

# Bioindicators of soil quality of open shrubland and vineyards

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## Abstract

Land-use effects on microbial communities may have profound impacts on agricultural productivity and ecosystem sustainability because they are critical to soil quality and health. Within this context, soil microbiological properties such as microorganism abundance, total microbial biomass, and enzymatic activities have been used as soil quality indicators. However, these properties are very sensitive to changes triggered by agronomic management. The aim of this work was to evaluate the effect of agricultural practices in the vineyards from the Central Monte Desert, San Juan, Argentina including the effects on soil microbial biomass using soils from the open shrubland as a reference. The microbial biomass carbon was significantly greater in soils from the open shrubland than in the vineyards during April and November. The abundance of cultivable soil microorganisms (bacteria and filamentous fungi) in soil samples from vineyards and open shrubland was statistically similar. Vineyard soils showed increased enzymatic activities (both in rows and between rows) in both seasons. We used multivariate analysis of all data measured here to propose a data set of variables (amylase, cellulase, and xylanase activities, bacterial abundance, microbial biomass and water content, pH and electric conductivity) for use in future studies of soil quality in the Central Monte Desert.

**Keywords:** Enzyme activity, microbial biomass carbon, arid systems.

## 1. Introduction

In arid environments, soils with sandy texture have poorly-developed profiles, fragile structure, and low organic matter content (Caravaca *et al.*, 2002; Cao *et al.*, 2011). This soil fragility increases the possibility of degradation and productivity losses due to extreme soil vulnerability, which is directly or indi-

rectly affected by anthropogenic activities including agricultural land management practices (Cao *et al.*, 2011). The assessment of changes in the quality of these arid soils due to the conversion of natural areas into productive agricultural areas requires adapting the definition of soil quality. Although there is no

clear consensus, prior work (Montecchia *et al.*, 2011). Karlen *et al.* (1997) has proposed the following definition of soil quality: “the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivities, maintain or enhance water and air qualities, and support human health and habitation”.

Montecchia *et al.* (2011) extended this definition and proposed that the quality of agricultural soils should consider not only the plant productivity but also soil microbial community composition and activity because the functional stability and health of soils depends on microbial activities. Within this context, soil microorganisms play a preponderant role in ecological processes especially the nutrient cycle (Forlan Amaral *et al.*, 2012). Biochemical properties related to the distinct biological cycle of elements (C, N, P and S) are generally used to assess soil quality. These properties include biochemical parameters to monitor microbial biomass carbon as well as specific enzymatic activities related to the degradation of plant material (Ushio *et al.*, 2010). Microbial biomass carbon can be more sensitive to changes in the condition of the soil's organic matter (even more than total organic carbon level). In contrast, the soil's enzymatic activity (primarily micro-biotic in origin) can swiftly respond to changes in soil management. This occurs much faster than other variables. These properties are useful as early indicators of the biological changes that occur due to traditional farming practices. Studying the relation between these two variables may explain the chemical and biological changes in the soil (García-Orenes *et al.*, 2010; Makhalyane *et al.*, 2015).

Soil organic carbon levels are significantly influenced by agricultural farming practices. Continuous soil tillage and the incorporation of organic residues or fertilization can alter soil properties such as microbial biomass, aggregate stability, and enzyme activity (Morugan-Coronado *et al.*, 2013). Several works have

studied the impact of different agricultural practices in crop rotation on the soil quality, but little is known about the response of soil microorganisms to the management used for perennial crops such as vineyards. Caravaca *et al.* (2002) evaluated the effects of crop management of *Olea europaea* L. and monitored changes in biochemical parameters. These authors determined that the tillage includes soil removal by agricultural machinery, and the use of agrochemicals decreases the enzyme activities and microbial biomass including soils treated with compost. Chaer *et al.* (2009) found that a shift from an undisturbed forest into a long-term cultivation was associated with the establishment of a less functionally stable microbial community.

One of the consequences of agricultural expansion is the change in land use from natural ecosystems (e.g. open shrubland or pasture) to croplands. In areas where monocultures prevail over crop rotations, it becomes difficult to maintain soil fertility and sustainability. In addition, certain agricultural practices associated with monocultures alter the soil's physical, chemical, and biological characteristics. This can degrade the microbial habitat and reduce the soil quality (Figueroa *et al.*, 2012).

The cultivation of *Vitis vinifera* L. is characterized by its susceptibility to plagues, pathogens, and weed competition. If vineyard cultivation is considered in isolation, i.e. unassociated with any type of ground cover, then it can be essentially described as a monoculture. Despite the importance of vineyard cultivation in San Juan Province, Argentina (second only to Mendoza Province in Argentina (INV, 2017)), there are no studies on the microbiological and biochemical quality indicators of vineyard soils compared to the natural uncultivated areas of this desert area. Soil quality indicators in both areas provide an opportunity to evaluate the effects produced by agronomic management. Thus, the objective of this study was to

evaluate the effects of agricultural practices in vineyards on soil microbial biomass; reference soils were from open shrubland adjacent to cultivated areas. We hypothesized that the conventional agro-management methods used in viticulture diminish the microbial biomass of soils.

