Soil Biology & Biochemistry 103 (2016) 39–45

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Environmental conditions shape soil bacterial community structure in a fragmented landscape

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ARTICLE INFO

Article history:
Received 15 March 2016
Received in revised form
4 August 2016
Accepted 6 August 2016
Available online 18 August 2016

Keywords:
Biogeography
Genetic and metabolic structure
Microbial community
Redundancy analysis
Relict forests
Variance partitioning

ABSTRACT

Several biogeographical hypotheses have been proposed to explain microbial distribution, but there is ongoing debate about the magnitude of the contribution of niche–based processes and historical contingencies in determining patterns of microbial structure. In this context, currently fragmented relict forests of olivillo (*Aextoxicon punctatum* Ruiz et. Pav.), which belonged to a continuous community along the coast of Chile during the Pleistocene, and their surrounding scrublands are ideally–suited for testing these hypotheses, since they remain as patches located at the northern tip of the distribution of the relicts. In each study site, edaphic and geographic variables were determined, and the bacterial structures were evaluated at the genetic and metabolic levels through fingerprint approaches along with multivariate analytical methods including redundancy (RDA) and variance partitioning (VPA) analyses. Forests possessed lower pH, and higher contents of moisture and organic matter. In addition, bacterial communities from both habitats differed, whereas the bacterial communities of the forests in different regions were very similar to each other. Our conclusion is that current abiotic soil factors, but not past events due to the historical connection of the forests, account for the variance in the structure of these soil bacterial communities.

1. Introduction

Processes underlying the generation and maintenance of microbial diversity have been widely–studied during recent decades (Fierer and Lennon, 2011). Currently, it is known that patterns in microbial biogeography exist; even so, the processes involved in shaping such arrangements are poorly understood (Hanson et al., 2012). However, unraveling them is critical for furthering our understanding of the factors that contribute to soil microbial diversity (Monroy et al., 2012). One hypothesis that has been put forward is that a structurally more complex and heterogeneous habitat is capable of sustaining a greater number of species (Bowman and McCuaig, 2003), demonstrating that the sorting of species by contemporary environmental conditions is important for shaping the community composition across space (Andersson et al., 2014). However, it has also been proposed that the distribution of microorganisms responds to historical events as a consequence of their limited dispersion and colonization capabilities (Fierer, 2008). The soil matrix thus acts as a physical barrier which limits passive dispersal of soil organisms and enhances the separation between communities (Vos and Velicer, 2008). In this context, it has been documented that spatial–distance effects related with past evolutionary events and adaption to past environmental conditions, among others, reflect the importance of historical processes (reviewed by Hanson et al., 2012). Furthermore, other researchers have proposed that both spatial heterogeneity and dispersion limitations explain the patterns of geographic diversity of the microbiota, suggesting that both historical and contemporary environmental factors are responsible for current bacterial distribution (reviewed by Hanson et al., 2012).

Consequently, there is substantial ongoing debate about the relative contribution of niche–based processes and historical contingencies in determining patterns of microbial structure. Interestingly, in Chile the existence of historically connected but currently fragmented olivillo (*Aextoxicon punctatum* Ruiz et. Pav.) forests that maintain today similar vegetational and climatic characteristics, allow the structure of their bacterial communities to be analyzed, and the various biogeographical hypotheses to be examined.

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http://dx.doi.org/10.1016/j.soilbio.2016.08.004
0038-0717/

The differences produced naturally in these fragmented areas of forest and the surrounding scrublands, with contrasting climate and soil properties as well as unique floral compositions, could create conditions that impact directly on soil microbiota (Reyes et al., 2011). The current communities of fragmented forests of olivillo belong to a community which continuously extended along the coast of central Chile (30°S – 42°S) during the last ice age of the Pleistocene (2.6 million years ago) (Villagrán et al., 2004). Discontinuous relict forests of northern and southern Chile currently maintain very similar climatic features and vegetation. In the Chilean semiarid region (Fray Jorge National Park, 30°S; and Santa Inés Hill, 32°S), these ecosystems are generally characterized by having hygrophilous forest areas present on top of the hills of the Cordillera de la Costa, with high relative humidity and low thermal oscillation, whilst the surrounding matrices have xerophytic scrubland with less influence of fog, greater diurnal temperature ranges and lower relative humidity (Pérez, 1994; Francois, 2004).

