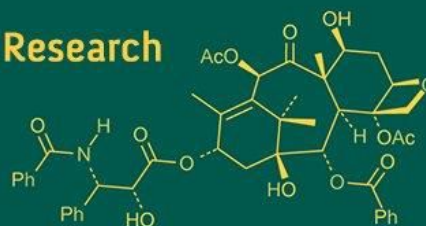


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Characterization of extracellular enzyme activities involved in organic matter decomposition and nutrient cycling in forest soils

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Abstract

While above-ground forest biodiversity has been widely documented, below-ground enzymatic processes driving organic matter breakdown remain less understood. This research characterized extracellular enzyme activities linked to carbon, nitrogen, and phosphorus cycling in three forest soil types within the Chaco-Pampa transition zone of central Argentina. Soil samples were collected at 0-20 cm depth across native dry forest, eucalyptus plantation, and mixed broadleaf woodland during wet and dry seasons between June 2023 and May 2024. Six enzymes were measured: β -glucosidase, cellulase, acid phosphatase, urease, dehydrogenase, and protease. Native forest soils had the highest overall activity, with β -glucosidase contributing 28.4% and acid phosphatase 22.1% of total activity. Eucalyptus plantations showed 31-39% lower activities than native forest, linked to litter quality differences. Wet season activities exceeded dry season values by 18-47%. Principal component analysis separated soil types along carbon- and phosphorus-cycling axes. Strong correlations emerged between organic carbon and β -glucosidase ($r = 0.84$, $p < 0.001$) and total nitrogen and urease ($r = 0.78$, $p < 0.01$). These findings indicate that conversion from native forest to monoculture plantations measurably reduces soil enzymatic capacity for nutrient cycling.

Keywords: Extracellular enzymes, forest soil, organic matter decomposition, nutrient cycling, β -glucosidase, acid phosphatase, seasonal variation, land use change, microbial biomass

Introduction

Forests cover roughly 31% of Earth's land surface and store an estimated 45% of terrestrial carbon, yet the biological machinery cycling that carbon through the soil remains poorly mapped in many regions [1]. Compared with extensive cataloguing of above-ground plant diversity, enzymatic processes controlling below-ground decomposition have received less attention in South American forest ecosystems [2].

Extracellular enzymes produced by soil microorganisms and plant roots are the main agents of organic matter breakdown [3]. They catalyze hydrolysis of complex polymers cellulose, proteins, and organic phosphorus compounds into molecules available for uptake [4]. Among the most commonly measured are β -glucosidase (carbon cycle), acid phosphatase (phosphorus cycle), urease (nitrogen cycle), and dehydrogenase as a general microbial activity indicator [5]. Their combined profiles serve as sensitive indicators of soil health [6].

Land use change is a major driver of enzyme alteration. Conversion of native forests to monoculture plantations has been linked to 20-55% reductions in key activities [7]. Argentina's Chaco region experienced rapid deforestation over recent decades, with native dry forest replaced by eucalyptus plantations and cropland [8]. But few investigations have tracked enzymatic consequences in the Chaco-Pampa transition zone [9]. Seasonal moisture also regulates enzyme production, with wet-dry cycles shifting microbial communities and expression patterns [10].

This research characterized six extracellular enzyme activities across three forest soil types in central Argentina over a full annual cycle, aiming to determine how land use and seasonal moisture affect nutrient cycling capacity [11, 12].

Research area description

Research sites were located in the Chaco-Pampa ecotone, approximately 45 km northwest of Córdoba city, Argentina (31°12'S, 64°28'W), at 480-540 m elevation. The sub-humid climate has mean annual temperature of 17.4°C and precipitation of 780 mm concentrated between October and March. Soils are *Typic haplustolls* with sandy loam texture. Three land cover types were selected within a 5 km radius: native dry Chaco forest (~80 years old), a 15-year-old *Eucalyptus camaldulensis* plantation, and a 40-year-old mixed broadleaf woodland. Each site covered at least 3 hectares with three 20 m × 20 m plots established randomly.

