U N I V E R S I D A D D E C O N C E P C I Ó N
PROGRAMA DE POSTGRADO
PROGRAMA MAGÍSTER EN CIENCIAS FORESTALES

“EFECTOS DE LA DEGRADACIÓN DE UN BOSQUE NATIVO TIPO FORESTAL RO-RA-CO SOBRE VARIABLES BIOGEOQUÍMICAS, RESERVORIOS DE CARBONO, NITRÓGENO Y FÓSFORO, DIVERSIDAD DE MICROORGANISMOS Y ACTIVIDAD ENZIMÁTICA EN SUELOS”

TESIS PARA OPTAR AL GRADO DE MAGÍSTER EN CIENCIAS FORESTALES

Por

ALEJANDRO ESTEBAN ATENAS NAVARRETE

PROFESOR GUÍA: FELIPE ABURTO G.
PROFESOR CO-GUÍA: GERARDO GONZÁLEZ R.
PROFESOR CO-GUÍA: CAROLINA MERINO G.

CONCEPCIÓN – CHILE
2022

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Comisión evaluadora

Felipe Aburto Guerrero. (Profesor guía) _____
Ingeniero Agrónomo, PhD.

Gerardo González-Rocha. (Profesor co-guía) _____
Biólogo, PhD.

Carolina Merino Guzmán (Profesor co-guía) _____
Biólogo, Dr.

Director de Postgrado:
Regis Texeira _____
Ingeniero Químico, PhD.

Decano Facultad de Ciencias Forestales:
Manuel Sánchez O. _____
Ingeniero Forestal, Dr.

“By myself,

but never alone”

AGRADECIMIENTOS

A mi profesor guía Felipe Aburto Guerrero, por su constante apoyo, confianza, comprensión y creer en mí durante el desarrollo de mi tesis y trabajo de analista durante estos años.

A mis profesores co-guías Gerardo González-Rocha y Carolina Merino Guzmán por su apoyo, comentarios y consejos que me brindaron durante este proceso.

A Foresta Nativa-ENEL por aportar con los fondos para hacer posible el desarrollo de esta investigación y mi postgrado. Así también al laboratorio AMESUVOS-UFRO por los análisis de enzimas realizados por su personal analítico.

A los profesores del postgrado de Ciencias Forestales, sus interesantes y constructivos cursos que cursé durante el desarrollo de mi Magister.

Al equipo y amigos del Laboratorio de Investigación en Suelos, Aguas y Bosques (LISAB) por todo el apoyo al debate de ideas, apoyo anímico y las risas durante las extensas jornadas de análisis. También a los estudiantes involucrados en labores de muestreo y procesamiento de muestras que involucró esta investigación.

A mi pequeño círculo cercano de amigos que siempre han estado ahí para dar una palabra de aliento, saber guardar espacio y por sobre todo comprender la falta de tiempo que muchas veces impidió juntas.

A mis padres Juan Carlos Atenas y Carmen Gloria Navarrete por nunca dejarme caer, por su constante apoyo, por interesarse en los temas que he decidido investigar y por siempre darme la libertad de decidir mi propio camino acompañado siempre de una buena conversación y consejos. También, a mi hermana Karen Atenas Navarrete, por estar siempre presente a pesar de la distancia, ya sea en una llamada o en un chat, siempre compartiendo con la familia perruna (Zaby y Poochie).

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2. GENERAL ABSTRACT

To evaluate the effect of forest degradation on soil biogeochemical properties we quantify the main C, N and P pools, soil microbial community (bacteria and fungi) via NGS-Illumina sequencing and microbiome analysis, and soil enzymes activities related to C, N, and P cycle along an anthropogenic forest degradation gradient that comprise four long term ecosystem research (LTER) plots: i) mature forest (MF), ii) secondary forest (SF), iii) degraded forest (DF), and iv) deforested site converted into a prairie (DP). Our research showed that prairie soils (DP) were more compacted and displayed greater amounts of TC, TN and TP than forested soils at topsoil (i.e., TN: 22.25 ± 2.34 Mg ha^{-1} and 11.7 ± 2.07 Mg ha^{-1} in average respectively). However, MF had greater TC and TN below 200 cm soil depth than DP soils (TC: 64.48 ± 21.87 Mg ha^{-1} and 9.52 ± 1.53 Mg ha^{-1}; TN: 6.86 ± 2.69 Mg ha^{-1} and 1.17 ± 0.47 Mg ha^{-1}). The bacterial microbiome was dominated by *Proteobacteria* (45.35±0.89%), *Acidobacteria* (20.73±1.48%), *Actinobacteria* (12.59±0.34%), and *Bacteroidetes* (7.32±0.36%) phyla in all sites. The soil fungal community was dominated by the phyla *Ascomycota* (42.11±0.95%), *Mortierellomycota* (28.74±2.25%), *Basidiomycota* (24.61±0.52), and *Mucoromycota* (2.06±0.43%). The DP soil microbiome was significantly less diverse in bacteria (D' = 0.47±0.04) and more diverse in fungi (H' = 5.11±0.33). The AOB community rose as a dominant group in the DP soils along with a reduction in the ECM fungi community. Only two out of ten enzyme activities (GAP and POD) showed a significant change after forest conversion to prairie (i.e, GAP: 6.35 ± 6.60 $x10^2$ µg g^{-1} soil in forested soils and 86.32 ± 5.04 $x10^2$ µg g^{-1} soil in DP); however, no consistent change was found among forested conditions. Forest degradation and especially land-use conversion altered soil C and nutrient pools depth distribution, stocks, microbial communities and soil enzyme activity. Forest degradation and especially land-use conversion altered soil C and nutrient pools depth distribution, stocks, microbial communities and soil enzyme activity. These profound changes have the potential to affect soil and forest ecosystem services and conservation.

3. GENERAL HYPOTHESIS

- Degradation of native forest by unregulated logging causes a decrease in carbon, nitrogen and phosphorus content, not only at the surface soil level, but also in deep soils (~ 300 cm depth), thus reducing soil quality and health.
- Mature forest soil will have higher total carbon, nitrogen and phosphorus pools than degraded forest soil, as well as a greater diversity of microorganisms and enzymatic activities.
- Degradation of native forest and land use change cause changes in microbial communities that negatively affect the provision of soil ecosystem services by the microbiota.

4. GENERAL OBJECTIVE

- To quantify the effect of the degradation of a native forest type RO-RA-CO forest on the carbon, nitrogen and phosphorus pools, the diversity of microorganisms (bacteria and fungi), and the activity of extracellular enzymes related to carbon, nitrogen and phosphorus cycling.

5. SPECIFIC OBJECTIVES

- Determine the carbon, nitrogen and phosphorus pools in soil profiles and their stoichiometric relationship along a gradient of native forest degradation type RO-RA-CO.
- To evaluate the diversity and richness of soil microorganisms (bacteria and fungi) in a degradation gradient of native forest type RO-RA-CO.
- Determine the enzymatic activity of extracellular soil enzymes linked to the biogeochemical cycling of carbon, nitrogen and phosphorus.

I. Anthropogenic Disturbances Alter Biogeochemical Pools and Microbial Diversity in surface soils of Andean Temperate Forests.[1]

Abstract

Soil microorganisms are a vital component and regulate a myriad of functions of forest ecosystems. Anthropogenic disturbances in natural forests could drive major shifts in plant and microbial communities resulting in substantial biogeochemical alterations. We evaluated the effect of anthropogenic disturbances in soils in an Andean temperate forest with different levels of degradation: i) mature forest (MF), ii) secondary forest (SF), iii) degraded forest (DF), and iv) deforested site converted into a prairie (DP). We quantified total carbon, nitrogen, and phosphorous (TC, TN, and TP) soil and nutrient available pools. Microbial communities' structure and diversity were assessed under each condition via NGS-Illumina sequencing and microbiome analysis. There were no significant differences in TC, TN, and TP across the forested states (MF, SF, DF). The deforested site condition presented significantly higher surface soil TC, TN, and TP and the lowest C:N, C:P, and N:P ratios. The bacterial microbiome was dominated by *Proteobacteria* (45.35±0.89%), *Acidobacteria* (20.73±1.48%), *Actinobacteria* (12.59±0.34%), and *Bacteroidetes* (7.32±0.36%) phyla in all sites. Yet, there were significant differences at the genus level across conditions. The soil fungal community was dominated by the phyla *Ascomycota* (42.11±0.95%), *Mortierellomycota* (28.74±2.25%), *Basidiomycota* (24.61±0.52), and *Mucoromycota* (2.06±0.43%). The DP soil microbiome was significantly less diverse in bacteria (D' = 0.47±0.04); however, it was significantly more diverse in fungi (H' = 5.11±0.33). Forest to prairie conversion facilitated the introduction of novel bacterial and fungal groups associated with livestock grazing. The AOB community rose as a dominant group in the DP soils along with a reduction in the ECM fungi community. The surface soil microbiome was surprisingly resistant to forest degradation and did not show a clear progression along the degradation gradient. However, the microbiome was strongly altered after forest conversion into grassland.

Keywords: Soil microbiome; soil carbon stocks, soil nutrients; forest degradation; Land-use change; deforestation.

[1]Alejandro Atenas Navarrete, Felipe Aburto Guerrero, Gerardo González-Rocha; Carolina Merino Guzmán, Radomir Schmidt, Kate Scow, and Enzo Álvarez Lara. In Revision. STOTEN.

1. Introduction

A healthy ecosystem is characterized by the integrity of its nutrient cycles and energy flows, stability, and resilience to disturbances. Soil health is determined by ecological characteristics and system functionality (Karlen et al., 1997; van Bruggen and Semenov, 2000). Thus, soil health is intrinsically associated with biological diversity and stability (van Bruggen and Semenov, 2000). Forests harbor numerous microhabitats, among which soil quantitatively represents the most important and diverse habitat for soil microorganisms (Baldrian, 2016). All food web members are dependent on soils as sources of nutrients for the degradation and cycling of organic compounds. The soil microbial community plays a crucial role in multiple ecosystem functions such as i) soil aggregate stabilization, structure – gas movement and storage, root growth (Garcia-Franco et al., 2015); ii) nutrient availability – nutrient cycling, nutrient mining, biodegradation (Glaser et al., 2018; Hu et al., 2016); iii) ecosystem stability – functional redundancy, both general (e.g., niche filling) and specific (e.g., biocides) resistance to invasion (van Bruggen and Semenov, 2000).

Disturbances or stress episodes change the structure of soil microbial communities leading to a succession in communities, where the extent and duration of successional changes depend on the intensity, frequency, and duration of the disturbances (Anderson, 2003; Domsch et al., 1983; van Bruggen and Semenov, 2000) which in turn directly affects ecosystem stability (Choi et al., 2017; Lladó et al., 2017; Lladó et al., 2018).

Understanding soil microbial community structural shifts following land-use change are critical to adjusting management and conservation practices and improving soil function and services (Acosta-Martínez et al., 2008). Molecular biology-based techniques have proven effective in assessing these changes (De Mandal and Panda, 2015; Eaton et al., 2017). Kim et al. (2013 used 454 pyrosequencing to show how soil bacterial diversity decreased as disturbance frequency increased, leading to community composition, abundance, and dominance changes. Mirza et al. (2014 described how the community composition of free-living nitrogen-fixing microorganisms changed because of the conversion from forest to grassland, associating these changes with soil acidity, total nitrogen, and C/N ratio.

In Chile, native forest covers approximately 13.4 million hectares of the national territory (19 % of the area), representing more than half of the temperate forest in the southern hemisphere (Miranda et al., 2017). The Roble - Raulí - Coigüe (*Nothofagus* spp.) forests stand out among Chilean forest types because of their high endemism, high productivity, wood quality, and the wealth of current knowledge about its basic ecology (CONAF, 2017; Rojas et al., 2012). Despite their relevance, these forests have been replaced by more intensively managed agricultural or forest plantations and are increasingly threatened by wildfires, uncontrolled timber extraction, livestock grazing, and the introduction of exotic species. These disturbances have degraded many of the remaining natural forests (Donoso and Promis, 2013; Echeverria et al., 2006) and soils in the region (Crovo et al., 2021a; Crovo et al., 2021b; Marquet et al., 2019; Nahuelhual et al., 2012). Most local studies have focused on the effect of degradation on C and nutrient cycling in soils due to forest degradation. For example, Dube and Stolpe (2016 showed a significant decrease in soil organic matter (SOM) and total carbon of forest biomass. However, there is minimal information about microbial diversity changes and their relation to nutrient and C pools in temperate forests (e.g., (Marín et al., 2017)).

The present study involved a sequencing analysis of topsoil samples from 4 Long Term Ecosystem Research plots (LTER) following a forest degradation gradient to explore how forest degradation of primary or secondary forests and forest conversion to typical mountainous grassland systems influence soil microbial communities and soil biogeochemical cycles. We hypothesized that increasing forest degradation progressively modifies soil microbiome, diversity, and soil elemental stoichiometry (C:N:P), displaying a potential ecological threshold after deforestation.

2. Materials and methods

2.1 Site description

The sampling sites were in the National Protected Area Ranchillo Alto, near Yungay, Ñuble Region, in the Andes Cordillera's piedmont (Figure 1A). Climate is Temperate Mediterranean with marked wet and dry seasons, a long-term MAP of 3000 mm (mostly fallen as snow), and a MAT of 13.5°C. Soils were formed from post-Last Glacial Maximum ash deposits overlying fluvial and fluvioglacial materials (Roa and Varela, 1985). The soils are mapped as a member of the Santa Barbara Soil Series (CIREN, 1999; Stolpe, 2011), and they are classified as members of the Pachic melanudands (Soil-Survey-Staff, 2014). Forests are dominated by the Roble-Raulí-Coigüe (RO-RA-CO) forest type (CONAF, 2017). These forests have been affected by unregulated logging (locally known as *floreo*) and livestock grazing. The conservation of this forest is critical as they are part of the Nevados de Chillán - Laguna del Laja biological corridor, which is recognized as a biodiversity hotspot by UNESCO (https://en.unesco.org/biosphere/lac/chillan-lajas).

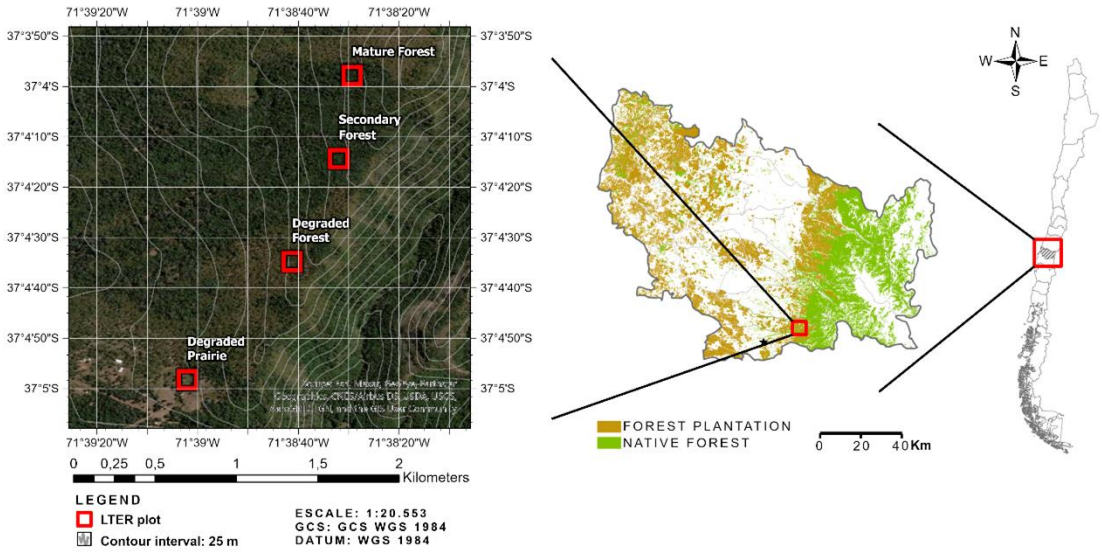

Figure 1. At left side map of BNP Ranchillo Alto site. Red square represents LTER plots that follows natural forest degradation gradient from north to south. At right side map of Ñuble Region, Yungay town (black star) and Ranchillo Alto sector (red square). In brown planted forest (*Pinus* and *Eucaliptus* spp.). In green natural forest. Datum WGS 1984, Huso 19S.

2.2 Permanent plots

Four squared 1 ha long-term ecosystem research plots (LTER) (Figure 1B) were established following standardized protocols described by (Marthews et al., 2014). These plots represent the most common forest conditions found within the study area. Three of the LTER plots are Andean temperate forest stands (Figure 2) with different levels of anthropogenic disturbance that have resulted in changes in vegetation composition, diversity, and structure. An additional LTER plot was established in a neighboring farm and corresponds to a deforested site used for grazing livestock (i.e., DP: Degraded Prairie condition) (see Table 1).

Table 1. Characteristics of the study sites.

Cover Type	**Site code**	**Condition**	**Vegetation**	**Coordinates**
Mature forest	MF	Non-degraded	*Nothofagus dombeyi*	-37.06626°S, -71.64149°W
Secondary forest	SF	Medium degraded	*Nothofagus alpina*	-37.07065°S, -71.64221°W
Degraded forest	DF	Highly degraded	*Nothofagus obliqua*	-37.07632°S, -71.64478°W
Degraded Prairie	DP	Forest to prairie conversion	Degraded prairie with few isolated tree patches	-37.082844°S, -71.650566°W

2.3 Soil sampling

We collected soil samples at each 1ha plot representing each state of forest degradation. In each permanent plot, nine sampling points were located 25 m from each other and the plot's borders with a soil auger (n = 9). Before soil sampling, the litter layer and organic horizons were carefully removed. Samples were taken at two depth intervals from 0 to 15cm and 15 to 30 cm. Only the upper 15 cm of soil was included in the soil microbial community analysis as this portion of soil corresponds to the topsoil. Soil samples were immediately stored in hermetically sealed bags and kept cold (approximately four °C) during transport to the Soil, Water and Forest

Research Laboratory (LISAB) of the Universidad de Concepción (UdeC). In the laboratory, soil samples were sieved at <2 mm to remove large roots and rocks fragments. These samples were split into two aliquots. The first was immediately

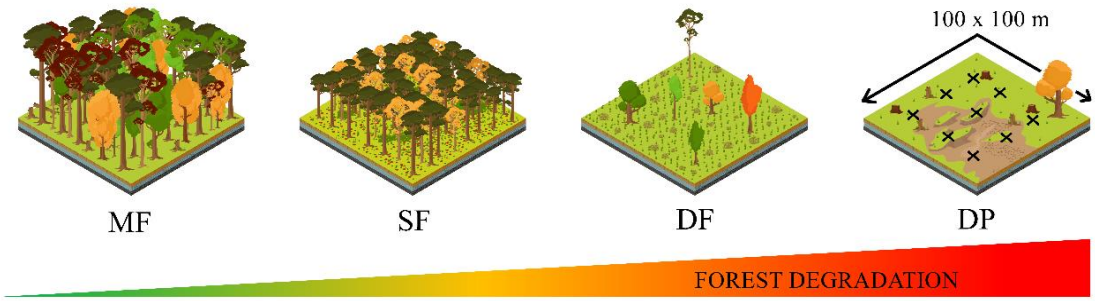

Figure 2. Scheme of 1 ha square permanent plots. From left to right MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie. Green to red bar indicates magnitude of forest degradation. The X marks on DP plot shows the relative position of auger sampling points (n = 9). Figure modified from Foresta Nativa (www.forestanativa.org).

stored at -80°C for DNA analysis (bacteria and fungi), while the second was air-dried at 25°C for nutritional analysis. An aliquot of the latter was oven-dried for moisture content determination and C, N, and P elemental analysis (see below).

2.4 Soil Nutrients and physical properties

Total carbon (TC) and total nitrogen (TN) were determined by the Dumas-TCD dry combustion method with an isotope ratio mass spectrometer (IRMS) coupled to a GSL solid sample preparation module (20-20 IRMS-GSL, SERCON® Limited, UK). Available nitrate (NO_3^-) and ammonium (NH_4^+) were determined according to Miranda et al. (2001. Total phosphorus (Pt), $P_{inorganic}$ (P_{ino}), $P_{organic}$ (P_{org}) were determined according to Bowman and Moir (1993. NO_3^-, NH_4^+, and all P fractions were determined colorimetrically with a Shimadzu UV-mini 1240 spectrophotometer. Physical properties such as bulk density (BD), porosity, moisture content (CH), and the textural class of each soil (sand, clay, and silt content) were determined according to Sadzawka et al. (2004. Soil C, N, and P stocks for topsoil were calculated using Eq. (1).

$$X_{stock} = \sum_{i=0}^{n} \frac{Xi}{100} \times BDi \times \frac{SLTi}{100} \qquad (1)$$

Where X_i represents the element (C, N, and P) concentration in % at a given soil horizon *i* (%), BD_i is the soil bulk density of the *i* depth interval (Mg m^{-3}), SLT_i is the

soil interval *i* thickness (cm). All elemental concentrations were expressed in megagrams per hectare of soil (Mg ha^{-1}). We also calculated C:N, C:P, N:P, and C:N:P ratios for elemental stocks.

2.5 Soil genomic DNA extraction and amplification

Soil genomic DNA was extracted from 12 soil mineral samples (3 composited replicates per LTER plot). According to the manufacturer's directions, total bacterial and fungal DNA extraction was performed from a 0.25 mg aliquot of soil (dry equivalent) using the PowerSoil DNA isolation kit (Mo Bio®). The integrity of the extracted DNA was evaluated by agarose gel electrophoresis, quality and concentration of extracted DNA were assessed based on absorbance ratios of 260/280nm (~1.8) and 260/230nm (>1.7) by using an EPOCH spectrophotometer (BioTek, USA), the yield of extracted DNA was determined as the ratio of the mass of total DNA obtained per extraction to the mass of soil used. Extracted DNA was stored at -20°C until sequencing. A region of the 16S rRNA gene was amplified for bacteria and the ITS1 region for fungi. For bacteria, the universal primer pair 27F (5'-AGA GAG TTT GAT CCT GGC TCAG-3') and 534R (5'-ATT ACC GCG GCT GGC TGC TGG-3') was used. For fungi, the ITS1F (5′-CTT GGT CAT TTA GAG GAA GTA A-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) primer pair was used. All samples were amplified in triplicate and then mixed; these were detected by 2% agarose gel electrophoresis. Twelve total amplicons were obtained (3 amplicons x 4 plots).

2.6 Sequencing and characterization of soil microbial community

Amplicons obtained from PCR were sequenced on an Illumina HiSeq 2500 platform (~400bp paired-end reads) at UC Davis Genome Center (Davis, CA, USA). Paired-end reads obtained were processed with the DADA2 package (Callahan et al., 2016) on R Project software (R-CoreTeam, 2019). The quality profile of reads was assessed and then trimmed to remove primers and sequences with more than one error per 100 bases, filtered to remove chimera sequences, and processed for identifying operational taxonomic units (OTU) with an identity level of ≥97%. OTUs taxonomic assignment was performed with two databases for Bacteria, the Ribosomal Database Project (RDP) and SILVA databases (Balvočiūtė and Huson,

2017; Van Elsas et al., 2019). Fungi OTUs taxonomy assignment was performed with UNITE database (Nilsson et al., 2018). Finally, the filtered OTU table obtained was processed to remove samples having reads below 150 bases, as they are usually uninformative and indicate low-quality reads (Baldrian, 2016).

