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Comparison of soil microbial communities inhabiting vineyards and native sclerophyllous forests in central Chile

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Abstract

Natural ecosystems provide services to agriculture such as pest control, soil nutrients, and key microbial components. These services and others in turn provide essential elements that fuel biomass productivity. Responsible agricultural management and conservation of natural habitats can enhance these ecosystem services. Vineyards are currently driving land-use changes in many Mediterranean ecosystems. These land-use changes could have important effects on the supporting ecosystems services related to the soil properties and the microbial communities associated with forests and vineyard soils. Here, we explore soil bacterial and fungal communities present in sclerophyllous forests and organic vineyards from three different wine growing areas in central Chile. We employed terminal restriction fragment length polymorphisms (T-RFLP) to describe the soil microbial communities inhabiting native forests and vineyards in central Chile. We found that the bacterial community changed between the sampled growing areas; however, the fungal community did not differ. At the local scale, our findings show that fungal communities differed between habitats because fungi species might be more sensitive to land-use change compared to bacterial species, as bacterial communities did not change between forests and vineyards. We discuss these findings based on the sensitivity of microbial communities to soil properties and land-use change. Finally, we focus our conclusions on the importance of naturally derived ecosystem services to vineyards.

Introduction

Changes in land cover (i.e., the biological and physical structures or attributes of land) and land use (i.e., how people use the land) are among the most important drivers of global change (Vitouseek et al. 1997). Particularly, agricultural activities are responsible for the conversion and subsequent loss of native habitats through the change of land cover and land use. These changes can alter the biological properties and the function of natural habitats because habitat fragmentation strongly modifies the species richness and the biophysical condition (e.g., edge effects) of a system. In turn, a change in the functionality of natural habitats consequently can have significant effects on their provided ecosystem services such as climate regulation, pollination, and natural pest control of crop fields (Foley et al. 2005; Turner et al. 2007a,b; Stefan-Dewenter and Westphal 2008; Martínez et al. 2009). Therefore, conservation of natural ecosystems and appropriate agricultural management can provide and maintain numerous ecosystem services provided to agricultural habitats, and subsequently, this can maximize agricultural production and reduce negative impacts on agriculture (Daily 1997; Power 2010; Palm et al. 2014).

Agricultural and other human-induced perturbations are occurring at unparalleled rates in Mediterranean

biomes (Cincotta et al. 2000; Underwood et al. 2008), most notable is the conversion of forests to cultivable fields (Lauber et al. 2008). Mediterranean ecosystems, which include the Mediterranean Basin, the Cape Floristic Province in South Africa, a portion of South Western Australia, the California Floristic Province, and central Chile, contain almost 20% of vascular plant species in the world with high rates of endemism. At the same time, these areas are highly threatened due to land-use change. Therefore, they have been classified as biodiversity hotspots highlighting the importance of prioritizing these areas for global biological conservation (Cowling et al. 1996; Myers et al. 2000). The Mediterranean climate is also suitable for viticulture, which has historically thrived in these areas (Hannah et al. 2013; Viers et al. 2013). During the last decades, the wine industry has been an important player in the land-use change. Between 1988 and 2010, the land occupied by vineyards has increased by 70%, and this is only in New World Mediterranean zones (Viers et al. 2013). The same pattern has been described for vineyards in Chile, where vineyard cropland increased by 75,969 hectares during the period of 1995–2013 (Servicio Agrícola Ganadero 2013). Detailed information indicates that some specific regions have lost natural habitats through the direct conversion of forests to vineyards such as in the case of the Leyda valley. In this emergent growing area in central Chile, vine growth coverage increased from zero hectares in 1996 to 2662 hectares in 2010. During the same period, the native forest and scrubland coverage decreased by 8878 hectares (Zepeda-Paulo 2013). Habitat conversion to vineyards has caused negative effects on ecosystem functions such as increase in pest abundance (Altieri and Nicholls 2002), alteration in nearby aquatic habitats (Deitch et al. 2009), and decreases in bird diversity (Laiolo 2005). However, there is also evidence demonstrating that preserving and restoring native habitats near croplands can have positive effects on insects (Chacoff and Aizen 2006) and macrofauna biodiversity (Hilty and Merenlender 2004; Schmitt et al. 2008).

Changes in the intensity of land use or land cover as a consequence of agricultural practices have profound effects on the physical and chemical properties of soil (Jangid et al. 2008), which can have negative and irreversible consequences on soil biological communities and their functions (Brussaard et al. 1997; Kennedy et al. 2004; Wardle et al. 2004a; Bardgett 2005; Hartmann and Widmer 2006; Chau et al. 2011). Indeed, the increased tillage and fertilizer use related to farming intensification are associated with an increased role of the bacterial relative to fungal-based soil metabolism (Hendrix et al. 1986; Wardle et al. 2004a; Bardgett 2005). Thus, intense agricultural practices can lead to faster, leakier soil nutrient cycling and a greater loss of nutrients and carbon in water (Wardle et al. 2004a; van der Heijden et al. 2008). Microbial diversity is essential for agroecosystem functioning, and the conservation of this diversity has economic and ecological relevance (Gardi et al. 2009; Köhl et al. 2014). As such, research on the interaction between soil microbial communities and the wine industry opens very exciting venues considering that soil is an important component of the concept of *terroir*, which is a very important feature for winemakers (van Leeuwen et al. 2004; Gilbert et al. 2014). In addition, there is scarce information about the role played by microorganisms in the interplay between wine, environment, and ecosystem services. Conversely, there is abundant evidence confirming the important role played by soil microorganisms in erosion control, soil formation, nutrient cycling, and plant health, all of which may be considered important ecosystem services provided by native habitats to the agricultural industry (Stoate et al. 2001; Fernández-Calviño et al. 2010; García-Orenes et al. 2013). Consequently, the study of forest microbial communities and their relationship with vineyards is of paramount importance to understand the ecosystem services provided by soil microorganisms. A better understanding of this could allow us to confer an "economic value" to native habitats, thereby quantitatively promoting their conservation. This is of great concern particularly in the Mediterranean region of Chile, which is underrepresented in the Chilean protected area network (Armesto et al. 1998; Marquet et al. 2004; Tognelli et al. 2008). In this region, a large fraction of the area is dedicated to vineyards and, where most of the land that could be protected in the future, is privately owned (Fig. 1).