To assess the magnitude of the impact of agricultural management, we studied samples from the rows (planting line) as well as between rows (inter-rows; serviced by agricultural machinery). We studied rows and inter-rows of vineyards and compared them with two contrasting nearby areas: 1) vineyards where rows and inter-rows are microsites and under bushes; and 2) the open spaces between them (open shrubland microsites).

## 2. Materials and Methods

### 2.1. Study sites

This study was performed in the Médanos Grandes dune field (Central Monte Desert), San Juan province (Argentina) in two contiguous areas: (1) open shrubland and (2) land cultivated with grapes (*Vitis vinifera* L.). The regional climate is arid-temperate with cold winters. The mean summer (January) and mean winter (July) temperatures over 10 years are 25.1 and 7.7 °C, respectively. However, there have been temperatures of up to 45 °C in summer and freezing temperatures in the winter. The soils are sandy with low organic matter content (Vega Ávila, 2012). These soils are classified as Entisoles (FAO-UNESCO, 1974). More specifically, they are typical torripsamentes (Regairaz, 2000). Médanos Grandes is characterized by two clearly distinct seasonal regimes: summer and winter; most rain is in December (early summer). The mean annual rainfall over the last 30 years is 90 mm ( $\pm 70$  mm). Data from the weather station of the National

Institute of Agricultural Technology (INTA) (San Juan, Argentina) indicate that the annual rainfall during 2010 was 17.8 mm from February to April and 0 mm from June to August.

*Médanos Grandes* is located on a flat area among the dunes. The vegetation is an open shrubland (cover ~15%) organized as a two-phase mosaic composed of shrub- or tree-dominated patches alternating in areas with sparse cover or bare soil (interspaces). The plant community is dominated by the family *Zygophyllaceae* and especially *Bulnesia retama* ('retamo'). This species is a slow-growing aphyllous shrub (up to 3 m high in our study area) that is highly tolerant to water stress. San Juan is the most important ecological area for *B. retama* because this species occupies 80% of the territory of the province. Other very abundant shrub species in this area are *Larrea divaricata*, *Capparis atamisquea*, *Lycium ciliatum*, and *Atriplex lampa*. The herbaceous vegetation is mostly composed of annual species although some perennial herbs (mostly *Heliotropium mendocinum*) are also abundant. Annual species can be roughly classified as cold-season 'winter' species (e.g. *Schismus barbatus* and *Chenopodium papulosum*) or as warm-season 'summer' species based on their vegetative period, (e.g. *Gomphrena martiana* and *Tribulus terrestris*) (Dalmaso & Llera, 1996).

Vineyards selected for this study correspond to the Cabernet Sauvignon variety with 15 years of cultivation. These vineyards were under a conventional tillage system (soil plowing twice a year) and were fertilized with calcium, magnesium, and zinc. They were treated with several agricultural chemicals: herbicide (glyphosate acid; Round-up) and the fungicides Caurifix® WG (copper oxychloride) and Kumulus® DF (sulfur). The vineyard irrigation system is gravitational (irrigation mantle) twice a month.

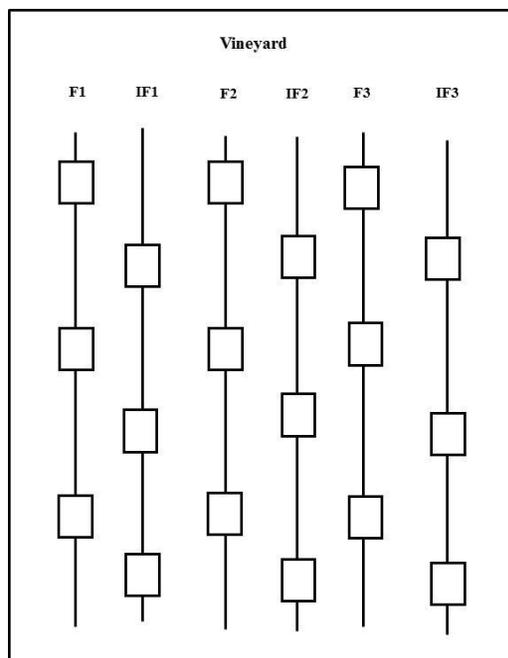
## 2.2. Experimental design

Samples were taken along three-line transect each approximately 100 m long with a sterilizable witness *in situ*. At each study site, soil sampling comprised three replicates per transect collected from the top soil (0-10 cm depth) in April (wet season) and November (dry season) 2010 using a soil core grid.

