

Comparative microbiology evaluation of bacterial biodiversity in rhizospheres of vegetative cover of exotic species of Australia (Eucalyptus), native to Chile (Quillay) and “badland” of Chile

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1. General framework

This research is part of the CONAF/JICA-Japan Watershed Restoration Project "Erosion Control and Forestry in Watersheds of the semi-arid zone of Chile", it has been extended 1994-2019, three 150 ha pilot areas have been located experimental and has allowed the successful development of nursery, reforestation and soil conservation .

Specifically, a micro-biological analysis of the evolution, biodiversity and quantification of bacterial strains are develop in the rhizosphere of:

1. Eroded soils (xeralf according to soil taxonomy classification).
2. Exotics coverages of *Eucalyptus camaldulensis* from Australia.
3. Native coverage of *Quillaja saponaria* endemic species of Chile.



2. Objectives

2.1 General objective

Evaluation comparative microbiological in the rhizosphere of vegetative cover of exotic species of Australia (Eucalyptus), native to Chile (Quillay) of “badland” soils in Chile.

2.2 Specific Objectives

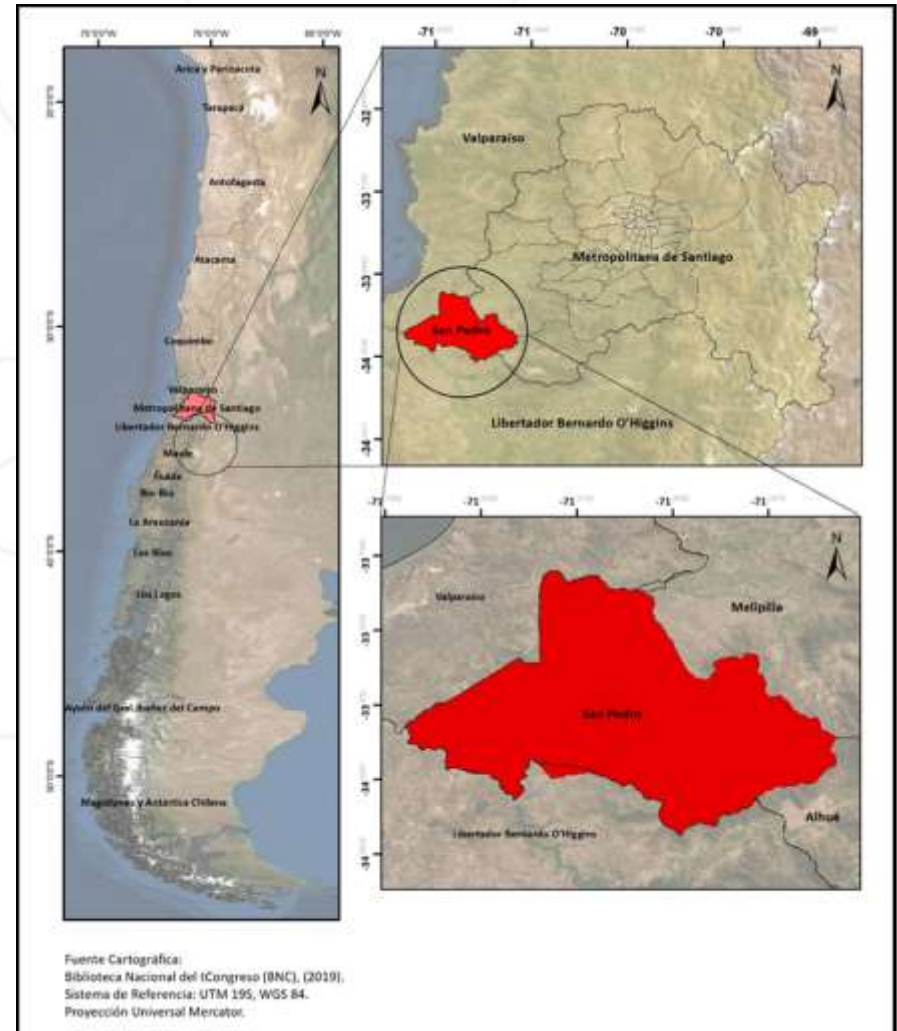
1. Evaluate the effects of comprehensive soil restoration treatments through the application of soil and water conservation techniques.
2. Evaluate the microbiological properties of the biodiversity of bacteria in rhizospheres of vegetative cover of exotic, native species and in eroded soils.
3. Propose a restoration model to provide ecosystem services through comprehensive approaches.