In this study, we employed terminal restriction fragment length polymorphisms (T-RFLP) to characterize and compare soil bacterial and fungal communities inhabiting sclerophyllous native forests and adjacent organic vineyards in central Chile. Diversity indices and community structure were compared between both habitats representing three different growing areas in central Chile. Because sclerophyllous forests are complex habitats with higher plant richness than vineyards, we expect that native habitats contain higher microbial richness and diversity than vineyards. We also expect that microclimate differences between growing areas have an important effect on microbial communities, inducing differences in diversity and community structures that should be detectable at geographical scales. T-RFLP is a culture-independent method used to characterize microbial community composition. This method has been shown to be effective when comparing diverse environments (Blackwood et al. 2003; Cregger et al. 2012). This method employs fluorescence-labeled primers to amplify taxonomically informative

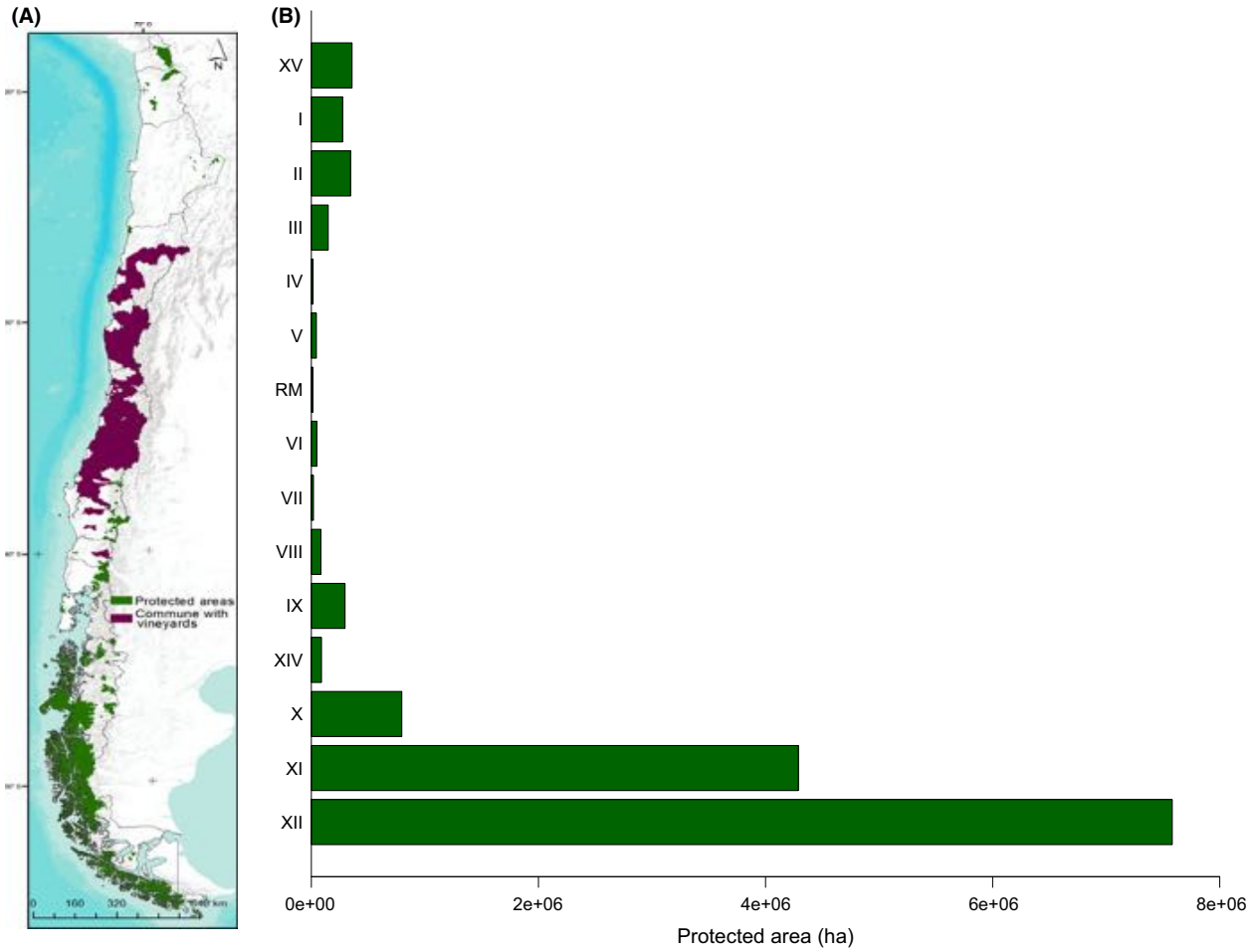

Figure 1. Map of Chile (A) showing vineyards (in purple) and protected areas (in green) and their respective total area in each administrative region (B), which are represented by Roman numerals and ordered from north to south. This plot shows that central Chile is the geographic region with the highest presence of vineyards and, at the same time, the region with few small wild protected areas.

genes (i.e., 16S rDNA for bacteria and ITS for fungi). The amplification products are then digested with restriction enzymes, and the size of the produced fragment can be used to determine certain taxonomic groups in an environmental sample (Tipayno et al. 2012). Despite the taxonomic resolution limitations of T-RFLPs, we chose this method because it (1) is sensitive to differences in environment; (2) is a low-cost molecular tool that can be easily implemented in any agrolaboratory; and (3) produces data with a short analysis pipeline compared to amplicon sequencing. Therefore, this study could also be useful to determine the feasibility of using a low-cost molecular technique (i.e., T-RFLP) to recognize changes in soil microbial diversity. Changes in microbial diversity due to land conversion can modify ecological functions with important consequences on grape and wine production. It is thus important to determine whehter theses effects of land-use change can be buffered by promoting the conservation and restoration of native habitats.

Materials and Methods

Sampling

Our study includes three different vine-growing areas in central Chile: the Aconcagua valley (north), the San Antonio valley (center), and the Colchagua valley (south). In each growing area, we identified one organic vineyard adjacent to a sclerophyllous forest patch. From each vineyard, we randomly selected three different plots and from each plot, we collected five soil samples at a depth of 15 cm and at a distance of 5 cm from a *Vitis vinifera* plant. From the adjacent forest, we collected five soil samples using the same methodology described before. Each soil sample was collected during the morning, stored in a sterile bag, and placed in a cooler with ice packs. During the afternoon, soil samples were transported to the laboratory where they were was homogenized, sieved, and split into two fractions: one fraction was stored at −80°C

until DNA extraction, and the other fraction was used to determine soil pH and texture at the Facultad de Agronomía of the Universidad Católica de Chile. This was performed as follows. A sample of 3 g of air-dry soil was mixed with 50 mL of deionized water, and then, the electrodes of the pH meter were immersed into the soil suspension to measure the pH (Zagal and Sadzawka 2007). Soil texture was determined from 40 g of soil dried at 40°C, which was then mixed with 50 mL of deionized water plus 10 mL of hydrogen peroxide and heated at 90°C for 1 h. After 24 h, the supernatant was discarded and the relative density and temperature were measured in the remaining solution with a hydrometer and thermometer, respectively. The formula used to calculate the clay, silt, and sand contents in each soil sample were taken from Zagal and Sadzawka (2007). Detailed information for forest and vineyard features from each growing area is provided in Table 1.

DNA extraction, PCR protocol, and T-RFLP profiles

DNA was extracted from 0.25 g of each soil sample using the Power Soil DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA,USA) following the manufacturer's instructions. DNA quality was determined by electrophoresis using a 0.8% agarose gel and also by DNA quantification using a NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA).

To study the bacterial community structure, 16S ribosomal DNA was amplified using the forward primer 8F (5′-AGA GTT TGATCC TGG CTC AG- 3′), which was labeled with the 6-carboxyfluorescein (FAM) fluorophore at its 5′-end, and the reverse primer 1392R (5′- ACG GGCGGT GTG TAC-3′). PCR amplification was performed according to Lane et al. (1985) and was followed by amplicon digestion with the restriction enzyme *Msp-1* according to the manufacturer's instructions. In order to study the fungi community structure, the ITS region of the genomic DNA was amplified using the forward primer ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′), which was labeled with the FAM fluorophore at its 5′-end, and the reverse primer ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′). PCR amplification was performed according to Gardes and Bruns (1993) and was followed by amplicon digestion with the restriction enzyme *Hinf-1* according to the manufacturer's instructions. Finally, bacterial and fungal digested fragments were separated by capillary electrophoresis and the fragment lengths were calculated using the ROX-1000 internal standard as a reference. Electrophoresis was performed by (Macrogen, Inc., Seoul, Korea).