2.7 Soil core microbiome analysis

The core microbiota, or core taxa, has been defined as the members shared by most microorganism assemblages from similar habitats. It has been suggested that the core taxa may play an essential role in community function (Shade and Handelsman, 2012). We assessed the soil core microbiota composition to the genus level in the three types of forests and the prairie. This information was used to identify core microorganisms in each soil and help determine soil ecosystem functions that may have been affected due to these changes.

2.8 Statistical analysis

A Shapiro-Wilk normality test ($\alpha = 0.05$) was performed on each of the nutritional variables to determine the type of statistical test to apply. TC, TN, C:N, C:P, N:P ratio, pH, and CH means of the different degradation states were evaluated through one-way ANOVA with a significance level $\alpha = 0.05$. NO3-, NH4+, PT, Pi and Po did not meet the normality requirement for ANOVA; thus, they were evaluated through a Kruskal-Wallis test with a significance level $\alpha = 0.05$ (Supplementary data 1). All analyses were performed using the R statistical software platform (R-CoreTeam, 2019).

Data obtained by mass sequencing were analyzed with R statistical software using mainly the DADA2 and Microbiome packages (Lahti et al., 2012 - 2017). The Microbiome package has more statistical tools to its advantage, which help to evaluate and plot the different alpha and beta diversity indices of the microbial community (bacteria and fungi) present in the soil samples, with UniFrac and Bray-Curtis metric distances (Lozupone and Knight, 2005), using similarity analysis (ANOSIM) implemented in the R package Vegan and Phyloseq according to Baldrian (2016 and Griffin et al. (2017.

3. Results

3.1 Soil biogeochemical reservoirs and physical properties

Soil C and nutrients did not significantly vary along with the natural forest degraded sites, but forested sites differed from the DP site. Soil pH was substantially more acidic in the DP (5.63±0.14) compared to the three forest sites (MF, SF, DF). Total carbon was similar across all forested areas. On the other hand, total carbon and nitrogen content was significantly higher in DP (9.13±1.20 Mg ha^{-1}) compared to the forest sites. Nitrate concentrations increased as forest degradation increased, becoming markedly higher in DP (12.37±4.32 g kg-1). Ammonium did not show differences between conditions. Total, inorganic, and organic phosphorus was significantly higher in DP but did not vary between the three forest sites (MF, SF, DF) (Table 2).

Our analysis reveals that deforestation has significantly affected C:N, C:P, and N:P stoichiometry (Table 2). C:N ratio was significantly lower in DP given the higher total carbon pool at this site. C:P ratio did not show differences among the three forest conditions; however, C:P ratio was considerably lower in the degraded prairie (significantly lower than MF and SF). The same trend was found for the N:P ratio.

The conversion to prairie also altered soil texture, which changed from sandy loam in the three forested sites (MF, SF, DF) to silt loam texture in the DP site (higher silt contents in the deforested site). Bulk density ranged from 0.49 to 0.59 g cm^{-3}, with lower bulk density observed in MF and DF than SF and DP. The latter resulted in lower soil porosity for these two sites (Table 2). Water content was greater in the MF and decreased as degradation progressed.

Table 2. Soil pH, nutrient stocks, soil C:N:P stoichiometry, and soil physical properties at the 0-15 cm soil depth interval in the forest degradation gradient.

Soil Properties	Sites			
	Matured Forest (MF)	**Secondary Forest (SF)**	**Degraded Forest (DF)**	**Degraded Prairie (DP)**
pH	5.36 ± 0.10a	5.41 ± 0.13a	5.49 ± 0.17ab	5.63 ± 0.14b
TC (Mg ha^{-1})	99.71 ± 12.69a	119.31 ± 20.88ab	92.99 ± 20.88a	128.77 ± 20.64b
TN (Mg ha^{-1})	5.66 ± 0.72a	5.87 ± 1.08a	5.44 ± 1.38a	9.13 ± 1.20b
NO_3^- (g kg^{-1})	1.70 ± 2.49a	1.22 ± 1.13a	3.44 ± 3.08a	12.37 ± 4.32b
NH_4^+ (g kg^{-1})	7.61 ± 2.82	8.95 ± 2.78	7.63 ± 1.61	6.86 ± 0.97
TP (Mg ha^{-1})	0.78 ± 0.13a	0.95 ± 0.22a	1.10 ± 0.47a	2.27 ± 0.38b
P_{ino} (Mgha^{-1})	0.07 ± 0.02a	0.06 ± 0.02a	0.07 ± 0.01a	0.18 ± 0.01b
P_{org} (Mg ha^{-1})	0.71 ± 0.14a	0.89 ± 0.24a	1.03 ± 0.47a	2.08 ± 0.37b
C:N	18±1.56a	20±2.03b	17±0.94a	14±0.61c
C:P	131±24.84a	133±43.24a	97±34.82ab	58±13.84b
N:P	7±0.88a	6±1.96a	6±2.09ab	4±0.85b
C:N:P	131:7:1	133:6:1	97:6:1	58:4:1
WC (%)	13.17 ± 0.95a	12.42 ± 1.23ab	11.43 ± 1.25b	12.13 ± 1.10ab
BD (g $cm^{3\ -1}$)	0.49 ± 0.05a	0.58 ± 0.00b	0.52 ± 0.05a	0.59 ± 0.02b
Porosity (%)	81.53 ± 2.04a	78.24 ± 0.05b	80.47 ± 2.00a	77.65 ± 0.86b
Sand (%)	68.07 ± 3.23a	56.26 ± 6.81b	63.67 ± 6.34a	47.80 ± 3.00c
Clay (%)	1.19 ± 0.37a	2.83 ± 0.86b	1.54 ± 0.73a	1.43 ± 1.15a
Silt (%)	30.74 ± 3.31a	35.75 ± 2.35b	34.80 ± 5.75b	50.77 ± 1.94c
Textural class	Sandy loam	Sandy loam	Sandy loam	Silt Loam

TC: total carbon; TN: total nitrogen; NO_3^-: nitrate; NH_4^+: ammonium; TP: total phosphorus; Pino: inorganic P; Porg: organic P; WC: Water content; BD: Bulk density. n = 9. Different letters in the same row show significant differences between conditions ($p \leq 0.05$).

3.3 Soil bacterial community structure and abundance

Reads obtained were grouped into 400 OTUs (97% identity) and were assigned to 18 bacterial Phyla, 60 orders, and 114 families. Across all sites, OTUs were mainly composed by the Phyla *Proteobacteria* (45.35%), *Acidobacteria* (20.73%), *Actinobacteria* (12.59%), and *Bacteroidetes* (7.32%) according to The Ribosomal Database Project (RDP) taxonomical classification. These four phylogenetic groups were found in all soils across the forested plots and accounted for more than 85% of

all sequences analyzed in this study (Figure 3); other phyla had a lower representation (≤ 5%). DP shows a lower relative abundance within the most abundant genus across all sites (Table 3).

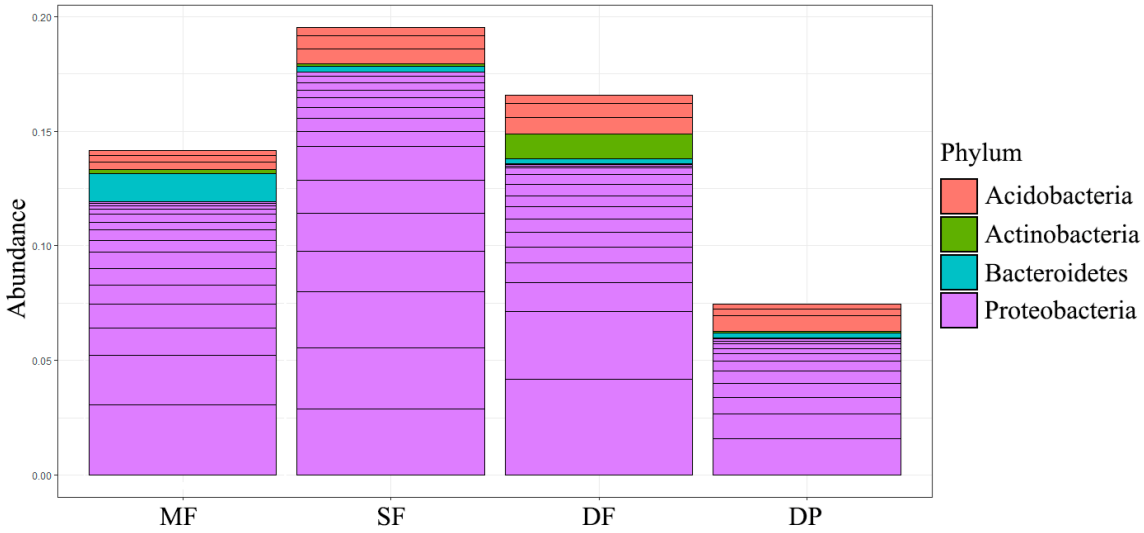

Figure 3. Major soil bacteria community phyla composition at the 0-15 cm depth interval at each site condition along the natural forest degradation gradient. Each bar comprises the top 20 OTUs most representatives at phylum level of each site. MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie.

Table 3. Relative abundance (%) of the most abundant genus in the soil Bacteria and Fungi communities at the 0 – 15 cm soil depth interval in the forest degradation gradient (site condition). Based on the top 20 most abundant and representative OTUs at each site (n = 12).

	Genus	Site Condition				Total OTUs
		MF	SF	DF	DP	
B	*Massilia*	6.39b	14.69a	5.68b	2.82b	7.39
a	*Bradyrhizobium*	7.62ab	6.06b	8.97a	4.24c	6.72
c	*Povalibacter*	2.34a	1.53b	2.62a	1.01b	1.88
t	*Glaciimonas*	0.30	5.02	1.68	<0.01	1.75
e	*Collimonas*	0.65	0.46	0.67	< 0.09	1.27
ri	*Phenylobacterium*	1.53	0.74	0.85	1.22	1.09
a						
	Flavitalea	1.85a	0.44b	0.47b	0.63	0.85
	Variovorax	0.81ab	1.06ab	1.07a	0.04b	0.74

	Kitasatospora	0.20	0.27	0.21	0.18	0.31
F	*Mortierella*	32.88	42.98	23.54	15.34	28.68
u	*Leohumicola*	4.54	1.32	0.45	4.8	2.78
n	*Umbelopsis*	0.57	2.47	2.52	2.11	1.92
g	*Cortinarius*	1.53	1.81	2.05	0.08	1.37
i	*Sebacina*	0.30	2.54	1.67	0.03	1.13
	Sagenomella	0.49	0.52	2.13	0.54	0.92
	Pseudogymnoascus	<0.01a	<0.01a	<0.03b	BLD	<0.01

OTUs: Operational Taxonomic Unit. Total OTUs represent the percentage of identified OTUs based on total reads according to the SILVA and UNITE databases. MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie. Different letters in the same row show significant differences between sites ($p \leq 0.05$). BLD: Below limit detection.

3.4 Soil bacterial community richness, abundance, and uniformity

Regarding the richness of identified OTUs (taxa), no significant differences were observed in soils across the forest conditions, both for observed OTUs and Chao Diversity Index. Shannon (H') and Fisher Diversity also showed no significant differences. However, the evenness of OTUs represented by Simpson's Index (D') displayed significantly lower values at DP (D' = 0.47±0.04) while MF, SF, and DF were not significantly different (D' = 0.29±0.01; 0.23±0.05 and 0.22±0.04 respectively). The proportion of the diversity observed at each site to the maximum expected diversity is represented by the Pielou's Diversity Index (J'). DP soils displayed a significantly higher Pielou's index than the SF plot. This index follows the trend DP > MF > DF > SF (Table 4). Most indices suggested that bacterial alpha diversity was similar between forested plots (MF, SF, DF) but increased significantly after forest conversion to prairie (DP).

Table 4. Microbial community richness and diversity indices of the 16S and 18S rRNA gene sequences for clustering at 97% sequence similarity from the 0 – 15 cm soil depth interval in the forest degradation gradient (n = 12).

Site	Bacteria						Fungi					
	Observed	[b]Chao	[c]H'	Fisher	[c]D'	[d]J'	Observed	[b]Chao	[c]H'	Fisher	[c]D'	[d]J'
MF	922	923	6.29	166	0.29	0.92	534	534	4.23	73.33	0.05	0.67
	±148	±148	±0.19	±28	±0.01a	±0.01ab	±30a	±30a	±0.30a	±4.98a	±0.01	±0.04
SF	724	724	5.86	127	0.23	0.89	427	427	3.91	58.75	0.05	0.65
	±197	±197	±0.45	±38	±0.05a	±0.03a	±117a	±117a	±0.66a	±16.71a	±0.03	±0.10
DF	867	863	6.12	151	0.22	0.90	448	448	3.93	59.11	0.04	0.64
	±14	±13	±0.05	±3	±0.04a	±0.01ab	±75a	±75a	±0.48a	±11.33a	±0.01	±0.06
DP	933	934	6.45	180	0.47	0.95	807	807	5.11	124.18	0.07	0.76
	±175	±176	±0.17	±32	±0.04b	±0.00b	±73b	±73b	±0.33b	±11.32b	±0.04	±0.04

Different letters in the same column show significant differences between sites ($p \leq 0.05$). MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie.
Operational taxonomic units (OTUs) according to the SILVA database.
[b]Chao richness estimator: the total number of OTUs estimated by infinite sampling. A higher number indicates a higher richness (Chao, 1984).
[c]Shannon (H') and Simpson (D') indexes characterize species diversity based on species richness as well as their relative abundance. A higher value represents more diversity.
[d] Pielou's Diversity Index (J') represents the proportion of the diversity observed at each site to the maximum expected diversity

3.5 Beta diversity of soil bacterial community

The beta diversity index of soil bacteria was not significantly affected as long the forest coverage was maintained. DP presented the most significant divergence across the evaluated conditions displaying a defined insolated phylogenetic cluster (Figure 6B). In addition, the high Pielou's equity index (J') for the bacterial community (J' = 0.9 mean) suggests that a few bacterial phyla dominate these clusters.

Soil bacteria community structure was affected by forest conversion, DP diverged significantly from all forested plots regardless of their degradation state (total OTUs identified, $p = 0.033$) (Figure 4A). Permutational MANOVA analysis (Adonis test) confirms that forest conversion to prairie affected bacteria community structure. All forested conditions (MF, SF, DF) are located further to the positive side of Axis 1. At the same time, DP is alone on the most negative side of Axis 1, which explains the most significant proportion of the divergence (28.2 %) (Figure 4B).

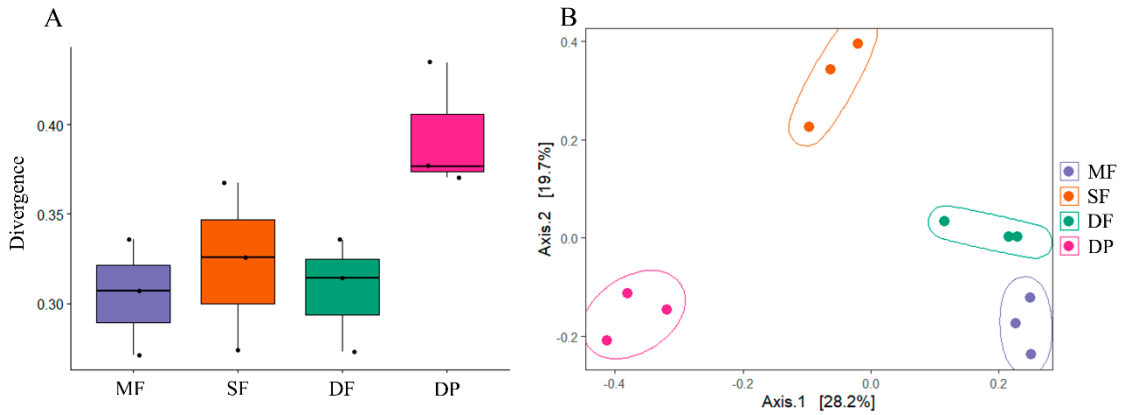

Figure 4. **A)** Divergence in total OTUs identified for each site in the 0-15 cm soil depth interval in the forest degradation gradient (n-3). Different letters indicate significant differences ($p < 0.05$) and dots outside the box are outliers. **B)** PERMANOVA for soil bacteria community. divergence of total identified OTUs at the 0-15 cm soil depth interval in the forest degradation gradient (n=12). MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie.

3.6 Soil fungal community structure and abundance

Fungi reads were grouped into 552 OTUs (97% identity) and assigned to 12 fungi phyla, 79 orders, and 178 families. Most of the fungal sequences belonged to the phyla *Ascomycota* (42.11%), *Mortierellomycota* (28.74%), *Basidiomycota* (24.61%), and *Mucoromycota* (2.06%) across conditions (Figure 5).

DP displayed the lowest relative abundance (15.37%) compared to the forested sites at the genus level. The forest sites showed decreasing abundance as degradation increased (MF: 32.94%; SF: 43.07% and DF: 23.56%, respectively). The phylum *Basidiomycota* was represented mainly by the class *Agaricomycetes* (21.52%), while the phylum *Mucoromycota* was dominated by the class *Umbelopsidomycetes* (1.86%). Many sequences could not be identified to the species level within this class (i.e., 19.31% of the total sequences).

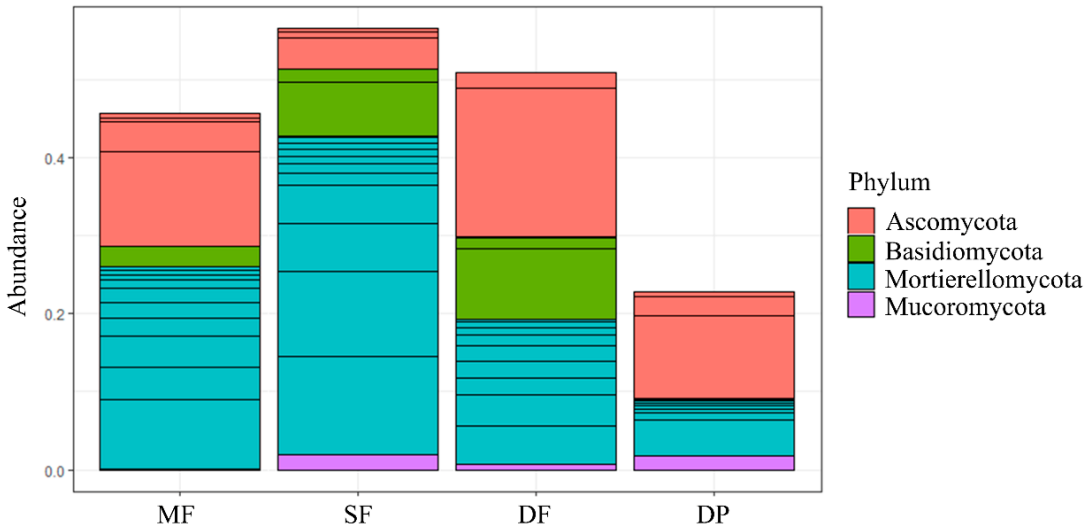

Figure 5. Fungi community composition at the 0-15 cm soil depth interval in the forest degradation gradient at Phylum level. Each bar contains the top 20 OTUs more representatives. MF: Mature forest; Secondary forest; DF: Degraded forest; DP: Degraded prairie.

3.7 Soil fungal community richness, abundance, and uniformity

The richness of identified fungi OTUs was significantly higher in DP (807±73) than under forested conditions. There were no significant differences among the forested sites (MF: 534±30; SF: 427±117; DF: 448±75). The Shannon (H') and Fisher diversity indexes showed that DP was significantly more diverse than the rest of the forested states (5.11±0.33 and 124.18±11.32, respectively). On the other hand, the Simpson's diversity index (D') and the Pielou's index did not show significant differences among states (Table 4).

3.8 Beta diversity of soil fungal community

As observed in soil bacterial communities, forest degradation altered fungi community structure. Among sites, the SF condition showed to be the most divergent community across the forested sites; however, the differences were not significant (*p = 0.063*) (Fig 6A). Permutational MANOVA analysis showed that the soil fungi community of MF and DF shared some OTUs abundances reflecting a similar structure as can be seen by the overlapped clusters formed. On the contrary, SF and DP displayed divergent phylogenetic clusters from MF and DF (Figure 6B).

Changes in soil fungal beta-diversity likely resulted from changes in its composition brought on by anthropogenic disturbances and changes in vegetation. The alpha fungi community diversity was only significantly altered after forest replacement by prairie (i.e., DP showed to be considerably richer (Chao: 807±73) and more diverse (H': 5.11±0.33) compared to all forested conditions). SF represented the most divergent community. In particular, the fungi communities of MF and DF showed similarities diverging from SF and DP. The latter two formed clearly separated phylogenetic clusters (Figure 6B).

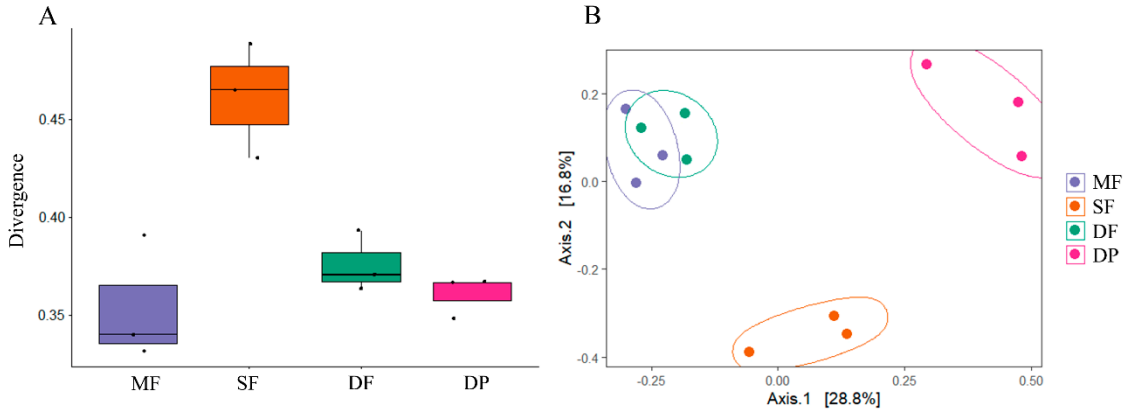

Figure 6. **A)** Total OTUs Fungal divergence identified for each site condition at the 0-15 cm soil depth interval in the forest degradation gradient (n=12). **B)** PERMANOVA for soil fungi divergence of total identified OTUs at the 0-15 cm soil depth interval in the forest degradation gradient (n=3). Different letters indicate significant differences between sites (999 permutations, Pr(>F) = 0.001). MF: Mature forest; SF: Secondary forest; DF: Degraded forest; DP: Degraded prairie.

3.9 Soil core microbiome community

We found a total of 51 bacterial taxa that were shared among all soils evaluated across the forest degradation gradient (ecological states). These taxa correspond to members of the genera *Bradyrhizobium*, *Acidobacteria* (grouped in GP1, GP2, GP6), *Massilia*, *Conexibacter*, and *Gemmatimonas*, with prevalence values > 70 %.

