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Responses of bulk and rhizosphere soil microbial communities to thermoclimatic changes in a Mediterranean ecosystem

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ABSTRACT

The effect of thermoclimatic changes on microbial communities in the rhizosphere of a wild thyme species, *Thymus zygis* L., and its surrounding bulk soil was studied along elevational gradients in the Sierra Nevada National Park (Spain). Multiplex amplicon sequencing of bacterial and fungal taxonomic markers revealed that the richness, diversity and structure of bacterial and fungal communities were affected by thermoclimatic changes, with environmental parameters (mean annual atmospheric temperature and precipitation) and edaphic properties (mainly pH and nutrients) as the major drivers. Although both bulk soil and rhizosphere communities were structured according to the thermoclimatic zones, the response of microorganisms to thermoclimatic changes was different depending on the rhizosphere effect. On the contrary, the microbial functional gene diversity was not affected by thermoclimatic changes suggesting functional redundancy in the microbial communities along the altitudinal gradients. However, the functional gene diversity was clearly different between bulk soil and the rhizosphere, with the latter harbouring a larger number of gene copies and more different functional genes than bulk soils. Finally, a set of microbial bioindicators are defined for the thermoclimatic zones as a starting point to develop improved biological tools and models to monitor and predict the effects of climate changes. To the best of our knowledge, this is the first study where the response of bulk soil and rhizosphere microbial communities to thermoclimatic changes has been studied in parallel.

1. Introduction

Microorganisms, including bacteria and fungi, exist in complex and dynamic communities in the soil where they form the basis for terrestrial ecosystems and biogeochemical cycles. The rhizosphere, which is the soil under the influence of plant roots (Hartmann et al., 2008), is particularly important for plant health and nutrition (Lakshmanan et al., 2014) and also plays roles in biogeochemical cycles (Philippot et al., 2008) and soil formation (Séguin et al., 2005; Drigo et al., 2008). In this biotope, the flow of nutrients released by plants as root exudates (Bais et al., 2006) affect microbial activity, richness and community composition so that microbial assemblages differ from those found in soil further away from the root, the so-called bulk soil. The microbial communities in the rhizosphere are influenced by the species and the physiological state of the plant but also by soil characteristics (Bulgarelli et al., 2013; Philippot et al., 2013; Chaparro et al., 2014; Lebeis, 2015; Llado et al., 2017). However, the effect of other environmental factors such as climate remains largely unknown, thereby limiting the accuracy of models formulated to predict and mitigate the possible adverse effects of climate change (Gärdenäs et al., 2011).

To determine the effect of climate change on microbial communities, altitudinal gradients in the mountain ranges that span multiple thermoclimatic zones in a short geographic distance are receiving much attention in recent years. As such, several studies have focused on taxonomic and functional changes in soil microbial communities along these gradients (Bryant et al., 2008; Fierer et al., 2011; Lin et al., 2015; Yasir et al., 2015; Lanzen et al., 2016; Siles et al., 2016; Siles and Margesin, 2017; among others). With the exception of Fierer and collaborators (2011), most of these studies have shown community changes at different altitudes although different tendencies were reported regarding the relationship between bacterial diversity and increasing elevation such as no trends (Yasir et al., 2015; Lanzen et al., 2016), decreasing trends (Bryant et al., 2008; Shen et al., 2013, 2015; Wang et al., 2015a; Wang et al., 2015b; Zhang et al., 2015), increasing trends (Siles and Margesin, 2016) hump-backed trends (Singh et al., 2012; Lin et al., 2015) or trends with a dip at lower mid elevations

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Table 1
Soil physicochemical properties and the estimated environmental factors. MAT, Mean Atmospheric Annual Temperature; MAP Mean Annual Precipitation.

Region	Thermoclimatic zone (Mediterranean)	Altitude (m)	pH	Total nitrogen (%)	Carbonates (%)	Oxidizable organic material (%)	Assimilable phosphorus (p.p.m.)	Assimilable potassium (p.p.m.)	MAT (ºC)	MAP (mm)
Capileira	Meso-	1100	7.08	0.1	1.2	1.5	3	65	17.2	494
	Supra-	1400	6.77	0.06	2.5	0.9	1	80	16.2	564
	Supra-	1700	5.86	0.13	1.6	2.1	3	75	14.8	639
	Oro-	2000	6.45	0.08	1.6	1.2	3	130	12.8	680
	Oro-	2300	5.72	0.48	1.5	6.4	5	180	12.0	728
Puerto de la Ragua	Meso-	1100	7.91	0.05	24	0.7	0	19	15.3	451
	Supra-	1400	6.36	0.12	1.6	1.9	2	89	14.9	472
	Supra-	1700	6.31	0.12	1.8	2	2	93	12.8	550
	Oro-	2000	6.41	0.14	1.6	1.7	3	59	11.5	603
	Oro-	2300	6.15	0.16	1.8	1.6	6	35	10.3	643

(Singh et al., 2014). With regard to fungal diversity along elevational gradients, these generally follow the same trends as those observed with bacterial diversity at the same location but not always (Wang et al., 2015a; Siles and Margesin, 2016). A possible explanation for the contradicting microbial diversity trends found in these studies could be due to the influence of local abiotic soil properties and biological factors which cannot easily be separated from those caused by climatic factors (Lanzen et al., 2016). As a result, obtaining additional data from other mountain systems could be of interest to carry out comparative studies (Hofmann et al., 2016). Moreover, more information of the functional gene diversity along altitudinal gradients may contribute to improve our understanding of how microbial communities respond to climate changes (Yang et al., 2014; Shen et al., 2016). As elevational gradient studies have so far been limited mostly to the microbial communities of bulk soil, expanding these studies to rhizosphere communities may give additional insights on the response of microbial communities harbored within this key ecological niche to climate change.

