Characterization and Comparison of Microbial Soil Diversity in Two Andean Peatlands in Different States of Conservation-Vega Tocorpuri

Carolina Belfiore¹, Ana Fernandez¹, Ana Paula Santos¹, Manuel Contreras², María Eugenia Farías¹*

¹Laboratorio de Investigaciones Microbiológicas de Lagunas Andinas (LIMLA), Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Tucumán, Argentina
²Centro de Ecología Aplicada (CEA), Suecia, Chile
Email: *mefarias2009@gmail.com

Abstract

Cerro Tocorpuri, belongs to the II region of Chile, in San Pedro de Atacama, on the border of Chile-Bolivia. The presence of a more or less constant supply of water conditions the existence of characteristic vegetation systems known as bogs (bofedales, vegas and marshes). These wetlands have a cultural, environmental and economic social importance. As a result of the exploitation of aquatic rights, peatlands began to dry up with the consequent loss of natural resources and damage to ancestral rights, and natural resources. The activities of microorganisms in wetlands play an important role in biogeochemical processes. The interaction between microbial diversity and soil, influences to the ability of the ecosystem to recover from stress (resilience). In the present work, the soil characteristics and the associated microbial biodiversity were studied, comparing samples of active and deteriorated peatland. It was seen that the loss of water causes great changes in the physical-chemical characteristics of the soil, which leads to a modification of the microbiota Proteobacteria decreased by 18% in deteriorated peatlands, which are evident more sensible to extreme conditions while Acidobacteria, Actinobacteria increased in these sample showing a better adaptation to the change of conditions. In view of the fact that high Andean Peatlands are exposed to increasing environmental impact, this preliminary comparative study of pristine and altered soil could guide the research directed to recovery of dead peatlands strategies.

Keywords

Peatland, Microbial Diversity, Altered Soil
1. Introduction

The Atacama Desert is the driest and oldest desert on Earth. Its surface conditions have remained relatively unchanged for millions of years [1] [2]. It has more than 100 basins with interior drainage, and most of them contain salt flats. These ecosystems are characterized by extreme aridity, strong winds, scarce but torrential rainfall, high rates of evaporation, high solar radiation, extreme daily temperature changes, negative water balance, combined with a wide range of salinity [1] [3] [4]. The salt composition is dominated by sulfate, chloride, sodium, and divalent cations [3] [5]. In spite of all of this, phototrophic and heterotrophic bacteria have recently been found in halite and gypsum evaporites and microbial mats [5] [6].

Cerro Tocorpuri, which belongs to the II region of Chile, is located at 5200 m a.s.l., in San Pedro de Atacama, on the border of Chile-Bolivia. Its coordinates are 22°25’60”S and 67°55’0”W in DMS (Degrees Minutes Seconds).

In regions I and II of Chile, the presence of a more or less constant supply of water, conditions the existence of typical vegetation systems known as peatland (bofedales, vegas and bogs), which technically belong to a humid environment [7]. Peatlands are plant formations that are established in an edaphic environment, mainly organic, characterized by a permanent saturated water condition, presenting a great biological diversity and a greater number of vegetal species with respect to the environment, which are characteristic of these systems. These wetlands are areas of valuable forage and watering hole threatened species conservation as vicuña, guanaco, llama, alpaca among others [7]. They are unique ecosystems, with a high vulnerability; the water is a fundamental factor that makes the development of the Andean biota possible in this high desert [8] (http://www.conaf.cl/humedales-chilenos-altoandinos-ecosistemas-estrategicos-de-importancia-internacional/).

The vegetation types correspond to biological ecological systems azonal, with characteristic vegetation due to the high and permanent soil moisture content [7] [9]. These wetlands have cultural, environmental and economic social importance, since it is the livelihood for communities [7].

The microbial processes in wetland are regulated for the hydrology [10] [11] [12] [13] [14]. Peatland began to dry up with the consequent loss of natural resources (flora and fauna) and the damages to the natural resources ancestral rights (including water) of indigenous communities, due to the acquisition and exploitation, the water rights by other non-agricultural uses (mainly mining) that are risking the sustainability and survival of these groups [7] [10].