The soil fungi core microbiome consisted of 16 taxa shared across sites with members of the genera *Mortierella*, *Pseudogymnoascus*, *Cortinarius*, *Umbelopsis*, *Leohumicola*, *Oidiodendron*, *Salicoccozyma*, *Tomentella*, *Hyaloscypha*, *Trichocladium*, *Chaetomium*, and *Geminibasidium*. One of the 16 taxa could not be identified (Figure S5B). As the abundances of some genera differed between forested soils and degraded prairie soils, there is also a difference in composition. A detailed list of core members and their relative abundance for bacterial and fungal communities can be seen in the heatmaps (Figure S5A and S5B, respectively). The implication of these differences will be discussed in section 4.3.

4. Discussion

4.1 Effect of forest degradation on surface soil biogeochemical pools

The examined forest ecological states represent a typical gradient of historical anthropogenic degradation and succession of a temperate RO-RA-CO forest type (*Nothofagus* spp.).

All soils across the gradient displayed acidic pH values (5.36 to 5.63), which are within the typical range for volcanic soils in the area (Ortiz et al., 2020; Tosso, 1985). The less acidic pH in the prairie condition is probably due to reduced plant productivity and litter decomposition, consequential decreased bicarbonate and organic acids production, and an alteration in base cation dynamics (Jobbágy and Jackson, 2003). In addition, prairie soils were enriched in N compared to forest states. Nitrate was the main available nitrogen form in prairie sites, while ammonium was for forested sites (4.5 times higher in MF than DP). Thus, it could be expected that forest sites could also have a higher contribution of acidity derived from nitrification.

Against what we expected, DP soils have the highest surface total carbon pool (128.77±20.64 Mg ha^{-1}). This relative carbon enrichment is most likely the result of

erosion of degraded upslope areas. Soil pits dug at the site revealed an evident thickening of organic-enriched surface horizons, particularly at lower slope positions (data not shown). This can also be validated by the significantly finer surface texture of the degraded prairie compared to forested sites (Table 2). This C enrichment in the most degraded state was also observed by Marín et al. (2017).

The higher concentration of nitrogen in the DP conditions is probably the result of former agricultural use and livestock grazing, in agreement with what was reported by Ortiz et al. (2020) in silvopastoral systems.

Total phosphorus tends to increase as forest degradation increases (MF < SF < DF < DP). This pattern may be associated with the addition of livestock dung, reduced accumulation in tree biomass due to logging, weakened extraction due to lower plant species diversity (i.e., tree diversity Shannon index (H') decreases as degradation increases, see supplementary data Table S1), and a higher bulk density in DP. Oelmann et al. (2021) showed that the more diverse a forest plant community is, the greater the exploitation of P resources by plants and soil microorganisms. Higher C and nutrients stocks on agricultural grassland often are associated with higher management intensities that commonly involve greater livestock numbers and plowing or traffic of heavy machinery that could increase soil bulk density (BD). As BD is used to calculate stocks (See methods Eq 1), increased soil BD could also translate into increased stocks.

The effects of selective logging of trees and livestock grazing directly impacted soils' C:N:P stoichiometries. C:N and C:P ratios decreased along the forest degradation gradient and were significantly lower in DP. This accumulation may be amplified due to the properties of the soils in our study, as Andosols' pseudo amorphous minerals have considerable capacity to protect organic carbon (and bound N) and fix phosphorus irreversibly (Crovo et al., 2021a).

Although a higher nutrient concentration is commonly seen as a positive aspect, this increase could also facilitate the establishment of fast-growing invasive plants and opportunistic microbial groups rather than native species and communities more adapted to the naturally limited nutrient availability.

4.2 Forest degradation and changes in the soil microbial community

The phyla *Proteobacteria* (mainly alpha- and betaproteobacteria) and *Acidobacteria* were dominant across all sites, as reported for most terrestrial environments (Bahram et al., 2018; Chu et al., 2020).

Our results suggest that forest degradation altered soil microbial community diversity but affected specific microbial groups to different extents. We did not find significant differences among conditions for most diversity indexes (only for D'). However, more apparent differences emerged when looking at abundances at the OTU level. Indeed, local Prokaryotic communities seem to be more ecologically adapted to the disturbances and the new conditions, and they may tolerate relatively well anthropic forest disturbances. On the other hand, changes in abundance and diversity of fungi communities may indicate greater susceptibility to environmental selection pressure and plant interrelation dependency. The soil properties evaluated (i.e., C, N, P stock and stoichiometry, and soil pH) were only weakly positively correlated to the Fungi diversity index and weakly negatively correlated with Bacteria diversity index (See Figure S4 supplementary data).

Similar to our findings, Liu et al. (2019) showed that the diversity of soil microbial communities increases from a natural coniferous forest to a grassland condition. It is important to note here that we only sampled the top layer of soil, and as result, we might have overlooked other changes in diversity in deeper soil horizons.

4.3 Soil core bacteria taxa

The soil core bacteria community of all sites comprises well-described rhizobacterial families and other plant-associated symbionts of plant roots (e.g., *Bradyrhizobiaceae, Massilia, Conexibacter*) (Pershina et al., 2018; Sylvia et al., 2005b). Deforestation into a prairie changed the microbial community, and some community members were entirely lost. In Table 5, we identify the main genera of bacteria and fungi (See section 4.4 below) and summarize the main related functions and causes for the disappearance. In our study, members of the genera *Burkholderia, Collimonas, Glaciimonas, Kitasatospora, Streptacidiphilus,* and *Variovorax* were present only in soils of forested sites independent of their degradation level (MF, SF, DF). However, they were virtually absent as core

members of the degraded prairie soils (DP) (Figure S6). The prevalence of these six genera as core members in the forested soils, despite differences in intensity of degradation (MF, SF, DF), indicates they are relatively resistant to disturbances caused by selective logging and grazing but highly susceptible to forest cover loss. Some of these genera participate or perform critical functions in the forested sites like lithogenic nutrient acquisition or degradation of complex organic compounds (Table 5).

Overall, most of the novel core microorganisms in DP play critical roles in decomposing organic matter, promoting nitrogen and phosphorus mineralization and uptake.

On the other hand, some genera arise in the core microbiome of DP soils over genera that dominates in forest soils. The presence of the *Nitrosospira* genus as core members in DP soil is noteworthy. Bacteria of this genus may be playing a distinct role, as some of these species are recognized as ammonia-oxidizing bacteria (AOB), promoting the conversion of ammonia to nitrite as their sole energy source (Kowalchuk and Stephen, 2001).

Table 5. Bacterial and fungal genera that have appeared or disappeared from the DP core microbiome compared to forested sites, and the most likely functions and potential causes of appearance or disappearance with their bibliographic references.

Genus	Function	References	Condition	Potential cause
Burkholderia, Collimonas	Lithogenic nutrient release, nutrient uptake from fungal organisms	(Leveau et al., 2010; Uroz et al., 2007)	Disappeared	Competition
Variovorax	Degrades complex organic structures (chitinolytic)	(Brabcová et al., 2016)	Disappeared	Competition, environmental disadvantage
Kitasatospora, Streptacidiphilus	Soil rhizobium, a potential antagonist of pathogens (*Streptomycetales* order)	(Cai et al., 2018)	Disappeared	Competition
Glaciimonas	Psychrophilic bacteria (specialist), nutrient release	(Huang et al., 2020)	Disappeared	Lower topsoil temperature in forested states

Inocybe, Cortinarius, Sebacina	ECMF, nutrient uptake, SOM mineralization, and soil aggregation	(Caiafa et al., 2021; McKenzie et al., 2000; Sylvia et al., 2005a; Weiss et al., 2004)	Disappeared	*Nothofagus* spp. Forest removal
Chaetomium	SOM decomposition, nutrient cycling (cellulolytic), and antagonist of pathogens	(Kaewchai et al., 2009; Matei et al., 2020)	Disappeared	Forest removal
Hyaloscypha	Wood debris saprophyte	(Baral et al., 2009; Cameron et al., 2019)	Disappeared	Reduction of tree cover, wood debris, and EMCF
Nitrospira	AOB bacteria	(Kowalchuk and Stephen, 2001)	Appeared	Forest to prairie conversion
Actinobacteria, Firmicutes, Chloroflexi	SOM decomposition, antagonist of pathogens	(Costa et al., 2022)	Appeared	Pasture conditions
Lactobacillus	Antagonist of pathogens	(Guan et al., 2016)	Appeared	Livestock and human activity
Rhizobacter	Pathogen of tuberous crops	(Kawarazaki et al., 2009)	Appeared	Livestock
Kofleria, Labilithrix, Terrimonas	Nutrient exploitation	(Haichar et al., 2008; Muñoz-Dorado et al., 2016)	Appeared	Competition for nutrient and space
Acidomelania, Clavaria	Biomarker of land management	(Tanney and Seifert, 2020; Walsh et al., 2014)	Appeared	New pasture conditions
Mycena, Saitozyma	Saprotrophic litter decomposition	(Li et al., 2020; Steffen et al., 2007)	Appeared	Change in vegetation and litter
Cadophora, Venturia	hemicellulolytic activity, pathogen	(Burr et al., 1996; Leung et al., 2016; Travadon et al., 2015)	Appeared	Proximity to fruit orchards
Calyptrozyma	Stress-tolerant oligotrophic fungi	(Pérez-Izquierdo et al., 2021)	Appeared	Fire event in the past
Archeorhizomyces	Colonize roots	(Menkis et al., 2014; Rosling et al., 2011)	Appeared	New favorable conditions, colonize ECMF habitats

Arnium, Sagenomella	Saprotrophic and coprophilic activity	(Gené et al., 2003; Melo et al., 2019; Mungai et al., 2012)	Appeared	Livestock and cattle dung
Salicoccozyma	Degradation of xenobiotic compounds, psychrotolerant	(Stosiek et al., 2019)	Appeared	DP soil colder temperature than forest sites

4.4 Soil core fungal taxa

Topsoil harbors many symbiont mycorrhizal fungi responsible for acquiring N and P and transporting it to their host plants and forming complex networks connecting multiple trees (Bahram et al., 2018). These organisms also act as plant growth promoters (PGPF) as they improve P and Fe availability (i.e., *Mortierella*) in deficient soils (Ozimek and Hanaka, 2020), like the ones in this study.

The soil core fungal community was substantially affected by deforestation and conversion to a prairie. The genera *Inocybe*, *Cortinarius* (*Agaricales* order); *Chaetomium* (*Sordariales* order); *Sebacina* (*Sebacinales* order), and *Hyaloscypha* (*Helotiales* order) are only present in the core microbiome of forested soils (MF, SF, DF). Forest clearing has been associated with a reduction of ECMF abundance, which could explain their disappearance in the deforested DP site (Hagerman et al., 1999)

Novel groups appear in the fungal core community after severe alteration (DP). Changes in vegetation from forest to a mix of shrubs and grasses favored *Acidomelania* and *Clavaria* genera. These genera are typical inhabitants of nutrient-poor, unfertilized, short-sward, and long-established grasslands and often occur on natural, alpine, and unmanaged pastures (Tanney and Seifert, 2020; Walsh et al., 2014). In a recent study, Praeg et al. (2020) found that land conversion between forests, meadows, and pastures strongly affected the fungal community. The *Clavaria* genus was identified as a biomarker of land management.

The relevance of the *Arnium* genus in DP soils could be related to cattle dung at this site. This genus is a cosmopolitan and coprophilous group that grows on various herbivore dung types (Mungai et al., 2012) and acts as wood and plant material degraders (Melo et al., 2019). Cattle also favored the presence of the *Sagenomella*

genus. Species of this genus can develop chlamydospores that survive under unfavorable conditions (Gené et al., 2003), like drier and hotter summers in the DP soils, as they are devoid of forest canopy cover. There is also abundant evidence of fire on the DP soils, which could explain the presence of the *Calyptrozyma* genus (Pérez-Izquierdo et al. (2021)

Overall, there is an evident higher abundance and dominance of some genera recognized as phytopathogens in the fungi core microbiome of DP soil like *Cadophora* and *Venturia* (Burr et al., 1996; Travadon et al., 2015). Marín et al. (2017) also found higher fungal phylogenetic richness in a clear-cut temperate rainforest of Chile where soils also had higher relative abundances of plant-pathogen fungi and lowered relative abundances of saprotrophic and ECMF fungi compared to pristine and managed forest.

The interpretations above of pathogenic activity without a proper pathogenicity test should be taken with caution. Some of the bacterial and fungal genera identified were omitted from this discussion as they have not yet been isolated or have been poorly studied (see Table 5 for summarized Fungi data mentioned above).

4.5 Threshold for soil microbial diversity alterations and implications for ecosystem functions

We have shown that forest degradation has altered surface soil properties like pH, biogeochemical pools, and stoichiometry. These changes are accompanied by changes in microbial diversity and shifts in the soil core microbiome diversity and composition. Across all forested conditions, the soil core microbiome was similar, with only slight differences in the abundances of specific OTUs (see 4.3.1 and 4.3.2). However, we found clear differentiation in the composition of the soil core microbiome between DF and DP. Certain soil microbial core groups are more sensitive to changes in their habitat, resulting in a drastic reduction of their population or even a complete disappearance (e.g., *Kitasatospora*, *Streptacidiphilus*, *Inocybe,* and *Cortinarius* genera). In contrast, others become more abundant (e.g., *Kofleria*, *Labilithrix,* and *Acidomelania* genera).

One of the most striking effects of the forest to prairie conversion was on communities associated with nitrogen cycling. Soil ammonia-oxidizing bacteria

(AOB) became core members of the soil microbial community in DP. The activity of AOB may be reflected in the lower concentrations of ammonia (substrate) in these soils and higher nitrate (complete oxidation byproduct) compared to the forested soils (see Table 2).

The introduction of grasses and shrubs selects specific rhizosphere microbial genera such as *Kofleria, Labilithrix, Terrimonas,* and fungi as *Acidomelania*, *Clavaria,* and *Mycena*.

The introduction of exotic livestock may have modified the core microbiome by introducing microbes related to cattle dung decomposition and pathogenic genera (e.g., *Labilithrix, Lactobacillus, Arnium,* and *Cadophora* genera). Introducing these pathogenic microbial groups can be detrimental to native animal species or the natural recovery or restoration of these ecosystems. In addition, livestock dung may have contributed to the unexpected increase of N and P at the DP site, further selecting these microbial groups.

Another significant change was the decline in the biodiversity (richness and abundance) of ECM fungi with the forest conversion to a prairie. As obligate symbionts of forest trees, ECM fungi are highly susceptible to forest removal. Evans et al. (2017) has identified thresholds in biodiversity and ecosystem function related to changes in the basal area in a forest undergoing dieback. They found that species richness of ECM fungi declines sharply with basal area and changes in soil respiration rate. Prescott and Grayston (2013) found that the specialization of microbial communities under different tree species may have more significant implications for physiologically narrow ecosystem processes, i.e., those performed by a more limited set of microorganisms.

We found that certain microbial groups were more sensitive to land-use change, such as AOB bacteria, ECM fungi, and *Clavaria* genus members. These groups may serve as biomarkers or early indicators to establish an ecological threshold for forest degradation to help conservation plans for these unique southern temperate forests. As has been detailed by Rompré et al. (2010), when forests are fragmented (such as habitat loss in DF and DP), there are critical habitat thresholds for specialist species that are sensitive to habitat changes (30 – 40 % habitat loss). For generalist

species, the threshold may be lower, and so these taxa might continue to exist within the new environment but at lower abundances than in the unfragmented environment.

5. Conclusions

Anthropogenic degradation of natural *Nothofagus* sp. forests progressively altered topsoil C, N, P stocks, and stoichiometry, which became significant after conversion to a prairie.

All forest states evaluated displayed a well-defined soil microbial community and shared principal core members, i.e., *Rhizobiales* order (N-fixing bacteria) and *Cortinarius* sp. (ectomycorrhizal fungi).

The conversion from forest to prairie represented a degradation threshold for the composition of the topsoil microbiome. Deforestation drove the disappearance and appearance of novel microbiome core groups. Potential ecosystem functionality losses or dysfunctionalities could result from losses of core groups. In either case, further research is needed to assess the magnitude of these changes in deeper soil layers and the implications of these disruptions for soil and forest ecosystem functions and health.

Acknowledgments

The authors thank all the students, colleagues, and friends who helped in the fieldwork and sample pre-processing and the reviewer's comments and thoughts that helped improve this article. We also would like to thank the public-private collaborative project between ENEL-Universidad de Concepción and the Foresta Nativa Initiative that supported this study and the Faculty of Forest Sciences of the University of Concepción for allowing us to carry out this study in the BNP Ranchillo Alto.

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II. Forest degradation drives deep changes in soil nutrients stock and enzymatic activity in volcanic soils.

Abstract

Unregulated logging and livestock grazing directly impact forest composition and structure and crucial soil ecosystem functions. Such adverse effects are direct threat to the conservation of forest and soils and the contributions of these ecosystems to society's needs. Here we evaluated changes deep soil C and nutrients (N and P) pool compartments (~ 300 cm) and the activities of ten extracellular enzymes associated with C, N and P cycle in soil (β-glucosidase (BG), Cellobiohydrolase (CBH), Dehydrogenase (DHA), Peroxidase (POD) and Polyphenol oxidase (PPO), Glycine aminopeptidase (GAP), Leucine aminopeptidase (LAP), β-N-acetylglucosaminidase (NAG) and Urease (UA), Acid phosphatase (AP)). Soils were sampled to a depth of ~300 cm in four LTER (Long Term Ecosystem Research) plots along a forest degradation gradient established in an Andean temperate forest. These included three forest stands: i) a mature forest stand *(MF)*, ii) a secondary forest stand (SF), and iii) a highly degraded forest stand (DF), and the previously forested area converted into a degraded prairie (DP). We found that more significant changes were associated with forest conversion to prairie than forest degradation. Differences between forested and prairie soils were mainly related to a higher bulk density (BD) (PC1); P fractions contents (TP and Pino), and POD, GAP, and LAP soil enzymes activities (PC2); and to N available forms (NO_3^- and NH_4^+) (PC3). Overall, prairie soils (DP) were more compacted (higher BD), TC, and nutrients stocks than forested soils at topsoil (i.e., TN: 22.25 ± 2.34 Mg ha^{-1} and 11.7 ± 2.07 Mg ha^{-1} in average respectively). However, MF had greater TC and TN below 200 cm soil depth than DP soils (TC: 64.48 ± 21.87 Mg ha^{-1} and 9.52 ± 1.53 Mg ha^{-1}; TN: 6.86 ± 2.69 Mg ha^{-1} and 1.17 ± 0.47 Mg ha^{-1}). Only two out of ten enzyme activities (GAP and POD) showed a significant change after forest conversion to prairie (GAP: 6.35 ± 6.60 $x10^2$ µg g^{-1} soil in forested soils and 86.32 ± 5.04 in DP; and POD: 23.14 ± 13.39 $x10^2$ µg g^{-1} soil in forested soils and 423.82 ± 46.15 $x10^2$ µg g^{-1} soil in DP); however, no consistent change was found among forested conditions. Soil enzymes activities of PPO, GAP, LAP, and POD correlated positively with TC content (r^2 >

0.5). Forest degradation and especially land-use conversion altered soil C and nutrient pools' depth distribution, stocks, and enzyme activity. The profound changes in biogeochemical pools could be long-lasting and affect soil functionality, representing a severe threat to soil and forest health and conservation.

Keywords: soil carbon persistence, deep soil, forest disturbance; land-use change; enzymes, C:N:P

Alejandro Atenas Navarrete, Felipe Aburto Guerrero, Gerardo González-Rocha, and Carolina Merino Guzmán. In preparation. STOTEN.

7. Introduction

Native forests are the most carbon-dense land cover in Chile, followed by plantation forests, shrublands, and pastures (Heilmayr *et al.* 2020). Andean temperate forests are essential in maintaining a significant number of ecosystem services. The Andean temperate forests of Chile are dominated by *Nothofagus* species locally recognized as the RO-RA-CO forest type that comprise *Nothofagus obliqua, Nothofagus alpina, and Nothofagus dombeyi* tree species. These *Nothofagus* spp. forests display high endemism, high wood productivity, and quality (CONAF 2017; Rojas *et al.* 2012). Because of the latter, these forests have experienced historical logging where the best individuals are rudimentarily harvested (locally known as *floreo*), fire, and grazing livestock. These kinds of disturbances have led to a degradation of natural forests sustained over time (Donoso & Promis 2013; Echeverria *et al.* 2006) and soil erosion in the region (Crovo *et al.* 2021a; Crovo *et al.* 2021b; Marquet *et al.* 2019).

Chemical and physical properties have been used as crude measures of soil productivity. Generally, the criteria for judging a level of degradation or revegetation success of disturbed ecosystems have been the visual distinguishable aboveground indicators, with little attention being given to the soil microbial communities and their functions related to C turnover and nutrient biogeochemical cycles (An *et al.* 2009; Mummey *et al.* 2002), an essential aspect of soil health (van Bruggen & Semenov 2000). In addition, most soil evaluations have focused on the upper 50 cm of soil (Dube & Stolpe 2016; Ortiz *et al.* 2020; Valle & Carrasco 2018), overlooking potential alteration of deeper soil compartments. This may lead to an incomplete understanding of the scale of alteration in nutrient cycling, organic matter quantity and quality (persistence), vegetative reestablishment, and long-term ecosystem stability and resilience.

Previous studies have reported that forest degradation would affect the soil physico-chemical and biological properties; and also, SOM dynamics, which subsequently alters the soil quality and fertility (Zhao et al. 2013). Further, forest degradation influences the soil microbial functions by affecting the soil C, N and P cycles (Sousa et al. 2012). Given that most organic inputs to soil are polymeric compounds, the decomposition of soil C depends on the microbial production of extracellular

enzymes that convert complex compounds and release nutrients that can be readily be used by plants, soil fauna, and microflora (Luo *et al.* 2017; Merino *et al.* 2016; Nannipieri *et al.* 2002). Disturbance of soil ecosystem that disrupts normal functioning alters the composition of soil microbial communities (Atenas et al., 2022), and it is potentially detrimental to both short- and long-term ecological stability, and thus for soil ecosystem functions (Mummey *et al.* 2002). Additionally, the variation in quality and availability of substrate, fine roots activity, litter quality, vegetation composition, plant biomass, and belowground processes also change the SOM content, hence, affect the soil microbial community functions, soil basal respiration, potentially mineralizable N, and soil extracellular enzyme activities (Nourbakhsh 2007). Several environmental factors, including soil moisture, oxygen availability, oxidation-reduction potential, pH, SOM content, depth of the soil profile, temperature, season of the year, heavy metal content, and soil fertilization or pesticide use can affect significantly extracellular enzymes in the soil environment (Wolińska & Stepniewska 2012). Given the facts, soil enzyme activities coupled with key nutrient cycling (C, N, and P) and oxidation–reduction processes have been used widely as a potential indicators of soil quality and health status (Dick 1994; Lagomarsino *et al.* 2009; Qin *et al.* 2021).

