# Microbial communities in Pampa soils : impact of landuse and climatic conditions

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# *Chapter 7*
# General discussion

The aim of the research described in this thesis was to assess the impact of land-use changes in conjunction with soil type and seasonal climatic variations on the composition, diversity and dynamics of the soil microbiome in the Pampa ecosystem. To reach this goal, microbial communities across different soil types and land use systems were analyzed. Furthermore, a microcosm experiment mimicking seasonal natural variation in soil moisture and temperature was performed. I used a combined approach of molecular fingerprinting, next-generation sequencing and network approaches to assess the soil microbiome.

In this chapter, I will discuss:

a) the methodology used to explore the total microbial community, including sampling strategy, molecular fingerprinting techniques, high-throughput sequencing, the separation of active and dormant populations, and network approach, which allows to assess microbial co-occurrence and keystone species;

b) the importance of soil type and soil climate to shape the structure of microbial communities and to determine their diversity;

c) how land-use changes through the removal of natural vegetation (grassland and forest) and introduction of anthropogenic uses may affect the diversity, structure and function of microbiome of the Pampa ecosystem;

d) ideas and directions for future studies on land-use changes in the Pampa ecosystem.

## *7.1 Methodological approaches: assessing the total microbial community in soil*

The field studies in *Chapters 2, 3, 4* and *6* can be classified as observational in which randomization is restricted solely to selecting samples from the population of interest and no manipulation of experimental conditions is performed. The approach used in this experimental design sometimes generates controversy among the scientific community as it indicates a specific limitation regarding the statistical concept of repetitions. Some researchers argue that this type of experiment cannot have true landscape level of replication but rather pseudoreplication and, therefore, it would not be a statistically valid experimental design for testing hypothesis. This view gained popularity ever since the work published by Hurlbert (1984), whose released his review and critique to ecologists, in matters of misconceptions in experimental design and statistical treatments emphasizing the need of genuine replication. However, after the publication of this study, the critical reevaluation of pseudoreplication has been discussed in a significant number of scientific articles. Hargrove and Pickering (1992) argue that, landscape-level experiments are often not possible and the nature of landscape-scale studies precludes replications in the way they are constructed in classical experimentation, which requires true replicates (Hurlbert 1984; 2009; Krebs 1999). However, Schank and Koehnle (2009) support the idea that pseudoreplication is a pseudoproblem that sets impossible standards for the majority of the experimental designs and analyses of experiments. My experiments, as case study, provided an unique opportunity to investigate the effects of land-use changes on microbial communities in areas that are otherwise nearly identical in terms of physiography and microclimate. Thus, the sampling strategy used in *Chapters 2, 3, 4* and *6* supported my conclusions on the effects of land-use changes on soil microbial community in the Pampa

biome at a local scale.

Cultured-based methods provided the base for the knowledge on microbial identification, physiology and ecology (Handelsman 2004). Not discarding the importance of standard culture techniques for investigation of the ecology of microbial communities (Bevivino et al. 2014), it is noteworthy that they are biased in their evaluation of microbial diversity. These methods select a certain set of microorganisms which are easily cultivable (*e.g., Proteobacteria, Firmicutes*) rather than the ones, that are abundant in soil and other ecosystems, but difficult to culture (*e.g.*, *Acidobacteria*) (George et al. 2011). Based on the most recent classification schemes, there are 30 phyla in the domain Bacteria and 5 phyla in the Archaea domain with cultivated representatives (Euzéby 1997). Thus, as the total number of phyla reaches more than 52 (Rappé & Giovannoni 2003) (candidate phyla included) many major groups cannot be cultured and are known solely via cultured independent methods (called candidate phyla) (Hugenholtz et al. 1998). These facts provide sufficient evidence that methods that circumvent the need for cultivation are preferable for the assessment of microbial communities in a highly diverse and complex ecosystem such as the soil. Aiming to obtain thorough understanding of the soil microbiome, I therefore chose to survey the soil microbial community in all of the work described in this thesis using uncultured-based approaches.