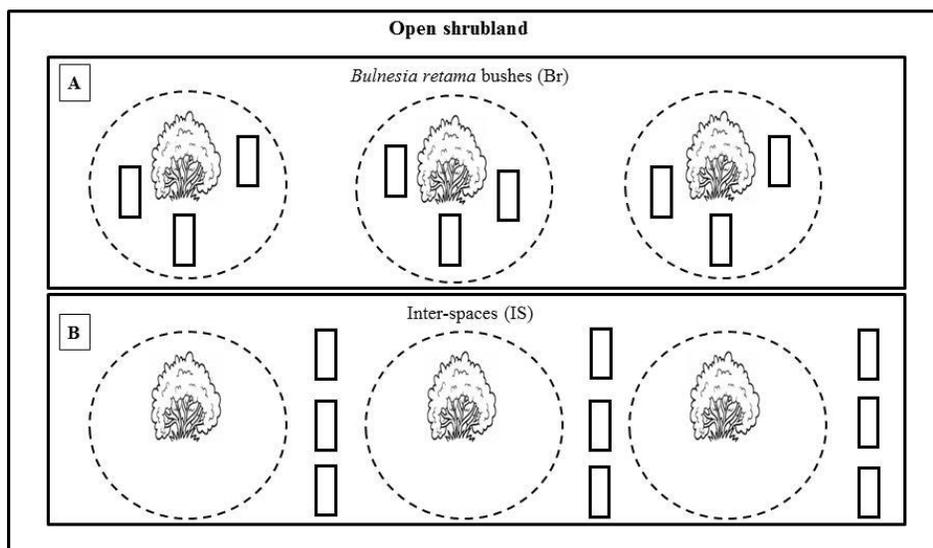
In the open shrubland (31°45' S and 68°12' W, 595 m altitude), soil samples were collected through three-line transects under *Bulnesia retama* bushes (Br) and in the open spaces between them (IS). The vineyard was located 31° 40' 51" S and 68° 12' 01.5" W, 472 m altitude, and soil samples were taken from the rows (R) of the crop under six distinct plants from each vineyard. These plants were located in the central portion of the stand. We also collected samples between rows (inter-rows (IR)). Although the sample sites were located in slightly different topographic po-

sitions, the corresponding soils were classified as the same type and showed similar soil texture. Therefore, we assumed that any difference in soil quality could be attributed to the different plant covers and to the impact of agricultural.

The samples were taken from 3 rows and 3 inter-rows. Three sub-samples were taken within each row and inter-row (Figure 1). In the pristine zone, samples were taken from the soils associated with 3 plants of *B. retama* as well as from 3 interspaces (bare soil). Three sub-samples were taken from each of these two natural sites (Figure 2). Subsamples were mixed and homogenized and subsequently placed in sterile containers and refrigerated for analysis in the laboratory. A portion of each soil sample was maintained at 4–5°C for subsequent biochemical and microbiological analyses, and the other soil portion was homogenized, air-dried, and sieved (2-mm sieve) to exclude plant roots. This was subsequently used for physicochemical analyses.



**Figure 1.** Soils sampling pattern. Vineyard box is shown with the microsites: 3 rows (F) and 3 inter-rows (IF) sampled. The boxes represent the subsamples (3) taken at random within each F and IF.



**Figure 2.** Soils sampling Pattern. A: The 3 circles represent the patches of vegetation (area under the canopy of the plant of Br). The boxes represent the sub-samples (3) taken at random points under the canopy of the bush. B: The boxes represent the sub-samples (3) taken at points located outside the vegetation patches of Br in the inter-space (IS) zone.

### 2.3. Physicochemical Analysis

The physicochemical parameters were soil texture, moisture, electrical conductivity (EC), pH, nitrogen (N), potassium (K), phosphorus (P), and total soil organic matter (SOM). Texture was determined as the quantitative proportion of each of the elementary size fractions comprising the mineral solid phase with a pipette. The weight of separate aliquots was controlled with gravimetry (Gee & Bauder, 1986). Soil moisture was gravimetrically determined by drying at 105 °C until the sample reached a constant weight (Editorial Committee, 1996). The EC and pH were determined using a glass electrode in a saturated solution of soil:water (1:5) (Editorial Committee, 1996). Total N was determined using the Kjeldahl digestion technique (Bremner & Mulvaney, 1982). The availability of K and P ( $P_2O_5$ ) was measured by their atomic absorption spectrum (Heldrich, 1990). Total SOM was

determined using the formula: SOM = total organic carbon (TOC) x 1.72 (Yeomans & Bremner, 1989); here, TOC was determined by the Walkley and Black wet-digestion method.

### 2.4. Microbiological Analysis

#### 2.4.1. Determination of the abundance of cultured bacteria and fungi

Microorganism abundance was determined via the plating count method (Reasoner & Geldreich, 1985). The abundance of cultivable microorganisms (bacteria and filamentous fungi) was determined by the agar plate method (three replicates). Samples were incubated at 28 °C for 3-5 days. The abundance was expressed as colony forming units (CFU) per gram of soil ( $CFU/g\ soil^{-1}$ ). For bacterial growth, the nutrient broth contained the following: beef extract

3.0 – 5.0 g/L; NaCl 5.0 g/L; Na<sub>2</sub>HPO<sub>4</sub> 1.0 g/L; agar 20.0 g/L; and pH = 7. For filamentous fungi, the brother contained the following: Czapeck: KH<sub>2</sub>PO<sub>3</sub> 1 g/L; NaNO<sub>3</sub> 30.0 g/L; KCl 5 g/L; MgSO<sub>4</sub> 7H<sub>2</sub>O 5 g/L; FeSO<sub>4</sub> 7H<sub>2</sub>O 0.1 g/L; sacarosa 30.0 g/L; and agar 20.0 g/L.