Overall, determining extracellular enzyme activities is a simple, quick, and precise way to assess changes in soil following disturbances (Nannipieri *et al.* 2017; Sofi *et al.* 2016). However, data on soil microbial indices for soil health (i.e., microbial diversity and extracellular enzyme activities) in Andean temperate forests are still scarce, even more from deeper soil compartments, limiting proper forest soil ecosystem degradation assessment. Coupling this with the application of emerging novel techniques such as microbiome analysis, like the previous chapter of this investigation, enables the opportunity for breakthroughs on the many functions of extracellular enzymes in microbial ecology on Andean temperate forest ecosystems. This study hypothesizes that soil microbial enzyme functionality varies across forest degradation gradient at ~300 cm soil depth, and therefore, could be used as sensitive indicators of soil health.

The aim of this study is to evaluate the effect of a degradation gradient on soil microbial activity of selected soil enzymes involved in soil nutrient cycling, i.e., carbon (β-glucosidases), nitrogen (ureases), phosphorus (acid phosphatase), and oxidation–reduction (dehydrogenase) under depth distribution. Lastly, the purpose is to understand the influence of forest ecosystem degradation gradient dominated by a *Nothofagus* spp on the relationship between soil physicochemical properties and microbial enzyme activity.

8. Materials and methods

8.1 Site description

The sampling sites were located in the National Protected Area (BNP) Ranchillo Alto, near Yungay, Ñuble Region, in the Andes Cordiellera's piedmont. Climate is Temperate Mediterranean with marked wet and dry seasons, a long-term MAP of 3000mm (mostly fallen as snow), and a MAT of 13.5 °C. Soils were formed from post-Last Glacial Maximum ash deposits overlying fluvial and fluvioglacial materials (Roa & Varela 1985). The soils are mapped as a member of the Santa Barbara Soil Series (CIREN 1999; Stolpe 2011), and they are classified as members of the Pachic melanudands (Soil-Survey-Staff 2014).

This research was conducted in four long-term ecosystem research plots (LTER) described in chapter one of this document. These previously established LTER plots comprise an anthropogenic degradation gradient (Figure 1) that consider three plots with different levels of anthropogenic disturbances that have changed the composition, diversity, and structure of the Andean temperate forest (RO-RA-CO forest type), and additionally, a degraded prairie (DP) site. The three forested sites correspond to a mature forest stand dominated by *Nothofagus dombeyii (MF)*, a secondary forest stand dominated by *Nothofagus alpina* (SF), and a highly degraded forest stand dominated by *Nothofagus obliqua* (DF). These forest plots are monitored to evaluate changes in critical ecosystem functions due to land degradation and land-use change processes common in Andean Forest in the region. For more details, see "Site description" and "permanent plots" please check the site description section in chapter one.

8.2 Soil sampling

Mineral soil samples were collected following the gradient of natural forest degradation. In each LTER plot, three sampling points were located along the slope in the east-west direction in the central section of the plot conforming at 25 m equidistant from the plot's borders and each other. Soil pits were hand-dug up to a depth of ~ 300 cm. Not all soil pits could be dug up to 300 cm because of physical limitations such as lithic contact or the presence of large boulders (minimum soil pit depth was recorded at 220cm). Each soil pit was fully described and sampled by soil genetic horizons following the standard protocol described by Schoeneberger *et al.* (2012). The litter was removed and not included in the analysis. After sampled soil samples were immediately stored in hermetically sealed bags and kept in a refrigerator at 4 °C during transport to the Soil, Water and Forest Research Laboratory (LISAB) of the University of Concepción (UdeC). In total, 12 soil profiles were sampled (Figure 1), yielding 130 individual soil mineral horizons depth samples (soil genetic horizons samples, see Table 1). In the lab, three subsamples were obtained from each sample. One subsample was immediately stored at 4 °C for soil enzyme activity analysis; a second subsample was air-dried at 25 °C, sieved at <2 mm to remove roots and rocks fragments for chemical analysis. A third subsample was obtained from fresh soil samples and immediately stored at -80 °C in an ultra-freezer until DNA extractions can be performed (work in progress that will not be addressed in this research).

Figure 1. Scheme of 1ha square LTER plots. At left-side from up to down MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie. The Green to red bar indicates the intensity of forest degradation. The X marks on the DP plot show the relative position of soil pits (n = 12, 3 per plot at 25 m equidistant, E-W orientation). At the right-side, photographs of one of the soil pits dug up at each LTER plot. A) Mature forest; B) Secondary Forest; C) Degraded Forest; D) Degraded Prairie. Figure modified from Foresta Nativa (www.forestanativa.org).

Table 1. General description of plots and central soil pits at BNP Ranchillo Alto. Soil pit descriptions were made by soil genetic horizons, according to Schoeneberger *et al.* (2012).

Site	Code	N° of identified horizons	Depth to bedrock (cm)	Detail	Location
Mature forest	MF	11	300	Forest dominated by *Nothofagus dombeyi*	-37.06626° -71.64149°
Secondary forest	SF	12	280	Forest dominated by *Nothofagus alpina*	-37.07065° -71.64221°
Degraded forest	DF	17	290	Forest dominated by *Nothofagus obliqua*	-37.07632° -71.64478°
Degraded prairie	DP	12	300	Degraded prairie with insolated tree patches	-37.082844° -71.650566°

8.3 Soil physical properties

We determine the principal physical properties of soils such as bulk density (BD), porosity, water content (WC), and the soil particle size (sand, clay, and silt content) of each soil according to Burt (2014). Three cylindrical soil cores (100 cm^3) were extracted from each soil horizon, which was subsequently oven-dried at 105 °C until constant weight to estimate bulk density (BD).

8.4 Soil C, N, and P stocks

We determine the major soil nutrients. Total carbon (TC) and total nitrogen (TN) were determined by the Dumas-TCD dry combustion method with an isotope ratio mass spectrometry equipment coupled to a GSL solid sample preparation module (IRMS-GSL, SERCON Limited, UK). Available nitrate (NO_3^-) and ammonium (NH_4^+) were determined according to Miranda *et al.* (2001). Total phosphorus (Pt), $P_{inorganic}$ (P_{ino}), $P_{organic}$ (P_{org}) were determined according to Bowman & Moir (1993). NO_3^-, NH_4^+, and all P fractions were determined colorimetrically with a Shimadzu UV-mini 1240 spectrophotometer.

As our soil samples were taken from soil genetic horizons and not fixed soil depth for replicates, we estimated the total C, N, and P stocks for the entire soil profile depth to better understand and represent the conditions at each LTER the plot. To achieve this, we measured each soil horizon sample individually and then analyzed them as the whole soil profile. Soil C, N, and P stocks for each soil horizon were calculated using Eq. (1).

$$\sum_{i=0}^{n} \frac{Xi}{100} \times BDi \times \frac{SLTi}{100} \times \left[1 - \left(\frac{CFi}{100}\right)\right] \qquad (1)$$

Where X_i represents the element (C, N, and P) concentration at a given soil horizon *i* (%), BD_i the soil bulk density of the depth interval soil horizon (Mg m^{-3}), SLT_i is the soil interval *i* thickness (cm), and CF (%) represents the percentage of coarse fragments (>2mm) at that interval. All sampled layers were summed to estimate the total stock of C, N, and P to the maximum depth of each sampled soil pit. We also assess the average concentration of C, N, and P for each soil pit to reflect the effect of the forest degradation gradient. All element concentrations were expressed in Megagrams per hectare of soil (Mg ha^{-1}). We also calculated C:N, C:P, N:P, and

C:N:P molar ratios for the whole soil profile using a depth weighted average for both elemental content and element stocks.

8.5 Soil enzyme activity

We measured the soil enzyme activities of ten of the most important soil enzymes related to the carbon, nitrogen, and phosphorus cycle. C cycle-related enzymes evaluated were β-glucosidase (BG), Cellobiohydrolase (CBH), Dehydrogenase (DHA), Peroxidase (POD), and Polyphenol oxidase (PPO); N cycle-related enzymes evaluated were Glycine aminopeptidase (GAP), Leucine aminopeptidase (LAP), β-N-acetylglucosaminidase (NAG) and Urease (UA); P cycle-related enzyme considered was Acid phosphatase (AP) (See Table 2 for details). NAG and UA were only evaluated in the forested soils (MF, SF, DF) without considering DP as a limitation of sample size.

Colorimetric enzyme assays were carried out at the Center of Amelioration and Sustainability of Volcanic Soils (AMESUVOS), Universidad de la Frontera, Temuco, according to adapted Allison's Lab protocols (Allison & Jastrow 2006). All soil enzyme activities were expressed in millimole of substrate oxidized per minute (mmol min^{-1} $x10^{-2}$ µg g^{-1} soil). Enzyme activities assay were performed in 50 mM sodium acetate buffer at pH 5.0 on six analytical replicates per soil sample (n = 6).

Table 2. Overview of soil enzymes evaluated with their respective fluorogenic substrates and ecological functions.

Enzyme	Code	Fluorogenic substrate	Predictor of soil function
β-Glucosidase	BG	pNP-β-D-glucopyranoside	Organic matter decomposition, Hydrolysis of simple sugars
β-Cellobiohydrolase	CBH	pNP-β-D-cellobioside	Hydrolysis of cellulose
Dehydrogenase	DHA	Triphenyl tetrazolium chloride (TTC)	A measure of any disruption caused by pesticides, trace elements, or management practices to the soil, as well as a direct action of soil microbial activity
Peroxidase	POD	L-DOPA	Catalyze oxidation reactions
Polyphenol oxidase	PPO	Pyrogallol and EDTA	Degrades lignin and other aromatic polymers

Glycine-aminopeptidase	GAP	Glycine p-nitroanilide	Catalyze the cleavage of amino acids from the amino terminus of protein or peptides
Leucine-aminopeptidase	LAP	Leucine p-nitroanilide	Cleaving of peptide bonds in proteins
β-*N*-acetylglucosaminidase	NAG	pNP-β-N-acetylglucosaminide	Hydrolysis of chitooligosaccharides into *N*-acetylglucosamine
Urease	UA	Urea	Transformation, biological turnover, and bioavailability of nitrogen
Acid phosphatase	AP	pNP-phosphate	P-acquisition via dissociation of phosphoric acid

8.6 Statistical analysis

All soil properties were plotted and statistically analyzed with R software (R-CoreTeam 2019). A Shapiro-Wilk normality test (α = 0.05) was performed on each variable to determine the type of statistical test to apply. To identify significant differences between the different degrees of forest degradation, the data for TC, TN, C:N, C:P, N:P ratio, and CH were evaluated through one-way ANOVA with a significance level α = 0.05, while the data for NO3-, NH4+, PT, Pi, and Po were assessed through a Kruskal-Wallis test with a significance level α = 0.05. Depth distributions were plotted using the AQP R package (Beaudette *et al.* 2013). To evaluate the effect of forest degradation and forest to prairie conversion on soil physical and biogeochemical properties (nutrients and enzymes activities), a principal component analysis (PCA) was performed for all soils sampled in the soil profiles, including all variables using the "FactoMineR" R package (Lê *et al.* 2008). Auto-correlated data was eliminated from the PCA analysis (i.e. PT and Po, since Po is calculated by subtracting the Pi from the PT pool chemical determination). The R package ggplot2 (Wickham 2016) were used to visualize and generate plots.

9. RESULTS

9.1 Soil physical properties

Soil bulk density (BD) at MF has values ranging from 0.38 Mg m^{-3} at topsoil to 1.28 Mg m^{-3} at deeper soil depths (~ 161 cm soil depth). SF has BD values ranging from 0.52 Mg m^{-3} at topsoil to 1.26 Mg m^{-3} at deeper soil depth (~ 126 cm soil depth). DF has BD values ranging from 0.43 Mg m^{-3} at topsoil to 1.18 Mg m^{-3} at deeper soil

depth (~ 140 cm soil depth). DP has BD values ranging from 0.48 Mg m^{-3} at topsoil to 1.12 Mg m^{-3} at deeper soil depth (~ 270 cm soil depth) (Figure 2).

Soil porosity showed the same pattern as BD, where SF displayed significantly more pore space between 200 – 250 cm soil depth intervals (Figure 2). MF, DF, and DP did not show differences in porosity. Bulk density increased with soil depth, while porosity tended to decrease, which is natural for most soils. Tree root presence also decrease concordantly with depth.

Soil particle size distribution in forested soils (MF, SF, and DF) had significantly higher sand content (63.73 ± 8.60 % in average) in the upper 100cm of soil depth compared to DP (46.95 ± 4.66 %), this latter with at least ~16% less of sand content. Below 100 cm soil depth sand content did not display significant differences between sites. Clay content was significantly higher at forested conditions (12.54 ± 5.00 % on average) between 150 - 170 cm soil than DP (1.85 ± 0.83 %). At deeper soil horizons, between 200 – 230 cm soil depth interval MF and DF had greater clay content (17.61 ± 0.11 and 15.05 ± 7.37 % respectively) than SF and DP (3.62 ± 2.32 and 2.03 ± 0.89 % respectively). Silt content showed significant differences in the upper 100 cm soil depth interval between DP and the forested sites. DP displayed a significantly higher average content of silt (50.54 ± 5.00 %) than forested sites (33.58 ± 7.99 % on average) (Figure 2).

Soils of forested sites (MF, SF, and DF) were predominantly sandy loam textural class, while soils of DP condition displayed silt loam textures. These differences in textural classes are more markedly on the first horizons of the soil profiles (0 to 100 cm soil depth interval).

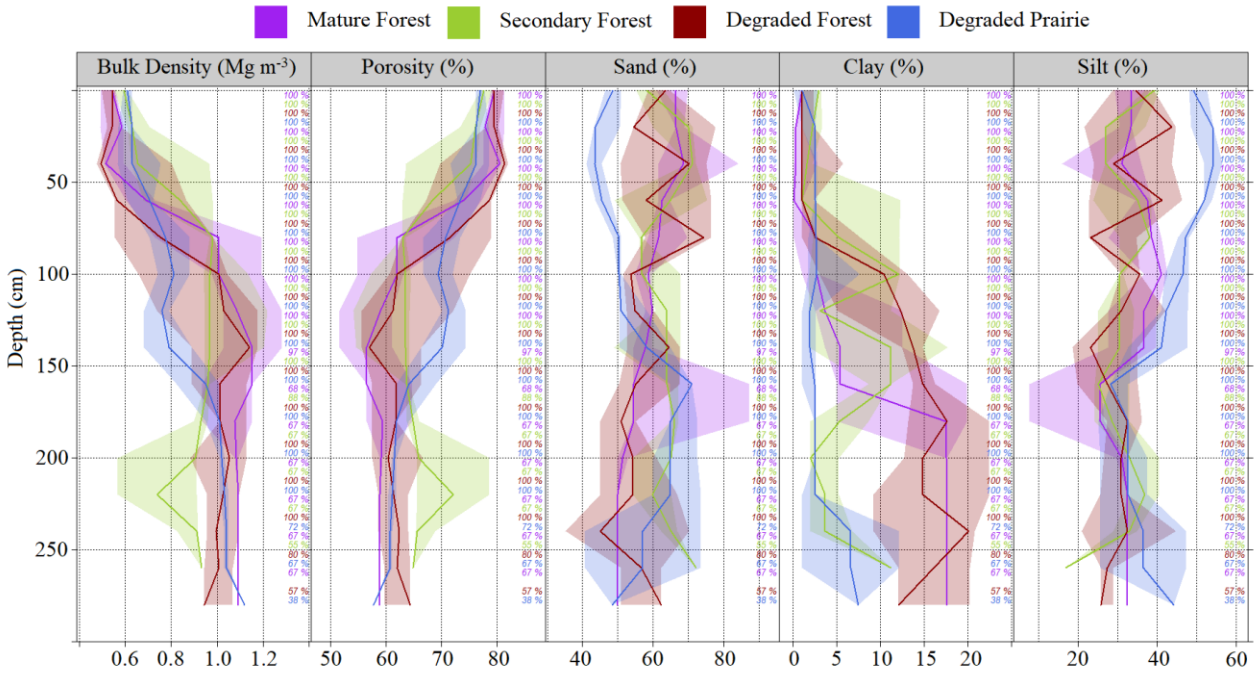

Figure 2. Soil physical properties depth distribution. MF: Mature Forest; SF: Secondary Forest; DF: degraded forest; DP: degraded prairie. The solid-colored line corresponds to the median; shadow shows the data range (25th and 75th percentiles) for each sampled depth. At the right side of each plot appears the percentage of data that contributed to the median. Significance: *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, • $p \leq 0.1$.

9.2 Soil total C, N, and P depth distribution

Overall soil total carbon (TC) and total nitrogen (TN) contents exponentially decrease with soil depth. There were significant differences only for TN and TP, mainly at topsoil (0 – 50 cm soil depth) between forested soils and degraded prairie soils, but not among forested soils (MF, SF, DF). However, SF was the only site that displayed significantly less content of TC (0.16 ± 0.08 %) between 50 – 100 cm soil depth intervals. Despite there were no significant differences between conditions, TC content follows the pattern DP > MF > DF > SF (Figure 3)

The total nitrogen (TN) contents showed to be only significantly different at topsoil (0 – 50 cm soil depth) between forested soils and DP soils (0.46 ± 0.22 % and 0.81 ± 0.22 % on average, respectively). Available forms of N, nitrate (NO_3^-), and

ammonium (NH_4^+) displayed high variance between replicates for the same conditions. Differences in soil available NO_3^- and NH_4^+ were only found in the upper 20 cm of the soil profile. All forested sites (MF, SF, DF) displayed higher contents of both N available forms (NO_3^- : 0.92 ± 0.73 mg kg^{-1} and NH_4^+ : 1.06 ± 0.38 mg kg^{-1} in average) compared to DP (NO_3^-: 0.11 ± 0.03 mg kg^{-1} and NH_4^+: 1.01 ± 0.16 mg kg^{-1}). No differences were found between forested soils (Figure 3).

At specific soil depth intervals, the total phosphorus (TP) content differs significantly between DP and forested soils. DP soils showed significantly greater TP contents than forested soils between 0 – 50 cm soil depth (0.25 ± 0.06 % and 0.11 ± 0.03 %, respectively), and also at ~100 cm soil depth and at soil horizons below ~ 240 cm soil depth. Below ~200 cm MF had the lowest TP content among all sites. The fraction of organic phosphorus (Po) follows the same depth-distribution trend as TP (Figure 3). In contrast, inorganic phosphorus (Pi) was greater in DP soils along all the depth distribution with a tendency to increase with depth. SF also displayed an increment in Pi content with depth specially between 100 – 150 cm soil depth interval. Po among forested soils were only significantly different at horizons below ~210 cm soil depth, at the same depth DP soils displayed its greater content of Po (Figure 3).

For C, N and P contents stock depth distribution see figure S9 in Supplementary data.

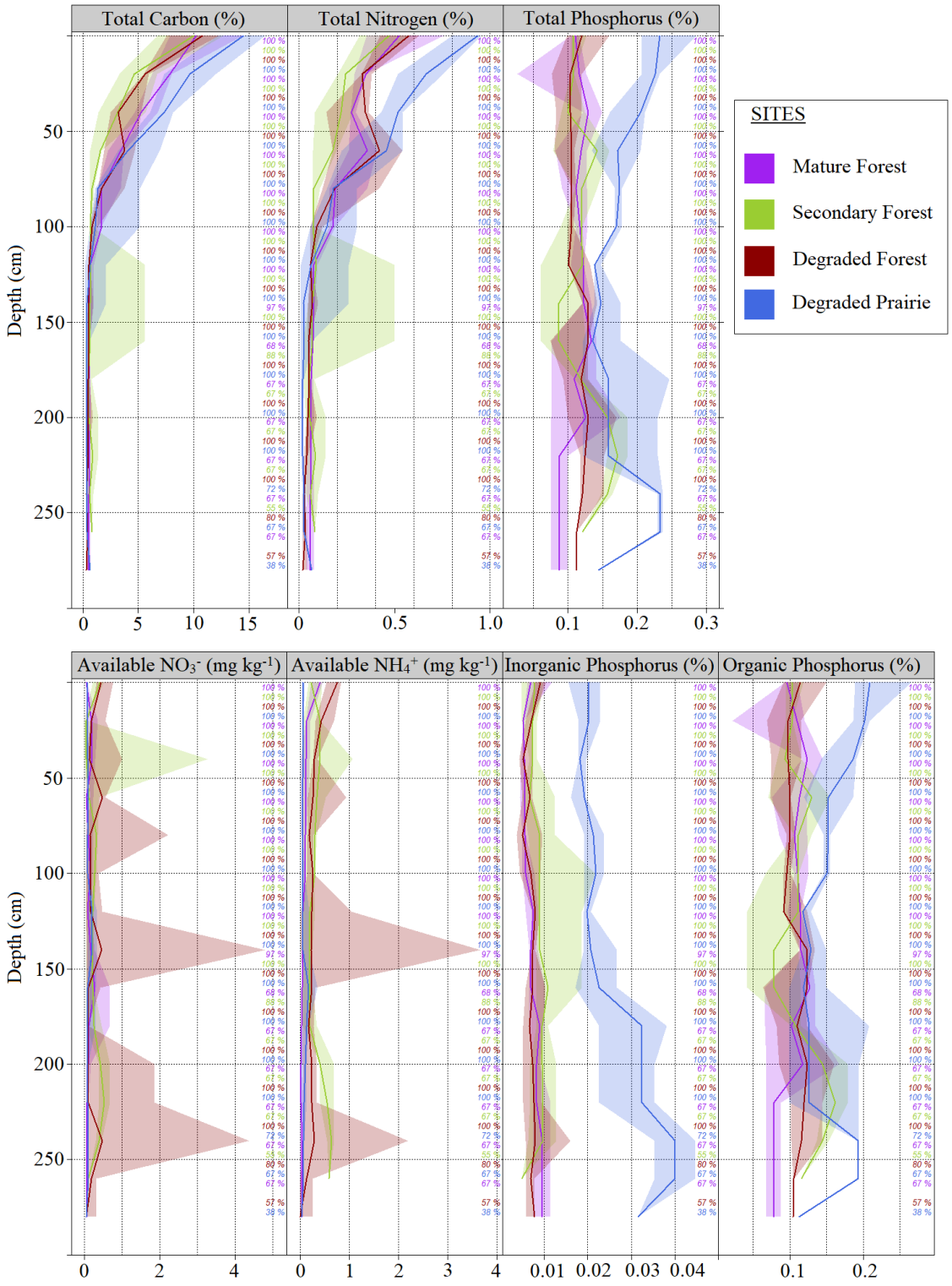

Figure 3. Total C, N, P, P_{ino} and P_{org} content and available Nitrate (NO_3^-) and Ammonium (NH_4^+) depth distribution in the forest degradation gradient. The solid-colored line corresponds to the median; shadow shows the data range (25^{th} and 75^{th} percentiles) for each sampled depth. At the right side of each plot appears the percentage of data that contributed for the median.

9.3 Soil C, N and P stocks, and stoichiometry

Our analysis reveals that C, N and P stock did not significantly vary among forest sites, but forest conversion to prairie significantly affects NH_4^+ (p = 0.027) and Pi stocks (p > 0.01). From forested sites only DF displayed a greater available ammonium stock than DP (6.77 ± 4.34 and 1.01 ± 0.53 Mg ha^{-1} respectively). On the other hand, DP displayed the largest Pi stock (5.95 ± 1.32 Mg ha^{-1}) than forested sites (1.88 ± 0.52 Mg ha^{-1} in average). The total C stock and the rest of the nutrients were not significantly affected (p < 0.1) with the forest degradation nor by forest conversion to prairie (Table 3 and Figure S8). There was a greater variability of stocks values in the degraded prairie and secondary forest sites.