After amplification by PCR, a variety of *fingerprinting* techniques are available for analysis of microbial communities (van Elsas & Boersma 2011). In *Chapter 2*, I used the RISA approach (Ribosomal Intergenic Spacer Analysis - (Borneman & Triplett 1997), to get insight into how soil type and land-use changes shift the structure and diversity of the archaeal and fungal communities. Based on length heterogeneity of the ITS (Internal Transcribed Spacers) region, the comparison of samples showed that each soil type or land use was characterized by a specific community profile, demonstrating the

effectiveness of this method to detect differences in the structure and diversity of the microbial community from different ecological niches. However, though this approach is suitable to study community shifts induced by land-use changes and soil types (as in *Chapter 2*), archaeal and fungal diversity and composition need to be explored in more details in order to obtain full understanding of the impact of these factors. The complexity of microbial communities often hampers the detection of subtle changes by *fingerprinting* approaches, since these methods do not provide taxonomic identities, and do not allow for the detection of populations present at low abundance (Ranjard et al. 2001; van Elsas & Boersma 2011). Although diversity indices based on molecular *fingerprinting* are generally much lower than based on high-throughput sequencing methods (Jami et al. 2014; Chen et al. 2015), both approaches are capable of recovering highly comparable trends related to shifts in community diversity and structure pointing to similar conclusions about the processes shaping microbial communities (Pilloni et al. 2012; Castro-Carrera et al. 2014; Verbruggen et al. 2012; Xia & Jia 2014; Cleary et al. 2012).

The high-throughput sequencing allows for significant steps forward in our understanding of complex microbial communities due to the efficiency and unbiased nature of the sequencing (Margulies et al. 2005; Lauber et al. 2009; Rodrigues et al. 2013). The massive sequencing enables the assessment of the microbiome from the top to the bottom, *i.e.,* from the most abundant species going down into the rare biosphere (Pester et al. 2010; Lynch & Neufeld 2015). As the main objective of this study was to obtain a deep understanding and taxonomic identification of the members of the microbiome, I surveyed the microbial community in *Chapters 3, 4, 5* and *6* using next-generation sequencing. Through the chapters, it is possible to see the evolution of sequencing technologies. In 2005, a breakthrough in massive DNA sequencing was announced (Margulies et al. 2005), introducing the pyrosequencing method associated with barcode indexing (Hamady et al. 2008) (used in *Chapters 3*, *4*

and *6*). In 2010, Ion Torrent approaches became available (used in *Chapter 5*), in which base-sequence composition is determined by measuring pH variation, thereby reducing the complexity of the equipment and the cost of sequencing reactions (Whiteley et al. 2012), but with the same accuracy to assess the microbial community as other sequencing methods (Yergeau et al. 2012).

Since the next-generating sequencing technologies are very recent, sequence processing and analysis approaches are still in progress and may fail by artifacts introduced by amplification and sequencing errors (Schloss et al. 2011). The most popular bioinformatic pipelines for sequence data processing, *i.e.*, QIIME (Caporaso et al. 2010) (used in *Chapters 3* and *4*) and mothur (Schloss et al. 2009) (used in *Chapter 6*) provide algorithms for reducing artifacts including steps such as quality filtering, denoising, chimera filtering and clustering (Quince et al. 2011). However, many biases and spurious OTUs often remain, confounding inferences of community structure and diversity (Bokulich et al. 2012). Recently, a new package has received considerable attention. The UPARSE pipeline (used in *Chapter 5*) generates a number of operational taxonomic units (OTUs) consistently closer to the expected number of species in a community (in comparison with a "mock" community), as compared with pipelines recommended by QIMME and mothur, in which the number of OTUs, often, far exceeds the number of real species (Kunin et al. 2010; Edgar 2013).

