

# Microbial communities of bulk and *Eschscholzia californica* rhizosphere soils at two altitudes in Central Chile

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## Abstract

Despite several reports point out a rhizosphere effect shaping soil microbial communities and others an effect of altitude on plant phenotypic features, currently little is known about the impact of elevational patterns on the diversity of soil microbial communities. In this study, diversity of soil microbial communities was assessed in samples derived of bulk and rhizosphere soils associated to Californian poppy (*Eschscholzia californica* Cham.) populations at 1000 and 2000 m.a.s.l in Central Chile. *E. californica*, a native plant of North America, is considered a successful invader in Mediterranean ecosystems worldwide but its effect on diversity of soil microbial communities is yet unknown. Microbial diversity was evaluated at genetic level through T-RFLP (terminal restriction fragment length polymorphisms) using bacterial, archaeal and fungal molecular markers, and at metabolic level using CLPP (community-level physiological profiles). At genetic level, microbial diversities of bulk and rhizosphere soils at lower altitude were similar, although at higher altitude microbial diversity of both types of soils was different, suggesting a plant filtering effect more notorious at higher altitude. At metabolic level, microbial diversity of rhizosphere soils were similar independently of the altitude, suggesting a plant filtering effect that exceeds the altitude effect observed in the case of the bulk soil.

**Keywords:** *Eschscholzia californica*, soil microbial community, metabolic diversity, genetic diversity, altitude, rhizosphere effect

## 1. Introduction

Genetic and metabolic diversity of soil microbial communities depends on multiples resources and numerous environmental factors such as organic material, moisture content, pH, among

others, including altitude, a factor whose effects on microorganisms are not yet well-known. The effect of elevational gradients on plant and animal diversity has been extensively documented (e.g. Lomolino, 2001;

Grytnes, 2003; McCain, 2005); however information on the effects of altitude on patterns of microbial diversity is newly emerging. Some reports pointed out that soil bacterial diversity patterns exhibit non-significant differences along an elevational gradient, in contrast to significant changes in diversity of macroorganisms (Fierer, *et al.*, 2011; Shen *et al.*, 2013). Conversely, other studies suggest that diversity of soil bacterial (Shen *et al.*, 2015; Singh *et al.*, 2014) and archaeal (Wang *et al.*, 2015) communities were affected by elevations or that at least the bacterial group acidobacteria was affected (Zhang *et al.*, 2014). On the other hand, the effect of altitude on soil microbial communities may be modulated by vegetation growing on these sites since rhizosphere provides a habitat different to bulk soil without plant cover for microbial communities (Kuske *et al.*, 2002; Orlando *et al.*, 2007). Several reports suggest that vegetation has an important role shaping the soil bacterial communities (Shen *et al.*, 2013), meanwhile the microbiota produces secondary metabolites that improve plant nutrition and protect against phytopathogens (Martínez-Viveros *et al.*, 2010).

Nowadays, some authors pointed out that invasive plants markedly alter the chemical and physical properties of soil (Weidenhamer and Callaway, 2010), which in turn would disturb the diversity of soil microbial communities (Batten *et al.*, 2006; Min *et al.*, 2013). Particularly, in Chile 700 species of invasive plants have been described (Quiroz *et al.*, 2009) within them *E. californica*, the Californian poppy, is an herbaceous plant native of the West Coast of North America and it is considered a successful invader in Mediterranean ecosystems worldwide (Leger and Rice, 2007). It was introduced in the Central Region of Chile in the late nineteenth century and 100 years later is widely distributed in the country from 30°S to 38°S and from sea level to 2200 m.a.s.l.

(Arroyo *et al.*, 2000). Even though *E. californica* is a very common plant in California and Chile, studies conducted to address its ecology are relatively scarce and particularly reported significant differences in reproductive and vegetative characteristics among *E. californica* populations located at different altitudes in Central Chile (Peña-Gómez and Bustamante, 2012; Castillo *et al.*, 2013).