It is known that the soil is a complex habitat with a large number of microbial populations [15]. The microbial diversity in soil ecosystems exceeds, by far, that of the eukaryotic organisms. Less than 1% of the billion microorganisms of the soil can be cultivated and characterized, soil ecosystems are, general, uncharted [16]. The activities of microorganisms in wetlands play an important role in biogeochemical processes and they are key to the functions of wetland [5] [17].
So it is necessary to know the microbial diversity to exploit the potential of the wetland ecosystems [18].

Although the interaction between the biodiversity and its function in the soil is not well studied, it is thought that has a positive influence on stability, productivity and resilience towards stress and disturbance [17]. They are responsible of regulating the dynamics of soil organic matter, carbon sequestration and emission of greenhouse gases. On the other hand, they modify the physical structure of the soil, enhancing the amount and efficiency nutrient acquisition for health of plants. Thereby, there is a conflict between industrial development and the protection of these natural resources [19]. Soil bacteria communities regulate wetland biogeochemical processes, however, there is little knowledge about their distribution and abundance [23].

Peatland degradation was associated with the changes occurred in the regional precipitation, the high water demand for lowland agriculture, urbanization and mining, all of them exceeding the availability of water [7] [10] [20] [21] [22]. In Atacama region water extraction for miner projects have increase in the last decades affecting peatlands. Their recovery is not a matter of water or vegetation restitution, since those strategies have failed, so the study of the microbiota is interesting.

The objective of this work was to study the soil characteristics and microbial biodiversity of soils associated to rhizosphere of wetlands, comparing a “deteriorated peatland (dead peatland)” with a “peatland in good condition (active peatland)”. The results would able to determine which are the essential biotic components in the rhizosphere in order to implement a recovery technology.

2. Material and Methods

2.1. Sample Sites and Sampling

Samples were taken in July 2015, from Tocorpuri Peatland, a place located in Second Region, Antofagasta, Chile.

Soils samples in different stages of preservation were collected, deteriorated soil (dead peatland) and soil in good condition (active peatland), as shown in Figure 1(a) and Figure 1(b) respectively.

2.2. DNA Extraction, PCR and Pyrosequencing

Total genomic DNA was isolated using the Power Biofilm DNA Isolation Kit (MO BIO Laboratories, inc.) according to the supplied protocol. Extracted DNA samples were amplified with F357 and R926 primers (NIH HMP Working Group, 2009). The reactions were performing according [81]. All sequences of the pyrosequencing runs were deposited in the NCBI Sequence Read Archive (SRA) database under the following accession number SRP120032.

2.3. Chemical Analysis and Moisture Content

The determination of organic material (OM), C (%), Nitrates (%), P (ppm), S
Figure 1. Photographs of peatland in different state of preserving: (a) dead and (b) active peatland.

(%) was carried out by standard methods in pH7 diagnóstico agrícola.

To calculate the % moisture for each sample, 10 g of the material was weighed into petri plate (pre-weighed), and the sample was dried for 24 hs in a 100°C oven (to constant weight). The moisture content was calculated using the following equation:
W = [(PP + wS) – (PP + DS)/(PP + DS) – PP] * 100

W: % moisture; PP: Petri Plate; wS: wet Sample; DS: Dry sample.

2.4. Scanning Electron Microscopy

The samples processed as described by [80] was observed in Zeiss Supra 55vp (Carl ZeissNTS GmbH, Germany) scanning microscopy in the Centro Integral de Microscopía Electrónica (CIME-CONICET-UNT).