Table 3. Depth weighted soil C and nutrient stock for the whole soil profiles in the forest degradation gradient.

Property	SITE			
	MF	**SF**	**DF**	**DP**
TC (Mg ha^{-1})	459.68 ± 14.95	340.79 ± 129.57	387.08 ± 6.36	576.65 ± 199.66
TN (Mg ha^{-1})	33.98 ± 2.62	24.03 ± 9.15	30.66 ± 3.58	41.58 ± 11.89
NO_3^- (g kg^{-1})	2.15 ± 1.84	3.79 ± 1.78	9.66 ± 6.69	1.42 ± 0.89
NH_4^+ (g kg^{-1})	2.02 ± 0.36ab	3.84 ± 1.57ab	6.77 ± 4.34a	1.01 ± 0.53b
TP (Mg ha^{-1})	25.41 ± 8.66	25.35 ± 8.10	27.46 ± 4.53	41.44 ± 8.43
P_{ino} (Mg ha^{-1})	1.73 ± 0.89a	2.12 ± 0.34a	1.80 ± 0.20a	5.95 ± 1.32b
P_{org} (Mg ha^{-1})	23.68 ± 7.81	23.23 ± 8.28	25.66 ± 4.37	35.49 ± 7.13

TC: total carbon; TN: total nitrogen; NO_3^-: nitrate; NH_4^+: ammonium; TP: total phosphorus; Pi: inorganic phosphorus; Po: organic phosphorus. n = 3. Different letters in the same row show significant differences between sites ($p \leq 0.05$). MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie. C:N, C:P and N:P of the whole mineral soil profiles were calculated as depth-weighted average of element contents in molar base. Our analysis reveals that there were not significant differences between sites for the profile as a whole for none of the ratios (Table 4).

Table 4. Depth-weighted average ratios of C:N, C:P and N:P of the four soils in the forest degradation gradient in molar base.

SITE	C:N	C:P	N:P	C:N:P
MF	20.71 ± 0.87	127.58 ± 12.26	6.15 ± 0.36	128:6:1
SF	21.04 ± 0.80	94.75 ± 8.80	4.50 ± 0.28	95:4:1
DF	17.54 ± 2.22	82.97 ± 6.58	4.81 ± 0.91	83:5:1
DP	17.13 ± 1.45	76.44 ± 20.31	4.46 ± 1.31	76:4:1

* Total carbon, nitrogen, and phosphorus weighted stock at the whole soil profile depth. MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie.

9.4 Soil enzyme activities depth distribution

After forest conversion to prairie, DP soils displayed a greater Peroxidase (POD) and Glycine aminopeptidase (GAP) enzyme activities compared to forested sites along the entire soil profile depth (Figure 4). Leucine aminopeptidase (LAP) enzyme activities only display significant differences between DP and forested sites between 100 – 150 cm soil depth interval, where DP displayed significantly higher activities of LAP (5.58 ± 3.81 $x10^{-2}$ µg g^{-1} soil) than forested sites (0.79 ± 0.71 $x10^{-2}$ µg g^{-1} soil in average).

Only at ~ 170 cm soil depth SF displayed greater Cellobiohydrolase (CBH) enzyme activities (0.22 ± 0.19 $x10^{-2}$ µg g^{-1} soil) compared to the other sites, DF and DP displayed lower activities (0.082 ± 0.076 $x10^{-2}$ µg g^{-1} soil and 0.089 ± 0.001 $x10^{-2}$ µg g^{-1} soil respectively). We could not detect CBH enzyme activities at MF soils (i.e., below technique's limit of detection). Behavior of Acid Phosphatase (AP) enzyme activities showed to be higher at topsoil and at the bottom of the soil profiles but did not display significant differences between sites.

Other C and N cycle related enzymes evaluated such as β-glucosidase (BG), Dehydrogenase (DHA), Polyphenol oxidase (PPO), β-Acetyl glucosamine (NAG), and Urease (UA) did not show consistent or significant differences neither among sites, nor by forest degradation or forest conversion to prairie. However, these soil enzymes showed greater enzyme activities at topsoil than deeper soil horizons. UA activities showed high variability along the entire soil profiles depth but did not show significant differences between forested sites. Due to an unexpected incident in the laboratory, we were unable to determine the enzymatic activities of UA and DHA in

DP soils at the time of this publication. It is important to note that studied pools do not represent fluxes or supplies.

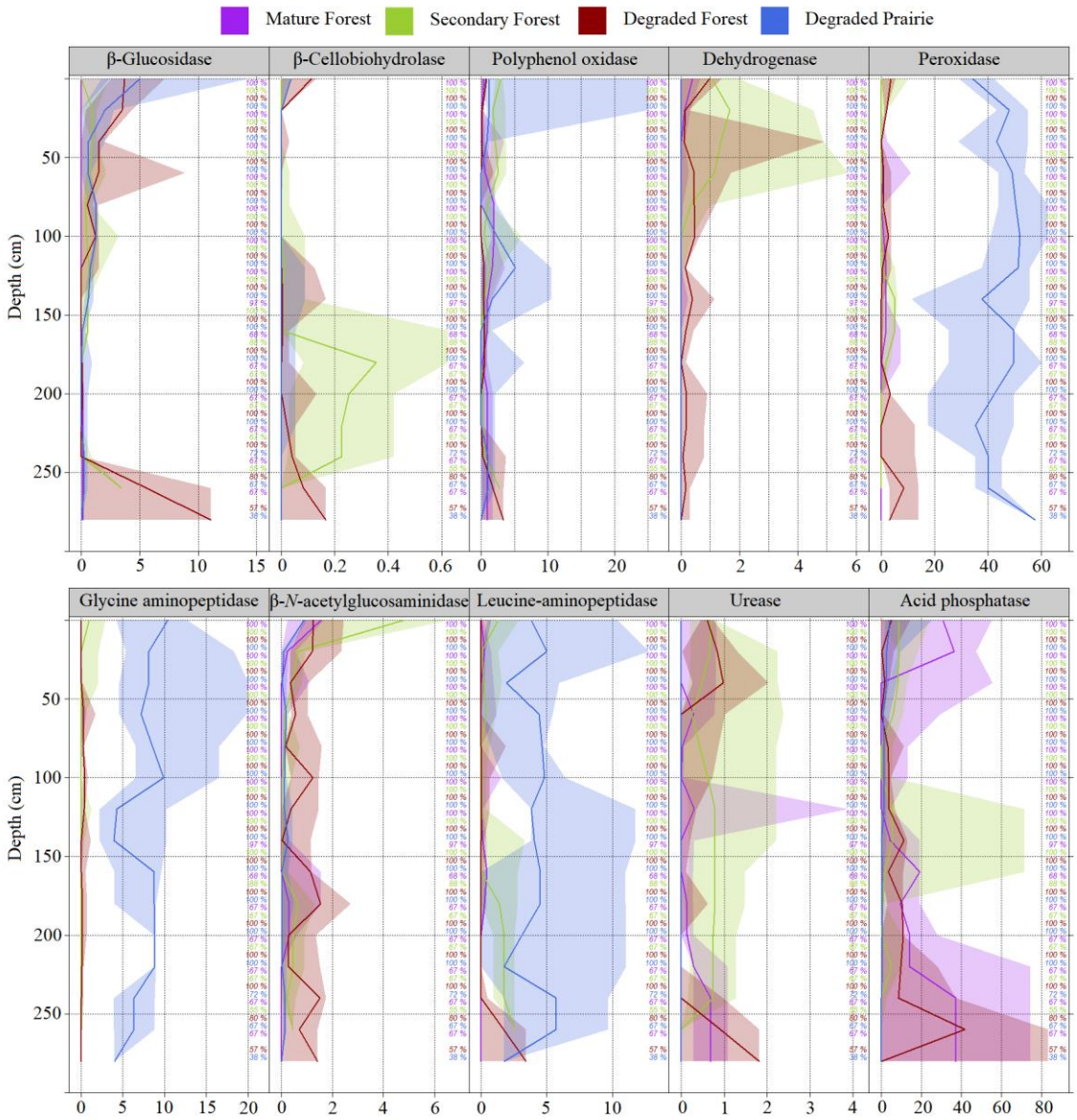

Figure 4. Soil extracellular enzyme activities expressed in millimole of substrate oxidized per minute (mmol x 10^{-2} µg g^{-1} soil) at depth distribution in the forest degradation gradient. C cycle related enzymes BG: ; CBH: ; PPO: ;DHA: ; POD: . Nitrogen cycle related enzymes: GAP: ; NAG: ; LAP: ; UA: . Phosphorus cycle related enzyme AP: The solid-colored line corresponds to the median; shadow shows the data range (25^{th} and 75^{th} percentiles) for each sampled depth. At the right side of each plot appears the percentage of data that contributed for the median.

9.5 Depth weighted soil enzyme activities

The depth-weighted soil enzyme activities showed that out of the ten soil enzymes evaluated, only two were significantly different, Peroxidase (POD, p = 0.038) and Glycine aminopeptidase (GAP, p = 0.048) enzyme activities. In both cases, DP soils displayed significantly higher soil enzyme activities for POD (423.82 ± 46.15 $x10^{-2}$ µg g^{-1} soil) and for GAP (86.32 ± 31.99 $x10^{-2}$ µg g^{-1} soil) compared to forested soils (23.14 ± 13.39 $x10^{-2}$ µg g^{-1} soil; and 6.35 ± 6.60 $x10^{-2}$ µg g^{-1} soil in average respectively) (Figure 5). However, other soil enzymes also display a tendency for higher activity values in the DP soil, like PPO and LAP (Figure S10 supplementary data). See table 5 for details of the complete ten soil enzymes depth weighted activities evaluated for the four soil profiles at each LTER site.

No significant differences were found for Dehydrogenase (DHA) activities between forest sites (p > 0.1), despite this it is important to note that both altered forest soils (SF and DF) displayed great variance in the enzyme activities than soils of mature forest (MF) (Figure 5).

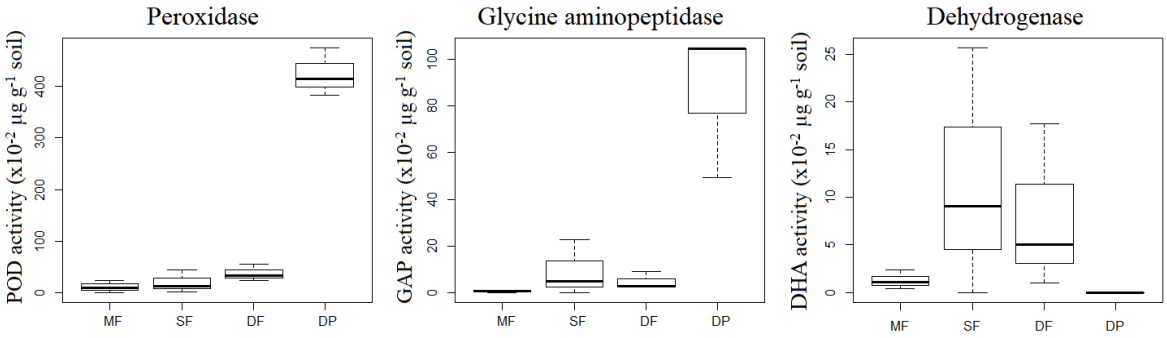

Figure 5. Depth weighted soil enzyme activities. From left to right-side Peroxidase (POD), Glycine aminopeptidase (GAP) and Dehydrogenase (DHA) expressed in millimole of substrate oxidized per minute (mmol $x10^{-2}$ µg g^{-1} soil) for the whole soil profiles. MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie.

Table 5. Depth-weighted soil enzyme activities for the whole soil profile depth of the four soils evaluated in the forest degradation gradient. Enzyme activities expressed in mmole of substrate oxidized per minute ($x10^{-2}$ µg g^{-1} soil).

SITE	BG	CBH	DHA	PPO	POD	GAP	NAG	LAP	UA	AP
MF	8.75 ± 8.07	BLD	1.25 ± 0.99	10.77 ± 7.24	11.63 ±11.64b	0.80 ± 0.17b	6.52 ± 2.39	2.82 ± 1.75	3.54 ± 2.10	267.46 ± 131.39
SF	12.79 ± 6.41	0.90 ± 0.89	17.36 ± 11.77	21.26 ± 13.34	19.96 ± 22.04b	13.65 ± 12.48b	21.35 ± 15.73	13.20 ± 11.04	7.35 ± 8.05	135.17 ± 146.15
DF	51.38 ± 50.07	0.53 ± 0.22	7.93 ± 8.77	8.81 ± 3.99	37.84 ± 16.47b	4.60 ± 3.8b0	15.57 ± 10.16	3.81 ± 3.76	6.50 ± 4.31	107.89 ± 97.61
DP	30.94 ± 28.81	0.23 ± 0.13	NA	61.44 ± 71	423.82 ± 46.15a	86.32 ± 5.04a	5.04 ± 3.65	50.48 ± 49.31	NA	44.43 ± 54.03

BG: β-glucosidase; CBH: Cellobiohydrolase; DHA: Dehydrogenase; PPO: Polyphenol oxidase; POD: Peroxidase; GAP: Glycine aminopeptidase; NAG: β-N-acetylglucosaminidase; LAP: Leucine aminopeptidase; UA: Urease; AP: Acid phosphatase. NA: Not applicable. Different letters at the same column shows significant differences between sites ($p \leq 0.05$). BLD: Below detection limit. NA: Not applicant.

9.6 Effect of forest degradation on soil biogeochemical properties

Principal components analysis (PCA) showed that the variables selected only explained 73.5% of the total variability in PC1, PC2, PC3, PC4, PC5, PC6, and PC7 (24.8 %, 18.6 %, 8.4 %, 6.3 %, 5.8 %, 4.9 %, and 4.7 % respectively; Table 6). PC1 variability was determined mainly by BD. The PC2 variability was related to TP, and some soil enzyme activities like POD, GAP, and LAP. The PC3 variability was related to N available fractions (NO_3^- and NH_4^+). The PC4 variability was related to clay content and to enzyme activities of CBH, NAG, and UA. The PC5 variability was determined by AP enzyme activity. The PC6 was related to Pi content and to C:N ratio. The PC7 was determined by BG enzyme activity and to C:P, and N:P ratios. Other principal components do not contribute enough to be included in this section. See supplementary data for extra PCA plots (Figures S13 – S16, Supplementary data).

It is important to note that there is an important difference between conditions, as all forested soils (MF, SF and DF) clustered together while degraded prairie soils (DP) grouped separately. Regarding this, forested soils were mainly correlated with NO_3^-, NH_4^+, and sand content as well as activities of some enzymes like DHA and UA; while DP soils were correlated to phosphorus fractions (TP and Pi) and silt content, and to the activities of GAP, LAP and POD soil enzymes. These aspects will be discussed in the next section.

In our soils LAP activity was positively correlated with TC content and also but to a lesser extent to GAP, POD and BG (See Figure S11 – S12 for further details on correlations, Supplementary data). In addition, this analysis showed that there is a synergic effect between a pair of enzymes like LAP:POD, LAP:PPO, LAP:GAP and GAP:POD ($r^2 \geq 0.5$). These activities are also well correlated with C and N, i.e. GAP and POD activities were correlated with TC ($r^2 = 0.71$ and $r^2 = 0.62$ respectively) and TN ($r^2 = 0.71$ and $r^2 = 0.61$ respectively) (Figure S12).

Table 6. Principal components values for the variables evaluated in the forest degradation gradient.

Variable	Principal Component						
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**
BD	0.35	0.02	0.19	0.10	0.10	0.11	0.20
Sand	-0.06	-0.27	-0.08	-0.34	-0.24	0.14	-0.04
Clay	0.23	-0.05	0.20	0.40	0.36	0.02	0.27
TC	-0.41	0.05	0.02	0.10	0.09	0.03	-0.02
TN	-0.39	0.10	-0.03	0.07	0.05	-0.12	0.00
NO_3^-	-0.03	-0.16	0.54	-0.32	-0.03	-0.12	0.12
NH_4^+	-0.08	-0.19	0.57	-0.21	0.05	0.02	0.03
TP	-0.05	0.39	0.18	0.00	0.02	-0.04	-0.19
Pino	0.17	0.20	0.03	-0.19	-0.09	0.63	0.26
C:N	-0.29	-0.02	0.03	-0.04	0.01	0.44	-0.19
C:P	-0.36	-0.13	-0.08	-0.02	0.02	0.10	0.29
N:P	-0.36	-0.13	-0.13	-0.04	-0.01	0.00	0.34
BG	-0.20	0.01	0.07	0.04	0.04	-0.21	0.52
CBH	-0.04	-0.03	0.29	0.27	-0.55	0.08	0.04
DHA	-0.05	-0.16	0.08	-0.25	0.22	-0.09	-0.41
POD	-0.02	0.41	-0.02	-0.19	0.01	0.11	0.08
PPO	-0.14	0.27	0.18	0.17	0.04	-0.27	-0.10
GAP	-0.08	0.41	0.01	-0.13	-0.04	0.02	0.07
NAG	-0.17	-0.04	0.29	0.37	-0.12	0.22	-0.25
LAP	-0.09	0.38	0.13	0.02	-0.10	0.02	-0.03
UA	0.00	-0.18	-0.11	0.36	-0.33	0.08	-0.06
AP	-0.15	-0.09	0.03	0.18	0.54	0.37	-0.09
Proportion of variance (%)	24.83	18.63	8.42	6.34	5.78	4.87	4.74
Cumulative (%)	24.83	43.46	51.88	58.22	64.00	68.87	73.62

Table X. BD: Bulk density; TC: Total carbon; TN: Total nitrogen; NO_3^-: Nitrate; NH_4^+: ammonium; TP: Total phosphorus; Pino: Inorganic phosphorus; BG: β-glucosidase; CBH: Cellobiohydrolase; DHA: Dehydrogenase; PPO: Polyphenol oxidase; POD: Peroxidase; GAP: Glycine aminopeptidase; NAG: β-N-acetylglucosaminidase; LAP: Leucine aminopeptidase; UA: Urease; AP: Acid phosphatase.

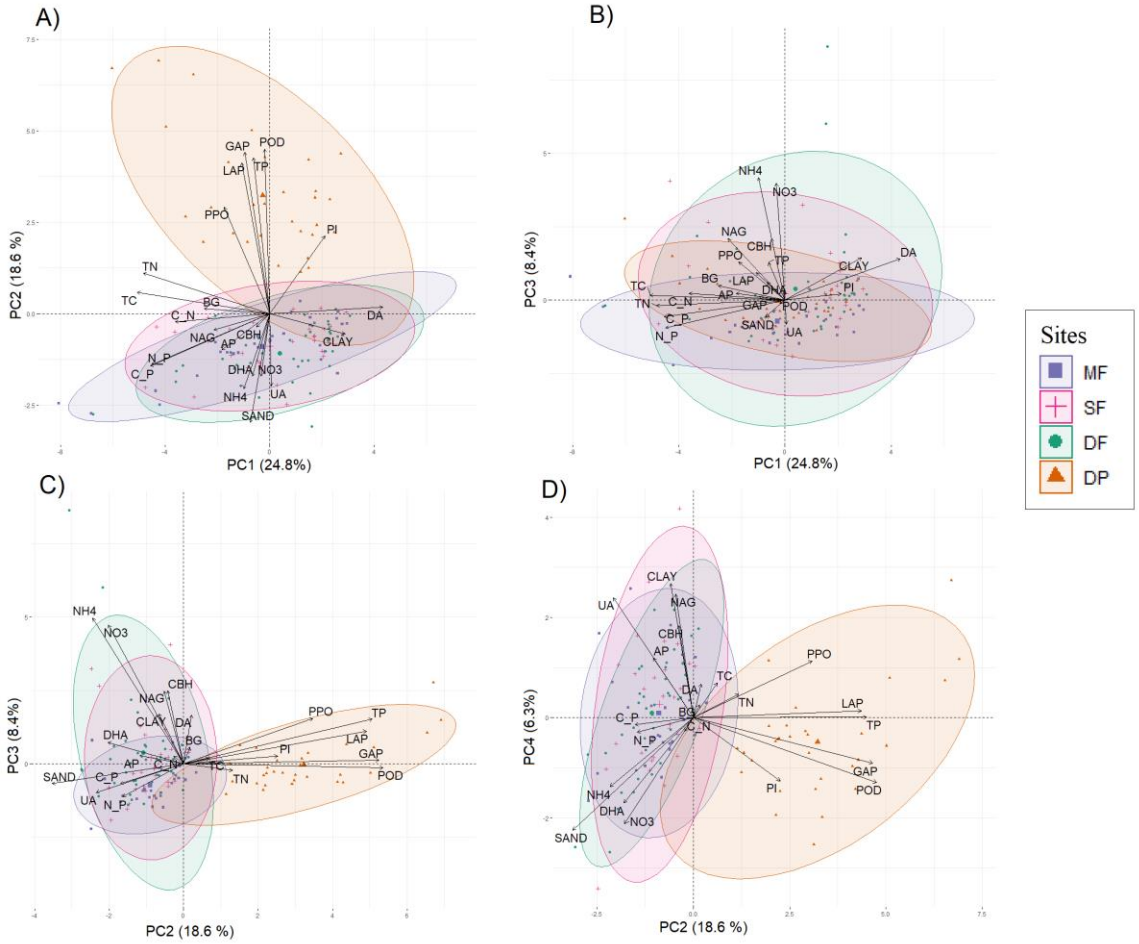

Figure 6. Principal component analysis (PCA) of soils in the forest degradation gradient. Arrows represent the principal component and symbols represent each soil horizon grouped by LTER plot. A) 2D plot of PC1 and PC2. B) 2D plot of PC1 and PC3. C) 2D plot of PC2 and PC3. D) 2D plot of PC2 and PC4. Ellipses correspond to 95% confidence. MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie; TC: Total carbon; TN: Total nitrogen; TP: Total phosphorus; PI: Inorganic phosphorus; NO3: Nitrate; NH4: Ammonium; C:N ratio, C:P ratio; N:P ratio; BD: Bulk density; BG: β-glucosidase; CBH: Cellobiohydrolase; DHA: Dehydrogenase; PPO: Polyphenol oxidase; POD: Peroxidase; GAP: Glycine aminopeptidase; NAG: β-N-acetylglucosaminidase; LAP: Leucine aminopeptidase; UA: Urease; AP: Acid phosphatase.

10. DISCUSSION

10.1 Effect of forest degradation on soil physical properties

BD showed typical values for Andisols within 0.45 to 1.21 g/cm^3 (Roa & Varela 1985; Tosso 1985). Soil bulk density increases with depth as typically subsurface layers are more compacted and have less organic matter, less aggregation, and lower porosity root penetration compared to surface layers. The highest topsoil BD at degraded prairie (DP) soils can be explained by the presence of cows and prior human activities i.e., use of agricultural machinery. Higher compaction in more heavily grazed forests increases soil erosion, consequently increasing topsoil mobility and carbon and nutrients losses (Crovo *et al.* 2021b). The DP condition showed the lowest content of sand and highest content of silt and clay, together with the highest BD and lower porosity among conditions. The infiltration rate is dependent on soil texture and clay mineralogy (Ditzler & Tugel 2002). Andisols typically display a strong resistance to water erosion and dispersion of soil aggregates due to a high permeability that reduces runoff (Delmelle *et al.* 2015), however these soil types are still susceptible to compaction and erosion after improper management (Dörner *et al.* 2009) like the ones experimented by DP soils (livestock grazing, fire episodes and use of agricultural machinery).