The effect of invasive plants on soil microbiota is a topic of current interest, as examples, in California the invasive yellow star-thistle, *Centaurea solstitialis* L., increases diversity of sulfur-oxidizing bacteria and sulfate-reducing bacteria (Batten *et al.*, 2006); the invasive cheat grass *Bromus tectorum* L., changes the composition of the soil bacterial community by decreasing denitrifying bacteria abundance in Utah (Kuske *et al.*, 2002); and the invasive blue wattle *Acacia dealbata* Link, increases bacterial richness and decreases fungal richness depending on the studied ecosystem in Northwest Spain (Lorenzo *et al.*, 2010). Moreover, it is proposed that invasive plants may have particularly strong effects on rhizosphere microbiota through their secondary metabolites (Vivanco *et al.*, 2004; Badri and Vivanco, 2009). In fact, *E. californica* produces various types of isoquinoline alkaloids (Fabre *et al.*, 2000), such as sanguinarine, a potent antimicrobial agent (Alcantara *et al.*, 2005). Since the rhizosphere is a well-defined microhabitat, different to the neighboring bulk soil, revealing the effect of the vegetation on the microbial community structure (Kuske *et al.*, 2002; Orlando *et al.*, 2007; Garbeva *et al.*, 2008), then we hypothesized that *E. californica* could act as an environmental filter influencing the rhizosphere microbial community and masking the altitudinal effect. Therefore this study was focused on evaluate genetic and metabolic diversity patterns of soil microbial communities growing in bulk soil without plant cover and in *E. californica* rhizosphere

soil. The aim of this work was to determine patterns of genetic and metabolic diversities of soil microbial communities at two altitudes, in samples derived of bulk and rhizosphere soils associated to *E. californica* populations located at 1000 and 2000 m.a.s.l in the Andes mountain range, Central Chile. The microbial genetic diversity was evaluated through T-RFLP (terminal restriction fragment length polymorphisms) (Liu *et al.*, 1997) using bacterial, archaeal and fungal molecular markers, whilst the metabolic diversity was assessed by CLPP (community-level physiological profiles) (Garland and Mills, 1991).

## 2. Materials and Methods

### 2.1. Study sites and soil sampling

Soil samples were collected in the locality of Farellones (33°21'S; 70°19'W) at two different altitudes: site 1 at 1000 m.a.s.l. and site 2 at 2000 m.a.s.l.; these sampling sites are located in the Andean range of Central Chile which is characterized by a dry Mediterranean climate with winter rains. At both altitudes linear transects within the area where the invasive plant was plentiful (*E. californica* rhizosphere soils, RS) and where there were not invasive or native plants (bulk soils, BS) were randomly disposed. The study was conducted with composite soil samples, in triplicate, in order to reduce the small-scale spatial heterogeneity; each composite sample was obtained mixing 10 independent samples, which were taken in the respective transect and separated from each other by 1 m. The sampling was performed during spring 2010, coinciding with the plant flowering. BS samples were obtained from the top soil to a depth of about 10 cm; whereas RS samples were collected from soil adhering to the roots (ca. 10 cm length) of *E. californica* flowering plants with a height of 20-30 cm. All samples were stored at 4°C until analyses.

### 2.2. Edaphic factors

For each, composite soil sample edaphic factors pH, moisture content (MC), organic matter (OM) and nitrate content ( $[\text{NO}_3^-]$ ) were determined. Briefly, pH was measured using potentiometry; MC and OM were calculated gravimetrically before and after desiccation and calcination, respectively; and  $[\text{NO}_3^-]$  was determined by colorimetry.