#### 2.4.2. Determination of microbial biomass carbon

Microbial biomass was determined indirectly by measuring the microbial biomass carbon (MBC) via the fumigation-extraction method (Vance *et al.*, 1987) using a conversion coefficient (kc = 0.4).

#### 2.4.3. Determination of soil enzymatic activities

All soil enzymatic activities were determined by incubating 1 g of soil and 1 mL 0.05 M Na-citrate buffer (pH 5.2) with the substrate.

Cellulolytic activity was determined using 2% w/v carboxymethyl cellulose. Amylolytic activity was measured with 1% w/v starch, and xylanolytic activity was measured with 1% w/v birchwood 4-o-methyl glucuronoxylan (Ghose & Bisaria, 1987).

The release of sugar-reducing groups (starch, cellulose, xylan) were determined by adding dinitrosalicylic acid to the mixture (Miller *et al.*, 1960) and boiling for 15 min. The samples were cooled, and absorption was measured at 540 nm. The results are expressed as nkatales. Samples were analyzed in triplicate and averaged for each enzymatic activity. A sample control was prepared by adding the substrate after stopping the reaction.

#### 2.5. Statistical analysis

Differences between physicochemical and microbiological parameters from open shrubland soils (Br and IS) and agricultural soils (R and IR) were evaluated via analysis of variance (repeated-measures ANOVA over time); the averages were compared with a Tukey Test ( $P < 0.05$ ).

Data on physicochemical parameters, microbial biomass, microorganism abundance, and enzymatic activities determined from vineyard soils and open shrubland soils were included in a single Principal Component Analysis (PCA) to assess the interactions between them and to choose variables that better discriminate between agricultural and open shrubland soils. All statistical analyses were performed using INFOSTAT (version 1.1, 2011, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina).

### 3. Results

#### 3.1. Physicochemical parameters

All soil samples were sandy-loam (87.5% sand; 8.1% silt; 4.4% clay). The pH in the vineyards was significantly higher between rows; the EC and water content was higher in the row soil samples than in open shrubland soils (Br and IS) in both seasons (Table 1).

In the wet season, the SOM did not differ significantly between vineyard and open shrubland soils. The N and P contents were higher in the vineyard R soil samples than in all open shrubland soils ( $P < 0.01$ ) (Table 1). The SOM and N were significantly higher in Br soils samples than in vineyard soils in the dry season ( $P < 0.01$ ) (Table 1).

**Table 1.** Soil physicochemical parameters of vineyards and open shrubland environments in the wet and dry seasons in 2010. The mean  $\pm$  SD is shown for each study site. Different letters indicate significant differences in pH, SM, EC, SOM, N, P, and K content between soils of both environments using Tukey's test,  $P < 0.05$ .