Table 1. Descriptive information of each sampled growing area: geographic location, mean ambient temperature, precipitation, soil pH, soil content, soil taxonomy, vine variety and planting year.

	Aconcagua valley (north)	Leyda valley (center)	Colchagua valley (south)
Latitude	32° 52′ S	33° 34′ S	34° 36′ S
Longitude	71° 7′ W	71° 22′ W	71° 7′ W
Altitude	307 m	216 m	268 m
Mean temperature	14.7°C	16.2°C	14.6°C
Precipitation	354 mm	457 mm	731 mm
pH forest soil[1]	7.87	6.86	6.34
pH vineyards soil[2]	8.1 ± 0.1	7.8 ± 0.5	7.5 ± 0.4
Forest soil content (sand, silt, and clay)	73% – 16% – 11%	67% – 22% – 11%	47% – 37% – 15%
Vineyard soil content (sand, silt and clay)	56% – 38% – 16%	61% – 26% – 13%	61% – 27% – 12%
Soil taxonomy	Alfisol	Alfisol	Alfisol
Vine variety	Cabernet Sauvignon	Sauvignon Blanc	Syrah
Planting year (± SD)	2002 ± 3	2006 ± 1	2001 ± 4

[1]pH in forests was determined from a single soil sample.
[2]pH in vineyards was determined in each plot and the mean (± standard deviation) is shown.

T-RFLP analysis

Each T-RFLP profile includes information about the height and the area of each peak in relation to a DNA fragment of a specific size (T-RFs). Profiles with fragments shorter than 50 bp were removed to avoid presence of "false" peaks (i.e., primer dimmers). In addition, the ROX-1000 internal standard does not provide length information for fragments longer than 946 bp; hence, fragments longer than 900 bp were removed. Further to this, T-RFLP profiles were processed according to the method described by Abdo et al. (2006) to bin, align, and remove background noise greater than three standard deviations. This was implemented using an R-script written by Ingo Fetzer (http://www.ufz.de/index.php?en=22174). Each T-RF area was standardized in relation to the total area calculated for the complete profile. Thus, a total of 53 T-RFLP profiles were obtained for bacterial communities and 75 profiles were obtained for fungal communities.

Statistical analyses

Diversity indices such as richness (S), the Shannon index (H′), and evenness (J) were estimated using the vegan package for R (Oksanen et al. 2013). Because the soil samples were obtained from different plots in each habitat

(vineyards and native forest) and growing area, we analyzed the diversity indices using a linear model with plots nested within habitat, and habitat nested within location. These analyses were performed using the lmerTest package for R (Kuznetsova et al. 2014) to test both the significance of the growing area (fixed effect) using a F-ratio test and the significance of the habitat and plot (random effects) using likelihood ratio tests. A permutational multivariate analysis of variance (PERMANOVA) was employed to test differences between communities in the statistical package Primer version 6 (Anderson et al. 2008). We carried out a PERMANOVA analysis employing Bray–Curtis distance matrices based on the square root-transformed abundance of each T-RF. The statistical significance of the growing area (fixed effect), habitat nested within location (random effect), and plot nested within habitat (random effect) were evaluated using 9999 permutations. Additionally, Bray–Curtis distance matrices were calculated with the *vegdist* function ("vegan" R-package). These matrices were employed to perform a nonmetric multidimensional scaling (NMDS) using the *metaMDS* function ("vegan" R-package) in order to visualize the community structure of each habitat within each growing area. The effect of pH on spatial structure of soil microbial communities was explored using the *envfit* function implemented in the vegan R-package. This function fits an environmental variable onto a given NMDS ordination, thereby maximizing the correlation between community structure and pH (Oksanen et al. 2013).

In order to assess differences in the contribution of specific operational taxonomic units (OTUs) to the diversity, we selected the 15 most abundant OTUs (equaled here to T-RFs) from the bacterial and fungal communities. Because PERMANOVA analysis only allowed us to detected a difference in bacterial community structure between growing areas and a difference in fungal community structure between habitats (see Results section), we compared the bacterial OTU relative abundances between growing areas and the fungal OUT relative abundances between habitat using univariate Kruskal–Wallis tests. Because post hoc tests following a Kruskal–Wallis test are not straightforward, Tukey pairwise comparisons were performed only when the ANOVA output did not differ from the Kruskal–Wallis output.

Results

Soil bacterial community

Using the presence and abundance of bacterial OTUs, we calculated richness, diversity (Shannon–Weaver index), and evenness for each soil sample collected from the sampled forests and vineyards in central Chile. Regarding the diversity indices, we did not find significant differences between the growing areas and habitats (Table 2). Conversely, PERMANOVA tests indicated that there were significant differences in the bacterial community structure of different growing areas ($P = 0.004$). The pairwise PERMANOVA comparisons showed that the bacterial community structure was significantly different between the north-center ($P = 0.013$), north-south ($P = 0.034$), and center-south growing areas ($P = 0.033$). Soil pH had a strong effect on the differentiation between bacterial communities from northern versus central and southern locations, and correlations of 0.80 with NMDS1 and 0.63 with NMDS2 were found ($P_{OVERALL} = 0.001$, Fig. 2A). Differences between growing areas were also confirmed comparing OTU abundances. For instance, OTUs 93 and 486 were significantly more abundant in the northern growing area, whereas OTUs 65 and 153 were significant

Table 2. Diversity indices (mean ± standard deviation) for soil bacterial communities inhabiting sclerophyllous forests and vineyards from three Mediterranean growing areas in central Chile. The table also shows the output of a linear-mixed model testing the significance of location (fixed effect) using a *F*-ratio test. Habitat is nested within location, and plot is nested within habitat (random effects), which were tested using likelihood ratio tests. ⊂ denotes "nested within".

	Location			Linear-mixed model		
	North	Center	South	Location	Habitat ⊂ Location	Plot ⊂ Habitat
Richness (S)						
Forest	4.8 ± 1.9	3.2 ± 1.8	3.0 ± 1.4	$F_{2,2.84} = 2.54$	$\chi_1 = 0$	$\chi_1 = 2.14$
Vineyard	6.1 ± 1.6	3.9 ± 1.6	3.9 ± 1.3	$P = 0.23$	$P = 1$	$P = 0.36$
Diversity (H′)						
Forest	1.32 ± 0.39	0.97 ± 0.61	0.99 ± 0.41	$F_{2,8.4} = 1.68$	$\chi_1 = 0$	$\chi_1 = 2.14$
Vineyard	1.59 ± 0.28	1.20 ± 0.42	1.17 ± 0.36	$P = 0.24$	$P = 1$	$P = 0.14$
Evenness (J)						
Forest	0.89 ± 0.05	0.96 ± 0.04	0.98 ± 0.02	$F_{2,10.48} = 2.67$	$\chi_1 = 0$	$\chi_1 = 0.84$
Vineyard	0.89 ± 0.05	0.94 ± 0.04	0.89 ± 0.01	$P = 0.12$	$P = 1$	$P = 0.36$