### 2.3. DNA extraction and PCR conditions

DNA was extracted from 0.25 g of each composite soil sample using the Power Soil™ DNA Isolation kit (MoBio Laboratories Inc., CA, USA) according to the manufacturer's instructions. The quality and integrity of the extracted DNA were visualized in 0.8 % (w/v) agarose gels in TAE 1X buffer (40 mM Tris-acetate, 1 mM EDTA [pH 8.0]) stained with GelRed™ (Biotium, CA, USA). All DNA samples were stored at -20°C until analysis. Therefore, the bacterial 16S rRNA genes (B-16S rDNA) were amplified with primers fD1-FAM (carboxyfluorescein) and rP2 (Weisburg *et al.*, 1991). The archaea 116S rRNA(A-16S rDNA) and the fungal 18S rRNA (F-18S rDNA) genes were amplified using nested PCR approaches. The A-16S rDNA amplifications were carried out using in the first reaction primers 23FPL (Bintrim *et al.*, 1997) and UA1204R (Baker *et al.*, 2003) and in the second reaction primers A571F and UA1204R-FAM (Baker *et al.*, 2003). The F-18S rDNA amplifications were performed using in the first reaction primers NS1 and EF3 (Hagn *et al.*, 2003) and in the second reaction primers EF4 and EF3-FAM (Smit *et al.*, 1999). All the amplifications were performed according to the cited literature recommendations and using the GoTaq® Green Master Mix (GoTaq® DNA polymerase in 1X Green GoTaq® Reaction Buffer [pH 8.5], 200 μM of each dNTP and 1.5 mM  $\text{MgCl}_2$ ) (Promega, WI, USA)

in a Maxygene thermo cycler (Axygen, CA, USA). The concentration and quality of the amplicons were determined electrophoretically as described above except that 1.2% (w/v) agarose gels were used.

#### 2.4. Terminal restriction fragment length polymorphisms (T-RFLP)

Terminal restriction fragment length polymorphisms (T-RFLP) were used to evaluate genetic diversity of microbial communities in each soil sample. The amplicons from each molecular marker were hydrolyzed overnight with the restriction enzymes *Hae*III and *Msp*I in the cases of B-16S rDNA and F-18S rDNA, and *Hae*III and *Bst*UI in the case of A-16S rDNA (Fermentas, NY, USA). Terminal restriction fragments (T-RFs) were separated on an automated Genetic Analyzer ABI3730XL (Applied Biosystems; Macrogen Inc., Seoul, Korea). The length of the fluorescently labeled T-RFs was determined by comparison with the GeneScan™ 1200 LIZ® size-standard using the GeneScan 3.71 software (Applied Biosystems, Darmstadt, Germany). Peaks with a fluorescence over 100 U and larger than 60 bp were analyzed by peak height. Patterns from different samples were normalized to identical total fluorescence units by an iterative standardization procedure. Relative abundance of T-RFs, as percentage, was determined by calculating the ratio between the height of a given peak and the normalized total peak height of each sample. Shannon ( $H'$ ) indices were calculated for each sample using the PAST software (available at: <http://nhm2.uio.no/norlex/past/download.html>). In addition, in order to relate the data of the three molecular markers and the edaphic factors, a canonical correspondence (CCA) analysis was conducted using the PAST software.

#### 2.5. Community-level physiological profiles (CLPP)

The community-level physiological profiles (CLPP) were used to evaluate the metabolic diversity of the microbial communities in each soil sample using the BIOLOG Eco-Plate™ system containing 31 different carbon sources and tetrazolium dye as redox indicator. Microbial suspensions were prepared from 1 g of fresh soil from each sample in 10 ml of sterile PBS buffer (137 mM NaCl, 2.7 mM KCl, 8 mM  $\text{Na}_2\text{HPO}_4$ , 2 mM  $\text{KH}_2\text{PO}_4$ ) and shaking at 150 rpm during 1 h. The plates were inoculated with 100  $\mu\text{l}$  of the soil suspensions per well, using one third of the plate for each composite sample replicate, and incubated at 25 °C during a week. Color development in the individual wells was registered every 24 h at a wavelength of 590 nm in an automated plate reader (Epoch, Biotek, Luzern, Switzerland). Absorbance data at the different reading times were corrected by subtracting the first reading (immediately after plate inoculation) to the corresponding response well. The absorbance value of the control well (without carbon source) was subtracted from the absorbance values of each well containing a carbon source and then only the values above 0.1 were considered. The average well color development (AWCD) for each substrate category in the plates was calculated as the mean of the absorbance values of each corresponding well. In order to know the plate incubation time corresponding to the exponential phase, the curves of AWCD versus time were fitted to a modified Gompertz equation using ORIGIN 8.0. Shannon ( $H'$ ) indices were calculated for each sample using the PAST software. In order to perform CCA using the PAST software, data were normalized dividing each absorbance value by the AWCD.