		WET	DRY
<b>pH</b>	<b>Br</b>	7.14 $\pm$ 0.19 <sup>(a)</sup>	7 $\pm$ 0.19 <sup>(a)</sup>
	<b>R</b>	8.22 $\pm$ 0.10 <sup>(b)</sup>	8.27 $\pm$ 0.10 <sup>(b)</sup>
	<b>IS</b>	7.24 $\pm$ 0.09 <sup>(a)</sup>	7.33 $\pm$ 0.18 <sup>(a)</sup>
	<b>IR</b>	8.40 $\pm$ 0.10 <sup>(c)</sup>	8.35 $\pm$ 0.16 <sup>(c)</sup>
<b>SM (g H<sub>2</sub>O g soil<sup>-1</sup>)</b>	<b>Br</b>	0.65 $\pm$ 1.55 <sup>(a)</sup>	0.03 $\pm$ 0.01 <sup>(a)</sup>
	<b>R</b>	8.16 $\pm$ 1.33 <sup>(b)</sup>	5.09 $\pm$ 1.89 <sup>(b)</sup>
	<b>IS</b>	0.01 $\pm$ 0.01 <sup>(a)</sup>	0.01 $\pm$ 0.01 <sup>(a)</sup>
	<b>IR</b>	0.54 $\pm$ 0.18 <sup>(a)</sup>	0.98 $\pm$ 0.17 <sup>(a)</sup>
<b>EC (dS m<sup>-1</sup>)</b>	<b>Br</b>	870.25 $\pm$ 76.96 <sup>(a)</sup>	850 $\pm$ 60 <sup>(a)</sup>
	<b>R</b>	1972.83 $\pm$ 509 <sup>(b)</sup>	1692.67 $\pm$ 693.30 <sup>(b)</sup>
	<b>IS</b>	500 $\pm$ 50 <sup>(a)</sup>	535 $\pm$ 49.35 <sup>(a)</sup>
	<b>IR</b>	879.00 $\pm$ 66.82 <sup>(a)</sup>	855 $\pm$ 75.83 <sup>(a)</sup>
<b>SOM (%)</b>	<b>Br</b>	0.63 $\pm$ 0.10 <sup>(a)</sup>	1.06 $\pm$ 0.2 <sup>(b)</sup>
	<b>R</b>	0.74 $\pm$ 0.22 <sup>(a)</sup>	0.70 $\pm$ 0.20 <sup>(a)</sup>
	<b>IS</b>	0.44 $\pm$ 0.13 <sup>(a)</sup>	0.45 $\pm$ 0.10 <sup>(a)</sup>
	<b>IR</b>	0.7 $\pm$ 0.2 <sup>(a)</sup>	0.5 $\pm$ 0.07 <sup>(a)</sup>
<b>N (ppm)</b>	<b>Br</b>	233.50 $\pm$ 61.30 <sup>(a)</sup>	355.67 $\pm$ 80 <sup>(c)</sup>
	<b>R</b>	394 $\pm$ 124.3 <sup>(b)</sup>	225.3 $\pm$ 38.4 <sup>(b)</sup>
	<b>IS</b>	122.6 $\pm$ 31.1 <sup>(a)</sup>	108.3 $\pm$ 9.8 <sup>(a)</sup>
	<b>IR</b>	225.3 $\pm$ 38.4 <sup>(a)</sup>	225.8 $\pm$ 18.7 <sup>(b)</sup>
<b>P (ppm)</b>	<b>Br</b>	63 $\pm$ 10.66 <sup>(a)</sup>	149.1 $\pm$ 24 <sup>(c)</sup>
	<b>R</b>	73.3 $\pm$ 10.6 <sup>(c)</sup>	72 $\pm$ 20 <sup>(b)</sup>
	<b>IS</b>	55 $\pm$ 6.26 <sup>(a)</sup>	47.1 $\pm$ 3.95 <sup>(a)</sup>
	<b>IR</b>	71.5 $\pm$ 20.07 <sup>(b)</sup>	90.83 $\pm$ 9.81 <sup>(b)</sup>
<b>K (ppm)</b>	<b>Br</b>	390.83 $\pm$ 59.28 <sup>(b)</sup>	484 $\pm$ 60 <sup>(b)</sup>
	<b>R</b>	244.7 $\pm$ 60.5 <sup>(a)</sup>	282 $\pm$ 40 <sup>(a)</sup>
	<b>IS</b>	250.8 $\pm$ 36.7 <sup>(a)</sup>	206.6 $\pm$ 60 <sup>(a)</sup>
	<b>IR</b>	281.8 $\pm$ 40.4 <sup>(a)</sup>	468.3 $\pm$ 32.6 <sup>(b)</sup>

The K content was higher in the Br soils in both sam-  
pling periods and in vineyard IR soil samples in the

dry season ( $P < 0.01$ ) with respect to the rest of the  
sampled sites from both environments.

### 3.2. Microbial biomass, enzymatic activities, and abundance of soil microorganisms

The abundance of soil microorganisms (bacteria and filamentous fungi) in soil samples from vineyards and open shrubland were statistically similar in both sea-

sons. However, the MBC content was higher in the patches of Br than in vineyard soils (R and IR) in both seasons ( $P=0.0001$ ) (Table 2). The three enzymatic activities (xylanolytic, cellulolytic and amylolytic) were higher in vineyard soils (R and IR) in both seasons (Table 2).

**Table 2.** Seasonal-spatial variation in microorganism abundance, microbial biomass carbon (MBC), and enzymatic activities in soils from vineyards and open shrubland environments in the wet and dry seasons in 2010. Mean  $\pm$  SD is shown for each study site. Different letters indicate significant differences in abundance of bacteria and fungi, MBC, and enzymatic activities of soils from both sites (Tukey's test,  $P < 0.05$ ).