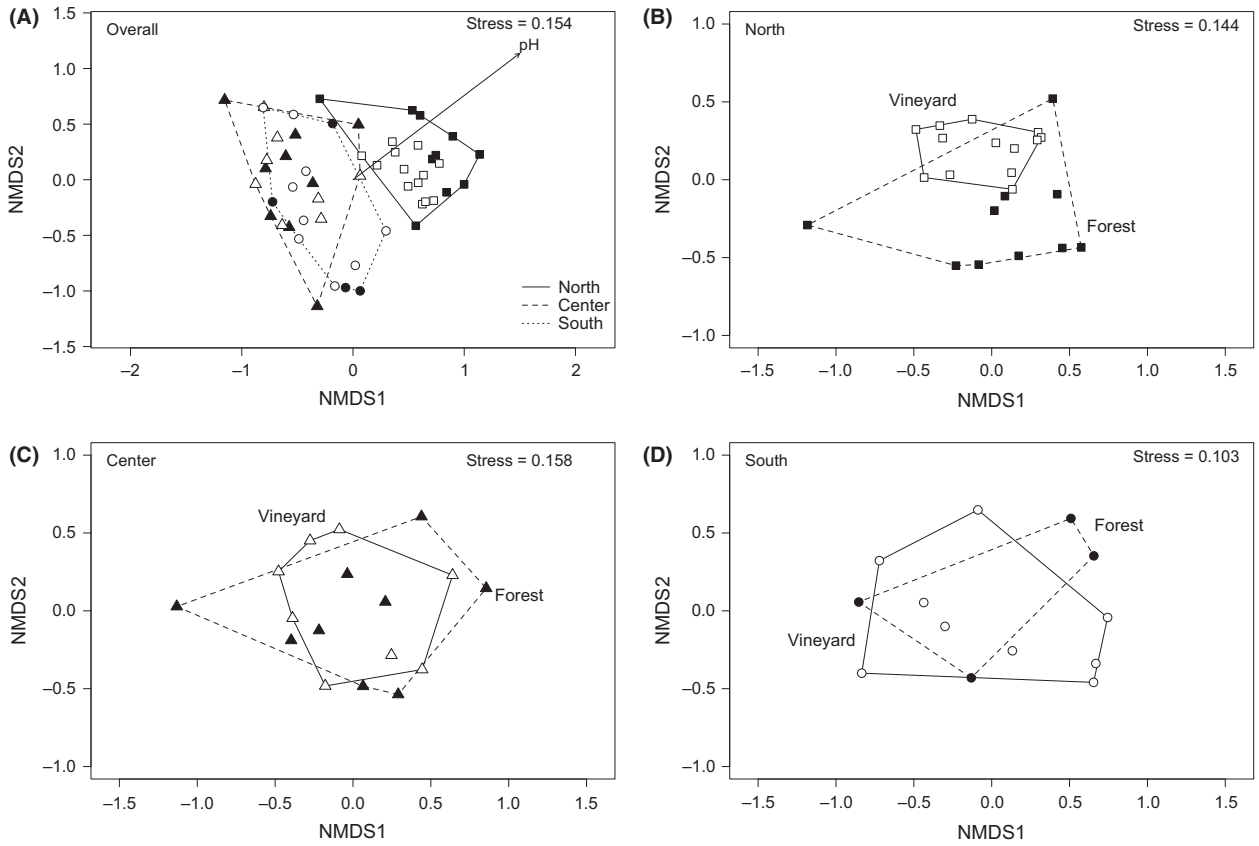

Figure 2. Non-metric multidimensional scaling plots of soil bacterial communities analyzed by T-RFLP show (A) geographical structuring of bacterial communities between northern (full squares: forest; open squares: vineyard), central (full triangles: forest; open triangles: vineyard), and southern (full circles: forest; open circles: vineyard) growing areas. Soil bacterial communities sampled from sclerophyllous forests (full symbols) and vineyards (open symbols) exhibit consistent overlap within the northern (B), central (C), and southern (D) growing areas. The arrows represent the correlation between pH and the community structure.

more abundant in the central and southern growing areas (Fig. 4A). Conversely, the bacterial communities were not different between the forests and vineyard habitats (PERMANOVA: $P = 0.47$). This is corroborated by the NMDS plots that show an overlap between bacterial communities from forests and vineyards in each growing area (Fig. 2B–D). We also found significant variation within habitats (PERMANOVA: $P = 0.008$).

Soil fungal communities

Using the presence and abundance of fungal OTUs, we calculated richness, diversity (Shannon–Weaver index), and evenness for each of the soil samples collected from the sampled forests and vineyards in central Chile. We did not find significant differences between the growing areas and habitats for the three diversity indices of the fungal communities (Table 3). PERMANOVA tests indicate that the fungal community structure did not differ between growing areas ($P = 0.21$). This is easily visualized in Figure 3A, where the fungal communities overlap for the three growing areas. Conversely, the PERMANOVA test indicates that the structure of the fungal communities were significantly different between forest and vineyard habitats ($P < 0.001$) and between plots within habitats ($P = 0.0001$). The NMDS plots show that the fungal communities inhabiting native forests have minimal overlap with those communities sampled from vineyards (Fig. 3B–D). Soil pH had a strong effect on the differentiation between fungal communities inhabiting in forest and vineyard soils. There was a strong correlation between soil pH and NMDS2 in the northern ($r = 0.92$, $P = 0.001$; Fig. 3B) and central growing areas ($r = 0.96$, $P = 0.002$; Fig. 3C). In the case of the southern growing area, soil pH was highly correlated with NMDS2 ($r = 0.99$, $P = 0.001$; Fig. 3C). This coincided with the direction of the separation between the forest and vineyard communities. Univariate tests for the most common fungal OTUs indicated that OTUs 256, 261, and 353 were significantly more abundant in forest habitats, whereas OTUs 259, 264, and

Table 3. Diversity indices (mean ± standard deviation) for soil fungal communities inhabiting sclerophyllous forests and vineyards from three Mediterranean growing areas in central Chile. Table also shows the output of a linear-mixed model testing the significance of location (fixed effect) using a *F*-ratio test. Habitat is nested within location, and plot is nested within habitat (random effects), which were tested using likelihood-ratio tests. ⊂ denotes "nested within".

	Location			Linear-mixed model		
	North	Center	South	Location	Habitat ⊂ Location	Plot ⊂ Habitat
Richness (S)						
Forest	24.2 ± 3.4	26.8 ± 4.0	27.7 ± 5.3	$F_{2,3.01} = 0.26$	$\chi_1 = 3.51$	$\chi_1 = 0.19$
Vineyard	20.9 ± 4.0	21.1 ± 3.5	22.6 ± 3.8	$P = 0.78$	$P = 0.06$	$P = 0.66$
Diversity (H′)						
Forest	2.58 ± 0.22	2.80 ± 0.43	2.97 ± 0.22	$F_{2,2.95} = 0.75$	$\chi_1 = 1.73$	$\chi_1 = 1.57$
Vineyard	2.16 ± 0.37	2.42 ± 0.25	2.49 ± 0.52	$P = 0.55$	$P = 0.19$	$P = 0.21$
Evenness (J)						
Forest	0.81 ± 0.05	0.85 ± 0.10	0.90 ± 0.02	$F_{2,2.85} = 1.13$	$\chi_1 = 0.42$	$\chi_1 = 2.53$
Vineyard	0.71 ± 0.09	0.80 ± 0.05	0.80 ± 0.11	$P = 0.43$	$P = 0.52$	$P = 0.11$

