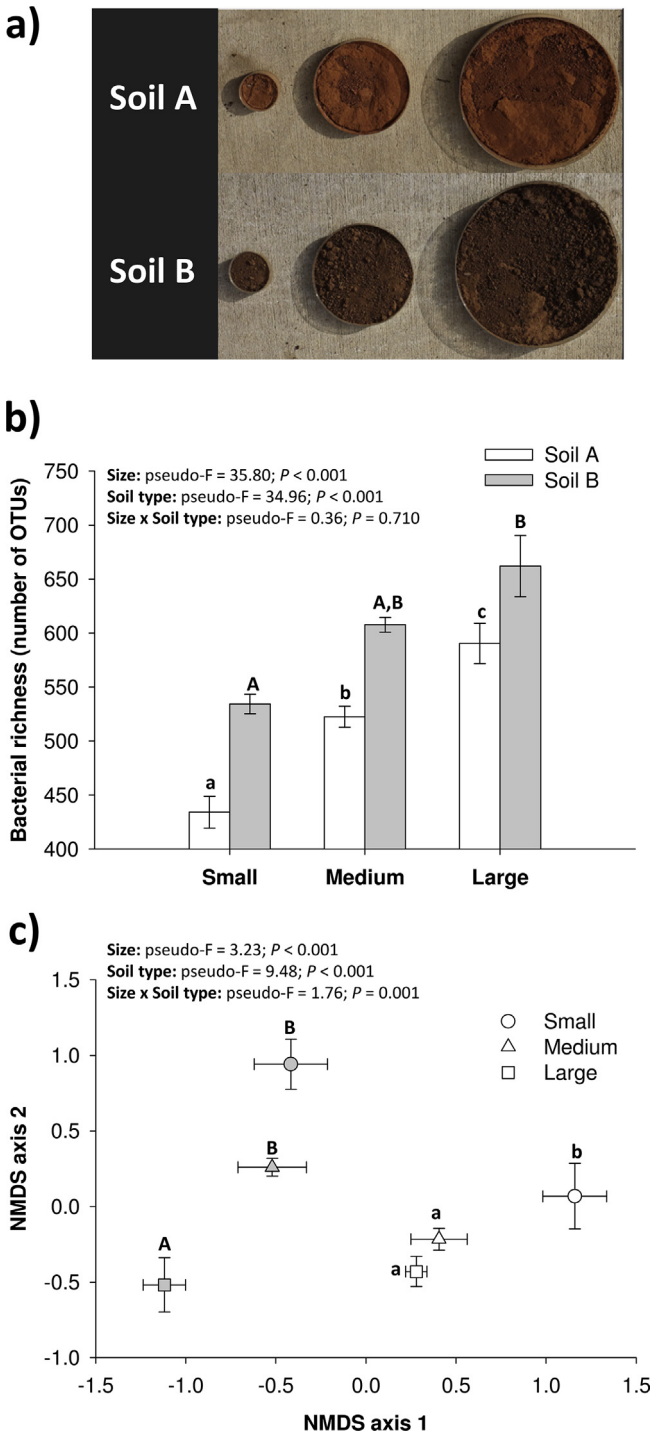

Fig. 1. Habitat area effects on the diversity and composition of bacteria. Panel (a) represents an example of the different island sizes used in this study for Soils A and B. Panel (b) presents mean values (± SE) for bacterial richness across different island sizes and soil types. Panel (c) presents results from a nMDS (mean ± SE) showing shifts in microbial composition at the OTU level across different island sizes and soil types. Different lower and upper-case letters indicate significant differences after post-hoc Tukey tests (only when applicable) for soils A and B, respectively.

to a few months (e.g., Edwards et al., 2015; Voříšková and Baldrian, 2013; Jurburg et al., 2017), we assume here that six months should be a reasonable incubation period over which to obtain a late successional microbial community in our soils. We collected all our soils after a six months incubation period, which ultimately allowed us to directly