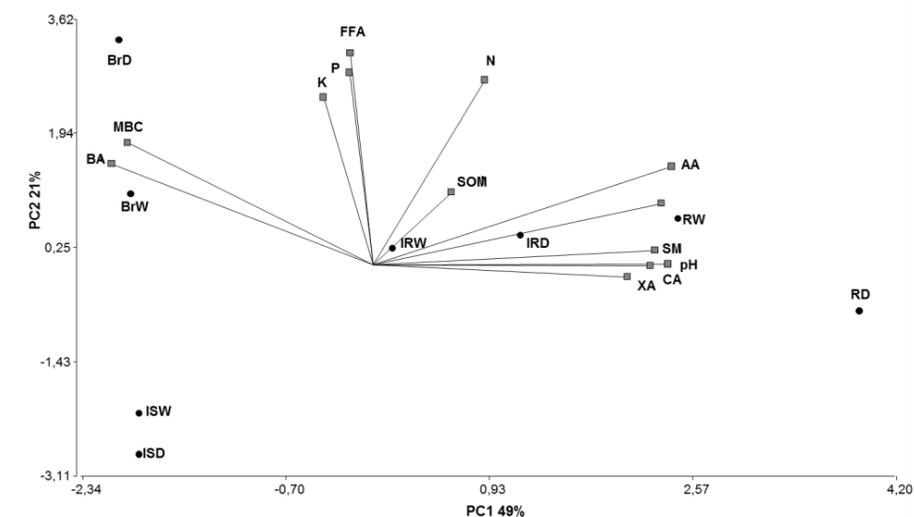
		WET	DRY
<b>MBC (ppm)</b>	<b>Br</b>	110.31 $\pm$ 11.75 <sup>(b)</sup>	120.59 $\pm$ 0.83 <sup>(b)</sup>
	<b>R</b>	24.1 $\pm$ 0.14 <sup>(a)</sup>	25.41 $\pm$ 0.58 <sup>(a)</sup>
	<b>IS</b>	47.85 $\pm$ 19.75 <sup>(a)</sup>	55.43 $\pm$ 0.61 <sup>(a)</sup>
	<b>IR</b>	29.35 $\pm$ 0.49 <sup>(a)</sup>	38.33 $\pm$ 0.46 <sup>(a)</sup>
<b>Xylanase (nkatales g soil<sup>-1</sup>)</b>	<b>Br</b>	3.97 $\pm$ 0.78 <sup>(a)</sup>	4.95 $\pm$ 1.44 <sup>(a)</sup>
	<b>R</b>	5.79 $\pm$ 0.78 <sup>(b)</sup>	34.2 $\pm$ 17.8 <sup>(b)</sup>
	<b>IS</b>	3.22 $\pm$ 1.86 <sup>(a)</sup>	4.85 $\pm$ 1.7 <sup>(a)</sup>
	<b>IR</b>	4.21 $\pm$ 0.8 <sup>(b)(a)</sup>	5.83 $\pm$ 2.25 <sup>(a)</sup>
<b>Amylase (nkatales g soil<sup>-1</sup>)</b>	<b>Br</b>	2.11 $\pm$ 0.5 <sup>(a)</sup>	2.03 $\pm$ 0.5 <sup>(a)</sup>
	<b>R</b>	3.92 $\pm$ 1.44 <sup>(d)(c)</sup>	3.96 $\pm$ 1.8 <sup>(d)</sup>
	<b>IS</b>	0.76 $\pm$ 0.21 <sup>(a)</sup>	0.51 $\pm$ 0.13 <sup>(a)</sup>
	<b>IR</b>	3.96 $\pm$ 1.80 <sup>(b)(c)</sup>	2.54 $\pm$ 0.21 <sup>(c)</sup>
<b>Cellulase (nkatales g soil<sup>-1</sup>)</b>	<b>Br</b>	2.73 $\pm$ 0.3 <sup>(a)</sup>	1.79 $\pm$ 0.6 <sup>(a)</sup>
	<b>R</b>	3.95 $\pm$ 0.51 <sup>(c)</sup>	21.6 $\pm$ 6.07 <sup>(c)</sup>
	<b>IS</b>	1.18 $\pm$ 0.7 <sup>(a)</sup>	0.54 $\pm$ 0.17 <sup>(a)</sup>
	<b>IR</b>	4.01 $\pm$ 0.57 <sup>(c)</sup>	14.45 $\pm$ 0.21 <sup>(b)</sup>
<b>Abundance of bacteria (Log 10 CFU g soil<sup>-1</sup>)</b>	<b>Br</b>	5.68 $\pm$ 0.29 <sup>(a)</sup>	5.71 $\pm$ 0.35 <sup>(a)</sup>
	<b>R</b>	5.5 $\pm$ 0.15 <sup>(a)</sup>	5.19 $\pm$ 0.19 <sup>(a)</sup>
	<b>IS</b>	5.53 $\pm$ 0.48 <sup>(a)</sup>	5.44 $\pm$ 0.48 <sup>(a)</sup>
	<b>IR</b>	5.43 $\pm$ 0.07 <sup>(a)</sup>	5.27 $\pm$ 0.25 <sup>(a)</sup>
<b>Abundance of filamentous fungi (Log 10 CFU g soil<sup>-1</sup>)</b>	<b>Br</b>	4.37 $\pm$ 0.53 <sup>(a)</sup>	4.42 $\pm$ 0.44 <sup>(a)</sup>
	<b>R</b>	3.57 $\pm$ 0.43 <sup>(a)</sup>	3.33 $\pm$ 0.56 <sup>(a)</sup>
	<b>IS</b>	2.74 $\pm$ 2.24 <sup>(a)</sup>	2.43 $\pm$ 1.82 <sup>(a)</sup>
	<b>IR</b>	3.58 $\pm$ 0.31 <sup>(a)</sup>	3.42 $\pm$ 0.28 <sup>(a)</sup>

Br: Bulnesia retama; R: Rows; IS: inter-spaces; IR: inter-rows. MBC: microbial biomass carbon.

The PCA of the integrated data set (including physicochemical parameters and enzymatic activities) clearly separated vineyard soils from nearby open shrubland soils. The first two principal components captured a significant portion (69%) of the total variance (Figure 3). Along the PC1 axis, the Br and IS were separated from R and IR. Br in the wet and dry seasons was distinct from IS in both seasons along the PC2 axis.