## 2.6. Statistical data analyses

Statistical significant differences of edaphic variables and Shannon ( $H'$ ) indices were assessed using one-way ANOVA and the post-hoc Tukey HSD test (GraphPad Software, Inc). The CANOCO software v.4 was used to test the significance of the canonical axes and the influence of each edaphic factor on the variance of the diversity data in the CCA.

## 3. Results

### 3.1. Edaphic factors

At 1000 m.a.s.l. (site 1) statistically non-significant differences were found between both types of soils, bulk (BS) and *E. californica* rhizosphere (RS) for any of the edaphic variables measured; however, at 2000 m.a.s.l. (site 2), RS had a statistically significant higher moisture content (MC) and pH than BS (Table 1). Comparing the soil variables of BS at both altitudes, MC and pH at site 2 were lower than at site 1, although statistically non-significant. Moreover, when comparing RS at both altitudes, pH at site 2 was statistically significantly higher than at site 1, but statistically non-significant differences in MC were observed despite it was also higher in site 2 than in site 1 (Table 1).

### 3.2. Genetic diversity of the soil microbial communities

In order to determine the genetic diversity of microbial communities of bulk soils (BS) and *E. californica* rhizosphere soils (RS), T-RFLP analyses of the archaeal and bacterial 16S rRNA genes and fungal 18S rRNA genes of each soil sample at both latitudes (sites 1 and 2) were performed. The highest number of T-RFs was obtained with *HaeIII* for the bacterial (8 T-RFs) and fungal (6 T-RFs) groups,

while for the archaeal group the highest number of T-RFs was observed with *BstUI* (5 T-RFs). The same T-RFs were found in both soil types, for the three molecular markers and the two enzymes used. Assuming that different T-RFs represent distinct operational taxonomic units (OTUs) and considering that differences in the richness of OTUs were not observed, by altitude or soil type, these soils would have a similar microbial composition, at least at the level of the most abundant T-RFs. However, some statistically significant differences in Shannon diversity indices ( $H'$ ) of bacterial and fungal groups were observed due to inequality in the relative abundance of some OTUs (Figure 1a), while archaeal diversity indices showed statistically non-significant differences at both altitudes or in both soil types. Thus, diversity indices of the fungal group from RS at site 2 were statistically significantly higher than the others. Moreover, the lowest values of bacterial genetic diversity indices were observed in RS at both altitudes (sites 1 and 2) (Figure 1a).

The relationship between edaphic parameters and genetic structure of microbial communities in BS and RS at both altitudes was explored using CCA. A sample separation in function of soil type (BS and RS) at site 2 along the axis 1 (CCA1; 88.3%) was observed; being MC and pH the most relevant factors explaining this scattering, the influence of the latter being in addition statistically significant (Figure 2a, Table 2). On the other hand, soil samples from both altitudes were separated along the axis 2 (CCA2; 8.2%), being the most influential factor OM (Figure 2a, Table 2). The canonical first axis and the sum of all the canonical axes presented statistical significance (Table 2). Interestingly, at genetic level soil samples at site 1 were grouped together independently of the soil type, although BS and RS samples formed two well-defined groups at site 2, suggesting that the plant filtering effect is more notorious at higher altitude.

**Table 1.** Edaphic variables. Values are means  $\pm$  standard deviation (n=3). In a row, means values followed by the same letter are not significantly different according to the Tukey HSD test ( $P \leq 0.05$ ).