Material and Methods

Material

This research was conducted at the Environmental Biochemistry Laboratory, Department of Biological Sciences, Córdoba Institute of Applied Sciences, Córdoba, Argentina, from June 2023 to May 2024. Soil samples were collected bimonthly from nine plots (three sites × three replicates). At each plot, five cores (0-20 cm depth) were taken along a diagonal transect using a 5 cm auger and composited. Samples were sieved through 2 mm mesh within 24 hours, stored at 4°C for enzyme assays (within one week), and at -20°C for chemical analyses. All reagents were analytical grade from Sigma-Aldrich Argentina [13].

Result

Methods

β-Glucosidase and acid phosphatase were measured by incubating 1 g soil with p-nitrophenol-linked substrates at 37°C for 1 hour, read at 410 nm [14]. Cellulase used carboxymethylcellulose with DNS reagent at 540 nm after 24-hour incubation at 50°C [15]. Urease was assessed by ammonium release from urea (2 h, 37°C) using indophenol blue at 630 nm. Dehydrogenase measured TTC reduction to TPF (24 h, 37°C) at 485 nm [16]. Protease used casein hydrolysis (2 h, 50°C) with Folin-Ciocalteu at 700 nm. Organic carbon was determined by Walkley-Black oxidation, total nitrogen by Kjeldahl, and available phosphorus by Bray-1. Microbial biomass carbon was estimated by chloroform fumigation-extraction [17]. All measurements were triplicated. Data were analyzed by two-way ANOVA (land use × season), Tukey's HSD, Pearson correlations, and PCA using R v4.3.1 [18].

Seasonal sampling conditions

Sampling spanned six bimonthly campaigns covering dry season (June-September 2023) and wet season (October 2023-March 2024), with final collection in May 2024. Soil moisture ranged from 8.3% during peak dry (August) to 27.6% during peak wet (January). Soil temperature at 10 cm varied between 9.2°C (July) and 24.8°C (January), recorded using portable TDR probes and thermocouples.

Table 1: Mean extracellular enzyme activities (μg product g⁻¹ soil h⁻¹) across three forest types during wet (W) and dry (D) seasons

Enzyme	Native (W)	Native (D)	Eucal. (W)	Eucal. (D)	Mixed (W)	Mixed (D)
β-Glucosidase	187.3±14.2	142.8±11.6	135.6±12.8	98.4±9.3	162.4±13.1	121.7±10.4
Cellulase	124.6±9.8	96.3±8.1	88.2±7.4	67.1±6.9	108.5±8.6	84.2±7.7
Acid Phosph.	148.2±11.3	118.4±9.7	102.7±8.9	79.6±7.2	131.8±10.2	103.5±8.8
Urease	72.4±6.1	58.3±5.4	49.8±4.7	38.2±4.1	63.7±5.8	50.6±5.0
Dehydrogenase	93.1±7.8	78.9±6.5	64.3±5.9	52.1±5.3	81.4±7.1	68.7±6.2
Protease	31.8±3.4	21.6±2.8	22.4±2.6	14.7±2.1	27.3±3.1	19.2±2.5

Table 1 shows mean enzyme activities across forest types and seasons. Native forest had significantly higher activities for all six enzymes versus eucalyptus (*p*<0.05). β-

Glucosidase in native forest averaged 187.3 μg PNP g⁻¹ h⁻¹ in wet season, 38% above the eucalyptus value of 135.6.

Table 2: Soil chemical properties and microbial biomass carbon (annual mean ± SD)

Parameter	Native Forest	Eucalyptus	Mixed Woodland
Organic Carbon (%)	3.84 ± 0.31	2.17 ± 0.24	3.12 ± 0.28
Total Nitrogen (%)	0.34 ± 0.04	0.19 ± 0.03	0.27 ± 0.03
Available P (mg kg ⁻¹)	18.7 ± 2.3	11.4 ± 1.8	15.2 ± 2.1
pH (H ₂ O)	6.42 ± 0.18	5.87 ± 0.21	6.18 ± 0.16
MBC (μg C g ⁻¹)	487.3 ± 42.1	298.6 ± 35.4	401.8 ± 38.7
C:N Ratio	11.3	11.4	11.6

Table 2 presents soil chemical properties. Organic carbon was highest in native forest (3.84%) and lowest in

eucalyptus (2.17%), with mixed woodland intermediate at 3.12%.