The PCA allowed us to select the set of variables

that best discriminated between vineyard soils and open shrubland soils. This selection was based on the correlation among the measured variables shown graphically in Figure 1 and the correlations of the variables to each axis (Table 3). The pH, EC, amylolytic, cellulolytic, and xylanolytic activities as well as the water content were generally higher in vineyard soils (R and IR). The soil bacterial abundance and MCB were higher in Br soils in both the wet and the dry seasons.



**Figure 3.** Principal components analysis (PCA) of the physicochemical and microbiological properties analyzed during wet/dry periods in vineyards and open shrubland soils. SM: soil moisture; EC: electrical conductivity, SOM: organic soil matter; N: nitrogen; K: potassium; P: phosphorus; MBC: microbial biomass carbon; BA: bacterial abundance; FFA: filamentous fungi abundance; CA: cellulase activity; XA: xylanase activity; AA: amylase activity; BrD: *Bulnesia retama*, dry season; BrW: *Bulnesia retama*, wet season; ISD: inter-spaces, dry season; ISW: inter-spaces, wet season; RD: rows, dry season; RW: rows, wet season; IRD: inter-rows, dry season; and IRW: inter-rows, wet season.

**Table 3.** Correlations of original variables to ordination axes derived from the PCA of the soils analyzed.

Variable <sup>a</sup>	r <sup>b</sup>	
	PC1	PC2
<b>SOM</b>	0.1	0.17
<b>N</b>	0.14	0.43
<b>P</b>	-0.03	0.44
<b>K</b>	-0.06	0.39
<b>pH</b>	0.37	2.00E-03
<b>EC</b>	0.36	0.14
<b>XA</b>	0.32	-0.03
<b>CA</b>	0.35	-2.30E-03
<b>AA</b>	0.38	0.23
<b>SM</b>	0.35	0.03
<b>BA</b>	-0.33	0.23
<b>FFA</b>	-0.03	0.49
<b>MCB</b>	-0.31	0.28

<sup>a</sup>: SM: soil moisture; EC: electrical conductivity, SOM: organic soil matter; N: nitrogen; K: potassium; P: phosphorus; MBC: microbial biomass carbon; BA: bacterial abundance; FFA: filamentous fungi abundance; CA: cellulase activity; XA: xylanase activity; AA: amylase activity. <sup>b</sup> A positive correlation indicates greater value in soils with higher coordinate scores for the axis whereas a negative correlation indicates greater value in soils with lower coordinate scores for the axis (see Figure. 3).

#### 4. Discussion

Analysis of the soil's physicochemical parameters showed that while vineyard soils are irrigated throughout the year, the *B. retama* soils showed more organic matter, N, and P in the dry season (Table 1). This is attributed to the effect of “nurse plants” in arid environments. Annual plants grow underneath these plants and take advantage of the rain pulses. These plants disappear during the dry periods leaving a deposit of organic matter. Thus, under the canopy of these plants, reservoirs of organic matter establish “fertility islands” that promote microbiological activity (Muro-Perez *et al.*, 2014). In the dry season, the low content of organic

matter and nutrients in vineyard soils (R and IR) with respect to Br soils could be the result of the farming methods implemented.

Several authors have suggested that conventional farming practices (which can cause structural soil damage) are disadvantageous. These farming practices accelerate organic carbon decomposition processes by triggering premature oxidation of organic matter. This decrease this parameter and affects the chemical properties of the soils (Caravaca *et al.*, 2002; Holland *et al.*, 2013).

In an arid region of Chile, Seguel *et al.* (2015) evaluated the physicochemical properties of some vineyard soils in inter-rows and rows (7-year establishment)

and compared the results with soil properties from a natural vegetation site (used as a control). Rows and inter-rows showed no significant differences, but the authors determined that the soil-use change had decreased the organic matter content (Seguel *et al.*, 2015). In a perennial crop in an arid Mediterranean environment, Caravaca *et al.* (2002) found a higher organic matter content and macro-nutrients in the topsoil and the rhizosphere of *Retama spherocarpa* L. (native woodland species) than in soils implanted with *Olea europaea sp. sylvestre* L. These authors suggested that the differences observed here could be due to conventional crop management (Caravaca *et al.*, 2002).

We observed higher values of MBC in the patches of *B. retama* than in the other soil sites (IS, R and IR) in both seasons. The lower MBC values observed in vineyard soils are consistent with the literature (Liu *et al.*, 2006; Montecchia *et al.*, 2011) and reflect the lower supply of labile C and other nutrients provided by litter and crop residues. It is remarkable that while the vineyard was irrigated twice a month, the area with natural vegetation (lacking that extra hydric input) had higher MBC values.