Edaphic factor <sup>(a)</sup>	BS <sup>(b)</sup>			RS		
	Site 1	Site 2		Site 1	Site 2	
MC	4.05 $\pm$ 1.01 ab	2.01 $\pm$ 0.41 a		4.26 $\pm$ 0.37 ab	5.74 $\pm$ 1.71 b	
OM	9.20 $\pm$ 4.64 a	5.10 $\pm$ 1.55 a		7.25 $\pm$ 2.33 a	4.93 $\pm$ 0.88 a	
[NO <sub>3</sub> <sup>-</sup> ]	25.21 $\pm$ 4.60 a	31.66 $\pm$ 8.72 a		28.15 $\pm$ 6.81 a	30.02 $\pm$ 5.46 a	
pH	7.51 $\pm$ 0.06 ab	7.39 $\pm$ 0.05 a		7.39 $\pm$ 0.11 a	7.60 $\pm$ 0.02 b	

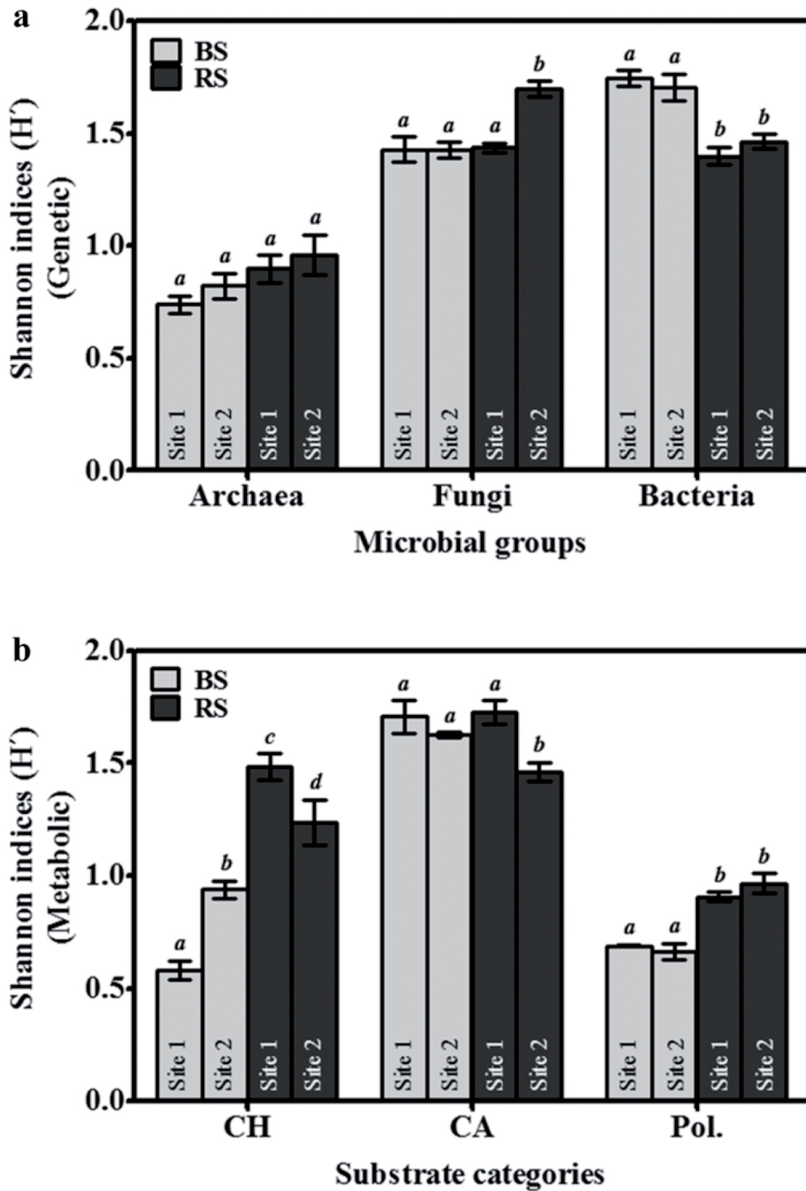
(a) MC: moisture content (%), OM: organic matter (%) and [NO<sub>3</sub><sup>-</sup>]: nitrate content ( $\mu\text{g/g}_{\text{dw}}$ ).