Therefore, our main aim is to understand the balance between long–term historical legacies versus more recent climate and vegetation patterns in structuring the assembly of bacterial communities. Considering the conditions found in the aforementioned sites, as a case study, we examined the bacterial communities in the soil of relict inner olivillo forests and the adjacent scrublands (habitats) in both Fray Jorge National Park and Santa Inés Hill (regions), locations separated by 200 km, which correspond to soils that have retained unique features over the years.

2. Materials and methods

2.1. Sampling sites and experimental design

In August 2013, soil samples from relict inner olivillo forests and from the surrounding scrublands in which they are immersed, were collected in Fray Jorge National Park and Santa Inés Hill (Table 1). Each sampling site is represented by the corresponding initials: FJF, Fray Jorge forest; FJS, Fray Jorge scrubland; SIF, Santa Inés forest; SIS, Santa Inés scrubland. In each of the four sites, 3 plots of 10 m × 10 m, separated by 20–25 m were defined. In each plot, 10 simple soil samples were collected from the top layer (0–10 cm) with 6 cm diameter corers. Subsequently, the 10 samples were sieved through a 2–mm mesh and pooled to form one composite soil sample per plot, thus obtaining 3 composite soil samples per site which were considered biological replicates. Samples were stored at 4 °C for bacterial metabolic profiling and edaphic physicochemical analyses, and frozen at −20 °C for bacterial genetic profiling.

2.2. Soil analyses

For each composite soil sample, we measured the soil parameters that are normally described as the most influential in determining soil bacterial community diversity (Lauber et al., 2008; Docherty et al., 2015; Cao et al., 2016): pH, moisture content (MC), organic matter (OM), nitrogen from ammonium ($[N-NH_4^+]$) and nitrate ($[N-NO_3^-]$) content. Briefly, pH was measured using potentiometry; MC and OM were calculated gravimetrically before and after desiccation and calcination, respectively; $[N-NH_4^+]$ was measured using an ion selective electrode and $[N-NO_3^-]$ was determined by colorimetry (Sadzawka et al., 2006).

2.3. DNA extractions and PCR amplification

From each composite sample, 0.25 g of soil were extracted using the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, 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). The primers used for amplification of the bacterial 16S rRNA gene were fD1 labeled with 6–carboxyfluorescein (6–FAM) at the 5′ end, and rP2 (Weisburg et al., 1991). All the amplifications were performed according to the cited literature recommendations, using the GoTaq® Green Master Mix (GoTaq® DNA polymerase in 1× Green GoTaq® Reaction Buffer [pH 8.5], 200 μM of each dNTP and 1.5 mM $MgCl_2$) (Promega, WI, USA) in a Maxygene thermocycler (Axygen, CA, USA). The quality of amplicons was determined by electrophoresis in 1.2% (w/v) agarose gels in 1× TAE buffer stained with GelRed™. PCR products were then purified using the Wizard SV PCR Clean–Up System (Promega, WI, USA) and spectrophotometrically quantified.

2.4. Terminal restriction fragment length polymorphism (TRFLP)

TRFLPs were used to evaluate the genetic structure of microbial communities in each composite soil sample. The amplicons from PCR products of the 16S rRNA marker were hydrolyzed independently with the restriction enzymes *Hae*III and *Alu*I (Fermentas, NY, USA), according to the manufacturer's specifications, and then digestion fragments were purified by ethanol precipitation. Terminal restriction fragments (TRFs) were separated on an automated Genetic Analyzer ABI3730XL (Applied Biosystems; Macrogen Inc., Seoul, S. Korea). The length of the fluorescently labeled TRFs was determined by comparison with the GeneScan™1200 LIZ® size–standard using the GeneMapper 3.7 software (Applied Biosystems, CA, USA). Patterns from different samples were normalized to identical total fluorescence units by an iterative standardization procedure (Dunbar et al., 2001). The relative abundance of TRFs, as percentage, was determined by calculating the ratio between the height of a given peak and the normalized total peak height of each sample (Yeager et al., 2004). Peaks with a relative abundance of less than 2% were discarded. The communities were characterized by

Table 1
Explanatory spatial (geographic) and environmental (edaphic) variables of each site (± standard error). The same lowercase letters in a row represent no significant differences ($p < 0.05$).