The Sierra Nevada National Park in southern Spain, is located in the Sierra Nevada mountain range within the Baetic System. The Park hosts predominantly high mountain shrublands and oak forests with flora which follow well described thermoclimatic zones found at different altitudes (Rivas-Martinez et al., 1997). These thermoclimatic zones are associated with the Mediterranean macrobioclimate consisting of high temperatures and low rainfall during the summer months and low temperatures during the winter. This Mediterranean biodiversity hotspot is a biosphere reserve integrated into national and international programs dedicated to monitoring the ecological impact of climate change (Zamora et al., 2015), which together with its marked stratification converts Sierra Nevada to be an ideal location for studies of subsoil microbial communities. Amongst the flora found in the Sierra Nevada, the wild thyme species, *Thymus zygis* L., is a cosmopolitan plant capable of growth at different altitudes and different soil types which along with its ecological importance in Mediterranean shrublands and its economic importance for the pharmaceutical, culinary and cosmetic industries (Pascual et al., 2016), makes it an interesting candidate for use as a model plant for rhizosphere studies.

In this study, the microbial communities in bulk soil and in the rhizosphere soil under the influence of *T. zygis* were analyzed across three thermoclimatic zones (Meso-, Supra- and Oro-Mediterranean), along two thermoclimatic transects in spatially distant regions in the Sierra Nevada Mountains during two consecutive years. The aim of this study was (i) to determine how the bacterial and fungal communities respond to thermoclimatic changes from a taxonomic and functional point of view and (ii) whether the responses are similar in bulk and rhizosphere soils. Furthermore, microbial bioindicators of the thermoclimatic zones were sought. We hypothesize that bulk and rhizosphere soil microbial communities are driven by deterministic factors associated with the thermoclimatic zonation. As a consequence of the selective ecological forces to which microorganisms are exposed at each thermoclimatic zone, we expect to find several bacterial and fungal species which could be used as potential bioindicators for future studies.

2. Material and methods

2.1. Sampling, processing and soil physicochemical characteristics

Soil and rhizosphere samples were taken in the spring of 2013 and 2014 along elevational gradients at two different regions within the Sierra Nevada National Park, Granada, Spain. Specifically, samples were collected at five different altitudes comprising three thermoclimatic zones (Meso-Mediterranean, 1107 ± 5.0 m; Supra-Mediterranean, 1402 ± 5.7 m, 1732 ± 27.3 m; and Oro-Mediterranean, 1998 ± 6.0 m, 2288 ± 33.2 m), on the south facing slopes of two regions within the Sierra Nevada Mountains; one near to the municipality of Capileira and another near to the mountain pass Puerto de la Ragua (Table S1). Each year, at each sampling area and at each altitude three adult and apparently healthy *Thymus zygis* L. plants of 10–15 cm height were collected within a maximum distance of 36 m from each other. Collected plants with intact roots and adhering soil were transported to the laboratory in sterile bags where the rhizosphere soil was separated from the roots as described in Pascual et al. (2016). At the same time as plants were collected, three soil samples were taken within approximately 1 m of each extracted plant by excavating to a depth of 10–15 cm. Soil samples were stored in sterile 50 ml Falcon tubes and transported to the laboratory. A total of 60 bulk and 60 rhizosphere soil samples were collected and further processed (3 bulk/rhizosphere soil replicates x 2 transects x 5 altitudes x 2 years).

Alongside the other soil samples, a large soil sample comprising of 1 dm^3 of thoroughly mixed bulk soil was taken at each sampling point during the first year and used for physicochemical characterization at the Andalusian soil analysis laboratory (Laboratorio Agroalimentario de Atarfe, Granada, Spain) using standard international methods. Each soil sample was characterized regarding soil pH, oxidizable organic matter, total nitrogen, assimilable phosphorous and potassium and carbonates. All collected soils have a sandy loam texture (Table 1). The physicochemical parameters of rhizosphere soil could not be measured due to the confounding effect of the buffer used to obtain this soil fraction as well as the limited quantities of rhizosphere soil obtained which were insufficient for proper analyses. Therefore, in this study, comparison of microbial diversity and composition with regard physicochemical edaphic parameters were limited only to bulk soils. Climatic factors MAT (Mean Atmospheric Annual Temperature) and MAP (Mean Annual Precipitation) were estimated for each sampling point using the tools available at https://cambia.climasig.es/# within the CNCM3 model and low emissions for the period 2011-2040 (Table 1).