(b) BS: bulk soils; RS: *E. californica* rhizosphere soils.

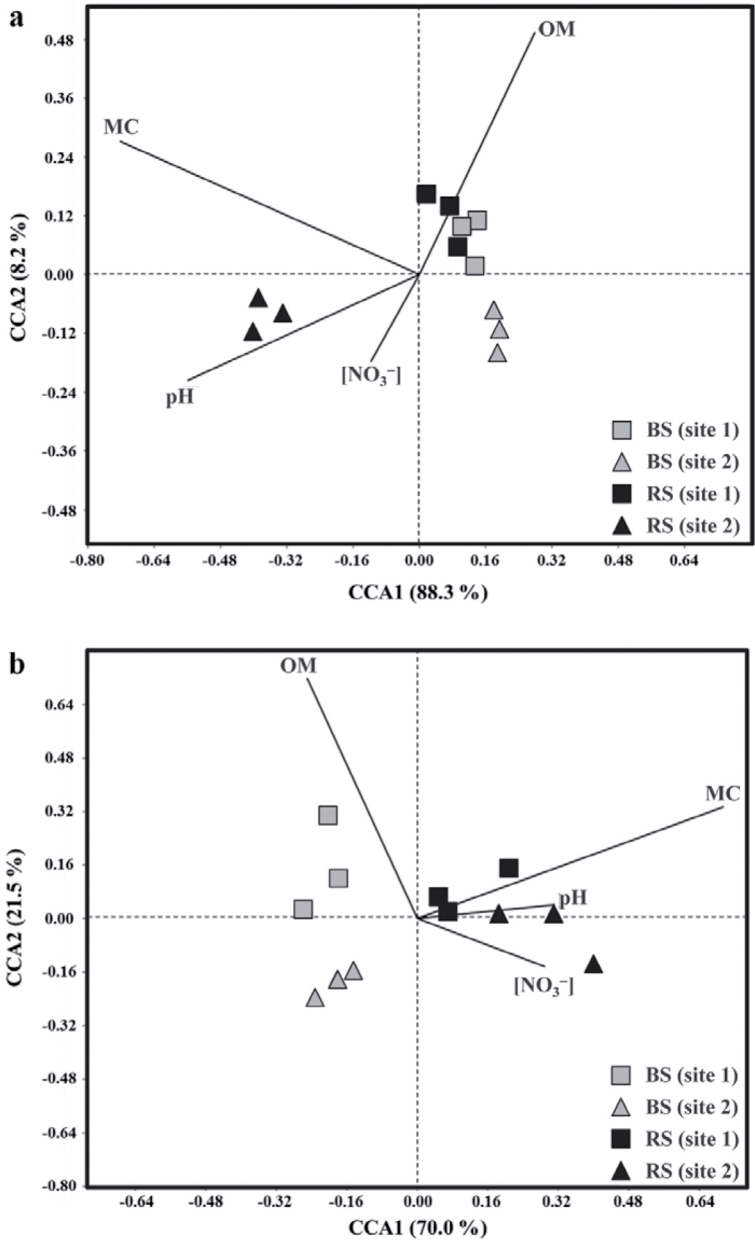
**Table 2.** Relationship between the environmental factors and the genetic and functional structures as determined by the canonical correspondence analyses (CCA). CCA scores of both canonical axes (CCA1 and CCA2) and the total variance (%) were determined using the PAST software. The tests of significance for the canonical axes and the influence of each edaphic factor were calculated using CANOCO.

Edaphic factor <sup>(a)</sup>	Genetic structure			Metabolic structure		
	CCA scores		p	CCA scores		p
	CCA 1	CCA 2		CCA 1	CCA 2	
MC	-0.7231	0.2724	0.051	0.6950	0.3341	0.001
OM	0.2790	0.4949	0.427	-0.2504	0.7197	0.285
[NO <sub>3</sub> <sup>-</sup> ]	-0.1172	-0.1779	0.521	0.2900	-0.1438	0.711
pH	-0.5600	-0.2163	0.001	0.3118	0.0411	0.001
Total Variance (%)	88.3	8.2	-	70.0	21.5	-
Test of significance	First canonical axis		0.010	First canonical axis		0.010
	All canonical axes		0.010	All canonical axes		0.010

(a) MC: moisture content (%), OM: organic matter (%) and [NO<sub>3</sub><sup>-</sup>]: nitrate content ( $\mu\text{g/g}_{\text{dw}}$ ).