2.5. Microbial Analysis

Ten grams of active and dead peatland samples were suspended in 90 ml of peptone water (bacteriological peptone, 0.1%) and vortexed thoroughly for 10 min. From these stock solutions, serial dilutions were performed and plated in duplicated on each culture media: total counts on Agar nutritive incubated for 48 h at 30˚C. On the other hand, BG11 medium [24] was used to culture cyanobacteria, the Erlenmeyer flasks were incubated under Light: Dark cycles (16:8 h) at 28˚C ± 1˚C during 14 days. The algal specimens will be observed under binocular microscopes Leitz SM Lux and Zeiss Axio 1. Determination to gene level will be based on [25].

3. Results and Discussion

Many times result difficult to separate soil functions into chemical, physical, and biological processes due of the dynamic nature of these processes. Chemical properties of numerous soil directly influence microbiological processes, and these, together with physical-chemical processes determine, the capacity to hold, supply, and cycle nutrients and on the other hand, the movement and availability of water [26].

Comparative physical chemical analysis between both samples showed a clear evidence of a higher percentage of moisture present in active peatland (92%) compared with dead peatland (47%). Soil pH is an important factor that affects directly the availability of nutrients and the chemical characteristics of the environment. The microorganisms have optimal pH ranges for their growth, when altered in the environment; significantly modify microbial density [27]. Soil pH levels near 7 are optimal for overall nutrient availability and crop tolerance [28]. On the other hand, the most of microorganisms grow better in mineral soils with neutral pH values, and they significantly reduce their activity when the pH is below 5.5 or above 9.0 [29]. In our studies to the pH in active peatland, the values were slightly alkaline (7.8) while in dead peatland they were strongly alkaline (9.1). The causes of change in pH are not clear but, probably the decrease of the vegetation in the dead peatland is influencing the increase of the pH since it is known that the Cation exchange may be the most important mechanism for the generation of acidity in peatlands. There is a direct relationship between pH and the exchangeable metabolic activity of the plants [30].

The conductivity measurements show that in active and dead peatland, they
have low conductivity values (0.25 dS/m and 0.28 dS/m respectively). The electrical conductivity was used to classify the samples as slightly saline soils because the values obtained in both cases were among 0 - 0.2 dS/m. As shown in Table 1 all physical parameters were higher in active sample. Soil organic matter (OM) is a key indicator of soil quality, intervenes in multiple functions in the soil, such as the ability to include nutrient retention, water holding capacity, and soil aggregation [28] [31]. This value is higher in the conserved peatland. With regard to the content of S, P and N-NO₃ as shown in Table 1, are higher in the active sample. In general, in soils with pH 6.5 to 8, these nutrients would be more available [28], therefore, in active peatland, they are more readily available than dead peatland where the pH value was higher.

Nitrogen is an important, growth-limiting nutrient in many peatlands [32] [33]; it can be added to the peat surface through atmospheric deposition or fixed by microbes [34] [35]. Our result showed that N-NO₃ content in active sample is significantly higher than in dead peatland. Moreover, sulfur plays an important role in the oxidation-reduction biogeochemistry of peatlands, influencing the production and emission of methane to the atmosphere [36] [37] [38] [39] and the mineralization of C [39] [40]. It can be supplied to peatlands by groundwater or like nitrogen through atmospheric deposition [41]. Peat S concentrations range greatly from 10 to 100 ppm in a Minnesota bog [42], to 800 - 7000 ppm in peatlands in West Virginia, Czechoslovakia and Alberta [40] [43] [44]. In our work, values of 502 ppm were obtained in the active peat, values that are higher than that found in the dead sample (102 ppm).

The microbiological state of the soil is a critical element in the studies of microbial ecology. The total viable counts on agar nutritive showed differences between analyzed samples. The number of cultivable microorganisms was higher in active peatland 8.89 ± 0.18 log CFU ml⁻¹ compared with 6.85 ± 0.3 log CFU ml⁻¹ in dead peatland.