Explanatory variables		Fray Jorge		Santa Inés	
		Forest	Scrubland	Forest	Scrubland
		FJF	FJS	SIF	SIS
Geographic data	Latitude	30°40′14.5″S	30°40′08.4″S	32°09′47.5″S	32°09′41.8″S
	Longitude	71°40′36.8″W	71°40′22.8″W	71°29′41.6″W	71°29′44.9″W
Edaphic data	pH	5.2 ± 0,1 (a)	6.0 ± 0.1 (b)	5.2 ± 0.1 (a)	5.6 ± 0.1 (c)
	Moisture Content (g H_2O/100g_{dw})	39.2 ± 1.6 (a)	3.8 ± 0.5 (b)	61.4 ± 12.5 (c)	2.9 ± 0.9 (b)
	Organic Matter (g/100g_{dw})	24.8 ± 1.7 (a)	7.5 ± 1.0 (b)	23.5 ± 1.4 (a)	5.3 ± 0.9 (b)
	Nitrate Content (μg N/g_{dw})	33.1 ± 2.7 (ac)	43.8 ± 1.9 (a)	59.3 ± 6.2 (b)	21.9 ± 0.8 (c)
	Ammonium Content (μg N/g_{dw})	0.6 ± 0.1 (ac)	1.2 ± 0.1 (b)	0.9 ± 0.1 (a)	0.4 ± 0.1 (c)

dw: dry weight.

the number of peaks (richness) and the peak heights (abundance) of each electropherogram. The estimation of the diversity of the bacterial community at the genetic level was performed using the richness (S), evenness (J) and Shannon diversity (H′) indices (Shannon and Weaver, 1949) using the PAST software (http://nhm2.uio.no/norlex/past/download.html).

2.5. Community level physiological profile (CLPP)

CLPPs were used to evaluate the metabolic structure of the microbial communities in each composite soil sample using the BIOLOG Eco–Plate™ (BIOLOG Inc., CA, USA), as described by Garland (1997). A composite soil sample suspension (1:10 w/v) was prepared with PBS buffer (137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4) and shaken at 150 rpm during 1 h. Each plate was inoculated with 100 μL per well of this suspension and incubated at 25 °C. The optical density at 590 nm was read every 24 h for a week using an automated plate reader (Epoch, Biotek, VT, USA). Absorbance values for each of the 31 substrates were corrected by subtraction of the blank control value and time 0 (Insam and Goberna, 2004). Negative values were set to zero. To minimize the effects of different inoculum densities, data were normalized by dividing the values obtained by their respective average well color development (AWCD) values (Garland and Mills, 1991). The AWCD versus time curves were fitted to a modified Gompertz equation (Zwietering et al., 1990) using ORIGIN 8.0 to obtain kinetic parameters (λ: latency, μ_m: maximum rate of color development, and A: maximum absorbance) in order to determine which data correspond to the exponential phase of each curve. These data were used to conduct comparative analysis of metabolic profiles at the community level and to calculate the diversity indices of the different samples.

2.6. Statistical analyses

Statistically–significant differences of edaphic variables pH, $[N–NO_3^-]$ and $[N–NH_4^+]$ (N=9 per sampling site) were assessed first with the Kolmogorov–Smirnov test for normality (Massey, 1951) and then analyzed by one–way ANOVA and the Bonferroni-Dunn post hoc test (Dunn, 1961) (GraphPad Software Inc).

In the case of edaphic variables OM and MC, diversity indices (H′, J and S) and kinetic parameters (λ, μ_m and A) (N=3 per sampling site), statistically–significant differences were determined by non–overlapping confidence intervals of measurements at each site after 10,000 random re–samplings conducted using the program R v. 2.2–1 and the *boot* package (Canty and Ripley, 2015).

2.7. Multivariate analyses

The influence of environmental and geographic factors was assessed by segregating the total microbial community structure variation (Peres–Neto et al., 2006) using a redundancy analysis (RDA) (Van Den Wollenberg, 1977) and a variance partitioning analysis (VPA) (Borcard et al., 1992).