3. Material and method

3.1 Material

The area where the project is developed corresponds to the Metropolitan Region, in the central zone of Chile, parallel $33^{\circ} 46'$ and $34^{\circ} 04'$ South Latitude and meridians $71^{\circ} 12'$ and $71^{\circ} 42'$ West Longitude, 38 km Direct distance from the Pacific and 120 km from Santiago. It is located in the semi-arid zone of the inland coastal dryland of the central zone of Chile in the Maipo river basin



3.2 Methodological approach

Medium to low levels of organic matter are recorded, pH in the weakly acidic range, low electrical conductivity, low levels of N and P, medium and high levels of K , cation exchange capacity that varies from 1.8 (very low) to 16.5 (low).

Micro-watershed	Horizont	M.O %	pH	C.E mmhos/cm	N ppm	N Total %	P ppm	K ppm	CIC C mol/kg
MC-1	Ap	2.3	6.0	0.45	5	0.065	5	75	15.2
MC-2	Ap	1.2	5.8	0.27	10	0.066	8	38	16.5
MC-3	Ap	2.8	6.0	0.48	7	0.042	4	169	8.5
MC-4	Ap	2.8	6.5	0.63	6	0.092	4	111	1.8

Table 1. Analytical values of base fertility of eroded xeralf granitic soils of San Pedro Melipilla Chile.



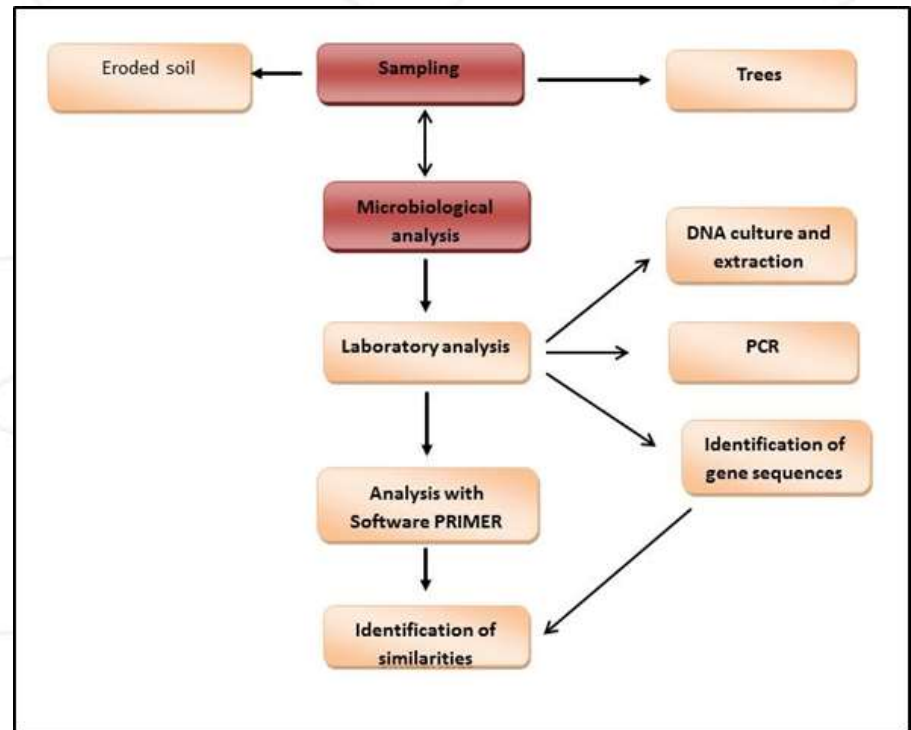
3.3 Methodology for soil microbiological analysis