Based on evidence that the rRNA content correlates well with growth rate and activity (Fegatella et al. 1998; Muttray & Mohn 1999; Campbell et al. 2011), calculating the rRNA:rDNA ratio is an effective approach to differentiate the response of the active and dormant fractions of a microbial community to environmental variations (DeAngelis and Firestone, 2012; Hugoni et al. 2013; Schostag et al. 2015). This approach has been proved to be sensitive to detect the dynamics of the active community rather than the single assessment by rDNA (Dlott et al. 2015) and to predict the contribution of

dormant cells to the maintenance of microbial diversity (DeAngelis et al. 2010; Jones & Lennon 2010). In *Chapter* 5, I showed that habitat selective factors may exert different effects on active and dormant communities as the rRNA:rDNA ratio differed along moisture and temperature variations in soil, which shows the potential of this approach to get an accurate picture of the dynamics within microbial communities. However, more studies with cultured representatives of the microbial communities in different ecosystems are needed to further confirm the usefulness of this approach and the functional relevance that it represents (Dlott et al. 2015).

When properly constructed, network analyses linked to ecological questions can reveal co-occurrence patterns and keystone species ("hubs") thus providing a robust tool to develop and test hypotheses on within-community processes. As an alternative approach to previous studies described in this thesis, where I checked for shifts in microbial diversity and abundance, in *Chapter 6*, I explored the potential of network approaches to predict keystone species and examine microbial interactions in a range of natural and anthropogenic land usages. First of all, the network topology may provide answers to questions about the level of connectance of microbial members of the network, *i.e.,* the proportion of possible interactions between species that are realized in a specific time and space frame (Bissett et al. 2014). The resultant network topology in *Chapter 6* conforms to the scale-free and small-world model (Chaffron et al. 2010), where most of the nodes (representing microbial genera) are not neighbors (small degree, *i.e.,* few connections between near-by nodes) but most of them can be reached from another one by a small number of connections (positive and negative links between nodes). The level of connectivity, assessed by closeness centrality (denote the distance of one node to another one) indicated that most soil bacterial members were important to the connectance of the whole microbial community, making the microbial community robust to changes, which may explain the resistance of the soil

microbiome to disturbances (Montoya et al. 2006).

Analysis of the networks in *Chapter 6* also highlighted that each land-use system presented a different and specific set of potential key species mainly belonging to different genera of the phyla *Proteobacteria, Actinobacteria*, *Chloroflexi*, *Bacteroidetes,* and *Firmicutes.* These key members of the microbial communities may act as the main intermediaries between microbial groups - they may have fewer connections, but mediate more group associations. The keystones have a large impact on community composition shaping the interactions with other members and could be more important to soil processes than species richness and abundance *per se*, especially in soil ecosystems. Eiler et al. (2012) also detected numerous "hubs", representing phylogenetic groups with strong interdependencies with other taxa. They suggest that in highly complex environments, like the soil, there may be hundreds of such keystone species. If important members in a community get lost by anthropogenic disturbances, the patterns of the microbial interactions would change dramatically and important soil process may be altered (Zhou et al. 2011; Montoya et al., 2006; Steele et al. 2011; Berry & Widder 2014).

## *7.2 Soil type and climate: overriding determinants of the soil microbiome*

Previous studies have found soil type as one of the most important factors determining the structure and functioning of soil microbial communities (Girvan et al. 2003; Suzuki et al. 2009; Takada Hoshino et al. 2011). Bossio et al. (1998), ranked the impact of various environmental variables governing the composition of microbial communities, with soil type ranked as the most important variable explaining the abundance of microbial groups, followed by time (representing different seasons) of sampling, farming operation, management system and spatial variation in the field.