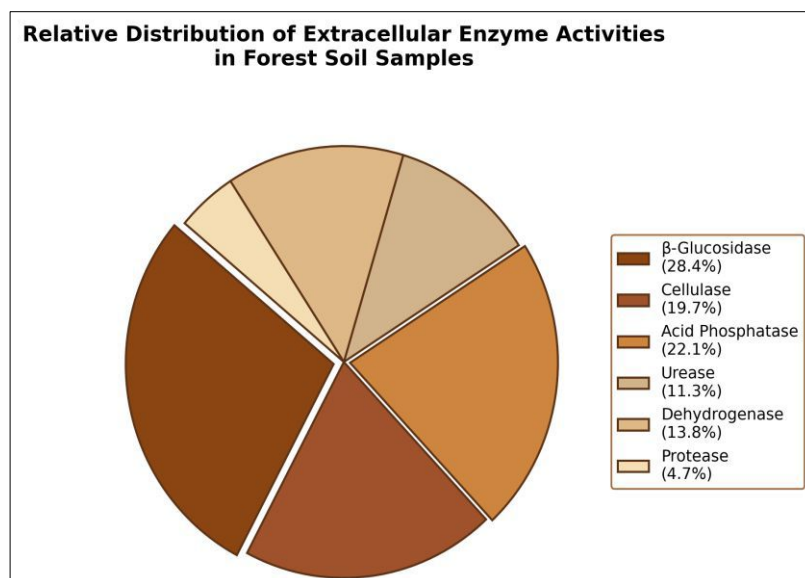


Fig 1: Proportional contribution of six extracellular enzymes to total activity across all forest soil types and seasons.

Figure 1 shows each enzyme's proportional contribution. β-Glucosidase was largest at 28.4%, followed by acid phosphatase at 22.1%, indicating carbon and phosphorus cycling dominated.

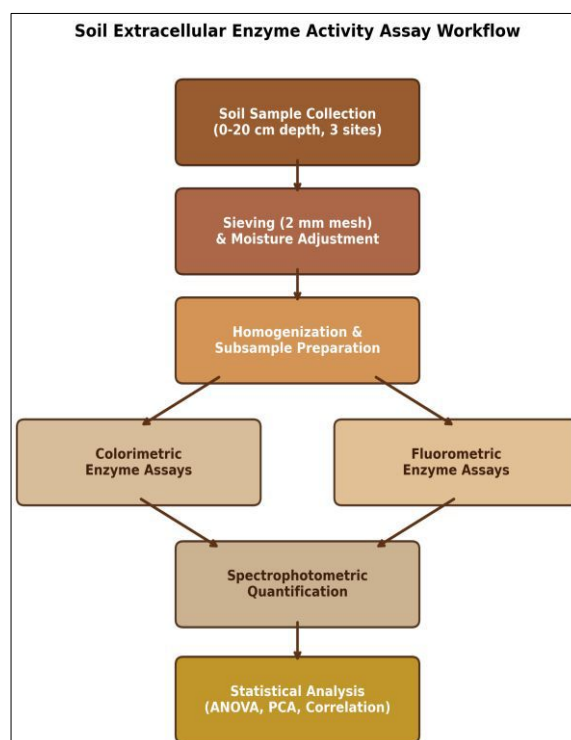


Fig 2: Analytical workflow for soil extracellular enzyme activity characterization.

Comprehensive Interpretation

PCA separated soil types along axes explaining 71.3% of variance. PC1 (46.8%) reflected carbon-cycling enzymes, PC2 (24.5%) phosphorus-cycling. Pearson correlations showed organic carbon-β-glucosidase $r = 0.84$ ($p < 0.001$), nitrogen-urease $r = 0.78$ ($p < 0.01$), and microbial biomass-dehydrogenase $r = 0.81$ ($p < 0.001$).

Discussion

Higher enzyme activities in native Chaco forest align with reports from subtropical dry forests showing undisturbed ecosystems maintain more active microbial communities [1, 3]. The 31-39% reduction under eucalyptus likely reflects

lower quality leaf litter containing elevated phenolic compounds that slow microbial colonization [7].