- Soil samples are obtained in the rhizosphere of native tree species ("quillay"), an exotic tree (Eucalyptus camaldulensis) and eroded soil samples to establish comparative patterns. Samples are analyzed in the laboratory by PCR amplifying the 16S rRNA and subsequently, the analysis is carried out with the PREMIER software for quantitative analysis of the results.
- For the microbiological study, samples of the rhizosphere of a native tree are obtained, which in this case is "Quillay" (Quillaja saponaria), an exotic tree from Australia Eucalyptus (Eucalyptus cameldulensis) and from eroded soil (without vegetation) to establish comparison patterns.
- 5 samples of the rhizosphere of 2 trees (Quillay and Eucalyptus) and 5 samples of eroded soil are obtained; the 5 samples of each zone are obtained to homogenize the prospecting of samples. The methodology includes 15 samples in total, are analyzed in the laboratory and the nucleotide sequences that identify the respective bacterial families are determined.



The microbiological analysis in the laboratory is carried out based on three logical and successive steps:

- Extraction and cultivation of soil microorganisms (Molano, 2004).
- Extraction and purification of microbial DNA.
- Extraction and purification of microbial DNA (Department of Biology, Faculty of science, Puerto Rico's University).
- Application of PCR (Cienfuegos, et al, 2008 and Espinoza, 2007).



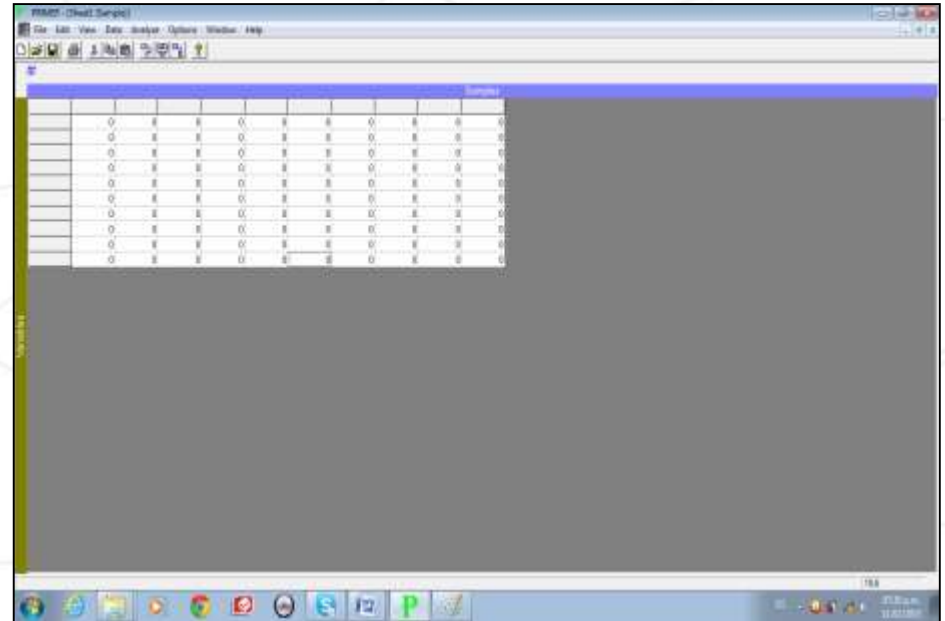
Graphic 1. Applied methodological sequence



3.4 Premier software template

Multivariate analysis, software analyzes information to perform statistical tests and graphical analyses of dendrograms are used. Information about bacteria genes is analyzed using PREMIER software.