Results from my study showed that soil type and associated soil properties may, indeed, be important explanatory factor for the structure of archaeal and fungal communities (*Chapter 2*). The data obtained from different soils along the toposequence also indicate that archaeal and fungal communities may be differentially controlled by soil type likely due to physiological and ecological contrasts between prokaryotic and eukaryotic groups (Wakelin et al. 2008; Pereira e Silva et al. 2012). While archaeal communities are largely influenced by soil type and associated chemical properties (Taketani & Tsai 2010; Chen et al. 2010; Takada Hoshino et al. 2011), fungal communities are less dependent of soil type and more dependent of environmental changes, such as land-use changes (*Chapter 2*), fertilization and management intensity as shown earlier by Oehl et al. (2010) and Suzuki et al. (2009).

Distinct bacterial and fungal communities have been associated with soils of varying pH (Lauber et al. 2009), moisture (Rogers & Tate 2001), P content (Faoro et al. 2010) and texture (Girvan et al. 2003). Overall, as described in *Chapter 2*, the soil properties did not vary consistently with soil type in the toposequence (*e.g.*, pH around 5 and clay around 95-100 g $kg^{-1}$). However, soil moisture ranged from well-drained to saturated conditions and also P-content was different between soil types, which, therefore, may be the most important factors explaining the divergence in community structure along the toposequence. Similarly to our results, also Rogers and Tate (2001) found that the topographic position can influence microbial community structure and activity through effects on moisture and vegetation along a toposequence.

Yet, as detected in *Chapter 3,* the bacterial community in the same soil type was not affected much after removing the natural vegetation and introducing agricultural crops and/or exotic tree plantation. Each soil type may harbor a specific bacterial community able to grow and proliferate in the particular environment of specific combinations of chemical and physical

characteristics (Delmont et al. 2014). Therefore, it may have a large buffering capacity to conserve its diversity against the impact of land usages or management practices (Wieland et al. 2001; Reeve et al. 2010).

One of the likely explanations of this resistance of the soil microbiome structure and functioning to disturbance is the existence of a large and dominant inactive fraction within the soil microbial community. In *Chapter 5*, I showed that, indeed, the dormant community comprises an important source of microbial diversity in these soils, reflecting in the diversity patterns of the total community. In environments exposed to strong fluctuations, as in my soils, dormancy or the ability to enter a state of reduced metabolic activity is supposed to be a life history strategy for the majority of microorganisms, preventing the extinction of microbial species (Jones & Lennon 2010).

The active community is more affected by moisture as well as by temperature, whereas the dormant and the total community were affected solely by moisture, indicating the dominance of water content regulating the diversity, structure and composition of microbial assemblages in soil. Overall, our results are consistent with previous studies showing that water content plays an important role in the composition and diversity of microbial communities over seasons (Valverde et al. 2014; Waldrop & Firestone 2006; Bouskill et al. 2012). Temperature has long been recognized to be a determinant for the microbial assemblages at global scales (Lipson 2007; Ding et al. 2015). However, temperature appears to be of less importance at local scales, particularly in subtropical and temperate ecosystems where the community might contain a widely adaptive (*e.g.,* functional plasticity and dormancy) capacity to variations in temperature as compared to tropical organisms that are adapted to little seasonal variability (Bardgett et al. 1999; Schindlbacher et al. 2011; Wallenstein & Hall 2011).

Despite being potentially different in biological traits, active and dormant communities are controlled by the same assemblage process along

moisture regimes. My results suggest that the relative influence of niche and neutral processes vary along the moistures gradient, with niche-based mechanisms being more influential at low water content, *i.e.,* at greater environmental stress. At low water content (8% moisture), phylogenetic clustering may be the rule, resulting in lower diversity due to habitat selection (Valverde et al. 2014). At high water content (16% and 23% moisture), there is a decline in phylogenetic clustering, indicating that this community is more influenced by random or neutral-based processes. It has been demonstrated, indeed, that in more benign environments (*e.g.*, "wet" habitats), the presence of abundant water and heterogeneities in substrate supply may lead to distinct niches increasing the diversity and richness and decreasing the importance of the habitat filtering (Chase 2007; Valverde et al. 2014).