In a study performed in a semi-arid Mediterranean environment, Garcia-Orenes *et al.* (2010) studied the properties of soils cultivated with another perennial crop similar to *Olea europaea* sp. These soils had both organic and conventional treatment. They then compared those samples to an area with natural vegetation. The values of MBC in the organically treated soils and in the area with native vegetation were similar. Soils with conventional treatment (tillage, agrochemicals, no amendment) had lower MBC values possibly due to crop management and herbicide use (Garcia-Orenes *et al.*, 2010). In vineyard soils from La Rioja (a semi-arid region in Spain), Peregrina *et al.* (2016) studied the impact of different management regimes (conventional tillage and two treatments with cover crops) on MBC. The traditional practice

of monoculture caused a decrease in MBC values because both cover crop treatments led to a higher MBC value than conventional tillage treatment.

Fungicides are one factor that can influence the microbiological properties of vineyard soils. Copper has accumulated in vineyard soils due to the long-term use of these fungicides since the end of the 19th century (Babcsányi *et al.*, 2016). Fernandez-Calviño *et al.* (2016) argued that the application of copper-based fungicides causes soil acidification and alters the microbial biomass.

While the vineyard soils studied here showed low values of MBC, they also showed increased levels of xylanase and cellulase. This could be due to a selective effect on the degrading microorganisms that tolerate the farming methods used here. This could be because the soil bacterial diversity is vast and is a reservoir of genetic resources. Adaptive genotypes may already exist and can facilitate rapid acclimatization to a variety of conditions (Montecchia *et al.*, 2011).

From an ecological point of view, these results could be explained by the soil resilience (Seybold *et al.*, 1999). In other words, vineyard soils can recover from agricultural disturbances. Griffiths & Philippot (2013) found no definite response of the soil microbiota to the disturbance because soil stability results from a combination of many biotic and abiotic soil characteristics. Another possible explanation of these results would be the presence of extracellular enzymes in vineyard soils that can catalyze these xylanolytic and cellulolytic reactions. These enzymes are not only available in dead cells they can also be absorbed in clays or can be integrated in humic substances. These enzymes play a vital role in agriculture and the nutrient cycle in particular because they are constantly being synthesized, accumulated, inactivated, and decomposed in the soil (Adetunji *et al.*, 2017).

Costantini *et al.* (2015) evaluated the effect of deep earthwork activities carried out before planting.

They studied an older vineyard planted in 2000 and a new one planted in 2011 in a semiarid environment of Italy. During the 2010–2014 period, the new vineyard had a lower TOC, and the microbiological analysis revealed a different structure of eubacterial communities between vineyards. The authors concluded that more time was necessary for recovery of soil functions.

The periodic irrigation used in these cultivated soils can also favor more filamentous fungi and can increase the cellulolytic and xylanolytic activities. These samples remain in a latent state during periods of minimal soil humidity. These findings coincide with a previous study (Holland *et al.*, 2013) that found an increase in the fungal biomass of vineyards with a low-frequency irrigation regime. While irrigation can modify the structure of microbial communities in *Vitis vinifera* L. soils, this paper found that the irrigation system does not microbial populations including fungal populations. Conversely, we found that all the remaining organisms (bacteria, protozoa, nematodes, collembola, and mites) were more abundant in continuous irrigation regimes. This suggests that the manipulation of irrigation in viticulture systems can affect the structure and function of the soil microbial communities (Holland *et al.*, 2013). This was observed in our multivariate analysis where enzyme activities, soil moisture, and electrical conductivity were associated with vineyard soils. Variables such as MBC and the abundance of filamentous fungi and bacteria were associated with soil patches of Br and IS. This analysis provides indicators, which represent the sites under study. Concurrently, it also reflects their sensitivity to the agricultural management practices that distinguish soils cultivated with *Vitis vinifera* L. from the arid zones of San Juan.

In the wet season, the organic matter content did not differ significantly between vineyard and open shrubland soils. This suggests the ability of soils from

pristine areas to quickly recycle nutrients resulting in organic matter content similar to vineyard soils. Moreover, it was surprising that the organic matter content was equivalent in both areas—the cultivated area does not receive organic amendments. However, unlike the pristine area, the vineyard is irrigated, which could explain these results.

The results suggest that differences between cultivated and non-cultivated soils can reflect decreases in soil quality due to the intrinsic environmental fragility of this region. However, enzyme activities were higher in these cultivated soils. This makes the idea of fragile soils questionable. Thus, soil quality parameters should be assessed over a longer time scale to determine whether it is possible to discuss deterioration of soil quality or rather we should describe soil resilience.

## 5. Conclusions

Enzymatic activities and microbial biomass carbon can explain the response of the soil microbiota of vineyard soils with respect to anthropic management. Xylanase, amylase, and cellulase activities increased in vineyard soils, and PCA analyses clearly showed that these activities were associated with vineyard soils. The results also suggest that microbial biomass was the variable most vulnerable to the anthropic management of vineyard soils. Therefore, microbial biomass carbon is a potential indicator of crop management and can discriminate non-sustainable agricultural methods in arid regions. However, additional surveys and comparisons at other locations and agricultural management practices are needed to validate this proposed set of soil quality indicators. Further research is required to explore the ecological context including the influence of environmental characteristics, temporal and spatial changes, and the intensity of management practices on crops.

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