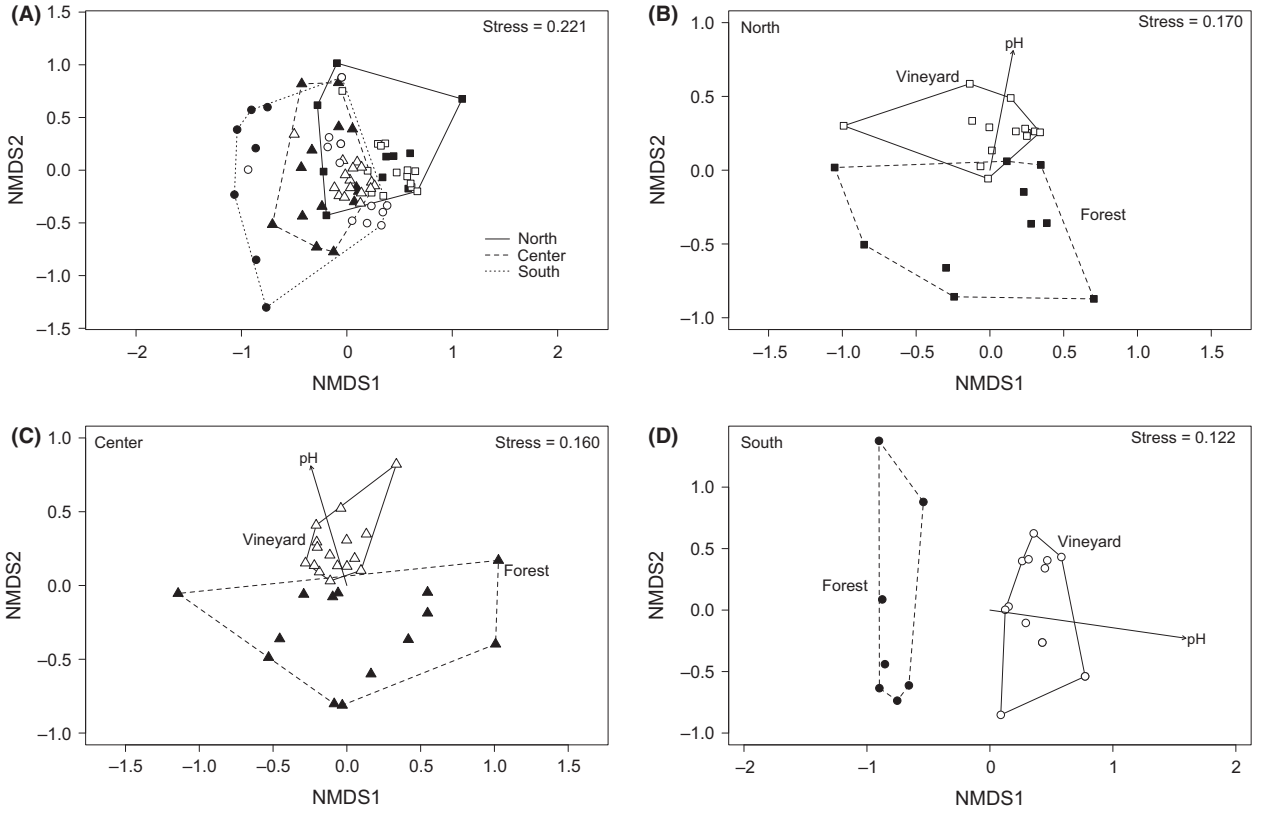

Figure 3. Nonmetric multidimensional scaling plots of soil fungal communities analyzed by T-RFLP show (A) little geographical structuring of habitat-pooled fungal communities between northern (full squares: forest; open squares: vineyard), central (full triangles: forest; open triangles: vineyard), and southern (full circles: forest; open circles: vineyard) growing areas, whereas soil fungal communities sampled from sclerophyllous forests (full symbols) and vineyards (open symbols) exhibit no overlap within the northern (B), central (C,) and southern (D) growing areas. The arrows represent the correlation between pH and the community structure.

283 were significantly more abundant in vineyards (Fig. 4B). Also of note is that some fungal OTUs, including the second most dominant OUT 97, exhibit similar abundance in forest and vineyard soils.

Discussion

The concept of *terroir* includes climatic, edaphic, and management conditions where wine is produced (van

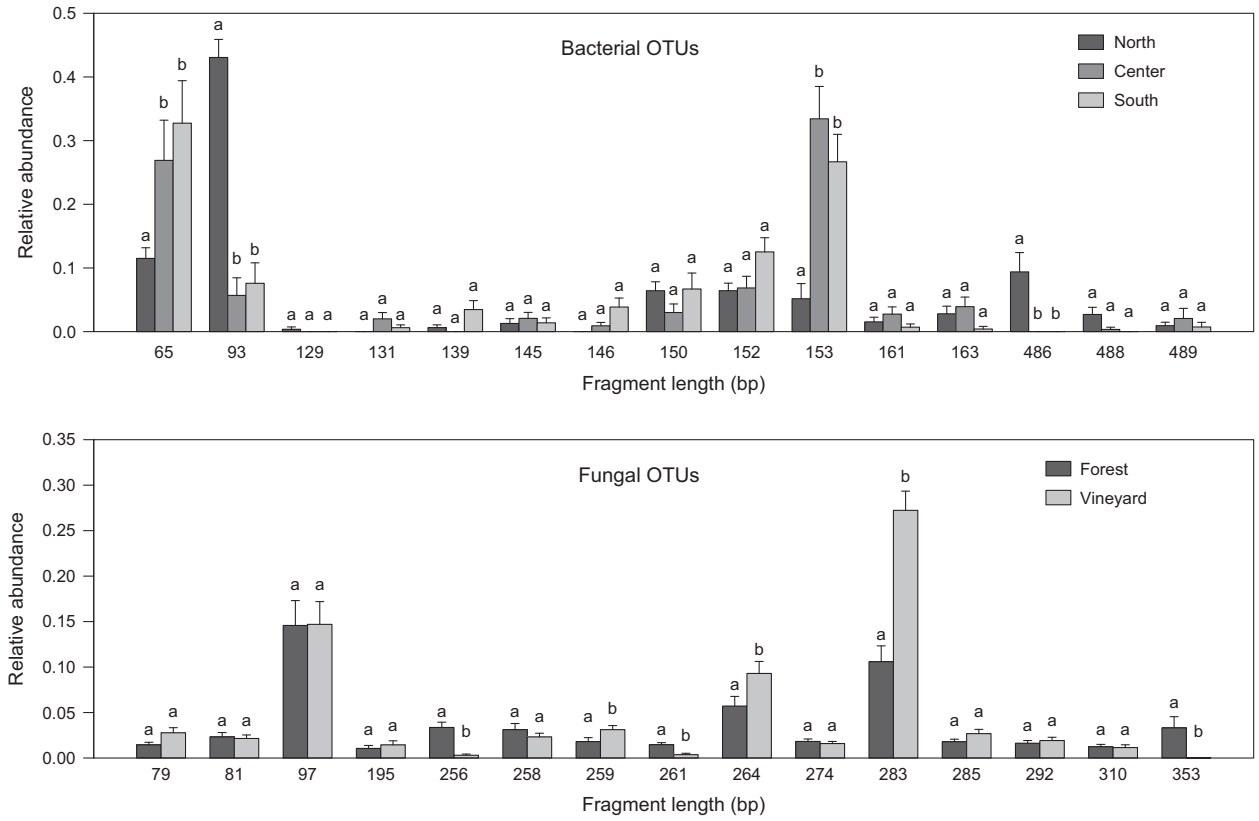

Figure 4. Means (± standard error) of relative abundance of dominant bacterial operational taxonomic units (OTUs) in different growing areas (top), and dominant fungal OTUs in different habitats (bottom). The first 15 most abundant fragments are shown (bp: base pairs). Letters indicate a posteriori significant differences between growing areas (top) and habitats (bottom).