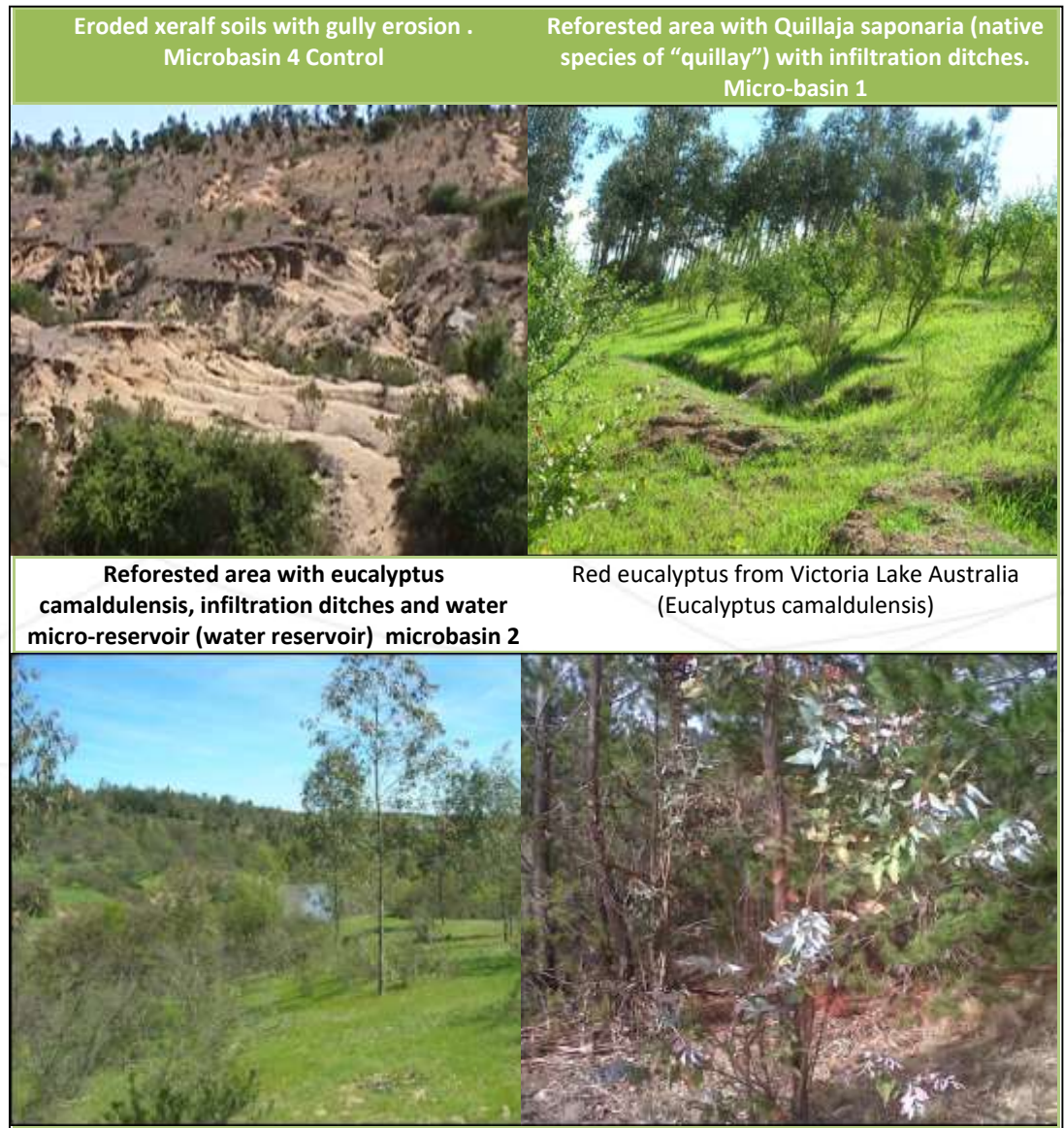
The application of the PCR technique allows to establish genetic differences between one family and another according to differentiation based on the application of an RFLP. The PCR results are analyzed with FIRST software, statistical test differences are established and quantified.



Picture 1. Premier software application



4. Presentation of results



4.1 Evolution of vegetative coverage in the treatments applied

The evolution of hydrographic basins in the last three decades has been a process characterized by a recovery in terms of vegetative biomass and edaphic conditions, reducing soil loss in reforested areas under soil conservation treatment from 30 ton/ha/year at 100 kg/ha/year ,based on evaluation of the USLE model applied to soil conservation works.

Tree biomass formed in the microwatershed under restoration according to the comparative values of NDVI (Normalized Difference Vegetation Index), between 1994 and 2010 clearly show a substantial increase in biomass and plant.

Microbasin description	Description	NDVI year 1990	NDVI year 2010	increase %
1	Afforestation	0.09	0.21	57.1%
2	Afforestation and erosion control	0.06	0.22	72.7%
3	Afforestation and erosion control	0.08	0.20	60%
4	Control witness	0.07	0.10	30%

Table 2. Microbasin description after NDVI 1990-2010



4.2 Bacterial microbiological evaluation according to bacterial similarity matrix

Of the 15 samples, 9 achieve acceptable levels of measurement, samples of 54 genes of 16S rRNA are obtained, and the similarity matrix is presented in table 3.