The response dataset consisted of the profiles of TRFLP (i.e. the number of peaks (richness) and the peak heights (abundance) for each community) and CLPP (i.e. number of used carbon sources (richness) and the associated absorbance (abundance) for each community). A similarity profile test (SIMPROF) (Clarke et al., 2008) was used to determine whether multivariate structure is present in the response dataset, based on a Bray–Curtis similarity matrix and groupings examined at the 5% significance level (<0.05) using a maximum of 1000 random permutations of the raw data employing the *clutsig* package(Whitaker and Christman, 2010) with R v. 2.2–1 software.

On the other hand, the explanatory variables included in these analyses were the edaphic parameters (pH, OM, MC, $[N–NH_4^+]$ and $[N–NO_3^-]$) and the geographic coordinates. The latter were transformed to UTM coordinates (x: latitude, and y: longitude) (http://www.latlong.net/lat–long–utm.html) to calculate second order variables ($x + y + x^2 + xy + y^2$) with the aim of modeling nonlinear simple spatial patterns (Legendre and Legendre, 1998).

Firstly, matrix variables were analyzed to check the distribution of the data (linear or normal) through detrended correspondence analysis (DCA) (Hill and Gauch, 1980). When data were not adjusted to the linear model, a Hellinger transformation was applied (Borcard et al., 2011). Secondly, a RDA with forward selection (Peres–Neto et al., 2006) using the stopping rules introduced by Blanchet et al. (2008) was applied, using the *packfor* package (Dray et al., 2013) with R v. 2.2–1 software, to select a subset of soil and geographic variables that parsimoniously explain the variance of the community. Briefly, since the global test (permutation test under reduced model) was significant, the stepwise selection process was performed, adding variables only if (i) it added a significant portion of explained variance, and (ii) the R^2_{adj} of the model did not exceed the R^2_{adj} of the overall model. In addition, during RDA calculations, if explanatory variables were complete linear combinations of previous constraints, they were removed from the estimation. Lastly, a type II scaling-correlation plot (response variable focused) was obtained. In order to determine the significance of the correlations between axes and explanatory variables, a Monte Carlo permutation test was performed on the basis of 999 random permutations, under a direct model.

Finally, with a VPA, the proportions of microbial community structure variation (R^2_{adj}) that were attributed to purely environmental variance [a], spatially structured environment variance [b], purely spatial variance [c] and residual variance [d] were estimated. All multivariate analysis and mentioned transformations, unless otherwise specified, were carried out using the *vegan* package (Oksanen et al., 2015) with R v. 2.2–1 software.

3. Results

3.1. Edaphic parameters

Soils of forest (FJF) and scrubland (FJS) in Fray Jorge National Park presented significant differences in pH, moisture content (MC), organic matter (OM) and ammonium content ($[N–NH_4^+]$), while differences in nitrate content ($[N–NO_3^-]$) were not observed. On the other hand, in the Santa Inés Hill, soils of forest (SIF) and scrubland (SIS) differed significantly in all soil parameters analyzed (Table 1).

The soil of the Santa Inés forest (SIF) had significantly higher MC and $[N–NO_3^-]$ content than the Fray Jorge forest (FJF); no differences were detected in the other parameters. Furthermore, the Fray Jorge scrubland (FJS) showed significantly higher values of pH, $[N–NO_3^-]$ and $[N–NH_4^+]$ contents than the Santa Inés scrubland (SIS).

Finally, in general, forest soils had significantly higher values of OM and MC compared with scrublands, and was more acidic. The $[N–NO_3^-]$ and $[N–NH_4^+]$ contents were variable between forests and scrublands.

3.2. Genetic structure of soil bacterial communities

In the analysis of restriction profiles of the 16S rRNA marker, 42 TRFs were obtained. Of these, 16 were generated with the *Alu*I enzyme and 26 using the *Hae*III enzyme. Most of the TRFs determined by this approach were present in all the analyzed communities, but with differences in abundance.

In the case of the *Alu*I enzyme, the TRF of 711 bp was only present in forests at both sites analyzed (FJF and SIF), whilst using the *Hae*III enzyme, the TRFs of 61 bp and 135 bp were absent in the Santa Inés forest (SIF). When the genetic structure of the soil bacterial community at each site was analyzed, in general no significant differences were found, except for the diversity (H′) index when comparing the Fray Jorge scrubland (FJS) and the Santa Inés forest (SIF), and for the evenness (J) index when comparing the Fray Jorge scrubland (FJS) with the forest and scrubland of Santa Inés (SIF and SIS, respectively) (Table 2).