The strong organic carbon-β-glucosidase correlation ($r = 0.84$) reinforces established links between substrate availability and hydrolytic activity. β-Glucosidase catalyzes the final cellulose breakdown step and responds directly to carbon input quality [5]. Native forest's highest values for both organic carbon and enzyme activity support a positive feedback between litter inputs and decomposition capacity. Seasonal moisture markedly affected all enzymes, with wet season values 18-47% higher. This matches findings from semi-arid forests where soil rewetting triggers microbial growth pulses [10]. Protease showed the largest seasonal

contrast (47%), possibly because protein mineralization depends on moisture-mediated substrate diffusion^[16].

PCA separation of land use types along functional axes suggests enzyme profiling can serve as a diagnostic tool for assessing soil degradation after forest conversion^[9].

Conclusion

This research demonstrated that land use conversion from native Chaco forest to eucalyptus plantation reduced extracellular enzyme activities by 31-39%, with carbon-cycling enzymes showing the greatest declines. Native forest soils maintained the highest organic carbon, microbial biomass, and enzymatic capacity across all seasons.

Seasonal moisture played a strong role, with wet season activities 18-47% higher. Protease and β -glucosidase were most moisture-responsive, highlighting decomposition dependence on water availability in sub-humid environments.

Enzyme activity profiles analyzed through PCA effectively distinguished land use types and may serve as practical soil health indicators. Strong correlations between specific enzymes and soil properties support a targeted enzyme approach for nutrient cycling assessment. Future work should track enzyme recovery in abandoned plantation soils reverting to secondary forest.

References

- Baldrian P. Microbial enzyme-catalyzed processes in soils and their analysis. *Plant, Soil and Environment*. 2009;55(9):370-378.
- Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, *et al.* Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biology and Biochemistry*. 2013;58:216-234.
- Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, *et al.* Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*. 2008;11(11):1252-1264.
- Allison SD, Vitousek PM. Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biology and Biochemistry*. 2005;37(5):937-944.
- Tabatabai MA, Dick WA. Enzymes in soil: research and developments in measuring activities. In: Burns RG, Dick RP, editors. *Enzymes in the environment*. New York: Marcel Dekker; 2002. p. 567-596.
- Nannipieri P, Giagnoni L, Landi L, Renella G. Role of phosphatase enzymes in soil. In: Bütemann E, Oberson A, Frossard E, editors. *Phosphorus in action*. Berlin: Springer; 2011. p. 215-243.
- Waldrop MP, Balser TC, Firestone MK. Linking microbial community composition to function in a tropical soil. *Soil Biology and Biochemistry*. 2000;32(13):1837-1846.
- Grau HR, Gasparri NI, Aide TM. Agriculture expansion and deforestation in seasonally dry forests of north-west Argentina. *Environmental Conservation*. 2005;32(2):140-148.
- Nogueira MA, Albino UB, Brandao-Junior O, Braun G, Cruz MF, Dias BA, *et al.* Promising indicators for assessment of agroecosystems alteration among natural, reforested and agricultural land use in southern Brazil. *Agriculture, Ecosystems and Environment*. 2006;115(1-4):237-247.
- Austin AT, Yahdjian L, Stark JM, Belnap J, Porporato A, Norton U, *et al.* Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia*. 2004;141(2):221-235.
- Caldwell BA. Enzyme activities as a component of soil biodiversity: a review. *Pedobiologia*. 2005;49(6):637-644.
- Zak DR, Holmes WE, White DC, Peacock AD, Tilman D. Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology*. 2003;84(8):2042-2050.
- Kandeler E, Eder G. Influence of cattle slurry in grassland on microbial biomass and on activities of various enzymes. *Biology and Fertility of Soils*. 1993;16(4):249-254.
- Eivazi F, Tabatabai MA. Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry*. 1988;20(5):601-606.
- Miller M, Palojarvi A, Rangger A, Reeslev M, Kjoller A. The use of fluorogenic substrates to measure fungal presence and activity in soil. *Applied and Environmental Microbiology*. 1998;64(2):613-617.
- Casida LE, Klein DA, Santoro T. Soil dehydrogenase activity. *Soil Science*. 1964;98(6):371-376.
- Vance ED, Brookes PC, Jenkinson DS. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*. 1987;19(6):703-707.
- R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2023.
- Treseder KK, Vitousek PM. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology*. 2001;82(4):946-954.
- German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biology and Biochemistry*. 2011;43(7):1387-1397.