	1x	3x	5x	6x	7x	9x	12x	13x	14x
14x	52.385	60.982	36.250	61.331	61.744	63.350	80.620	72.478	
13x	55.013	63.623	42.357	56.332	57.020	57.916	76.525		
12x	57.471	63.227	42.481	63.654	61.322	60.311			
9x	48.218	58.758	36.706	73.710	79.280				
7x	51.907	62.965	37.461	69.597					
6x	53.151	53.606	44.912						
5x	66.167	51.673							
3x	64.157								
1x									

Table 3. Bacterial Similarity Matrix



According to the NMDS (non-metric multidimensional scaling) of bacteria, communities exhibit high similarity when they grow in the same environment. In this case, the bacterial communities of the rhizosphere of eucalyptus, quillay and bare (eroded) soil are significantly different depending on the vegetation cover.

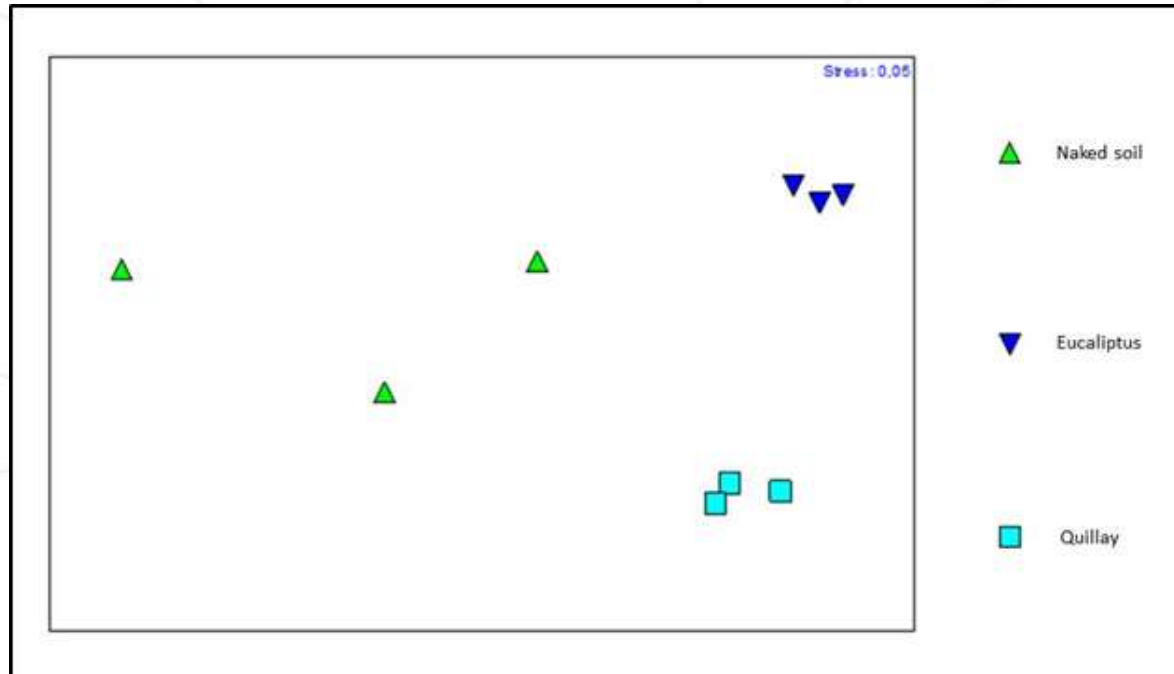
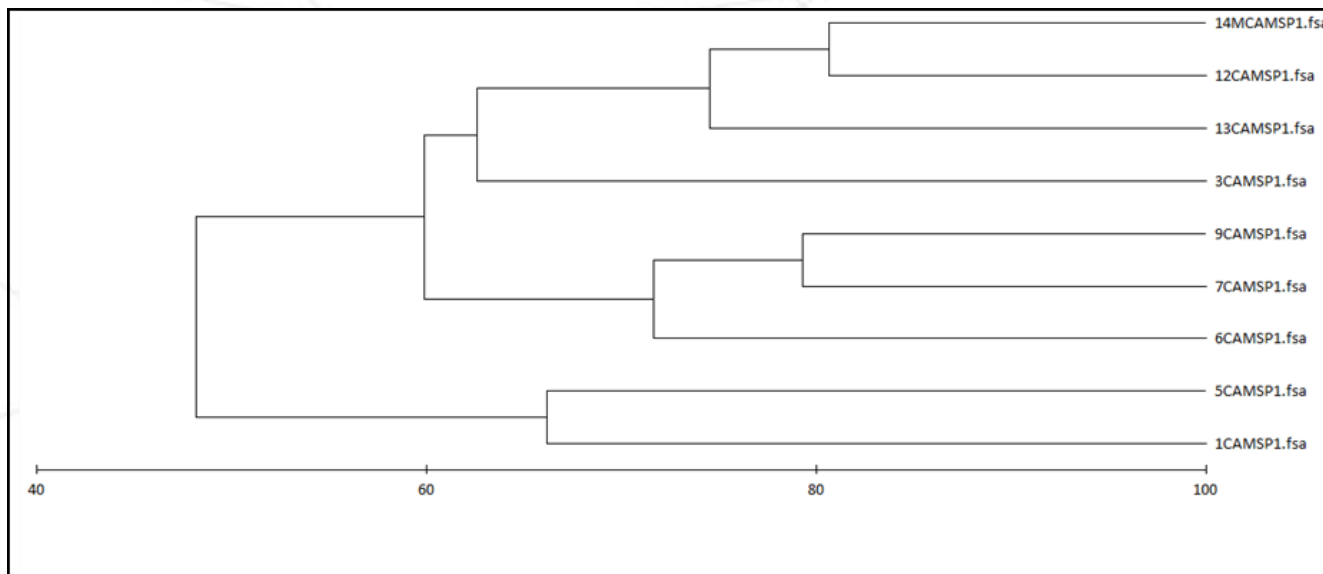