Leeuwen et al. 2004). Plants acquire resources and nutrients to live directly from the soil. The availability of these resources and nutrients can be modified by the interaction between plants and microorganisms, and it is known that soil microorganisms control these functions (Brimecombe et al. 2007). Soil microbial communities are very sensitive to environmental variability, and they are also useful indicators of soil quality (Hartmann and Widmer 2006). Traditionally, studies have been focused on whether microbial community structure is affected by environmental variability, land use, or agricultural management, but few studies have investigated the community relationships between crop systems and native habitats (see Jangid et al. 2008; Upchurch et al. 2008). Therefore, the main motivation behind the present work was to make a preliminary assessment of the microbial communities inhabiting native forest and vineyards.

The findings of the present work reveal that bacterial community structure differs between growing areas but does not differ between sampled habitats. In general, microorganisms and their community structure are very sensitive to environmental conditions such as carbon and nitrogen content, soil texture, and pH among other edaphic features (Kennedy et al. 2004; Chau et al. 2011; Tipayno et al. 2012). Microbial communities also change across geographic gradients such as altitude, and these gradients can covary with multiple climatic and edaphic factors (Corneo et al. 2013). Furthermore, other studies have shown that microbial communities can differ across different stages of soil development (Wardle et al. 2004b). Despite the lack of detailed environmental data for each sampled growing area, the geographic structure of soil bacterial communities inhabiting forests and vineyards in central Chile could be related to geographic variation in temperature and/or pH. According to the literature, pH covaries significantly with latitude and evidence suggests that this edaphic parameter is a good predictor of microbial richness, diversity, and community composition at local and biogeographical scales (Fierer and Jackson 2006; Fernández-Calviño et al. 2010). The correlations generated in this study show that increasing pH is correlated with the spatial structuring of northern versus central and southern growing areas. As such, environmental pH might explain differences between bacterial communities

inhabiting different growing locations. On the other hand, rainfall and soil moisture are important environmental variables influencing soil microbial communities (Angel et al. 2010; Cregger et al. 2012). In our case, differential rainfall between localities may produce differential moisture in the soil. This, in turn, could have a profound effect on the soil bacterial communities. However, further studies are required to determine the direct effect of rainfall on the geographic pattern of bacterial communities in the sclerophyllous forests and vineyards. Indeed, seasonal variation of bacterial communities in the sclerophyllous matorral from central Chile has been attributed to differential precipitation between seasons (Farías et al. 2009). Furthermore, it is known that bacteria can be passively transported by soil water (Chapin et al. 2002) and thus, they can be transported by rain runoff from high-elevation forests to low-elevation vineyards, thereby homogenizing the bacterial communities between both habitats.

Conversely to bacteria, fungal communities did not differ between growing areas but were significantly different between habitats. Kasel et al. (2008) reported a weak effect of location on fungal communities from southern Australia but a very strong influence of land use on fungal community composition (but see Green et al. 2004). Variation in land use may involve different agricultural management strategies such as the use of pesticides and/or fungicides. Commonly, vineyards employ fungicides against plant diseases (e.g., powdery mildew, noble rot). These fungicides are directly applied to vines but may eventually accumulate in vineyard soils and modify fungal diversity. For instance, copper has been widely recognized as an efficient fungicide with high accumulation rate in cultivable soils exceeding the concentration in forest soils as a factor of ten (Viti et al. 2008). An increased copper concentration in vineyard soils has a negative effect on fungal biomass and activity (Probst et al. 2008; Viti et al. 2008) but a marginal effect on fungal community structure (Fernández-Calviño et al. 2010). On the other hand, sulfur is a very popular fungicide widely used in Chile to control powdery mildew of grapes particularly in organic vineyards that do not employ copper-based fungicides. As sulfur does not target a specific fungal process to prevent spores from germinating, both harmful and beneficial fungi might be killed with the addition of this fungicide (Köller 1999). Therefore, this could be responsible for the differences seen between fungal communities in native forests and vineyards. However, further studies would be required to assess the role of fungicides on the observed patterns. Multiple factors can be involved in the structuring of microbial communities; for instance, soil pH is an important factor affecting microbial structure at different spatial scales (Fierer and Jackson 2006; Fernández-Calviño et al. 2010). Specifically, Corneo et al. (2013) reported that pH influences the fungal community structure in vineyards across an altitudinal gradient. Additionally, fungal growth has been found to be negatively correlated with pH (Rousk et al. 2009). However, the most significant difference between sclerophyllous forests and vineyards is the vegetation type. Whereas vineyards are a woody-perennial monoculture, the adjacent sclerophyllous forests have high biodiversity and have among the highest rates of endemism in the world (Cowling et al. 1996). These forests are mainly composed of peumo (*Cryptocarya alba*), boldo (*Peumus boldus*), quillay (*Quillaja saponaria*), litre (*Lithrea caustica*), and espino (*Acacia caven*), among other tree and shrub species. Plant diversity has an important effect on soil microbial communities mainly because the diversity of litter composition and mycorrhizal associations may facilitate or constrain fungal establishment (Kasel et al. 2008; McGuire et al. 2012).

Sustainable agricultural management and conservation of natural habitats can affect the soil microbial communities and, consequently, influence ecosystem functioning (Bevivino et al. 2014; Köhl et al. 2014). In the case of vineyards, similar bacterial communities between habitats imply that native forests may support some ecosystem functions or bacterial communities that are more resilient to land-use change than are fungal communities. In several crop systems, the presence of adjacent native forests delivers important ecosystems services such as high pollinator diversity (Chacoff and Aizen 2006), and even it has been shown that adjacent forests increase yields in coffee crops (Ricketts et al. 2004; Brosi et al. 2008). Indeed, in these crop systems where the ecological function can be easily translated into a final ecosystem service, conservation strategies developed with the agroindustry are common (Wong et al. 2015). Although the wine industry does not rely on ecosystem services such as pollination, the fundamental concept of terroir opens an interesting avenue in order to explore the extent to which microbial communities are characteristic of particular growing areas and therefore are essential components that the wine industry should seek to preserve. Indeed, recent work by Bokulich et al. (2014) found that grape-associated microbial diversity depends on regional, varietal, and climatic factors. This supports the idea that microbial terroir could be determining some wine properties. In this regard, information provided by T-RFLPs can be a useful and low-cost approach to determine the biological properties of native habitats. This information can be used to help grape growers to determine the effect of agricultural management, and also, it can be used to recognize the ecological functions provided by natural ecosystems. For instance, fungicide application can be an agricultural practice that influences the abundance and diversity of indigenous yeasts associated with grapevines. These yeasts,

in turn, are very important during spontaneous fermentation processes (Barata et al. 2012). The obvious next step is to consider the use of metagenomic tools to clearly determine the taxonomic and functional diversity of the soil microbiota and to relate this diversity to soil ecological functions. Finally, once generated, this information can be linked to specific ecosystem services in agroecosystems.