10.2 Effect of forest degradation on soil biogeochemical pools

We did not find significant differences in C pools along the degradation gradient in forested sites (MF, SF, DF). However, these conditions were significantly different to the degraded prairie soil (DP). The greater TC and nutrients stocks (TN and TP) in DP soils are most likely explained by the accumulation of eroded topsoil material removed from higher areas in the slope and presence of pyrogenic carbon that was widespread in this condition. Evidence for erosion could be found in the significant enrichment of fine material, specially silt in the upper 1m of soil. The higher C stock in this condition can also be partially explained because of the higher values of BD. BD is used during the calculations of stocks (See methods Eq 1), and higher soil BD will also translate into higher stocks (See supplementary data figure S2).

The significantly higher phosphorus fraction at DP soils are probably the result of the augmented accumulation of eroded organically enriched materials and of very particular characteristics of these volcanic soils. Andisols rich in short range order

(SRO) minerals display high phosphate retention due to direct mineral P complexation or stabilization of organic compounds rich in organic matter by SRO minerals. However, the DP soils may show higher TP or Po values, this does not necessarily translate into higher P availability. In fact, Andisols display very low P availability, which can become a limiting factor for plant productivity. The additional lower diversity and reduced tree biomass due to logging and slash burning (See supplementary data Table S1) reduced the amount of P associated with standing biomass leaving much of this P pool retained in soil. Phosphate presents a heterogenous distribution (patches), given its high affinity for the soil matrix and, as the rhizosphere quickly becomes depleted of P, the roots must continually grow to find new available P hotspots. The litter and topsoil layer (0 – 10 cm) are the most active zone for P acquisition and cycling for plants and microorganisms because of the poor P mobility in soil (Borie *et al.* 2019). Crovo *et al.* (2021a) described that the presence of non-crystalline minerals in Andisols has a higher nutrient stabilization for C, N and P, due to a strong organo-mineral interaction, even under intense perturbations. This agrees with our observations, where DP also displayed higher TC and TN in the shallower soil depth in comparison to forested soils (MF, SF and DF). However, it is important to note that the concentrations of C and nutrients at deeper soil horizons (below 200 cm soil depth) tend to more consistently decrease as a result of forest degradation in DF and DP.

10.3 Changes in soil enzyme activities and implications for soil C, N and P cycle dynamics

Merino *et al.* (2020) has showed that SOM mineralization can be increased by the activities of peroxidase enzymes (POD) like LiP and MnP in volcanic-allophanic soils, and that POD enzymes activities could potentially be preserved in the soil matrix (clay minerals). In our soils peroxidase activities (POD) were significantly affected by the forest conversion, these higher activities displayed in all DP soil profile could be translated into higher CO_2 respired by soil and thus potentially C losses from this ecosystem (Figure 4 and 5). The higher concentration of C in the surface of DP could also explain the higher activity of this enzyme, but further analysis is needed to determine the quality of the carbon and SOM present in these soils.

In the same line, the big change registered for Glycine aminopeptidase (GAP) activities were also attributable to deforestation and growing dependence in mineral associated OM. As GAP enzyme activities are related to degradation of proteins, here higher activities were positively correlated with TC and TN contents registered at DP soils. Allison & Jastrow (2006) found that degradative enzymes (i.e. GAP and POD) were greatest in C-rich fractions of grassland soils and that this could be explained by a mechanism of physical isolation of enzymes and their C substrates at the molecular scale, thus sorption on mineral surfaces probably reduces actual enzyme activities in the soil well below the potential activities that were measured under assay conditions (Allison & Jastrow 2006). Enzymes could accumulate on clay surfaces via the same stabilization mechanism that decreases C turnover. It has been suggested that both enzymes and C are immobilized on or within soil minerals, thus enzymes could coexist with C substrates without degrading them (Zimmerman *et al.* 2004).

Dehydrogenase (DHA) activities has been widely used as bioindicator of soil disturbance. This enzyme breaks down SOM by oxidize them, and acts as a good indicator of overall microbial respiratory activity of soils. Our research showed that DHA enzyme activities at topsoil tends to be lower at soil of undisturbed condition (MF) and higher at disturbed forest soils (SF and DF), contrary to what was summarized by Utobo & Tewari (2015) where DHA activities were lower in devegetated soils than in undisturbed soils, and also between . This increment also could be related to a better SOM quality in this soils, water content or pH changes, however further research needs to be done to assess these changes in soil properties.

Overall, this suggests that soil enzyme activities (and soil microbiome, see chapter 1) are suitable indicators of the early changes in soil biogeochemical conditions after forest degradation and more important of forest land conversion. Among the enzyme activities that we assayed, Peroxidase (POD) and Glycine aminopeptidase (GAP) were the more sensitive to forest conversion, and that alterations in Dehydrogenase (DHA) activities reflects the effects of soil disturbance due to forest degradation,

hence these enzymes can be a useful bioindicators of LUC induced changes in soil quality and soil health.

11. CONCLUSIONS

Anthropogenic degradation of natural *Nothofagus* spp. forests decrease the soil TC, TN and P fractions belowground deeper than 200 cm soil depth.

Soil C:N:P stoichiometry tends to decrease with forest degradation and with forest to prairie conversion.

Extracellular Peroxidase (POD) and Glycine aminopeptidase (GAP) activities increase significatively in soil after forest conversion to prairie, but forest degradation does not show to has significant effect on their activities.

ACKNOWLEDGMENTS

The authors thank all the students, colleagues, and friends who helped in the fieldwork and sample pre-processing and the reviewer's comments and thoughts that helped improve this article. We also would like to thank the public-private collaborative project between ENEL-Universidad de Concepción and the Foresta Nativa Initiative that supported this study and the Faculty of Forest Sciences of the University of Concepción for allowing us to carry out this study in the BNP Ranchillo Alto.

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13. General Conclusions

Our research on the effect of native forest degradation by unregulated logging and conversion from forest to prairie allows us to evaluate and understand the state of quality and health of the soils of Andean temperate *Nothofagus* spp. forests. With this, it is evident that forest degradation causes alterations in the C, N and P reservoirs not only in the topsoil but also has effects as deep as 300 cm soil depth. Our research shows no significant differences in the C:N, C:P and N:P ratios along the forest degradation gradient, however a decrease in the C:N:P stoichiometry along the degradation gradient is evident, indeed this is more evident in the soils of forest to prairie conversion.

It is also evident that the forest degradation alters the vertical distribution of C, N and P in the evaluated sites at topsoil, and that this becomes stronger with the conversion from forest to prairie, being P the one that responds with greater strength.

Is also evidenced that each one of the forest states evaluated displayed a well-defined soil microbial community, which by one hand forested soils shared principal core members like *Rhizobiales* spp. and *Cortinarius* spp. wich are essencial microorganisms involved in the N cycle and SOM degradation respectively. On the other hand, the degraded prairie soils showed a disappearance of key microorganisms present in the forested soils and the incorporation of new microorganisms absent in these soils, mainly linked to livestock-grazing activity.

In addition to the mentioned above, a significant increase in the activities of two soil enzymes, Peroxidase (POD) and Glycine aminopeptidase (GAP) were evidenced due to the change from forest to prairie, and also an increase in Dehydrogenase (DHA) activity was observed due to forest degradation which is a signal of soil alterations.

Our research contributes relevant information to the understanding of soils affected by native forest degradation due to unregulated logging and ultimately their

conversion to prairie, a world common problem being experienced in south-central Chile. Our results indicate that forest degradation does not significantly affect some of the soil properties evaluated, but the tendency shows a decrease in soil quality and health associated properties, the ones that conversion to prairie strongly does.

Overall, our result remarks the importance of know the status of soils affected by forest degradation and LUC as an assessment tool for the evaluation of soil quality and health. Future research on this topic should consider the quantity and quality of C and N present in the litter and the SOM content as they are essential components of the extracellular soil enzymes activities and microorganism metabolism.

14. Supplementary data

Table S1. Alpha diversity index of trees in the natural forest degradation gradient (MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest). n = 100. DP did not meet analysis requirements. Table adapted from Cynthia V. et al. in preparation.

Diversity indexes	SITE		
	MF	SF	DF
Observed	30	29	33
Abundance	1468	2323	4195
H' (Shannon)	2.08	2.21	1.77
Fisher	5.3	4.7	4.89
D' (Simpson)	0.78	0.83	0.74
J' (Pielou)	0.61	0.66	0.50

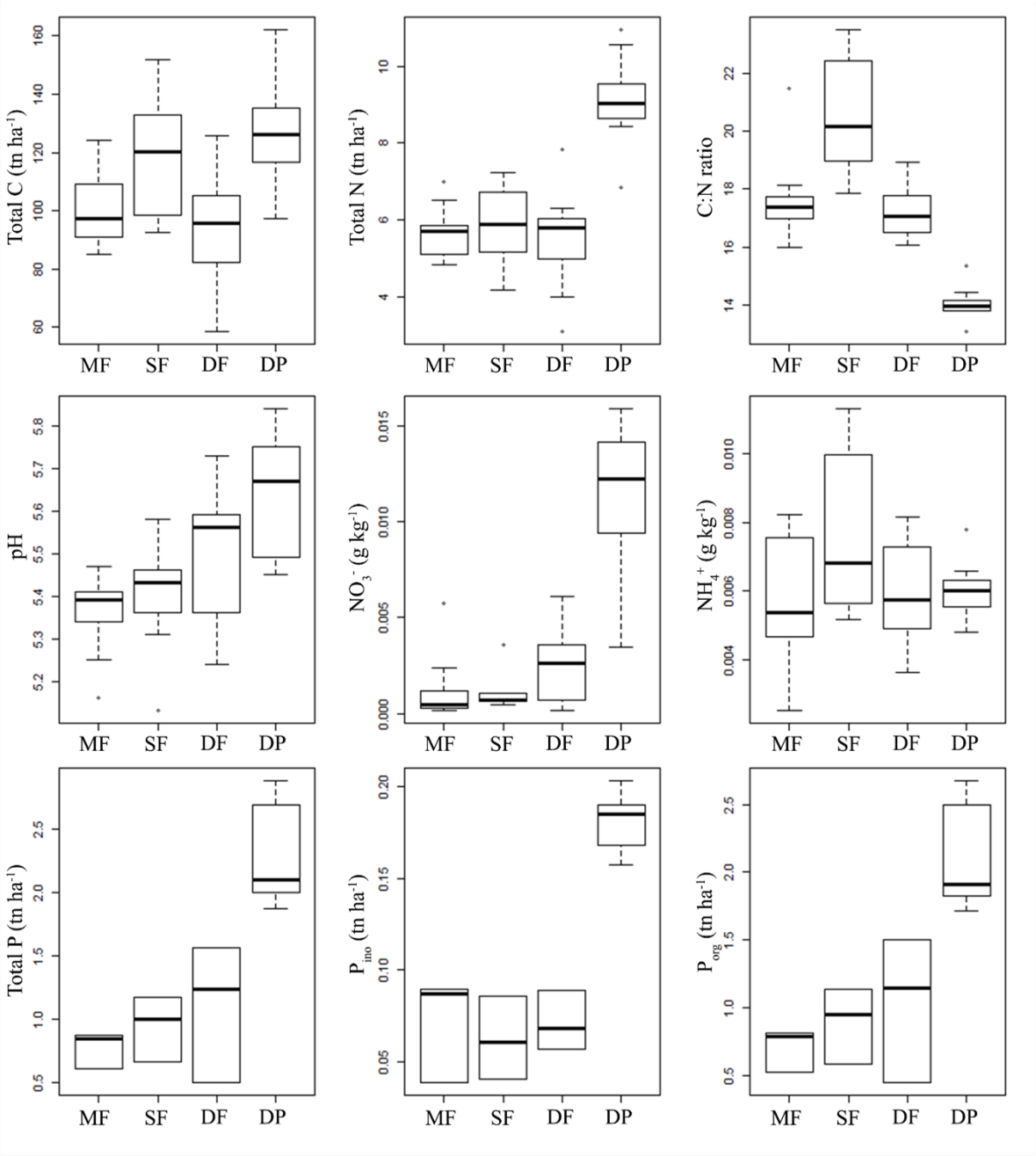

Figure S1. Soil nutrients content comparison at the 0-15cm soil depth interval in the forest degradation gradient (n=9). MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie. Dots outside the boxes are outliers.

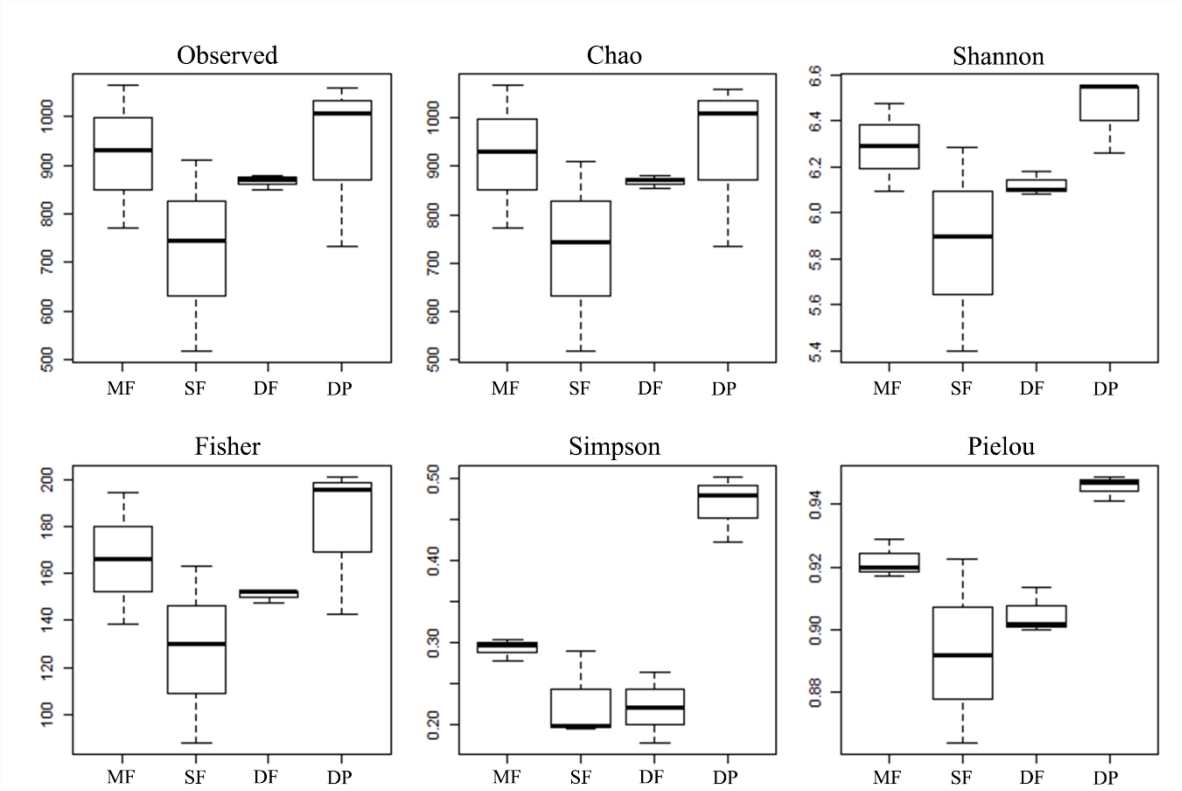

Figure S2. Alfa diversity index for soil bacteria community at the 0-15cm soil depth interval in the forest degradation gradient (n = 3). MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie.

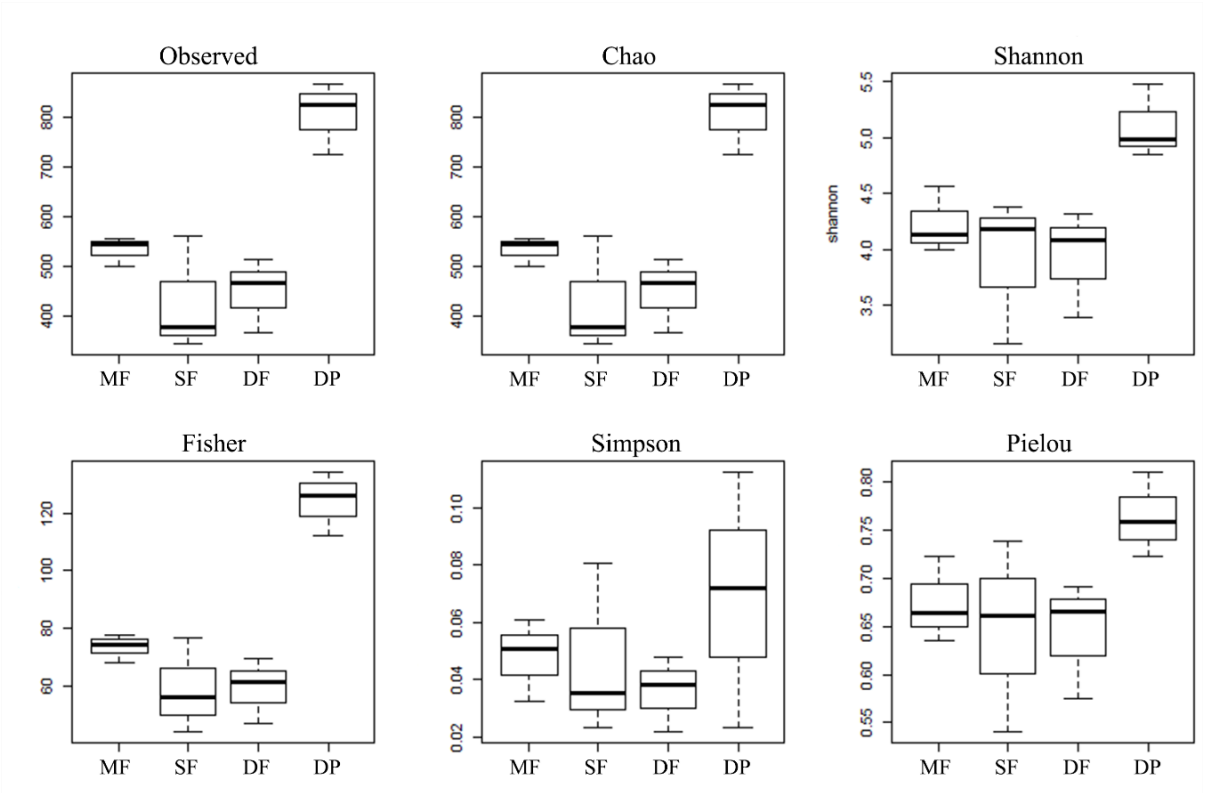

Figure S3. Alfa diversity index for soil fungi community at the 0-15cm soil depth interval in the forest degradation gradient (n = 3). MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie.

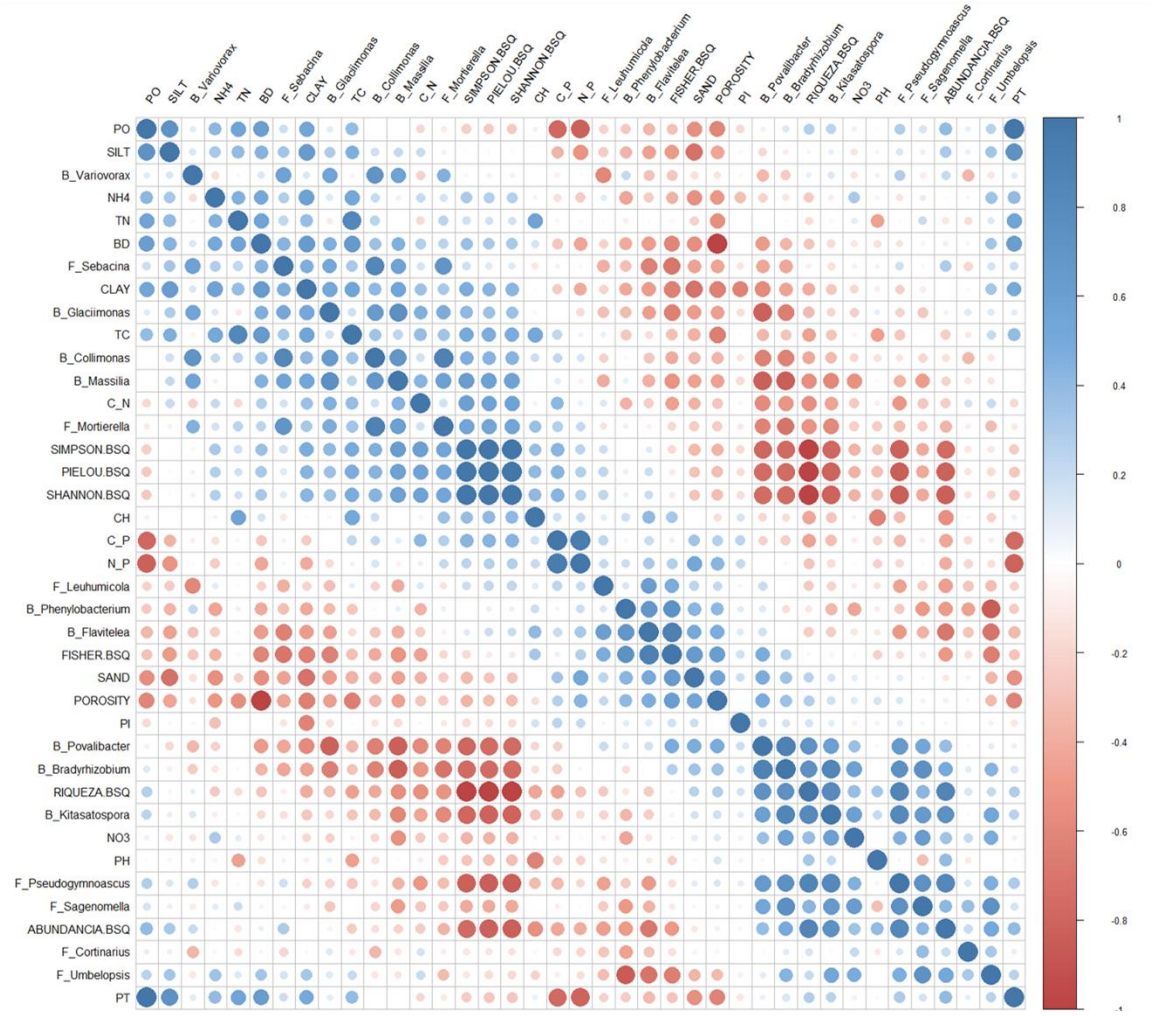

Figure S4. Correlation matrix for all data mesured in this research. BSQ: Forest; BAC: Bacteria; PT: Total phosphorus; Pi: Inorganic phosphorus; Po: Organic phosphorus; DB: Bulk density; TN: Total nitrogen; TC: Total carbon; C:N ratio; C:P ratio; N:P ratio; CH: Humidity; NO3: Nitrate; NH4: ammonium. Correlation matrix also contains the most abundant genera identified for Bacteria (B_) and Fungi (F_). Color scale at the right side indicates a positive (blue) or negative (red) correlation between data, while intensity and size of the color circle indicates if the correlation is strong or weak.