**Figure 1.** Shannon diversity ( $H'$ ) of microbial communities in bulk soils (BS) and *Eschscholzia californica* rhizosphere soils (RS) at 1000 m.a.s.l. (site 1) and 2000 m.a.s.l. (site 2). (a) Genetic diversity indices calculated from T-RFLP. (b) Metabolic diversity and equitability indices calculated from CLPP for specific carbon sources: carbohydrates (CH), carboxylic acids (CA) and polymers (Pol.). Means and standard deviation of triplicate measures are shown. Within each data set, different letters represent significantly different values according to the Tukey HSD test ( $P \leq 0.05$ ).



**Figure 2.** Correspondence canonical analyses (CCA) relating diversity of microbial communities and edaphic factors (MC, moisture content; OM, organic matter; [NO<sub>3</sub><sup>-</sup>], nitrate content and pH). (a) CCA based on the genetic structure of the microbial communities, (b) CCA based on the metabolic diversity of the microbial communities.



### 3.3. Metabolic diversity of the soil microbial communities

In order to determine the metabolic diversity of microbial communities of bulk soil (BS) and *E. californica* rhizosphere soil (RS), CLPP analyses were determined according to the number of carbon sources used by each microbial community.

The different carbon sources were grouped into chemical groups and Shannon diversity indices ( $H'$ ) for each set of substrates were calculated using the AWCD at the plate incubation times corresponding to the exponential growth phase. The most outstanding differences regarding metabolic diversity were found in carbohydrates (CH), carboxylic acids (CA) and polymers (Pol.) (Figure 1b). These differences in the use of CH presented an inverse behavior at both altitudes depending on whether they are BS or RS, increasing their utilization the former and decreasing the latter at higher altitude. On the other hand, respecting CA, RS presented a lower utilization of them but just at site 2. Finally, there were not differences in the use of Pol. considering the different altitudes, although RS presented a statistically significantly higher consume of these carbon sources, being the glycogen only used by these communities (Figure 1b). However, this was not the only substrate exclusively degraded by RS communities, consumption of  $\beta$ -methyl-D-glucoside and D-cellobiose was also observed only in this soil type, suggesting a different metabolic behavior of microbial communities of BS and RS.

The CCA, based on metabolic data, showed anew a separation of the microbial communities according to the soil type (BS and RS) along the axis 1 (CCA 1; 70.0%), being MC and pH the edaphic variables which significantly explained the sample ordination (Figure 2b, Table 2). Once again, soil samples from both altitudes were separated along axis 2 (CCA 2; 21.5%),

being the most influential factor OM (Figure 2b, Table 2). Also in this case, the canonical first axis and the sum of all the canonical axes exhibited statistical significance (Table 2). Strikingly, at metabolic level, RS samples were grouped together independently of the altitude, suggesting a plant filtering effect that exceeds the altitude effect observed in the case of BS samples.

## 4. Discussion

Several studies have shown that both, soil physicochemical characteristics and plant features affect the structure of microbial communities in the rhizosphere (e.g. Lorenzo *et al.*, 2010; Min *et al.*, 2013; Philippot *et al.*, 2013), however few studies have been focused on the effect of environmental factors such as altitude on the genetic and metabolic diversity of microbial communities and how it could be modulated by the plant. In this study, the effect of altitude on microbial communities from two habitats, bulk and rhizosphere soils, were compared. Particularly, *E. californica* plants were chosen because of some reports in the literature suggesting that as invasive plants they strongly alter the chemical and physical properties of soils (Weidenhamer and Callaway, 2010); for example they roots produce isoquinoline alkaloids with antimicrobial activity (Fabre *et al.*, 2000) which in turn may disturb the structure of soil microbial communities (e.g. Kuske *et al.*, 2002; Batten *et al.*, 2006; Lorenzo *et al.*, 2010; Min *et al.*, 2013). Interestingly, our results showed that archaeal and bacterial genetic diversities of rhizosphere soils (RS) were similar at both altitudes, but the fungal diversity increased at higher altitude. In contrast, bulk soils (BS) revealed no changes with altitude in the genetic diversity of any of the three microbial groups, which is in accordance with Shen *et al.* (2013) who did not find any elevational gradient