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Conflict of Interest

None declared.

References

Abdo, Z., U. M. E. Schüette, S. J. Bent, C. J. Williams, L. J. Forney, and P. Joyce. 2006. Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. Environ. Microbiol. 8:929–938.

Altieri, M. A., and C. J. Nicholls. 2002. The simplification of traditional vineyard based agroforests in northwestern Portugal: some ecological implications. Agroforest. Syst. 56:185–191.

Anderson, M. J., R. N. Gorley, and K. R. Clarke. 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth, UK.

Angel, R., M. I. M. Soares, E. D. Ungar, and O. Gillor. 2010. Biogeography of soil archea and bacterial along a steep precipitation gradient. ISME J. 4:553–563.

Armesto, J. J., R. Rozzi, C. Smith-Ramírez, and M. T. K. Arroyo. 1998. Conservation targets in South American temperate forests. Science 282:1271–1272.

Barata, A., M. Malfeito-Ferreira, and V. Loureiro. 2012. The microbial ecology of wine grape berries. Int. J. Food Mircobiol. 153:243–259.

Bardgett, R. D. 2005. The biology of soil: a community and ecosystem approach. Oxford Univ. Press, Oxford, U.K..

Bevivino, A., P. Paganini, G. Bacci, A. Florio, M. S. Pellicer, M. C. Papaleo, et al. 2014. Soil bacterial community response to differences in agricultural management along with seasonal changes in a Mediterranean region. PLoS ONE 9:e105515.

Blackwood, C. B., T. Marsh, S. H. Kim, and E. A. Paul. 2003. Terminal restriction fragment length polymorphism data analysis for quantitative comparison of microbial communities. Appl. Environ. Microbiol. 69:926–932.

Bokulich, N. A., J. H. Thorngate, P. M. Richardson, and D. A. Mills. 2014. Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. Proc. Natl Acad. Sci. USA 111:E139–E148.

Brimecombe, M. J., F. A. A. M. De Leij, and J. M. Lynch. 2007. Rhizodeposition and microbial populations. Pp. 73–110 *in* R. Pinton, Z. Varanini and P. Nannipieri, eds. The rhizosphere: biochemistry and organic substances at the soil-plant interface, 2nd ed. CRC Press, Boca Raton, FL.

Brosi, B. J., P. R. Armsworth, and G. C. Daily. 2008. Optimal design of agricultural landscapes for pollination services. Conserv. Lett. 1:27–36.

Brussaard, L., V. M. Behan-Pelletier, D. E. Bignell, V. K. Brown, W. Didden, P. Folgarait, et al. 1997. Biodiversity and ecosystem functioning in soil. Ambio 26:563–570.

Chacoff, N. P., and M. A. Aizen. 2006. Edge effects on flower-visiting insects in grapefruit plantations bordering premontane subtropical forest. J. Appl. Ecol. 43:18–27.

Chapin, F. S., P. A. Matson, and H. A. Mooney. 2002. Principles of terrestrial ecosystem ecology. Springer, New York.

Chau, J. F., A. C. Bagtzoglou, and M. R. Willig. 2011. The effect of soil texture on richness and diversity of bacterial communities. Environ. Forensics 12:333–341.

Cincotta, R. P., J. Wisnewski, and R. Engelman. 2000. Human population in biodiversity hotspots. Nature 4040:990–992.

Corneo, P. E., A. Pellegrini, L. Cappellin, M. Roncador, M. Chierici, C. Gessler, et al. 2013. Microbial community structure in vineyard soils across altitudinal gradients and in different seasons. FEMS Microbiol. Ecol. 84:588–602.

Cowling, R. M., P. W. Rundel, B. B. Lamont, M. K. Arroyo, and M. Arianoutsou. 1996. Plant diversity in Mediterranean climate regions. Trends Ecol. Evol. 11:362–366.

Cregger, M. A., C. W. Schadt, N. G. McDowell, W. T. Pockman, and A. T. Classen. 2012. Response of the soil microbial community to change in precipitation in a semiarid ecosystem. Appl. Environ. Microbiol. 78:8586–8594.

Daily, G. C. 1997. Nature's services: societal dependence on natural ecosystems. Island Press, Washington, DC.

Deitch, M. J., G. M. Kondolf, and A. M. Merenlender. 2009. Hydrologic impacts of small-scale instream diversions for frost and heat protection in the California wine country. River Res. Appl. 25:118–134.

Farías, F., J. Orlando, L. Bravo, R. Guevara, and M. Carú. 2009. Comparison of soil bacterial communities associated with actinorhizal, non-actinorhizal plants and the interspaces in the sclerophyllous matorral from Central Chile in two different seasons. J. Arid Environ. 73:1117–1124.

Fernández-Calviño, D., A. Martín, M. Arias-Estévez, E. Bååth, and M. Díaz-Raviña. 2010. Microbial community structure of vineyard soils with different pH and cooper content. Appl. Soil Ecol. 46:276–282.

Fierer, N., and J. A. Jackson. 2006. The diversity and biogeography of soil microbial communities. Proc. Natl Acad. Sci. USA 103:626–631.

Foley, J. A., R. DeFries, G. P. Asner, C. Barford, G. Bonan, S. R. Carpenter, et al. 2005. Global consequences of land use. Science 309:570–574.

García-Orenes, F., A. Morugán-Coronado, R. Zornoza, and K. Scow. 2013. Changes in soil microbial community structure influences by agricultural management practices in a Mediterranean agro-ecosystem. PLoS ONE 8:e80522.

Gardes, M., and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. Mol. Ecol. 2:113–118.

Gardi, C., L. Montanarella, D. Arrouays, A. Bispo, P. Lemanceau, C. Jovilet, et al. 2009. Soil biodiversity monitoring in Europe: ongoing activities and challenges. Eur. J. Soil Sci. 60:807–819.

Gilbert, J. A., D. van der Lelie, and I. Zarraonaindia. 2014. Microbial *terroir* for wine grapes. Proc. Natl Acad. Sci. USA 111:5–6.

Green, J. L., A. J. Holmes, M. Westoby, I. Oliver, D. Briscoe, M. Dangerfield, et al. 2004. Spatial scaling of microbial eukaryote diversity. Nature 432:747–750.

Hannah, L., P. R. Roehrdanz, M. Ikegami, A. V. Shepard, M. R. Shaw, G. Tabor, et al. 2013. Climate change, wine, and conservation. Proc. Natl Acad. Sci. USA 110:6907–6912.

Hartmann, M., and F. Widmer. 2006. Community structure analysis are more sensitive to differences in soil bacterial communities than anonymous diversity indices. Appl. Environ. Microbiol. 72:7804–7812.

van der Heijden, M. G. A., R. D. Bardgett, and N. M. van Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11:296–310.

Hendrix, P. F., R. W. Parmelee, D. A. Crossley, D. C. Coleman, E. P. Odum, and P. M. Groffman. 1986. Detritus food webs in conventional and no-tillage agroecosystems. Bioscience 36:374–380.

Hilty, J. A., and A. M. Merenlender. 2004. Use of riparian corridors and vineyards by mammalian predators in northern California. Conserv. Biol. 18:126–135.

Jangid, K., M. A. Williams, A. J. Franzluebbers, J. S. Sanderlin, J. H. Reeves, M. B. Jenkins, et al. 2008. Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. Soil Biol. Biochem. 40:2843–2853.