The soil is considered ecologically habitat extremely stable [45]. The number and types of microorganisms present in a soil depend on various environmental factors such as nutrients, moisture, aeration, temperature, pH, agricultural practices, etc. [46]. Some microorganisms of a habitat may be dominant. The populations and the structure of the community tend to be kept constant, however, this homeostasis and dominance of certain populations can be disrupted when

<table>
<thead>
<tr>
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<th>Dead peatland</th>
<th>Active peatland</th>
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<tbody>
<tr>
<td>Organic material (%)</td>
<td>3.78</td>
<td>29.18</td>
</tr>
<tr>
<td>C (%)</td>
<td>1.89</td>
<td>14.59</td>
</tr>
<tr>
<td>N-NO₃ total (%)</td>
<td>24.30</td>
<td>260</td>
</tr>
<tr>
<td>P (ppm)</td>
<td>17.80</td>
<td>21.10</td>
</tr>
<tr>
<td>S (ppm)</td>
<td>150</td>
<td>502</td>
</tr>
</tbody>
</table>

Table 1. Analysis of soil samples.
environmental conditions are altered. In the case of “dead peatland” samples, water is the main factor that affects the structure of microbial communities, affected mainly by human activity. To elucidate differences in microbial community structure between wet and dry soils, pyrosequencing was undertaken. The results obtained reveal that the most of the 16 S rRNA gene sequences were affiliated with *Proteobacteria, Actinobacteria* and *Acidobacteria* in both samples, and they were the most responsive phyla to change in study samples. As shown in Figure 2, *Proteobacteria* decreases in dead peatland while *Actinobacteria, Acidobacteria* and *Gemmatimoidetes* increases in dead peatland. *Bacteroidetes, Firmicutes, Chloroflexi, Chlorobi, Latescibacteria, Cyanobacteria* and *Nistriospira* were found in the same proportion in all samples.

![Figure 2. Comparison of the microbial diversity associated with active (a) and dead (b) peatland.](image-url)
Regarding proteobacteria phylum there is a decrease of the 18% in dead soils, comparing with active soils. The classes Alpha, Gamma, Beta and Delta are in all samples; however abundance in each sample was different. In dead soils there is a decrease of the percentage (33%) comparing with proteobacteria present in active soils (51%). The Alphaproteobacteria were more abundant 26% and 18.8% in active and dead sample respectively, while Gammaproteobacteria in active peatland was 7% higher that their presence in dead peatland. These results are in concordance to that published by [47]; who reported that Proteobacteria, mainly Gammaproteobacteria, decreased in abundance with desiccation in Chinese and Japanese soils.

The Actinobacteria are a group of bacteria with a highly active secondary metabolism, they are found in large amounts in the peat layers, decomposing cellulose and other plant polymers [48]. They are important in the decomposing and degrading of mixture of organic polymers and plays an interesting role to the recycling nutrients associated with recalcitrant polymers [49] [50] [51]. An advantage over Gram-negative soil bacteria is their ability to spread through relatively dry soil and to survive in adverse conditions [52]. Previous studies have demonstrated that this phylum was drought resistant and able to grow under extreme dry conditions [53] [54]. Our results shown, that Actinobacteria increased in relative abundance with desiccation. This results is agrees with the response of bacterial communities in California grassland soil study [55] and the results obtained by [47] [56] who shown that this phylum were one of the most responsive to change in water availability; increased with dry-down and decreased with wet-up.

One of the most abundantly distributed bacterial groups in the environment corresponds to Acidobacteria [57] [58] [59]. Its members have been detected in numerous 16S rRNA gene surveys from different environments as soil, sediment, fresh-water, marine and extreme environments [57] [58] [60]. It is known that access to nutrients becomes more limited when the water film thickness is reduced by drought [61]. Bacteria have strategies to surviving desiccation [62] [63], for example recently it was seen that Acidobacteria produces exopolysaccharides. Our results showed that Acidobacteria increased 5% in dry peatlands, which shows the resistance of this group to environmental conditions.

DeBruyn [64] observed that the highest proportions of Gemmatimonadetes were found in arid soils, suggesting an adaption to low-moisture environments, which explains the higher percentage of these phyla, found in the dead samples.