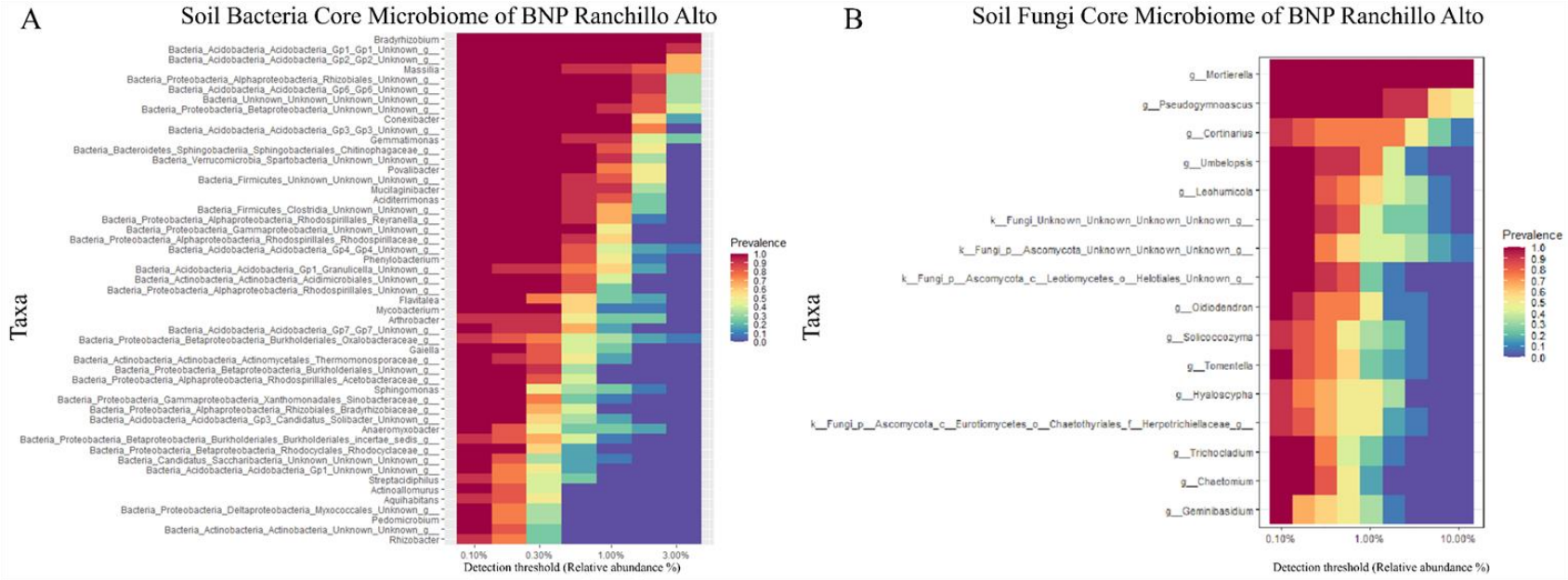

Figure S5 Soil core microbiomes for all the soils evaluated at the 0-15 cm soil depth interval in the forest degradation gradient at BNP Ranchillo Alto (MF, SF, Df and DP). OTUs identified at genus level with minimum detection limit adjusted to 0.1%. Heatmap color represent the prevalence value as percentage of the detected OTUs. **A)** Soil Bacteria core microbiome. **B)** Soil Fungi core microbiome. This heatmaps represent the most representative OTUs at genus level that are shared among all the soils in the degradation gradient.

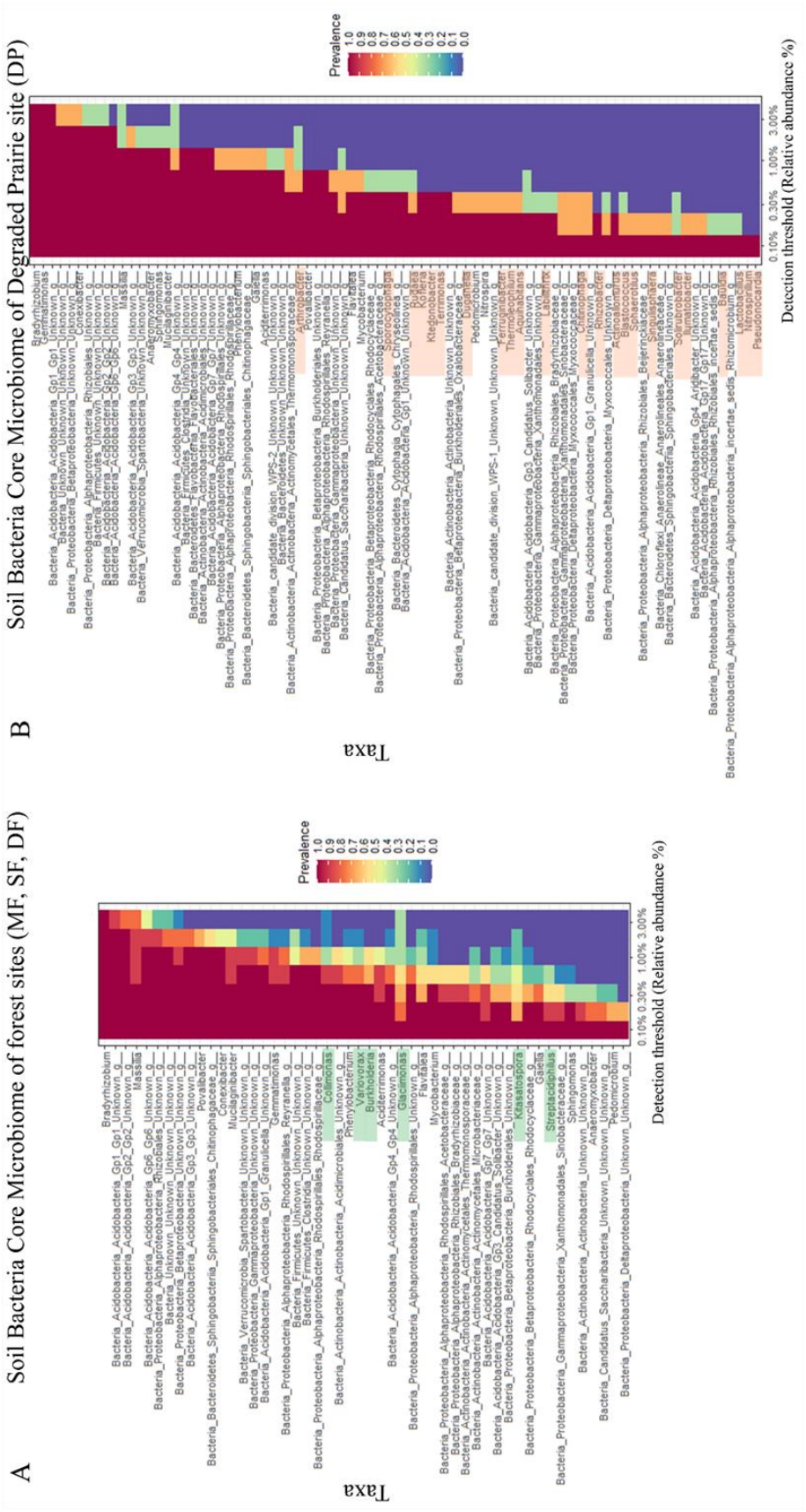

Figure S6. Soil core microbiome for Bacteria OTUs identified at genus level in the 0-15 cm soil depth in forest degradation gradient at BNP Ranchillo Alto. Minimum detection limit adjusted to 0.1%. **A)** Soil core microbiome of forest sites (MF, SF, DF). Green color highlights the bacterial genera that are only present as core members of the forest soils. **B)** Soil core microbiome of prairie site (DP). The orange color highlights the bacterial genera that are only present as core members of the degraded prairie soils.

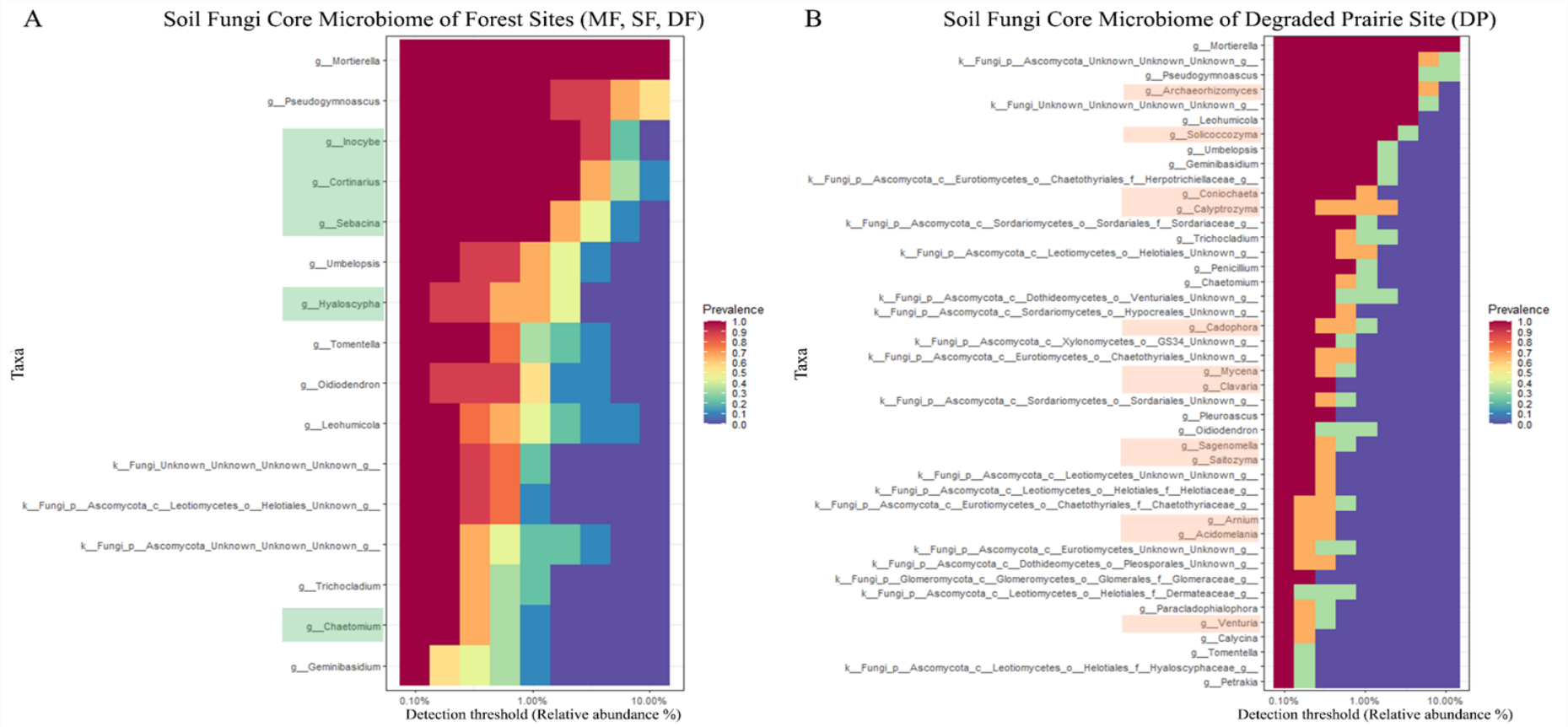

Figure S7. Soil core microbiome for Fungi OTUs identified at genus level in the natural forest degradation gradient at BNP Ranchillo Alto. Minimum detection limit adjusted to 0.1%. **A)** Soil core microbiome of forest sites (MF, SF, DF). Green color highlights the bacterial genera that are only present as core members of the forest soils. **B)** Soil core microbiome of prairie site (DP). The orange color highlights the bacterial genera that are only present as core members of the degraded prairie soils.

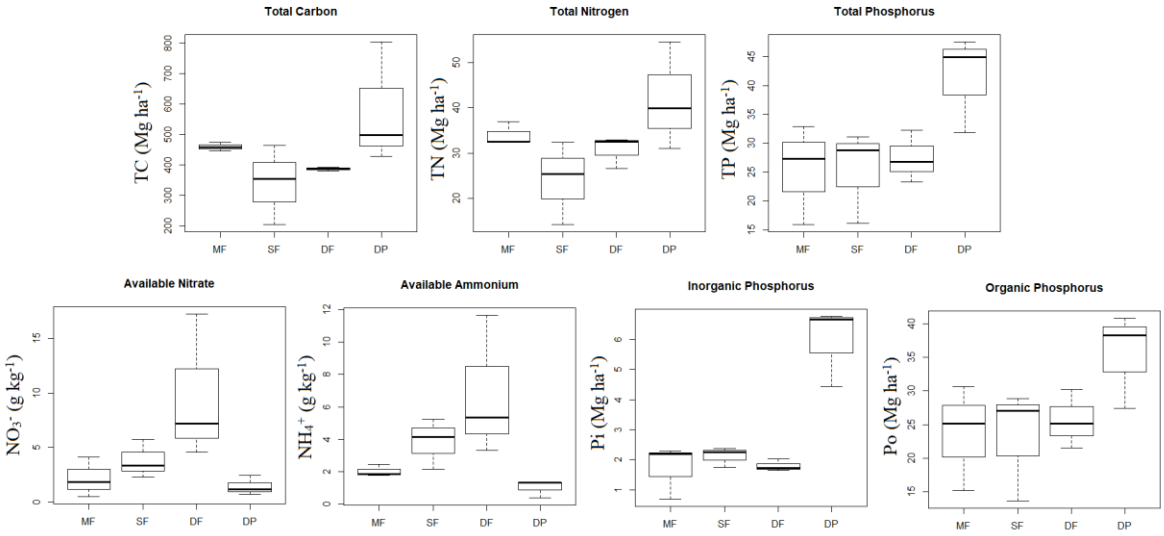

Figure S8. Soil TC and nutrients represented as total stock in the whole soil profiles in the forest degradation gradient. MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie; TC: Total carbon; TN: Total nitrogen; TP: Total phosphorus; Pi: Inorganic phosphorus; Po: Organic phosphorus; NO3: Nitrate; NH4: Ammonium. (N = 12).

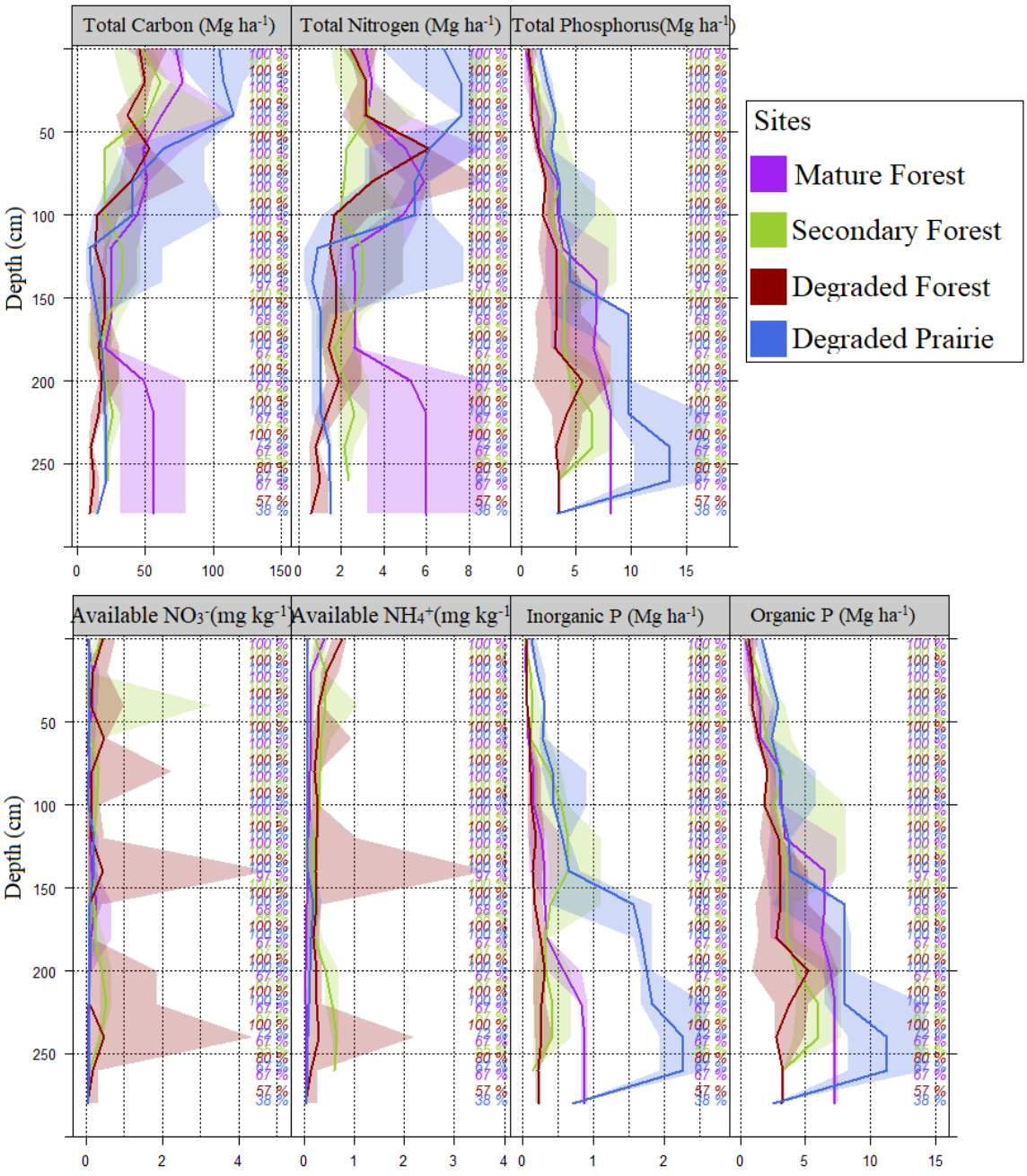

Figure S9. Total C, N, P, Pi and Po content and available Nitrate (NO_3^-) and Ammonium (NH_4^+) depth distribution in the forest degradation gradient represented as stocks (Mg ha^{-1}) . The solid-colored line corresponds to the median; shadow shows the data range (25^{th} and 75^{th} percentiles) for each sampled depth. At the right side of each plot appears the percentage of data that contributed for the median.

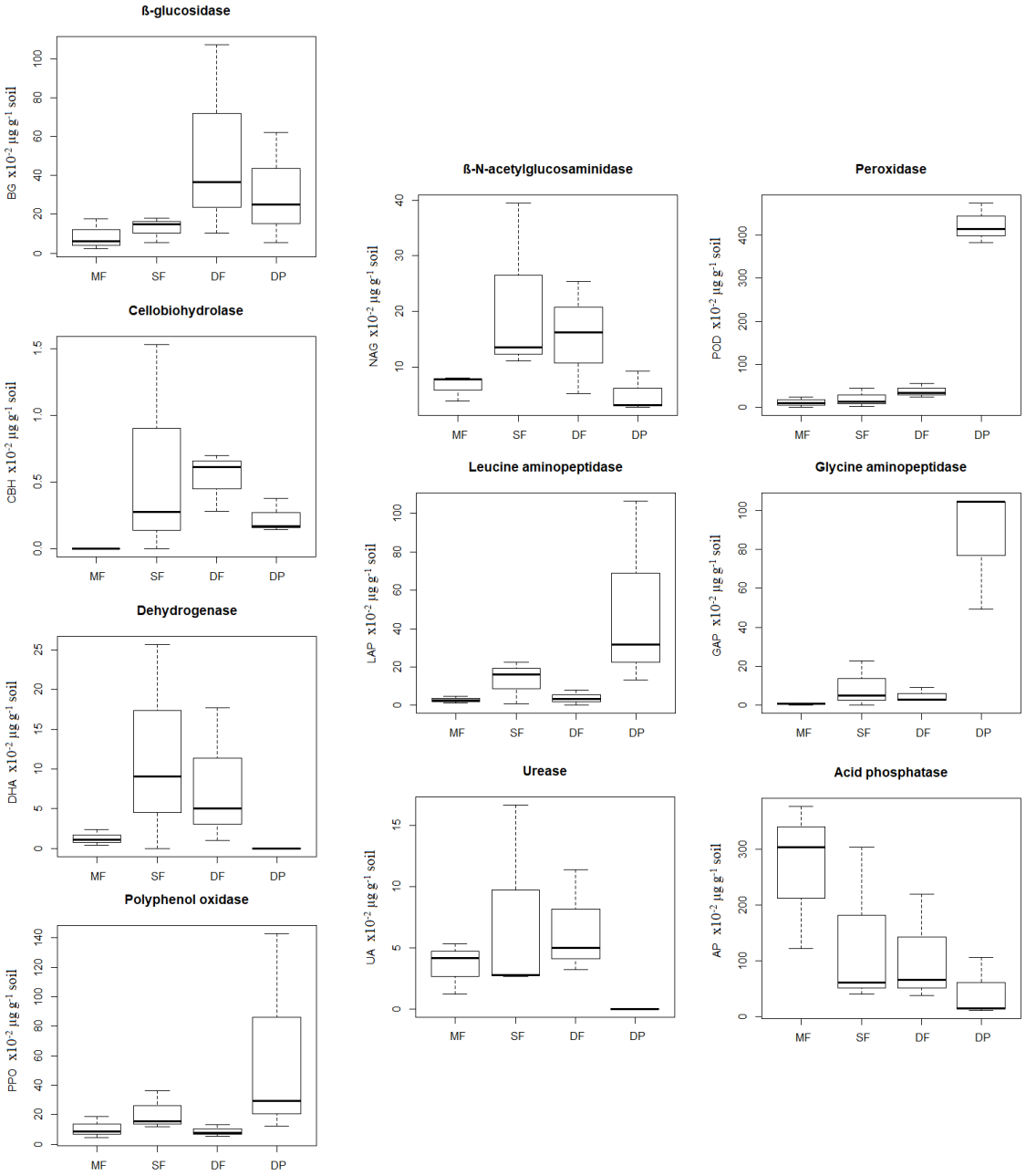

Figure S10. Soil extracellular enzymes activities expressed in millimole of substrate oxidized per minute ($x10^{-2}$ µg g^{-1} soil) in the forest degradation gradient. MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie; BG: β-glucosidase; CBH: Cellobiohydrolase; DHA: Dehydrogenase; PPO: Polyphenol oxidase; POD: Peroxidase; GAP: Glycine aminopeptidase; NAG: β-N-acetylglucosaminidase; LAP: Leucine aminopeptidase; UA: Urease; AP: Acid phosphatase. (N = 131)