3.3. Metabolic structure of soil bacterial communities

The metabolic profiles of soil bacterial communities were first analyzed by comparing kinetic parameters using CLPP. The lag phase (λ, in hours) of the scrublands (FJS: 46.0 ± 3.4, SIS: 49.7 ± 1.8) was significantly higher than in forests (FJF: 27.8 ± 1.1, SIF: 24.8 ± 1.3). On the other hand, the maximum rate of color development, i.e. the consumption rate of carbon sources (μ_m, $A_{590\ nm}$/h) was significantly higher in forests (FJF: 0.023 ± 1.4e^{-3}, SIF: 0.022 ± 2.8e^{-4}) than in scrublands (FJS: 0.015 ± 9.0e^{-4}, SIS: 0.008 ± 2.4e^{-3}), a finding that was also significant between scrublands. Finally, when analyzing the maximum color development, i.e. the maximum consumption of carbon sources (A, $A_{590\ nm}$), forests stand out with significantly higher values (FJF: 1.74 ± 0.15, SIF: 1.74 ± 0.12) than the surrounding scrublands (FJS: 1.14 ± 0.03, SIS: 0.69 ± 0.17). According to the curves, comparative analyses of the metabolic profiles were carried out at 72 h of incubation of Eco–Plates™, when the metabolism of soil bacterial communities of each sampling site was in the exponential phase.

In general, soil bacterial communities in the forests were metabolically more diverse (H′), with an ability to metabolize significantly more carbon sources (richness, S) and in a significantly more homogeneous intensity (evenness, J) compared with the communities present in the scrublands (Table 2).

3.4. Influence of explanatory variables on the structure of bacterial communities

In multivariate RDA, the structure of soil bacterial communities (TRFLP and CLPP) was correlated with edaphic and geographic variables of the different sampling sites (Fig. 1). From a total of 10 explanatory variables ($pH + MC + OM + [N{-}NO_3^-] + [N{-}NH_4^+] + x + y + x^2 + xy + y^2$), those that significantly explained the variance of the structure of bacterial communities were selected (Table 3).

In the structure ordination, when analyzing together the genetic and metabolic data, the global RDA test was significant ($p = 0.001$) and the first two axes accumulated 68.0% of the total variance (Fig. 1). Communities were mainly separated according to forests and scrublands, with the replicates in forests more similar to each

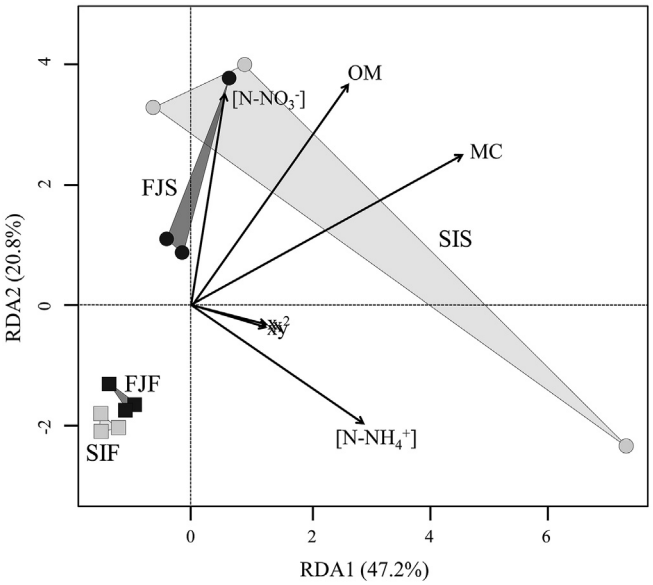

Fig. 1. Redundancy analysis (RDA) between the genetic and metabolic structure of bacterial communities and the edaphic and geographic explanatory variables. Sampling sites: Fray Jorge scrubland (FJS, black circles); Fray Jorge forest (FJF, black squares); Santa Inés scrubland (SIS, gray circles); Santa Inés forest (SIF, gray squares). Explanatory variables: (i) Edaphic data: pH, moisture content (MC), organic matter (OM), ammonium ($[N{-}NH_4^+]$) and nitrate ($[N{-}NO_3^-]$) contents. (ii) Geographic data: latitude (x), longitude (y) and second–order variables (xy, x^2 and y^2). Only the variables remaining after forward selection are shown.