With regard the phylum Bacteroidetes, have colonized virtually all types of habitats on Earth [65]. They are frequent members of microbial mats, due to their ability to degrade organic compounds [66]. Their presence is similar in wetter soil (10%) compared to dry soil (9%). About Chlamydiae, this group has not been previously associated with arid soil communities [67]. A recent study, that evaluated growth rates of soil bacteria found that Chloroflexi is a slow-growing bacteria [68]; this type of bacteria typically have a good tolerance to drought conditions.
which would explain its presence in dead peatland samples. It is known that Firmicutes produce endospores under stressful environmental conditions [70], this group appear en both samples in the same proportions, as well as Nitrosi- rae that was found in equal proportions in both samples.

Latescibacteria with cyanobacteria are present in low proportions (1%). Latescibacteria, little is known about this phylum; however Youssef et al., 2015 suggest that latescibacteria transform algal detritus sinking from sunlit surface waters into fermentation products to contribute to microbial food webs in waters bellow. On the other hand some members of Latescibacteria may be capable of forming greigite magnetosomes, and play unrecognized roles in iron and sulfur cycles [71].

Although, cyanobacteria dominated the bacterial populations of many extreme environments [72], and many studies reveal the ability of this phylum to withstand large periods of drying [62] [73] [74] [75] [76] [77]. Nevertheless the scarcity of cyanobacteria in the present work is in accordance to the observations of a low cyanobacterial presence from the Salar de Atacama [5] [78] [79] [80] [81].

From the active peatland samples, it was possible to isolate different genera of cyanobacteria (Figure 2), and they could be observed under a microscope as shown Figure 3(A). However, in the samples from the dead peatland, they were absent. Our results are in accordance with [82], who reported a low or absent cyanobacterial signatures in arid soils of the McKelvey Valley, and on the other hand with the molecular-based studies by [83] [84] [85] [86], inform that cyanobacteria are being absent at all arid studies sites. Electron microscopy revealed a clear difference between active and dead samples (Figure 3). It can be observed strong association between roots and Cyanobacteria (Figure 3(A)) with the presence of diatoms (Figure 3(C)), and Prokaryotes (Figure 3(B)). On the other hand, in the dead sample, inorganic remains were observed and there were no roots as in the active sample (Figure 3(D)).

Diatoms and cyanobacteria were isolated from preserved peatland using BG11 medium. Four type of cyanobacteria were identified: Oscillatoria sp.; Lyngbya sp.; Nostoc sp. and Dolichospermum sp. and with regard diatoms five type grew: Ulnaria sp.; Cocconeis placentula; Pinnularia sp.; Nitzschia sp., and Rhopalodia sp. (Figure 4).

It is known that the diatoms respond quickly to any subtle changes in the environmental conditions and hence they are the most promising tools in biomonitoring [87] [88]. Diatoms are the only unicellular partners to form associations with heterocyst forming cyanobacteria [89]. Heterocysts are specialized nitrogen fixing cells present in some filamentous cyanobacteria, such Nostoc, Cylindrospermum, Anabaena and Dolichospermum. Some of them are present in the samples studies.

This work began the studies of soils associated with peatland in different states of preservation. Although this is only a first approach to the microbial ecology of
these environments, it can be proposed that the loss of water causes great changes in the physical-chemical characteristics of the soil, which leads to a modification of the microbiota.

As conclusion, the results showed that, in the dead peatland samples there was a decrease of Proteobacteria mainly Alpha and Gamma, moreover an increase of Acidobacteria and Gematimoidetes in approximately 5% was observed. On the other hand Actinobacteria phyla increased markedly in dead peatland samples.
Evidently is more resistant to the extreme conditions. The microbial response to desiccation would reflect adaptation strategies. The recovery of these lands should not only consist on the hydration of the soils but also in the bioinoculation of the native organisms. In that way, proteobacteria isolated from healthy soils would be the main candidates to develop a bioinoculation starter.

Ongoing research is developing inoculums with microorganisms isolated from “healthy soils” in order to produce a biofertilizer for the recovery of peatland dried by miner activities in Andean Altiplane.

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