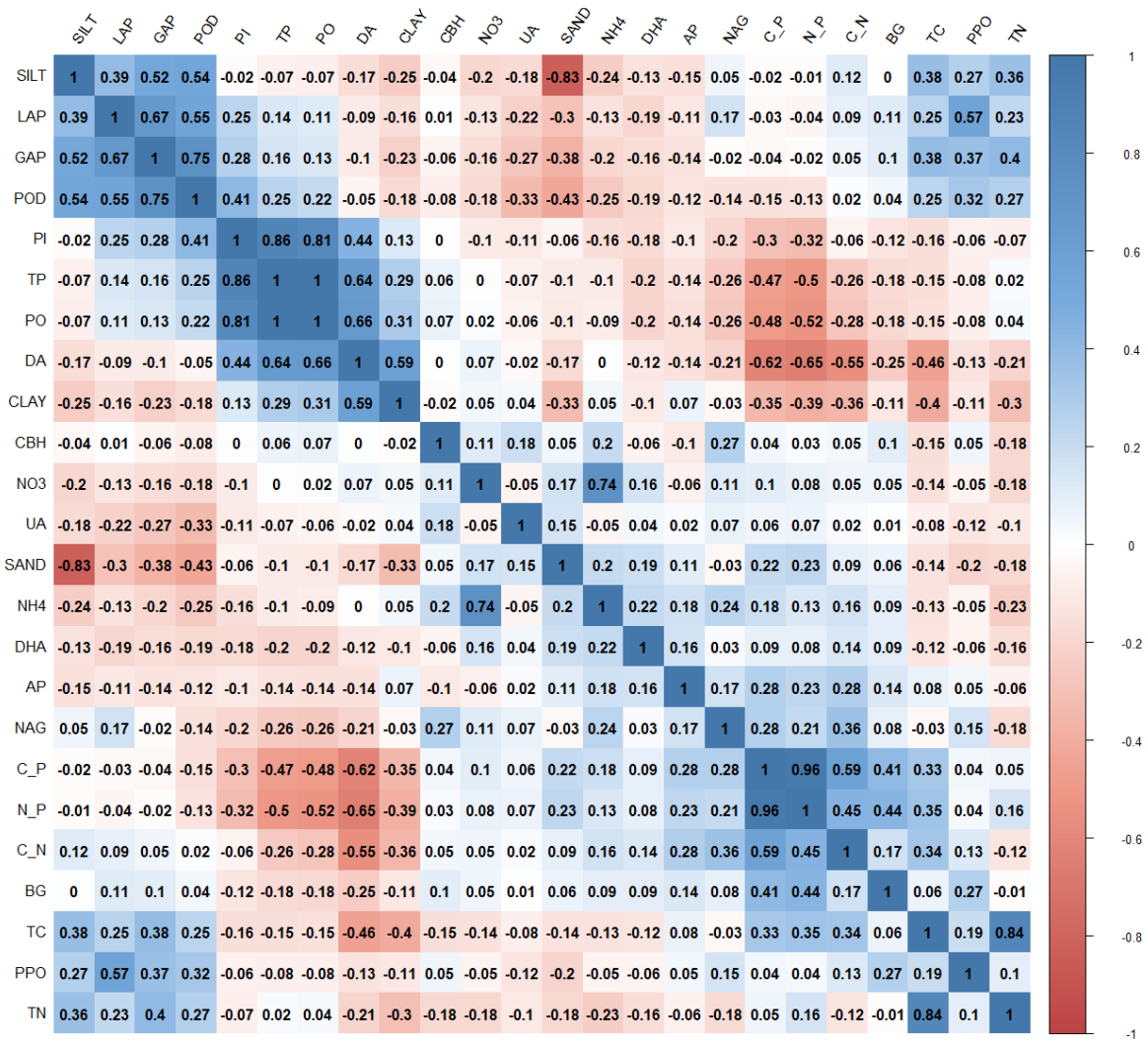

Figure S11. Correlation matrix for all variables measured in all the soil samples (n = 131) in this research. BSQ: Forest; BAC: Bacteria; PT: Total phosphorus; Pi: Inorganic phosphorus; Po: Organic phosphorus; DB: Bulk density; TN: Total nitrogen; TC: Total carbon; C:N ratio; C:P ratio; N:P ratio; CH: Humidity; NO3: Nitrate; NH4: ammonium. BG: β-glucosidase; CBH: Cellobiohydrolase; DHA: Dehydrogenase; PPO: Polyphenol oxidase; POD: Peroxidase; GAP: Glycine aminopeptidase; NAG: β-N-acetylglucosaminidase; LAP: Leucine aminopeptidase; UA: Urease; AP: Acid phosphatase. Color scale at the right side indicates a positive (blue) or negative (red) correlation between data, while intensity and size of the color circle indicates if the correlation is strong or weak.

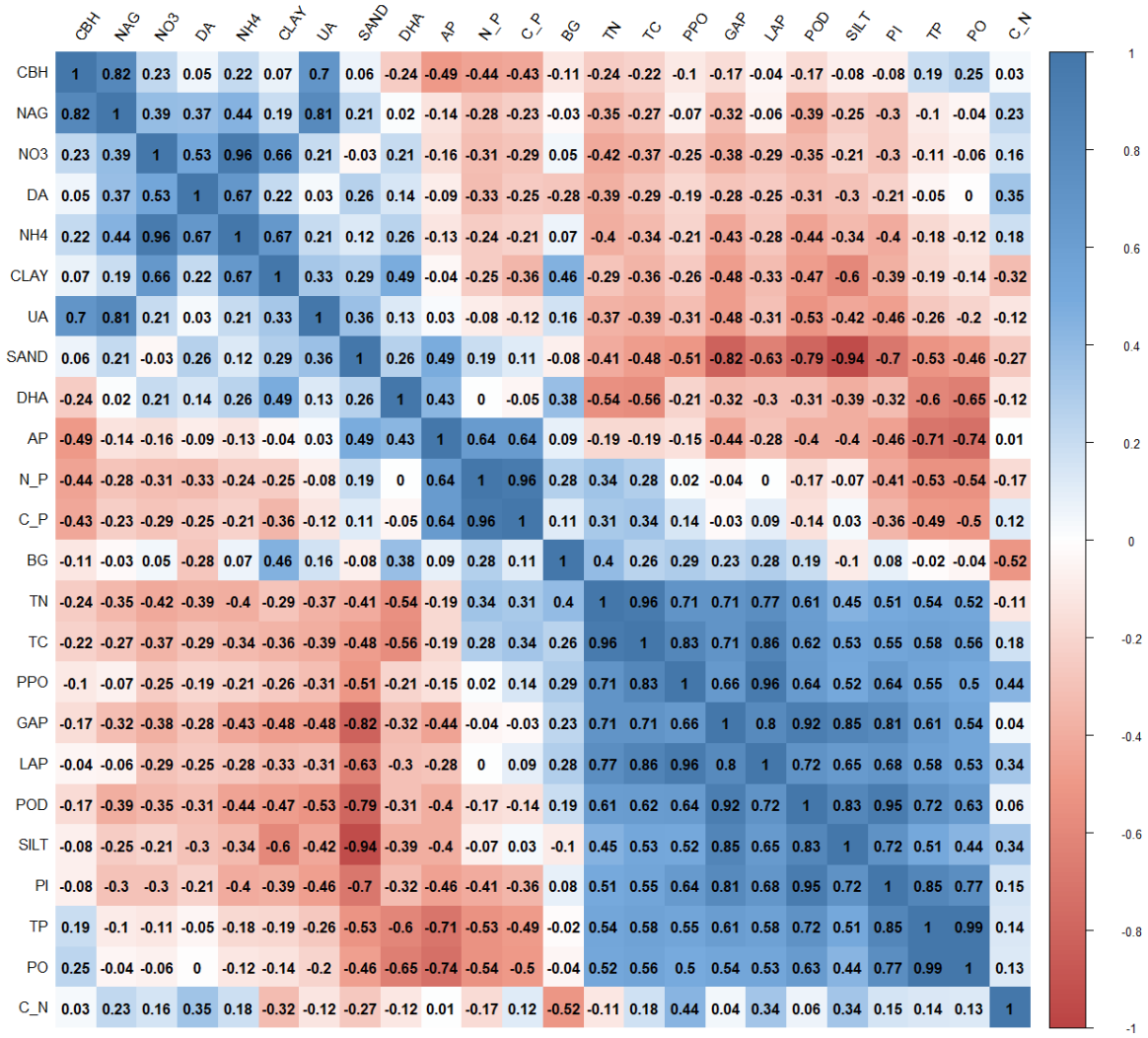

Figure S12. Correlation matrix for all variables measured expressed as a total stock in the whole soil profiles (n = 12) in this research. BSQ: Forest; BAC: Bacteria; PT: Total phosphorus; Pi: Inorganic phosphorus; Po: Organic phosphorus; DB: Bulk density; TN: Total nitrogen; TC: Total carbon; C:N ratio; C:P ratio; N:P ratio; CH: Humidity; NO3: Nitrate; NH4: ammonium. BG: β-glucosidase; CBH: Cellobiohydrolase; DHA: Dehydrogenase; PPO: Polyphenol oxidase; POD: Peroxidase; GAP: Glycine aminopeptidase; NAG: β-N-acetylglucosaminidase; LAP: Leucine aminopeptidase; UA: Urease; AP: Acid phosphatase. Color scale at the right side indicates a positive (blue) or negative (red) correlation between data, while intensity and size of the color circle indicates if the correlation is strong or weak.

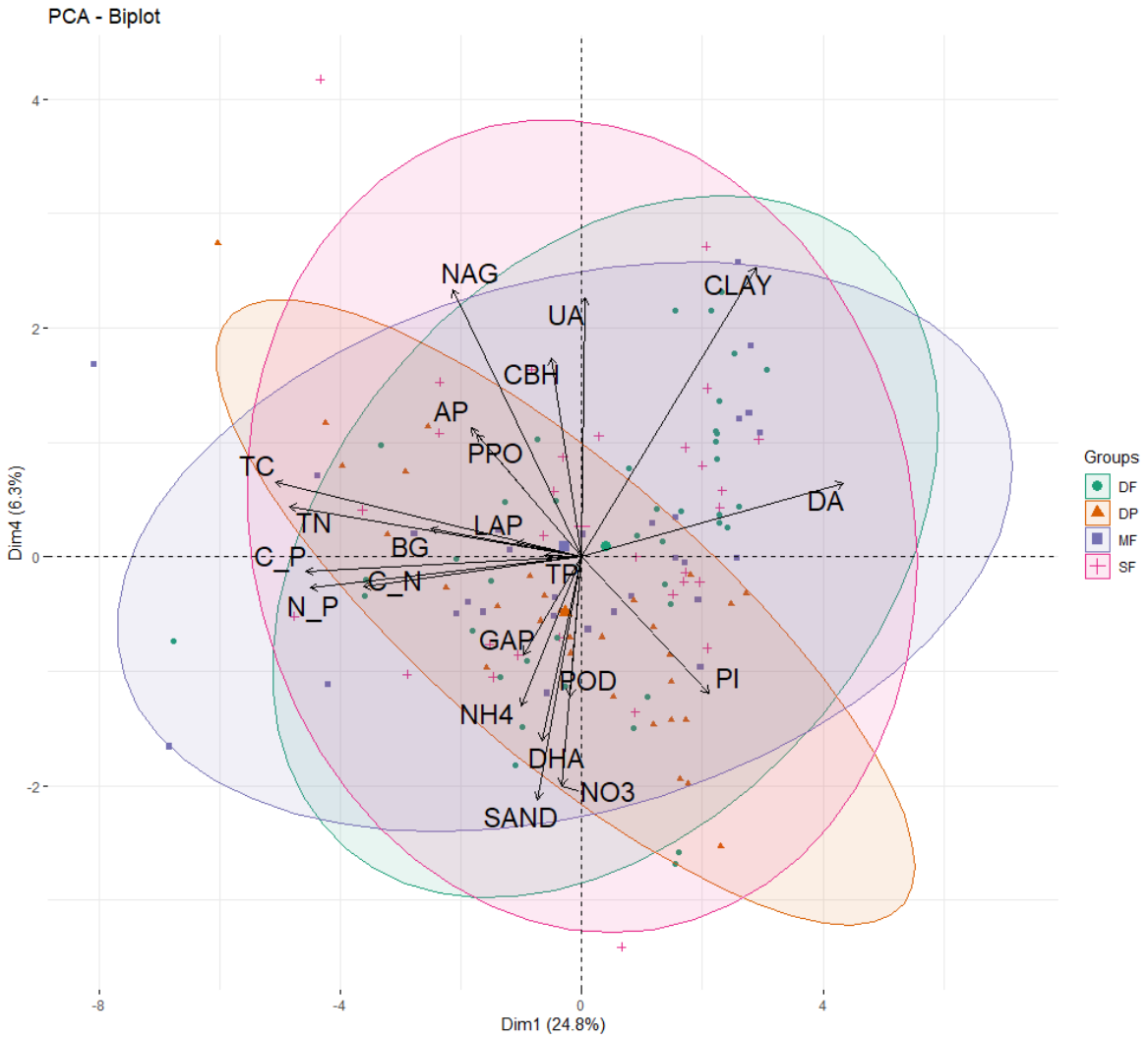

Figure S13. Principal component analysis (PCA) considering PC1 and PC4 of soils in the forest degradation gradient. Arrows represent the principal component and symbols represent each soil horizon grouped by LTER plot. Ellipses correspond to 95% confidence. MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie; TC: Total carbon; TN: Total nitrogen; TP: Total phosphorus; PI: Inorganic phosphorus; NO3: Nitrate; NH4: Ammonium; C:N ratio, C:P ratio; N:P ratio; BD: Bulk density; BG: β-glucosidase; CBH: Cellobiohydrolase; DHA: Dehydrogenase; PPO: Polyphenol oxidase; POD: Peroxidase; GAP: Glycine aminopeptidase; NAG: β-N-acetylglucosaminidase; LAP: Leucine aminopeptidase; UA: Urease; AP: Acid phosphatase.

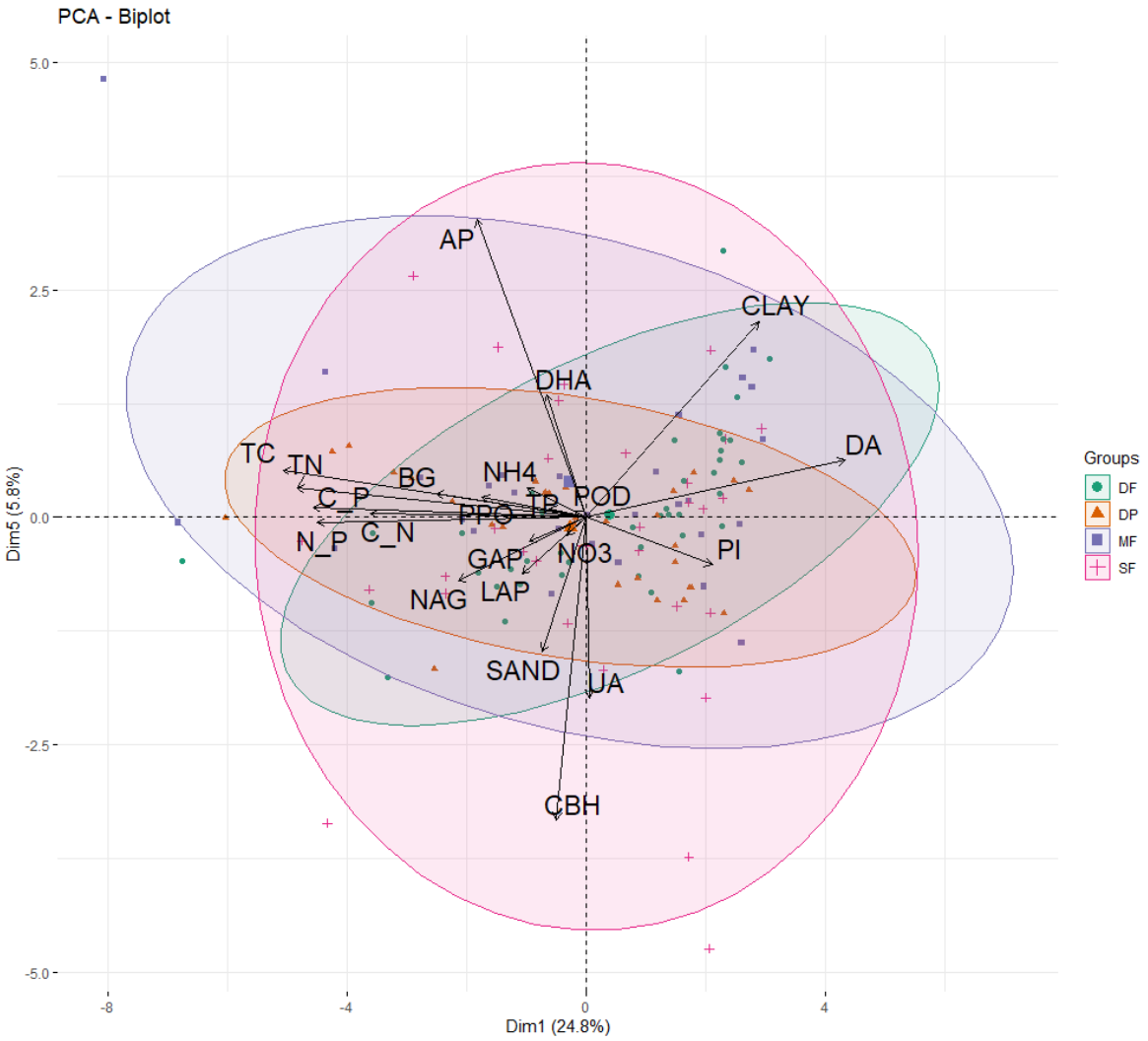

Figure S14. Principal component analysis (PCA) considering PC1 and PC5 of soils in the forest degradation gradient. Arrows represent the principal component and symbols represent each soil horizon grouped by LTER plot. Ellipses correspond to 95% confidence. MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie; TC: Total carbon; TN: Total nitrogen; TP: Total phosphorus; PI: Inorganic phosphorus; NO3: Nitrate; NH4: Ammonium; C:N ratio, C:P ratio; N:P ratio; BD: Bulk density; BG: β-glucosidase; CBH: Cellobiohydrolase; DHA: Dehydrogenase; PPO: Polyphenol oxidase; POD: Peroxidase; GAP: Glycine aminopeptidase; NAG: β-N-acetylglucosaminidase; LAP: Leucine aminopeptidase; UA: Urease; AP: Acid phosphatase.

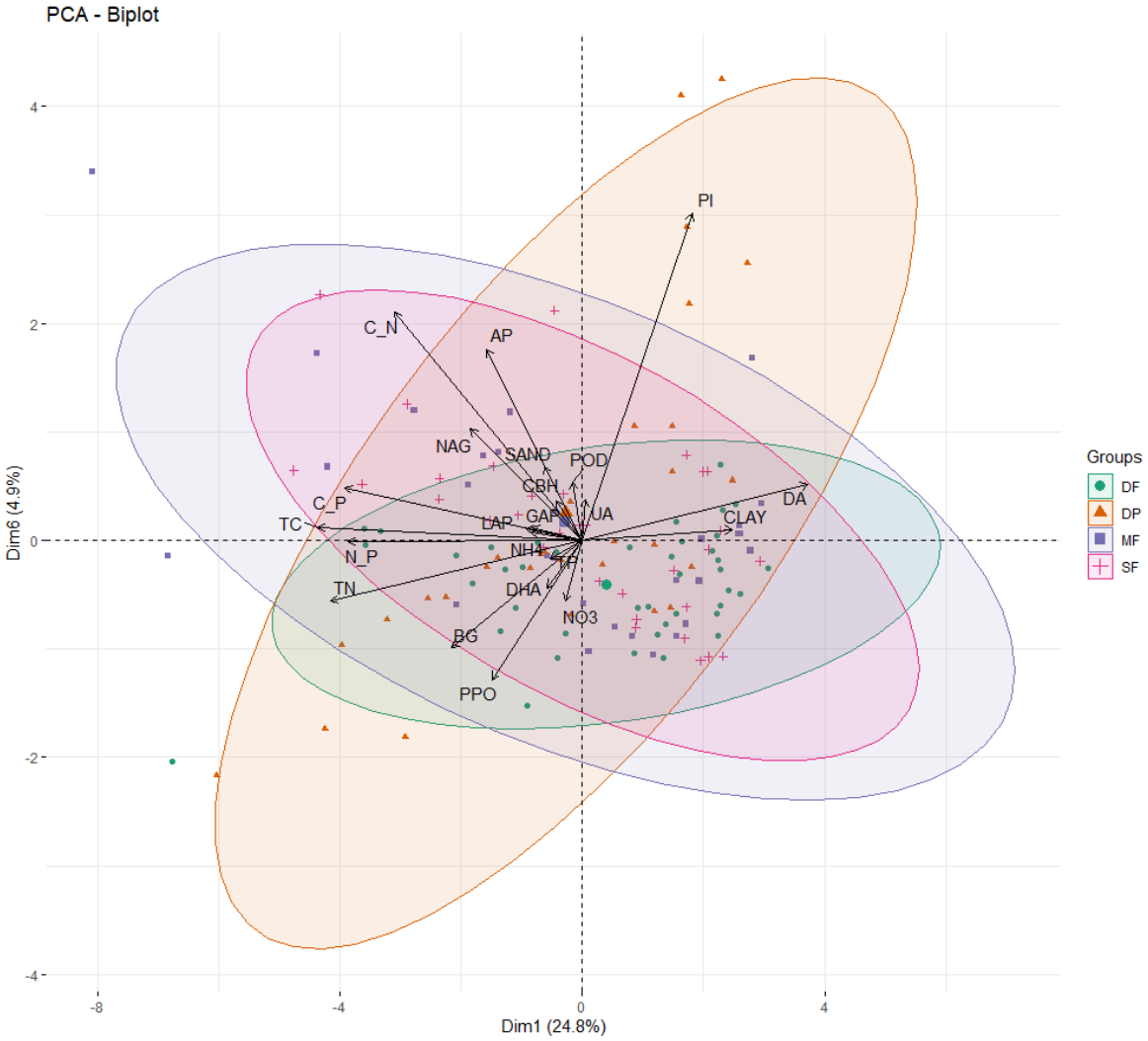

Figure S15. Principal component analysis (PCA) considering PC1 and PC6 of soils in the forest degradation gradient. Arrows represent the principal component and symbols represent each soil horizon grouped by LTER plot. Ellipses correspond to 95% confidence. MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie; TC: Total carbon; TN: Total nitrogen; TP: Total phosphorus; PI: Inorganic phosphorus; NO3: Nitrate; NH4: Ammonium; C:N ratio, C:P ratio; N:P ratio; BD: Bulk density; BG: β-glucosidase; CBH: Cellobiohydrolase; DHA: Dehydrogenase; PPO: Polyphenol oxidase; POD: Peroxidase; GAP: Glycine aminopeptidase; NAG: β-N-acetylglucosaminidase; LAP: Leucine aminopeptidase; UA: Urease; AP: Acid phosphatase.

Figure S16. Principal component analysis (PCA) considering PC1 and PC7 of soils in the forest degradation gradient. Arrows represent the principal component and symbols represent each soil horizon grouped by LTER plot. Ellipses correspond to 95% confidence. MF: Mature Forest; SF: Secondary Forest; DF: Degraded Forest; DP: Degraded Prairie; TC: Total carbon; TN: Total nitrogen; TP: Total phosphorus; PI: Inorganic phosphorus; NO3: Nitrate; NH4: Ammonium; C:N ratio, C:P ratio; N:P ratio; BD: Bulk density; BG: β-glucosidase; CBH: Cellobiohydrolase; DHA: Dehydrogenase; PPO: Polyphenol oxidase; POD: Peroxidase; GAP: Glycine aminopeptidase; NAG: β-N-acetylglucosaminidase; LAP: Leucine aminopeptidase; UA: Urease; AP: Acid phosphatase.