Table 3
Redundancy coefficients for the first two axes (RDA1 and RDA2) and contribution to the variance of the explanatory variables (geographic and edaphic). Analysis based on genetic and metabolic structures using TRFLP and CLPP, respectively. Significant *p*–values are highlighted in bold.

Explanatory variables[a]	General structure			
	RDA1	RDA2	*p*	Variance
MC	0.8570	0.4732	**0.001**	39.5%
OM	0.5013	0.7053	**0.002**	18.6%
$[N{-}NO_3^-]$	0.1088	0.6694	**0.018**	10.8%
$[N{-}NH_4^+]$	0.5523	0.3783	0.051	7.1%
xy	0.2442	−0.0773	0.182	5.1%
x^2	0.2436	−0.0745	0.163	4.8%
x	0.2433	−0.0749	0.399	2.6%
Total variance (%)	47.2	20.8	–	88.5%
Test of significance	First canonical axis	**0.001**		
	All canonical axes	**0.001**		

[a] Explanatory variables: (i) Edaphic data: pH, moisture content (MC), organic matter (OM), ammonium ($[N{-}NH_4^+]$) and nitrate ($[N{-}NO_3^-]$) contents. (ii) Geographic data: latitude (x), longitude (y) and second–order variables (xy, x^2 and y^2). Only the variables remaining after forward selection are shown.

Table 2
Diversity indices (± standard error) calculated using genetic (TRFLP data) and metabolic (CLPP data) structures. The same lowercase letters in a row represent no significant differences ($p < 0.05$).

Diversity indices		Fray jorge		Santa inés	
		Forest	Scrubland	Forest	Scrubland
		FJF	FJS	SIF	SIS
Genetic structure	Richness (S)	38.3 ± 0.7 (a)	37.7 ± 1.2 (a)	38.0 ± 0.6 (a)	38.0 ± 1.0 (a)
	Diversity (H′)	3.32 ± 0.08 (ab)	3.30 ± 0.04 (a)	3.37 ± 0.01 (b)	3.38 ± 0.03 (ab)
	Evenness (J)	0.73 ± 0.04 (ab)	0.72 ± 0.01 (a)	0.76 ± 0.01 (b)	0.77 ± 0.01 (b)
Metabolic structure	Richness (S)	27.0 ± 0.7 (a)	19.0 ± 1.3 (b)	27.0 ± 0.7 (a)	13.0 ± 3.2 (b)
	Diversity (H′)	3.17 ± 0.03 (a)	2.69 ± 0.10 (b)	3.21 ± 0.03 (a)	2.26 ± 0.30 (b)
	Evenness (J)	0.87 ± 0.01 (a)	0.80 ± 0.03 (b)	0.90 ± 0.01 (c)	0.77 ± 0.01 (b)

other. Constraints accounted for 88.5% of the total variance, being moisture content (MC), organic matter (OM) and nitrate content [N–NO_3^-] the variables which accounted significantly to the variance (Table 3).

Accordingly, the SIMPROF test determined three significant groupings within the response dataset (TRFLP and CLPP). The structure profiles of the microbial communities of the forests differed significantly from those of the scrublands, with the SIS2 sample forming an independent group.

Finally, in order to quantify the variance of the structure of bacterial communities explained by edaphic (environmental) and geographical (spatial) factors, a VPA was carried out (Fig. 2). The variation of the metabolic and genetic structure of bacterial communities was explained in 69.4% by the explanatory variables included in the analysis ([a]+[b]+[c], $R^2_{adj} = 0.694$, $p = 0.002$). In particular, 51.9% of the variation was accounted for only by environmental variables ([a], $R^2_{adj} = 0.519$, $p = 0.009$) and 7.3% was explained only by spatial variables ([c], $R^2_{adj} = 0.073$, $p = 0.123$). Then, considering the variation accounted for by spatially structured soil variables [b], the environment explained in total 62.2% of the variance ([a]+[b], $R^2_{adj} = 0.622$, $p = 0.001$), while the spatial influence reached 17.6% ([b]+[c], $R^2_{adj} = 0.176$, $p = 0.059$). Therefore, most of the variation was explained by environmental variables; however, 30.5% of the variation of the structure of the communities was not accounted for by the explanatory variables included in the analysis ([d]).