## *7.3 Effects of land-use changes on the soil microbiome*

### *7.3.1 Diversity*

In the last decade, the loss of biodiversity has become a global concern as more and more evidences becomes available showing that it will negatively affect the functionality of the ecosystem (Wagg et al. 2014), such as nutrient cycling for plant growth (Bartelt-Ryser et al. 2005), plant productivity and soil health (*e.g.*, disease suppression) (Mazzola 2004), being key to a sustainable society. For soil ecosystems, recent global predictions indicate that grassland ecosystems likely will experience the greatest change in biodiversity because of large scale land-use changes being the main drivers of biodiversity losses (Sala et al. 2000; Souza et al. 2013). Not only above-ground, but also below-ground organisms are affected severely by land-use changes, as shown earlier (Lorenzo et al. 2010; Carbonetto et al. 2014).

Because most soils with agricultural crops and exotic tree plantations receive considerable chemical inputs which change directly or indirectly the

soil environment, it is conceivable that microbial diversity in these soils will be altered and potentially reduced compared to the native soils of the Pampa ecosystem (Cardinale et al. 2012; Carbonetto et al. 2014). Moreover, studies linking microbial assemblages and above-ground plant communities provide empirical support to the prediction that a decrease in plant diversity will lead to a decrease of microbial diversity (Lange et al. 2015). However, contrary to these predictions, in *Chapters 2, 3* and *4*, I demonstrated that relatively short-term disturbances (*i.e.,* less than 15 years of changes in land usage through removal of grassland and forest vegetation) of plant diversity and composition did not deplete archaeal and bacterial richness and diversity. This was in line with earlier observations that in agriculture sites or in sites with low vegetation diversity (generally as a result of converting a natural site into agricultural land) bacterial diversity was not reduced as compared to native sites (da C Jesus et al. 2009; Ding et al. 2013). The clearest decrease in diversity was observed for the soil fungal communities at *Eucalyptus* and *Acacia* plantations (*Chapter 2*). The differences in quality and quantity of litter compounds generated by these two exotic species may be the main factors acting as selective pressure towards specific fungal species, such as specific decomposers, capable of degrading specific substrates (Macdonald et al. 2009; Lauber et al. 2013). Furthermore, the decrease of light availability, decrease in microhabitat heterogeneity, and the proliferation of strong competitors may also have contributed to the observed decrease in fungal richness (Jacquemyn et al. 2003). It has been suggested that the fungal community recovers slowly after disturbances, because these microorganisms tend to be less resistant and resilient to disturbances than *e.g*., bacteria are (Hedlund et al. 2004; de Vries et al. 2012).

However, it is also important to recognize that similarity in diversity or richness does not mean that species composition is the same: the same diversity, but different microbial structure and composition, as found in this

studies, might indicate that, under the influence of environmental changes, microbial communities have gradually been replaced by another community composed of species with different abundances and phylogenetic relatedness that survive better under the new conditions (which I called substitution hypothesis in *Chapter 3*). Moreover, the apparent diversity stability to disturbance might be explained by microbial dormancy. Only a fraction of the bacterial community appeared to be metabolically active (*Chapter 5*) and the large dormant portion of microorganisms may contribute to diversity stability via generation of a long-lived seed bank (Chesson 2000) and niche complementation (intraspecific differences in resource and habitat use by active and dormant members) (Cordero & Polz 2014).

### *7.3.2 Structure and core microbial community*

Environmental disturbances caused by land-use changes such as changes in nutrient availability (Bardgett et al. 1999), pH (da C Jesus et al. 2009), soil density (Jiao et al. 2012), soil temperature (Zogg et al. 1997) and moisture (Drenovsky et al. 2010) lead to alteration in the soil microbiome composition and functioning in grasslands (Wakelin et al. 2013). Based on the differences between natural (*e.g.,* grassland and forest) and anthropogenic (*e.g.,* soybean and *Eucalyptus*) usages and associated management I expected a strong effect on the microbial structure associated with the shifts in soil properties. However, this was not the case for all land uses examined here. The results described in *Chapter*s *2*, *3* and *4*, indicate that the differences in microbial community structures of the land use systems evaluated here were primarily based on differences in relative abundance of specific microbial groups rather than on their presence/absence (*Chapters 3* and *4*).