Kasel, S., L. T. Bennett, and J. Tibbits. 2008. Land use influences soil fungal community composition across central Victoria, south-eastern Australia. Soil Biol. Biotechnol. 40:1724–1732.

Kennedy, N., E. Brodie, J. Connolly, and N. Clipson. 2004. Impact of line, nitrogen and plant species on bacterial community structure in grassland microcosms. Environ. Microbiol. 6:1070–1080.

Köhl, L., F. Oehl, and M. G. A. van der Heijden. 2014. Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota. Ecol. Appl. 24:1842–1853.

Köller, W. 1999. Chemical approaches to managing plant pathogens. Pp. 337–376 *in* J. R. Ruberson, ed. Handbook of pest management. Marcel Dekker Inc, New York, NY.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen 2014. lmerTest: test for random and fixed effects for linear mixed effect models (lmer objects of lme4 packages). R package version 2.0-11. Available via http://CRAN.R-project.org/package=lmerTest.

Laiolo, P. 2005. Spatial and seasonal patterns of bird communities in Italian agroecosystems. Conserv. Biol. 19:1547–1556.

Lane, D. J., B. Pace, G. J. Olsen, D. A. Stahl, M. L. Sogin, and N. R. Pace. 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl Acad. Sci. USA 82:6955–6959.

Lauber, C. L., M. S. Strickland, M. A. Bradford, and N. Fierer. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol. Biochem. 40:2407–2415.

van Leeuwen, C., P. Friant, C. Chone, O. Tregoat, S. Koundouras, and D. Dubourdieu. 2004. Influence of climate, soil, and cultivar on terroir. Am. J. Enol. Vitic. 55:207–217.

Marquet, P. A., M. Tognelli, I. Barria, M. Escobar, C. Garin, and P. Soublette. 2004. How well are Mediterranean ecosystems protected in Chile? Insights from gaps in the conservation of Chilean vertebrates. Pp. 1–4 *in* M. Arianoutsou and V. P. Papanastasis, eds. Proceedings 10th MEDECOS Conference, Rhodes, Greece. Millpress, Rotterdam, the Netherlands.

Martínez, M. L., O. Pérez-Maque, G. Vásquez, G. Castillo-Campos, J. García-Franco, K. Mehltreter, et al. 2009. Effects of land use change on biodiversity and ecosystem services in tropical montane cloud forests of Mexico. Forest Ecol. Manag. 258:1856–1863.

McGuire, K. L., N. Fierer, C. Bateman, K. K. Treseder, and B. L. Turner. 2012. Fungal community composition in neotropical rain forest: the influence of tree diversity and precipitation. Microb. Ecol. 63:804–812.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature 403:853–858.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, et al. 2013. vegan: community ecology package. R package version 2.0-10. Available via http://CRAN.R-project.org/package=vegan.

Palm, C., H. Blanco-Canqui, F. DeClerck, L. Gatere, and P. Grace. 2014. Conservation agriculture and ecosystem services: an overview. Agric. Ecosyst. Environ. 187:87–105.

Power, A. G. 2010. Ecosystem services and agriculture: tradeoffs and synergies. Philos. Trans. R. Soc. B 365:2959–2971.

Probst, B., C. Schüler, and R. G. J. Joergensen. 2008. Vineyard soils under organic and conventional management: microbial biomass and activity indices and their relation to soil chemical properties. Biol. Fertil. Soils 44:443–450.

Ricketts, T. H., G. C. Daily, P. R. Ehrlich, and C. D. Michener. 2004. Economic value of tropical forest to coffee production. Proc. Natl Acad. Sci. USA 101:12579–12582.

Rousk, J., P. C. Brookes, and E. Baath. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl. Environ. Microbiol. 75:1589–1596.

Schmitt, T., B. Augenstein, and A. Finger. 2008. The influence of change in viticulture management on the butterfly (Lepidoptera) diversity in a wine growing region of southwestern Germany. Eur. J. Entomol. 105:249–255.

Servicio Agrícola Ganadero. 2013. Catastro Vitícola Nacional – Chile. Available via http://www.sag.cl/content/catastro-viticola-nacional-2013.

Stefan-Dewenter, I., and C. Westphal. 2008. The interplay of pollinator diversity, pollination services and landscape change. J. Appl. Ecol. 45:737–741.

Stoate, C., N. D. Boatman, R. J. Borralho, C. Rio Carvalho, G. R. de Snoo, and P. Eden. 2001. Ecological impacts of arable intensification in Europe. J. Environ. Manag. 63:337–365.

Tipayno, S., C. G. Kim, and T. Sa. 2012. T-RFLP analysis of structural changes in soil bacterial communities in response to metal and metalloid contamination and initial phytoremediation. Appl. Soil Ecol. 61:137–146.

Tognelli, M. F., P. I. Ramirez de Arellano, and P. A. Marquet. 2008. How well do the existing and proposed reserve networks represent vertebrate species in Chile?. Divers. Distrib. 14:148–158.

Turner, B. L., E. F. Lambin, and A. Reenberg. 2007a. Land change science spatial feature: the emergence of land change science for global environmental change and sustainability. Proc. Natl Acad. Sci. USA 104:20672–20677.

Turner, W. R., K. Brandon, T. M. Brooks, R. Costanza, G. A. B. da Fonseca, and R. Portela. 2007b. Global conservation of biodiversity and ecosystem services. Bioscience 57:868–873.

Underwood, E. C., J. H. Viers, K. R. Klausmeyer, R. L. Cox, and M. R. Shaw. 2008. Threats and biodiversity in the Mediterranean biome. Divers. Distrib. 15:188–197.

Upchurch, R., C. Y. Chiu, K. Everett, G. Dyszynski, D. C. Coleman, and W. B. Whitman. 2008. Differences in the composition and diversity of bacterial communities from agricultural and forest soils. Soil Biol. Biochem. 40:1294–1305.

Viers, J. H., J. N. Willimas, K. A. Nicholas, O. Barbosa, I. Kotzé, L. Spence, et al. 2013. Vinecology: pairing wine with nature. Conserv. Lett. 6:287–299.

Viti, C., D. Quaranta, R. De Philippis, G. Corti, A. Agneli, R. Cuniglio, et al. 2008. Characterizing cultivable soil microbial communities from copper fungicide-amended olive orchard and vineyard soil. World J. Microbiol. Biotechnol. 24:309–318.

Vitouseek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth's ecosystems. Science 227:194–499.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H. Wall. 2004a. Ecological linkages between aboveground and belowground biota. Science 304:1629–1633.

Wardle, D. A., L. R. Walker, and R. D. Bardgett. 2004b. Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305:509–513.

Wong, C. P., B. Jiang, A. P. Kinzig, K. N Lee, and Z. Ouyang. 2015. Linking ecosystem characteristics to final ecosystem services for public policy. Ecol. Lett. 18:108–118.

Zagal, E., and A. Sadzawka. 2007. Protocolo de métodos de análisis para suelos y lodos. Servicio Agrícola Ganadero, Ministerio de Agricultura, Gobierno de Chile.

Zepeda-Paulo, R. 2013. Dinámica espacio-temporal del bosque nativo y matorral esclerófilo y la industria vitivinícola: Una aproximación para la conservación en el Valle de Leyda, Región de Valparaíso. Agronomic Engineer Thesis. Available via http://cybertesis.uach.cl/tesis/uach/2013/fifz.57d/doc/fifz.57d.pdf.