Figure 1. NMDS of bacteria



4.3 Evaluation of the degree of genomic variability of the bacterial edaphic communities

Samples that have a point in common correspond to similar bacterial communities; This is established by the similarity between the nucleotide sequences of 16S rRNAs. The 9 samples have a high diversity, which is visualized in the bifurcation of the tree dendrogram.



Graphic 2. Dendrogram of bacterial microbial communities "genomic variability" of the soils of San Pedro Melipilla Chile



4.4 Microbiological evaluation of bacterial activity according to similarity test

Type of cover	Abundance average (%)	Similarity average (%)	Similarity Sd	Contribution %
Badland xeralf	66.12	26.41	3.33	43.54
Soil of eucalyptus (exotic species)	62.72	20.94	10.11	28.22
Soil of quillay (native species)	51.30	18.93	9.32	24.73

Table 4. Percentages of similarity and contribution according to bacterial species



5. Conclusions and recommendations

- The *Eucalyptus camaldulensis*, a tree native to Australia of lake albacutia and the Quillay (*cryptocaria alba*) endemic tree species of Chile have evolved for thousands of years adapting to the various zone-climatic edafo-climatic conditions and “genetic memory”, which has led to a parallel evolution of the species bacterial soil.
- Key elements for bacterial adaptation have been the root exudates released by “Quillay”, which allows inferring that the root exudates differ from the Eucalyptus species as they belong to a different ecological and edaphic original environment.
- Bacterial biological soil crusts have demonstrated high efficacy to recover eroded and degraded soils. In this context, they contribute to the recovery and recarbonization of eroded soils through the induction of potential recovery processes and the generation of pedogenetic eco-system services, support and provision, in the interaction and synergy with soil and water conservation practices.



6. Thanks and bibliographic references

- We sincerely thank PhD. Marcela Wilkens, an academic from the Faculty of Chemistry and Biology of the University Of Santiago of Chile, conducted the research of environmental microbiology.
- Otherwise, PhD. Marlene Manzano, a biochemist at the Faculty of Biology of the Pontifical Catholic University of Chile, conducted genomic research.
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