tendency in soil bacterial diversity, being pH the main factor controlling bacterial diversity and community composition. In our study, CCA revealed that pH and moisture content were the most influential edaphic factors in soil samples ordination according to altitude, but a major separation of RS samples was observed suggesting that the rhizosphere effect could increase these differences probably due to physiological changes of the plant at higher altitude (2000 m.a.s.l.) rather than altitude *per se*.

Indeed, changes on diversity of soil microbial communities could be consequences of environmental limitations imposed by altitude on plants (Körner, 2007). Despite molecular mechanisms involved in the feedback loops between *E. californica* and soil microbial diversities remain unknown, it is important to note that pH has been reported as the best descriptor of soil microbial diversity and richness beyond other edaphic factors (Fierer and Jackson, 2006; Lauber *et al.*, 2009).

Curiously, in the case of metabolic diversity, differences in the utilization of carbohydrates were observed in both soil types, RS samples from both altitudes were clustered together suggesting a plant filtering effect that reduces the differences observed between BS communities. Rhizosphere is a micro-environment strongly influenced by secondary metabolites such as alkaloids secreted by the plant root system, which have a significant influence on soil properties and microbial communities (Garbeva *et al.*, 2008; Haichar *et al.*, 2014; Bais *et al.*, 2006) and whose biosynthesis can be triggered by biotic and abiotic elicitors (Badri and Vivanco 2009; Ribera and Zuñiga, 2012).

Although a high diversity of alkaloids for *E. californica* has been described (Hagel and Facchini, 2013), altitude has not been reported as an elicitor in exudate production by this exotic species as in other plants whose exudate production was sensitive

to factors related to the altitude, i.e. UV radiation (Rinnan *et al.*, 2006), temperature (Kuzyakov *et al.*, 2007), or soil moisture (Reid, 1974).

However, differences in some plant features, mainly floral and vegetative characters, have been observed among *E. californica* populations located at different altitudes in the same sampling area of our study (Peña-Gómez and Bustamante, 2012). Some differences of these phenotypic traits related with a decrease in reproductive effort (biomass/flower size and flower number) would suggest that populations at higher altitude could have limitations in obtaining resources (Hiraga and Sakai, 2007) and this stress condition could indirectly affect rhizosphere microbial assemblages.

In general, our results suggest that metabolic diversity of the bulk soil communities is more sensitive to altitude than genetic diversity, but the plant is able to modulate this effect, probably as a consequence of altitude-related environmental factors that affect the physiology of the plant. In this sense, rhizosphere could be a relevant factor to moderate the indirect effect of altitude on soil microbial communities through edaphic factors such as soil moisture or pH which cannot be considered *per se* as altitude-dependent environmental factors (Körner, 2007).

Considering that plant-soil microorganisms interaction plays a relevant role in colonization, adaptation and propagation of plant species and there is a growing interest in identifying the factors that determine the success of invasive plants; the impact of exotic plants on the structure of soil microbial communities is a topic of current interest. Nevertheless, specific effects produced by altitude on soil microorganisms through factors such as radiation or atmospheric pressure should be examined in further studies.

## 5. Conclusions

Altitude modifies genetic and metabolic diversity patterns of soil microbial communities, although environmental factors such as pH and soil moisture, which appeared affecting microbial communities, would be mainly related with a rhizosphere effect. Therefore, *E. californica* would act as an ecological filter decreasing the altitude effect observed in the case of the bulk soil.

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