Relatively short-term disturbances as in the studies described here may not always drive strong alterations in microbial structure. Marshall et al.

(2011) already indicated that microbial communities are relatively insensitive to short-term changes in plant composition, as they may respond only gradually to associated changes in rhizodeposition, litter quantity and quality and soil properties. The apparent stability of the microbial community structure as observed in *Chapters 2*, *3* and *4*, have also been reported previously by Araújo et al. (2013). The current community structure may have been determined largely by historical events (*e.g.,* prevalence of vegetation type, weather conditions as well as soil type) rather than by the current disturbances (Martiny et al. 2006). It is also possible that the apparent stability of the soil microbiome is conferred by the intra-community interactions and the presence of microbial key species (*Chapter 6*), as argued by some authors (Bissett et al. 2013; Peura et al. 2015).

Although a large fraction of the soil microbiome did not show great shifts, alterations in the abundance of specific microbial groups were detected (*Chapters 3* and *4*). These groups were not dominant but collectively represented a considerable part of the differences observed among samples. According to Bissett et al. (2013), this pattern occurs because the degree of resistance and resilience differs among microbial groups and may vary according to (i) the sensibility to environmental disturbances, *e.g., Acidobacteria* may be sensitive to alteration in Ca and Mg - (*Chapter 2*) (Navarrete et al. 2013), (ii) the effects of these disturbances on other organisms which microorganisms interact with *e.g.,* rhizobia are linked with the presence of leguminous plants in the soybean field (*Chapter* 3) (Sugiyama et al. 2014), (iii) differences in ecological functions *e.g.,* higher concentration of $NH_4^+$ in natural forest (*Chapter 3*) may have favored the growth and activity of the *Nitrospira* group, resulting in alteration of the nitrification process, as depicted by the higher amounts of $NO_3^-$ + $NO_2^-$ in soils (Schramm et al. 1998; Meyer et al. 2013) and, (iv) their abundance, *e.g.,* rare taxa are more sensitive to changes, since they are more vulnerable to extinction than abundant ones in

the short term disturbances caused by human activities (Gaston 2008).

I detected a large overlap of microbial taxa between soil types and land usages within sites, suggesting the existence of a resistant or even resilient "core microbiome" that did not suffer any changes related to shifts in soil properties or plant cover (*Chapters 2, 3* and *4*). The core microbiome can be defined as the fraction of microorganism shared among habitats and presumed to play key roles in ecosystem functioning (Shade & Handelsman 2012). Since the soil microbiome is the result of thousands of years of soil formation processes, climate dynamics and vegetation developments (Tarlera et al. 2008; Berg & Smalla 2009), it is plausible that the current microbiome is comprised of a core of species completely adapted to the prevailing natural environmental conditions of the Pampa ecosystem and largely resistance against short- or even longer- term anthropogenic disturbances of the biotic and abiotic ecosystem properties (Yasir et al. 2015; Montecchia et al. 2015).