4. Discussion

We studied the structure of bacterial communities through genetic and metabolic fingerprint–based approaches in order to determine the contribution of long–term historical legacies and more recent climate and vegetation patterns in shaping them. In particular, since fragmented forests, such as those in this work, reflect historical connections by maintaining similar current environmental conditions, the impact on soil microbial communities includes both the past and present conditions of the relict forests and the surrounding scrublands.

The results obtained in this work by the genetic and metabolic analysis of soil bacterial communities (TRFLP and CLPP) as well as soil parameters, showed patterns that clearly distinguish habitats. The structure of the communities in forests was more similar to each other than with scrublands. The relevance of including analyses at genetic and metabolic levels is that the functional diversity of soil microbial communities cannot be totally predicted based on the taxonomic structure (Green et al., 2008), probably because most soil organisms are metabolically inactive (Fierer and Lennon, 2011), many are functionally redundant (Allison and Martiny, 2008) and only a few taxa are essential for ecosystem functioning (Marzorati et al., 2008). In this regards, Orlando et al. (2007) suggest that differences in phylogenetic composition of bacterial communities do not exclude a high similarity of physiological capacities to metabolize the carbon sources available on Eco–Plates™, mainly because certain microbial taxa are able to modify their metabolism as an adaptive strategy in fluctuating environments (Fierer and Lennon, 2011). Bacterial communities from all the sampling sites are potentially active and each one has specific characteristics; those from both forests showed similar kinetics of metabolism of carbon sources, which were different from those observed in surrounding scrublands, having these in turn, unique characteristics that differentiate them from one other.

Although fingerprinting approaches have some caveats and only detect the most abundant taxa in a community, they are valuable tools to assess changes in microbial community diversity (van Dorst et al., 2014), including when performed in combination with similar analyses used in our study (e.g. Hovatter et al., 2011; Lekberg et al., 2011; López-García et al., 2013). In addition, it has been shown that molecular fingerprinting and pyrosequencing methods share a similar potential to monitor community structural changes, as they are capable of detecting spatial (van Dorst et al., 2014), temporal (Gillevet et al., 2009) and treatment (Cleary et al., 2012) shifts in communities. However, it cannot be ruled out that rare microbial taxa can exhibit biogeographic patterns which could contribute to the discrimination of environmental samples (Lynch and Neufeld, 2015). Therefore, by employing fingerprinting methods, we could compare relative changes in bacterial richness and abundance levels (i.e. community structure) across individual samples, even if the taxonomic identity of community members is unknown, as long as multivariate analyses (RDA and VPA) were conducted to detect differences in community structure (Blackwood et al., 2007).

The multivariate analysis showed that bacterial communities from the forests showed higher similarity between replicas, generating more compact discrete groups when compared to the replicas of the scrublands. This effect could be due to the historical closeness of the forests (Pérez, 1994; Francois, 2004; Villagrán et al., 2004) and to the constant supply of certain carbon sources within them, unlike the scrublands where microbial communities have had to diversify their metabolic capacities. More information is obtained by including the VPA, which confirms that the major variation in structure of bacterial communities is explained by environmental variables (62.2%). Therefore, soil bacterial communities from the fragmented olivillo forests and the surrounding scrublands in the Cordillera de la Costa (Chile) are not randomly distributed, but respond to biotic, abiotic, genetic and metabolic factors shaping their structures. In any case, it must be considered that a fraction of 30.5% of the variance of the diversity of the studied communities is not explained by the variables included in the analyses, which indicates that other soil, geographical, or even biological variables not included in our analysis could be playing a role in defining the communities in the studied soils.

The hypothesis that the distribution of communities is modulated by contemporary environmental and historical factors is

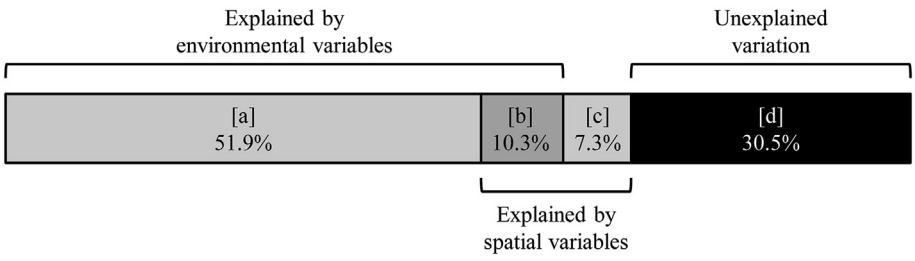

Fig. 2. Variance partitioning analysis (VPA) results illustrating the proportion of variation in the bacterial diversity data explained by forward-selected environmental variables, forward-selected spatial variables, and unexplained variation.