Similar to the findings described in this thesis, Montecchia et al. (2015) detected the existence of a core microbiome, composed of a large proportion of OTUs persistent at three land usages (forest, short- and long-term agriculture), and resistant to disturbances caused by changes in land use. Similarly, Orgiazzi et al. (2013) found ubiquitous fungal taxa present in different ecosystems (soil types and land usages), which they classified as generalist fungi, with oligotrophic and chitinolytic abilities, again suggesting the presence of a stable core adapted to nutrient-poor soil conditions and with the ability to exploit organic resources broadly distributed in soils. However, the function and importance of the core microbiome is far away from being understood as only few studies have been directed to the understanding of this stable compartment of the microbial community in soil (Bacci et al. 2015; Orgiazzi et al. 2013). Nevertheless, the core microbiome may become a vital strategic factor in the development of sustainable agricultural practices in future *e.g.*, for management of plant diseases, in a similar way as proposed in some reports as

"transfer therapy" realized in gut and rhizosphere (Gopal et al. 2013; Mendes & Raaijmakers 2015). Even though microbial diversity and structure can be shifted by ecosystem disturbances, the core microbiome would allow for maintaining at least some of the key soil functions after conversion of natural vegetation to agricultural land or tree plantations (Montecchia et al. 2015).

### *7.3.3 Relationship between structure and functions of microbial communities*

The measures of broad-scale functions, such as biomass production and potential microbial activity (based on the metabolic quotient - $qCO_2$) did not converge with the 16S rDNA sequence (*Chapters 3*, bacterial and archaeal communities) and ITS profile (*Chapter 2*, archaeal and fungal community) data, supporting the idea that structure and functioning of microbial communities are not necessarily correlated. There are several reasons for the contrasting patterns in structure and functioning of microbial communities as the result of land use changes. One is that only function but not community structure responds to a disturbance (Frossard et al. 2012). Another reason, confirmed here, and by others (Allison & Martiny 2008; Bowen et al. 2011), is that once a certain level of microbial diversity is reached in soil under either natural or anthropogenic conditions, all key functions exist within the different communities, and so differently structured communities may exert similar functions. Also functions may be limited by other factors such as water content and temperature which do not directly affect significantly composition or diversity (Bissett et al. 2011). And in case of intensive management, as in agricultural lands and tree plantations, there may be a time lag in the structural responses of the microbial community while functional responses may be faster (Berga et al. 2012).

## *7.4 Final conclusions and future directions*

I showed that changes in land usages by the removal of natural vegetation and introduction of crops and exotic trees do not always lead to a reduction of microbial diversity or to extreme modifications in community structure and composition. Yet I showed that specific microbial groups, key species and patterns of microbial co-occurrence were sensitive to anthropogenic impacts, but how these shifts influence the soil ecosystem functioning remains obscure. It was not possible to assess all combination of crops and exotic tree plantations on the Pampa ecosystem, but even although no potential high risk level on the Pampa ecosystem was observed, we should be concerned about the long-term impacts of disturbances and how it will influence soil microbial diversity and, as a consequence, functioning.

Several factors may act as drivers of communities in a natural ecosystem, including soil type, land usages and seasonal climatic variations. To better understand the emergent patterns of microbial structuring and functioning observed here, ecological aspects of the soil microbiome such as the level of resistance/resilience, physiological status and microbe-microbe interactions in combination with the type, level and time of perturbations have to be considered in more details. Bacteria seem not to be the most responsive group of microorganisms to short-term disturbances, whereas the fungal community showed to be more sensitive to variations in the above ground plant community, which calls for deeper investigations in this and others disturbance sensitive groups.

The studies described here are useful for identification of the ecological processes that link biodiversity and ecosystem services, assisting in future restoration, monitoring programs and to meet policy objectives regarding the consequence of changes in the Pampa ecosystem. Future studies should address how the patterns of microbial community dynamics within the Pampa

biome and other grasslands are linked to key ecosystem processes such as nutrient cycling, plant growth promotion, and overall ecosystem health. In order to better investigate the potential threats of land-use changes, the development of a more integrative and multidisciplinary research approach encompassing biotic and abiotic ecosystem processes combined with analyses of "meta-omics" processes (metagenome, transcriptomics, proteomics and metabolomics) will improve our ability to decipher how environmental traits moderate changes in the soil microbiome and the above-below ground feed-back mechanisms operating in the Pampa biome.

## *7.5 References*


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