increasingly evaluated. Several studies have shown that free–living microorganisms vary in abundance, distribution and diversity, at several taxonomic and spatial scales, considering ranges from 2 m to 20,000 km (Martiny et al., 2006). For example, changes in the structure and composition in bacterial communities along an altitudinal gradient in the Atacama Desert (Chile) have been observed, where communities differ between the driest sites without plant coverage, and less arid sites with vegetation cover (Drees et al., 2006). On a larger scale, there is similar evidence comparing microbial diversity along a gradient of precipitation and vegetation cover. For example, Angel et al. (2010) observed that groups of bacteria and archaea were unique to each climate region in Israel (arid, semi–arid and Mediterranean), and on a similar scale, Burns et al. (2015) assessed the hierarchy of drivers of the soil bacterial community structure in the wine grape vineyards in the Napa Valley (California), where location and edaphic variables were the strongest explanatory factors. In view of this, some authors have proposed that microbial groups are correlated with spatial heterogeneity, suggesting that there is an environmental filter (Kraft et al., 2015; de Armas-Ricard et al., 2016). In addition, these groups would also be conditioned by the historical characteristics of the study sites, which would have an important role in determining patterns of microbial species richness (Fierer and Lennon, 2011). In fact, recently it has been reported that in arctic soils, historical events are the major drivers of microbial community distribution (Shi et al., 2015). On the other hand, Monroy et al. (2012) extend the drivers of the soil bacterial community structure, and suggest that factors other than soil chemistry, plant community composition and site history may play significant roles in structuring communities of soil bacteria. This shared influence between current and historical conditions was also reflected in a meta–collection of 54 studies where evidence of the effects of contemporary and historical selection processes was determined. Most of the studies (92.6%) had a significant correlation between the microbial composition and at least one environmental or habitat parameter, which demonstrates that the selection imposed by the prominent contemporary setting has a role in the formation of microbial biogeographic patterns (Hanson et al., 2012). Consistent with the studies reviewed by Hanson et al. (2012), contemporary selection had a higher effect, compared to historical processes, on the structure of the bacterial communities from the fragmented olivillo forests and the surrounding scrublands in the Cordillera de la Costa (Chile).

Consequently, although there are interesting ecological questions that can be addressed by examining biogeographic patterns within individual habitat types, the specific diversity patterns observed are highly dependent on the environment in question (Fierer and Lennon, 2011). However, the magnitude of the contribution of niche–based processes and historical contingencies should be calculated in order to achieve an overview of which factors contribute to the structuring of microbial communities.

5. Conclusions

Finding suitable sites for addressing the biogeographical hypotheses that have been put forward to explain soil microbial community structure is a significant challenge. Here, we show that soil bacterial communities present in the fragmented olivillo relict forests and the surrounding scrublands of two Chilean geographic regions separated by 200 km have unique characteristics in terms of structure. Much of this diversity is influenced by current soil conditions. Nevertheless, nearly a third of the bacterial diversity variance remains unexplained with the variables included in our analyses. Consequently, these bacterial communities are structured by environmental factors and there is no evidence for the influence of historical contingencies in structuring them.

Acknowledgements

We wish to thank Francisca Díaz, Aliosha Figueroa and Marlene Henríquez for assistance in sample collection. In addition, we wish to thank the editor's and reviewers' comments for significantly improving previous versions of this work. R. Almasia acknowledges a scholarship from the National Commission for Scientific and Technological Research (CONICYT, 2012) 22121214. Sample collection in the Fray Jorge National Park was authorized by the National System of Protected Wild Areas of the State (SNASPE) of the National Forestry Corporation (CONAF). The research was funded by the projects PAIFAC (Support Program for Research of the Faculty of Sciences, Universidad de Chile) led by J. Orlando, FONDECYT (National Fund for Scientific and Technological Development) 1140527 led by M. Handford and VIU (Valorization of University Research) 110031 led by R. Almasia.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.08.004.

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